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Cholesterol Content and Formation of Oxidized Cholesterols in Processed Squids

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Abstract

The effect of cooking(boiling, steaming and baking) and drying on the cholesterol content and formation of oxidized cholesterols in squid(Japanese flying squid, *Todarodes pacificus*) was studied. Cholesterol content of live squid meat varied with the portion sampled, and results from spectrophotometric assay ranged from 263.2mg/100g(mantle) to 315.8mg/100g(tentacle). The cholesterol levels analyzed by gas chromatography(GC) for squid samples were lower by 7% of total cholesterol for live squid meat and 24% for processed meat than those results by spectrophotometric assay. Cooking resulted in the decrease of the initial total cholesterol content of raw meat from 10%(boiling for 5min.) to 25%(steaming for 5min.). The amounts of cholesterol remaining after baking were 68% for microwave oven samples and 64% for convection oven samples. Drying of raw tissue caused the greater reduction in cholesterol content than cooking but showed no significant difference in samples stored for 6 weeks at 4°C and 20°C. Raw squid meats contained essentially no oxidized cholesterols, while the 22-hydroxychoesterol was detected in frozen meats. The additional oxidized cholesterols as cholestane-triol was indentified with 22-hydroxycholesterol in cooked samples. Sun dried meat stored at 4°C and 20°C for 6 weeks had the three kinds of oxidized cholesterols such as 22-hydroxycholesterol, cholesta-3,5-dien-7-one and cholestane-triol.

Key words: squid, cholesterol, cooking and drying, oxidized cholesterol

INTRODUCTION

In Korea where squid are widely sold either raw or dried form traditionally, most of consumers have recently been sensitized to the importance of diet in maintaining a healthy lifestyle and regard squid as a representative of high cholesterol seafood. Not only is concerning for cardiovascular disease, but trend of preference for low calorie food also added to the lower squid consumption than past decades. The confusion from therapeutic value of fish oil and high-cholesterol seafood diet has rendered a need for better information about the "true" cholesterol value for squid, or an explanation for its changes during processing.

A wide range of cholesterol value can be expected for marine animals which consume plants, such as the vivalves, depending on the methodology used for the measurement of sterols. Some methods do not distinguish among the various sterols that can be present, but report total sterols as total cholesterol regardless of the inclusion of plant sterols(1). Before the advent of gas chromatographic methods, cholesterol determined by a precipitation method which worked well for finfish and those shellfish such as lobsters and some crabs, because almost 100% of their sterols are present as cholesterol(2). Therefore, squid cholesterol data reported using precipitation method are not capable for dietary recommendation for the ingestion of cholesterol. Along with "true" cholesterol value for squid, the effect of heat on the cholesterol content of squid and what happens to the tissue cholesterol of squid after cooking, drying and preservation also attract our concern. Although the Korean people have commonly consumed squid as dried and roasted products for many years, little information is available on the occurrence of cholesterol oxidation products in these food items.

In an attempt to provide nutritional data for dietary planning, true cholesterol value for squid and their changes during cooking and processing were determined using gas chromatographic method compared with precipitation method. In this study, we checked the oxidized cholesterol products in squid meats on the

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frozen, cooked, dried and stored products.

MATERIALS AND METHODS

Sample preparation

All Japanese flying squids(*Todarodes pacificus*, mantle length 29±4cm, weight 145±14g) were freshly caught from Young-Il Bay, Pohang, Korea on October 1994 and shipped frozen at -20°C. They were analyzed within 2 month of capture. Frozen retail pack comprising 30~33 squids was thawed in 4°C refriegerator for 19 hours. The thawed samples were cleaned(skin, tentacles and viscera removed) and cut open along the dorsal surface.

Cooking was done by heating the mantles and tentacles in distilled water(1 : 10 squid to water, w/w) at 100°C for 1, 2.5 and 5 minutes. The surface water was wipped off lightly with filter paper and then ground in a tissue grinder(Food Mixer, FIC-100A). Steamed samples were prepared in steam cooker for 1, 2.5 and 5 minuted using same mantles and tentacles as described in boiled samples. Prior to microwave cooking (2,450 MHz. 1~5min.), samples were cut into small pieces (1cm×1cm, mantle) and a rod shape(1cm in length, tentacle). Oven cooking was run in convection oven(24-5-TKX) with 100g of same squid samples as microwave samples at 160°C for 5, 15 and 25 minutes.

