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Fisheries and Aquatic Sciences

Original Article
Fish Aquat Sci 17(2), 189-195, 2014

Analysis of Headspace Volatile Compounds in Cold-stored and Freeze-dried Krill *Eupausia superba*

Jin-Yong Park, Ye-Joo Kim and Yang-Bong Lee*

Department of Food Science and Technology, Pukyong National University, Busan 608-737, Korea

Abstract

Headspace volatile compounds of cold-stored and freeze-dried Krill *Eupausia superba* were analyzed to investigate their flavor qualities using a system combining a dynamic headspace isolator, an automatic thermal desorber, and a gas chromatograph-mass-selective detector. Levels of oxidation products of polyunsaturated fatty acids such as aldehydes, alcohols, and ketones, which are known to give seafood a nasty smell because of their low flavor threshold values, increased during cold storage of krill. Notably, levels of 2-methylpropanal, 3-methylbutanal, 2-methylbutanal and 2-butanone increased during its storage. They can be considered index compounds of off-odor according to freshness degradation during storage. By contrast, in freeze-dried krill powder, levels of aldehydes, ketones, and aromatic compounds decreased rapidly. Only alcohols, which did not greatly affect the food flavor, were isolated in large amounts. It was confirmed that levels of oxidized compounds of krill increased during cold storage, but decreased in freeze-dried krill.

Key words: Krill, Headspace volatile compound, Oxidation

Introduction

Krill *Eupausia superba* are among the biggest resource species that are mainly caught in the Antarctic Ocean. The possible fishing yield amount is estimated to be about 4 million tons. Krill contain proteins, essential amino acids, minerals, and polyunsaturated fatty acids (Kim et al, 2012). They are usually used as fishing bait, and farmed fish feed, as well as in oil and other products in Korea. They are not often used in food products as ingredients because of their nasty smell after fishing due to their self-digestion by enzymes (Kim and Kim, 1999). Research on krill edibility has progressed in many countries including Russia, where research began in 1961. Poland, Japan, Chile, and China have studied krill and their use (Kim, 2011). Further research is still required for their use as ingredients in food products.

The good-quality krill caught in the Antarctic Ocean need to be treated when transported to processing facilities to maintain freshness. However, krill show short-term growth at low temperatures in seawater. As activities of enzymes such as proteases, tyrosinase, and lipases are strong, deterioration of the cephalothorax and disintegration of body organization proceed within 1–2 hours when krill are piled intact on deck after fishing. Off-flavors such as fishy odor of krill due to self-digestion restrict krill use. The fish odor is a sensory quality factor for fish. Therefore, krill are immediately treated by rapid freezing after fishing in most pelagic fishing vessels to preserve their freshness (Kim, 2011).

Crustaceans such as crabs and shrimps have characteristic tastes, and their flavors are different from those of fish. Most people like their taste, and their flavor components have been of interest for processed marine products for a long time (Oh et al., 2001). Volatile compound analyses of crustaceans include flavor characteristic analysis of volatile compounds

Received 08 January 2014; **Revised** 24 February 2014
Accepted 06 March 2014

***Corresponding Author**
E-mail: yblee@pknu.ac.kr

from shrimps by gas chromatography (GC) olfactometry (Lee et al., 2002) and analysis of the compounds that affect the odor of cooked shore-swimming crab flesh (Oh, 2002). Studies for use, such as enzymatic hydrolysis of crawfish processing by-products for bioflavor production (Baek, 1994), reaction technology for making natural crab-like flavors from snow crab processing by-products (Anh, 2005), and headspace volatile compound analysis of a krill reaction flavor and its application to teriyaki sauce (Kim et al., 2013), have also been conducted. However, few papers on volatile compound analysis of krill have been published so far.

Volatile compounds contributing to the odor of refrigerated krill were isolated using the headspace method, a certified method of flavor analysis to obtain basic data for quality evaluation and maintenance. Also, volatile compounds in freeze-dried krill powder were identified to compare its quality with that of fresh krill and to provide basic data for its application as an ingredient of other foods.

