

Characterization of important odorants in four steamed *Coilia ectenes* from China by gas chromatography–mass spectrometry–olfactometry

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Abstract Odorants were extracted from four *Coilia ectenes* (Yangtze River-*Coilia*, East China sea-*Coilia*, Chao Lake-*Coilia* and Yellow River-*Coilia*) by MonoTrap™ and analyzed by the headspace-monolithic material sorptive extraction technique combined with gas chromatography–mass spectrometry–olfactometry (GC–MS–O). A total of 63 volatile compounds were identified. The results of GC–MS–O analysis associated with odor intensity revealed that trimethylamine (fishy, ammonia-like), 1-penten-3-ol (mushroom, cabbage), *N,N*-diethyl-formamide (roasted meaty), hexanal (grassy, earthy), ethylbenzene (nutty, floral), (*Z*)-4-heptenal (fishy, boiled potato), benzaldehyde (almond, metallic), 1-octen-3-ol (mushroom, cabbage), nonanal (oily), and decanal (green, oily) were the important odorants in the four species of *Coilia ectenes*. Furthermore, trimethylamine and 1-penten-3-ol were common to four *Coilia ectenes*. TMA (fishy, ammonia-like), 1-penten-3-ol (mushroom, cabbage), *N,N*-diethyl-formamide (roasted meaty), hexanal (grassy, earthy), (*Z*)-4-heptenal (fishy, boiled potato), and benzaldehyde (almond, metallic) were included in Yangtze River-*Coilia*; TMA, 1-penten-3-ol, ethylbenzene (nutty, floral), (*Z*)-4-heptenal, 1-octen-3-ol (mushroom, earthy), and nonanal (oily) in East China sea-*Coilia*; TMA, 1-penten-3-ol, ethylbenzene, 1-octen-3-ol and nonanal in Chao Lake-*Coilia*; and TMA, 1-penten-3-ol,

hexanal, (*Z*)-4-heptenal, 1-octen-3-ol and decanal (green, oily) in Yellow River-*Coilia*.

Keywords *Coilia ectenes* · Headspace-monolithic material sorptive extraction · GC–MS–O · MonoTrap™ · Important odorants

Introduction

Coilia ectenes related to Osteichthyes, Clupeiformes, and Engraulidae, is an important fish species that migrates from near-ocean waters to freshwater rivers every spring. In China, *Coilia ectenes* is mainly classified into the Jianghai migratory type and Lake settlement type according to its survival mode. The Jianghai migratory type migrates periodically between river and sea for some reason, which includes physiological requirements, genetic, and environment factors. The Jianghai migratory type includes Yangtze River-*Coilia* and East China sea-*Coilia*. The lake settlement type is a kind of *Coilia ectenes* living in lakes all through its lifetime, including Chao Lake-*Coilia* and Yellow River-*Coilia* [1]. It was reported that some *Coilia ectenes* species do not migrate to freshwater rivers due to genetic or environment factors, so there are significant differences between the river-anchovy and sea-anchovy [2]. *Coilia ectenes* has a high economic value for its distinctive aroma, intensive umami taste, and high nutritional value. However, its declining production fails to meet the market demands due to overfishing and environmental pollution, thereby contributing to the rise in prices (Yangtze River-*Coilia* is 8000–12000 yuan/kg, i.e., approximately 1333–2000 US\$/kg) and to the development of *Coilia ectenes* farming.

MonoTrap™ (MT) is a novel absorbent composed of silica gel, activated carbon, and octadecyl silane (ODS).

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It is widely used in the extraction of polar and non-polar compounds as well as those with high and low boiling points. When the samples pass through the pores of the silica monolithic structure, they are trapped by the ODS groups, which are bound to the silica surface or the activated carbon present inside and outside the structure. This mode of absorption is called monolithic material sorptive extraction (MMSE), which is similar to solid-phase micro extraction (SPME) and stir bar sorptive extraction (SBSE). The headspace MMSE (HS-MMSE) with a larger specific surface area and porous surface [3], has higher adsorption efficiency than the headspace SPME (HS-SPME) and the headspace SBSE (HS-SBSE). HS-MMSE has been applied to plants, coffee, tea, sesame oil, and dairy products [4].

The GC–MS–O technology, which was first proposed by Fuller in 1964, is a useful method for evaluation of odorants from complex materials, including the frequency detection method, the dilution method and the direct intensity method [5]. The frequency detection method was used to detect the odor frequency by assessors and described by Linssen in 1993 [6]. The dilution method is the dilution level at which flavor compounds cannot be smelled by the human nose [7]. The direct intensity method could offer several advantages of reducing sniffing time and errors by recording the changes of odor intensities directly, which is superior to frequency detection and the dilution method [8]. Several researchers have analyzed odor-active compounds in seafoods by GC–MS–O, such as the New Zealand sea urchin *Evechinus chloroticus* [9], rainbow trout *Oncorhynchus mykiss* [10], and Yangtze River-*Coilia* [11]. However, few works have focused on the study of flavor compounds in *Coilia ectenes* by GC–MS–O associated with odor intensity.

The objective of this study was to identify the important odorants in steamed Yangtze River-*Coilia*, East China sea-*Coilia*, Chao Lake-*Coilia* and Yellow River-*Coilia* by GC–MS–O (direct intensity method) as associated with the odor intensity method. This study may provide a theoretical basis for further research and development in breeding technologies of *Coilia ectenes* or other aquatic products.

