Analysis of Volatile Compounds Formed from Fish Oil Heated with Cysteine and Trimethylamine Oxide

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Volatile compounds formed in the headspace of menhaden fish oil heated with cysteine or cysteine and trimethylamine oxide (TMAO) were analyzed by gas chromatography and gas chromatography/ mass spectrometry. Among >150 gas chromatographic peaks, 105 compounds were positively identified. Among the 45 compounds identified in the headspace of heated fish oil without TMAO or cysteine, the major compounds identified were aldehydes. When fish oil was heated with cysteine, the number of volatiles formed increased (77 compounds identified), and the addition of TMAO further increased the number of volatiles formed (87 compounds identified). The major compounds identified were 26 heterocyclic compounds: 8 furans, 8 pyridines, 1 pyrrole, 4 thiazoles, 3 thiazolines, and 2 thiophenes. TMAO acted as a promoter of the oxidative degradation of fish oil and cysteine rather than as a reactant for the nitrogen sources. The heterocyclic compounds identified are believed to contribute to the flavor of cooked fish.

INTRODUCTION

Cooked food flavors, including a cooked fish flavor, evolve from browning reaction products. A browning reaction, which is an interaction between carbonyl compounds and amine compounds, produces many volatiles, including cooked flavor compounds (Shibamoto, 1983). Among the precursors of carbonyl compounds, lipids have been reported to be an important ingredient in the formation of browning-flavor compounds in lipid-rich foods such as fish (Shahidi and Cadwallader, 1997). Fish flesh contains high levels of lipid, ranging from 0.5 to 25% (Kinsella, 1987). Polyunsaturated fatty acids (PUFAs) are the major lipids found in fish oil. For example, the levels of palmitoleic acid (16:1), oleic acid (18:1), and eicosapentaenoic acid (20:5) in menhaden oil are 11.2−17.9, 10.7−23.4, and 10.2−14.1%, respectively (Stansby, 1981). These PUFAs degrade into various carbonyl compounds or radicals such as $^{\bullet}$CHO, $^{\bullet}$CH$_3$, CH$_3$O$^{\bullet}$, $^{\bullet}$CO, $^{\bullet}$CH$_2$O, and $^{\bullet}$OH and subsequently undergo a secondary reaction with amine compounds to produce a cooked flavor (Ohnishi and Shibamoto, 1984; Lee et al., 1991; Macku and Shibamoto, 1991).

Trimethylamine oxide (TMAO) is reported to be present, often at high levels, in fish, particularly in fish living in cold water. For example, fish oils of Pacific herring (Alaska) and surf smelt (Alaska) contain 46.6 and 31.8 mmol/L TMAO, respectively (Raymond, 1994). TMAO has been predicted to form several amine compounds upon thermal degradation (Reece, 1983). Therefore, it was hypothesized that carbonyl compounds formed from lipids reacted with amines produced from TMAO to form flavor compounds, in addition to flavor compounds formed from the interaction between proteins and carbohydrates.

In the present study, volatile compounds formed in the headspace of heated menhaden fish oil with cysteine

or cysteine and TMAO were isolated using the apparatus previously descibed by Umano and Shibamoto (1987) and analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (CG/MS).

EXPERIMENTAL PROCEDURES

Materials. Menhaden fish oil was purchased from Sigma Chemical Co. (St. Louis, MO). TMAO and cysteine were bought from Aldrich Chemical Co. (Milwaukee, WI). Dichloromethane was purchased from J. T. Baker Chemical Co. (Phillipsburg, NJ). Authentic chemicals were obtained from reliable commercial sources or were gifts from Takata Co., Ltd. (Osaka, Japan).

Sample Preparation. Menhaden fish oil (10 g) was mixed with cysteine (0.1, 0.25, 0.5, or 1.0 g) or cysteine (0.1, 0.25, 0.5, or 1.0 g) and TMAO (0.25 g) in a 50 mL two-neck, round-bottom flask. The flask was connected to a simultaneous purging and solvent extraction apparatus (SPE) previously described (Umano and Shibamoto, 1987). The samples were heated at 200 °C, and the headspace volatiles were purged into 250 mL of deionized water by a steam of purified air (flow rate = 7.2 mL/min) for 6 h. The volatiles dissolved in the water were simultaneously and continuously extracted with 50 mL of dichloromethane. After the extracts were dried over anhydrous sodium sulfate, they were condensed using a rotary flash evaporator and then further condensed under a purified nitrogen stream to 0.3 mL (0.4 mg). The total amount of volatiles in a sample was estimated from GC peak area percent. The concentrated extracts were placed in vials and stored under argon at − 4 °C until analyzed.

