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Static headspace analysis-olfactometry (SHA-O) of odor impact components in salted-dried white herring (*Ilisha elongata*)

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Abstract

The odorous components of salted-dried white herrings from different treatment conditions were determined by static headspace analysis-olfactometry (SHA-O). Fish samples were purchased from two locations, manufactured by two methods, purchased for two consecutive years, and investigated at two conditions (raw and steamed). Twenty-seven odorous compounds were found among the fish products including alcohols (3), aldehydes (7), miscellaneous compounds (2), ketones (3), other *N*-containing compounds (4), other *O*-containing compounds (1), and *S*-containing compounds (7). Seven potent components including 3-methylbutanal (almond-like), methanethiol (decay vegetable-like), dimethyl trisulfide (fried garlic, onion-like), hydrogen sulfide (rotten egg-like), trimethylamine (fishy, fish oil-like) bis(methylthio)methane (fried garlic/onion-like), and (*Z*)-4-heptenal (fatty, grease-like) were identified. The majority of them did not have significant difference among the different treatments ($p > 0.05$). Other less potent components may contribute to the odor of a fish due to their differences in distribution and potency. Statistically, only location and method × period interaction have major impacts on the magnitude of the odorants found in salted-dried white herrings ($p < 0.05$).

Keywords: Herrings; Odor; Preservation

1. Introduction

Fish preservation is common in a number of developed and developing countries (Infofish, 1983). Among various preservation methods, the combination of salting and drying of fish has a long history. Salted-dried fishes have a "salty, characteristic flavor and odor" (Subba Rao, 1967). Numerous articles have reviewed the odorous components found in seafood as well as their mechanisms of formation (Baek & Cadwallader, 1997; Boyle, Lindsay, & Stuiber, 1993; Durnford & Shahidi, 1998; Lindsay, 1990; Shahidi & Cadwallader, 1997; Spurvey, Pan, & Shahidi, 1998). Josephson (1991) categorized the mechanisms of odor formation in seafood into four groups including: (1) enzyme-mediated conversion of lipids and carotenoids, (2) autoxidative degradation of free fatty acids, (3) enzymatic conversion of sulfur-nitrogen-containing precursors, and (4) thermal decomposition of species-specific precursors.

White herring (*Ilisha elongata*) is an important commercial marine fish dwelling in the Indo-Pacific region which includes the East China Sea, Korea, and southern Japan (AFCD, 2004). It is considered as a pelagic fish, abundant from February to May, and has a maximum length of 40.5 cm with a compressed and elongated body covered with large scales. It has large eyes and an upward pointing mouth. The colors of its dorsal and ventral bodies are silvery white and pale yellowish green, respectively, with pale yellowish white fins.

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Salted-dried white herring is a traditional dried seafood product produced in Hong Kong, particularly in the old fishing site known as Tai O in the Lautau Island. This is where some fishermen still produce salted-dried fishes and other dried seafood products for sale to customers. Whole-sale dried seafood shops are also located in another area known as Sai Wan in the Hong Kong Island where shoppers often sell imported salted-dried fishes produced from other countries using the same traditional production steps from Hong Kong. Similar to the kench curing of fish used in the western culture where fish flesh is rubbed and solid salt is made to penetrate the meat, the fish is stacked and the extracted moisture is drained away (Poulter, 1988); after removal of the internal organs and the gills in the preparation of salted-dried fish, both the fish body and gill cavities are filled with dry salt and are layered alternatively with fish and salt (Horner, 1997). Depending on the season, the amount of salt used and the number of fermentation days are adjusted accordingly. Solar drying is commonly practiced after the salting step. In the past when cooling facilities were not readily available to refrigerate fishes caught from the ocean, the rate of autolysis was high before landing. The texture of these salted-dried fish products were often softer than those that were refrigerated before being prepared as salted-dried fishes (Hsu, 1994). For food preparation, a small chuck of salted-dried fish is baked and/or steamed before serving (Herklots & Lin, 1960). Although the salted-dried fish is a traditional food and is enjoyed by consumers, not much information has been reported on the odorants that are responsible for these fish products.

Therefore, by using the mild static-headspace analysis-olfactometry (SHA-O) technique, this investigation focused on the headspace volatile components that were responsible for the characteristic odorants of the salted-dried white herring. In addition, the effects of the fish product in raw and steamed (s) conditions, the processing methods – delayed (D) and refrigerated (R), the purchase locations – Sai Wan (S) and Tai O (T), as well as the purchase periods – year 2001 (a) and year 2002 (b) on the overall odor strength of the headspace components were also evaluated.

2. Material and methods

2.1. Sample preparation

Salted-dried Chinese herrings (*I. elongata*) prepared by both delayed salting (D) and refrigerated salting (R) methods were bought from two famous dried-seafood locations, namely, Tai O (T) on Lantau Island and Sai Wan (S) in Hong Kong Island, during the years 2001–2002. Both places are located in Hong Kong.

The samples were transported back to the Biology Department at the Chinese University of Hong Kong in their original package and were stored temporarily in a cool and dry location. Within two days of purchase, the fishes were grouped and beheaded. They were then kept in an air-tight glass container stored in a freezer (−20 °C) before further treatment and analysis were carried out.