Materials and methods

Materials

Ten Yangtze River-*Coilia* (average weight and length 109.5 ± 7.7 g and 26.3 ± 0.6 cm, respectively) and ten East China sea-*Coilia* (average weight and length 106.1 ± 4.5 g and 25.9 ± 1.1 cm, respectively) were purchased from the Xiaohu Aquatic Products Market (Jiangsu, China) in April 2012; ten Chao Lake-*Coilia* (average weight and length 25.2 ± 9.4 g and 20.4 ± 2.0 cm, respectively) were purchased

from Fuhuang Sungem Food Company (Anhui, China) in October 2012; and ten Yellow River-*Coilia* (average weight and length 91.5 ± 6.4 g and 29.9 ± 1.7 cm, respectively) were purchased from Dongying Jingming Aquatic Products Company (Shandong, China) in April 2012.

All specimens were transported to the laboratory with dry ice, fishbone and liver were removed, and then stored at -80 °C until required for analysis.

C₆–C₃₀ *n*-alkanes standards were purchased from Sigma–Aldrich Trading Co., Ltd (Shanghai, China). Other reagents used were of analytical grade and purchased from Anpel Laboratory Technologies Co., Ltd (Shanghai, China).

HS-MMSE for volatile compounds extraction

Yangtze River-*Coilia*, East China sea-*Coilia*, Chao Lake-*Coilia*, and Yellow River-*Coilia* were rinsed under running water, eviscerated, steamed for 20 min to guarantee the *Coilia ectenes* was fully cooked, and minced. After cooling to room temperature, the *Coilia ectenes* were homogenized in an ice-bath condition (Model JZ-II, Tianjin Sifang Equipment Ltd, China). Prior to use, MonoTrap™ rods were conditioned in an oven at 250 °C for 30 min to remove any impurities and inserted into the stainless steel MT holders using a set of clean tweezers to avoid contamination, and the stainless steel MT holder was inserted into the MT stand. Nine MT rods were applied in this study for achieving the greatest extraction of volatile compounds in *Coilia ectenes*. A clean septum was passed through the end of the MT holder using clean tweezers, and a cap was placed on the top of the holder. MT rods were placed at a fixed position in the headspace of a 15 mL-vial (item Nr. VAAP-38015E-1760A-100, Shanghai Anpu Scientific Instrument Co., Ltd.) containing approximately 5.0 g homogenized sample, and a septum was hermetically sealed on the vial, which was kept in a heat-gathering style magnetism mixer for 60 min at 80 °C. Volatile compounds were exposed and adsorbed to both side surfaces of the MT rods. Following adsorption, the MT rods were removed and immediately placed carefully in an adsorption tube, then desorbed via a thermal desorption unit (TDU, Gerstel, Baltimore, Md., USA) and injected via a cold injection system (CIS, Gerstel, Baltimore, Md., USA). The TDU temperature was programmed at 180 °C/min from 50 to 270 °C and maintained for 5 min; the CIS temperature was programmed at 12 °C/s from -40 to 250 °C and maintained for 0.5 min.

Gas chromatography–mass spectrometry–olfactometry (GC–MS–O)

A gas chromatography–mass spectrometer (Model 6980, Agilent Inc., USA) was coupled to an ODP 2 sniffing

port (Gerstal). The effluent from the capillary column was split (1:1.5 v/v) between the mass spectrometry detector (MSD) and the ODP-2. Separations in the GC were performed on a DB-5MS capillary column (60 m length \times 0.25 mm internal diameter \times 0.25 μ m film thickness; Agilent Inc., USA), using helium (99.999 % space purity) as the carrier gas (1.2 mL/min). The oven temperature was programmed from 40 to 100 °C at a rate of 5 °C/min, then increased to 180 °C at a rate of 2 °C/min, followed by an increase at 5 °C/min to 240 °C and maintained for 5 min.

MS conditions were as follows: detector interface temperature was 250 °C, ion source temperature was 230 °C, ionization energy was 70 Ev, mass range was 40–450 amu, electron multiplier voltage was 1576 V, and scan rate was 1.8/s. Samples were desorbed at 270 °C with a TDU (Gerstal) directly into the hot injector (250 °C) of the CIS (Gerstal) with simultaneous cryofocusing using liquid nitrogen.

In the GC–MS–O study, ten experienced assessors with previous sniffing experience (five male and five females, 22–30 years of age) were chosen as candidates in the GC–MS–O panelist selection. Based on the guidelines established by Pollien and others [12], three assessors (one male and two females) were required to take part in GC–MS–O detection due to their high sensitive olfaction and recorded the aroma characteristics and aroma strength of each sample. Each sample was smelled twice by the assessors. The mean of odor intensity was calculated and the final odor intensities were obtained from the mean values of at least two assessors. During a 50-min sniffing period per sample, assessors ranked the intensity of each odor on a four-point intensity scale: 1 = weak, 2 = moderate, 3 = strong, and 4 = very strong [8].

Statistical analyses

All detections had been replicated three times in this study. Results were expressed as mean \pm standard deviation (SD) ($n = 3$). The SPSS 19.0 software (SPSS Inc., Chicago, Ill., USA) was applied to check if there were significant differences ($p < 0.05$) between four *Coilia ectenes* based on one-way analysis of variance (ANOVA).

