Quantitative Analysis of Volatile Aldehydes Formed from Various Kinds of Fish Flesh during Heat Treatment

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Volatile aldehydes (formaldehyde, acetaldehyde, propanal, isobutanal, butanal, isopentanal, pentanal, hexanal, heptanal, octanal, nonanal) formed in a headspace from various kinds of fish flesh during heat treatment were collected in a cysteamine solution. Aldehydes derivatized to corresponding thiazolidines were subsequently analyzed by a gas chromatograph with a nitrogen–phosphorus detector. Amounts of formaldehyde found ranged from 0.48 to 5.31 μg/g of fish flesh and amounts of acetaldehyde found ranged from 1.70 to 15.47 μg/g of fish flesh. These two highly volatile aldehydes generally made up the largest quantities of aldehydes. The total aldehydes (except formaldehyde and acetaldehyde) formed ranged from 1.06 to 51.09 μg/g of fish flesh. Aldehydes were in decreasing order in sardine, herring, red salmon, mackerel pike, mackerel, croaker, smelt, yellow-tail, horse mackerel, and squid. Propanal was the most abundant aldehyde found in mackerel pike, red salmon, and sardine.

Keywords: *Acetaldehyde; cooked fish; formaldehyde; volatile aldehydes*

INTRODUCTION

Indoor air quality has received relatively little attention compared to that given to outdoor air. Indoor air quality is also important, however, since most people in developed countries spend more than 90% of their time inside buildings such as offices, schools, stores, and their own homes (Leeper, 1981). Among the numerous chemicals found in indoor air, most organic compounds are formed from foods during cooking (Schulte, 1964). It is well-known that cooking food produces a tremendous number of chemicals, including some toxic compounds (Nagao et al., 1983; Ohnishi and Shibamoto, 1984). There are many reports on volatile chemicals found in foods such as cooked meats (Mussinan and Walradt, 1974), roasted nuts (Walradt et al., 1971), and cooked eggs (Umano et al., 1990). There is, however, little information on the volatile chemicals formed in cooked fish.

Fish flesh contains high levels of lipids ranging from 0.5% to 25% (Kinsella, 1987). Lipids occur primarily as triglycerides with polyunsaturated fatty acids (PUFAs), including eicosapentenoic acid ($C_{20:5}$) and docosahexenoic acid ($C_{22:6}$) (Kinsella, 1987). The highly unsaturated fatty acids render fish flesh extremely susceptible to oxidation and rapid degradation. PUFAs produce volatile aldehydes (including formaldehyde, acetaldehyde, and propanal) by action of heat via the process of lipid peroxidation (Esterbauer, 1982; Frankel, 1982). These volatile aldehydes have received much attention, not only as flavor chemicals but also as toxicants (Esterbauer, 1982). Also formaldehyde formed in fish reacts with protein and subsequently causes muscle toughness (Erickson, 1993).

There have been many reports on the toxicity of volatile aldehydes over the past decade. In one instance, squamous cell carcinoma in the nasal cavity of rats resulted from repeated inhalation of formaldehyde (Albert et al., 1982). Acetaldehyde is also capable of inducing nasal carcinomas in experimental animals (Feron et al., 1982). According to the technical support document prepared by the California Environmental Protection Agency/Air Resources Board, ambient acetaldehyde is an air pollutant which may cause or contribute to an increase in mortality or serious illness or which may pose a present or potential hazard to human health on the basis of the results of risk assessment (California EPA/ARB, 1993).

Quantitative analysis of aldehydes formed in a vapor phase from heated foods is one avenue to assess indoor air quality. It is, however, extremely difficult to analyze highly volatile and reactive aldehydes such as formaldehyde and acetaldehyde. Most commonly used methods for these aldehydes involve derivatization with 2,4-dinitrophenylhydrazine (Papa and Turner, 1972; Selim, 1977; Kuwata et al., 1979). However, this derivatization requires a strong acidic condition which may alter the chemicals of interest.

Recently, a simple and specific method that involves derivatization of aldehydes with cysteamine to form stable thiazolidines has been reported (Umano and Shibamoto, 1987; Yasuhara and Shibamoto, 1989a). The resulting thiazolidines were analyzed by a gas chromatograph equipped with a fused silica capillary column and a nitrogen–phosphorus detector (NPD). Cysteamine reacts with carbonyl compounds readily under mild conditions of room temperature and neutral pH. The only drawback of this method is that cysteamine does not react with α,β-unsaturated aldehydes such as acrolein and β-dicarbonyl compounds such as malonaldehyde.

