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Influence of Sulfur Amino Acids on the Volatile and Nonvolatile Components of Cooked Salmon (*Salmo salar*)

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Volatile and nonvolatile compounds, which could contribute to flavor, were analyzed in salmon. One hundred twenty-three volatile compounds were identified in the headspace of two different samples of cooked salmon, including lipid-derived volatiles, Maillard-derived volatiles, sulfur volatiles, Strecker aldehydes, nitrogen heterocyclic compounds, terpenes, and trimethylamine. Significant differences between samples were found for 104 of the volatiles. Although the levels of free cysteine and methionine were low in the salmon, sulfur volatiles were formed in the cooked fish, demonstrating that there were sufficient sulfur amino acids present for their formation. Notable differences in sulfur compounds between the samples suggested that small changes in sulfur amino acids could be responsible. When this hypothesis was tested, salmon heated with cysteine had increased levels of many thiophenes, thiazoles, alicyclic sulfides, and nitrogen heterocycles. With the addition of methionine, levels of dimethyl sulfides, two alicyclic sulfides, pyrazines, some unsaturated aldehydes, and alcohols and 2-furanmethanethiol increased. The largest difference found among the nonvolatile (low molecular weight water-soluble) compounds was in inosine monophosphate.

KEYWORDS: Aroma volatiles; salmon flavor, cooked fish flavor; sulfur volatiles; tastants; cysteine; methionine; inosine monophosphate

INTRODUCTION

Fish flavor is derived from nonvolatile taste-active compounds and volatile compounds, which may stimulate the odor receptors. In a previous study of haddock, the nonvolatile low molecular weight components were found to be responsible for major flavor notes of the cooked haddock (*1*). The nonvolatile low molecular weight water-soluble components can also be the precursors of the volatile aroma compounds. The aroma of raw fish is the result of a complex mixture of volatile compounds, which result from many processes such as microbial enzymic breakdown and lipid degradation (enzymic and oxidative). In the case of cooked fish, the Maillard reaction and Maillard−lipid interactions are also important (*2*). Much of the literature has concentrated on lipid degradation and the effects of storage on fish flavor due to the highly unsaturated nature of fish polyunsaturated fatty acids (PUFAs) (*2*−*5*). Typical lipid-derived volatiles in fish are unsaturated C8 and C9 alcohols, aldehydes, and ketones, which give fresh plantlike aromas (*4*). Trimethylamine (TMA) is the most characteristic volatile in fresh saltwater fish (*2*) and is responsible for "old fishy, ammonia" odors (*4*). It is formed by the reduction of trimethylamine oxide (TMAO) by bacterial enzymes (*3*, *4*). TMAO can also act as an oxidizing agent, for example, promoting the degradation of both cysteine and fish oils (*6*). Cooked fish are generally low in typical Maillard reaction flavor volatiles, such as pyrazines (*6*), in comparison to levels in most meats, probably due to lower levels of free sugars (*7*, *8*). There are some aroma volatiles that are specific to certain fish. Canned tuna fish has a meatlike aroma thought to be due to 2-methyl-3-furanthiol (*9*). Salmon is high in carotenoid pigment, resulting from either crustacea in the diet in the case of wild fish or added carotenoids in the case of reared fish, which leads to the pink flesh and also to specific flavor volatiles with alkyl furanoid type structures (*10*). However, little is known regarding the contribution of volatile sulfur compounds to fish flavor. The level of volatile sulfur compounds in fish is typically low in comparison to the lipid-derived compounds (*2*), but as many sulfur volatiles have very low odor thresholds, they can be important to the overall flavor perception (*11*). The sulfur volatiles are generally thought to result from reactions involving cysteine or methionine despite the very low levels of these free amino acids in fish (*11*, *12*). Whereas methionine is an essential amino acid in the fish diet, cysteine is considered nonessential as it can be synthesized from the methionine (*13*). Methional and thiophenecarboxaldehyde have been identified as odor-active compounds of raw salmon (*14*). Methional has been found to be a potent odorant in boiled cod and boiled salmon, while other methionine-derived compounds such as methanethiol and the dimethyl sulfides were potent odorants in boiled cod alone (*15*). Dimethyl

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sulfides, thiophene derivatives, and dihydro-2*H*-thiopyran-2(4*H*)one have previously been identified from the headspace of canned salmon (*16*). It appears that more sulfur compounds have been found in shellfish. In shrimps and prawns, for example, dimethyl sulfides and derivatives of thiophene, thiazole, trithiolane, and dithiazine were identified. The seafood was "uncooked", although the number of sulfur compounds found increased where heating was involved in the extraction technique (*12*).

Of the nonvolatile components in fish, nucleotides and related compounds are important in fish flavor because of their umami properties. Following the death of a fish and during subsequent storage, adenosine triphosphate (ATP) is dephosphorylated to adenosine diphosphate and then to adenosine monophosphate, which undergoes oxidative deamination to form inosine monophosphate (IMP). Further degradation to inosine and hypoxanthine may occur (*17*). In most fish species, ATP degrades very quickly to IMP. Fresh fish contains more IMP than would normally be required to cause flavor enhancement, and the slow conversion of IMP to inosine that occurs when fish flesh is stored is thought to be an important cause of flavor loss (*18*). IMP itself does not readily act as a flavor precursor in the formation of volatile flavor compounds. However, hydrolysis of IMP releases both hypoxanthine and ribose-5-phosphate, the latter being a reactive Maillard precursor. Hence, despite the loss of flavor enhancement as fish ages and IMP is degraded, there is an increase in reactive Maillard precursors, which can form aroma compounds on cooking. Creatine and creatinine are guanidine compounds that are also characteristic constituents of muscle tissue (*19*). Both constituents have been found to be taste-active in previous studies (*20*).

This paper reports a study of the volatile and nonvolatile compounds in salmon that may contribute to flavor in canned fish, with particular emphasis on sulfur compounds. To determine the role of the sulfur amino acids in aroma generation, volatiles were also analyzed from samples to which cysteine and methionine had been added.

EXPERIMENTAL PROCEDURES

Materials. Fresh filleted and boned farmed salmon were purchased from local retail suppliers and stored at 4 °C overnight before use. Nonvolatile compounds were analyzed in fillets from four separate salmon. One of these fillets was also used for volatile analysis (denoted salmon 4). A fifth separate salmon was purchased for volatile analysis only (salmon 5).

For capillary electrophoresis (CE), all reference compounds (amino acids, nucleotides, creatine, creatinine, anserine, and carnosine) were purchased from Sigma-Aldrich Co. Ltd. (Dorset, United Kingdom) and were ≥99% purity. Reagent grade phosphoric acid, disodium hydrogen phosphate, and sodium tetraborate were also from Sigma-Aldrich Co. Ltd.. The EZ-Faast amino acid analysis kit (Phenomenex, Macclesfield, United Kingdom) was used for the analysis of amino acids by gas chromatography−mass spectrometry (GC-MS). Norvaline, used as an internal standard, was from Sigma-Aldrich Co. Ltd. For sugar analysis, high-performance liquid chromatography (HPLC) electrochemical-grade sodium hydroxide solution and HPLC gradient water were purchased from Fisher Scientific UK (Loughborough, United Kingdom), and anhydrous sodium acetate was from Sigma-Aldrich Co. Ltd.