Materials and Methods

Preparation of cold-stored krill and freeze-dried krill powder

Frozen krill were obtained from Dong-Won Industries (Busan, Korea). Cold-stored krill were kept at 5°C for 0, 2, or 4 days. Freeze-dried krill were prepared by freeze-drying for 3 days. They were then crushed using a Philips HR-136 grinder (Philips, Amsterdam, Netherlands), sealed, and kept at -40°C.

Adsorption and desorption of headspace volatile compounds

Samples (5 g each) were put into 250-ml dark brown bottles, which were sealed, were heated in a dry oven at 50°C for 30 min, and cooled at room temperature for 1 h. The headspace volatile compounds were adsorbed for 5 min in a 90-cm stainless steel adsorption pipe (Agilent, Santa Clara, CA, USA) of compacted Tenax- TA (Supelco, Bellefonte, PA, USA) using a VPC-10 vacuum pump (Shimadzu, Kyoto, Japan) and a mass-flow controller (MFC; Shimadzu). Desorption of the stainless steel pipe was carried out using an ATD 400 automatic thermal desorber (Perkin Elmer, Waltham, MA, USA). Desorption occurred in the opposite direction to adsorption. The ATD desorption conditions were 350°C for 4 min. The desorbed gas was focused at -30°C. Then, a second desorption step (350°C for 1 min) was performed. Desorption flow was maintained at 50.2 ml/min.

Operating conditions for GC-MSD

The desorbed volatile compounds were automatically injected into a gas chromatograph (Shimadzu) using an automatic thermal desorber, separated, and identified by a Shimadzu QP-2010 plus mass-selective detector (MSD).

The AT-1 GC column (60 m × 0.32 mm × 1.0 µm; Alltech, Portland, ME, USA) was used. The temperature conditions of the oven were divided into four steps. In the first step, the temperature was kept at 35°C for 10 min; in the second step, it was increased to 120°C at a rate of 8°C/min and then maintained for 10 min. In the third step, it was increased up to 180°C at a rate of 12°C/min and maintained for 7 min. Finally, it was increased to 230°C at a rate of 15°C/min and then maintained for 10 min. The temperature of the GC interface was kept at 230°C. The carrier gas was helium of 99.9999% purity. The MSD temperature was 250°C. The mass range was set at 20 to 350 m/z. The ionization voltage was 70 eV. Carrier gas was helium of 99.9999% purity. The other analysis conditions were the same as for the GC.

The compounds for each peak in the GC-MSD total ionization chromatogram (TIC) were identified by comparing the mass spectrum with the Wiley (Hoboken, NJ, USA) and National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA) mass spectra databases. Then, the Kovats retention indexes were compared. Quantitative analysis of the identified volatile compounds was carried out with the relative ratios, based on the full total peak areas for all the identified compounds.

Results and Discussion

Cold-stored krill

Headspace volatile compounds isolated from krill stored for 0, 2 and 4 days at 5°C after thawing using dynamic headspace analysis and their TICs are shown in Fig. 1. Total amounts of volatile compounds increased during the cold storage periods, and most volatile compounds were detected within 30 min. Interference of volatile compounds occurred at a retention time of 3–7 min, and peaks were increased or decreased. Notably, some peaks in the range of 17 to 22 min of retention time were increased.

Identified headspace volatile compounds from cold-stored krill are shown in Table 1. Among a total of 33 volatile compounds, 10 aldehydes, five alcohols, four ketones, three acids, two furans, seven aromatic compounds, one ester, and one aliphatic compound were identified in the cold-stored krill.

Aldehydes have been reported to be generated by oxidation of polyunsaturated fatty acids (Lee et al., 1997). Levels of 3-methylbutanal, 2-methylbutanal, 2-methylpropanal, and nonanal increased during the storage period. Branched aldehydes such as 3-methylbutanal and 2-methylbutanal are considered to be generated by Strecker degradation of amino acids (Oh et al., 2001). These compounds were increased during the storage period in Alaska pollock and mackerel (Lee,