In the present study the formation of volatile aldehydes from the flesh of various kinds of fish upon heat treatment was investigated using this cysteamine derivatization method.

EXPERIMENTAL PROCEDURES

Chemicals. Cysteamine hydrochloride was purchased from Aldrich Chemical Co. (Milwaukee, WI). Anhydrous sodium sulfate and reagent grade sodium hydroxide were obtained

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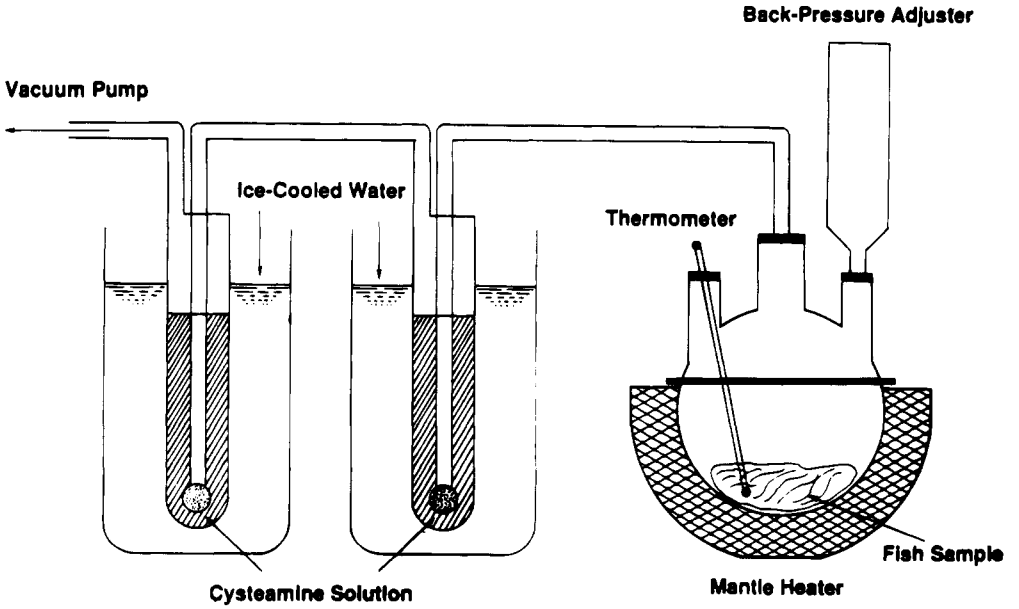

Figure 1. Apparatus used to collect aldehydes from headspace volatiles of various kinds of fish flesh heated at 200 °C.

from Wako Chemical Industry Co., Ltd. (Osaka, Japan). All standard thiazolidines were synthesized from cysteamine and corresponding aldehydes according to the method reported previously (Yasuhara and Shibamoto, 1989a).

Fish Samples. Mackerel (*Scomber japonicus*), smelt (*Osmeridae mordax*), horse mackerel (*Trachurus japonicus*), croaker (*Micropogon undulatus*), yellow-tail (*Seriola quinqueradiata*), mackerel pike (*Esox lucius*), red salmon (*Oncorhynchushegh sockeye*), sardine (*Sardinops caerulea*), herring (*Clupea harrengus pallasi*), and squid (*Loligo opalescens*) were bought from a local market and used immediately. The backbone, head, tail, and internal organs were removed from the mackerel, yellow-tail, and red salmon before use. The soft bones were removed from the squid. The other fish were used after the head and tail were removed.

Sample Preparations. Figure 1 shows the apparatus used to collect aldehydes from heated fish flesh. A fish sample (100 g) was placed in a 3-L three-neck round-bottom separable flask (bottom part was stainless steel). The heating temperature was measured by a thermocouple placed between the flask and a mantle heater. The temperature of the sample was determined by a mercury thermometer as shown in Figure 1. When the flask temperature reached 200 °C (approximately 5 min after the heater was switched on), 7 L of vapor in the headspace of the flask was drawn into the two impingers connected in a series at 300 mL/min and then the solutions in the impingers were combined. Each impinger contained 50 mL of a cysteamine solution (20 mg/mL). After the solution was allowed to stand for 30 min at room temperature, it was extracted three times with 10 mL of dichloromethane each time. The extracts were combined and then poured onto a column packed with anhydrous sodium sulfate to remove the water. The column was washed with 20 mL of dichloromethane, and the eluate was added to the extract. The extract was condensed to 2 mL by distillation through a Vigreux column. The thiazolidine derivatives were purified using column chromatography. The condensed extract was placed on top of a column packed with silica gel and then eluted with 10 mL of dichloromethane followed by a 150 mL solution of ethyl ether/dichloromethane (1/9). The dichloromethane fraction that contained mainly hydrocarbons and neutral compounds was discarded. The ethyl ether/dichloromethane fraction was condensed to approximately 9 mL by distillation through a Vigreux column and transferred to a volumetric flask. The volume of this fraction was adjusted to exactly 10 mL with the addition of dichloromethane, and then a 10 μL benzene solution of *N*-methylacetamide (10 mg/mL) was added as an internal standard for GC analysis.