2.2. Gas chromatography static headspace analysis and olfactometry (GC-SHA-O)

Odor evaluations of the samples were carried out using the GC-SHA-O technique. Twenty grams of fish were obtained by cutting across the dorsal and ventral fins of each fish with a pair of stainless steel scissors. The chuck of meat was then divided vertically into two halves. One halve (10 g) was used in its raw state without steaming, while the other was steamed (s). The steamed samples were prepared by steaming raw samples above boiling water in a small stainless steel pot covered with a lid for 20 min. Three randomly picked halves from a batch of fishes of the same production conditions were combined into one sample. For both steamed and non-steamed samples (consists of three fish halves), they were each homogenized with a household blender (MX-T2GM, Matsushita Electric Co. Ltd., Taipei, Taiwan) for 10 s, followed by manually mixing with a stainless steel spatula. A 20 g homogenized sample was used for aroma analysis by transferring the fish sample to a 285 ml gas-tight glass culture vessel (Wheaton culture vessel, model no. 991760, Wheaton Scientific, NJ, USA). The vessel with the sample was left to equilibrate at 50 °C in an incubator (model 132000, Boekel Scientific, Cambridge, England) for 2 h before the headspace volume was withdrawn for evaluation. For the steamed portion, randomly picked fish samples of the same conditions were first steamed. The pooled samples were blended immediately, and a 20 g portion was transferred to a 285 ml gas tight glass jar and equilibrated at 50 °C for 2 h as done previously.

Then 25, 5, 1, or 0.2 ml of headspace was collected from the incubated culture vessel with a gas-tight syringe (SGE International Pty Ltd, Victoria, Australia) and was injected into the temperature programmable cooled injection system (CIS 4, Gerstel, Mülheim an der Ruhr, Germany) of a gas chromatography (model 6890, Hewlett–Packard Co., Palo Alto, CA, USA) coupled to a mass selective spectrometor (model 5973, Hewlett–Packard Co., Palo Alto, CA, USA), a flame ionization detector (Hewlett–Packard Co., Palo Alto, CA, USA), and an olfactometor (Gerstel, Mülheim an der Ruhr, Germany) (GC-MS-FID-O) system for analyses. The CIS was cryogenically cooled down to −150 °C with liquid nitrogen and was held at that temperature during the headspace injection to cryofocus the volatile compounds in the injector. During injection, it was heated to 230 °C at a ramp rate of 10 °C/s and held at 230 °C for 10 min.

A fused silica open tubular column (Supelcowax-10, 60 m length × 0.25 mm i.d. × 0.25 μm film thickness, nominal; Supelco, Inc., Bellefonte, PA, USA) was used as the analytical column. Helium gas (ultrahigh purity grade, 99.999%) was used as the carrier gas with a constant linear

velocity of 30 cm/s. Oven temperature was programmed from 30 °C to 195 °C at a ramp rate of 6 °C/min. The initial and final hold times were 5 and 30 min, respectively. Mass selective detector (MSD) interface, ion source, and MS quadrupole temperatures were set at 250 °C, 230 °C, and 106 °C, respectively. The ionization voltage was 70 eV, and the electron multiplier voltage was 1200 V.

The column flow was split into the three detectors (MSD, FID, and O) at a split ratio of 1:1:1 using deactivated capillary columns of different diameters and lengths. Results from FID facilitate the identification of the perceived odorants by matching their elution time between the olfactory and the FID data, as well as their eluted peak between the MS and FID data. The transfer line of the sniff port for the olfactometor was set at 200 °C. Three panelists separately evaluated the effluence of the same sample from a sniff port and then reported the sensation, time range, and intensity of each odorant perceived in a report sheet provided during a 30-min session. Until a panelist reported no perceived odor for a sample, the same procedures would be repeated at the next smaller volume (which is 1/5 of the previous volume).

2.3. Compound identification for GC-SHA-O

Tentative identifications of compounds were done by matching the mass spectra of unknowns with those suggested by the Wiley Chemical database (7Nth edition, Hewlett–Packard Co., Palo Alto, CA, USA) or their characteristic odors. The compounds were positively identified by comparing their mass spectra, retention times/indices, and odor descriptors with those of the authentic standards (van den Dool & Kratz, 1963) under the same experimental conditions.

2.4. Calculation of the volume ratio (VR)

The volume ratio (VR) of a compound is defined as the largest headspace volume used as a reference in the evaluation divided by the smallest effective headspace volume perceived by a panelist. The higher the calculated VR of a compound, the stronger its flavor intensity will be. The highest reference volume used was designed at 125 ml, since a panelist might miss an odorant at 25 ml (the highest injection volume), while other panelists might be able to perceive it at 25 ml. The mean VR of an odorous compound was calculated by the geometric mean of VRs from the three panelists.

2.5. Statistical analysis

One-way analysis of variance (ANOVA) was carried out to determine the differences among treatment groups at $p = 0.05$. Multiple comparisons using the Tukey test were carried out for ANOVA with significance. Multivariate analysis of variance (MANOVA) and univariate analysis of variance (ANOVA) were used to test the variances of the volume ratios (VRs) of the odorants from raw or steamed conditions, the purchase locations, the purchase periods, and the processing methods. Pillar's criterion, Hotelling's trace, and Wilks' λ were used as the statistical selection criteria for the dependant variables in the MANOVA. The statistical analysis was performed using a Minitab Version 14.13 software package (State College, PA, USA).