Volatile compounds were analyzed by matching the mass spectra (Wiley/NIST 2008) and the linear retention index (LRI) with reference values and odor properties. The LRI was calculated by the following equation,

$$LRI = \left(\frac{Rt(x) - Rt(n)}{Rt(n+1) - Rt(n)} + n \right) \times 100,$$

where $Rt(x)$ is the retention time of each volatile compound (x), $Rt(n)$ and $Rt(n + 1)$ are the retention times of

n -alkanes eluting directly before and after the compound (x) under identical chromatographic conditions.

Results

The total ion chromatogram obtained from *Coilia ectenes* samples is shown in Fig. 1. A total of 63 volatile compounds were identified in steamed meat of Yangtze River-*Coilia*, East China sea-*Coilia*, Chao Lake-*Coilia*, and Yellow River-*Coilia* on a DB-5ms column (Table 1): including 19 aldehydes, 11 ketones, 11 alcohols, 8 aromatics, 5 hydrocarbons, 6 N- and S- containing compounds, and 3 furans. Among them, 49 were found in Yangtze River-*Coilia*, 31 in East China sea-*Coilia*, 30 in Chao Lake-*Coilia*, and 43 in Yellow River-*Coilia*. On comparing the compounds found in *Coilia ectenes*, we found that 20 volatile compounds were common to all four *Coilia ectenes*. Thirteen volatile compounds (propanal, 2-methyl-2-butenal, (E)-2-pentenal, 2-ethyl-4-pentenal, 2-heptanone, 2,3-octanedione, 5-methyl-2-heptanone, heptanol, (E)-2-octen-1-ol, 2-ethyl-4-methyl-1-pentanol, undecane, *N,N*-dimethyl-formamide, and *N,N*-diethyl-formamide) were found exclusively in Yangtze River-*Coilia*, two volatile compounds (2-butanone, 1-pentadecene) were found in Chao Lake-*Coilia*, and 9 volatile compounds (2,2-dimethyl-propanal, heptanal, 1H-pyrrole-2,5-dione, 5-ethyl-2(5H)-furanone, limonene, 2-ethyl-1H-pyrrole, 2-propyl-furan, 2-(2-pentenyl)furan, hexadecanoic acid, methyl ester) were found in Yellow River-*Coilia*.

A total of 35 odorants (including 33 volatile compounds and 2 unknown compounds) were identified in four *Coilia ectenes* by the olfactometric analysis (Table 2). Among all the odorants, there were 19 compounds in Yangtze River-*Coilia*, 21 compounds in East China sea-*Coilia*, 16 compounds in Chao Lake-*Coilia* and 20 compounds in Yellow River-*Coilia*. Based on the direct intensity method, assessors use a scale to measure the perceived intensity of a compound as it elutes, those odorants whose intensities were greater than or equal to 3 could be regarded as important odorants. Results showed that the important odorants including TMA (fishy, ammonia-like), 1-penten-3-ol (mushroom, cabbage), *N,N*-diethyl-formamide (roasted meaty), hexanal (grassy, earthy), (Z)-4-heptenal (fishy, boiled potato) and benzaldehyde (almond, metallic) in Yangtze River-*Coilia*; TMA, 1-penten-3-ol, ethylbenzene (nutty, floral), (Z)-4-heptenal, 1-octen-3-ol (mushroom, earthy) and nonanal (oily) in East China sea-*Coilia*; TMA, 1-penten-3-ol, ethylbenzene, 1-octen-3-ol and nonanal in Chao Lake-*Coilia*, TMA, 1-penten-3-ol, hexanal, (Z)-4-heptenal, 1-octen-3-ol and decanal (green, oily) in Yellow River-*Coilia*.

Fig. 1 Total ion chromatograms of volatile compounds of four steamed *Coilia ectenes* **a** Yangtze river-*Coilia*, **b** East China sea-*Coilia*, **c** Chao lake-*Coilia*, **d** Yellow river-*Coilia*