Quantitative Analysis of Aldehydes as Thiazolidines. A Hewlett-Packard Model 5890A gas chromatograph equipped with a DB-Wax fused silica capillary column (30 m × 0.25 mm i.d., d_f = 0.25 μm) and an NPD was used. The oven temperature was held at 40 °C for 2 min and then programmed to 200 °C at 6 °C/min and held for 12 min. The GC peak areas were integrated with a System Instruments Model 7000B integrator. The injector temperature was 250 °C, and the detector temperature was 280 °C. A linear velocity of helium carrier gas was 33.7 cm/s at 40 °C. The injector split ratio was 1:20. A GC standard calibration curve for 2-methylthiazolidine was prepared according to the method reported previously (Ettre, 1967). Quantitative analysis of thiazolidines was performed using a standard calibration curve for 2-methylthiazolidine.

Table 1. Deviations in Relative Response Factors (RRF) of 2-Alkylthiazolidines

	deviation of RRF within a day							
	1st day		2nd day		3rd day		4th day	
2-alkylthiazolidine	RRF	RSD[a]	RRF	RSD	RRF	RSD	RRF	RSD
unsubstituted	0.731[b]	6.1	0.735	3.2	0.767	3.8	0.821	1.6
2-ethylthiazolidine	0.913	0.34	0.902	1.2	0.902	0.47	0.897	0.79
isopropylthiazolidine	0.911	0.81	0.882	2.3	0.81	1.8	0.869	1.6
propylthiazolidine	0.74	0.75	0.768	0.60	0.771	0.89	0.764	1.1
isobutylthiazolidine	0.710	0.72	0.694	1.3	0.694	1.3	0.686	1.4
butylthiazolidine	0.690	0.79	0.679	0.85	0.682	1.2	0.678	1.1
pentylthiazolidine	0.504	1.4	0.502	1.6	0.508	1.7	0.505	1.1
hexylthiazolidine	0.459	1.8	0.457	2.4	0.469	2.5	0.466	1.8
heptylthiazolidine	0.370	3.5	0.376	3.4	0.385	3.3	0.385	1.5
octylthiazolidine	0.275	9.0	0.284	6.0	0.294	5.0	0.299	2.3

[a] Relative standard deviation (%). [b] Values are the average of five replications.

Measurement of NPD Relative Response Factor. A relative response factor (RRF) for each thiazolidine derivative was defined by the equation

$$\mathrm{RRF} = S_a/S_i$$

where S_a is the peak area of each thiazolidine derivative per unit weight and S_i is the peak area of 2-methylthiazolidine per unit weight. Measurement was performed on four consecutive days and five runs were carried out each day.

RESULTS AND DISCUSSION

The RRFs of 2-alkylthiazolidines are shown in Table 1. The deviation in RRF is significantly small, indicating that use of a calibration curve of 2-methylthiazolidine to determine other thiazolidine derivatives is acceptable. In this study, the NPD detection limits of

Table 2. Amounts of Formaldehyde and Acetaldehyde Found in the Headspace of Various Kinds of Fish Flesh Heated at 200 °C

