Oxidation of Polyunsaturated Fatty Acids & Antioxidative Action of Astaxanthin in Krill (*Eupausia superba*)

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Krill (*Eupausia superba*) is harvested with lots of amounts, but it is mainly used as animal feed except few food ingredients. Even though it contains such functional components as polyunsaturated fatty acids of eicosapentaenoic acid and docosahexaenoic acid, taurin, and astaxanthin, the reason that it is not widely applied to food products is thought to be the disadvantages of small sizes, rapid browning and formation of bad smells. The detail study on the bioactive component of krill is expected to more application of krill to food ingredients and products. First of all, poly unsaturated fatty acids of krill and its oxidized and bad smell were studied. Also, the astaxanthin which is color pigment and has antioxidative activity were investigated.

Key words : krill, polyunsaturated fatty acid, EPA, DHA, oxidation, volatile compounds, astaxanthin,

Introduction

Krill (*Eupausia superba*) is called as Antarctic krill and the amount of krill is estimated to be 1 ～ 2 billion tones and the possible annual harvesting amount is thought to be 50～200 million tones[1]. Krill has several kinds of bioactive components which are polyunsaturated fatty acids, taurin, chitin, astaxanthin, and others[2]. First of all, the polyunsaturated fatty acids of EPA and DHA are discussed. While these polyunsaturated fatty acids are nutritionally important, its main problem for food application is its bad smell[3,4]. Also, the oxidation mechanism was investigated and their degradation compounds were measured by dynamic headspace analysis with purge & trap, solid phase microextraction and solvent trapping. Krill was applied to sausage. The amounts of DHA and headspace volatile compounds were analyzed for quality evaluation. Finally, astaxanthin of antioxidative agent was investigated.

Chemical structures and nutritional values of polyunsaturated fatty acids

Fat has a basic structure which combines fatty acids to glycerol backbone. When one, two and three fatty acids are added, the fats are called mono-, di- and tri-acylglycerols. Table 1 shows the main fatty acids which are commonly found in food. The position of double bond from methyl end indicates the letter and numbers of $\omega-3$, $\omega-6$ and $\omega-9$ which are separated to be useful in the aspect of human nutrition[5]. EPA and DHA which contain lots in krill are $\omega-3$ fatty acids. The amounts of DHA were compared with those of other foods as shown in Table 2 (*web data*). Krill ingredient was added to Korean hot pepper soybean sauce (“Gochujang”) and sausage.

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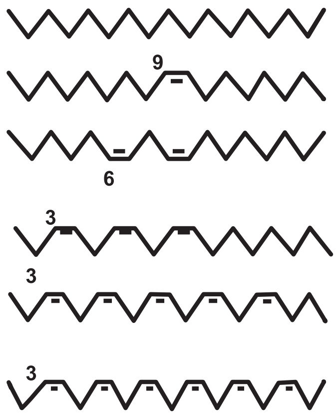
Table 1. Chemical structures of main ω-3, 6, 9 fatty acids



Nomenclature	Methyl-end	Carboxyl-end	Saturation
Stearic 18:0		COOH	Saturate
Oleic 18:1 ω-9		COOH	Monoene
Linoleic 18:2 ω-6		COOH	PUFA
Linolenic 18:3 ω-3		COOH	PUFA
EPA 20:5 ω-3*		COOH	PUFA
DHA 22:6 ω-3**		COOH	PUFA

* EPA = eicosapentaenoic acid
** DHA= docosahexaenoic acid

Table 2. Contents of docosahexaenoic acid (DHA) of foods

Food	DHA (mg)	Food	DHA (mg)
Pink salmon	638	Trout, rainbow, cooked	44
White tuna	535	Tuna, white, packed in water	54
Herring, Pacific, cooked	75	Crab, dungeness, cooked	10
Salmon, chinook, cooked	62	Shrimp, cooked	12
Salmon, Atlantic, cooked	95	Cod, Pacific, cooked	15
Oysters, Pacific, cooked	43	Fish oil, menhaden	9
Salmon, sockeye, cooked	60	Fish oil, salmon	18

