# **Aroma-Active Compounds in Skipjack Tuna Sauce**

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Volatile compounds in skipjack tuna ( $Katsuwonus\ pelanis$ ) viscera (STV) and tuna sauce (TS) made from STV were analyzed by vacuum simultaneous distillation—solvent extraction/gas chromatography/mass spectrometry/olfactometry and aroma extract dilution analysis. Predominant odorants in STV were lipid-derived compounds, such as (E,E)-2,4-heptadienal (stale/peanut-like), (E,Z)-2,6-nonadienal (cucumber-like), (E,E)-2,4-decadienal (fatty/rancid fat-like), (E,Z)-nonenal (stale, bitter), and (Z)-4-heptenal (fishy/rancid), and unidenified compounds having grassy, fresh fish-like odors. In contrast to STV, potent odorants in TS were mostly thermally generated compounds such as 3-(methylthio)propanal (baked potato-/soy sauce-like), dimethyl trisulfide (cooked cabbage-like), and 3-methylbutanal (dark chocolate-like). Additional potent odorants in TS were (E,E)-2,4-heptadienal, (E)-2-nonenal, phenylacetaldehyde (honeysuckle-like), and two unidentified compounds having nutty, baked potato-, vitamin-, and cooked rice-like odors. Two amino acids, glutamic acid and aspartic acid, were predominant in both samples.

Keywords: Skipjack tuna viscera; tuna sauce; tuna flavor; aroma-active; AEDA

### INTRODUCTION

Fish sauce, which is known by various names depending on the country of orign, is a clear brown liquid hydrolysate of salted fish and is a popular fermented fish product used in Asia. In particular, fish sauce is an important ingredient of kimchi, a Korean fermented vegetable product. The characteristic aroma and taste of fish sauce are primarily due to degradation of protein and lipid by autolytic and bacterial enzymes during fermentation (Beddows et al., 1976; Saisithi et al., 1966). Traditional methods of producing fish sauce take from 6 to 12 months of fermentation time (Sanceda et al., 1996). A number of people have shown that the fermentation process can be accelerated by use of fish viscera (Yoshinaka et al., 1983; Kim and Ha, 1995; Lee and Woo, 1992) and/or commercial enzymes (Beddows et al., 1976; Beddows and Ardeshir, 1979; Lee et al.,

Increased environmental awareness and more stringent regulations have prompted the Korean seafood industry to seek alternative uses of their processing byproducts. Potential exists for the production of fish sauce from byproducts, such as viscera, heads, and frames, from tuna canning factories (Lee et al., 1989; Lee and Woo, 1992; Kim and Ha, 1995). Lee et al. (1989) reported that the quality of tuna sauce made by adding koji was equivalent to that of soy sauce. They further reported that the major taste compounds of this product were free amino acids and nonvolatile organic acids.

Several reports have been published on the aroma of fish sauces, which have been described as a blend of ammoniacal, cheesy, and meaty aromas (Dougan and Howard, 1975; Beddows et al., 1976). Limited studies have reported on the predominant flavor compounds in fish sauces. Triqui and Reineccius (1995a,b), Cha et al. (1997), and Triqui and Guth (1997) studied the aroma-active components in fermented anchovy. Compounds that contribute to the characteristic odor of fish sauce made from tuna byproduct using a commercial protease have not yet been elucidated.

Aroma extract dilution analysis (AEDA) has been successfully employed to establish predominant aroma compounds in foods (Gasser and Grosch, 1988; Chung and Cadwallader, 1994; Cadwallader et al., 1995). In AEDA, a flavor dilution (FD) factor is determined for each odorant in a flavor extract. The FD factor is the highest dilution of the extract at which an odorant is detected by gas chromatography/olfactometry. FD factors are used to indicate the most intense odorants in the flavor extract. Our objectives were to use AEDA to identify and compare aroma-active compounds in skipjack tuna viscera (STV) and tuna sauce (TS) made from STV and to compare free amino acid profiles of STV and

## MATERIALS AND METHODS

**Materials.** Skipjack tuna (*Katsuwonus pelanis*) viscera (STV) and TS made from STV with commercial protease were obtained from Dongwon Industry Co. (Changwon, Korea). The samples were stored at  $-20\,^{\circ}\text{C}$  until analyzed. Soluble solids content (measured by a refractometer; Atago Co., Ltd., Japan) and pH (Fisher Scientific pH meter model 630, Pittsburgh, PA) of TS were 36.4 and 6.0 °Brix, respectively. Amino nitrogen in TS was 0.89 (±0.01 SD) g% (w/w) as measured by using the formol method (AOAC, 1980). Proximate compositions (AOAC, 1980) of STV and TS were as follows: moisture, 79.21 (±0.09) and 68.42 (±0.05)%, lipid, 1.56 (±0.04) and 0.16 (±0.01)%; protein, 16.02 (±0.04) and 4.96 (±0.03)%; ash, 3.60 (±0.06) and 21.52 (±0.04)%; salinity, 2.11 (±0.02) and 21.09 (±0.04)%, respectively.

