Cholesterol Oxidation in Some Processed Fish Products

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ABSTRACT: Numerous foods of animal origin are reported to contain considerable levels of cholesterol oxidation products (COP); however, very few reports are available on fish products. Levels of COP were assessed in samples of fish roe, fish oil, and fish meal. Among the fish roe samples, the smoked cod roe had the highest amount of COP, 93 μg/g lipids. Refined and bleached menhaden oil had 8 μg/g, and two experimental alkali-refined, bleached, and deodorized herring fish oil samples contained similar amounts of COP. The range of total COP in the three fish meal samples ranged from 50 to 78 μg/g fish meal. Generally, processed fish roe contained high amounts of COP compared with refined fish oils, which had very low amounts of COP. Fish meal samples had very high amounts of COP.

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KEY WORDS: Cholesterol oxidation products, COP, fish meal, fish oil, fish products, fish roe.

The sterols of fish are mainly composed of cholesterol with various amounts in different fish organs (1). Cholesterol is prone to oxidation, and some cholesterol oxidation products (COP) have been shown to have a wide variety of effects both *in vitro* and *in vivo* and have been linked to human diseases and cholesterol metabolism (2). Considering the similarity in lipid metabolism between fish and mammals (1), it is feasible that toxic effects of COP found in humans also may apply to fish. Reports on the levels of COP in fish and fish products come mainly from Japan and Taiwan; this area has recently been reviewed (3).

Fish eggs contain highly unsaturated FA, which are oxidation-sensitive, as well as high amounts of cholesterol compared with other fish tissue (1). In a study of pickled and spiced Alaskan pollack (*Theragra chalcogramma*) roe, the total amount of COP was about 209 μg/g, and the dominating COP were the epimers of 7-hydroxycholesterol and 7-ketocholesterol (4).

World production of fish oil is rather steady and contributes less than 2% of the world production of fats and oils (5,6). No published data are available on the levels of COP in commercial fish oil. However, cholesterol oxidation in a model system with added cholesterol was greatly enhanced by coexisting highly PUFA in fish oil (4,7).

Fish meal is produced from dried ground tissue of undecomposed whole fish or fish cuttings, with or without extraction of part of the oil. There are two methods of manufacturing fish meal: the dry process and the wet process. In the first process, fish with low oil content are generally used, whereas in the wet process the fish meal is made out of fish with higher oil content. Heat treatment is required to produce the final fish meal product (8). Lipids constitute *ca.* 10% of fish meal, which is rich in PUFA. In addition, lipids extracted from fish meal may contain more than 5% cholesterol (9), compared with less than 1% cholesterol in the corresponding fish oil (10). Thus, fish meal is possibly a rich source of COP, as well as oxidized lipids.

The level of 7β-hydroxycholesterol and 7-ketocholesterol ranged from 0.4 to 9.4 μg/g and from 0.2 to 5.0 μg/g fish meal, respectively, in 22 samples of commercial fish meals from several countries (11). The authors pointed out that as a result of difficulties in determining PV, the COP content is the more accurate figure to use for describing the oxidative status of fish meal and fish feeds. However, no other study has been reported, to our knowledge, on the content of COP in fish meals.

The objective of this study was to determine the COP content in some common fish products consumed in Sweden. A few samples of herring fish oil and refined menhaden oil from Norway and Sweden were also analyzed. For the first time, detailed analysis on the levels of COP in commercial and experimental fish oils and in some experimental samples of fish meal from Norway was also undertaken, because of the importance of fish meal as feed for farmed aquatic and terrestrial animals.

EXPERIMENTAL PROCEDURES

Samples. Samples of fish roe were collected either from live specimens of lumpsucker (*Cyclopterus lumpus*) and Atlantic salmon (*Salmo salar*), or as processed products of lumpsucker and Atlantic cod *(Gadus morhua).* The lumpsucker roe samples were prepared from one batch of fresh roe collected on 1 d from a local fisherman on the Swedish west coast. The fish were approximately 2–3 kg mature females. The roe from several fishes was pooled and randomly sampled. The fresh roe was frozen immediately at −80°C. For the black-colored roe (Black PN, E151, and FD&C Yellow No. 6, E110) , originating from the same roe batch, the processing procedures were performed in the food plant at the end of the ordinary production line. The lumpsucker roe was salted in brine, stored, and thereafter colored and packed. The salted cod roe samples were collected in a production plant. The Atlantic cod was obtained from the North Sea fishery, and the roe sample was from egg sacs originating from one barrel. One batch of the same mixture of the salted roe was smoked separately

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and thereafter analyzed. The smoking temperature did not exceed 22°C, and the inner temperature of the roe mass was approximately 10°C. The salmon roe was collected fresh from a salmon hatchery. The females (4–7 kg), first spawners, were stripped the same day, and the roe was sampled and frozen at −80°C. The samples of fish meals and alkali-refined, bleached, and deodorized fish oils (oil samples 1 and 2) were submitted by the Norwegian Herring Oil and Meal Industry Research Institute (SSF) (Bergen, Norway), and they were experimental products. A sample of refined and bleached menhaden oil (oil sample 3) was also used in this investigation (Karlshamns Oils & Fats AB, Karlshamn, Sweden). All samples were stored at −20°C until analyses were performed, unless otherwise stated.