3. Results and discussion

3.1. Aroma composition of salted-dried white herring

Salted-dried white herrings belonging to different treatment conditions were analyzed using the static headspace analysis-olfactometry (SHA-O) in order to determine the odorants that were responsible for their characteristic flavor. Twenty-seven compounds were found and distributed among seven chemical classes (Table 1). Aldehydes had the highest count of seven compounds, whereas the lowest count of one was with the oxygen-containing compound. Other chemical classes included sulfur-containing compounds (7), nitrogen-containing compounds (4), alcohols (3), ketones (3), and miscellaneous compounds (2).

3.1.1. Aldehydes

Among the aldehdyes, both propanal and 3-methylbutanal were found in all the treatments in the raw and steamed fishes. Meanwhile, (*Z*)-4-heptenal was found only in the steamed samples but not in the raw ones. The rest of the aldehydes were missing among the treatment groups. Described as almond-like, 3-methylbutanal was found to be the most potent component by SHA-O within the aldehydes as well as in all other chemical classes. Moreover, 3-methylbutanal has been reported in steamed distillated extracts in salted fermented fish and shrimp pastes (Cha & Cadwallader, 1995). It could be formed by the Strecker degradation of leucine in the course of Maillard reaction when the samples were subjected to solar-drying operation (Collin, Osman, Delcambre, El-Zayat, & Doufour, 1993). It might also be formed via the microbial degradation of amino acids. Propanal was described as a nutty, Maillard reaction-like odor, and was likely produced by the autoxidation of polyunsaturated fatty acids or by the retro-aldol condensation of 2,4-heptadienal which was also identified in some of the treatment groups (Josephson & Lindsay, 1987). These compounds were probably formed when the fish died and during the drying step.

Carbon-6, -8, and -9 alcohols and carbonyls were reported to contribute to the fresh fish flavor (Josephson & Lindsay, 1986). In the current experiment, only hexanal belongs to such classes. A green odor was described by the panelists for the component, respectively. It could be formed by the controlled oxidation of the long chain polyunsaturated fatty acids mediated by the lipoxygenase system in live fishes (Lindsay, 1990). On the other hand, (*Z*)-4-heptenal has a fatty grease odor and might be formed

by the retro-aldol condensation of (*E*,*Z*)-2,6-nonadienal which was derived from lipid autoxidation after the fish died (Josephson & Lindsay, 1987).

3.1.2. Nitrogen-containing compounds

One prominent member in the nitrogen-containing compound group was the trimethylamine which had a very high VR and was found in all treatment groups in both raw and steamed samples. Trimethylamine was described by the panelists as fishy, salty, and with a deterioration-like aroma. It was generally believed to be produced by microbial metabolism in the presence of the precursor trimethylamine oxide (Herberd, Flick, & Martin, 1985). During the salted-dried fish production, a sufficient amount of time and substrate was available for the contaminating microorganisms to break down the substrate and produce the odorous compound. It was reasonable to find trimethylamine among those potent odorants, though it did not always have the highest impact as shown from some of the treatment groups (Tables 1a and 1b).

Two pyrazines, 2,3-dimethylpyrazine and 2-ethyl-6-methylpyrazine, were found to be randomly distributed among the treatment groups. The former has been reported in dried scallops and cooked crabmeats, whereas the latter was identified in cocoa products, potato chips, and so on (Buttery, Seifert, Guadagni, & Ling, 1971; Chung, 1999; Chung, Yung, & Kim, 2001; Reineccius, Keeney, & Weissberger, 1972). Pyrazines are products formed between sugars and amino acids under suitable conditions (Maga & Sizer, 1973). High temperature generally promotes the rate of formation of the Maillard reaction products (Lane & Nursten, 1983). However, when the temperature is mild, as in the solar drying of salted-dried fish, the rate of Maillard product formation may be low. This could lead to the low amount and odor intensity of the pyrazines found in the samples.

3.1.3. Other oxygen-containing compounds, alcohols, and ketones

None of the compounds found among the three chemical classes including other oxygen-containing compounds, alcohols, and ketones were identified in all treatment groups. In addition, their average VR ranged from zero to six. Furfural had a potato-like aroma as described by the panelists and was the only component found in the other O-containing compound class. It is a product of Maillard reaction (Fors, 1983) and could be produced from melanoidins at a high temperature (Tehrani, Kersiene, Adams, Venskutonis, & De Kimpe, 2002). Meanwhile, 3-methylbutanol and (*Z*)-2-penten-1-ol were found in the frozen and fresh Mackerels, respectively, and were likely produced by the decomposition of the secondary hydroperoxides of fatty acids (Alasalvar, Quantick, & Grigor, 1997). Cyclopentanol was formed when polyasparagine was subjected to irradiation (Ahn, 2002), and it had bug-like and nutty aromas as described by the panelists. Furthermore, 2,3-butanedione and 2,3-pentanedione possessed a sour note and a fruity note, respectively, and were products from either the fragmentation of sugar in Maillard reaction or in lipid oxidation (Frankel, 1980; Yaylayan, 1997). Finally, 3-hexanone was reported to have a fruity flavor in the fish samples. It was also described to provide an ethereal, grape, and wine-like aroma elsewhere (Sigma–Aldrich, 2004). Crab meat, European dry-cured ham, and microwaved or boiled potatoes were reported to contain 3-hexanone (Chung, 1999; Chung & Cadwallader, 1993; Oruna-Concha, Bakker, & Ames, 2002a, Oruna-Concha, Bakker, & Ames, 2002b; Sabio, Vidal-Aragón, Bernalte, & Gata, 1998). During fish processing, lipid degradation could lead to the formation of 3-hexanone, particularly when the fish was subjected to solar drying (Oruna-Concha et al., 2002b).