L-Cysteine (97%) and L-methionine (≥98%) used in solution for addition to the fish were purchased from Sigma-Aldrich Co. Ltd. 1,2-Dichlorobenzene in methanol (130.6 μg/mL) and alkane standard C6−C25 (100 μg/mL in diethyl ether), used as GC-MS standards, were also obtained from Sigma-Aldrich Co. Ltd. Authentic samples of reference flavor compounds were either purchased from a range of laboratory chemical suppliers or obtained as gifts from flavor laboratories.

Preparation of Sample Extracts for Analysis of Nonvolatile Components. Fresh salmon (0.3 kg) was blended in a food processor, and portions of 5 g were weighed into polypropylene copolymer NalgeneR Oak Ridge centrifuge tubes (Nalge Nunc International, Rochester, NY). Cold water (10 mL) and 300 μL of an aqueous solution of norvaline (1 mg/mL) and 500 μL of a purine solution (1 mg/mL) were added as internal standards. Where amino acids solutions were added to the salmon (see preparation of extracts for volatile analysis), the homogenate was sampled and prepared as above. The tubes were shaken for 2 min prior to centrifugation at 10000*g* for 20 min at 4 °C in a RC-5C Plus SorvallR centrifuge. The supernatant was decanted in a 50 mL polypropylene tube, and the residue was re-extracted with 5 mL of cold water. The two extracts were combined and filtered through a 0.2 μm membrane filter (Millipore) to remove any fat and/or tissue particles. Finally, 10 mL of the filtrate was transferred to a CentriplusR YM-3 ultrafiltration tube with 3000 MWCO regenerated cellulose membrane (Millipore Corp., Bedford, MA) and centrifuged at 5000*g* for 4 h at 4 °C. The filtrate was stored at −18 °C until further analysis.

Determination of Peptides and Other Amino Compounds by CE. The method used for the determination of peptides and other amino compounds was a modification of the one described by Flores et al. (*21*). The samples were analyzed by CE using a HP3D CE with diode array detection and a HP3D Chemstation for instrument control (Agilent, Palo Alto, CA). Electrophoretic separation was performed at constant pressure (50 mbar) with a 5 s injection of the sample onto an extended light path capillary of 48.5 cm total length (40 cm to detector) × 50 μm i.d. × 3 bubble factor, maintained at 25 °C. A 100 mM phosphate (Na_2HPO_4) buffer adjusted to pH 2.5 was used for the separation. (The buffer was changed every run.) Preconditioning consisted of 3 min with 0.1 M sodium hydroxide followed by 3 min with buffer. A constant voltage of 15 kV was applied with a positive to negative polarity. Detection was at 200 nm, and full spectra were collected between 195 and 600 nm.

Determination of Nucleotides by CE. A modification of the method described by Uhrova et al. (*22*) was used for the determination of nucleotides and related compounds in the salmon samples. Aqueous fish extracts were analyzed by CE (instrument as above). Electrophoretic separation was performed at constant pressure (50 mbar) with a 5 s injection of the sample onto an extended light path capillary of 64.5 cm total length (56 cm to detector) × 75 μm i.d. × 2.7 bubble factor, maintained at 25 °C. A 0.02 M phosphate−borate buffer adjusted to pH 9.2 was used for the separation (the buffer was changed every run). Preconditioning consisted of 5 min with 0.1 M NaOH followed by 5 min with buffer. A constant voltage of 20 kV was applied with a positive to negative polarity. Detection was at 254 nm for 30 min, and full spectra were collected between 195 and 600 nm.

Determination of Free Amino Acids by GC-MS. The free amino acids were measured using the EZ-Faast amino acid derivatization technique (Phenomenex, Torrance, CA) followed by analysis on the Clarus 500 GC-MS system as described by Elmore et al. (*23*).

Determination of Monosaccharides and Disaccharides by Anion Exchange Chromatography HPIC-PAD. This was carried out on an 8220i Dionex high-performance anion exchange chromatography system with pulsed amperomeric detection (Dionex Corp., Sunnyvale, CA) using the method described by Elmore et al. (*23*).

Preparation of Sample Extracts for Analysis of Volatile Compounds. Samples from both salmon 4 and salmon 5 (200 g) were chopped, and each was blended (20 s) with 200 mL of required solution and placed into autoclave bottles (100 mL). Solutions were as follows: distilled water (control), cysteine at 0.01% (w/v) (low cysteine) or 0.1% (w/v) (high cysteine), and methionine at 0.01% (w/v) (low methionine) or 0.1% (w/v) (high methionine). The additions of 0.01 (w/v) and 0.1% (w/v) were equivalent to 5 and 50 mg amino acid/100 g fish, respectively.

All samples were cooked in an autoclave (Certoclav, Austria) for 40 min at 125 °C. Samples were reweighed postcooking to ensure no moisture loss. Informal sensory analysis of the samples was carried out by three experienced panelists sniffing the samples.

Samples were rehomogen*ize*d postcooking, and 25 g of each mixture was placed in a 250 mL conical flask with a Dreschel head for the collection of volatile compounds. Oxygen-free nitrogen was passed over

the sample for 1 h at a rate of 40 mL/min. The volatiles were swept onto a preconditioned glass trap (4 mm i.d., 1/4 o.d. × 3.5 mm long), packed with Tenax TA (Supelco, Poole, United Kingdom). Throughout the collection, the sample was maintained in a water bath at 60 °C. Following extraction, the trap was flushed with nitrogen for 10 min to remove moisture. 1,2-Dichlorobenzene in methanol (1 μL containing 130.6 ng/μL) was added as a standard to the trap. Four replicates of each sample were extracted for analysis by GC-MS.

GC-MS Analysis of Volatile Compounds. After the headspace collection of volatiles, the samples were analyzed using a Perkin-Elmer Clarus 500 GC-MS system (Perkin-Elmer, Beaconsfield, United Kingdom) equipped with an automated thermal desorber (Turbomatrix ATD). The Tenax tubes were desorbed at 300 °C (heating rate 40 °C/s) and cryofocused onto a packed cold trap at −30 °C. GC separation was carried out on a DB5 nonpolar column (60 m × 0.32 mm id, 1 μm film thickness; J&W Scientific, Agilent). The temperature program employed was 2 min at 40 °C, a ramp of 4 °C/min to 260 °C, and hold for 10 min. The mass spectrometer was operated in the electron impact mode with a source temperature of 230 °C, an ionizing voltage of 70 eV, and a scan range from *m*/*z* 29 to 350. The data were controlled and stored using TurboMass software (Version 4.5, Perkin-Elmer).

The identification of the compounds was based on the comparison of their mass spectra with spectra from authentic compounds analyzed in our laboratory, spectra from the NIST/EPA/NIH Mass Spectral Database (Version 2.0a, 2002), or spectra published elsewhere. To confirm the identification, the linear retention index (LRI) was calculated for each volatile using the retention times of a homologous series of C6−C25 *n*-alkanes and by comparing the LRI with those of authentic compounds analyzed under similar conditions. The approximate quantification of volatiles was calculated from GC peak areas, by comparing with the peak area of the 1,2-dichlorobenzene standard, using a response factor of 1. Although this does not allow for accurate quantification, it does allow for comparison between samples. The mean coefficient of variation for individual components was 24%.