Oxidation of polyunsaturated fatty acids

Polyunsaturated fatty acids including EPA and DHA have 5 and 6 double bonds, respectively. The 1,4-diene structures of polyunsaturated fatty acids easily lose hydrogen ion (H^+) which is attached to the 3rd carbon position. The formed fatty acid free radical is easily oxidized with oxygen and the peroxy free fatty acid radicals is degraded to produce the volatile compounds which are related to bad and oxidized smells[6]. The general scheme is shown in Fig. 1. The main volatile compounds have several groups of aldehyde, alcohol and ketone. As mentioned earlier, krill has lots of EPA and DHA, so its headspace volatile compounds which are thought to be related with bad and oxidized smells are analyzed as the followed section.

Headspace volatile compounds of krill during refrigerated storage

Flavor is one of important attributes for food quality. Flavor can be separated to two aspects of taste and aroma. While the kinds of taste expression are a few, the expressions and compounds of aroma are too many to be fully understood[7]. The main instrument for analysis of aroma compounds is gas chromatography. The more important one is the isolation of the volatile compounds which are related

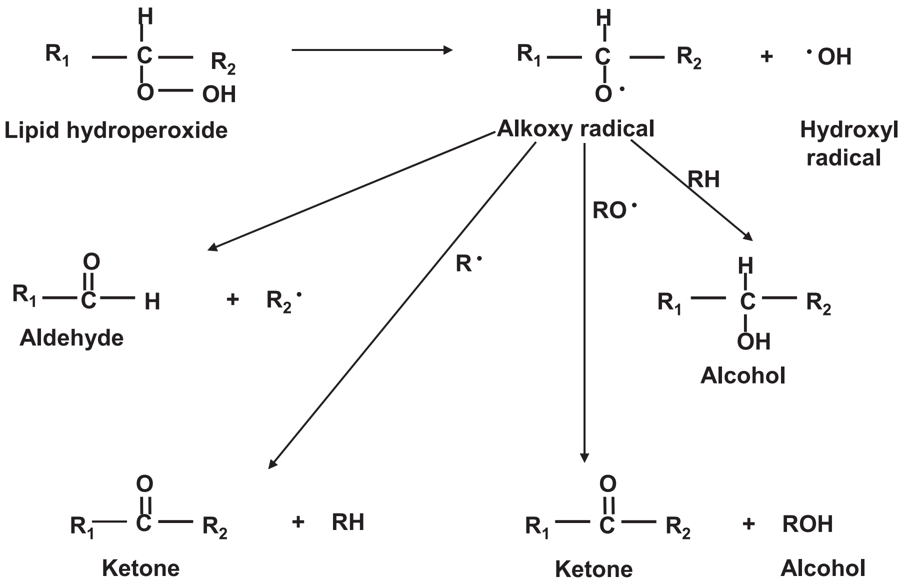


Fig. 1. General scheme of oxidation of fatty acids and the formation of their degradation products.

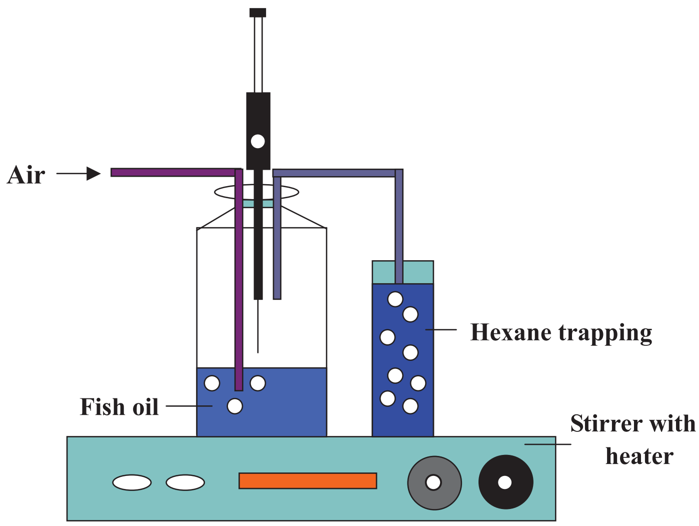


Fig. 2. The newly developed device for isolating headspace volatile compounds of oxidized fish oil with solid phase microextraction and hexane adsorption.

