STUDIES ON WAX ESTERS IN FISH

Until 1978, New Zealand only had access to fisheries close to the shore, but since then there has been exclusive access to anywhere within 320 km of the coastline. This has made available many deep-water fisheries, and many fish are commonly caught in these that were rarely able to be caught previously. Although these fish are mainly caught for their flesh, it has been found that they have very high levels of substances known as wax esters among their oils.

Wax esters are esters (RCOOR') with up to thirty carbon atoms and at most one double bond. They form a lens on water instead of spreading out like other oils, and are less viscous than hydraulic oil although they share many other properties with it. Wax esters are usually obtained either from spermaceti oil or the oil of the Jojoba plant, but as these sources are both highly limited New Zealand deep-water fish are a valuable source of these esters.

These esters have a variety of uses in many industries. They can be used as high pressure lubricants, replacing hydraulic oil, and in the pharmaceutical, cosmetic, printing and leather industries, as well as in candles and polishes.

The commonest fish oil used to obtain wax esters is that of the orange roughy, although other fish are also suitable. New Zealand is currently the only country extracting wax esters from fish oil, although some other countries also have suitable fish in their waters.

INTRODUCTION

The term 'wax esters' includes all esters of long chain carboxylic acids with long chain alcohols. These include the liquid waxes used for lubricants, cosmetics, linoleum and printing inks, and the solid waxes used for candles, polishes etc. Until recently, the only source of these products were the protected sperm whale and the jojoba plant. However, since 1815 when M. Chevreul showed that spermaceti oil from the sperm whale contained wax esters, these lipids have been found in a wide range of marine animals. It is assumed that wax esters function as energy reserves which are used up during long periods of starvation, as is common among deep-water and cold-water forms. When wax esters occur in epipelagic (mid-water) fish, they are usually associated with the roe, while deep-water fish have wax esters in the muscle and other tissues. The very different composition of wax esters in roe (mainly unsaturated) and in muscle (saturated and unsaturated) may be because roe wax esters function as an energy reserve for developing fry, whereas muscle wax may help to control buoyancy.

New Zealand has access to large reserves of deep-water fish (such as the orange roughy and the dories) which contain these saturated and unsaturated esters. Research is currently being done into how best to tap this resource.

A NEW SOURCE OF WAX ESTERS

The catching of the deep sea teleost fish orange roughy (*Hoplostethus atlanticus*), black oreo (*Allocyttus sp.*) and smooth oreo (*Pseudocyttus maculatus*) from depths of up to 1 200 m has recently been commercialised within the New Zealand 200 mile Exclusive Economic Zone (EEZ). The discovery and exploitation of the orange roughy, black oreo and smooth oreo, compared to our traditionally caught species such as snapper (*Chrysophrys auratus*), trevally (*Caranx georgianus*), etc. which are shallow to mid-water depth fish, have led to a number of potentially interesting developments in the fishing industry. The orange roughy is caught primarily for its white edible flesh, but increasing importance is being placed on the oil from this and other species. The fish is largely being trawled at about 600-1100 metres off the Chatham Rise (between the Chatham Islands and the South Island) and has become the most successful New Zealand fish in Europe. It is largely sold in Australia and the USA.

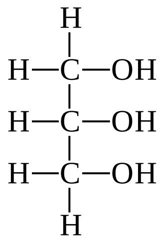
Comparison of the composition of the in-shore mid-water fish with the deep water fish shows a trend towards higher lipid (oil) content and lower moisture content (**Table 1**).

Table 1 - Composition of some New Zealand fish species (%w/w)

Species			**Protein**	**Lipid**	**Moisture**	**Ash**
In-shore / Mid-water	**Jack mackerel (whole fish)**		22.9	3.8	72.3	1.4
	Red cod (whole fish)		15.5	0.7	82.0	1.5
	Hoki (whole fish)		20.8	1.6	76.5	1.4
	Southern blue whiting (whole fish)		15.9	0.8	82.6	0.7
Deep water	**Orange roughy**	**(whole fish)**	12.2	17.6	67.4	2.8
		(fillet)	13.3	9.9	75.8	1.0
	Black oreo	**(whole fish)**	15.2	14.5	66.6	3.7
		(fillet)	17.6	7.6	73.8	1.0
	Smooth oreo	**(whole fish)**	13.7	5.7	78.3	2.3
		(fillet)	11.4	4.1	83.5	1.0

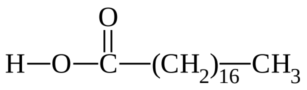
Marine Lipids

As can be seen from **Table 1**, lipids make up between a fifth and a half of the dry weight of fish. This is mainly composed of triglycerides (fats) and wax esters. Fats are esters of the trihydroxyl alcohol glycerol with three long chain monocarboxylic acids (fatty acids). The structures of glycerol and some common fatty acids are given below.