Statistical Analysis. For the nonvolatile compounds, the data were analyzed by one-way analysis of variance (ANOVA) using the STATGRAPHICS Plus 4.1 package (Herndon, VA). Means were compared using the Fisher's least significant difference (LSD) test at $p < 0.05$. To analyze the results of volatile compounds, multifactor ANOVA was carried out for each volatile compound. The factors used were the salmon, level of cysteine, and level of methionine. The STATGRAPHICS Plus 4.1 software was also used to carry out principal component analysis (PCA).

RESULTS AND DISCUSSION

Nonvolatile Compounds. The concentrations of free amino acids, other amino compounds, and nucleotides in four different samples of raw salmon are shown in **Table 1**. There were significant differences between the samples of salmon for the majority of nonvolatile compounds. The differences between free amino acids were statistically significant but relatively small. However, it is thought that small differences in free amino acids, such as cysteine, may have an effect on the flavor compounds formed. Regarding nucleotides, it is known that their concentration can vary significantly between fish, due to different physiological conditions, as a result of differences in capture methods and postmortem storage conditions (*24*). This helps to explain the highly significant differences between the concentrations of nucleotides in this study. The concentrations of hypoxanthine, inosine, and IMP, which are products of nucleotide degradation, can be used to determine the freshness of fish (*17*). The level of IMP formed by nucleotide breakdown in fish flesh reaches a peak within 1−2 days postmortem, and as it decreases, fish tend to become less flavorsome and less acceptable (*25*). The significant differences in IMP levels between the four salmon samples would indicate that the fish were bought at different postmortem ages. Such differences in IMP may have implications for the flavor quality of the salmon as perceived by the consumer. Creatine was found in much higher amounts in all salmon as compared to creatinine. In fish, creatine is often found in higher amounts than creatinine, the dehydration product of creatine (*4*). Sugar levels in raw fish were analyzed for one sample only (salmon 4). Glucose was found at substantially higher levels than ribose and fructose. Most fish contain some free glucose and ribose, whereas fructose occurs only in certain species of fish (*18*, *26*). Glucose and ribose form in fish muscle postmortem by degradation of glycogen and ATP, respectively (*18*).

Table 1. Mean Values[a] (mg/100 g) of Nonvolatile Compounds in Raw Samples of Salmon

compounds	salmon no. 1	2	3	4	sig[b]	LSD
free amino acids						
alanine	33 a	38 b	38 b	33 a	**	2.5
arginine	2.5 ab	3.1 c	2.7 b	2.5 a	***	0.21
aspartic acid	1.9 a	1.3 a	3.5 b	2.9 b	***	0.66
cysteine	0.04 ab	0.04 a	0.07 b	0.02 a	*	0.03
cystine	ND[c]	ND	ND	ND		
glutamic acid	26 bc	17 a	23 b	31 c	**	5.2
glycine	20 bc	17 a	21 c	18 ab	*	2.7
histidine	4.8 a	12.3 bc	9.8 b	14.1 c	***	2.5
isoleucine	4.3 ab	3.9 a	4.9 b	3.7 a	**	0.61
leucine	6.2 a	5.9 a	8.3 c	7.4 b	***	0.89
lysine	6.7 b	4.8 a	8.8 c	8.7 c	***	1.2
methionine	0.08 b	0.02 a	ND a	ND a	*	0.05
phenylalanine	2.5 a	3.3 a	5.0 b	5.0 b	***	0.99
proline	2.4 a	3.2 b	3.9 c	3.1 b	**	0.57
sarcosine	0.62 a	0.67 a	1.4 b	0.99 ab	*	0.49
tryptophan	0.14 a	0.15 a	0.39 b	0.25 a	**	0.12
tyrosine	4.0 a	4.4 a	7.7 b	6.6 b	**	1.5
β-alanine	4.1 a	7.7 b	4.5 a	9 c	***	0.91
other amino compounds						
creatinine	1.9 b	2.4 c	1.4 a	1.8 b	***	0.17
anserine + carnosine	809 c	1003 d	601 b	457 a	***	56
creatine	542 b	504 b	337 a	301 a	**	102
nucleotides						
hypoxanthine	14 c	22 d	12 b	9.4 a	***	1.34
inosine	159 d	133 c	66 a	91 b	***	11.3
5AMP	ND	ND	ND	ND		
5IMP	23 b	17 b	99 c	1.1 a	***	6.5

[a] Means with different letters within a row are significantly different at $p < 0.05$. [b] Significant at ***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$. [c] Not detected.

Changes in nonvolatile compounds occurring during cooking are shown in **Table 2** for both salmon 3 and salmon 4. Creatine was transformed to creatinine during the cooking procedure, as shown by a dramatic increase in creatinine in the cooked salmon and a decrease in creatine as compared to raw levels. This has been found in previous studies (*19*). Regarding the sugars, glucose and ribose significantly decreased on cooking, whereas fructose significantly increased, although the levels were very low (**Tables 2** and **3**). It is likely that glucose and ribose readily took part in Maillard reactions, where ribose is typically five times more reactive than glucose (*18*).

Changes in free amino acids on cooking were small and not significant. There is very little literature on levels of cysteine and methionine in fish during and postcooking. It is likely that some methionine and cysteine are released from the total protein on cooking. Cooking of shrimps was found to increase methionine from 0.28 to 0.46 g/100 g, dry weight (*12*). However, nonsignificant changes were also reported in a previous study of cooked haddock (*1*). It is possible that any release of these amino acids from cooking may be similar or slower than the rate of degradation or utilization of the amino acids.

Table 2. Mean Values (mg/100 g) of Nonvolatile Compounds in Raw and Cooked Salmon

	salmon 3			salmon 4		
compounds[a]	raw	cooked	sig[b]	raw	cooked	sig[b]
free amino acids						
cysteine	0.07	ND	**	0.02	ND[c]	NS
sarcosine	1.4	1.6	NS	1.0	1.8	**
tryptophan	0.39	0.31	*	0.25	0.41	NS
β-alanine	4.5	3.9	*	9.0	8.4	NS
other amino compounds						
creatinine	1.4	175	***	1.8	179	***
anserine + carnosine	601	542	*	457	443	NS
creatine	337	99	***	301	89	***
nucleotides						
inosine	66	76	**	91	87	NS
5AMP	ND	9.1	***	ND	8.6	***
5IMP	99	68	***	1.1	2.2	**
sugars						
glucose				19	11	***
fructose				0.81	2.0	***
ribose				1.6	0.40	***

[a] Only compounds where significant differences were found between raw and cooked samples are included in this table. Other compounds given in **Table 1** and not listed here were not significantly changed on cooking. [b] Significant at ***p < 0.001, **p < 0.01, and *p < 0.05; NS, not significant. [c] Not detected.