with the aroma. The isolation of the volatile compounds can be separated to two types. They are total volatile analysis and headspace analysis[8]. Total volatile analysis which uses such solvents as diethyl ether, hexane and other organic solvents is simple solvent extraction and simultaneous steam distillation & solvent extraction (SDE) which is used by Lickens-Nickerson Apparatus. The headspace analysis has static headspace isolation, dynamic headspace isolation of purge & trap and solid phase microextraction (SPME, Fig. 2). SPME is newly developed method which uses fibers of 5 kinds. The commonly used method is dynamic headspace method of purge & trap and the common trap is Tenax chemical trap[9]. The headspace volatile compounds during krill storage in a refrigerator were measured and the main volatile compounds are shown in Table 3.

DHA and EPA contents and volatile compounds of krill-added sausage

Krill is applied to sausage[10]. The fatty acids of krill-added sausage were analyzed and the amounts of EPA and DHA were analyzed and their amounts are shown in Fig. 3. The amounts of EPA and DHA of krill-added

Table 3 . Headspace volatile compounds of krill during storage in a refrigerator

	Retention time(min)	Peak area of volatile compounds of krill stored at a refrigerator		
		1 day	2 day	3 day
Aldehyde				
2-methylpropanal	6.02	9.3	28.0	55.7
3-methylbutanal	10.51	52.1	88.4	188.9
2-methylbutanal	11.17	13.9	15.9	53.4
pentanal	12.90	9.5	72.1	23.2
hexanal	17.84	24.1	19.7	9.2
furfural	18.80	1.6	2.2	3.2
2-methylhexanal	20.39	1.8	1.1	0.6
benzaldehyde	23.84	1.2	3.7	0.4
octanal	25.80	8.2	17.5	1.0
nonanal	31.59	23.2	32.7	37.7
decanal	35.71	48.2	53.6	34.6
Sum of total aldehyde		**193.1**	**334.9**	**407.9**
Alcohol				
2-methyl-1-propanol	9.31	2.1	3.2	3.3
1-butanol	11.55	0.2	0.2	0.8
1-penten-3-ol	12.58	104.6	140.9	157.8
2-ethyl-1-hexanol	28.32	0.8	1.5	0.7
Sum of total alcohol		**107.7**	**145.8**	**162.6**
Acids				
acetic acid	18.61	20.4	29.2	29.2
benzoic acid	33.96	1.1	1.3	0.8
Sum of total acid		**21.5**	**30.5**	**30.0**
Ketones				
2-butanone	7.27	29.7	38.7	186.6
2-hexanone	15.23	15.0	0.0	6.4
Sum of total ketone		**44.7**	**38.7**	**193.0**
Sum of total compounds		**367.0**	**549.9**	**793.5**