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All standard aroma compounds were purchased from Aldrich Chemical Co. (Milwaukee, WI) except for 2-acetyl-1-pyrroline, which was from Dr. R. Buttery (USDA, ARS, WRRC, Albany, CA), and (*Z*)-4-heptenal, which was purchased from Alfa (Ward Hill, MA).

**Vacuum Simultaneous Steam Distillation—Solvent Extraction (V-SDE).** V-SDE was conducted under vacuum (ca. 24–26 in. Hg) using the apparatus described by Chung and Cadwalladar (1994). Samples (500 g of homogenized STV or 1.0 L of TS) plus deodorized distilled water (1.0 L for STV or 0.5 L for TS) and 2,4,6-trimethylpyridine (TMP; 90.8  $\mu$ g) as an internal standard were extracted for 2.5 h with 200 mL of redistilled diethyl ether. Boiling point temperature of both samples was maintained at 60–65 °C during extraction. V-SDE extracts were kept at -20 °C overnight to facilitate water removal. The volume of the V-SDE extracts was reduced to 5 mL under a gentle stream of nitrogen; the extracts were dried over 2 g of anhydrous sodium sulfate and then further reduced under N<sub>2</sub> to 1.0 mL. Duplicate V-SDE extractions were prepared for both STV and TS.

Gas Chromatography/Mass Spectrometry (GC/MS). Two microliters of each V-SDE extract was injected into an HP 5890 Series II GC/HP 5972 mass selective detector (MSD) (Hewlett-Packard Co., Palo Alto, CA) (splitless mode; 30 s valve delay; injector temperature 200 °C; helium carrier gas at 0.96 mL/min constant flow) equipped with a capillary column (DB-Wax, 60 m  $\times$  0.25 mm i.d.  $\times$  0.25  $\mu m$  film; J&W Scientific Inc., Folsom, CA). Oven temperature was programmed from 40 to 200 °C at 3 °C/min, with initial and final hold times of 5 and 60 min, respectively. MSD conditions were as follows: capillary direct MS interface and ion source temperature, 280 °C; ionization energy, 70 eV; mass range, 33–350 amu; scan rate, 2.2 scans/s; electron multiplier voltage, 200 V above autotune. Duplicate analyses were performed on each V-SDE extract.

AEDA. Prior to dilution, the concentration of each extract was adjusted to possess the same abundance of the internal standard peak. Serial dilutions (1:3) of V-SDE extracts were prepared using diethyl ether as diluent. The gas chromatography/olfactometry (GC/O) system consisted of a Varian 3400 GC (Varian Instrument Group, Walnut Creek, CA) equipped with a flame ionization detector (FID) and a sniffing port. One microliter of each dilution extract was injected (splitless mode) into a capillary column (DB-Wax or DB-5ms,  $30 \text{ m} \times 0.32 \text{ mm}$ i.d.  $\times$  0.25  $\mu m$  film; J&W Scientific Inc.). Effluent from the end of the GC column was split 1:1 between the FID and the sniffing port. Further details of the procedure have been reported elsewhere (Chung and Cadwallader, 1994). Oven temperature was programmed from 40 to 200 °C at a rate of 6 °C/min with initial and final hold times of 5 and 30 min, respectively. FID and injector temperatures were 250 and 200  $^{\circ}$ C, respectively. Sniffing port and transfer line temperatures were maintained at 200  $^{\circ}$ C. GC/O was performed on each extract dilution by two trained panelists familiar with fish sauce flavor. Panelists were asked to assign odor properties to each compound detected. Odor descriptions were assigned by panelists using free choice vocabulary. Further details of this procedure can be found elsewhere (Baek et al., 1997).

Compound Identification and Quantification. Compound identifications were based on comparison of GC retention indices (RI) (van den Dool and Kratz, 1963), mass spectra, and odor properties of unknowns with those of authentic standards analyzed under identical GC conditions. Tentative identifications were based either on matching mass spectra of unknowns with those in the Wiley 138k mass spectral database (John Wiley and Sons, Inc., 1990) or on matching RI values and odor properties of unknowns with those of authentic standards. Relative concentrations of positively identified compounds were determined using MS response factors for each compound relative to the internal standard. Response factors were determined by analyzing standard compounds at three levels. Quantitation of coeluted compounds was achieved by mass chromatography (Hites and Biemann, 1970) as described by Cadwallader et al. (1995) and Baek and Cadwallader (1996).

Analysis of Free Amino Acids. Free amino acids were determined according to a modification of the method of Choi et al. (1996). Namely, 15 g of homogenized STV or TS was gently mixed with 50 mL of 10% TCA solution in a mortar. The mixture was transferred to a 100 mL volumetric flask, diluted to 100 mL with distilled water, and centrifuged (4000g, 10 min; VS-5500, Vision Scientific Co. Ltd., Seoul, Korea). A 30 mL aliquot of the aqueous supernatant was extracted with redistilled diethyl ether to remove lipid and pigment materials and then dehydrated on a vacuum rotary evaporator (J. Bibby Science Products, Staffordshire, England). The dried material was dissolved in 0.02 N HCl and finally brought to a volume of 25 mL for analysis of free amino acids using an automatic amino acid analyzer (Pharmacia Biochrom 20, Pharmacia LKB Biotechnology, Cambridge, England). Duplicate analyses were performed on each sample.