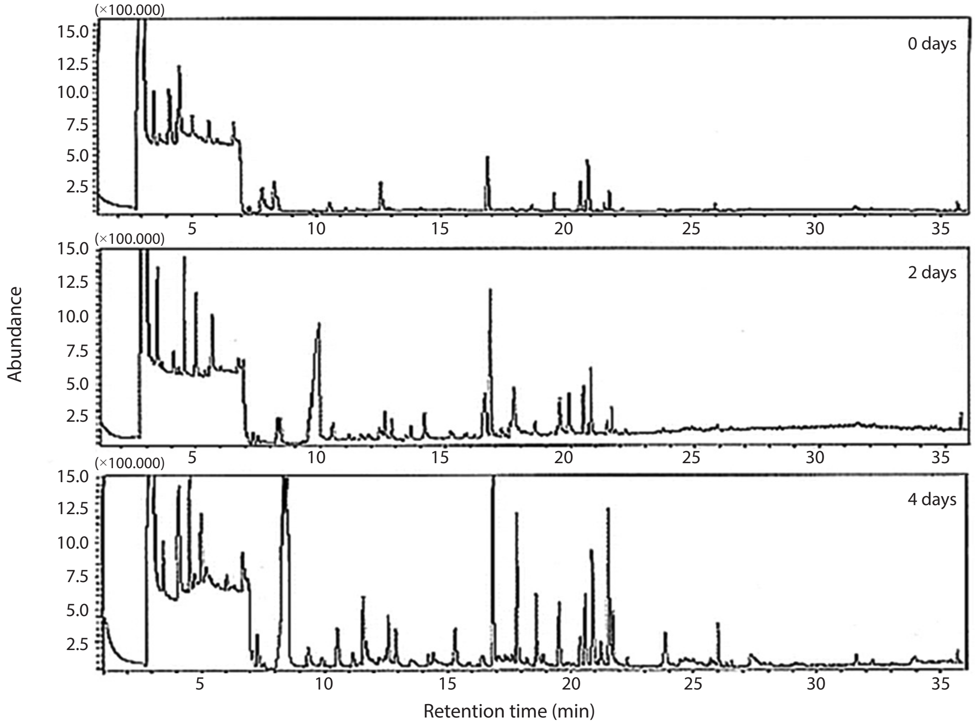

Fig. 1. Total ion chromatograms of headspace volatile compounds isolated from Krill for different storage periods of 0, 2 and 4 days at 5°C.

2011). Notably, 3-methylbutanal was identified in Korean salted and fermented anchovies (Cha, 1992). It can be predicted that 3-methylbutanal affects the odor of krill because its threshold value is very low (0.2 ppb; Lee, 2011). In fact, it was confirmed that 3-methylbutanal is directly related to off-odor according to freshness/degradation during storage (Lee, 2011). However, among C_6-compounds, hexanal has a fresh smell and a good flavor (Lee, 2011), and benzaldehyde is a distinguishing flavor component of steamed crab (Cha, 1992). Octanal and decanal in the stored krill disappeared during the storage period. Notably, benzaldehyde has been reported to be an important flavor component in blue crab meat, steamed crayfish, and salted fish (Cha et al., 2006). Also, benzaldehyde is known to act as a precursor to the formation of heterocyclic compounds, which are considered to be delectable flavors in food (Oh et al., 2001). However, its effect on krill odor was considered to be low because of its high threshold value and low concentration (Ahn et al., 2006).

Among alcohols, 1-penten-3-ol and 1-butanol were identified. 1-Penten-3-ol is known to have a fatty, hay, or grass flavor (Cha et al., 1999) or a rancid odor (Lee et al., 1997). 1-Butanol was detected in small amounts and is known to have a peculiar flavor, pungent and resembling a solvent (Lee, 2011). Generally, alcohols were reported to be decomposition products of secondary peroxides in fatty acids (Lee et al., 1997). Aromatic compounds were abundant among the identified volatile compounds of krill. Their levels were decreased during storage.

Ketones were identified as the second most abundant group among the headspace volatile compounds and were reported to give crustaceans a sweet or fruity flavor (Cha et al., 2006). However, in seafood, they were reported to involve a nasty smell (Lee et al., 2002). Ketones are produced by oxidation or pyrolysis of polyunsaturated fatty acids. Generally, it has been reported that ketones greatly affect the flavor of fresh fish because they have low threshold values (Cha et al., 1999). Acids are classed as wax components, and they produce a soft and mild aroma. The somewhat fishy flavor of the acids decreased during the storage period. Acetic acid was the most abundant acid. Acetic acid is reported to produce an off-flavor or a sour flavor at high temperatures (Lee et al., 2002). Aliphatic compounds, esters, and furans were identified, but only in small amounts.