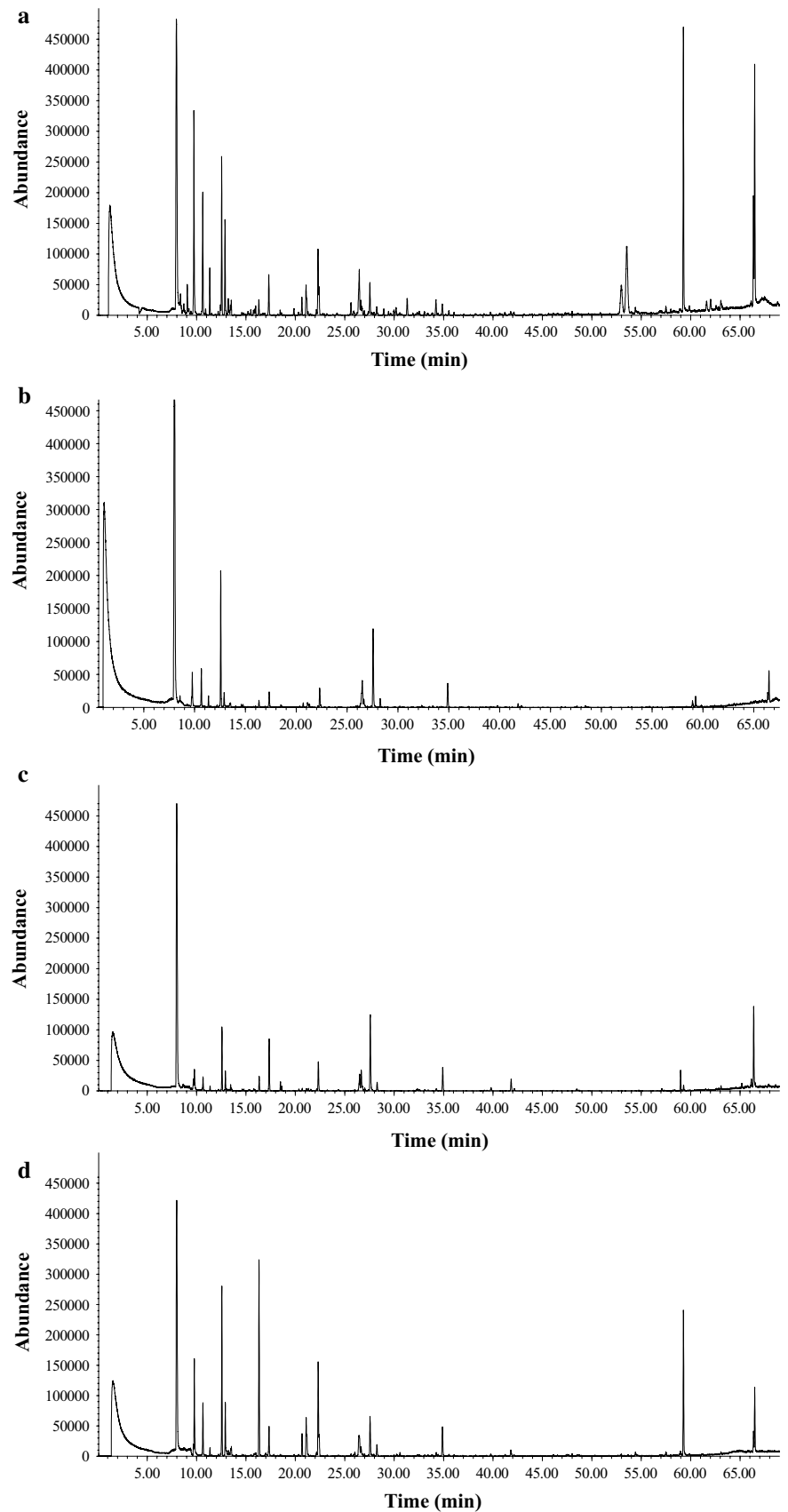


Table 1 Odor thresholds and peak area of volatile compounds identified in four steamed *Coilia ectenes* ($n = 3$)

LRI ^b	Compounds	Identification ^c	Odor threshold ($\mu\text{g}/\text{kg}$) ^d	Peak area (10^3)			
				A	B	C	D
Aldehydes (19)							
<600	Propanal	MS	0.69 [1]	17.44 \pm 2.02a	ND	ND	ND
657	3-Methylbutanal	MS, LRI	1.6 [2]	8.77 \pm 1.69a	3.24 \pm 1.04b	3.70 \pm 0.38b	4.79 \pm 0.35b
697	2,2-Dimethyl-propanal	MS, LRI	NA	ND	ND	ND	12.75 \pm 2.35a
700	2-Methylbutanal	MS	10 [3]	20.79 \pm 1.53a	9.85 \pm 1.66b	9.92 \pm 1.77b	ND
743	2-Methyl-2-butenal	MS, LRI	NA	2.86 \pm 0.36a	ND	ND	ND
754	(E)-2-Pentenal	MS	0.98 [4]	9.28 \pm 1.43a	ND	ND	ND
802	Hexanal ^a	MS,LRI	NA	91.99 \pm 6.82b	43.91 \pm 5.34c	103.83 \pm 7.44b	115.07 \pm 12.11a
853	(E)-2-Hexenal	MS, LRI	NA	20.00 \pm 3.74a	ND	ND	13.75 \pm 1.41b
895	2-Ethyl-4-pentenal	MS, LRI	NA	15.07 \pm 3.34a	ND	ND	ND
901	(Z)-4-Heptenal	MS, LRI	3.4 [5]	21.58 \pm 2.21a	2.98 \pm 0.54b	ND	24.05 \pm 4.25a
904	Heptanal	MS, LRI	6 [6]	ND	ND	ND	28.23 \pm 3.67a
975	Benzaldehyde ^a	MS, LRI	10 [7]	165.95 \pm 28.22a	116.10 \pm 6.15b	82.91 \pm 4.78c	171.87 \pm 17.53a
1005	Octanal ^a	MS, LRI	0.88 [8]	17.63 \pm 0.17c	28.51 \pm 2.11b	27.34 \pm 3.12b	46.26 \pm 2.74a
1016	(E,E)-2,4-Heptadienal	MS, LRI	57 [1]	16.85 \pm 1.96a	ND	ND	12.04 \pm 1.44b
1084	3-Methyl-benzaldehyde	MS, LRI	NA	6.14 \pm 0.52b	ND	9.31 \pm 1.03a	7.73 \pm 0.92b
1107	Nonanal ^a	MS, LRI	2 [9]	44.30 \pm 4.57c	99.33 \pm 5.43b	92.29 \pm 2.93b	141.73 \pm 5.46a
1179	4-Ethyl-benzaldehyde ^a	MS, LRI	NA	7.60 \pm 1.14c	2.58 \pm 0.66d	11.83 \pm 1.09b	16.94 \pm 3.11a
1208	Decanal ^a	MS, LRI	2.6 [9]	14.15 \pm 2.41c	36.70 \pm 3.12b	38.07 \pm 3.27b	64.04 \pm 5.79a
1370	2-undecenal	MS, LRI	NA	ND	18.51 \pm 3.89b	ND	21.04 \pm 2.45a
Ketones (11)							
<600	2-Butanone	MS, LRI	NA	ND	ND	3.96 \pm 0.75a	ND
693	2,3-Pentanedione	MS, LRI	NA	31.88 \pm 4.39a	4.36 \pm 1.01c	ND	16.22 \pm 2.14b
696	3-Pentanone	MS	0.06 [10]	7.95 \pm 1.46a	ND	ND	7.86 \pm 0.73a
825	3-Cyclohepten-1-one	MS, LRI	NA	8.77 \pm 1.10a	3.80 \pm 0.89b	ND	7.01 \pm 1.22a
888	2-Heptanone	MS, LRI	62 [11]	4.08 \pm 1.17a	ND	ND	ND
980	2,3-Octanedione	MS, LRI	NA	30.20 \pm 4.21a	ND	ND	ND
984	1H-Pyrrole-2,5-dione	MS, LRI	NA	ND	ND	ND	8.64 \pm 1.33a
1038	5-Ethyl-2(5H)-furanone	MS, LRI	NA	ND	ND	ND	9.53 \pm 2.03a
1079	Acetophenone	MS, LRI	NA	9.18 \pm 1.57a	ND	2.47 \pm 0.35c	8.03 \pm 1.13a
1091	5-Methyl-2-heptanone	MS, LRI	NA	5.94 \pm 1.82a	ND	ND	ND
1098	(E,E)-3,5-Octadien-2-one	MS, LRI	NA	34.45 \pm 5.76a	ND	6.99 \pm 1.23c	21.98 \pm 2.38b
Alcohols (11)							
<600	1-Propanethiol	MS	NA	ND	19.00 \pm 2.13b	ND	23.39 \pm 3.24a
681	1-Penten-3-ol ^a	MS, LRI	4300 [1]	196.10 \pm 4.22b	33.15 \pm 3.72c	43.91 \pm 5.31c	239.51 \pm 13.78a
735	2-Methyl-1-butanol	MS	0.33 [10]	7.22 \pm 1.95a	7.53 \pm 1.22a	6.48 \pm 1.13b	ND
761	1-Pentanol ^a	MS, LRI	360 [9]	10.76 \pm 1.57a	7.04 \pm 0.57b	5.33 \pm 0.86c	7.07 \pm 0.23b
765	(Z)-2-Penten-1-ol	MS	NA	12.52 \pm 1.86a	ND	ND	8.82 \pm 1.28b
825	(E)-3-Octen-2-ol	MS, LRI	NA	2.73 \pm 1.28b	ND	30.58 \pm 3.45a	ND
863	1-Hexanol	MS	1.61 [4]	9.20 \pm 0.14a	2.65 \pm 0.68c	6.93 \pm 0.61b	ND
966	Heptanol	MS	0.45 [10]	18.26 \pm 6.49a	ND	ND	ND
978	1-Octen-3-ol ^a	MS, LRI	48 [1]	43.05 \pm 4.13b	19.23 \pm 2.34c	63.10 \pm 4.21a	49.79 \pm 4.75b

