

Flavor and Taste-Active Compounds in Blue Mussel Hydrolysate Produced by Protease

Yong-Jun Cha[†], Hun Kim and Sung-Min Jang

Dept. of Food Science and Nutrition, Changwon National University, Changwon 641-773, Korea

Abstract

Volatile flavor compounds in hydrolyzed blue mussel(HBM) produced by OptimaseTM APL-440, with untreated blue mussel(UBM) were compared. A total of 100 volatile compounds were detected in both HBM and UBM, consisting mainly of 25 aldehydes, 16 ketones, 17 alcohols, 8 nitrogen-containing compounds, 11 aromatic compounds, 8 terpenes, and 15 miscellaneous compounds. Levels of aromatic compounds decreased after hydrolysis, whereas levels of 7 nitrogen-containing compounds increased. The compounds, 3-methylbutanal, (Z)-4-heptenal, and (E,Z)-2,6-nonadienal, had the highest odor values in both samples. Total free amino acids in HBM were 21.89%(w/w) and increased by 3.4 times higher than UBM. Glutamic acid and aspartic acid, having sour tastes, were the major taste-active compounds in HBM.

Key words: blue mussel, hydrolysate, volatile flavor compounds, taste-active compounds

INTRODUCTION

Coupled with rising energy costs and the problem of availability and conservation of natural resources, nowadays, this forces the food industry to constantly develop new technology and sources of raw materials to meet these needs. In addition, there is the desire for the raw materials to be natural and harmless to consumer. One of the increasingly important and widely sought sources of food is seafood, particularly shellfish.

Blue mussel, having a desirable taste and aroma, is an important marine resource in Korea(1). However, in recent years processing utilization of blue mussel has declined due to decreasing harvesting yields associated with marine environmental contamination. Therefore, an alternative use of blue mussel, such as production of flavorants via enzymatic hydrolysis, for having a supplementary value may be a fundamental benefit to seafood industry.

Several studies have addressed the production of seafood flavorings by enzymatic hydrolysis(2,3). Developing natural seasoning agents from blue mussel with commercial proteases was also attempted by many researchers(4-6). However, a better understanding of flavor compounds in blue mussel hydrolysate is needed if alternative products are to be produced.

The objective of this study was to compare taste-

active and volatile flavor compounds of enzyme hydrolyzed blue mussel(HBM) with those of untreated blue mussel(UBM).

MATERIALS AND METHODS

Materials

A fresh blue mussel(*Mytilus edulis*) was purchased from a fish market in Masan, Korea and transported on ice in polyethylene bags to the Flavor Lab., Changwon National University within 1 hr. The sample was refrigerated(4°C) until analysis. Blue mussel hydrolysate was produced by OptimaseTM APL-440(Solvay Enzymes, Inc., Elkhart, USA) at optimal hydrolysis conditions, e.g. pH 9.8, 58°C, 2.9 hr reaction time, 46.8%(w/v) substrate concentration, 0.34%(v/w) enzyme/substrate ratio, as described in previous paper(6). Standard flavor compounds were obtained from Aldrich Chemical Co.(Milwaukee, USA).

Analysis of free amino acids

Fifteen grams of untreated(UBM) or 30ml of hydrolyzed blue mussel(HBM) were treated with 50ml of 10% TCA solution and then rinsed with redistilled diethyl ether to remove lipid and pigments. The aqueous extract was evaporated to dryness on vacuum rotary evaporator

[†]Corresponding author

(Bibby Science Co., England), dissolved in buffer solution (pH 2.2), and then volume adjusted to 25ml prior to analysis of free amino acids (FAAs). FAAs were determined by an automatic amino acid analyzer (Pharmacia Biochrom 20, England) equipped with Li⁺ type high performance ultra pack. Duplicate analysis were performed on each sample.

Vacuum simultaneous steam distillation-solvent extraction (V-SDE)

V-SDE was conducted under vacuum (540~580atm, b.p. 55~60°C) using the apparatus described by Chung and Cadwallader (7). One liter of HBM plus 90.8µg of internal standard (2,4,6-trimethylpyridine, TMP) and 1L of distilled water (or 500g of UBM plus 1.5L of distilled water) were extracted for 2.5 hr with 200ml of redistilled diethyl ether. V-SDE extracts were kept at -20°C overnight to facilitate water removal. The details were described by Cadwallader et al. (8). Extracts were dried over 2g of anhydrous sodium sulfate and concentrated under a gentle stream of N₂ to 0.3 ml before analysis. Duplicate extractions were carried out on each sample.