### RESULTS AND DISCUSSION

Comparison of Odorants in STV and TS. FD chromatograms of STV and TS are shown in Figure 1. A total of 45 odorants were detected by GC/O (Table 1). Among these, 26 odorants were detected in STV and 38 in TS. Odor descriptions for these compounds are given in Table 1. Three odorants in STV (no. 20, 33, and 38) had FD factors equal to 38, while only two compounds (no. 16 and 20) were detected at this high FD factor in TS. Odorants with FD factors >36 in STV were described as metallic/earthy (no. 11), stale/bitter/peanutlike (no. 20), stale/bitter/hay-like (no. 21), cucumber-like (no. 23), grassy/fresh fish-like (no. 33), and fatty/fried/ rancid-like (no. 38). Most of these were presumably lipid-derived compounds. However, except for some odorants (no. 20, 21, and 33) also detected in STV, predominant odorants in TS (FD > 35) were not lipidderived but consisted of dark chocolate-like (no. 2), rotten/sulfury/cooked cabbage-like (no. 12), baked potato/ soy sauce-like (no. 16), nutty/baked potato/soy saucelike (no. 17), floral/honeysuckle-like (no. 28), and vitamin/ chicken broth-like (no. 29) odors. These thermally generated compounds probably contribute to the characteristic aroma of TS. Many odorants (no. 30, 31, 32, 36, and 37) having meaty/sulfury notes were detected in the RI regions (DB-Wax column) of 1660-1700 and 1740-1780. Odorants 31 and 32 were detected in TS only. These meaty aromas are characteristic of fish sauce (Dougan and Howard, 1975). In addition to odorants 31 and 32, many other odorants were detected in TS only and were described as dark chocolate-like (no. 1), buttery (no. 3), rotten/amine-like (no. 5), rancid/ fishy/crabby (no. 6 and 8), nutty/popcorn-like (no. 13, 14, 24, and 27), cigarette/tobacco-like (no. 39), butterscotch/caramel-like (no. 40), nutty/roasty/meaty (no. 34 and 42), iodine-like (no. 44), and indole/grape-like (no. 45). Odorants 5, 14, 29, 31, 39, and 42 might strongly contribute to the overall flavor of tuna sauce because of their high FD factors. Results of AEDA are in good agreement with those of Dougan and Howard (1975) and Beddows et al. (1976), who observed that the aroma of fish sauce was composed primarily of three characteristic types of odors such as ammoniacal, cheesy, and meaty.

**Identification of Aroma-Active Compounds.** More lipid-derived odorants having high FD factors ( $>3^5$ ) were detected in STV than in TS. These included (Z)-4-heptenal (no. 7), (E,E)-2,4-heptadienal (no. 20), (E)-2-nonenal (no. 21), (E,Z)-2,6-nonadienal (no. 23), and (E,E)-2,4-decadienal (no. 38). These compounds were described as rancid, stale, and fatty-like except for (E,Z)-2,6-nonadienal (cucumber-like). Choi et al. (1996) re-

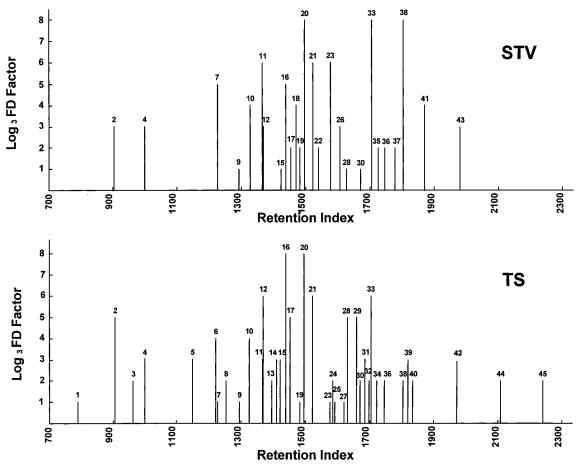


Figure 1. Flavor dilution chromatograms of volatiles isolated from STV and TS made from STV. Peak numbers correspond to those in Table 1.

ported that the content of  $\omega$  – 3 fatty acids, which are highly susceptible to lipid oxidation, was 22.72% of the total fatty acids in STV. Lipid-derived compounds including (Z)-4-heptenal, (E,Z)-2,6-nonadienal, and (E,E)-2,4-decadienal were detected with low FD factors in TS. Baek and Cadwallader (1996) reported that lipidderived compounds decreased significantly after enzymatic hydrolysis. This may be explained by the fact that lipid-derived compounds can react with Maillard reaction intermediates to form heterocyclic compounds (Ho et al., 1989; Shibamoto and Yeo, 1992). compound (E,Z)-2,6-nonadienal, which can be derived from  $\omega - 3$  fatty acids, is readily converted to (Z)-4heptenal through the retro-aldol degradation reaction (Josephson et al., 1984; Josephson and Lindsay, 1987). Two branched aldehydes, 2-methylpropanal (no. 1) and 3-methylbutanal (no. 2), both having dark chocolate-like odors, were detected in TS. In particular, the intensity of 3-methylbutanal was higher in TS than in STV. These compounds, which may positively affect the overall flavor of tuna sauce, have been known to originate from Strecker or microbiological degradation of amino acids (Collin et al., 1993).