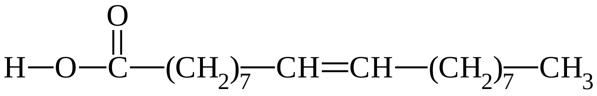
$$\begin{array}{c} H \\ | \\ H-C-OH \\ | \\ H-C-OH \\ | \\ H-C-OH \\ | \\ H \end{array}$$

Glycerol



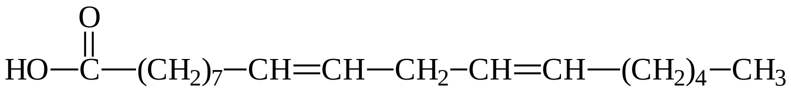
$$H-O-\overset{\overset{O}{\|}}{C}-(CH_2)_{16}-CH_3$$

Stearic acid (saturated) 18:0



$$H-O-\overset{\overset{O}{\|}}{C}-(CH_2)_7-CH=CH-(CH_2)_7-CH_3$$

Oleic acid (monounsaturated) 18:1



$$HO-\overset{\overset{O}{\|}}{C}-(CH_2)_7-CH=CH-CH_2-CH=CH-(CH_2)_4-CH_3$$

Linoleic acid (polyunsaturated) 18:2

(Note that fatty acids are often named with two numbers corresponding to the number of carbon atoms:number of double bonds).

Wax esters are fatty acids esterified to long-chain saturated or monoenoic (one double bond) alcohols.

Triglycerides are the major component of all plant and animal lipids, and include all of the many commercially significant fats and oils of animal and plant origin. Wax esters, on the other hand, occur as protective coatings on plant leaves and fruits, and in animal and insect secretions (e.g. wool wax and beeswax) and are generally considered minor components of animal and plant lipids. However, when the lipids of inshore and mid-water fish are compared with those of deep-water fish the compostion changes from almost wholly triglyceride to almost wholly wax esters (**Table 2**), indicating that deep-water fish could be used as a valuable source of wax esters.

Table 2 - Total lipid breakdown for mid-water and deep-water fish (% w/w)

	Inshore / Mid-water	**Deep-water**
Triglycerides	92.3	3.1
Wax esters	—	94.9
Cholesterol	2.4	1.0
Phospholipids	3.5	1.0
Others (Free fatty acids)	2.8	—

The deep water species are of particular interest as a commercial source of fish oil high in wax esters, and are essentially a liquid wax. The quantities of oil vary markedly, depending on the site of the fish being considered. **Table 3** shows details for orange roughy of the percentage of the total fish weight of various sites, the % of oil contained at those sites, and the % of the total oil in the fish that this amount represents.

Table 3 - Oil content of orange roughy

Site	% of body weight	oil content (%)	% of total oil
Whole fish	—	17.6	—
Head	38.6	20.2	44.1
Gut	8.0	24.1	11.0
Skin	3.4	43.9	8.4
Frame	13.1	21.2	15.9
Muscle	36.3	9.9	20.4

The striking feature of **Table 3** is the level of oil in the head, which is normally discarded at sea. The skin level is also very high, indicating that oil can easily be recovered from it in the processing plant. Generally about 2 or 3 mm of flesh is removed, and this has an oil content of about 25% . It is interesting to note that this type of fish is at present commercially fished only in New Zealand and Australian waters.

It is not clear why marine animals store neutral lipid as both triglycerides and wax esters instead of only triglycerides like terrestial animals. Wax ester formation may be a biochemical mechanism for making lipids at unusually high rates from amino acid and glucose precursors. Another possibility is that the physico-chemical properties of the wax esters themselves makes them superior to triglycerides under certain physiological conditions. There are no convincing examples to demonstrate this however.

Wax esters are more hydrophobic than analogous triglycerides. For each hydrocarbon chain in triglycerides there is one hydrophilic ester group, whereas in wax esters there is one half of an ester group. A consequence of this difference is that the two lipids interact differently with water - the triglyceride spreads to cover the surface, but wax ester floats as a lens. Thus long-chain wax esters are classed as non-polar lipids and triglycerides as polar lipids. Liquid wax esters have lower specific gravities and their viscosities are much less influenced by temperature variations.