Instrumental Analysis. A Hewlett-Packard (HP) model 5890 gas chromatograph (GC) equipped with a 60 m × 0.25 mm i.d. (d$_f$ = 1 μm) DB-Wax bonded-phase fused-silica capillary column (J&W Scientific, Folsom, CA) and a flame ionization detector (FID) was used. The linear velocity of the helium carrier gas was 30 cm/s. The injector and the detector temperatures were 250 °C. The oven temperature was programmed from 50 to 200 °C at 3 °C/min and held for 40 min.

An HP 5890 series II gas chromatograph interfaced to an HP 5791 A mass selective detector (GC/MS) was used for mass spectral identification of the GC components at an MS ionization voltage of 70 eV. Column and oven conditions were as stated above.

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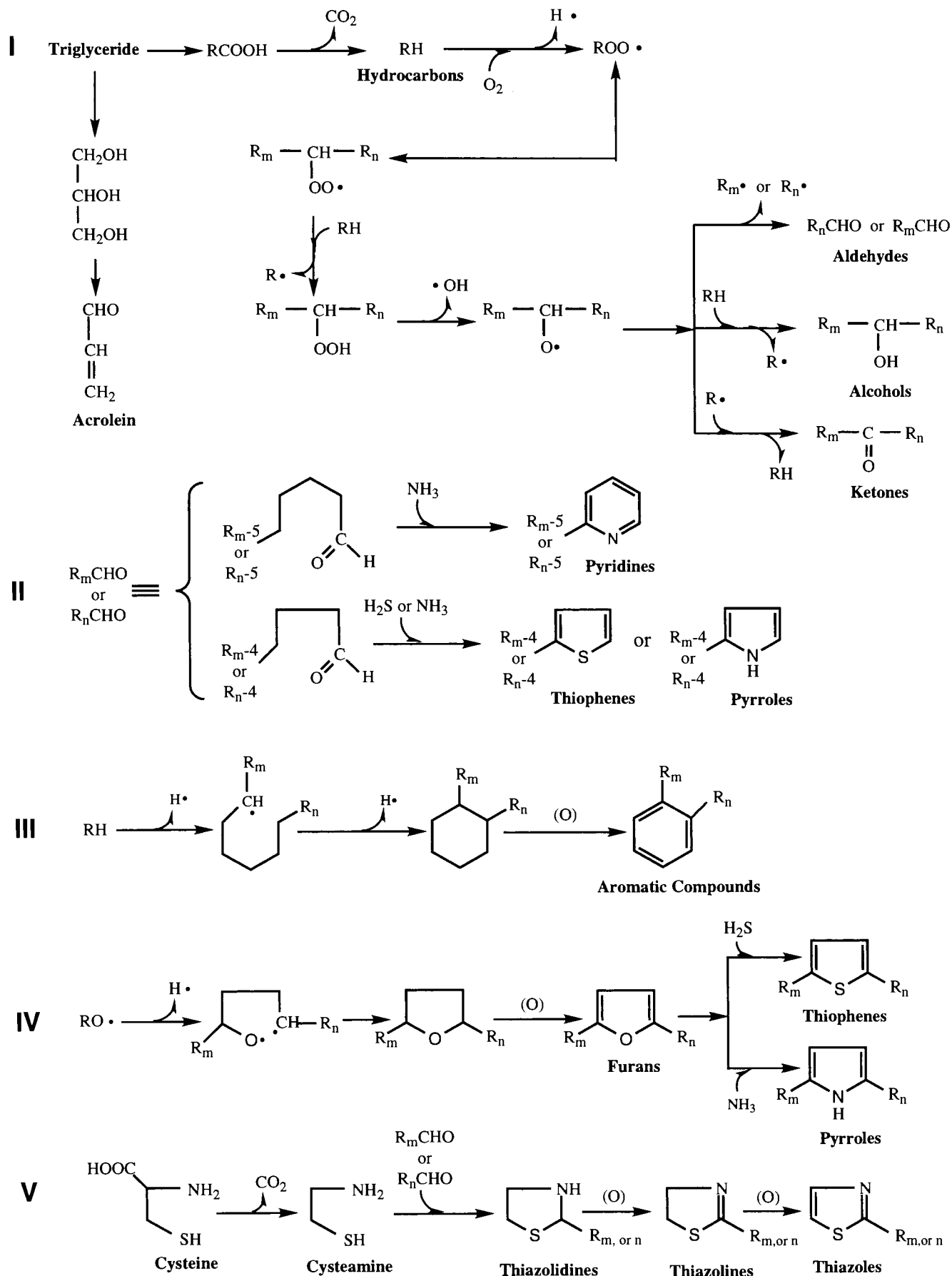
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Figure 1. Hypothesized formation pathways of compounds found in the headspace of fish oil heated with cysteine or cysteine with TMAO [extracted from Alencar et al. (1983), Buttery et al. (1977), Boelens et al. (1974), Shibamoto (1996), and Ohnishi and Shibamoto (1984)].