Table 3. Mean Values (mg/100 g) of Nonvolatile Compounds in Raw and Cooked Salmon (Salmon 4), with Added Cysteine or Methionine (50 mg/100 g Fish)

	with added cysteine			with added methionine		
compounds[a]	raw	cooked	sig[b]	raw	cooked	sig[b]
free amino acids						
cysteine	42	3.6	***	0.07	0.01	*
cystine	37	5.5	**	ND[c]	ND	
methionine	ND	1.2	**	64	62	NS
sarcosine	0.54	1.1	**	1.0	1.0	NS
tryptophan	0.28	0.74	**	0.43	0.37	NS
other amino compounds						
creatinine	2.2	196	***	2.1	195	***
creatine	351	97	***	345	96	**
nucleotides						
5AMP	ND	9.8	***	ND	9.5	***
5IMP	3.2	4.2	**	3.0	5.2	**
sugars						
glucose	19	12	***	18	12	**
fructose	0.77	2.1	***	0.58	2.3	*
ribose	1.6	0.44	**	0.39	0.31	NS

[a] Only compounds where significant differences were found between raw and cooked samples are included in this table. Other compounds given in **Table 1** and not listed here were not significantly changed on cooking. [b] Significant at ***p < 0.001, **p < 0.01, and *p < 0.05; NS, not significant. [c] Not detected.

The levels of free cysteine and free methionine were very low in all samples (0.02−0.07 and 0−0.08 mg/100 g, respectively). These values are lower than values recorded in the literature for most fish types. The level of free methionine found in fish (noncrustacean) is typically 1−6 mg/100 g (*1*, *27*−*29*). The level is higher in shell fish, varying from 9 to 60 mg/100 g in crab, lobster, and prawn (*27*). There are very few literature measurements of cysteine in fish partly because it is a difficult compound to quantify and second because the levels are very low in fish. The levels of cysteine and methionine chosen to spike the salmon samples were representative of typical and maximum levels that have been found in fish. Levels of 0.005 and 0.05 g amino acid/100 g fish were used.

For salmon 4, raw and cooked salmon spiked with cysteine and methionine are compared in **Table 3**. The addition of 50 mg/100 g amino acid gave measured levels of 80 mg/100 g cysteine plus cystine and 60 mg/100 g methionine in the spiked raw salmon samples. The salmon spiked with cysteine demonstrated a substantial and significant decrease of cysteine on cooking. Cysteine is readily degraded to hydrogen sulfide and other reactive intermediates on heating (*11*). The level of methionine was not significantly decreased on cooking of the methionine-spiked fish. This does not indicate that the methionine was not involved in the formation of sulfur volatiles since the amount of methionine required to form odor-active volatile compounds with low odor thresholds will be small.

Aromas of Heated Salmon Systems. One of the salmon samples used for nonvolatile analysis (salmon 4) was examined for headspace volatiles and aroma assessment after cooking. Another salmon sample was also used for comparison (salmon 5).

After cooking, the aromas of the salmon samples differed. The salmon 4 control sample generated "salmon, chicken, tinned salmon, nonfatty, poached salmon" notes. With the high level of added cysteine (50 mg/100 g fish), the aroma was described as "liver, old mutton, fatty, fried fish". The added methionine (50 mg/100 g fish) gave the fish sample "canned potato, onion, fish pie" notes. The salmon 5 sample, cooked and evaluated on a separate day, had a more fatty odor than salmon 4, described as "fatty, lamb fat, lamb stew, cod liver oil, cake crust, green, pungent, not very fishy". The addition of a low level of cysteine (5 mg/100 g fish) reduced the lamb fat aroma, producing a "less fatty" note as compared to the control fish. The high cysteine addition (50 mg/100 g fish) produced a sample that was "more fishy than control, roasted fish, sulfur, boiled eggs, less fatty, fried". The low level of methionine added (5 mg/100 g fish) produced a sample similar to the control with "slight onion, smoky, potato" notes.

Volatile Compounds. One hundred twenty-three volatile compounds were tentatively identified in two different samples of cooked salmon (**Table 4**). Among these, lipid-derived volatiles (alcohols, aldehydes, thiophenes, and furans), Maillard-derived volatiles (pyrazines, thiazoles, and thiophenes), other sulfur volatiles (methional, dimethyl sulfides, and alicyclic sulfides), N-heterocyclic compounds, terpenes, and TMA were identified, and of the 123 volatiles measured, the levels of 114 were significantly different between samples. Forty-two were significantly different between the only two fish samples used and were not significantly affected by the cysteine and methionine additions. Cysteine addition had a statistically significant effect on 47 volatiles, and methionine had an effect on 42.

A number of the volatiles were highly correlated. As expected, the lipid-derived volatiles were generally correlated to one another, as were Maillard-derived volatiles (pyrazines, Maillard thiophenes, and thiazoles). The data set was reduced by removing the highly correlated factors (correlation coefficients ≥0.97) prior to carrying out PCA using the mean values of the significant volatiles. A plot of the first two principal components (PCs), explaining 86% of the systematic variation within the data set, is given in **Figure 1**. PC1 separated the two salmon samples, with salmon 4 on the right side and salmon 5 on the left side of the plot. PC2 separated the salmon spiked with high methionine from the salmon spiked with high cysteine. Salmon spiked with low methionine and cysteine levels were not separated from control salmon. It can be seen from the component plot (**Figure 2**) that toward one side of PC1, which is closer to salmon 4 than salmon 5, lipid-derived volatiles

Table 4. Amounts of Volatile Compounds Identified in Headspace of Cooked Salmon (ng Collected from Headspace of 25 g of Sample)