Analytical. Total lipids from sample fish roe and fish meals were extracted following published methods (9,12). Determination of COP in all samples was done by cold saponification of lipids, enrichment of COP by solid-phase extraction (SPE). Quantification and identification were done by GC and GC–MS, essentially as described previously (13).

RESULTS AND DISCUSSION

The contents of COP from different fish roe samples are shown in Table 1. Fresh fish roe samples had relatively small amounts of COP compared with processed roe samples. Processed black-colored lumpsucker roe samples contained a total of 38 μg/g COP in the extracted lipids. This sample contained considerably higher amounts of cholestanetriol and 20α-hydroxycholesterol than other samples. The smoked cod roe sample had the largest amount of total COP. The major COP in this sample were 7β-hydroxycholesterol (50 μg/g lipids) and cholesterol epoxides, dominated by cholesterol-5β,6β-epoxide (22 μg/g lipids).

Among the different fish tissues, eggs have the highest content of cholesterol (1). Oxidative degradation of cholesterol during fish processing and subsequent storage proceeds in conjunction with the oxidative degradation of constituent PUFA of fish oils in the tissues (4,6). Cholesterol oxidation in fish oil is accelerated owing to coexisting TAG composed of highly PUFA (4,6,13,14).

Food preservation by drying, smoking, or marinating is applied worldwide. Oxidized cholesterol has been measured in spiced and pickled Alaskan pollack roe (4). The major COP were 7α-hydroxycholesterol (38 μg/g), 7β-hydroxycholesterol (58 μg/g), and 7-ketocholesterol (33 μg/g), whereas the fresh sample did not contain any quantifiable amounts of these compounds. Similarly, the fresh fish roe samples in this study contained negligible amounts of COP in the lipids. However, the processed fish roe samples contained considerable amounts of COP, but much less than the pickled and spiced Alaskan pollack roe (4). One reason, other than the high degree of unsaturation of the different fish roes, may be the pro-oxidative effect of the salt, as discussed in Hansen *et al.* (15). In addition, smoking, can, as shown by Tóth and Potthast (16), also have the opposite effect with antioxidative properties.

The samples of two commercial herring fish oils from Norway contained similar amounts of total and individual COP (Table 2). There were almost no qualitative and quantitative differences between the two herring fish oil samples. The refined menhaden oil had a total COP content of 8 μg/g oil. The main difference in this sample compared with the herring fish oil was that there were relatively higher amounts of cholestanetriol and 25-hydroxycholesterol, and lower amounts of cholesterol-5α,6α-epoxide and cholesterol-5β,6β-epoxide.

The content of COP in the herring fish oil samples and in the menhaden oil studied is low compared with many other animal products (17). Only one report is known where unspecified commercial fish oil was shown to contain COP at levels similar to those in this study (18). Fish oil generally contains lower amounts of cholesterol compared with fish tissue lipids (9,10). Even though fish oil is composed of highly unsaturated FA, it is probably this lower level of cholesterol that contributes to the low content of COP in fish oils. The contents of PUFA in herring and menhaden oil are *ca.* 15 and 32%, respectively (10). However, the amount of cholesterol in herring fish oil is higher than that of menhaden oil, *ca.* 0.8 and 0.5%, respectively (10). That COP levels in these two different fish oil samples were similar can possibly be explained in the following way: Although menhaden oil has a higher content of PUFA, the generation of COP due to the lower cholesterol content was minimized compared with herring fish oil, in which the opposite was true. This point needs further investigation.

The total amounts of COP in the three samples of Norwegian fish meal samples ranged from 50 to 77 μg/g (Table 3).

TABLE 1
Content of COP (means of duplicate ± SEM) in Some Samples of Lumpsucker, Salmon, and Cod Roe[a]

	COP (μg/g lipids ± SEM)								
Sample	7α-OH	7β-OH	7-Keto	5α,6α-epoxy	5β,6β-epoxy	Triol	20-OH	25-OH	Total
Fresh salmon roe	0.52 ± 0.24	0.77 ± 0.28	1.32 ± 0.87	0.94 ± 0.39	1.82 ± 0.20	Trace	0.80 ± 0.36	Trace	6.23 ± 1.42
Fresh lumpsucker roe	1.56 ± 0.02	1.10 ± 1.02	1.19 ± 1.11	2.28 ± 1.51	1.22 ± 0.73	0.72 ± 0.43	3.20 ± 0.18	0.29 ± 0.10	11.56 ± 2.24
Black-colored lumpsucker roe	3.08 ± 1.46	3.72 ± 0.87	4.36 ± 0.37	5.62 ± 0.29	2.72 ± 0.17	11.20 ± 2.09	6.22 ± 1.02	0.64 ± 0.15	37.56 ± 1.39
Smoked cod roe	5.70 ± 1.39	50.03 ± 18.53	2.80 ± 0.98	6.98 ± 5.74	21.66 ± 7.87	2.59 ± 0.10	2.16 ± 0.53	1.14 ± 0.04	93.06 ± 17.9