Freeze-dried krill

Fifty-three headspace volatile compounds identified in freeze-dried krill powder are shown in Table 1. Among these compounds, there were six aldehydes, six alcohols, four aromatic compounds, two esters, five ketones, 18 aliphatic compounds, three sulfur-containing compounds, two pyrazines, and one furan.

Compared with the cold-stored krill, levels of aldehydes,

Table 1. Headspace volatile compounds in krill according to different storage periods (days) at 5°C and freeze-dried krill

Compounds	RT*	RI†	Ratio‡				Rf§
			Storage periods (days)			Freeze-dried	
			0	2	4		
Aldehyde (12)¶							
Acetaldehyde	3.26	<500	-	-	-	1.63	c
2-Methyl propanal	5.19	549	0.54	1.11	2.55	0.32	c
Butanal[T(5)]	6.05	583	-	-	-	0.12	
3-Methyl butanal	8.65	647	3.05	3.50	8.66	0.23	b, c
2-Methyl butanal	8.84	651	0.81	0.63	2.45	-	a
Pentanal[T]	12.91	725	0.56	2.86	1.06	-	
Hexanal	15.95	788	1.41	0.78	0.42	-	b, c
2-Methylene hexanal[T]	20.39	910	0.11	0.04	0.03	-	
Benzaldehyde	21.60	947	0.07	0.15	0.02	0.29	a, c
Octanal	23.01	987	0.48	0.69	0.05	0.27	a
Nonanal	26.68	1084	1.36	1.49	1.50	-	b, c
Undecanal	35.71	1308	2.82	2.12	1.59	-	a
Subtotal			11.21	13.38	18.32	2.86	
Alcohol (10)							
Methanol	3.35	<500	-	-	-	7.76	b
2-Propen-1-ol	5.34	555	-	-	-	0.10	b
1-Propanol[T]	5.73	571	-	-	-	0.16	
2-Methyl-1-propanol[T]	7.97	634	9.84	7.40	9.42	-	
1-Butanol	10.25	676	0.01	0.01	0.04	-	c
1-penten-3-ol[T]	12.58	717	6.12	5.58	7.23	3.74	
2-ethoxy-ethanol[T]	14.42	758	0.02	0.02	0.01	-	
1-Pentanol[T]	15.04	770	-	-	-	16.84	
(R,R)-(+)-2,4-Dimethyl-1-heptanol[T]	21.14	933	-	-	-	0.16	
2-ethyl-1-hexanol[T]	27.33	1100	0.05	0.06	0.03	-	
Subtotal			16.04	13.07	16.73	28.76	
Furan (3)							
2,5-Dihydrofuran[T]	5.51	562	-	-	-	0.34	
Furfural	17.36	823	0.09	0.09	0.15	-	c
2-Pentyl furfural[T]	25.67	1059	0.03	0.03	0.04	-	
Subtotal			0.12	0.12	0.18	0.34	
Aromatic compound (8)							
Benzene	9.27	659	-	-	-	1.54	b
Methyl benzene	14.90	768	9.06	10.61	9.97	-	b
Ethyl benzene	18.76	864	4.76	3.62	3.21	0.29	b
Styrene	19.74	891	1.38	1.11	1.35	0.42	a
1,2-Dimethyl benzene	19.03	872	3.24	2.48	2.59	0.36	b
1,3-Dimethyl benzene[T]	19.89	894	10.39	7.66	7.32	-	
1,2,3-Trimethyl benzene[T]	25.99	1067	1.48	0.86	0.73	-	
Benzothiazole[T]	36.49	1339	6.92	6.23	5.21	-	
Subtotal			37.23	32.56	30.38	2.61	
Acid(3)							
Acetic acid[T]	8.34	641	7.00	11.38	4.78	-	
2-Methyl propanoic acid	15.60	781	0.01	0.02	0.06	-	b
Benzoic acid[T]	31.81	1192	0.06	0.05	0.04	-	
Subtotal			7.07	11.45	4.88	0.00	
Ester (3)							
Acetic acid, ethyl ester	7.25	618	-	-	-	4.47	a, b
2-Propenoic acid, 2-methyl-, ethenyl ester[T]	14.09	751	-	-	-	1.00	
Acetic acid, butyl ester[T]	18.61	860	1.19	1.16	1.34	-	
Subtotal			1.19	1.16	1.34	5.47	