3.1.4. Sulfur-containing compounds

Six sulfur-containing compounds were found in the fish samples, and majority of them had very high average VRs. They all had characteristic odors. Methional had a soy sauce, meaty, cooked corn-like odor (Tables 1a and 1b). It has been found in other seafoods such as blue crab meat and ripened anchovy (Chung & Cadwallader, 1994; Triqui & Guth, 1997). Methionine was able to undergo Strecker degradation to produce methional (Chan & Reineccius, 1994; Morton, Akroyd, & May, 1960). With long drying time and ambient temperature during production, the Strecker degradation of the amino acid was facilitated.

The odor of dimethyltrisulfide was described as cooked onion-like in the literature. It was considered as an off-flavor at high concentration when royal red prawns were spoiled by the natural, contaminating microbe flora (Whitfield, Freeman, & Bannister, 1981). When fish was subjected to similar conditions for contamination like those of prawns during processing, the generation of dimethyltrisulfide became highly possible. In the current investigation, the panelists described the aroma as fried garlic- and onion-like aromas, and had a relatively high odor impact on the salted-dried products. Although not found in all treatment groups, dimethyldisulfide possessed a fried garlic/town gas-like odor similar to dimethyltrisulfide. This compound was reported in the headspace of the refrigerated mackerel, but not in the fresh samples (Alasalvar et al., 1997). This compound could be formed by the oxidation of methanthiol or the microbial degradation of methanione (Christensen, Kjaer, & Madsen, 1981). Hydrogen sulfide was reported to have a rotten egg odor and was generated from meat composed mainly of sulfur-containing amino acids such as cystine and/or methionine, e.g., in beef when subjected to high temperature (Machiels, Istasse, & van Ruth, 2004).

Bis(methylthio)methane has a fried garlic/onion-like odor and was the second most potent component detected by the SHA-O. It was reported to be present in seafood, white truffle, and hop (Fiecchi, Galli-kienle, Scala, & Capella, 1967; Lermusieau, Bulens, & Collin, 2001; Whitfield, 1999;

Table 1a
Odorous compounds found in the headspace of the raw salted-dried white herring

No.[a]	Compound[b]	RI[c]	Confirmation[d]	Descriptor[e]	Mean volume ratio of salted-dried white herrings[f]								Mean[g]
					Non-steamed (raw)								
					RSa	DSa	RTa	DTa	RSb	DSb	RTb	DTb	
	Aldehydes												
1	Propanal	708	M,R,O	Nutty, Maillard reaction	15	9	9	125	9	25	125	25	43
2	3-Methylbutanal	864	M,R,O	Almond	125	366	73	366	73	43	366	625	254
3	Hexanal	1076	M,R,O	Green	3	3	3	5	0	0	25	43	10
4	2-Methyl-2-butenal[t*]	1104	M,O	Coffee	0^{a}	0^{b}	3	0^{c}	3	0^{d}	0^{e}	0^{f}	1
5	Heptanal	1180	M,R,O	Fishy, fish oil, town gas	0	5	9	43	25	9	0	0	11
6	(*Z*)-4-heptenal	1236	M,R,O	Fatty, grease	43	25	73	73	9	0	125	125	59
7	(*E*,*E*)-2,4-heptadienal[t*]	1495	M,O	Root, ginseng	15	9	15	5	0	5	0	9	7
	Miscellaneous compounds												
8	Unknown 1[*]	1018	–	Fruity	0^{a}	0^{b}	0^{c}	0^{d}	0^{e}	0^{f}	3	0^{g}	0
9	Unknown 2	1467	–	Moldy	0	3	5	0	3	0	0	0	1
	N-containing compounds												
10	Trimethylamine	610	M,R,O	Fishy, salty, deterioration	214	25	125	9	214	125	25	125	108
11	2,3-Dimethyl-pyrazine	1342	M,R,O	Pop corn, rice	3	5	5	9	0	0	3	3	3
12	2-Ethyl-4-methyl-1*H*-pyrrole[t*]	1369	M	Peanut skin, cooked rice	5^{a}	$366^{a–o}$	0^{b}	0^{c}	0^{d}	0^{e}	0^{f}	0^{g}	46
13	2-Ethyl-6-methylpyrazine[t*]	1390	M	Metallic, rusty	9	9	0	0	5	25	3	9	7
	Other O-containing compounds												
14	Furfural[*]	1482	M,R,O	Potato	0	5	5	0	0	15	9	0	4
	Alcohols												
15	3-Methyl-1-butanol[*]	1187	M,R,O	Metallic, sharp	0^{a}	$9^{a–o}$	0^{b}	0^{c}	3^{d}	5^{e}	0^{f}	0^{g}	2
16	Cyclopentanol	1284	M,R,O	Bug, nutty	0	3	0	0	9	3	5	3	3
17	(*Z*)-2-penten-1-ol[*]	1297	M,R,O	Mushroom, woody	0^{a}	0^{b}	5^{c}	3^{d}	0^{e}	0^{f}	0^{g}	$25^{a–o}$	4
	Ketones												
18	2,3-Butanedione	969	M,R,O	Sour	9	3	5	0	9	9	9	5	6
19	2,3-Pentanedione[*]	1053	M,R,O	Fruity	$0^{a,h}$	$0^{b,c}$	$5^{o,z}$	$15^{b,e–m,o,s,y}$	$0^{j,n}$	$3^{o,p}$	$0^{l,u}$	$0^{f,q}$	3
20	3-Hexanone	1057	M,R,O	Fruity	0	0	0	0	0	5	3	3	1
	S-containing compounds												
21	Hydrogen sulfide[t*]	614	O	Rotten egg	$9^{a,h,s}$	$366^{b–n}$	$125^{l,o,w}$	$366^{p–w,y,cc,ii}$	$0^{j,u,x}$	$0^{c,y,z}$	$15^{m,ee}$	$5^{f,q,aa}$	111
22	Methanethiol[t*]	672	M,O	Decay vegetable	214	366	73	366	125	125	25	25	165
23	Dimethyl disulfide	1075	M,R,O	Fried garlic, towngas	0	0	0	3	15	3	5	0	3
24	Bis(methylthio) methane[*]	1288	M,R,O	Fried garlic/onion	125^{a}	73^{b}	5^{c}	9^{d}	366	125^{e}	1^{f}	25^{g}	93
25	Dimethyl trisulfide[*]	1386	M,R,O	Fried garlic/onion	366	5	25	43	366	125	43	214	148
26	Methional	1456	M,R,O	Soy sauce	25	0	9	9	9	5	3	43	13
27	3-Thiophene-carboxaldehyde[t]	1685	M,O	Meaty, cooked corn, mint	25	0	15	15	25	15	0	0	12