[a]COP, cholesterol oxidation products; 7α-OH (7α-hydroxycholesterol), 5-cholesten-3β, 7α-diol; 7β-OH (7β-hydroxycholesterol), 5-cholesten-3β, 7β-diol; 7-keto (7-ketocholesterol), 5-cholesten-3β-ol-7-one; 5α,6α-epoxy (cholesterol-5α,6α-epoxide), 5-cholestan-5α, 6α-epoxy-3β-ol; 5β,6β-epoxy (cholesterol-5α,6α-epoxide), 5-cholestan-5β,6β-epoxy-3β-ol; triol (cholestanetriol), cholestan-3β,5α, 6β-triol; 20-OH (20α-hydroxycholesterol), 5-cholesten-3β,20α-diol; 25-OH(25-hydroxycholesterol), 5-cholesten-3β, 25-diol; trace, <0.10 μg/g lipids.

TABLE 2
Content of COP (means of duplicate ± SEM) in Some Samples of Fish Oil[a]

	COP (μg/g lipids ± SEM)								
Sample	7α-OH	7β-OH	7-Keto	5α,6α-epoxy	5β,6β-epoxy	Triol	20-OH	25-OH	Total
1	0.32 ± 0.03	0.58 ± 0.01	1.62 ± 0.21	0.44 ± 0.03	1.55 ± 0.02	0.12 ± 0.01	0.73 ± 0.06	0.59 ± 0.03	5.95 ± 0.33
2	0.53 ± 0.03	0.98 ± 0.07	1.37 ± 0.06	0.64 ± 0.01	2.77 ± 0.08	Trace	1.29 ± 0.12	0.61 ± 0.01	8.27 ± 1.12
3	0.95 ± 0.25	0.92 ± 0.24	0.49 ± 0.17	0.27 ± 0.07	0.47 ± 0.21	1.81 ± 0.85	0.87 ± 0.25	2.58 ± 1.15	8.36 ± 3.20

[a]See Table 1 for abbreviations. Sample 1, Norwegian experimental herring fish oil no. 1; sample 2, Norwegian herring fish oil no. 2; sample 3, menhaden oil.

TABLE 3
Content of COP (means of duplicate ± SEM) in Some Samples of Fish Meal[a]

	COP (μg/g lipids ± SEM)								
Sample	7α-OH	7β-OH	7-Keto	5α,6α-epoxy	5β,6β-epoxy	Triol	20-OH	25-OH	Total
1	9.35 ± 0.05	14.09 ± 3.47	24.96 ± 3.59	6.95 ± 1.42	19.82 ± 4.37	0.35 ± 0.01	0.80 ± 0.06	1.14 ± 0.20	77.46 ± 13.17
2	7.01 ± 0.16	12.62 ± 1.04	15.62 ± 2.23	3.03 ± 1.23	10.12 ± 1.83	0.27 ± 0.09	0.64 ± 0.17	0.74 ± 0.04	50.05 ± 5.70
3	6.90 ± 0.97	15.57 ± 3.63	12.51 ± 1.75	3.06 ± 0.61	11.47 ± 1.26	0.28 ± 0.01	0.71 ± 0.16	0.69 ± 0.02	51.19 ± 8.41

[a]See Table 1 for abbreviations. Samples: Norwegian experimental fish meal nos. 1, 2, and 3.

Samples 2 and 3 had nearly identical contents of total COP and individual COP. Sample 1 contained slightly higher amounts of total COP. Other differences, compared with samples 2 and 3, were the higher amounts 7-ketocholesterol and higher amounts of both cholesterol-5α,6α-epoxide and cholesterol-5β,6β-epoxide (Table 3).

The contents of COP in the fish meal samples were highest among all the fish and fish products analyzed in this study in terms of μg/g sample (Tables 1–3). About 80% of the total cholesterol remained in the fish meal; only *ca.* 20% was extracted with the oil (9). A recent study reported COP levels in 22 samples of commercial fish meals from several countries (11). It was demonstrated that the contents of 7β-hydroxycholesterol and 7-ketocholesterol ranged from 0.4 to 9.4 μg/g and 0.2 to 5.0 μg/g, respectively, in these fish meal samples. The results from the present study showed considerably higher amounts of these two COP, as well as their total amounts. However, the results cannot be compared because the samples were different, as were the methods of analysis: GC and HPLC were used in the present study and in the published report (11), respectively.

There are three major areas of utilization of fish meal, i.e., in terrestrial animal feeds, in feeds for aquaculture, and in terrestrial agriculture. Of the total production of fish meal, *ca.* 35% is used in aquaculture (6). A considerable proportion of fish lipids is composed of PUFA; the fish meal is possibly oxidized at various levels, depending on the technique used for industrial production and handling. No research has been published on the effects of different fish-meal qualities in nutritional studies for farmed aquatic and terrestrial animals and their impact on the overall quality of the resulting fish and meat products.

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