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Table 1. Volatile Compounds Identified in the Headspace of Fish Oil (10 g) Heated Alone or with Cysteine (0.25 g) or Cysteine (0.25 g) plus TMAO (0.25 g)

compound	Kovats[b] index	GC peak area %[a]		
		fish oil	fish oil + cysteine	fish oil + cysteine + TMAO
alcohols				
butanol	1142	c	0.18	c
1-penten-3-ol	1157	10.20	13.65	10.03
1-hexen-3-ol	1248	1.30	1.25	1.29
(E)-2-pentenol	1311	3.15	2.30	3.35
(Z)-2-pentenol	1318	0.72	0.47	0.47
hexanol	1356	0.05	c	c
1-octen-3-ol	1448	0.09	c	0.22
aldehydes				
propanal	790	0.20	0.43	0.16
acrolein	838	21.74	3.67	0.34
butanal	890	2.18	1.47	0.74
pentanal	971	1.61	0.97	0.79
(E)-2-butenal	1032	7.34	8.54	4.85
(Z)-2-butenal	1035	1.26	0.38	0.25
hexanal	1073	1.55	1.51	2.08
(E)-2-methyl-2-butenal	1084	c	0.41	0.74
(Z)-2-pentenal	1106	0.70	c	c
(E)-2-pentenal	1121	9.74	10.18	7.99
(E)-3-hexenal	1126	0.10	c	c
(Z)-3-hexenal	1131	0.15	c	c
heptanal	1176	1.73	0.80	2.38
(Z)-2-hexenal	1207	0.12	c	c
(E)-2-hexenal	1209	1.82	2.02	3.34
(Z)-4-heptenal	1228	c	c	0.12
octanal	1287	0.17	c	c
(E)-2-heptenal	1314	1.05	0.37	1.09
nonanal	1392	d	c	c
(E,E)-2,4-hexadienal	1395	0.14	c	0.05
(E)-2-octenal	1419	0.72	c	0.25
(E,Z)-2,4-heptadienal	1454	1.64	c	0.41
(E,E)-2,4-heptadienal	1483	5.53	0.25	2.08
(E)-2-nonenal	1532	d	c	c
benzaldehyde	1507	0.79	1.20	1.29
aromatic compounds				
benzene	932	0.94	2.70	2.74
toluene	1029	c	0.93	2.36
ethylbenzene	1116	0.11	2.22	2.00
propylbenzene	1196	c	0.68	0.78
butylbenzene	1301	c	0.24	0.56
1-phenyl-(Z)-1-butene	1391	c	0.29	0.90
pentylbenzene	1403	c	d	0.08
1-phenyl-(E)-1-butene	1479	c	0.07	0.40
heterocyclic compounds				
furans				
2-ethylfuran	944	1.90	3.95	3.02
2-vinylfuran	1063	c	0.08	0.14
2-butylfuran	1123	c	0.21	0.27
2-(1-propenyl)furan	1196	c	0.26	0.65
2-ethyl-5-vinylfuran	1209	c	0.25	0.35
2-pentylfuran	1220	0.37	0.82	1.35
2-hexylfuran	1323	c	c	0.28
5-methyl-2(5H)-furanone	1664	c	c	0.04
pyridines and pyrroles				
1-methylpyrrole	1127	c	0.01	0.14
1-ethylpyrrole	1168	c	0.20	0.18
pyridine	1176	c	0.90	0.96
2-methylpyridine	1214	c	0.30	0.44
2-ethylpyridine	1279	c	1.01	2.19
3-methylpyridine	1289	c	0.14	0.60
4-methylpyridine	1298	c	c	0.20
2,5-dimethylpyridine	1325	c	c	0.12
3-ethylpyridine	1375	c	c	0.13
5-methyl-2-methylpyridine	1412	c	c	0.06
thiazolines and thiazoles				
2-methylthiazole	1228	c	0.76	0.27
thiazole	1239	c	c	0.42
2-methyl-2-thiazoline	1292	c	0.53	0.44
5-methylthiazole	1318	c	c	0.29
2-methyl-3-thiazoline	1349	c	0.33	0.29
2-propylthiazole	1372	c	c	0.07
2-propyl-2-thiazoline	1442	c	d	0.08