Among three compounds having popcorn-like odors (no. 10, 14, and 27), two were identified as 2-acetyl-1pyrroline and 2-acetylpyrazine. 2-Aetyl-1-pyrroline, a thermally generated compound, had high FD factors in both samples, while 2-acetylpyrazine and no. 14 (unknown) were found only in TS at low intensities.

Compounds having desirable nutty, meaty, roasty, vitamin-, and baked potato-like odors (no. 13, 16, 17,

19, 24, 26, 29-32, 34, 36, 37, and 42) comprised a third of the total number of odorants detected in this study. Among these, 3-(methylthio)propanal (no. 16) had the highest FD factor in TS. 3-(Methylthio)propanal may be formed during heat treatment via Strecker degradation of methionine (Forss, 1979).

Two sulfur-containing compounds, methyl ethyl disulfide (no. 5) and dimethyl trisulfide (no. 12), were detected with high FD factors in TS and were described as rotten/amine-like and cooked cabbage-like, respectively. In particular, dimethyl trisulfide, which can be thermally generated from cysteine (Mussinan and Katz, 1973), may strongly contribute to the characterstic flavor of TS because of its high FD factor and low threshold (Milo and Grosch, 1996; Table 2). Other thermally generated odorants, including 2,3-butanedione (no. 3; buttery) and phenylacetaldehyde (no. 28; floral/honeysuckle-like), were identified. 2,3-Butanedione was detected only in TS, and phenylacetaldehyde was detected at a higher FD factor in TV than in STV. These compounds may make a positive contribution to the overall flavor of tuna sauce. Meanwhile, 1-octen-3-one (no. 9; mushroom-like) and 2-aminoacetophenone (no. 45; phenolic/grape-like) may play negative roles in the characteristic flavor of TS because of their undesirable odors. In particular, 2-aminoacetophenone, known as a secondary metabolite of Saccharomyces cerevisiae, is considered an off-flavor in wine (Ciolfi et al., 1995). Several unidentified odorants having undesirable earthy, metallic, and geosmin-like odors (no. 11, 18, and 25) were found with low FD factors in both samples.

Table 1. Aroma-Active Compounds in STV and TS Made from STV

			retention index $^b$			
peak no.a	compound	methods of indentification	DB-Wax	DB-5ms	odor description $^c$	
1	2-methylpropanal	MS, RI, odor	789	(2.49)	dark chocholate	
2	3-methylbutanal	MS, RI, odor	902	638	dark chocolate	
3	2,3-butanedione	MS, RI, odor	958	(2.69)	buttery	
4	unknown		999		canned tuna, green onion	
5	methylethyl disulfide	MS	1148		rotten, amine, putrid	
6	unknown		1222		rancid, fishy, crabby, rotten	
7	(Z)-4-heptenal	MS, RI, odor	1229	901	fishy, rancid	
8	unknown		1248		rancid, fishy	
9	1-octen-3-one	RI, odor	1292	977	mushroom, earthy	
10	2-acetyl-1-pyrroline	RI, odor	1326	921	popcorn, nutty	
11	unknown		1363		metallic, earthy	
12	dimethyl trisulfide	MS, RI, odor	1366	968	rotten, sulfury, cooked cabbage	
13	trimethylpyrazine	MS, RI, odor	1394	1004	nutty, stale, wet straw, earthy	
14	unknown		1412		nutty, popcorn	
15	(E)-2-octenal	MS, RI, odor	1421	1063	coffee, nutty, stale, baked potato	
16	3-(methylthio)propanal	MS, RI, odor	1439	908	baked potato, soy sauce	
17	unknown	, ,	1455		nutty, baked potato, soy sauce	
18	unknown		1475		mushroom, earthy, metallic	
19	unknown		1485		nutty, stale, baked potato	
20	(E,E)-2,4-heptadienal	MS, RI, odor	1494	1013	stale, bitter, peanut	
21	(E)-2-nonenal	MS, RI, odor	1524	1162	stale, bitter, hay	
22	octanol	MS, RI, odor	1537	1081	fruity, sour, spicy, floral	
23	(E,Z)-2,6-nonadienal	MS, RI, odor	1574	1156	sweet, cucumber, fresh	
24	unknown	, ,	1583		nutty	
25	unknown		1587		dirt, earthy, geosmin	
26	unknown		1606		burnt, nutty	
27	2-acetylpyrazine	MS, RI, odor	1618	1031	nutty, popcorn	
28	phenylacetaldehyde	MS, RI, odor	1628	1046	floral, honeysuckle	
29	unknown	, , , , , , , , , , , , , , , , , , , ,	1659		vitamin, chicken broth	
30	unknown		1669		meaty, sulfury, popcorn	
31	unknown		1683		meaty, sulfury	
32	unknown		1698		meaty, sulfury	
33	unknown		1704		grassy, fresh fish	
34	unknown		1721		roasty, cooked rice, meaty	
35	unknown		1726		rancid, crabby	
36	unknown		1741		meaty, sulfury, soy sauce	
37	unknown		1780		meaty, sulfury	
38	(E,E)-2,4-decadienal	MS, RI, odor	1804	1322	fried, fatty, rancid	
39	unknown	,,	1818		cigarette, tobacco (fresh), sweet	
40	unknown		1835		butterscotch, caramel	
41	unknown		1872		sweet, rancid, bug (dienal)	
42	unknown		1973		nutty, savory, roasty, meaty, chicker	
43	unknown		1977		fish sauce, sweet, rancid, soy sauce	
44	unknown		2106		iodine, disinfectant	
45	2-aminoacetophenone	MS, RI, odor	2237	1316	phenolic, indole, grape-like	