Table 1 continued

LRI ^b	Compounds	Identification ^c	Odor threshold ($\mu\text{g}/\text{kg}$) ^d	Peak area (10^3)			
				A	B	C	D
1000	(E)-2-Octen-1-ol	MS	0.04 [12]	8.24 \pm 2.27a	ND	ND	ND
1025	2-Ethyl-4-methyl-1-pentanol	MS, LRI	NA	8.39 \pm 1.10a	ND	ND	ND
Aromatics (8)							
669	Benzene ^a	MS, LRI	1500 [13]	329.21 \pm 31.58a	233.28 \pm 12.65c	192.86 \pm 16.35c	276.95 \pm 25.33b
776	Toluene ^a	MS, LRI	1300 [9]	44.74 \pm 1.81b	17.55 \pm 2.42c	57.33 \pm 3.22b	87.10 \pm 11.34a
870	Ethylbenzene ^a	MS, LRI	26 [8]	58.05 \pm 8.71b	11.89 \pm 1.43c	7.62 \pm 1.23c	112.12 \pm 6.91a
879	<i>p</i> -Xylene ^a	MS	1 [14]	38.33 \pm 8.71a	22.24 \pm 1.76b	8.18 \pm 0.48c	6.53 \pm 1.21c
904	Styrene ^a	MS	0.05 [14]	223.92 \pm 50.31b	46.80 \pm 4.23d	140.94 \pm 6.35c	370.70 \pm 24.67a
972	Phenol	MS	58.59 [15]	ND	10.43 \pm 1.31a	6.40 \pm 0.32b	ND
1041	Limonene	MS	0.2 [4]	ND	ND	ND	18.84 \pm 1.45a
1214	Naphthalene ^a	MS, LRI	40 [16]	9.51 \pm 0.33b	5.05 \pm 0.78c	7.67 \pm 1.23b	14.20 \pm 1.25a
Hydrocarbons (5)							
1400	Undecane	MS	10 [14]	15.83 \pm 4.84a	ND	ND	ND
1494	1-pentadecene	MS	3.6 [17]	ND	ND	127.78 \pm 3.54a	ND
1500	Pentadecane ^a	MS, LRI	NA	719.16 \pm 77.22b	161.56 \pm 6.23c	60.57 \pm 4.19c	1621.18 \pm 23.76a
1600	Heptadecane ^a	MS, LRI	NA	215.82 \pm 34.85b	68.63 \pm 3.55c	263.79 \pm 25.66b	333.33 \pm 21.15a
1703	2,6,10,14-tetramethyl-pentadecane	MS, LRI	NA	517.70 \pm 44.60b	304.21 \pm 7.89b	ND	1353.51 \pm 34.77a
<i>N</i> - or <i>S</i> - containing aromatic compounds (6)							
<600	Trimethylamine ^a	MS, LRI	2.5 [18]	1563.21 \pm 67.06d	4066.05 \pm 55.24b	2172.03 \pm 42.58c	4665.83 \pm 59.36a
785	<i>N,N</i> -Dimethyl-formamide	MS, LRI	NA	9.54 \pm 2.27a	ND	ND	ND
921	2-Ethyl-1H-pyrrole	MS, LRI	NA	ND	ND	ND	8.14 \pm 0.78a
936	<i>N,N</i> -Diethyl-formamide	MS, LRI	NA	5.23 \pm 1.60a	ND	ND	ND
991	Dimethyl trisulfide	MS, LRI	NA	11.43 \pm 0.75a	3.60 \pm 0.34b	ND	10.63 \pm 1.78a
1588	Hexadecanoicacid, methyl ester	MS	NA	ND	ND	ND	11.71 \pm 1.35a
Furans (3)							
702	2-Ethylfuran ^a	MS, LRI	NA	23.67 \pm 5.61b	5.71 \pm 1.02c	4.59 \pm 0.89c	30.52 \pm 2.54a
1001	2-Propylfuran	MS	NA	ND	ND	ND	10.25 \pm 0.88a
1002	2-(2-Penteny)furan	MS, LRI	NA	ND	ND	ND	7.12 \pm 1.67a
Total			49	31	30	43	