Gas chromatography/mass spectrometry (GC/MS)

One µl of each V-SDE extract was injected into an HP 5890A GC/HP 5971 mass selective detector (MSD) (Hewlett-Packard Co.) (splitless mode; 30s valve delay; injector temperature 155°C) with a fused silica open-tubular column (Supelcowax 10, 60m × 0.25mm i.d. × 0.25mm film thickness; Supelco, Inc., Bellefont, USA). Oven temperature was programmed from 40°C to 175°C at 2°C/min with initial and final holding times of 5 and 30 min, respectively; oven temperature was then further increased to 195°C at 5°C/min and held for 25 min. Other GC conditions and MSD parameters have been described elsewhere (9,10). Duplicate analysis were performed on each extract.

Identification and relative abundance of compounds

Volatile compounds were identified by matching retention indices (RI) (11) and mass spectra of unknowns with those of authentic standard compounds. Tentative identifications were based on standard MS library data (12). Concentration of each volatile compound was calculated by mean relative content (n=4) to TMP concentration per gram of sample (factor=1).

RESULTS AND DISCUSSION

Free amino acid compositions in UBM and HBM

Free amino acids in protease hydrolysed blue mussel (HBM) with those of untreated blue mussel (UBM) are shown in Table 1. Total free amino acids (FAAs) in HBM was 21.88g%(w/w) on a dry weight basis and was 3.4 times higher than UBM (6.36g%). As shown in Table 1, taurine was comprised over half of the total FAAs in UBM, and was unchanged after hydrolysis. However, taurine was reported to have essentially no taste impact (13), nor any effect on formation of aroma active components (14). In addition to taurine, glycine, alanine, arginine and glutamic acid also were found at high levels, and comprised 76.1% of the total amino acids in UBM. Konosu and Yamaguchi (13) reported that alanine and glycine were the major free amino acids in most species of mussel. After taurine, leucine was in the highest abundance in HBM, followed by arginine, alanine, lysine, asparagine, glutamic acid, and glycine. From the result of taste values (TVs) calculated, however, glutamic acid and aspartic acids, having sour tastes (15), had the highest TVs and the lowest thresholds in both samples, followed by arginine (bitter), lysine (sweet), valine (bitter), alanine (sweet), and methionine (bitter) in HBM. Glutamic acid, a known flavor enhancer, was as being in the highest abundance in various hydrolysates, such as hydrolysed vegetable protein (HVP) and soysauce (16). Cha and Cadwallader (17) reported that glutamic acid, aspartic acid, arginine, and lysine contribute greatly to the characteristic taste of skipjack tuna sauce through TVs test. Most amino acids, including glutamic acid, glycine, alanine, and lysine, are known to be important aroma precursors that react primarily through Maillard reactions with other compounds (14,16,18). A lot of reactive amino acids in Maillard reaction, especially basic amino acids, should be considered to play an important role in the development of the characteristic aroma in blue mussel hydrolysate (14).

Volatile flavor profiles in blue mussel hydrolysate

Volatile flavor compounds in HBM and UBM were compared. A total of 100 volatile compounds were detected of which 96 were positively identified (Table 2). Eighty-four compounds were detected in HBM and 75 in UBM. These compounds were composed of 25 aldehydes, 16