				salmon 4[c]			salmon 5[d]				significance[e] of		
code	identification	LRI[a]	ID[b]	control	high Cys	high Met	control	low Cys	high Cys	low Met	salmon	Cys	Met
					amines								
m01	TMA	<600	A	263	274	277	4	5	3	3	***	NS	NS
					alcohols								
a01	1-butanol	661	A	24	34	29	2	1	1	1	***	NS	NS
a02	1-pentanol	766	A	342	487	462	107	54	63	54	***	NS	NS
a03	1-hexanol	867	A	54	72	61	20	14	16	13	***	NS	NS
a04	1-heptanol	970	A	59	95	82	32	25	29	22	***	NS	NS
a05	1-nonanol	1172	A	8	11	9	2	7	4	2	***	***	NS
					unsaturated alcohols								
b01	1-penten-3-ol	686	A	23	29	28	225	184	167	150	***	NS	*
b02	2-penten-1-ol (*Z*)	768	A	97	96	114	74	45	38	52	***	*	NS
b03	1-hexen-3-ol	779	B(*34*)	44	64	62	20	12	14	12	***	NS	NS
b04	1,5-octadien-3-ol (*E* or *Z*)	977	B(*34*)	87	87	128	29	30	25	28	***	NS	***
b05	1-octen-3-ol	980	A	87	97	118	54	47	44	44	***	NS	*
					aldehydes								
d01	butanal	<600	A	181	290	261	25	17	15	11	***	NS	NS
d02	3-methylbutanal	655	A	122	123	152	33	26	12	17	***	NS	NS
d03	2-methylbutanal	664	A	55	72	92	10	9	5	6	***	NS	**
d04	pentanal	699	A	103	78	124	131	88	68	70	NS	*	*
d05	hexanal	804	A	411	486	513	456	352	279	320	NS	NS	NS
d06	heptanal	903	A	258	336	323	130	91	94	96	***	NS	NS
d07	octanal	1005	A	112	135	139	72	66	66	56	***	NS	NS
d08	nonanal	1107	A	172	192	182	120	105	101	101	***	NS	NS
d09	decanal	1207	A	25	37	45	12	13	11	9	***	NS	*
					unsaturated aldehydes								
e01	2-butenal (*E*)	648	A	48	24	53	2	1	1	1	***	NS	NS
e02	2-methyl-2-butenal (*E*)	745	A	16	15	34	9	8	5	7	***	NS	***
e03	2-pentenal (*E*)	757	A	83	74	129	23	17	13	16	***	NS	*
e04	2-hexenal (*E*)	856	A	84	43	94	24	21	13	21	***	*	NS
e05	4-heptenal (*Z*)	900	A	132	112	212	53	51	31	49	***	*	***
e06	2-heptenal (*E*)	960	A	46	37	41	21	14	7	17	***	**	NS
e07	2,4-heptadienal (*Z,Z*)	999	A	52	66	79	18	25	25	20	***	*	***
e08	2,4-heptadienal (*E,E*)	1015	A	87	104	123	45	59	63	52	***	**	***
e09	2-octenal (*E*)	1063	A	18	24	24	12	10	10	8	***	NS	NS
e10	3,6-nonadienal (*Z,Z*)	1076	B(*15*)	6	7	10	2	2	1	1	***	NS	***
e11	2,4-octadienal (*E, E*)	1115	A	13	16	21	7	9	9	7	***	*	***
e12	2,6-nonadienal (*E,Z*)	1159	A	8	5	13	8	9	5	8	NS	***	**
e13	2-nonenal (*E*)	1163	A	15	19	18	8	7	6	6	***	NS	NS
e14	2-decenal (*E*)	1266	A	11	15	12	7	5	6	4	***	NS	NS
					aromatic aldehydes								
h01	benzaldehyde	971	A	97	100	133	41	43	40	32	***	NS	*
h02	benzeneacetaldehyde	1053	A	7	3	8	7	6	3	6	NS	***	NS
h03	4-ethylbenzaldehyde	1175	B(*34*)	8	9	12	6	8	6	7	***	**	**
					ketones								
k01	2-butanone	<600	A	269	150	275	27	22	33	7	***	NS	NS
k02	2,3-butanedione	<600	A	85	48	74	5	4	1	2	***	NS	NS
k03	2-pentanone	686	A	35	46	49	11	9	9	6	***	NS	NS
k04	2,3-pentanedione	697	A	75	88	97	54	49	31	35	**	NS	NS
k05	3-hydroxy-2-butanone	709	A	74	40	116	24	24	11	48	***	*	**
k06	2-heptanone	891	A	15	19	20	6	5	6	5	***	NS	NS
k07	2,3-octanedione	983	B(*35*)	5	5	8	3	3	2	2	***	NS	*
k08	2-nonanone	1091	A	14	31	17	5	5	9	4	***	**	NS
					unsaturated ketones								
l01	3-penten-2-one	739	B(*36*)	34	36	48	7	7	7	6	***	NS	*
l02	6-octen-2-one	985	C	30	38	43	10	8	8	7	***	NS	NS
l03	3,5-octadien-2-one (isomer)	1072	C	15	11	15	8	6	4	6	***	**	NS
l04	3,5-octadien-2-one (*E,Z*)	1096	B(*37*)	40	47	52	27	21	16	21	***	NS	NS
					cyclic ketones								
c01	cyclopentanone	797	A	4	6	6	1	1	1	1	***	NS	NS
c02	2-cyclohexene-1,4-dione	1032	B(*38*)	6	5	7	2	2	2	2	**	NS	NS
					furans								
f01	2-methylfuran	605	A	5	4	13	1	1	1	1	**	NS	NS
f02	2-ethylfuran	720	A	274	250	249	241	170	278	258	NS	NS	NS
f03	2-propylfuran	793	A	10	9	14	8	4	9	8	NS	NS	NS
f04	2-ethyl-5-methylfuran	802	A	1	2	4	1	1	3	2	NS	*	**
f05	2-furfural	835	A	10	3	15	10	9	2	7	NS	***	**
f06	2,5-diethylfuran	888	C	2	3	5	1	1	3	2	NS	*	**
f07	2-acetylfuran	914	A	4	6	5	2	2	2	1	***	NS	NS
f08	5-methylfurfural	954	A	30	20	28	15	13	9	13	***	**	NS
f09	2-pentylfuran	994	A	34	39	46	35	17	37	30	NS	NS	NS
f10	2-(2-pentenyl)furan (*E* or *Z*)	1003	C	107	137	171	110	57	115	104	NS	NS	NS
f11	5-ethyl-5-methoxy-2(5*H*)furanone	1080	C	1	4	0.2	1	1	1	2	NS	NS	NS

dominated, particularly, lipid-derived alcohols (saturated and unsaturated, a- and b-coded), lipid aldehydes (saturated and unsaturated, d- and e-coded), and ketones (k-coded). On the opposite side of PC1 (closer to salmon 5), few volatiles were positioned. These were 3-methyl-1,2-dithian-4-one (s10), 2-methyl-3-furyl-1-methyl-2-oxopropyl disulfide (j02), 1-penten-3-ol