Different letters in a row represent significant differences ($p < 0.05$)

ND not detected

NA Not available

A Yangtze river-Coilia B East China sea-Coilia C Chao lake-Coilia and D Yellow river-Coilia

^a Volatile compounds identified in all four steamed Coilia ectenes

^b Linear retention index (LRI) on a DB-5MS column

^c Means of identification: MS mass spectra (identified from the mass spectra deposited in a database); LRI linear retention index (compared with the LRI in the literature)

^d Odor thresholds were cited from the following 25 studies in the literature, all using air as the matrix: [Hall and Andersson 44], Bedborough and Trott [45], Bartschat and Mosandl [46], Tamura and others [47], Von Ranson and others [48], Deadman and Prigg [49], Khiari and others [50], Cometto-Muñiz and Abraham [51], Nagata [52], Schnabel and others [53], Cain and others [54], Pyysalo and Suihko [55], Nauš [56], Zoeteman and others [57], Giri and others [58], Sävenhed and others [59], Tamura and others [60], and Amoore [61]

Table 2 Odorants (Odor intensity >1) in four steamed *Coilia ectenes* by GC–O detection ($n = 3$)

LRI	Compounds	Identification	Odor description ^a	Odor intensity ^b			
				A	B	C	D
<600	Trimethylamine ^c	MS, LRI, Odor	Fishy, ammonia-like	4	4	4	4
<600	1-Propanethiol	MS, Odor	Sulfurous	–	2	–	1
<600	Propanal	MS, Odor	Green, fruity	1	–	–	–
<600	2-Butanone	MS, LRI, Odor	Roast	–	–	2	–
657	3-Methylbutanal ^c	MS, LRI, Odor	Dark chocolate	2	1	1	2
669	Benzene	MS, LRI, Odor	Floral	–	2	–	–
681	1-Penten-3-ol ^c	MS, LRI, Odor	Mushroom, cabbage	3	3	4	3
693	2,3-Pentanedione	MS, LRI, Odor	Roasted meaty, caramel	2	–	–	1
700	2-Methylbutanal	MS, LRI, Odor	Nutty, sweet	1	–	–	–
702	2-Ethylfuran	MS, LRI, Odor	Sweet corn	–	2	–	–
735	2-Methyl-1-butanol	MS, Odor	Fatty	–	1	1	–
754	(E)-2-Pentenal	MS, Odor	Marine, fishy	2	–	–	–
761	1-Pentanol	MS, LRI, Odor	Floral	–	–	1	–
765	(Z)-2-Penten-1-ol	MS, Odor	Mushroom, cabbage	–	–	–	2
776	Toluene	MS, LRI, Odor	Painty, dusty	–	–	1	2
785	<i>N,N</i> -Diethyl-formamide	MS, LRI, Odor	Roasted meaty	3	–	–	–
802	Hexanal ^c	MS, LRI, Odor	Grassy, earthy	3	2	2	3
825	(E)-3-Octen-2-ol	MS, LRI, Odor	Mushroom	–	–	2	–
825	3-Cyclohepten-1-one	MS, LRI, Odor	Roasted meaty, caramel	2	2	–	1
853	(E)-2-Hexenal	MS, LRI, Odor	Green, fishy	2	–	–	–
870	Ethylbenzene	MS, LRI, Odor	Nutty, floral	–	3	3	2
901	(Z)-4-Heptenal	MS, LRI, Odor	Fishy, boiled potato	3	3	–	4
904	Heptanal	MS, LRI, Odor	Green	–	2	–	–
975	Benzaldehyde ^c	MS, LRI, Odor	Almond, metallic	3	2	1	2
978	1-Octen-3-ol ^c	MS, LRI, Odor	Mushroom, earthy	1	3	3	3
991	Dimethyl trisulfide	MS, LRI, Odor	Cabbage, sulfurous	2	1	–	2
1005	Octanal	MS, LRI, Odor	Grassy, oily	–	1	2	1
1016	2,4-Heptadienal	MS, LRI, Odor	Green, fishy	2	–	–	–
1079	Acetophenone	MS, LRI, Odor	Nutty	–	–	2	–
1098	(E,E)-3,5-Octadien-2-one	MS, LRI, Odor	Sweet, roasty	1	–	–	1
1107	Nonanal	MS, LRI, Odor	Oily	–	3	3	2
1163	Unknow	–	Corn, fruity	2	1	–	–
1179	4-Ethyl-benzaldehyde	MS, LRI, Odor	Marine	–	1	–	1
1208	Decanal ^c	MS, LRI, Odor	Green, oily	2	2	2	3
1300	Unknow	–	Garlic, toasted meaty	–	1	–	1