[a] Compound number.
[b] Identified compound. t: tentatively identified compound. [*]: statistical significance, $p < 0.05$, One-way ANOVA.
[c] Linear retention index (van den Dool & Kratz, 1963).
[d] Method of confirmation. M: mass spectrum; R: retention index; O: odor.
[e] Descriptor(s) used by panelists.
[f] Volume ratio (VR) of salted-dried herring under various conditions. D: delay treatment; R: refrigerated treatment; T: purchase location at Tai O; S: purchase location at Sai Wan; a: purchase period in year 2001; b: purchase period in 2002; s: steamed sample if specified, otherwise, it is not steamed or raw. Same letter across treatments of a compound indicates significantly different ($p < 0.05$). $n = 3$ for each condition.
[g] Overall mean VR of a compound from the raw treatment groups.

Table 1b
Odorous compounds found in the headspace of steamed salted-dried white herrings

No.[a]	Compound[b]	RI[c]	Confirmation[d]	Descriptor(s)[e]	Mean volume ratio of salted-dried white herrings[f]								Mean[g]
					Steamed								
					RSas	DSas	RTas	DTas	RSbs	DSbs	RTbs	DTbs	
	Aldehydes												
1	Propanal	708	M,R,O	Nutty, Maillard reaction	15	15	43	43	15	73	25	43	34
2	3-Methylbutanal	864	M,R,O	Almond	366	366	366	366	73	214	366	366	310
3	Hexanal	1076	M,R,O	Green	3	5	9	5	0	5	15	25	8
4	2-Methyl-2-butenal[t*]	1104	M,O	Coffee	0^{g}	0^{h}	0^{i}	0^{j}	3	5	5	$9^{a–j}$	3
5	Heptanal	1180	M,R,O	Fishy, fish oil, town gas	0	0	5	5	25	5	3	5	6
6	(*Z*)-4-heptenal	1236	M,R,O	Fatty, grease	25	25	73	125	43	25	73	125	64
7	(*E*,*E*)-2,4-heptadienal[t*]	1495	M,O	Root, ginseng	3	15	3	3	0	0	0	5	4
	Miscellaneous compounds												
8	Unknown 1[*]	1018	–	Fruity	0^{h}	0^{i}	$5^{a–n}$	0^{j}	0^{k}	0^{l}	0^{m}	0^{n}	1
9	Unknown 2	1467	–	Moldy	5	0	0	0	3	0	0	0	1
	N-containing compounds												
10	Trimethylamine	610	M,R,O	Fishy, salty, deterioration	214	15	43	125	125	125	125	214	123
11	2,3-Dimethyl pyrazine	1342	M,R,O	Pop corn, rice	5	15	25	0	3	9	5	9	9
12	2-Ethyl-4-methyl-1*H*-pyrrole[t*]	1369	M	Peanut skin, cooked rice	0^{h}	0^{i}	0^{j}	15^{k}	3^{l}	0^{m}	0^{n}	0^{o}	2
13	2-Ethyl-6-methylpyrazine[t*]	1390	M	Metallic, rusty	3	3	0	0	0	0	15	9	4
	Other O-containing compounds												
14	Furfural[*]	1482	M,R,O	Potato	0	3	5	3	9	0	25	3	6
	Alcohols												
15	3-Methyl-1-butanol[*]	1187	M,R,O	Metallic, sharp	0^{h}	0^{i}	0^{j}	0^{k}	3^{l}	0^{m}	0^{n}	0^{o}	0
16	Cyclopentanol	1284	M,R,O	Bug, nutty	0	3	0	3	5	0	0	5	2
17	(*Z*)-2-penten-1-ol[*]	1297	M,R,O	Mushroom, woody	0^{h}	0^{i}	9^{j}	0^{k}	0^{l}	0^{m}	0^{n}	0^{o}	1
	Ketones												
18	2,3-Butane dione	969	M,R,O	Sour	3	3	5	3	0	3	3	5	3
19	2,3-Pentane dione[*]	1053	M,R,O	Fruity	$0^{i,r}$	$0^{s,t}$	$25^{a,c,d,n,p,q,t–x,z,aa}$	$0^{e,w}$	$0^{k,x}$	$0^{y,z}$	$0^{m,v}$	$0^{g,aa}$	3
20	3-Hexanone	1057	M,R,O	Fruity	0	0	0	0	0	0	9	3	1
	S-containing compounds												
21	Hydrogen sulfide[t*]	614	O	Rotten egg	$3^{i,t,bb}$	$73^{b,cc,dd}$	$625^{a,o,x,z,aa–hh,jj,kk}$	$0^{e,p,gg}$	$3^{k,v,hh}$	$0^{d,ii,jj}$	$25^{n,ff,m,cc,ll}$	$25^{g,r,kk}$	94