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Table 1. (Continued)

compound	Kovats[b] index	GC peak area %[a]		
		fish oil	fish oil + cysteine	fish oil + cysteine + TMAO
thiophenes				
2-ethylthiophene	1162	c	0.09	0.18
3-vinylthiophene	1332	c	c	0.09
hydrocarbons				
heptane	705	0.53	0.25	0.99
1-heptene	740	0.21	0.11	0.03
2,3-dimethyl-1,3-butadiene	757	c	0.06	c
ethylcyclopentane	773	c	0.03	c
octane	800	1.51	2.31	0.87
1-octene	850	c	0.04	c
(Z)-2-octene	862	c	0.35	0.11
nonane	900	c	1.20	0.27
(E)-1,3-octadiene	955	c	c	0.02
butylcyclopentane	971	c	0.29	0.18
(E,Z)-2,4-octadiene	991	c	0.09	0.09
decane	1000	0.48	0.82	0.66
(E,E)-1,3,5-heptatriene	1005	c	c	0.37
(E,Z)-1,3,5-heptatriene	1020	c	c	0.37
(E)-1,3-nonadiene	1046	c	d	0.10
1,3,5-octatriene	1094	c	0.35	0.56
(E,Z)-1,3,5-octatriene	1097	c	0.69	0.82
undecane	1100	c	0.61	0.49
(E,Z)-2,4,6-octatriene	1151	c	c	0.09
dodecane	1200	0.28	c	c
(Z,E,E)-2,4,6-nonatriene	1239	c	c	0.09
pentadecane	1500	d	0.13	c
ketones				
2-butanone	911	0.20	d	0.32
1-buten-3-one	938	c	c	0.04
2-pentanone	971	c	0.92	0.69
1-penten-3-one	1014	1.63	2.75	1.40
3-hexanone	1042	c	0.13	0.15
2-hexanone	1068	c	0.07	c
3-penten-2-one	1120	0.14	c	c
2-heptanone	1171	c	d	0.35
3-hexen-2-one	1209	0.61	0.71	0.70
2-nonanone	1381	c	0.05	0.17
miscellaneous compounds				
ethylene sulfide	899	c	0.12	0.15
1H-indene	1466	c	c	0.04
3,5-dimethyl-1,2,4-trithiolane	1583	c	c	0.03
3,5-dimethyl-1,2,4-trithiolane isomer	1604	c	c	0.02
γ-butyrolactone	1618	0.26	c	c
2,4,6-trimethyl-1,3,5-dithiazine	1745	c	c	0.02

[a] Solvent peak excluded. [b] On DB-Wax. [c] Not found. [d] Peak area % <0.001.

Volatile compounds were identified by comparison with the Kovats gas chromatographic retention index I (Kovats, 1965) and by the MS fragmentation pattern of each component compared with those of authentic chemicals.