Table 1. Free amino acids and taste values in UBM and HBM¹⁾

Compound	Concentration ²⁾		Taste threshold ³⁾ (g/dl)	Taste value ⁴⁾	
	UBM	HBM		UBM	HBM
Aspartic acid	0.067	0.285	0.003	22.3	95.0
Threonine	0.122	1.117	0.26	0.5	4.3
Serine	0.095	1.148	0.15	0.6	7.7
Asparagine	0.139	1.407	0.1	1.4	14.1
Glutamic acid	0.294	1.307	0.005	58.8	261.4
Proline	0.046	0.073	0.3	0.2	0.2
Glycine	0.647	1.241	0.13	5.0	9.5
Alanine	0.424	1.706	0.06	7.1	28.4
Valine	0.049	1.142	0.04	1.2	28.6
Cystine	0.123	N/D ⁴⁾	N/A ⁷⁾		
Methionine	0.064	0.736	0.03	2.1	24.5
Isoleucine	0.040	0.989	0.09	0.4	11.0
Leucine	0.060	1.899	0.19	0.3	10.0
Tyrosine	0.088	1.237	N/A		
Lysine	0.119	1.592	0.05	2.4	31.8
Arginine	0.368	1.725	0.05	7.4	34.5
Histidine	0.068	0.327	0.02	3.4	16.4
Ammonia	0.011	0.024	N/A		
Ornithine	0.023	0.026	N/A		
Phosphoserine	0.056	0.160	N/A		
Taurine	3.102	3.221	N/A		
Urea	0.328	0.380	N/A		
Anserine	0.001	0.070	N/A		
Hydroxyproline	0.001	0.020	N/A		
3-Methylhistidine	- ⁶⁾	N/D	N/A		
α -Aminoadipic acid	-	0.007	N/A		
β -Alanine	0.024	0.028	N/A		
DL ⁺ -Allohydroxylysine	0.001	0.021	N/A		
Total	6.360	21.888			

¹⁾UBM, Untreated blue mussel; HBM, enzyme hydrolyzed blue mussel with OptimaseTM APL-440

²⁾Concentrations are on a dry weight basis in g/100g and g/100ml of UBM and HBM, respectively

³⁾Kato et al.(15), ⁴⁾Taste value : compound concentration divided by taste threshold

⁵⁾N/D : Not detected, ⁶⁾Trace(<1mg), ⁷⁾N/A : Not available

ketones, 17 alcohols, 8 nitrogen-containing compounds, 11 aromatic hydrocarbons, 8 terpenes, and 15 miscellaneous compounds.

As shown in Table 2, the number and relative abundance of aldehydes were higher than the other classes of compounds in both samples. Levels of aldehydes increased 1.8 fold in HBM compared with UBM. Certain aldehydes, such as (E)-2-methyl-2-butenal, 2-furancarboxaldehyde, (E,E)-2,4-heptadienal, decanal, 4-ethylbenzaldehyde, and (E,E)-2,4-decadienal, were detected only in HBM. Three compounds, 3-methylbutanal, (Z)-4-heptenal, and benzaldehyde were notably high in both samples. As shown in Table 2, however, benzaldehyde may not contribute to the characteristic flavor of blue mussel with its high threshold. On the other hand, the compounds,

3-methylbutanal(dark chocolate-like)(17), (Z)-4-heptenal (rancid, crab-like)(8), and (E,Z)-2,6-nonadienal(cucumber-like)(17), having lower thresholds, may positively affect to the overall flavor of HBM. The alkanals and alkenals detected in both samples might have been due to oxidation of PUFA during sample treatment and hydrolysis (19-21). These compounds are known to contribute fatty-oily, slightly rancid odors, while the dienals contribute pleasant fried-fatty aromas(22,23). Levels of 2-methyl butanal, 3-methylbutanal, and (Z)-4-heptenal increased after hydrolysis. The compound (Z)-4-heptenal was reported to be produced from (E,Z)-2,6-nonadienal by retro-aldol condensation(24).

Levels of ketones were 2.8 fold high in HBM compared with UBM. The compounds, 2-nonanone, 6-methyl-5-