Table 1. Continued

Compounds	RT*	RI†	Ratio‡				Rf§
			Storage periods (days)			Freeze-dried	
			0	2	4		
Ketone (8)							
2-Propanone	4.53	519	19.81	25.80	17.13	-	c
1-(Acetyloxy)-2-propanone^T	5.90	578	-	-	-	0.28	
2-Butanone	6.37	595	1.74	1.53	8.64	1.55	c
2-Pentanone^T	11.04	688	-	-	-	0.69	
2-Hexanone	15.32	776	0.70	-	0.29	-	c
4-Octen-3-one^T	17.16	817	-	-	-	0.81	
Cyclohexanone^T	21.22	936	0.01	-	0.03	-	
6-Methyl-5-hepten-2-one^T	22.63	977	-	-	-	0.43	
Subtotal			22.26	27.34	26.10	3.76	
Aliphatic compound (19)							
n-Hexane	6.52	600	-	-	-	4.86	a
Methyl cyclopentane^T	9.90	670	4.88	0.91	2.07	-	
n-Heptane	11.87	700	-	-	-	0.75	a
1-Pentene^T	14.20	753	-	-	-	0.79	
Hex-3-ene^T	14.45	759	-	-	-	0.68	
2,5-Dimethyl hexane^T	15.23	774	-	-	-	0.28	
3-Methyl heptane^T	15.58	781	-	-	-	0.26	
1-Octene^T	16.22	793	-	-	-	0.87	
Octane	16.61	800	-	-	-	1.03	a
Cycloheptane	16.83	807	-	-	-	0.11	b
(Z,Z)-3,5-Octadiene^T	17.39	824	-	-	-	0.29	
2,2,4,6,6-Pentamethyl heptane^T	19.23	877	-	-	-	0.17	
Nonane	20.07	900	-	-	-	0.21	a
1-(Hexyloxy)-3-methyl hexane^T	21.08	931	-	-	-	0.08	
3-Ethyl-1-octene^T	21.56	946	-	-	-	0.15	
5-Methyl nonane	22.13	962	-	-	-	0.69	b
2,2,3-Trimethyl decane^T	22.56	975	-	-	-	0.26	
Undecane	27.33	1100	-	-	-	0.38	a
5-butyl nonane^T	27.56	1105	-	-	-	0.30	
Subtotal			4.88	0.91	2.07	12.15	
Sulfur containing compound (3)							
Dimethyl sulfide	4.50	517	-	-	-	20.71	a
Dimethyl disulfide	13.70	743	-	-	-	1.35	c
1-Propanethiol^T	20.67	919	-	-	-	0.22	
Subtotal			0.00	0.00	0.00	22.28	
Pyrazine(2)							
4-Vinyl-tetrahydro-4-pyranol^T	19.73	891	-	-	-	0.41	
2,5-Dimethyl pyrazine	20.80	923	-	-	-	0.42	a
Subtotal			0.00	0.00	0.00	0.83	
Others (6)							
Acetonitrile	4.01	<500	-	-	-	19.10	b
Dimethyl amine^T	5.07	544	-	-	-	0.03	
N,N-Dimethyl acetamide	17.16	817	-	-	-	0.88	b
N,N-Dimethyl formamide^T	20.60	917	-	-	-	0.17	
α-Pinene	21.38	940	-	-	-	0.14	a
Copaene^T	40.47	1481	-	-	-	0.61	
Subtotal			0.00	0.00	0.00	20.94	
Total			100.00	100.00	100.00	100.00	

"-" indicates 'not detected'; the superscript of "T" of the identified compounds means tentatively identified compound by matching mass spectrum data of sample with reference one; the other compounds are positively identified by using MS and RI.

*,† RT and RI stand for retention time and Kovat retention index, respectively.

‡ Identified volatile components are showed in relative ratio thereby total peak area is 100 percent.