22	Methanethiol[t*]	672	M,O	Decay vegetable	125	73	625	73	125	366	15	15	177
23	Dimethyl disulfide	1075	M,R,O	Fried garlic, towngas	0	0	0	0	9	15	0	0	3
24	Bis(methylthio)-methane[*]	1288	M,R,O	Fried garlic/onion	73^{h}	5^{i}	43	0^{j}	$625^{a–k}$	214	43^{k}	73	134
25	Dimethyl trisulfide[*]	1386	M,R,O	Fried garlic/onion	214	25	366	9	366	214	73	73	167
26	Methional	1456	M,R,O	Soy sauce	25	9	43	15	15	9	5	25	18
27	3-Thiophene-carboxaldehyde[t]	1685	M,O	Meaty, cooked corn, mint	5	0	15	5	25	9	0	5	8

[a] Compound number.
[b] Identified compound. t: tentatively identified compound. [*]: statistical significance, $p < 0.05$, One-way ANOVA.
[c] Linear retention index (van den Dool & Kratz, 1963).
[d] Method of confirmation. M: mass spectrum; R: retention index; O: odor.
[e] Descriptor(s) used by panelists.
[f] Volume ratio (VR) of salted-dried herring under various conditions. D: delay treatment; R: refrigerated treatment; T: purchase location at Tai O; S: purchase location at Sai Wan; a: purchase period in year 2001; b: purchase period in 2002; s: steamed sample if specified, otherwise, it is not steamed or raw. Same letter across treatments of a compound indicates significantly different ($p < 0.05$). $n = 3$ for each condition.
[g] Overall mean VR of a compound from the steamed treatment groups.

Whitfield, Freeman, Last, & Bannister, 1981). Whitfield (1999) suggested that the compound could originate from the diet of marine animals. Alternatively, in the presence of suitable microorganisms such as *Pseudomonas* or *Achromobacter* sp. and trimethylamino oxide (TMAO) demethylase in the animial's digestion system, substrates including cysteine and trimethyloxide could eventually transform to form the potent component (Whitfield & Tindale, 1984). Similarly, bis(methylthio)methane found in salted dried fish could be accumulated from the fish diet in live fish or could be generated from the microbial mediated enzymatic reaction in both alive and dead fish.

Meanwhile, 3-thiophenecarboxaldehyde was reported among the products when lipid interacted with products from the Maillard reaction (Elmore, Campo, Enser, & Mottram, 2002). In the current evaluation, it was described as having a meaty, cooked corn, mint-like odor.

Overall, 27 odor compounds were found, and the mechanisms by which the odorants were formed in the salted dried fishes included, when the fish was alive, the natural lipoxygenase-mediated lipid oxidation, and when the fish died and during salting and drying, microbial contamination and degradation, lipid autoxidation, Strecker degradation, Maillard reaction, and interaction between different substrates. In addition, some compounds might originate from the diet of the animal. Based on the observed results, many odorants were formed when the fish died, and their amounts depended on their formation mechanisms which were influenced by temperature, concentration of substrates, microbial load, and so on. Adequate control of these parameters during production may produce products with optimal flavor quality.