Table 2. Volatile flavor compounds and odor values in UBM and HBM

Compound name by class	Retention index	Concentration(ng/g) ¹⁾		Odor threshold (ng/g) ²⁾	Odor value ³⁾	
		UBM	HBM		UBM	HBM
Aldehydes(25)						
Butanal	858	12.85(5.51)	53.24(25.70)	N/A ⁵⁾		
2-Methylbutanal	908	11.02(3.67)	141.37(60.59)	3 ⁶⁾	3.67	47.12
3-Methylbutanal	911	47.74(20.20)	216.65(121.18)	0.2 ⁶⁾	238.68	1083.24
Pentanal	971	22.03(14.69)	34.88(23.87)	12 ⁶⁾	1.84	2.91
2-Butenal	1032	36.72(11.02)		N/A		
Hexanal	1071	18.36(9.18)	29.38(23.87)	5 ⁷⁾	3.67	5.88
(E)-2-methyl-2-butenal	1083		20.20(12.85)	N/A		
(E)-2-Pentenal	1119	77.11(16.52)	11.02(7.34)	N/A		
2-Methyl-(E)-2-pentenal	1146	7.34(7.34)	9.18(7.34)	N/A		
Heptanal	1175	20.20(11.02)	40.39(11.02)	3 ⁷⁾	6.73	13.46
(E)-2-Hexenal	1206	69.77(23.87)	29.38(11.02)	17 ⁸⁾	4.10	1.73
(Z)-4-Heptenal	1231	69.77(3.67)	112.00(58.75)	0.04 ⁶⁾	1744.20	2799.90
Octanal	1278	18.36(7.34)	45.90(18.36)	0.7 ⁷⁾	26.23	65.57
Nonanal	1388	16.52(7.34)	53.24(31.21)	1 ⁷⁾	16.52	53.24
(E,E)-2,4-Hexadienal	1394	11.02(7.34)		60 ⁹⁾	0.18	
(E)-2-Octenal	1415	34.88(11.02)		3 ⁸⁾	11.63	
2-Furancarboxaldehyde	1454		27.54(14.69)	N/A		
(E,E)-2,4-Heptadienal	1483		23.87(12.85)	778 ¹⁰⁾		0.03
Decanal	1496		16.52(5.51)	N/A		
Benzaldehyde	1513	64.26(5.51)	183.60(73.44)	350 ³⁾	0.18	0.52
(E,Z)-2,6-Nonadienal	1576	18.36(16.52)	29.38(14.69)	0.01 ¹¹⁾	1836.00	2937.60
(E,E)-2,4-Octadienal	1579	66.10(12.85)	100.98(18.36)	N/A		
3-Thiophenecarboxaldehyde	1666		11.02(3.67)	N/A		
4-Ethylbenzaldehyde	1690	40.39(25.70)		N/A		
(E,E)-2,4-Decadienal	1798	9.18(5.51)		0.07 ⁸⁾	131.14	
Ketones(16)						
2-Butanone	894	5.51(5.51)		N/A		
1-Penten-3-one	1014	25.70(14.69)	31.21(31.21)	N/A		
1-(2-Furanly)-ethanone	1022	3.67(3.67)	25.70(25.70)	N/A		
2,3-Pentanedione	1053	11.02(11.02)	36.72(31.21)	N/A		
3-Penten-2-one	1116	9.18(5.51)		1.5 ⁷⁾	6.12	
2-Heptanone	1171		29.38(20.20)	140 ¹¹⁾		0.21
2-Octanone	1273		18.36(12.85)	50 ⁷⁾		0.37
(E)-3-Hepten-2-one	1288		64.26(42.23)	56 ¹¹⁾		1.15
6-Methyl-5-heptene-2-one	1325	23.87(11.02)	77.11(23.87)	N/A		
2-Nonanone	1376	25.70(7.34)	86.29(34.88)	200 ⁷⁾	0.13	0.43
(E)-3-Octen-2-one	1395		16.52(12.85)	N/A		
2-Decanone	1485		47.74(20.20)	N/A		
(E,E)-3,5-Octadien-2-one	1560	14.69(3.67)	40.39(25.70)	150 ⁹⁾	0.10	0.27
2-Undecanone	1584	23.87(11.02)	22.03(12.85)	N/A		
1-Phenylethanone	1638		14.69(9.18)	N/A		
5-Ethenyl-dihydro-5-methyl-2(3H)-furanone ⁴⁾	1656	18.36(12.85)		N/A		
Alcohols(17)						
Ethanol	923	459.00(425.95)	339.66(372.71)	100000 ⁹⁾	0.00	0.00
Propanol	1034	211.14(60.59)	47.74(40.39)	N/A		
Butanol	1133	11.02(5.51)	3.67(3.67)	500 ³⁾	0.02	0.01
1-Penten-3-ol	1151	576.50(99.14)	400.25(123.01)	400 ³⁾	1.44	1.00
Pentanol	1238	23.87(9.18)	23.87(9.18)	4000 ⁹⁾	0.01	0.01
1-Hexen-3-ol	1240	42.23(5.51)	16.52(14.69)	N/A		
(Z)-2-Penten-1-ol	1299	45.90(27.54)	71.60(25.70)	N/A		
(E)-2-Penten-1-ol	1309	660.96(99.14)	602.21(227.66)	N/A		
Hexanol	1340	27.54(5.51)	14.69(12.85)	2500 ¹²⁾	0.01	0.01
(Z)-3-Hexen-1-ol	1370	33.05(5.51)	14.69(7.34)	70 ³⁾	0.47	0.21
(E)-2-Hexen-1-ol	1401	12.85(7.34)	14.69(9.18)	400 ⁹⁾	0.03	0.04
1-Octen-3-ol	1439	75.28(7.34)	42.23(7.34)	1 ⁷⁾	75.28	42.23

