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**IAFMM**  
**International Association of Fish Meal**  
**Manufacturers**

**ANALYSIS OF FISH OILS FOR FREE AND  
ESTERIFIED CHOLESTEROL**

**PROJECT C11.90**

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# **ANALYSIS OF FISH OILS FOR FREE AND ESTERIFIED CHOLESTEROL**

## **EXECUTIVE SUMMARY**

Food companies see a marketing advantage in being able to label their products as "low in cholesterol". The cholesterol levels in 32 samples of commercial fish oils was measured. Because it is technically easier to remove free cholesterol from food products, the contents of both free and esterified cholesterol was measured in the oils.

The results do not suggest that fish oils from any particular origin are higher or lower than any other origin. Levels of free cholesterol ranged from 0.18% to 1.17% and esterified cholesterol from 0.01% to 0.60%. The level of cholesterol ester appears to be related to the freshness of the fish.

## **INTRODUCTION**

Because of health reasons various national regulatory agencies are allowing a declaration of cholesterol content on food products. Food companies see a marketing advantage in being able to label their products as "low in cholesterol".

Fish oil contains cholesterol but the levels vary. The first objective of this study was to measure the cholesterol content in fish oils made from fish caught in different geographical areas at different times of the year.

It is technically easier to remove free cholesterol from food products compared with the more difficult esterified cholesterol. Thus the second objective of this work was to measure the content of both free and esterified cholesterol.

## **METHODOLOGY**

### **Samples of Oil**

Commercially produced fish oils were obtained from Norway, U.S.A., Chile and South Africa. The oils were obtained from fish caught at different times of the year from three countries of origin. The samples were sent to the Fishing Industry Research Institute in South Africa for analysis.

200-250g of each sample was sent to South Africa packed in fish cans or in a suitable plastic bottle filled to the brim before sealing. The samples were air-freighted to the analysing laboratory and analysed within one month of receipt.

## Method of Analysis

Total cholesterol was determined by gas chromatography after the addition of stigmasterol as an internal standard and saponification. Free cholesterol was separated from esterified cholesterol after the addition of stigmasterol by thin layer chromatography. Free cholesterol and stigmasterol were removed from the TLC plate and the mixture was then analysed by gas chromatography. Esterified cholesterol content was calculated by subtracting the free cholesterol from the total cholesterol.

The FFA contents of the oils were determined by titration in hot ethanol with 0.02M sodium hydroxide using phenolphthalein as indicator.

## RESULTS AND DISCUSSION

Table 1 shows the content of free and esterified cholesterol and free fatty acid in the fish oils from different origins. The level of free cholesterol ranged from 0.18% (oil 2) to 1.17% (oil 28). The content of esterified cholesterol ranged from 0.01% (oil 15) to 0.60% (oil 22). The results do not suggest that fish oils from any particular origin were significantly higher or lower than any other origin. There appears to be a wide spread of values within origins.

Lovern and coworkers (J. Sci. Food Agric 10 (1959) 327) found that esterification of free cholesterol took place during spoilage of cod on ice. It seems therefore that contrary to expectations, cholesterol esters like free fatty acids (FFA) are a spoilage product of fish. Inspection of the results in table 1 and figure 1 clearly shows that high FFA contents are found together with high cholesterol ester contents, except in the case of oil 17. Calculations show that FFA and cholesterol esters show a close relationship with a correlation coefficient of  $r = 0.683$ . For 32 oils this is significant to the 0.001% test level. Mathematically expressed the relationship is :

$$\text{Cholesterol ester (\%)} = 0.056 \text{ FFA (\%)} + 0.14$$

It is uncertain whether the Chilean oils were pre-refined\*. The South African, Norwegian and American oils were definitely not. Pre-refining may effect the relationship since it will reduce the FFA content but its effect on cholesterol ester levels (free and esterified) is unknown.

The evidence therefore seems convincing that the cholesterol ester content of a fish oil is directly related to the freshness of the fish at the time of processing. However other factors will affect the absolute levels, for example the oil content of the fish, the feeding situation, the development of gonads.

An interesting area of research would be to see the effect of refining on the total cholesterol level and on the ratio of esterified to free cholesterol of an oil.

\* No. *Carna oils*

TABLE 1

## FREE ESTERIFIED CHOLESTEROL AND FFA CONTENT OF FISH OILS FROM DIFFERENT COUNTRIES

SAMPLE	COUNTRY OF ORIGIN	SPECIES	PRODUCTION DATE	CATCH AREA	CHOLESTEROL FREE	CHOLESTEROL ESTERIFIED (%)	FFA (% oleic)
1	Norway	Capelin (crude)	27.07.88	Island/Jan Mayen	0.37	0.38	3.94
2		Capelin (crude)	18.02.90	Island/Jan Mayen	0.18	0.46	5.59
3		Herring (crude)	24.10.88	North Sea	0.44	0.14	2.11
4		Mackerel (crude)	27.08.87	North Sea	0.25	0.08	0.94
5		Horse Mackerel (crude)	17.10.90	North Sea	0.36	0.06	1.05
6		Sandeel (crude)	29.06.87	North Sea	0.65	0.32	2.10
7	Chile	Jurel	11.90	Zone 8 <sup>a</sup> , Chile	0.73	0.08	1.57
8		Sardina Espanola	11.90	Zone 4 <sup>a</sup> , Chile	0.62	0.11	2.81
9		Anchoveta/Sardina	11.90	Zone 2 <sup>a</sup> , Chile	0.64	0.22	2.21
10		Anchoveta	11.90	Zone 1 <sup>a</sup> , Chile	0.76	0.27	1.99
11	United States of America	Menhaden (crude)	10.90	Central Gulf	0.49	0.11	2.49
12		Atlantic Menhaden	10.90	Chesapeake Bay	0.35	0.10	1.88
13		Menhaden (crude)	10.90	Western Gulf	0.34	0.11	2.00
14		Menhaden (crude)	10.90	Eastern Gulf	0.32	0.19	3.24
15	Norway	Herring (crude)	04.06.91	North Sea	0.48	0.01	1.27
16		Sandeel (crude)	20.05.91	North Sea	0.50	0.14	2.10
17		Capelin (crude)	02.02.91	Barentz Sea	0.51	0.40	1.64
18	United States of America	Menhaden (crude)		Eastern Gulf	0.47	0.08	2.87
19		Menhaden (crude)		Central Gulf	0.39	0.02	1.89
20		Menhaden (crude)		Western Gulf	0.43	0.04	2.35
21		Menhaden (crude)		Chesapeake Bay	0.72	0.05	2.88
22	South Africa	Red-eye	24.01.90		0.94	0.60	7.19
23		Anchovy	06.03.90		1.14	0.43	7.95
24		Anchovy	26.04.90		0.98	0.17	2.66
25		Pilchard	10.05.90		0.56	0.17	2.06
26		Pilchard	02.07.90		0.72	0.08	1.77
27		Red-eye	10.04.91		1.12	0.14	1.85
28		Red-eye	11.04.91		1.17	0.08	1.33
29		Anchovy	24.04.91		0.51	0.23	7.10
30		Anchovy	02.05.91		0.60	0.06	2.70
31		Anchovy	14.05.91		0.59	0.03	2.43
32		Anchovy	17.05.91		0.51	0.15	3.77

FIGURE 1