3.2. Contribution of the odorants to the salted-dried fish products

When the compounds in Table 1 were arranged according to the order of their retention times, the majority of the components with high VR were clustered at the early retention index followed by compounds that were low in VR. Then another two clusters were observed. The initial first cluster mainly contains trimethylamine, hydrogen sulfide, methanethiol, propanal, and 3-methylbutanal. Majority of these compounds were very potent in odor in both raw and steamed samples with average VRs of 108, 123; 111, 94; 165, 177; 43, 34; and 254, 310, respectively. Interestingly, both bis(methylthio)methane and dimethyl trisulfide had similar odor sensations and were described as fried garlic- and fried onion-like odors by the panelists. Moreover, if their VRs are summed up in each treatment group, their total values generally come close to the highest value in each of their respective treatments. This may suggest that the fried onion-like odor is generally an important odor in salted-dried white herring based on the observed results.

A careful inspection of the magnitude of the odorants in each treatment group in Table 1 shows that each group has its own compound profiles with different average VRs, though the three clusters of potent components are often present. In the raw, salted-dried white herrings, based on the mean VR of each component (Table 1), the decreasing odor strength (with mean VR > 40) of the components is shown: 3-methylbutanal, methanethiol, dimethyltrisulfide, hydrogen sulfide, trimethylamine, bis(methylthio)methane, (*Z*)-4-heptenal, 2-ethyl-4-methyl-1*H*-pyrrole, and propanal. Similarly, for the steamed samples (Table 1), the ranking (with average VR > 40) is as follows: 3-methylbutanal, methanethiol, dimethyltrisulfide, bis(methylthio)methane, trimethylamine, hydrogen sulfide, and (*Z*)-4-heptanal. Apparently, due to the relatively high VR of the first seven compounds, they were generally more important in contributing to the aroma of the salted-dried white herring of different treatments. Slight variations in the odor strength of any components in each treatment group would probably only be perceived by very sensitive consumers. Table 1 also shows that potent components are not consistently maintained at similar magnitudes. In fact, statistical analyses using one-way ANOVA and Tukey test at $p = 0.05$ level showed that most variations were due to random error. This suggested that their VR were similar among different treatment groups. For components with low VR, the majority of them did not differ significantly. Often, significance was found only in one treatment group but was not found in the rest of the treatments. In short, most treatment groups of both potent and less potent components were statistically similar, suggesting that the odorants contributed similarly to different treatments. Moreover, differences in odor perception were mostly contributed by a small number of odorants and their distributions in different treatments.

Table 2
Summary of the significance of the treatment factors in the 2 × 2 × 2 × 2 factorial design MANOVA

Treatment factors	*F*-Value[a]	*p*-Value	Significance[b]
Method	1.682	0.268	–[c]
Location	4.128	0.042	*
Period	3.585	0.058	–
Steam	2.955	0.090	–
Method × location	3.224	0.074	–
Method × period	5.724	0.019	*
Method × steam	3.452	0.063	–
Location × period	2.286	0.153	–
Location × steam	1.815	0.235	–
Period × steam	2.430	0.135	–
Method × location × period	3.705	0.054	–
Method × location × steam	1.957	0.205	–
Method × period × steam	3.708	0.054	–
Location × period × steam	1.350	0.378	–
Method × location × period × steam	1.247	0.423	–

[a] Degree of freedom.
[b] Statistical selection criteria based on Pillai's criterion, Hotelling's trace and Wilks' λ.
[c] Statistically insignificance.
* Significant difference at $p < 0.05$.

3.3. Influence of treatments (raw/steamed, periods, locations, methods) on the overall odor intensity perceived

MANOVA was used to evaluate if differences existed among the treatment groups. Table 2 shows that only the location effect and the method × period interaction were significantly different ($p < 0.05$). This suggested that the mean vectors of the different treatment groups involved in the locations or the method × period interactions were not equal, and that their dependent variables, i.e. the VR of odorants, varied across the locations as well as the method × period interactions.

In order to further understand the influence of different purchase locations on the strength of the odorants, additional univariate ANOVA on each compound was carried out, and the results related to the factor – locations are shown in Table 3. No significant difference was found for more than half of the compounds in the table, suggesting that the odorous compounds have similar odor contribution from the two locations ($p > 0.05$). This observation has an important implication to the potent components, particularly trimethylamine and methanethiol which were very high in their mean VRs, but were not statistically significant ($p > 0.05$). These compounds maintained similar and strong odor strengths in the two locations. For the remaining 11 compounds having significance ($p < 0.05$) in Table 3, highly potent compounds including hydrogen sulfide, 3-methylbutanal, bis(methylthio)methane, and trimethyl trisulfide were found and were expected to have a major influence on the odor perception of the fish product.

Collectively, location S has a slightly higher overall mean VR than that of location T which is indicative of the higher influence of location S on the odorant strength. However, a careful inspection of these significant odorants showed that a majority of them (seven compounds) had higher mean VRs in location T than those in location S. Furthermore, potent odorants including hydrogen sulfide, 3-methylbutanal, and (*Z*)-4-heptanal were found with a higher VR in location T. Conversely, two potent odorants including bis(methylthio)methane and dimethyl trisulfide were found to have a significantly higher odor impact in location S than that in location T (Table 3). Both locations did not have much influence in the odor strength in both trimethylamine and methanethiol. Based on both magnitude and distribution of the potent odorants, salted-dried white herrings from location S had a much stronger odorant described as a fried garlic- and onion-like aroma contributed by the two S-containing compounds, whereas those from location T were