Table 4. Continued

				salmon 4[c]			salmon 5[d]				significance[e] of		
code	identification	LRI[a]	ID[b]	control	high Cys	high Met	control	low Cys	high Cys	low Met	salmon	Cys	Met
	thiophenes (hypothesized to be lipid-derived)												
t01	2-methylthiophene	775	A	23	31	34	7	6	10	5	***	NS	*
t02	2-ethylthiophene	871	A	2	4	4	1	1	2	1	***	**	*
t03	2-ethyl-(2*H*)-thiapyran	1022	A	6	15	10	2	2	6	3	***	***	NS
	thiophenes (hypothesized to be Maillard-derived)												
t04	thiophene	672	A	19	28	26	2	2	4	1	***	NS	NS
t05	3-methylthiophene	786	A	2	2	4	1	1	1	1	***	NS	***
t06	4,5-dihydro-2-methyl-3(2*H*)thiophenone	994	A	14	11	26	4	4	3	4	***	NS	***
t07	2-thiophenecarboxaldehyde	1010	A	4	27	8	4	5	18	3	***	***	NS
t08	3-methyl-2-thiophenecarboxaldehyde	1133	A	0.2	3.3	0.2	0.2	0.5	2.2	0.2	**	***	NS
t09	5-methyl-2-thiophenecarboxaldehyde	1135	A	0.01	4.5	0.02	0.1	0.3	4.1	0.04	NS	***	NS
t10	2,3-dihydro-6-methylthieno[2,3c]furan	1176	A	1	16	3	1	3	7	1	***	***	NS
t11	dimethylthiophenecarboxaldehyde	1210	C	2	2	6	1	2	1	2	**	***	***
	pyrazines												
p01	pyrazine	734	A	24	15	35	8	8	4	7	***	*	**
p02	methylpyrazine	827	A	15	14	24	8	10	6	7	***	*	***
p03	ethylpyrazine	920	A	9	7	13	nd	nd	nd	nd	***	NS	**
p04	2,5(or 6)-dimethylpyrazine	915	A	3	2	5	1	2	1	1	***	***	***
	thiazoles												
z01	thiazole	735	A	5	67	8	3	15	19	2	***	***	NS
z02	4-methylthiazole	802	A	1	1	1	nd	nd	nd	nd	***	NS	NS
z03	2-methylthiazole	808	A	nd	8	nd	nd	nd	nd	nd	**	*	NS
z04	4,5-dimethylthiazole	936	A	2	2	4	1	1	1	1	***	*	***
z05	5-ethylthiazole	951	B(*39*)	nd	0.1	nd	nd	0.03	0.1	nd	NS	***	NS
z06	2-acetylthiazole	1025	A	4	96	7	4	27	42	3	**	***	NS
z07	1-(2-thiazolyol)-1-propanone	1132	C	0.3	14	1	0.2	2	4	0.2	**	***	NS
	dimethyl sulfides												
s01	methanethiol	<600	B(*34*)	14	13	27	0.3	0.3	0.2	0.2	***	NS	***
s02	dimethyl disulfide	747	A	5	5	38	2	2	1	3	***	NS	***
s03	3-(methylthio)propanal (methional)	910	A	0.9	0.2	84	0.4	0.6	0.2	2.0	NS	NS	***
s04	dimethyl trisulfide	982	A	3	3	11	1	1	1	2	*	NS	***
	alicyclic sulfides												
s05	3-methyl-1,2-dithiolan-4-one	1090	A	0.7	4.4	1.5	0.5	0.7	1.7	0.4	**	***	NS
s06	3,5-dimethyl,1,2-dithiolan-4-one (isomer)	1115	B(*40*)	0.4	1.4	0.9	0.3	0.4	0.6	0.2	***	***	NS
s07	3,5-dimethyl,1,2-dithiolan-4-one (isomer)	1124	B(*40*)	0.4	1.2	0.8	0.3	0.4	0.5	0.3	***	***	NS
s08	1,2-dithian-4-one	1194	B(*40*)	3.1	3.2	5.3	2.1	2.3	1.8	2.2	***	NS	***
s09	4-methyl-1,2-dithiepane	1231	B(*41*)	0.6	0.9	1.4	0.5	0.6	0.4	0.4	***	NS	***
s10	3-methyl-1,2-dithian-4-one	1241	B(*42*)	nd	Nd	nd	0.2	0.2	0.4	0.2	***	***	NS
	furan thiol derivatives												
j01	2-furanmethanethiol	915	A	0.38	0.23	1.15	0.68	0.49	0.38	0.69	NS	NS	*
j02	2-methyl-3-furyl 1-methyl-2-oxopropyl disulfide	1518	B(*43*)	nd[f]	Nd	nd	nd	nd	0.21	nd	**	***	NS
	nitrogen heterocycles												
n01	pyridine	747	A	7	19	8	2	1	2	1	***	*	NS
n02	1*H*-pyrrole	751	A	6	13	11	2	2	3	2	***	**	NS
n03	2-methylpyridine	821	A	1	5	2	1	0.5	1	0.4	***	**	NS
n04	2-ethylpyridine	906	A	6	13	9	2	3	3	2	***	**	NS
n05	3-ethylpyridine	964	A	1	17	2	1	2	9	1	***	***	NS
	unsaturated hydrocarbons												
q01	2,4-octadiene (isomer)	815	C	41	40	58	10	11	12	13	***	NS	*
q02	2,4-octadiene (isomer)	822	C	31	32	43	7	7	8	8	***	NS	*
q03	1,3-octadiene	826	C	3	8	5	0.4	1	2	1	***	**	NS
q04	a 1,3,5(or 6)-octatriene	873	C	1	1	1	6	4	6	6	***	NS	NS
q05	a 1,3,5(or 6)-octatriene	883	C	8	5	7	2	1	1	2	***	NS	NS
q06	a 1,3,5(or 6)-octatriene	885	C	15	10	15	4	3	3	4	***	NS	NS
	terpenes												
r01	α-pinene	942	A	43	29	32	28	16	22	23	NS	NS	NS
r02	camphene	961	A	1	1	1	12	7	10	10	***	NS	NS
r03	α-terpinene	1026	B(*34*)	10	7	7	8	6	11	9	NS	NS	NS
r04	*m*-cymene	1033	A	4	3	2	7	5	7	7	***	NS	NS

(b01), an octatriene (q04), and a terpene (r04). Toward one side of PC2, which is close to salmon spiked with high methionine solution, were found 2-furanmethanethiol (j01), methional (s03), and dimethyl trisulfide (s04) and also the correlated factors methanethiol (s01) and dimethyl disulfide (s02). All of these volatiles (except j01) are known to be formed from the degradation of methionine. On the opposite side of PC2, thiazoles, thiophenes, alicyclic sulfides (z-, t-, and s-coded), and N-heterocycles (n-coded) were found close to salmon spiked with high cysteine solution. From the PCA, it appears that the largest effect on the volatile profile of the cooked salmon samples was the two different samples of salmon and that relatively large additions of cysteine or methionine were necessary to cause changes in the profile.

TMA. TMA was identified in both salmon samples. It was present at substantially higher quantities in salmon 4 than salmon 5. TMA originates from the bacterial breakdown of TMAO, present in marine fish where it contributes to osmoregulation (*30*). TMA has been shown to increase over storage as noted in a recent study of cultured and wild sea bream (*3*). Salmon caught in freshwater (parr and smolt) were previously found to contain no TMAO (*30*). Accumulation of TMAO has also been found to depend on whether the fish's diet contains a source of TMAO (*30*). TMAO is not heat-stable. It degrades to TMA during heat

Table 4. Continued

code	identification	LRI[a]	ID[b]	salmon 4[c] control	salmon 4[c] high Cys	salmon 4[c] high Met	salmon 5[d] control	salmon 5[d] low Cys	salmon 5[d] high Cys	salmon 5[d] low Met	significance[e] of salmon	significance[e] of Cys	significance[e] of Met
				aromatic hydrocarbons									
x01	toluene	771	A	16	11	13	9	7	7	8	**	NS	NS
x02	*m*-xylene	875	A	24	20	21	10	8	9	10	***	NS	NS
x03	*p*-xylene	877	A	7	4	5	3	2	3	3	***	NS	NS
x04	1-ethyl-3-methylbenzene	969	A	6	5	5	4	3	3	3	**	NS	NS
				miscellaneous									
g01	2-hydroxybenzaldehyde	1057	B (*44*)	2	3	3	4	4	2	4	NS	NS	NS
g02	acetophenone	1076	A	15	17	15	6	6	13	5	*	NS	NS
g03	4-(methylthio)phenol	1230	C	1	4	1	0.4	1	2	0.4	***	***	NS
				unidentified compounds									
u01	MW 124: 41 (56),[g] 53 (45), 55 (37), 79 (92), 81 (100), 95 (19), 109 (10), 124 (57)	943		54	47	58	24	12	13	16	***	NS	NS
u02	a hexenylcyclopentene: 41 (95), 67 (38), 79 (100), 105 (15)	1070		34	47	47	16	14	13	13	***	NS	NS
u03	MW 150: 41 (49), 54 (84), 67 (63), 79 (100), 81 (81), 91 (35), 96 (50), 107 (23), 121 (29), 150 (4)	1094		86	74	81	32	26	38	32	***	NS	NS
u04	MW 150: 41 (34), 54 (47), 67 (47), 79 (100), 81 (55), 91 (30), 96 (27), 107 (22), 121 (17), 150 (3)	1103		60	56	58	28	25	28	27	***	NS	NS
u05	MW 176: 41 (75), 43 (80), 57 (91), 71 (52), 79 (100), 81 (57), 91 (76), 108 (58), 120 (44), 176 (2)	1302		14	26	30	12	10	13	12	*	NS	*