Sun-dried samples were prepared outdoors every day at 20±3°C for 6 hours during 3-days processing. Samples were removed from drying hangers and kept at 4°C in air-sealed polyethylene bags to homogenize for moisture profiles. The final sun-dried squid measured about 65% moisture. Indoor drying was done in dark and well air-condtioned laboratory at 25°C for 4 days. The moisture content of those squids was about 50%. Both dried samples were packed in polyethylene bags and kept in 4 and 20°C incubators for 6 weeks.

Colorimetric procedure for cholesterol content

Quantitation of cholesterol contents of squid sample was employed the procedure included extraction of total lipid and reaction of nonsaponifiable fraction with a color-developing reagents introduced by Ichida(3) with a slight modification. Each ground squid sample (50g for cooked sample and 12g for dried sample) was mixed with 75ml of ethanol, warmed in a 35~50°C water bath for 30min., and filtered firstly. Second lipid extraction was done with solid residue in 50ml of ethanol-ethyl ether(3 : 1, v/v) at 35~50°C for 30min. again. The extract from sample was made up to 100ml with ethanol. 1ml of those made up exract was quantitated for cholesterol using 5ml of color reagent of sulfosalicylic acid solution(50g sulfosalycylic acid/1L acetic acid)-anhydroacetic acid-H_2SO_4(35 : 65 : 10, v/v/v). The absorbance of colored mixture was read at 625nm against the blank.

GC assay for cholesterol and its oxidized derivatives

Prior to quantitate cholesterol and its oxidized derivatives from squid samples, 1g of extracted lipid was saponified by 25ml of 1N KOH in methanol at 85°C for 1 hour(4), and the unsaponifiables were extracted three times, each time with 20ml of isopropyl ether. Pooled isopropyl ether extracts were washed twice with 10ml of hot distilled water, followed by drying with anhydrous sodium sulfate. The extracts were concentrated in a rotary vacuum evaporator and dissolved in 4ml of ethylacetate at -10°C. 230μl of stored unsaponifiables was freed of solvent on a nitrogen flash evaporator. The dried extracts of nonsaponifiables were redissolved into 250μl of pyridine-Sylon BTZ mixture (5 : 1, v/v). About 1μl of reaction mixture was applied to capillary gas chromatography (GC) at the conditions summarized in Table 1. 4% of 5α-cholestane in pyridine was used as an internal standard and those derivatization was run at room temperature for at least 10 minutes. Quantitation of cholesterol and identification of individual oxidized cholesterol were carried out by comparison with GC-MS spectra(Hewlett-Packard MS-59 827A) and the retention time of the authentic standards as follows; cholesterol(Sigma), 7α-hydroxycholesterol (Steraloids), 7β-hydroxycholesterol(Sigma), 5α-epoxycholesterol(Sigma), 5β-epoxycholesterol(Steraloids) and 7-ketocholesterol(Sigma).

RESULTS AND DISCUSSION

Total cholesterol contents of squid products

Mean cholesterol values of squid products obtained by colorimetric and gas chromatographic(GC) procedures are presented in Table 2. Cholesterol content of live squid ranged from 263.2mg/100g meat with mantle and 315.8mg/100g with tentacle, and those results were not beyond the range of cholesterol content from earlier

Table 1. Operation condition of gas-chromatography(GC) and mass-spectrum(MS) for analysing the cholesterol and its oxidized derivatives in squid

Item	Operation condition	
	GC	GC-MS
Instrument	Shimadzu GC-14B	Hewlett-Packard GC-MS (GC-5890 series-2, MS : 59827A)
Column	Capillary column (SE-30 fused silicacapillary column, 30m×2.5μm, Supelco)	Capillary column (HP-5 fused silicacapillary column, 50m×0.2mm, Hewlett-Packard)
Carrier gas	Gas of Nitrogen 2.5ml/min (15psig=100KPa)	
Oven temperation profile		
Initial temperature	190°C	280°C
Initial hold time	2min	
Final temperature	280°C	280°C
Final hold time	21min	15min
Program rate	10°C/min	
Total run time		70min
Injector temperature		270°C
Detector temperature		280°C
Spilit ratio		15 : 1
Mass range		29-500m/e