A Yangtze river-*Coilia*B East China sea-*Coilia*C Chao lake-*Coilia* D Yellow river-*Coilia*

– Date not detected or available

^a Odor description described by three experienced assessors during GC–O detection

^b Odor intensity reported by three experienced assessors during GC–O detection (1 = weak intensity, 2 = moderate intensity, 3 = strong intensity, and 4 = very strong intensity)

^c Odorants identified in all four steamed *Coilia ectenes*

Discussion

Volatile compounds in four steamed *Coilia ectenes*

A total of 19 aldehydes were found in four *Coilia ectenes*. The 3-methyl-butanal and 2-methyl-butanal mainly

contribute to the nutty almond notes, and may be formed by the Strecker degradation of leucine in the course of the Maillard reaction [13]. This reaction is known to be enhanced at high temperatures [14]. Benzaldehyde is a predominant compound in Yangtze River-*Coilia*, East China sea-*Coilia* and Chao Lake-*Coilia*, which originated from

the oxidation of carbon–carbon double bonds in styrene and have been detected in fresh-baked sockeye salmon [15], roasted peanuts [16], and algae [17]. There are high levels of hexanal and nonanal in Chao Lake-*Coilia*. Hexanal, generally imparting a grassy odor, originates from the degradation of *n*-6 PUFA oxides and could be used as a degradation indicator of seafood products and meat from terrestrial animals [18]. Nonanal, imparting grassy and oily odors, is the predominant constituent in the autoxidation of linoleic acid [19]. Octanal and decanal may contribute to the more desirable aromas as well as the rancid odors and flavors that appear during the spoilage of fat and fatty foods [20]. Unsaturated straight chain aldehydes mainly contribute to the grassy, fatty, and fishy odors. For example, (*Z*)-4-heptenal is characterized by a fatty and creamy odor, and this compound may be formed by a retro-aldol condensation of 2,6-nonadienal, which has been shown to be a major odor impact compound in a number of fresh marine creatures [21]. Other saturated aliphatic aldehydes such as octanal and decanal have grassy and oily odors.

Ketones, which are derived from PUFA oxidative degradation [22], have a higher threshold value compared to aldehydes and a relative small contribution to fish aroma [23, 24]. However, the flavor differences among fish were mainly attributed to carbonyl compounds, so ketones have certain influences on flavor development [25]. Yangtze River-*Coilia*, Chao Lake-*Coilia* and Yellow River-*Coilia* have high levels of (*E, E*)-3, 5-octadien-2-one, which resulted from PUFA auto-oxidation and imparting sweet and roasted odors [4]. The compound 2, 3-pentanedione is an indicator of lipid oxidation in chilled fish muscle [26]. The 2, 3-pentanedione may contribute to roasted meat, caramel, and buttery odors, especially in cooked fillet of European catfish [27]. The compound 3-cyclohepten-1-one presents a high concentration in East China sea-*Coilia*, and has native and intense aromas that might be associated with the high fat content of East China sea-*Coilia*.

Alcohols are divided into two main classes: saturated alcohols and unsaturated alcohols. Saturated alcohols have higher flavor threshold values than unsaturated alcohols, therefore the contributions to fish odors is small [28, 29]. Unsaturated alcohols have been identified as the major volatile alcohols in shellfish and cooked alligator meat [30] as well as many other aquatic products. Eleven alcohols were detected in *Coilia ectenes*; three alcohols (1-penten-3-ol, 1-pentanol, and 1-octen-3-ol) were common to all four species. The compound 1-penten-3-ol is the most abundant alcohol in Yangtze River-*Coilia* and Yellow River-*Coilia*, which was generated from PUFA oxidation [31]. It has been reported that 1-penten-3-ol presented in fresh whitefish at high concentration [32]. The compound 1-octen-3-ol presents in Chao Lake-*Coilia* at high concentration,

imparting desirable mushroom-like odors, mainly derived from the hydrogen peroxide degradation of linoleic acid [33].

Eight aromatics were identified in *Coilia ectenes* and six aromatics (benzene, toluene, ethylbenzene, *p*-xylene, styrene, and naphthalene) were common in all four species. Among them, toluene and *p*-xylene are generally believed to originate from environmental pollutants [34], which contributing to unpleasant off-flavors.

Hydrocarbons do not have any odor activities due to their high threshold values [35]. Nevertheless, intermediate alkanes, alkenes, arenes, and a small fraction of heterocyclic compounds may enhance the overall fish flavor [36].