[a] Linear retention indices. [b] A, mass spectrum and LRI agree with those of an authentic compound; B, mass spectrum agrees with reference spectrum in the NIST/EPA/NIH mass spectral database and LRI agree with those in the literature (reference given); and C, tentative identification where mass spectrum agrees with reference spectrum in the NIST/EPA/NIH mass spectral database. [c] Salmon 4 was a salmon fillet to which water (control), 50 mg/100 g cysteine (high Cys), or 50 mg/100 g methionine (high Met) was added prior to cooking. [d] Salmon 5 was a salmon fillet to which water (control), 5 mg/100 g cysteine (low Cys), 50 mg/100 g cysteine (high Cys), or 5 mg/100 g methionine (low Met) was added prior to cooking. [e] Significant at ***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$; NS, not significant. [f] Not detected. [g] *m/z* (relative intensity).

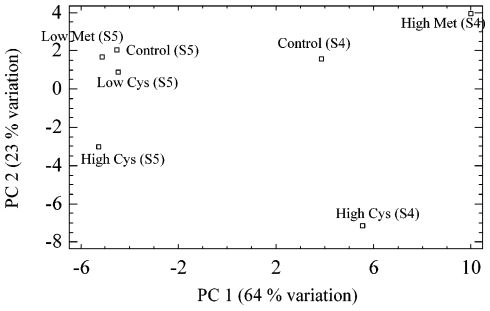

Figure 1. PC scatter plot (PCs 1 and 2) of cooked salmon. S4 and S5 represent the two salmon samples analyzed. The control represents the salmon samples cooked with water; low Cys and low Met represent the salmon samples cooked with 0.01% w/v cysteine and methionine, respectively; and high Cys and high Met represent the salmon samples cooked with 0.1% w/v cysteine and methionine, respectively.

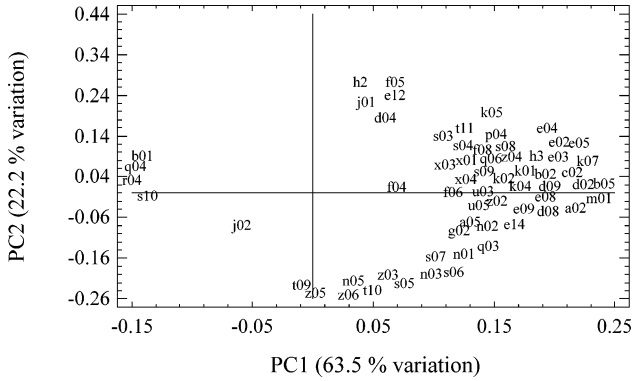

Figure 2. PC plot of component weightings (PCs 1 and 2) for cooked salmon (see **Table 4** for volatile codes).

processing, such as canning (*30*). Therefore, storage, diet, and stage of lifecycle may have influenced the TMA in these two fish. TMA is responsible for the characteristic off-flavor and ammonia-like odor of raw fish (*2*). It can react with other compounds, such as lipid degradation products, to produce further aroma compounds (*6, 30*).

Lipid-Derived Volatiles. Many lipid-derived volatiles were identified in the salmon, particularly saturated and unsaturated alcohols, aldehydes, and ketones as well as lipid-derived thiophenes. There were high levels of many of these volatiles and significant differences between the salmon samples (**Table 4**). Lipid-derived alcohols, aldehydes, and ketones are degradation products of PUFAs and are known to increase during fish storage. A study of the changes in volatile compounds of raw sardine (*Sardina pilchardus*) over 9 days of storage showed significant increases of 1-octen-3-ol, 4-heptenal, and 2,4-heptadienal after 4 days of storage (*5*). These volatiles were found at higher levels in salmon 4 as compared to salmon 5. 4-Heptenal formation is accelerated with increased temperatures and is commonly found in cooked, stored seafood (*4*). It has been found to be an odor-active compound in fish, usually associated with deterioration (*4*). 4-Heptenal forms from the retro-aldol condensation of 2,6-nonadienal (*2*), which itself can be formed from the enzymic breakdown of eicosapentaenoic acid (EPA) via 12-lipoxygenase (*31*). The headspace concentrations of 2,6-nonadienal (e13) measured from the salmon samples were low, particularly as compared to the high concentrations of 4-heptenal (e03) (**Table 4**), suggesting that the retro-aldol condensation of 2,6-nonadienal was faster than the lipoxygenation of EPA or that the levels of EPA was limiting. 1-Octen-3-ol can also be enzymically derived from the degradation of linoleic acid hydroperoxide. It is known to be one of the major volatile alcohols in shellfish (*26*) and was also found to vary during storage of sea bream (*3*). The eight-carbon alcohols and ketones are responsible for the mushroom and geranium-like

aromas in fish (*31*). The eight-carbon alcohols normally occur in greater abundance than the corresponding ketones (*31*). In this study, 1-octen-3-ol and 1,5-octadien-3-ol were detected at higher levels than the 3,5-octadien-2-one and 6-octen-2-one. 2-Nonenal has also been found as an enzymically derived carbonyl in fresh fish (*31*). Nine-carbon carbonyls are responsible for cucumber and melon-like aromas in fish.

2,4-Heptadienal and 3,5-octadien-2-one can form from the autoxidation of EPA (*2*). Generally, the oxidatively derived carbonyls are found at lower levels in fish (*31*) than those that are enzymically derived, if the fish has not been stored for a long period. In this study, the measured headspace concentrations of 2,4-heptadienal and 3,5-octadien-2-one were lower than those of 4-heptenal.

Most of the lipid-derived volatiles in this study were significantly higher in salmon 4 than salmon 5. This, along with the higher TMA levels, suggests that salmon 4 was probably stored for a longer period. The exception was 1-penten-3-ol (b01), which was much higher in salmon 5 than salmon 4.

Several thiophenes that were derived from lipid (**Table 4**) showed slightly higher levels in added cysteine samples. These can be formed from the reaction of lipid-derived aldehydes with hydrogen sulfide formed from cysteine via hydrolysis or Strecker degradation. 2-Ethylthiophene showed a significant, but small, increase with high-added cysteine. There was a high level of 2-ethylfuran in the salmon. This, or its precursors, can react with hydrogen sulfide released from cysteine to form 2-ethylthiophene (*6*). A previous study found that the level of 2-ethylthiophene in fish oil increased when it was heated with cysteine (*6*).