Table 2. Cholesterol content in various squid samples determined by colorimetric and gas chromatographic(GC) procedures (mg/100g sample(mg/100g soild))

	Mantle		Tentacles	
	Colorimetric	GC	Colorimetric	C
Live[1)]	263.2(1174.9)		315.8(1619.4)	
Frozen[2)]	181.8(883.9)		227.2(1183.8)	292.4(1499.7)
Thawed[3)]	269.2(1244.4)	243.22(1150.4)	315.8(1619.4)	
Dried[4)]				
Indoor dried	526 1(1015.1)	464.9 (897.4)	651.1(1562.5)	563.7(1211.9)
Sun-dried	384.1(1035.2)	348.7 (985.5)	598.5(1484.2)	648.5(1284.7)

1)Same species of live squid sample were obtained from local live-seafood market to compare with the frozen squids on the fishing boat

2)Frozen live squids stored at -40°C for 3 days

3)Thawed at 4°C for 19 hours using shipped frozen(-20°C) squid retail pack

4)Dried with the thawed squids from shipped frozen retail pack

reports(5-7). Shimma et al.(5) reported cholesterol value for edible portion of *Loligo* spp. for 350mg/100g, and Krzynowek et al.(7) reported *Illex spp.*(108~315 mg/100g) contained significantly less($p \leq 0.01$) cholesterol than *Loligo* spp.(171~449mg/100g) regardless of portion sampled.

Our squid(Japanese flying squid, *Todarodes pacificus*) mantle samples had significantly less cholesterol than tentacles using both cholesterol assays as described in the other report(7). The gas chromatographic(GC) data resulted in 7% lower in total cholesterol value with live squid and 24% lower with processed squids than those values by colorimetric assay. A similar trend in cholesterol content from procedure variation could be found in the previous report(8) that cholesterol values could be overestimated by colorimetric assay procedure not employing saponification with antioxidant protection compared with the values obtained by GC procedure. Those discrepancy might be expected that colorimetric method did not distinguish true cholesterol among the various sterols present including plant sterols, and the value reported for cholesterrol could be erroneously high(7,9,10). The large range of cholesterol values found for squid could be derived from species and seasonal variation, since cholesterol is necessary for the production of hormones, perhaps there is a great physiological demand for cholesterol during certain periods of their rapid maturation process(7).

The amount of cholesterol in processed squid meats would be influenced by total weight losses and drippings during drying and thawing. The present data demonstrated that frozen storage caused a decrease, while thawing resulted in either no change(GC) or an increase(colorimetric). In general, frozen storage and thawing resulted an increase in those cases where a change occurred. The reason for these changes must be sought in the alterations occurring in protein-cholesterol complexes of tissues after freezing(11). Our different results in changing cholesterol content after freezing might be due to the severe frozen denaturation (-40°C) of proteins thereby refolding the more protein-cholesterol complexes compared with the other reports (12). On the other hand, there was some decrease in cholesterol of both dried products as solid bases. A similar trend in cholesterol content of dried squid was found in the study of Osada et al.(13). They reported that dried squid had a 36% lower cholesterol content than that of raw sample. Those were suspected in the complexing of oxidized cholesterol products during drying.

Effect of cooking on cholesterol content

The effect of heating condition on cholesterol level was evaluated on boiled and steamed squids(mantle and tentacles)(Fig. 1). Eventhough there was not consistent trend in decreasing cholesterol content determined by colorimetric procedure, a decrease in cholesterol level of both boiled and steamed squid mantle could be observed with heating time by GC procedure. However, cholesterol content of all cooked(boiled and steamed) tentacles decreased rapidly in 1 minute and changed little thereafter. On the other hand as wet bases, boiled samples(5min.) had a 16% lower cholesterol content(25.3mg%) with tentacles and 18% lower (26.3mg%) than raw. Similar results were revealed in steamed sample(5min.), and most of steamed samples contained less cholesterol content than boiled samples processed for same heating time. Osada et al.(13) also observed a 56% lower cholesterol decrease in canned squid. Those results indicated that steaming could lead more moisture loss in raw samples and forming protein-lipid complex including cholesterol due to its high heat penetration effect than boiling(11). Also, both baking (microwave and convection oven) caused in a decreased cholesterol level compared with boiled or steamed samples, and those baked squids had a lower cholesterol content than steamed squids(Fig. 2). Those reducing cholesterol level in convection oven baked samples than microwave oven baked samples might be accounted for more cholesterol loss in drip and decomposition of cholesterol(14). This is in accord with the findings of Prusa and Hubhes.(15) for cholesterol content in pork tenderloin steaks heating by convection and microwave oven. Fig. 3 and 4 indicated the changes in cholesterol content in dried squids during storage at 4°C and 20°C.