RESULTS AND DISCUSSION

Table 1 shows the volatile compounds identified in the headspace of fish oil heated with cysteine or cysteine and TMAO. On the basis of radicals formed from a triglyceride upon oxidative degradation, Figure 1 shows the hypothesized formation pathways of volatile compounds identified in the present study. As shown in pathway I, a triglyceride produced glycerol and fatty acids. Acrolein, which was the major compound formed in the headspace of heated fish oil (relative GC peak area % = 10.20), is known to form from oxidative homolytic fission of C−O bonds of glycerol moiety in lipids (Loefroth et al., 1989). When 120 g of beef fat and various cooking oils were heated, 50−110 mg of acrolein was recovered from their headspace (Umano and Shibamoto, 1987). The addition of cysteine and TMAO decreased considerably the recovery of acrolein, suggesting that acrolein underwent a secondary reaction. Hydro-

carbons (RH) were formed readily from fatty acid moieties of triglyceride. Many unsaturated hydrocarbons were identified in the present study, suggesting that menhaden fish oil contains many unsaturated fatty acid moieties as mentioned above.

In a previous study (Shibamoto and Horiuchi, 1997), the addition of TMAO increased the number of hydrocarbons recovered from 6 to 17. An attempt was made to use TMAO as a source of amines for the browning reaction because, as mentioned above, fish oil contains a fairly large amount of TMAO. Unexpectedly, only two nitrogen-containing compounds (N,N-dimethylformamide and N-methylpyrrole) were recovered. Therefore, it was hypothesized that TMAO worked as an oxidative agent rather than as a precursor of flavor compounds. In fact, TMAO has been reported as a prooxidant (Knolker, 1996). The location of the hydroperoxide radical (ROO•) on carbon atoms in a fatty acid moves readily, and many isomeric hydroperoxides [$R_mCH(O•)$-R_n] were formed. These hydroperoxides produced various aldehydes, alcohols, and ketones (deMan, 1992). Aldehydes and ketones are primarily derived from the thermal oxidation of fatty acids (Frankel, 1982), and
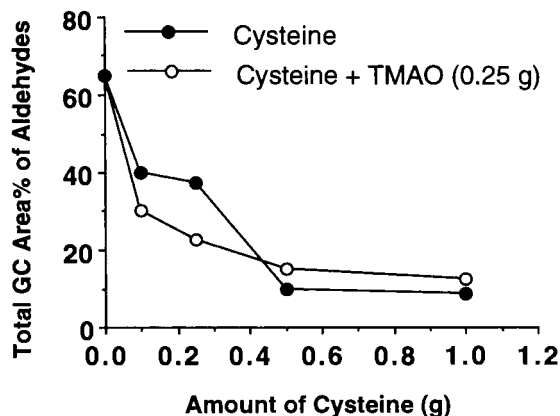
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Figure 2. Concentration of total volatiles recovered from fish oil heated with different amounts of TMAO and/or cysteine.

Figure 3. Relative amounts of aldehydes recovered from fish oil heated with different amounts of TMAO and/or cysteine.

they were the most abundant products from fish oil in the present study. Because air was used to purge the headspace of samples (200 °C) for a prolonged time period (6 h), oxygen in the air stream might also play an important role in the oxidative degradation of vapor-phase lipids.

The addition of cysteine and cysteine with TMAO increased the number of volatile compounds from 45 to 77 and 87, respectively. However, the number of volatiles produced from fish oil did not significantly influence the different amounts of cysteine added. Therefore, results from only the most representative samples are reported in Table 1. The major compounds identified were heterocyclic compounds: 8 furans, 8 pyridines, 2 pyrroles, 4 thiazoles, 3 thiazolines, and 2 thiophenes. Nitrogen- and/or sulfur-containing heterocyclic compounds were the major products in the above two reaction systems. Production of these heterocyclic compounds has been reported in many Maillard reaction systems that consisted of a lipid and an amino acid (Mottram, 1996). For example, sulfur-containing heterocyclic compounds were the most predominant of all the volatile compounds isolated from the headspace of peanut oil heated with cysteine (Chung et al., 1994).