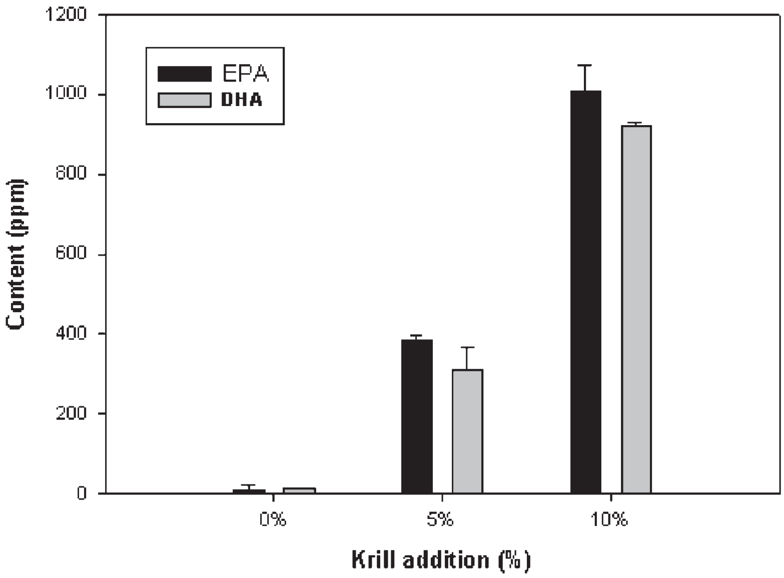


Fig. 3. Contents of EPA and DHA of 0 %, 5 % and 20% krill-added sausage.

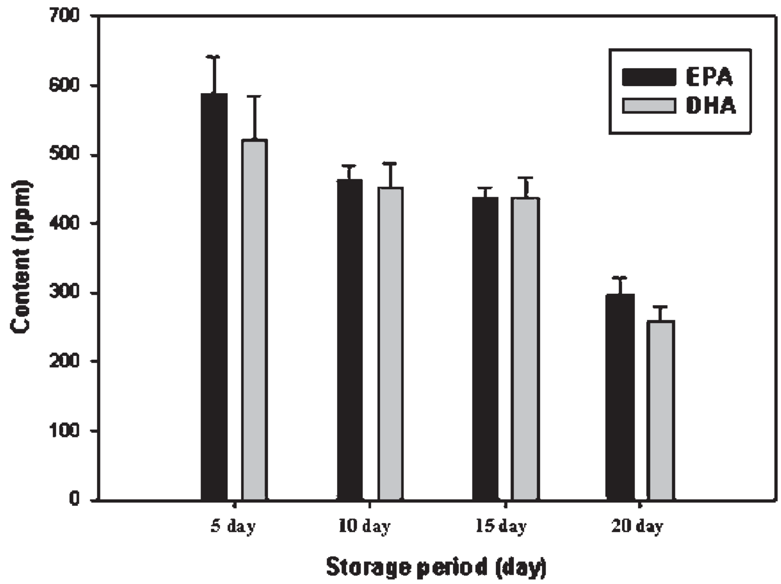


Fig. 4. Changes of EPA and DHA during storage of 10% krill-added sausages at a refrigerator.

sausages were increased as the added amounts of krill increased. Also, the changes of EPA and DHA, and headspace volatile compounds of krill-added sausages during storage at a refrigerator were measured and their results are shown in Fig. 4 and Table 4, respectively. The amounts of EPA and DHA were still remained up to about 70% for 15 day storage in a refrigerator. The decreased EPA and DHA were thought to produce a few oxidized volatile compounds. The reason was thought that the spices which were added during sausage processing might act as antioxidants and the direct contact of oxygen to DHA and EPA might be reduced. Krill itself may protect EPA and DHA with its astaxanthin from oxidation. Next section will discuss the chemical structure and distribution of astaxanthin.

Chemical structures and nutritional values of astaxanthin

Astaxanthin gives its distinctive red colour to krill, shrimps, salmon, crawfish and crabs and its amount of natural foods was shown in Table 5. Astaxanthin is a

Table 4 . Changes of headspace volatile compounds of krill-sausages during storage at a refrigerator

Compound name	RT (min)	Storage period (day)			
		5	10	15	20
acetaldehyde	3.46	3.6	5.8	3.9	3.1
ethanol	4.08	3.6	5.2	6.8	5.8
1-propanol	6.17	-1)	-	4.0	-
methyl propyl disulfide	23.23	3.3	10.9	3.0	11.4
benzaldehyde	23.63	8.6	12.7	11.8	78.4
camphene	24.23	39.4	46	71.9	32.0
beta-phellandrene	25.09	12.0	27.8	17.4	32.0
beta-pinene	25.42	13.7	32.6	32.0	46.0
beta-myrcene	25.57	26.0	49.3	47.8	17.0
alpha-phellandrene	26.53	10.2	18.3	16.3	5.8
3-carene	26.96	4.9	8.3	5.7	14.9
6-methyl alpha-ionone	27.14	6.6	13.4	14.6	10.2
hexyl t-butyl ether	27.29	9.8	13.3	10.8	11.1
D-limonen	27.83	11.7	12.7	11.0	83.9
2-acetyl-cycloheptanone	29.36	50.0	101.1	80.3	20.5
beta-terpinol	29.78	16.9	26.7	22.0	8.4
3,4-dimethylanisole	31.32	7.6	5.3	8.0	11.8
eucalyptol	31.66	15.4	10.8	10.1	4.1
dipropyl disulfide	31.97	7.4	6.3	4.0	8.5
4-methyl-1,3-cyclohexen-1-ol	35.01	3.3	10.9	6.5	12.8
decanal	35.21	-	-	11.8	4.4
caryophyllene	45.18	3.9	7.6	5.4	6.4