Table 2. (Continued)

Compound name by class	Retention index	Concentration(ng/g) ¹⁾		Odor threshold (ng/g) ²⁾	Odor value ³⁾	
		UBM	HBM		UBM	HBM
Heptanol	1444	33.05(12.85)	36.72(11.02)	N/A		
2-Ethyl-1-hexanol	1480	20.20(9.18)	189.11(49.57)	N/A		
Octanol	1548	33.05(14.69)	27.54(16.52)	110 ¹²⁾	0.30	0.25
2-Furanmethanol	1650	11.02(5.51)	22.03(20.20)	N/A		
Benzenemethanol	1866		12.85(3.67)	N/A		
N-Containing compounds(8)						
Pyridine	1167	185.44(18.36)	47.74(42.23)	2000 ⁷⁾	0.09	0.02
Pyrazine	1200		9.18(5.51)	N/A		
2-Methylpyridine	1202		3.67(3.67)	N/A		
Methylpyrazine	1249		22.03(20.20)	N/A		
2,5-Dimethylpyrazine	1304		33.05(16.52)	1700 ⁶⁾		0.02
2,3-Dimethylpyrazine	1330		7.34(5.51)	2500 ⁶⁾		0.00
Trimethylpyrazine	1390		18.36(22.03)	23 ⁶⁾		0.80
3-Ethyl-2,5-dimethylpyrazine	1428		12.85(3.67)	N/A		
Aromatic hydrocarbones(11)						
Toluene	1028	42.23(34.88)	99.14(80.78)	N/A		
Ethylbenzene	1114	5.51(3.67)	5.51(5.51)	N/A		
<i>p</i> -Xylene	1123	9.18(5.51)	7.34(7.34)	N/A		
<i>m</i> -Xylene	1129	12.85(9.18)	5.51(7.34)	N/A		
<i>o</i> -Xylene	1170	18.36(7.34)		N/A		
4-Ethyltoluene	1212	3.67(3.67)		N/A		
Styrene	1244	11.02(7.34)		N/A		
1,2,4-Trimethylbenzene	1266	14.69(1.84)	11.02(9.18)	N/A		
Naphthalene	1721	23.87(11.02)	16.52(5.51)	N/A		
2-Methylnaphthalene	1838	20.20(14.69)		N/A		
<i>o</i> -Cresol	2066	36.72(3.67)	47.74(16.52)	N/A		
Terpenes(8)						
α -Pinene	1009	3.67(1.84)	11.02(5.51)	N/A		
Limonene	1195		12.85(9.18)	10 ¹¹⁾		1.29
(E)-Caryophyllene ⁴⁾	1584	29.38(9.18)	44.06(69.77)	N/A		
(Z)-Citri	1668		60.59(89.96)	N/A		
α -Murolene ⁴⁾	1709	89.96(14.69)		N/A		
(E)-Citral	1716	38.56(16.52)	38.56(16.52)	N/A		
δ -Cadinene ⁴⁾	1749	130.36(77.11)		N/A		
Geranylacetone	1845	33.05(22.03)	12.85(9.18)	60 ⁶⁾	0.55	0.21
Micellaneous compounds(15)						
Ethylacetate	867	218.48(99.14)	370.87(302.94)	5000 ⁹⁾	0.04	0.07
Nonane	891	3.67(5.51)	11.02(9.18)	N/A		
2-Ethylfuran	943	11.02(11.02)	9.18(5.51)	N/A		
Decane	993	5.51(5.51)	12.85(5.51)	N/A		
Dimethydisulfide	1053		27.54(27.54)	12 ⁹⁾		2.30
Undecane	1095	9.18(9.18)	7.34(3.67)	N/A		
Dodecane	1196	29.38(18.36)	18.36(1.84)	N/A		
2-Pentylfuran	1221	5.51(1.84)	7.34(5.51)	6 ⁸⁾	0.92	1.22
Tridecane	1295	22.03(25.70)	20.20(11.02)	N/A		
Dimethyltrisulfide	1363		27.54(20.20)	0.01 ⁹⁾		2754.00
Tetradecane	1396	38.56(14.69)		N/A		
Pentadecane	1502	14.69(14.69)	20.20(20.20)	N/A		
Hexadecane	1599	22.03(3.67)		N/A		
2-Acethylthiazole	1635		150.55(108.32)	10 ¹³⁾		15.06
Hexanoic acid	1842		20.20(5.51)	3000 ¹⁴⁾		0.01