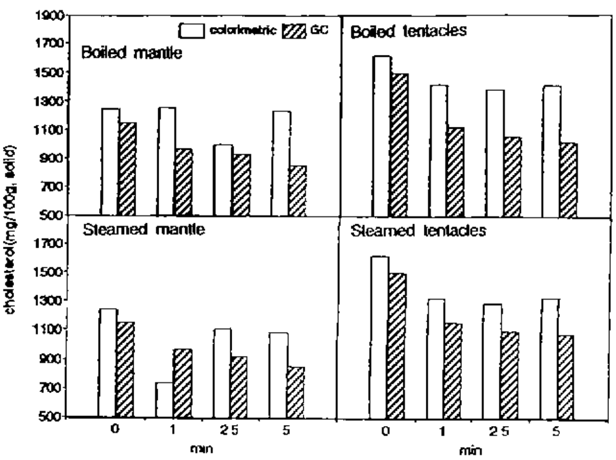

Fig. 1. Effect of cooking on the total cholesterol content of squids as soild bases.

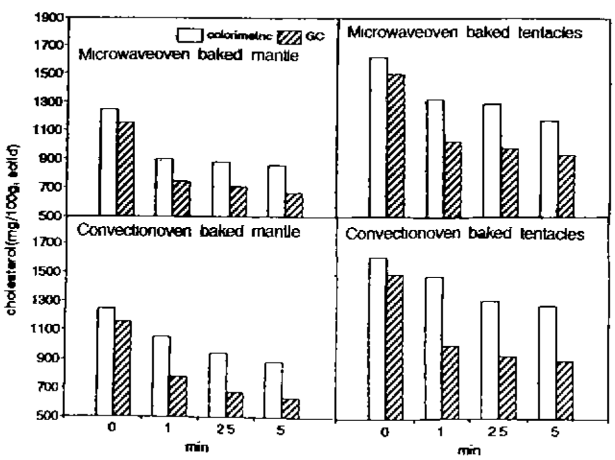

Fig. 2. Changes in cholesterol content of squids during baking.

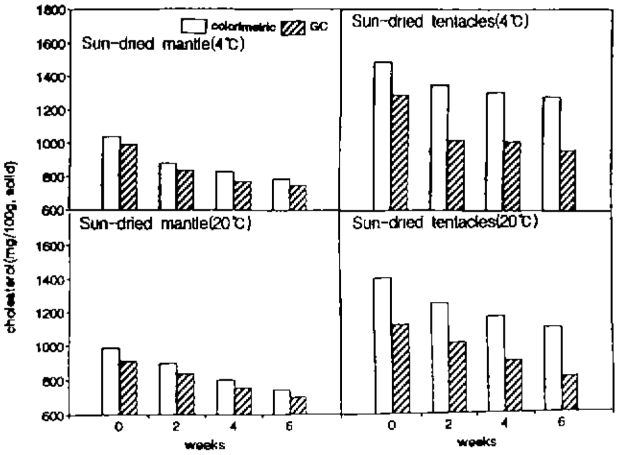

Fig. 3. Cholesterol content of sun-dried two squid samples during storage at 4°C and 20°C.

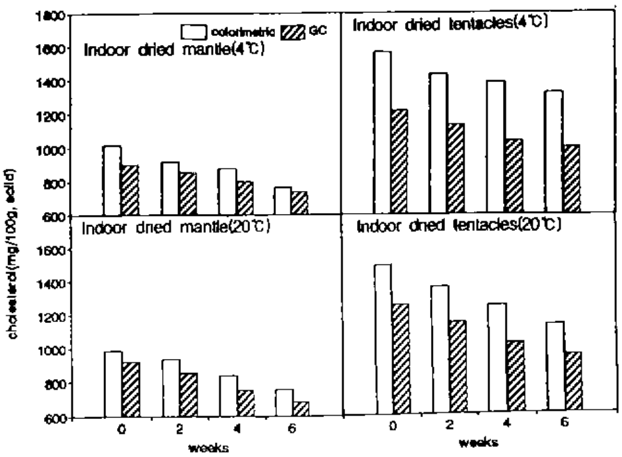

Fig. 4. Cholesterol content of indoor dried squid samples during storage at 4°C and 20°C.