<sup>a</sup> Numbers correspond to those in Figure 1. <sup>b</sup> Retention indices on DB-Wax and DB-5ms capillary colums. Numbers in parentheses represent retention times. <sup>c</sup> Odor description as perceived by panelists during gas chromatography/olfactometry.

Levels and Odor Values of Positively Identified **Odorants.** Concentrations and odor values (OVs) for positively identified odorants are presented in Table 2. Most compounds were present at levels exceeding their odor detection thresholds except for 2,3,5-trimethylpyrazine, (*E,E*)-2,4-heptadienal, and 2-acetylpyrazine. With the exception of (*E*)-2-octenal, the OVs for each odorant in STV and TS were consistent with their respective FD factors. However, OVs were not necessarily comparable to FD factors between the different odorants in each sample. For example, dimethyl trisulfide had the highest OV in TS, followed by 3-methylbutanal, 3-(methylthio)propanal, 2-methylpropanal, (E)-2-nonenal, and (*E,E*)-2,4-decadienal, whereas 3-(methylthio)propanal and (E,E)-2,4-heptadienal had the highest FD factors in TS followed by dimethyl trisulfide and (*E*)-2-nonenal. A similar trend was observed for STV. The disparity between OVs and FD factors cannot be readily explained but might be due to errors in quantitation and/or in the odor detection thresholds values used for calculation of OVs. In any event, results of quantitative analysis and AEDA indicate dimethyl trisulfide, 3-methylbutanal, 3-(methylthio)propanal, (E,E)-2,4-heptadienal, (E)-2nonenal, and phenacetaldehyde to be the predominant aroma-active components of TS. Results of AEDA further indicate the importance of several unknown odorants (e.g., no. 17, 29, and 33).

Free Amino Acid Composition. Free amino acid composition of STV and TS is shown in Table 3. The amount of free amino acids in TS was about 53 g% (w/w on a dry weight and salt-free basis) and was 2.4-fold greater than in STV. Seven amino acids, including glutamic acid, leucine, lysine, arginine, alanine, valine, and taurine, were found in high abundance and accounted for 52 and 48% of the total amino acids in STV and TS, respectively. In particular, the flavor enhancer glutamic acid was found in the highest abundance in both samples. Glutamic acid is among the most abundant free amino acids in protein hydrolysates, such as hydrolyzed vegetable protein (Weir, 1986). Other amino acids, such as taurine, may have little or no taste impact (Konosu and Yamaguchi, 1982). The contents of three amino acids, alanine, lysine, and serine, increased 3-fold in TS. Lysine and alanine are known to impart sweet tastes (Kato et al., 1989). Konosu and Yamaguchi

Table 2. Concentrations and Odor Values for Positively Identified Odorants in STV and TS Made from STV

	compound	$concn^b$			odor value $^d$	
peak no.a		STV	TS	odor threshold $^c$ (mg/L)	STV	TS
1	2-methylpropanal	$ND^e$	182	$0.7^f$		260
2	3-methylbutanal	140	1395	$0.4^{g}$	350	3488
3	2,3-butanedione	ND	274	$2.6^h$		105
5	methylethyl disulfide	ND	9.2	$NA^i$		
7	( <i>Z</i> )-4-heptenal	37	2.1	$0.04^{j}$	925	53
9	1-octen-3-one	ND	ND	$0.01^{f}$		
10	2-acetyl-1-pyrroline	ND	ND	$0.1^{k}$		
12	dimethyl trisulfide	5.0	70	$0.008^f$	625	8750
13	2,3,5-trimethylpyrazine	ND	37	$400^{I}$		0.09
15	(E)-2-octenal	26	8.3	$3^k$	8.7	2.8
16	3-(methylthio)propanal	23	187	$0.2^{m}$	115	935
20	(E,E)-2,4-heptadienal	41	6.0	778 <sup>n</sup>	0.05	0.00
21	(E)-2-nonenal	21	12	$0.08^{f}$	263	150
22	octanol	46	ND	$110^{o}$	0.42	
23	(E,Z)-2,6-nonadienal	20	1.5	$0.02^f$	1000	75
27	2-acetylpyrazine	ND	15	$62^{h}$		0.24
28	phenylacetaldehyde	18	67	$4^k$	4.5	17
38	(E,E)-2,4-decadienal	13	4.4	$0.03^f$	433	147
45	2-aminoacetophenone	ND	9.2	NA		