carotenoid consisting of 18 carbon polyene chain with a ring at each end. The isoprene unit forms its basic structure as shown in the below Fig. 5. Astaxanthin is called as 3,3'-dihydroxy-4,4'-diketo-β-carotene. Compounds which have a structure similar to astaxanthin are β-carotene, rutein, and violaxatnin. As shown in Fig. 5, astaxanthin has two rings which have R-S isomers. The OH groups are decided to R-S isomers, so there are three different isomers[11]. The main distribution of these three isomers are S,S'-isomer, but the differences of antioxidative activities and nutritional values between these compounds are thought to be significantly different. Also, the main structure in natural foods is S-S isomers. Astaxanthin has free form and the mono- or di-esterified forms with fatty acids. RS isomer composition of esterified and unesterified astaxanthin of *Pleuroncodes planipes* were studied by Coral-Hinostroza & Bjerkeng (2002)[12]. The main optical form was 3S, 3'S isomer, followed by 3R, 3'R and 3R, 3'S isomers. The amounts of 3R, 3'R were close to those of 3S, 3'S isomer and the amounts of 3R, 3'S isomers were much less with the approximate range of 1/4-1/8. Astaxanthin and crude lipid extract were isolated from Langostilla meal[13]. The range of fatty acids was C12 to C22 for saturated fatty acids and C16 to C20 for unsaturated fatty acids. The ratios of $(\omega-3)/(\omega-6)$ are very high in the range of 5.9 to 58.7.

Conclusion

Oxidized products of polyunsaturated fatty acids in krill were successfully identified by using the combined instruments of purge & trap, gas chromatography and mass selective detector for isolating, separating and identifying the headspace volatile compounds during storage. Also, the oxidation mechanism of polyunsaturated fatty acids in krill and changes of the volatile compounds during storage of krill-added sausages were investigated. A developed processing technology for reducing oxidized compounds in krill is required. Also, further study for using astaxanthin as a natural antioxidant is needed.

Acknowledgement

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Table 5. Content of astaxanthin of natural foods

Kind	Content (mg/kg)	Kind	Content (mg/kg)
Black tiger prawn	10-150	Pink salmon	4-6
Sockeye salmon	26-37	Chum salmon	3-8
Coho salmon	9-21	Red seabream	2-14
Chinook salmon	8-9	Salmon eggs	0-14
Atlantic salmon	3-11	Rainbow trout	1-3

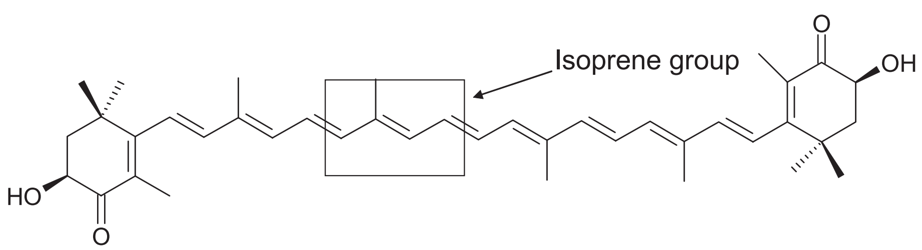

Fig. 5. Chemical structure of astaxanthin.

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