¹⁾Concentration of each compound was calculated with relative content to internal standard concentration(factor=1), numbers in parentheses represent relative standard deviations

²⁾Odor thresholds in water, ³⁾Odor value: compound concentration divided by odor thresholds

⁴⁾Compound tentatively identified by mass spectrum, ⁵⁾Not available

⁶⁾Buttery et al.(30) ⁷⁾Buttery et al.(31) ⁸⁾Buttery et al.(32) ⁹⁾Hansen et al.(33) ¹⁰⁾Tamura et al.(34)

¹¹⁾Buttery et al.(35) ¹²⁾Takeoka et al.(36) ¹³⁾Schutte(37) ¹⁴⁾van Germert and Nettenbreijer(38)

heptene-2-one, (E)-3-hepten-2-one, 2-decanone, and (E,E)-3,5-octadien-2-one were the most abundant ketones in HBM. These ketones may have resulted from thermal oxidation/degradation of PUFA(20). Kubota et al.(25) also reported that carbonyl compounds, resulting mainly from lipid and amino acid degradation, contributed strong seaweed-like odor to cooked krill.

Levels of alcohols in UBM decreased after hydrolysis. Ethanol, 1-penten-3-ol, and 2-ethyl-1-hexanol were the most abundance in both samples. 1-Penten-3-ol was reported to contribute a heavy and plant-like aroma to oyster(26). Except for 1-octen-3-ol, having mushroom-like odor, however, alcohols may not contribute to the overall flavor of blue mussel hydrolysate because of their high threshold values(Table 2)(23).

Six pyrazines detected were only found in HBM. Methylpyrazine, 2,5-dimethylpyrazine and trimethylpyrazine were found in high abundance. However, these compounds may not greatly contribute to the overall flavor of HBM because of their low odor values. These heterocyclic compounds might have to be formed from various nitrogen sources by Maillard and pyrolysis reactions during hydrolysis and subsequent heat treatment (19,27).

Eleven aromatic hydrocarbons were detected in UBM and 7 in HBM. Toluene was found high abundance in both HBM and UBM. Levels of several hydrocarbons were reduced after hydrolysis and heat treatment.

Two compounds, δ -cadinene and α -muurolene, were in high abundances in UBM. These compounds, generally derived from essential oils of plants, might have originated from algae via the food chain. Among the miscellaneous compounds identified, three sulfur compounds, consisting of dimethyldisulfide(DMD), dimethyltrisulfide(DMTS), and 2-acetylthiazole(2-AT) were found only in HBM. DMD and DMTS are known to produce strong sulfurous odors(28) and may be undesirable to the flavor profile of HBM, while 2-AT, having a nutty/popcorn-like aroma(29) was thought to make a positive contribution.

Blue mussel hydrolysate could be converted a valuable alternative product such as flavoring agent on the basis of flavor profiles, e.g. producing of Maillard reaction flavor. However, further research is needed to determine the relative significance of the sensory components in this interesting flavor system.

ACKNOWLEDGEMENTS

This research was funded by the SGRP/PTRP (Problem-Oriented Technology Development Project for Agriculture, Forestry and Fisheries) in Korea. We thank Dr. Cadwallader in Mississippi State University for revising this manuscript.