<sup>a</sup> Numbers correspond to those in Table 1 and Figure 1. <sup>b</sup> Mean values of duplicates. Values in mg/kg of STV and mg/L of TS. <sup>c</sup> Odor thresholds in water. <sup>d</sup> Odor value = compound concentration divided by odor threshold. <sup>e</sup> ND, concentration not determined. <sup>f</sup> Milo and Grosch (1996). <sup>g</sup> Guth and Grosch (1994). <sup>h</sup> Fors (1983). <sup>j</sup> NA, Not available. <sup>j</sup> McGill et al. (1977). <sup>k</sup> Buttery et al. (1988). <sup>j</sup> Maga (1982). <sup>m</sup> Guadagni et al. (1972). <sup>n</sup> Tamura et al. (1995). <sup>o</sup> Takeoka et al. (1992).

Table 3. Free Amino Acids in STV and TS Made from STV

	concn <sup>a</sup>		taste threshold <sup>b</sup>	taste value <sup>c</sup>	
compound	STV	TS	(g/dL)	STV	TS
aspartic acid	0.90	2.65	0.003	301	885
threonine	0.90	2.59	0.26	3.5	9.9
serine	0.92	2.70	0.15	6.1	18.0
asparagine	0.01	1.15	0.1	0.2	11.5
glutamic acid	1.94	4.58	0.005	389	917
proline	0.91	1.31	0.3	3	4.4
glycine	0.60	1.80	0.13	4.6	13.9
alanine	1.10	3.63	0.06	18.3	60.6
valine	1.14	3.40	0.04	28.5	84.9
cystine	0.07	0.03	$\mathbf{N}\mathbf{A}^d$		
methionine	0.81	1.58	0.03	26.9	52.5
isoleucine	1.04	2.78	0.09	11.6	30.9
leucine	1.67	4.49	0.19	8.8	23.6
tyrosine	1.26	1.12	NA		
lysine	1.38	4.31	0.05	27.6	86.1
arginine	1.46	3.96	0.05	29.2	79.1
histidine	0.86	1.32	0.02	42.9	66.2
phenylalanine	1.28	2.33	0.09	14.2	25.9
$\beta$ -alanine	0.02	0.01	NA		
γ-aminobutyric acid	0.19	0.27	NA		
ammonia	0.09	0.28	NA		
ornithine	0.21	0.23	NA		
phosphoserine	0.17	0.28	NA		
taurine	1.79	3.14	NA		
urea	0.04	0.45	NA		
3-methylhistidine	0.03	0.01	NA		
anserine	0.77	2.47	NA		
carnosine	0.10	$ND^e$	NA		
sarcosine	0.03	0.02	NA		
α-aminoisobutyric acid	0.06	0.01	NA		
cystathionine	0.04	0.02	NA		
total	21.8	52.9			

 $^a$  Values are on a dry weight and salt free basis in g/100 g and g/100 mL of STV and TS, respectively. The data represent mean vlues of duplicates.  $^b$  Kato et al. (1989).  $^c$  Taste value = compound concentration divided by taste treshold.  $^d$  Not available.  $^e$  Not detected.

(1982) reported that the dipetides anserine, carnosine and sarcosine were abundant in tuna and some species of sharks.

Taste values (TVs) were calculated for the amino acids from concentration and taste threshold data (Table 3). Glutamic and aspartic acids, having sour tastes (Kato

et al., 1989), had the highest TVs and the lowest thresholds in both samples, followed by lysine, valine (bitter), arginine (bitter), histidine (bitter), and alanine. Sanceda et al. (1990) reported that glutamic acid might make an important contribution to the taste of fish sauce. Hayashi et al. (1981) also reported that glycine, glutamic acid, and arginine contribute to the characteristic flavor of boiled crab. On the basis of the TVs, specific free amino acids having sweet, sour, and bitter tastes may play a prominant role in the overall taste of TS. Furthermore, these free amino acids, along with free fatty acids liberated from tuna viscera by enzymatic hydrolysis, may be thermally degraded during processing of TS, and these degradation products may contribute to the characteristic aroma of TS.

## LITERATURE CITED

AOAC. Official Methods of Analysis, 13th ed.; Association of Official Analytical Chemists: Washington, DC, 1980.

Baek, H. H.; Cadwallader, K. R. Volatile compounds in flavor concentrates produced from crayfish processing byproducts with and without protease. *J. Agric. Food Chem.* **1996**, *44*, 3262–3267.