Table 3
Summary of the univariate ANOVA results for location effect and method × period interaction on the odorants in salted-dried white herrings

No.[a]	Compound	Location			Mean[d] VR of location		Method × period		
		F-Value[b]	*p*-Value	Significance[c]	S	T	*F*-Value[b]	*p*-Value	Significance[c]
10	Trimethylamine	0.40	0.530	–	132	99	2.33	0.137	–
21	Hydrogen sulfide	9.38	0.004	**	57	148	1.41	0.244	–
22	Methanethiol	0.36	0.551	–	190	152	0.69	0.411	–
1	Propanal	6.46	0.016	*	22	55	0.07	0.797	–
2	3-Methylbutanal	8.00	0.008	**	203	361	0.13	0.716	–
18	2,3-Butanedione	0.61	0.440	–	5	4	0.73	0.398	–
8	Unknown 1	3.45	0.073	–	0	1	0.78	0.383	–
19	2,3-Pentanedione	24.67	0.000	***	0	6	4.21	0.048	*
20	3-Hexanone	3.23	0.082	–	1	2	0.24	0.630	–
23	Dimethyl disulfide	0.42	0.523	–	5	1	1.64	0.210	–
3	Hexanal	9.79	0.004	**	2	16	1.98	0.169	–
4	2-Methyl-2-butenal	1.29	0.265	–	1	2	1.29	0.265	–
5	Heptanal	0.01	0.908	–	9	9	3.75	0.062	–
15	3-Methylbutanol	12.74	0.001	**	2	0	5.53	0.025	*
6	(*Z*)-4-heptenal	14.17	0.001	**	24	99	0.01	0.934	–
16	Cyclopentanol	0.75	0.394	–	3	2	1.81	0.188	–
24	Bis(methylthio)methane	7.71	0.009	**	201	26	1.52	0.227	–
17	(*Z*)-2-penten-1-ol	25.33	0.000	***	0	5	18.53	0.000	***
11	2,3-Dimethylpyrazine	0.36	0.555	–	5	7	2.48	0.125	–
12	2-Ethyl-4-methyl-1*H*-pyrrole	7.55	0.010	*	47	2	8.09	0.008	**
25	Dimethyl trisulfide	4.97	0.033	*	210	106	2.53	0.121	–
13	2-Ethyl-6-methylpyrazine	2.43	0.129	–	7	4	0.63	0.432	–
26	Methional	2.94	0.096	–	12	19	5.85	0.021	*
9	Unknown 2	1.96	0.172	–	2	1	0	0.949	–
14	Furfural	1.00	0.326	–	4	6	2.7	0.110	–
7	(*E*,*E*)-2,4-heptadienal	1.08	0.307	–	6	5	3.69	0.064	–
27	3-Thiophenecarboxaldehyde	3.00	0.093	–	13	7	0	0.968	–

[a] Compound number arranged in ascending order of retention index of each compound.
[b] Degree of freedom.
[c] Significant difference at *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, –: not significant at $p = 0.05$.
[d] VR: volume ratio.

dominated by the rotten egg, almond-like odor. Herrings from both locations processed similar decaying vegetable-like, fishy, salty, and deterioration-like odors (Table 1).

There were significant interactions for method × period in some components ($p < 0.05$). The results of univariate ANOVA show that five compounds were significantly different including 2,3-pentanedione, 3-methylbutanol, (*Z*)-2-penten-1-ol, 2-ethyl-4-methyl-1*H*-pyrrole, and methional (Table 3), but none of them belonged to the potent components discussed earlier. Among them, the first four compounds were affected by location. The magnitudes of these five compounds were also influenced by both the processing methods and the year in which the white herrings were purchased. However, the rest of the compounds were not affected by the interaction (Table 3). Due to the significant interaction, the main effects of both method and period on the strength of the odorants could not be interpreted directly. Moreover, MANOVA shows collectively that there were no differences among the odor strengths of the components when the salted-dried fish was in either raw or steamed condition (Table 2).

4. Conclusion

In conclusion, a combined total of 27 compounds were found in the salted-dried white herrings of different treatment groups. Among them, seven compounds were very prominent and were found in most fishes. These included 3-methylbutanal (almond-like), methanethiol (decay vegetable-like), dimethyl trisulfide (fried garlic, onion-like), hydrogen sulfide (rotten egg-like), trimethylamine (fishy, fish oil-like), bis(methylthio)methane (fried garlic/onion-like), and (*Z*)-4-heptenal (fatty and greasy). The majority of them and many less potent components showed no significance (one-way ANOVA and Tukey test, $p > 0.05$). Statistical analyses using MANOVA showed that among the four treatment factors (purchase locations, purchase periods, processing methods, and raw-steamed conditions), only purchase location was found to be significant ($p < 0.05$) and was due mainly to the treatment groups which involve 11 odorants including four of the seven potent ones (hydrogen sulfide, 3-methylbutanal, bis(methylthio)methane and dimethyl trisulfide). The three remaining factors (method, period, and raw-steamed condition) did not have an impact on the overall odor strength. Similarly, all interactions except method × period did not show any impact on the strength of the odorants. It is hoped that the current findings would provide specific markers and general conditions to researchers and manufacturers for the further improvement of the product so that salted-dried white herrings will have better quality and nutritional value.

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