REFERENCES

1. National Fisheries Research and Development Agency : Chemical Composition of Marine Products in Korea. National Fisheries Research and Development Agency in Republic of Korea, p.56, 88(1989)
2. In, T. and Lyraz, I. : Seafood flavorants produced by enzymatic hydrolysis. In "Making profits out of seafood Wastes" Keller, S.(ed.), Proceedings of the International Conference on Fish By-Products, April 25-27, Anchorage, AK, USA, p.197(1990)
3. Baek, H. H. and Cadwallader, K. R. : Enzymatic hydrolysis of crayfish processing byproducts. *J. Food Sci.*, **60**, 929 (1995)
4. Lee, Y. C., Kim, D. S., Kim, Y. D. and Kim, Y. M. : Preparation of oyster(*Carssostrea gigas*) and sea mussel (*Mytilus coruscus*) hydrolysates using commercial protease. *Korean J. Food Sci. Technol.*, **22**, 234(1990)
5. Choi, I. J., Nam, H. S., Shin, Z. I. and Lee, B. M. : A study on the proteolysis of mussel protein by a commercial enzyme preparation. *Korean J. Food Sci. Technol.*, **24**, 519(1992)
6. Cha, Y. J., Kim, H. and Kim, E. J. : Development of blue mussel hydrolysates as a flavouring. *J. Food Sci. Nutr.*, **3**, 10(1998)
7. Chung, H. Y. and Cadwallader, K. R. : Aroma extract dilution analysis of blue crab claw meat volatiles. *J. Agric. Food Chem.*, **42**, 2867(1994)
8. Cadwallader, K. R., Baek, H. H., Chung, H. Y. and Moody, M. W. : Contribution of lipid-derived components to the flavor of alligator meat. In "Lipid in food flavors" Ho, C. T. and Hartman, T. G.(eds.), ACS Symposium Series 558, American Chemical Society, Washington, D. C., p.186 (1994)
9. Cha, Y. J., Cadwallader, K. R. and Baek, H. H. : Volatile flavor components in snow crab cooker effluent and effluent concentrate. *J. Food Sci.*, **58**, 525(1993)
10. Cha, Y. J. and Cadwallader, K. R. : Volatile components in salt-fermented fish and shrimp pastes. *J. Food Sci.*, **60**, 19(1995)
11. van den Dool, H. and Kratz, P. D. : A generalization of the retention index system including linear temperature programmed gas liquid partition chromatography. *J. Chromatogr.*, **11**, 463(1963)
12. Hewlett-Packard Co., Wiley/NBS Database(PBM format). Palo Alto, CA.(1988)
13. Konosu, S. and Yamaguchi, K. : The flavor components in fish and shellfish. In "Chemistry & biochemistry of marine food products" Martin, R. E., Flick, G. J., Hebard,