The *N*- and *S*-containing aromatic compounds, resulted from Maillard reactions between amino acids and reducing sugars, pyrolysis of amino acids (such as proline) and thiamine, dicarbonyl compounds from Maillard intermediate products, and degradations of thiamine via aldol condensation and aldehyde amine polymerization [31]. The *S*-containing aromatic compounds originate from the thermal degradation of methionine and cysteine [37], imparting meaty, maotai, and onion garlic flavors [31]. Even though some differences in the types and levels of *N*- and *S*-containing aromatic compounds in *Coilia ectenes*, TMA (53.82 %) was present in all four species. TMA is generally believed to be produced by microbial metabolism in the presence of the precursor TMA oxide (TMAO) [38]. The presence of high levels of TMA in seafoods is undesirable, while TMA may add a pleasant crustacean-like odor at low levels [39].

Furans are a family of substances that play an important role in the flavor formation of *Coilia ectenes*, three furans have been identified in this study. The 1st odorant is 2-ethyl-furan, which was detected in all four *Coilia ectenes*, having a sweet corn odor. It was reported that 2-ethyl-furan is the oxidative degradation products of linolenic acid and commonly in crab [40–42]. The other furans, which were only detected in Yellow River-*Coilia* in this study, have also been reported in crab [43].

Above all, the main compounds associated with fish flavor are C6–C9 olefine aldehydes, enols and olefine ketones. There are more aldehydes, ketones, and alcohols in Yangtze River-*Coilia* and Yellow River-*Coilia* than in East China sea-*Coilia* and Chao Lake-*Coilia*. It is possible that Yangtze River-*Coilia* (15.7 ± 1.2 %) and Yellow River-*Coilia* (18.1 ± 0.5 %) have a higher fat content than East China sea-*Coilia* (13.6 ± 2.6 %) and Chao Lake-*Coilia* (8.6 ± 1.0 %), aldehydes, ketones, and alcohols are produced from the oxidation and degradation of lipids. Additionally, environmental factors, e.g., water quality, sediment, algae and microbial species, may have significant effects on the flavor of *Coilia ectenes* [17]. The aromatic and hydrocarbon levels are similar to the four *Coilia*

ectenes, other flavor compounds (e.g., TMA, dimethyl trisulfide compounds, and alkyl furans) are abundant in Yangtze River-*Coilia* and Yellow River-*Coilia*.

Important odorants in four steamed *Coilia ectenes*

A total of 35 odorants were identified in four *Coilia ectenes* (Table 2). Most of the odors are present at low levels but have important functions in the flavor development of *Coilia ectenes*. The characteristic flavors may originate from lipids, mainly short chain aldehydes and ketones [62]. Even though odorants are different among the four species, the main odor characteristics are fishy, grassy, mushroom, fatty, oily and meaty, which are consistent with a previous report [11]. TMA (fishy, ammonia-like), 1-penten-3-ol (mushroom, cabbage), *N,N*-dimethyl-formamide (roasted meaty), hexanal (grassy, earthy), ethylbenzene (nutty, floral), (*Z*)-4-heptenal (fishy, boiled potato), benzaldehyde (almond, metallic), 1-octen-3-ol (mushroom, earthy), nonanal (oily), and decanal (green, oily) were the important odorants in four *Coilia ectenes*.

TMA levels are inversely proportional to fish quality [39]. TMA was perceived by the assessors to have average odor intensity values of 4 in four *Coilia ectenes*, showing the fishy and ammonia-like odors.

Unsaturated alcohols have low odor threshold values and with mushroom and metallic-like odors. The 1-penten-3-ol and 1-octen-3-ol are commonly found in freshwater and seawater fish with strong odor activities [31]. The 1-penten-3-ol has an odor intensity value more than 3 in four *Coilia ectenes*, imparting mushroom and cabbage odors. The 1-octen-3-ol imparted the mushroom and earthy odors, mainly derived from the hydrogen peroxide degradation of linoleic acid [33], and has the lowest value in Yangtze River-*Coilia*.

Hexanal, contributed to the grassy, earthy scent of the four *Coilia ectenes*. It has been reported that hexanal mainly derived from the oxidation of linoleic acid and provides the “green” and fatty characters of fish and other seafoods [63]. Ethylbenzene, which has a nutty, floral odor, was previously identified with a relatively high intensity. The (*Z*)-4-heptenal, is a volatile flavor compound with low odor threshold value, which is characterized by a cooked fish, potato-like odor, might be derived from the water-mediated retro-aldol condensation of (*E,Z*)-2,6-nonadienal [64]. Chao Lake-*Coilia* has the highest levels of (*E,Z*)-2,6-nonadienal. Benzaldehyde, which was assigned an odor intensity of 3 in Yangtze River-*Coilia*, mainly contributed to the almond and metallic odors. It is derived from the oxidation of benzyl alcohol, which is catalyzed by dehydrogenases [65]. Decanal, which resulted from the oxidation of oleic and linoleic acids [11], has the higher intensity in Yellow River-*Coilia*

than the other three *Coilia ectenes*, imparting unpleasant green and oily odors.

The compound 2, 3-pentanedione contributes to roasted meaty and caramel odors and buttery and caramel odors in salmon and trout [35]. The (*E, E*)-3, 5-octadien-2-one, which has a sweet, roast odor, has previously been detected in several seafood products [43].

In addition to known odorants, two unknown odorants were detected by the assessors. The first unknown odorant was detected as having a corn, fruity odor. The second unknown odorant had a garlic, roasted meaty odor. Although these odors could be detected by the assessors, they could not be identified by MS due to their weak signals. Nevertheless, even with weak signals and low estimated concentrations, these compounds may impart significant aromas in steamed *Coilia ectenes*, and should be evaluated in further studies.

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