Baek, H. H.; Cadwallader, K. R.; Marroquin, E.; Silva, J. L. Identification of predominant aroma compounds in muscadine grape juice. *J. Food Sci.* **1997**, *62*, 249–252.

Beddows, C. G.; Ardeshir, A. G. The production of soluble fish protein solution for use in fish sauce manufacture. 1. The use of added enzymes. *J. Food Technol.* **1979**, *14*, 603–612.

Beddows, C. G.; Ismail, M.; Steinkraus, K. H. The use of bromelain in the hydrolysis of mackerel and the investigation of fermented fish aroma. *J. Food Technol.* **1976**, *11*, 379–388.

Buttery, R. G.; Turnbaugh, J. G.; Ling, L. C. Contribution of volatiles to rice aroma. *J. Agric. Food Chem.* **1988**, *36*, 1006–1009.

Cadwallader, K. R.; Tan, Q.; Chen, F.; Meyers, S. P. Evaluation of the aroma of cooked spiny lobster tail meat by aroma extract dilution analysis. *J. Agric. Food Chem.* **1995**, *43*, 2432–2437.

Cha, Y. J.; Chang Lee, G. H.; Cadwallader, K. R. Aroma-active compounds in salt-fermented anchovy. In *Flavor and Lipid Chemistry of Seafoods*; Shahidi, F., Cadwallader, K. R., Eds.; ACS Symposium Series 674; American Chemical Society: Washington, DC, 1997; pp 131–147.

- Choi, Y. J.; Kim, I. S.; Lee, K. W.; Kim, G. B.; Lee, N. G.; Cho, Y. J. Available compounds of cooking drips, dark muscle, head, and raw viscera from skipjack. *J. Korean Fish. Soc.* **1996**, *29*, 701–708.
- Chung, H. Y.; Cadwallader, K. R. Aroma extract dilution analysis of cooked crab claw meat volatiles. *J. Agric. Food Chem.* **1994**, *42*, 2867–2870.
- Ciolfi, G.; Garofolo, A.; Di Stefano, R. Identification of some *o*-aminophenones as secondary metabolites of *Saccharomyces cerevisiae*. *Vitis* **1995**, *34*, 195–196.
- Collin, S.; Osman, K.; Delcambre, S.; El-Zayat, A. I.; Dufour, J.-P. Investigation of volatile flavor compounds in fresh and ripened domiati cheeses. *J. Agric. Food Chem.* **1993**, *41*, 1659–1663.
- Dougan, J.; Howard, G. E. Some flavoring constituents of fermented fish sauces. *J. Sci. Food Agric.* **1975**, *26*, 887–894.
- Fors, S. Sensory properties of volatile Maillard reaction products and related compounds. In *Maillard Reaction in Food and Nutrition*, Waller, G. R., Feather, M. S., Eds.; ACS Symposium Series 215; American Chemical Society: Washington, DC, 1983; pp 185–286.
- Forss, D. A. Review of the progress of dairy science: mechanisms of formation of aroma compounds in milk and milk products. *J. Dairy Res.* **1979**, *46*, 691–706.
- Gasser, U.; Grosch, W. Identification of volatile flavour compounds with high aroma values from cooked beef. Z. Lebensm. Unters. Forsch. 1988, 186, 489–494.
- Guadagni, D.; Buttery, R. G.; Turnbaugh, J. G. Odor thresholds and similarity ratings of some potato chip components. *J. Sci. Food Agric.* **1972**, *23*, 1435–1444.
- Guth, H.; Grosch, W. Identification of the character impact odorants of stewed beef juice by instrumental analyses and sensory studies. *J. Agric. Food Chem.* **1994**, *42*, 2862–2866.
- Hayashi, T.; Furukawa, H.; Yamaguchi, K.; Konosu, S. Comparison of taste between natural and synthetic extracts of snow crab meat. *Bull. Jpn. Soc. Sci. Fish.* **1981**, *47*, 529–534
- Hites, R. A.; Biemann, K. Computer evaluation of continuously scanned mass spectra of gas chromatography effluents. *Anal. Chem.* **1970**, *42*, 855–860.
- Ho, C.-T.; Bruechert, L. J.; Zhang, Y.; Chiu, E.-M. Contribution of lipids to the formation of heterocyclic compounds in model systems. In *Thermal Generation of Aromas*, Parliament, T. H., McGorrin, R. J., Ho, C.-T., Eds.; ACS Symposium Series 409; American Chemical Society: Washington, DC, 1989; pp 105–113.
- Josephson, D. B.; Lindsay, R. C. Retro-aldol degradations of unsaturated aldehydes: role in the formation of c4-heptenal from t2,c6-nonadienal in fish, oyster, and other flavors. *J. Am. Oil Chem. Soc.* **1987**, *64*, 132–138.
- Josephson, D. B.; Lindsay, R. C.; Stuiber, D. A. Variations in the occurrence of enzymically derived volatile aroma compounds in salt- and freshwater fish. *J. Agric. Food Chem.* 1984, 32, 1344–1347.
- Kato, H.; Rhue, M. R.; Nishimura, T. Role of free amino acids and peptides in food taste. In *Flavor Chemistry: Trends and Developments*; Teranishi, R., Buttery, R. G., Shahidi, F., Eds.; ACS Symposium Series 388; American Chemical Society: Washington, DC, 1989; pp 158–174.
- Kim, S. M.; Ha, J. U. Utilization of the protein hydrolysates of skipjack tuna viscera. *Korean J. Food Sci. Technol.* **1995**, 27, 141–146.
- Konosu, S.; Yamaguchi, K. The flavor components in fish and shellfish. In *Chemistry and Biochemistry of Marine Food Products*; Martin, R. E., Flick, G. J., Hebard, C. E., Ward, D. R., Eds.; AVI Publishing: Westport, CT, 1982; pp 367–404
- Lee, E. H.; Lee, T. H.; Kim, J. S.; Ahn, C. B. Processing and taste compounds of the fish sauce from skipjack scrap. *Bull. Korean Fish. Soc.* **1989**, *22*, 25–35.
- Lee, S. C.; Woo, K. L. A study on development of effective utilization method of skipjack tuna viscera. *Korean J. Food Sci. Technol.* **1992**, *24*, 86–91.