- C. E. and Ward, D. R.(eds.), AVI Publishing Co., Connecticut, p.367(1982)
14. Ohshima, T., Yokoyama, T., Wada, S., Lee, E. H. and Koizumi, C. : Studies on precursors of the odor evolved from cooked squid mantle flesh. *Nippon Suisan Gakkaishi*, **57**, 1743(1991)
 15. Kato, H., Rhue, M. R. and Nishimura, T. : Role of free amino acids and peptides in food taste. In "*Flavor chemistry : Trends and developments*" Teranishi, R., Buttery, R. G. and Shahidi, F.(eds.), ACS Symposium Series 388, American Chemical Society, Washington, D. C., p.158(1989)
 16. Weir, G. S. D. : Protein hydrolysates as flavourings. In "*Developments in food proteins-4*" Hudson, B. J. F. (ed.), Elsevier Applied Science Pub., New York, p.175 (1986)
 17. Cha, Y. J. and Cadwallader, K. R. : Aroma-active compounds in skipjack tuna sauce. *J. Agric. Food Chem.*, **46**, 1123 (1998)
 18. Hwang, H. I., Hartman, T. G. and Ho, C. T. : Relative reactivities of amino acids in pyrazine formation. *J. Agric. Food Chem.*, **43**, 179(1995)
 19. Bailey, M. E. and Einig, R. G. : Reaction flavors of meat. In "*Thermal generation of aromas*" Parliment, T. H., McGorin, R. J. and Ho, C. T.(eds.), ACS Symposium Series 409, American Chemical Society, Washington, D. C., p.421(1989)
 20. Karahadian, C. and Lindsay, R. C. : Role of oxidation in the formation and stability of fish flavors. In "*Flavor chemistry: Trends and developments*" Teranishi, R., Buttery, R. G. and Shahidi, F.(eds.), ACS Symposium Series 388, American Chemical Society, Washington, D. C., p.60(1989)
 21. St. Angelo, A. J. : Lipid oxidation in foods. *CRC Crit. Rev. Food Sci. Nutr.*, **36**, 175(1996)
 22. Vejaphan, W., Hsieh, T. C. Y. and Williams, S. S. : Volatile flavor components from boiled crayfish(*Procambarus clarkii*) tail meat. *J. Food Sci.*, **53**, 1666(1988)
 23. Heath, H. B. and Reineccius, G. : Off-flavors in foods. In "*Flavor chemistry and technology*" Heath, H. B. and Reineccius, G.(eds.), MacMillan Pub. LTD, Basingstoke, England, p.121(1986)
 24. Josephson, D. B. and 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.*, **64**, 132(1987)
 25. Kubota, K., Kobayashi, A. and Yamanishi, T. : Basic and neutral compounds in cooked odor from Antarctic krill. *Agric. Biol. Chem.*, **46**, 2835(1982)
 26. Josephson, D. B., Lindsay, R. C. and Stuber, D. A. : Volatile compounds characterizing the aroma of fresh Atlantic and Pacific oysters. *J. Food Sci.*, **50**, 59(1985)
 27. Shibamoto, T. and Bernhard, R. A. : Effect of time, temperature, and reactant ratio on pyrazine formation in model systems. *J. Agric. Food Chem.*, **24**, 847(1976)
 28. Shankaranarayana, M. L., Raghavan, B., Abraham, K. O. and Natarajan, C. P. : Sulphur compounds in flavors. In "*Food Flavours, Part A. Introduction*" Morton, I. D. and Macleod, A. J.(eds.), Elsevier, New York, p.169(1989)
 29. Ho, C. T. and Carlin, J. T. : Formation and aroma characteristics of heterocyclic compounds in foods. In "*Flavor chemistry: Trends and developments*" Teranishi, R., Buttery, R. G. and Shahidi, F.(eds.), ACS Symposium Series 388, American Chemical Society, Washington, D. C., p.92(1989)
 30. Buttery, R. G., Ling, L. C. and Stern, D. J. : Studies on popcorn aroma and flavor volatiles. *J. Agric. Food Chem.*, **45**, 837(1997)
 31. Buttery, R. G., Turnbaugh, J. G. and Ling, L. C. : Contribution of volatiles to rice aroma. *J. Agric. Food Chem.*, **36**, 1006(1988)
 32. Buttery, R. G., Seifert, R. M., Guadagni, D. G. and Ling, L. C. : Characterization of additional volatile components of tomato. *J. Agric. Food Chem.*, **19**, 524(1971)
 33. Hansen, M., Buttery, R. G., Stern, D. J., Cantwell, M. I. and Ling, L. C. : Broccoli storage under low-oxygen atmosphere: Identification of higher boiling volatiles. *J. Agric. Food Chem.*, **40**, 850(1992)
 34. Tamura, H., Nakamoto, H., Nakamoto, H., Yang, R. H. and Sugisawa, H. : Characteristic aroma compounds in green algae(*Ulva pertusa*) volatile. *Nippon Shokuhin Kagaku Kaishi*, **42**, 887(1995)
 35. Buttery, R. G., Seifert, R. M., Guadagni, D. G. and Ling, L. C. : Characterization of some volatile constituents of Bell peppers. *J. Agric. Food Chem.*, **17**, 1322(1969)
 36. Takeoka, G. R., Flath, R. A., Mon, T. E., Teranishi, R. and Guentert, M. : Volatile constituents of Apricot(*Prunus armeniaca*). *J. Agric. Food Chem.*, **38**, 471(1990)
 37. Schutte, L. : Precursors of sulfur-containing flavor compounds. *CRC Crit. Rev. Food Technol.*, **4**, 457(1974)
 38. van Germert, L. G. and Nettenbreijer, A. H. : Compilation of odor thresholds values in air and water. RID, CIVO, Central Institute for Nutrition and Food Research TNO, Zeist, The Netherlands(1997)

(Received January 24, 1998)