fish	no. of expts	formaldehyde (μg/g)	acetaldehyde (μg/g)
mackerel	1	0.64	5.62
	2	0.20	1.36
	3	0.61	8.58
	b	0.48 ± 0.20	5.19 ± 2.96
smelt	1	0.82	2.50
	2	0.89	5.54
	3	1.37	6.78
	b	1.02 ± 0.24	4.94 ± 1.80
horse mackerel	1	0.81	1.68
	2	0.86	3.27
	3	0.25	*a*
	b	0.64 ± 0.28	2.47
croaker	1	0.86	6.05
	2	2.51	7.27
	3	1.75	4.16
	b	1.71 ± 0.67	5.83 ± 1.28
yellow-tail	1	*a*	2.66
	2	1.09	4.37
	3	0.94	4.98
	b	1.09	3.52 ± 0.98
mackerel pike	1	1.66	11.24
	2	1.29	7.20
	3	0.80	6.24
	b	1.25 ± 0.35	8.23 ± 1.88
red salmon	1	*a*	8.43
	2	2.32	6.35
	3	3.98	5.66
	b	3.15	7.39 ± 1.17
sardine	1	3.07	11.35
	2	4.79	15.36
	3	8.07	19.70
	b	5.31 ± 2.07	15.47 ± 3.41
herring	1	1.09	13.27
	2	1.69	16.68
	3	3.27	16.20
	b	2.02 ± 0.92	15.38 ± 6.28
squid	1	3.08	1.57
	2	2.69	1.11
	3	3.01	2.42
	b	2.93 ± 0.17	1.70 ± 0.54

[a] Value was ignored. [b] Mean ± standard deviation ($n = 3$) or average ($n = 2$).

each aldehyde were 5.8 pg for formaldehyde, 7.1 pg for acetaldehyde, 10.0 pg for propanal, 12.5 pg for isobutanal, 14.4 pg for butanal, 24.7 pg for hexanal, and 36.5 pg for octanal. When the flask temperature reached 200 °C, the temperature of the fish flesh inside was 90 °C.

Among the aldehydes identified in the present study, only formaldehyde and acetaldehyde were reportedly toxic to experimental animals. Therefore, these two aldehydes will be discussed separately from the others. Table 2 shows amounts of formaldehyde and acetaldehye recovered from a headspace of fish flesh during heat treatment. The values are from three replicate experiments. Quantitative analysis of highly volatile aldehydes, particularly formaldehyde and acetaldehyde, in vapor phase is difficult. Formaldehyde tends to be adsorbed onto a glass surface (Yasuhara and Shibamoto, 1989a). Fish flesh also has nitrogen-containing compounds such as trimethylamine oxide that increase the background of an NPD chromatogram. It has been shown that trimethylamine oxide decomposes into trimethylamine and formaldehyde by heat treatment (Usuki et al., 1971). It was, therefore, extremely difficult to obtain consistent results from replicate experiments on formaldehyde and acetaldehyde analysis. However, it is obvious that considerable amounts of formaldehyde and acetaldehyde were formed in the headspace of each fish flesh during heat treatment. The approximate amounts of formaldehyde found ranged from 0.48 (mackerel) to 5.31 μg/g (sardine), and the approximate amounts of acetaldehyde found ranged from 1.70 (squid) to 15.47 μg/g (sardine). Among the aldehydes found, these two highly volatile aldehydes were generally found in the largest quantities. Sardine produced the largest amounts of both formaldehyde and acetaldehyde. Herring produced the second largest amount of acetaldehyde, but it did not produce as much formaldehyde as sardine. Acetaldehyde is probably derived from the oxidation of fish lipid ω−3 fatty acids (Boyd et al., 1992).

Table 3 shows the amounts of propanal, isobutanal, butanal, isopentanal, pentanal, hexanal, heptanal, octanal, and nonanal found in the headspace of heated fish flesh. The values are mean ± standard deviation of three replicate experiments. On a few occasions, one of the three outcomes was as much as 5 times larger or 5 times smaller than the two other outcomes; in these cases, the anomalous results were simply ignored. The results of replicate experiments from the aldehydes larger than C_3 (C_3−C_9) showed slightly more consistency than those from formaldehyde and acetaldehyde. The total amounts of aldehydes (except formaldehyde and acetaldehyde) formed ranged from 1.06 (squid) to 51.09 μg/g (sardine). The total amounts of aldehydes were formed in decreasing order in sardine, herring, red salmon, mackerel pike, mackerel, croaker, and smelt. The total amounts of aldehydes formed from horse mackerel, yellow-tail, and squid were relatively low