- Maga, J. A. Pyrazines in food: an update. *CRC Crit. Rev. Food Sci. Nutr.* **1982**, *16*, 1–115.
- McGill, A. S.; Hardy, R.; Burt, J. R. Hept-*cis*-4-enal and its contribution of the off-flavour in cold stored cod. *J. Sci. Food Agric.* **1974**, *25*, 1477–1489.
- Milo, C.; Grosch, W. Changes in the odorants of boiled salmon and cod as affected by the storage of the raw material. *J. Agric. Food Chem.* **1996**, *44*, 2366–2371.
- Mussinan, C. J.; Katz, I. Isolation and identification of some sulfur chemicals present in two model systems approximating cooked meat. J. Agric. Food Chem. 1973, 21, 43–45.
- Saisithi, P.; Kasemsarn, B.-O.; Liston, J.; Dollar, A. M. Microbiology and chemistry of fermented fish. *J. Food Sci.* **1966**, *31*, 105–110.
- Sanceda, N. G.; Kurata, T.; Arakawa, N. Overall quality and sensory acceptance of a lysine-fortified fish sauce. *J. Food Sci.* 1990, 55, 983–988.
- Sanceda, N. G.; Kurata, T.; Arakawa, N. Accelerated fermentation process for the manufacture of fish sauce using histidine. *J. Food Sci.* **1996**, *61*, 220–222, 225.
- Shibamoto, T.; Yeo, H. Flavor compounds formed from lipids by heat treatment. In *Flavor Precursors. Thermal and Enzymatic Conversions*, Teranishi, R., Takeoka, G. R., Guntert, M., Eds.; ACS Symposium Series 490; American Chemical Society: Washington, DC, 1992; pp 175–182.
- Takeoka, G. R.; Buttery, R. G.; Flath, R. A. Volatile constituents of Asian pear (*Pyrus serotina*). *J. Agric. Food Chem.* **1992**, *40*, 1925–1929.
- Tamura, H.; Nakamoto, H.; Yang, R. H.; Sugisawa, H. Characteristic aroma compounds in green algae (*Ulva pertusa*) volatiles. *Nippon Shokuhin Kagaku Kogaku Kaishi* **1995**, 42, 887–891.
- Triqui, R.; Guth, H. Determination of potent odorants in ripened anchovy (*Engraulis encrsicholus* L.) by aroma extract dilution analysis and by gas chromatographyolfactometry of headspace samples. In *Flavor and Lipid Chemistry of Seafoods*; Shahidi, F., Cadwallader, K. R., Eds.; ACS Symposium Series 674; American Chemical Society: Washington, DC, 1997; pp 31–38.
- Triqui, R.; Reineccius, G. A. Flavor development in the ripening of anchovy (*Engraulis encrasicholus* L.). *J. Agric. Food Chem.* **1995a**, *43*, 453–458.
- Triqui, R.; Reineccius, G. A. Changes in flavor profiles with ripening of anchovy (*Engraulis encrasicholus*). J. Agric. Food Chem. 1995b, 43, 1883–1889.
- van den Dool, H.; Kratz, P. D. A generalization of the retention index system including linear temperature programmed gas liquid partition chromatography. *J. Chromatogr.* **1963**, *11*, 463–471.
- Weir, G. S. D. Protein hydrolysates as flavourings. In *Developments in Food Proteins—4*; Hudson, B. J. F., Ed.; Elsevier Applied Science: New York, 1986; pp 175–217.
- Yoshinaka, R.; Sato, M.; Tsuchiya, N.; Ikeda, S. Production of fish sauce from sardine by utilization of its visceral enzymes. *Bull. Jpn. Soc. Sci. Fish.* **1983**, *49*, 463–469.

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