§ Rf represents references; a is http://flavornet.org/, b is http://pherobase.com/, c is a thesis (Makkhen K, 2012).

¶ Numbers in the parenthesis represent the number of volatile compounds in each class.

aromatic compounds, ketones and acids were decreased in freeze-dried krill powder. Compounds with low boiling points are thought to be volatilized by the freeze-drying process, so levels of nasty compounds were rapidly decreased by heat treatment. By contrast, levels of alcohols and aliphatic compounds were rapidly increased. Alcohols have been reported to not affect the flavor of food greatly, unless they are present in large amounts, because of their high threshold values (Lee et al., 1997).

Hydrocarbon compounds such as aliphatic compounds also have high threshold values, and are thus not considered to greatly affect krill powder flavor (Oh et al., 2001). Esters are produced by esterification with alcohols and carboxylic acid. Their levels were slightly increased. Ethyl acetate was the ester with the highest content, and it was previously identified in processed fish products (Cha, 1992). Esters of low molecular weight, but not those of high molecular weight, greatly affect the sweet, fruity, and candy-like flavor of salted and fermented anchovies (Cha et al., 2006).

Unlike in cold-stored krill, sulfur-containing compounds such as dimethyl sulfide and dimethyl disulfide that are considered to be important flavor compounds in heated food were identified in freeze-dried krill (Anh, 2005). They have strong sulfur or cooked cabbage flavors in seafood products (Lee et al., 1997). Dimethyl sulfide was the most abundant sulfur-containing compound, and it is thought to be important for the flavor of sweet corn. It is produced by oxidation of methionine to methionine sulfoxide in oxidized lipids. It is reported to give onion, garlic, and cabbage flavors at very low concentrations (Lee, 2011). Dimethyl disulfide was reported to have a decayed onion flavor (Ahn et al., 2006). Sulfur-containing compounds such as dimethyl disulfide are reported to adversely affect food flavor because, at high concentrations, they mask good flavors (Ahn et al., 2006). Also, they are called aroma-active compounds because they have very low odor threshold values. Straight- chain sulfur-containing compounds are known to be produced by pyrolysis of unsaturated fatty acids and sulfur-containing amino acids (Cha et al., 2006). They have been isolated from salted and fermented anchovies (Cha, 1992).

Furans are known to be associated with burnt, sweet, and bitter flavors in processed meat and fish products (Cha, 1992). Pyrazines were reported to have a nutty, roasted, or toasted aroma in most foods (Anh et al., 2006). Their levels were slightly increased by freeze-drying. Pyrazines are generally known to be the important flavor components in crustaceans such as shrimps and crayfish (Ahn et al., 2006). Nitrogen-containing compounds such as pyrazines are considered to have distinguishing flavors because of their sensual characteristics and threshold values (Oh et al., 2001). Pyrazines are produced by Maillard or pyrolysis reactions through Strecker degradation during heating. It was reported that pyrazines levels were increased by high reaction temperatures and high concentrations (Anh, 2005). 2,5-Dimethyl pyrazine was identified in freeze-dried krill. It was previously found in potato chips, roasted peanuts, cocoa beans and baked potatos (Anh, 2005), and in red snow crab concentrate (Ahn et al., 2006). 2,5-Dimethyl pyrazine and dimethyl disulfide are known to contribute to the characteristic flavor of crab (Anh, 2005). Sulfur- or nitrogen- containing heterocyclic compounds were reported to be good flavors in crustaceans such as the red snow crab and in crayfish by-products (Lee et al., 1997).

Changes in volatile compounds in cold-stored and freeze-dried krill

Aldehydes, alcohols, and ketones are volatile compounds whose levels were increased by oxidation during cold storage. Notably, branched aldehydes such as 3-methylbutanal and 2-methylbutanal are volatile compounds whose levels in Alaska pollock and mackerel were increased by cold storage, and they were identified in salted and fermented anchovies. Therefore, these compounds are thought to be directly related to off-flavor according to freshness degradation during storage. By contrast, in freeze-dried krill, levels of aldehydes and ketones were dramatically decreased, while alcohol levels were increased. This is thought to have affected the off-flavor of krill because of the high threshold values for alcohols.

Acknowledgments

This work was supported by a Research Grant of Pukyong National University (year 2013).

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