As shown in those figures, the cholesterol content was reduced steadily with storage period, and sun-dried and stored samples at relatively high temperature(20°C) showed a lower cholesterol level than indoor dried and stored samples at low temperature(4°C). This suggested that some of the cholesterol might have been oxidized effectively and produced cholesterol oxidized products (COPs) due to longer exposure to air, as mentioned by Osada et al.(13).

Cholesterol oxidized products(COPs) in squid

In general, oxidized derivatives of cholestero(oxidized cholesterols) exert a wide range of biological activities such as disturbance of arachidonic acid metabolism(16), inhibition of cholesterol synthesis(17), carcinogenesis(18) and cytotoxicity(19). But the available information regarding oxidized cholesterol in high cholesterol seafoods such as shrimps and squids is still limited, and the effects of processing on cholesterol oxidation are almost unknown. However, the present study was initiated to investigate whether cholesterol in squid will undergo oxidation when it is frozen, dried and heated, and concerned appearance of oxidized products after processing. Findings were confirmed by mass spectra of trimethylsilyl(TMS) derivatives of synthetic cholesterol and COPs(Fig. 5) and listed those COPs in Table 3. While no oxidized cholesterol was detected in live squid, 22-hydroxycholesterol could indentified

Table 3. Detected cholesterol oxidized products(COPs) in the various squid products

Sample	Cholesterol oxidized products(COPs)			
Live squid[1)]	Cholesterol			
Frozen squid[2)]	Cholesterol	22-hydroxycholesterol		
Boiled squid[3)]	Cholesterol	22-hydroxycholesterol	Dholestane-triol	
Steamed squid[4)]	Cholesterol	22-hydroxycholesterol	Dholestane-triol	
Baked				
Convection oven[5)]	Cholesterol	22-hydroxycholesterol	Dholestane-triol	
Microwave oven[6)]	Cholesterol	22-hydroxycholesterol	Dholestane-triol	
Dried[7)]				
14 days	Cholesterol	22-hydroxycholesterol	Cholest-3,5-dien-7-one	Cholestane-triol
28 days	Cholesterol	22-hydroxycholesterol	Cholest-3,5-dien-7-one	Cholestane-triol
42 days	Cholesterol	22-hydroxycholesterol	Cholest-3,5-dien-7-one	Cholestane-triol

1)Some species of live squid sample were obtained from local live-seafood market to compare with the frozen squids on the fishing boat
2)Frozen live squids stroed at -40°C for 3 days
3)Boiled at 100oC in water for 1min, 2.5min and 5min
4)Steamed at 100oC for 1min, 2.5min and 5min
5)Baked in convection oven at 160°C(320°F) for 5min, 15min, 25min
6)Baked microwave oven at 2450Mhz for 1min, 2.5min, 5min
7)Sun-dried squid were stored at 4°C and 20°C