High levels of added methionine increased levels of a number of lipid-derived volatiles, in particular 1,5-octadien-3-ol (b04), 4-heptenal (e05), 2,4-heptadienal (e07 and e08), and 2,4-octadienal (e11). Methionine readily undergoes degradation. In doing so, it may have increased the redox type reactions in the heated system.

Maillard-Derived Volatiles. Four pyrazines, eight Maillard-derived thiophenes, and seven thiazoles were identified in the salmon samples. The pyrazines were affected more by the fish sample than by the addition of cysteine or methionine. Although pyrazines are typical products of Maillard reaction systems and levels of pyrazines are generally high in meat products, they are generally found at relatively low levels in fish (*6*). The limiting factor in the generation of pyrazines in fish as compared to meat may be the lower levels of glucose and ribose (*7, 8*) in fish. The higher level of pyrazines in salmon 4 as compared to salmon 5 suggested that salmon 4 had a higher level of sugars. Glucose in salmon 4 was quantified at 19 mg/100 g and ribose at 1.6 mg/100 g (**Table 2**). In beef muscle, glucose levels are typically between 260 and 800 mg/100 g and ribose up to 200 mg/100 g (*8*). The addition of the high level of cysteine led to a small decrease in pyrazines, and high methionine led to a small increase in pyrazines. The addition of cysteine may have led to other reactions competing with the formation of pyrazines. Cysteine readily degrades to hydrogen sulfide and ammonia, which may then react with dicarbonyls that would have otherwise led to pyrazine formation. Methionine, however, may have acted to increase the overall amino acid pool required for the initial step of the Maillard reaction, hence leading to more pyrazine formation.

The Maillard-derived thiophenes were significantly affected by both the fish sample and the addition of the sulfur amino acids. Five thiophenes were significantly increased by cysteine and three by methionine addition (**Table 4**). Thiophenes may be formed by the action of hydrogen sulfide, released from cysteine, for example, on sugar degradation products (*11*).

Thiazoles were significantly affected by both fish sample and added cysteine and not generally affected by methionine. Undoubtedly, the greatest effect was the substantial increase in thiazole and 2-acetylthiazole caused by the addition of both low and high levels of cysteine. Thiazoles have previously been found in cooked crustacea (*12*) and baked canned salmon (*10*). They are considered to be formed using cysteine via the Strecker degradation, which releases both hydrogen sulfide and ammonia. The route to alkylthiazoles probably involves the reaction of α-dicarbonyls, such as 2,3-butanedione or 2-oxopropanal, with ammonia and hydrogen sulfide (*11*). 2-Acetylthiazole was found at high levels in tiger prawns, and this was attributed to formation from cysteine. Even though cysteine was not detected in the prawns, it was thought that it might have reacted quickly to form the cyclic S-containing compounds (*12*). 2-Methylthiazole was only detected in salmon 4 and only with the addition of high cysteine. Previous research found 2-methylthiazole to increase during heat treatment of fish oil with cysteine (*6*).

Methional and Dimethyl Sulfides. Methional, methanethiol, dimethyl disulfide, and dimethyl trisulfide were all significantly increased by methionine addition, generally at both the low and the high levels of addition. These compounds have low odor thresholds and contribute to both desirable and undesirable aromas, depending on their concentrations (*26*). Methional is a degradation product of methionine, and it can be reduced to methanethiol, and spontaneous oxidation of the latter yields dimethyl disulfide (DMDS) and dimethyl trisulfide (DMTS) (*32*). In a previous study, methanethiol, DMDS, and DMTS were found in cultured and wild sea bream and increased during the storage period (*3*).

Alicyclic Sulfides. Five of the alicyclic sulfides identified were slightly higher in salmon 4 as compared to salmon 5. However, these compounds were more substantially affected by the addition of sulfur amino acids. 3-Methyl-1,2-dithiolan-4-one, the two 3,5-dimethyl-1,2-dithiolan-4-one isomers, and 3-methyl-1,2-dithian-4-one were all significantly increased by cysteine, both at low and high levels of addition. However, 4-methyl-1,2-dithiepane and 1,2-dithian-4-one were significantly increased by high methionine addition and not significantly increased by cysteine.

Furanthiols. Only one 2-methyl-3-furanthiol derivative was identified (the 2-methyl-3-furyl 1-methyl-2-oxopropyl disulfide), and this was found only in the high cysteine sample of salmon 5. 2-Furanmethanethiol was found in all of the samples of both fish, the only small significant difference being an increase with high methionine addition.

N-Heterocycles. All five of the N-heterocyclic compounds identified were significantly increased by cysteine addition; they were also generally at slightly higher levels in salmon 4 as compared to salmon 5. In a previous study, pyrroles and pyridines, particularly 2-ethylpyridine, were found to increase when fish oil was heated with cysteine (*6*). All proposed formation pathways utilized the ammonia released from Strecker degradation of cysteine. Pyridines were thought to derive from the reaction of fatty aldehydes with ammonia. This might explain the formation of pyridine, 2-methylpyridine, and 2-ethylpyridine in this study; however, their respective C5, C6, and C7 aldehydes were not systematically lower at high cysteine addition. It is more difficult to explain the formation of 3-ethylpyridine via this route as this would suggest formation from a branched C5 or C6 aldehyde.

Referring back to the aromas of the cooked salmon samples, it is clear that the addition of methionine, which led to high methional levels, gave recognizable potato odor notes. Despite the higher levels of most lipid volatiles in salmon 4 as compared to salmon 5, it was salmon 5 that was described as having more fatty odors. However, the overall volatile profile should be considered. Salmon 5 was substantially higher in one lipid-derived alcohol (1-penten-3-ol) whereas salmon 4, although high in other lipid-derived volatiles, was particularly high in TMA, a potent "fishy" odorant, which may have had a noticeable effect on the overall aroma.

The volatiles identified in the salmon fillets during this study have not all been previously found in salmon. Among the 35 seafood products referred to in the TNO listing, none refer specifically to salmon (*33*). Many of the lipid-derived volatiles identified in this study have been previously found in salmon by Josephson et al. (*10*, *31*) or Girard and Durance (*16*). Dimethyl sulfides have been found previously in canned salmon (*16*) and methional in raw and cooked salmon (*14*, *15*). However, most of the thiazoles [except 4,5-dimethylthiazole (*10*)], most of the thiophenes [except the methyl and ethyl derivatives in canned salmon (*10*, *16*) and thiophenecarboxaldehyde in raw salmon (*14*)], the alicyclic sulfides, and the pyridines do not appear to have been reported previously in salmon.

This study has demonstrated that the volatile profile of cooked salmon can be altered by the addition of sulfur amino acids, cysteine, and methionine. Such differences brought about by the amino acid additions could occur due to natural variability of amino acid levels in salmon. Even in samples where amino acids were not added, there were differences in key compounds, such the thiazoles, thiophenes, dimethyl sulfides, alicyclic sulfides, and nitrogen heterocycles. Although the sources of variability between the retail-bought salmon were unknown, the results do suggest that small differences in the quantities of methionine and cysteine in the fish could be important in volatile formation. Future projects could involve diet intervention of farmed salmon, aiming to modify and control the levels of methionine and cysteine.

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Supporting Information Available: Correlations between the volatiles identified in the cooked salmon. This material is available free of charge via the Internet at http://pubs.acs.org.

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