Table 3. Amounts of Propanal (PA), Isobutanal (IBA), Butanal (BA), Isopentanal (IPA), Pentanal (PA), Hexanal (HA), Heptanal (HPA), Octanal (OA), and Nonanal (NA) Found in the Headspace of Various Kinds of Fish Flesh Heated at 200 °C

	amount of aldehyde (μg/g of fish flesh)									
fish	PA	IBA	BA	IPA	PA	HA	HPA	OA	NA	total
mackerel	3.03 ± 1.14	3.16 ± 1.66	0.44 ± 0.10	4.00 ± 1.70	0.27 ± 0.14	0.50 ± 0.16	0.37 ± 0.08	0.23 ± 0.02	0.34 ± 0.11	12.34 ± 4.92
smelt	2.48 ± 1.00	2.40 ± 460	0.32 ± 0.08	2.18 ± 0.42	0.22 ± 0.07	0.24 ± 0.09	0.26 ± 0.14	0.11 ± 0.04	0.23 ± 0.05	8.44 ± 0.52
horse mackerel	0.49 ± 0.13	0.39 ± 0.07	0.05 ± 0.02	0.26 ± 0.02	0.02 ± 0.01	0.06 ± 0.03	*a*	*a*	*a*	1.27 ± 0.24
croaker	2.36 ± 0.37	2.32 ± 0.76	0.39 ± 0.11	2.09 ± 0.45	0.26 ± 0.10	0.34 ± 0.15	0.29 ± 0.12	0.25[b]	0.27[b]	9.38 ± 1.60
yellow-tail	2.22 ± 0.92	0.20[b]	0.15[b]	0.34[b]	0.06[b]	0.29[b]	0.05[b]	0.05[b]	0.05[b]	3.39[b]
mackerel pike	8.50 ± 1.25	3.31 ± 0.69	1.53 ± 0.43	4.27 ± 0.23	0.98 ± 0.24	1.24 ± 0.25	0.62 ± 0.15	0.51 ± 0.12	0.88 ± 0.18	21.83 ± 3.39
red salmon	9.65[b]	4.69[b]	1.48[b]	4.81[b]	1.18[b]	1.29[b]	1.38[b]	0.71[b]	1.81[b]	26.96[b]
sardine	16.96 ± 3.36	5.16 ± 1.94	5.51 ± 2.53	6.25 ± 1.56	3.44 ± 1.44	5.42 ± 2.31	3.48 ± 1.49	1.58 ± 0.62	3.29 ± 1.30	51.09 ± 16.58
herring	9.73 ± 1.61	7.53 ± 1.37	2.17 ± 0.43	7.43 ± 0.98	1.27 ± 0.30	1.46 ± 0.38	1.15 ± 0.25	1.02 ± 0.11	2.02 ± 0.46	33.78 ± 2.27
squid	0.35 ± 0.06	0.26 ± 0.06	0.01 ± 0.00	0.44 ± 0.07	*a*	*a*	*a*	*a*	*a*	1.06 ± 0.16

[a] Not detected. [b] One value was ignored (average of two experiments).

among the various kinds of fish examined. Herring and sardine produced over 3 mg of total aldehydes in a headspace from 100 g of flesh. Horse mackerel did not produce aldehydes larger than C_7. Aldehydes larger than C_5 were not found in the case of squid. However, it may not be relevant to discuss squid as a fish.

Propanal was the most abundant aldehyde found in the headspace of heated fish flesh from mackerel pike, red salmon, and sardine. Propanal might be formed mainly from $\omega-3$ fatty acids, such as eicosapentaenoic acid and docosahexaenoic acid, which make up 10–20% of all fish lipids (Ackman, 1989). Tamura et al. (1991) reported that propanal formed from $\omega-3$ fatty acid linolenic acid in relatively large amounts upon oxidation. They proposed that oxidative cleavage of a double bond at C-3 produced propanal. It was also found in autoxidized fish oil (Ke and Ackman, 1975).

As mentioned above, there are few papers on analysis of volatile compounds formed from cooked fish. Kasahara and Nishibori (1982) reported 11 volatiles, including 8 carbonyl compounds, in the headspace of smoked sardine. Carbonyl compounds were derivatized to hydrazones with 2,4-dinitrophenylhydrazine and then analyzed with gas chromatography. However, formaldehyde was not recovered by this method. Propanal and isopentanal were found as the most abundant carbonyl compounds in the headspace of dried sardine, which is consistent with the results obtained in the present study. Hexanal, heptanal, and pentanal were recovered as major aldehydes from the headspace of heated pork fat in the previous study (Yasuhara and Shibamoto, 1989).

Many different substances contribute to the pollution of indoor air. However, because people are exposed to many different vapors formed from cooking foods, it is important to determine the details of the formation of suspected toxicants, such as formaldehyde, that are formed during cooking.

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