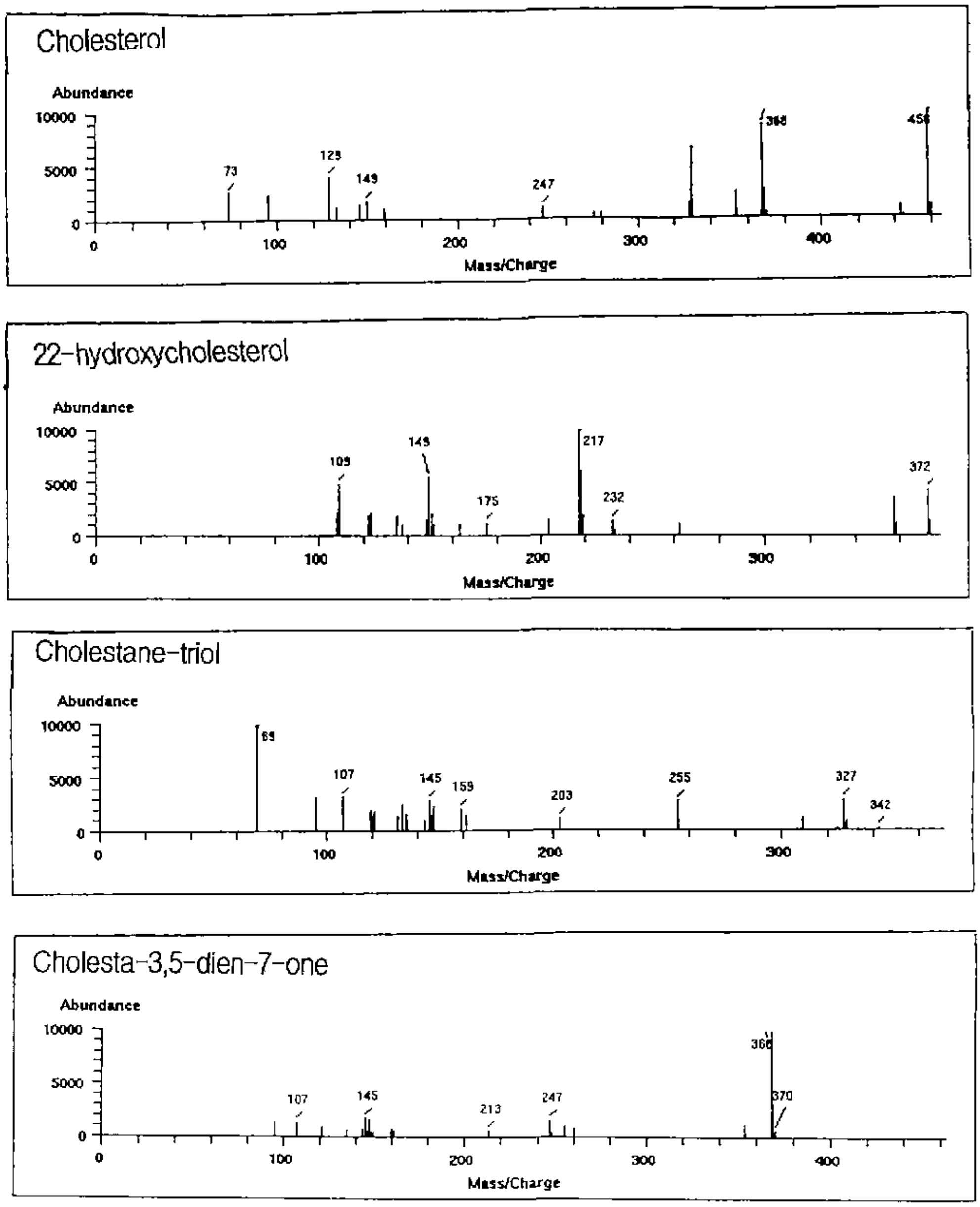

Fig. 5. Mass spectra of trimethylsilyl(TMS) derivatives of synthetic cholesterol and compound isolated from sampled squid.

in frozen products. The other oxidized cholesterol as cholestane-triol was identified with 22-hydroxycholesterol in boiled, steamed and baked squids. When squids were dried and then stored long time, additional COP like cholest-3,5-diene-7-one was produced. Osada et al.(13) reported that dried squid contained 11.0mg/100g of sample of oxidized cholesterol. Among the oxidized cholesterols detected, 7β-hydroxy-cholesterol, 5β-epoxy-cholesterol, 5α-epoxy-cholesterol, 7-keto-cholesterol and unknown oxidized cholesterols were indentified. As shown in ours and result of Osada et al.(13), results for COPs found in squid may not be reliable due to variations in methodology such as hot vs. cold saponification, choice of analytical tools(20) and processing condition. Researchers suggested that of the common COPs, cholest-3,5-diene-7-one is artifactually derive from 7-ketocholesterol, and formation of cholestane-triol results from degradation of epimeric epoxide during the analytical procedure(21). On the other hand, cholesterol was heated with unsaturated fat, cholestrol was readily decomposed even in 1 hour of heating. Such results reflect the instability of unsaturated fatty acids in squid accelerate oxidation of cholesterol at high temperature. However, if the squid had been harsh condition capable of inducing dehydration(sun-drying), cholesterol oxidation increased.

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