As shown in pathway II (Figure 1), thiophenes or pyrroles were hypothesized to form from fatty aldehydes and hydrogen sulfide or from ammonia formed from cysteine via Strecker degradation, respectively (Schönberg et al., 1948). Pyridines were the most predominant heterocyclic compounds found in the present study. They were hypothesized to form from the corresponding *n*-aldehyde with ammonia produced from cysteine (Buttery et al., 1977). Relative amounts of certain aldehydes such as 2-butenal, hexanal, 2-pentenal, and 2,4-heptadienal decreased when the amount of cysteine increased, suggesting that these aldehydes underwent a secondary reaction with ammonia formed from cysteine to give pyridines.

A series of alkylbenzenes that might be formed from alkylcyclohexanes via oxidation were found, particularly in the samples with cysteine and TMAO (pathway III in Figure 1). Alkylcyclohexanes were hypothesized to form from alkyl radicals $(R_mCH(\cdot)R_n)$ (Alencar et al., 1983). Many alkylcyclohexanes were found in heated beef fats (Ohnishi and Shibamoto, 1984), but they were not recovered in the present study. This may be because of a lack of saturated fatty acid moieties in the particular fish oil used. A benzene ring must be formed readily from an unsaturated fatty acid moiety upon oxidation.
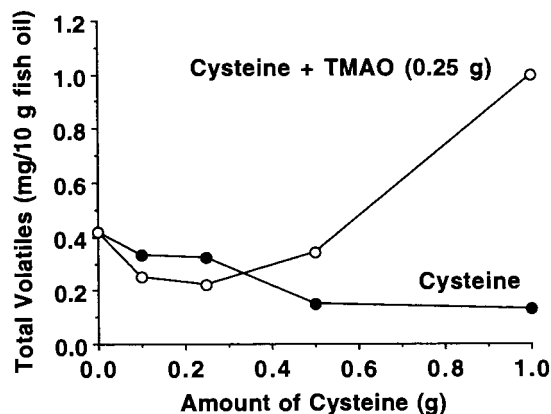
Furans, which are known as sugar degradation products (Hodge, 1967), may form from a RO• as shown in pathway IV (Figure 1). Furans were reportedly converted into thiophenes and pyrroles via exchange of an oxygen atom with a sulfur atom of hydrogen sulfide and with a nitrogen atom of ammonia, respectively (Boelens et al., 1974).

As shown in pathway V, thiazolines and their dehydrogenation product thiazoles were hypothesized to form from thiazolidines upon oxidation. Cysteamine, which formed from cysteine upon decarboxylation, reacted with aldehydes to yield thiazolidines (Fujimaki et al., 1969). Recovery of cyclic ethylene sulfides such as trithiolanes and a dithiazine suggests that radicals with a smaller number of carbon units such as $CH_2\cdot$ and $CH_2CH_2\cdot$ were formed, and these radicals may also produce thiazolines and thiazoles with ammonia and hydrogen sulfide.

Figure 2 shows the estimated total amounts of volatiles produced from fish oil with different amounts of cysteine or cysteine with TMAO. Estimated total amounts of volatiles decreased when the amounts of cysteine increased. However, when the amount of cysteine was increased over 0.25 g with 0.25 g of TMAO, the amount of volatiles increased considerably. Therefore, it may be confirmed that TMAO promoted oxidative degradation of cysteine to yield hydrogen sulfide and ammonia (Fujimaki et al., 1969), as well as the degradation of fish oil to yield many carbonyl compounds (Frankel, 1982). These secondary products reacted with each other to yield many heterocyclic compounds. Figure 3 shows the total GC peak area percent of aldehydes recovered from fish oil heated with various amounts of cysteine or cysteine with TMAO. It is obvious that the relative amount of aldehydes decreased when cysteine increased, suggesting that the aldehydes formed were consumed to produce secondary compounds.

Cooked fish possesses a particular flavor which is different than that of other cooked foods or beverages such as meats, bread, and coffee. In the present study, pyrazines (which are known as chemicals possessing a roasted or toasted flavor and which give cooked flavors to heat-treated foods and beverages) were not found. Pyrazines are also the most abundant heterocyclic compounds found in the Maillard reaction model systems (Shibamoto, 1983). However, pyridines were the most predominant flavor chemicals found in fish oils heated with cysteine. Therefore, instead of pyrazines, pyridines may play an important role in the flavor of cooked fish.

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