Catabolism

Catabolism is the breakdown of complex nutrient molecules such as lipids, carbohydrates and proteins to simpler molecules such as lactic acid, CO_2, NH_3 and urea with the production of energy. Hydrolysis by lipase initiates the catabolism of wax esters. Although little is known about the lipases that hydrolyse wax esters in animal tissues, there is some information on the intestinal digestion of wax esters and the lipase responsible for the wax ester hydrolysis in developing jojoba seed. After surveying a variety of invertebrates and fish for their ability to digest wax esters, it has been concluded that there is no wax ester lipase which is distinct from normal triglyceride lipases. Wax ester digestion is much slower in comparison with triglyceride digestion. It is uncertain why wax esters are such poor substrates for lipases. In triglyceride hydrolysis, water molecules must flow freely into the active site. The insoluble mixture of products of triglyceride digestion, monoglycerides and protonated fatty acids, spontaneously interact with water forming product phases that ensure a steady flow of water molecules to the lipase molecules. However, the products of wax ester ingestion, long-chain alcohols and protonated fatty acids, both form an oil or solid phase with water, and may inhibit the reaction. The wax ester molecule itself, because of its greater hydrophobicity, may not interact with the active site of the lipase as quickly as the more polar triglyceride molecule.

Comparison with other sources of wax esters

The major current sources of liquid waxes are the protected sperm whale (*Physter macrocephalus* and *P. catadon*) and the desert shrub jojoba (*Simmondsia chinensis*). **Table 4** shows the total lipid composition of these sources compared with deep-water fish caught in New Zealand waters.

Table 4 - Analysis of lipids in various oils (% w/w)

	Orange roughy	Black oreo	Small spined oreo	Sperm whale	Jojoba
Wax esters	94.9	91.5	95.6	65.8	97.1
Triglycerides	3.1	4.8	2.5	30.1	—
Cholesterol/alcohols	1.0	2.7	1.5	4.0	2.5
Phospholipids	1.0	1.0	0.4	0.1	—
Other (free fatty acids)	—	—	—	—	0.4

It can be seen that the levels of wax esters in the fish compare closely with jojoba oil.

The principal components of the wax esters of orange roughy are of the C34-C42 chain length, contrasting with the shorter C28-C36 found in sperm whale oil and C38-C44 from jojoba oil (**Table 5**).

Table 5 - Gas chromatographic composition of wax esters (%)

Chain length	26	28	30	32	34	36	38	40	42	44	46	48
Orange roughy	—	—	0.2	2.1	11.4	16.7	24.8	23.4	14.8	5.5	1.1	—
Sperm whale	4.7	14.0	21.1	23.2	19.9	11.7	4.4	—	—	—	—	—
Jojoba	—	—	—	—	—	1.6	6.2	30.6	49.5	8.1	0.9	0.2

The fatty acid components are primarily 16: 1, 18:1, 20:1 and 22:1 acids. The alcohols are mainly 18:1, 20:1, and 22:1 but some 16:1 also. Jojoba oil consists mainly of 20:1 and 22:1 alcohols and 20:1 and 22:1 fatty acids.

Potential uses of orange roughy oil

In view of the similarity of orange roughy oil to jojoba oil and sperm whale oil, and because there are problems as to the supply of oil from these sources which tend to keep the oil cost high, there is a commercial potential for the oils from orange roughy and the oreo species (**Table 6**).

Table 6 - Possible end uses of orange roughy oil and wax

Oil	Solid wax (hydrogenated)
lubricant (high pressure) cosmetics leather industry pharmaceuticals factice (linoleum, printing inks etc.) source of long-chain fatty acids and alcohols	candles polishes cosmetics protective coatings pharmaceuticals

In the hydrogenated form, it could substitute for hydrogenated jojoba oil, or other waxes of commercial importance such as carnauba and candelilla, for use in tanning, pharmaceuticals, cosmetics etc. The sulfurized form[1] could substitute for sulfurized spermaceti oil for use as an extreme pressure and antiwear additive in differential and transmission lubricants, or hydraulic fluids with a low coefficient of friction, and in cutting and drawing oil.

The physical and mechanical properties of refined and deodorised orange roughy oil are shown in **Table 7**. Also shown are the properties for jojoba oil and rapeseed oil, where available. The results show the close comparison of mechanical and lubricational properties between orange roughy and jojoba oils. In particular, the similarity of the results in the extreme pressure and wear tests and oil stability are very similar.

The significance of the high viscosity index of orange roughy is demonstrated as the orange roughy oil is only half as viscous as the hydraulic oil at 20°C although their viscosities at 100°C are similar. Thus although wax esters are not currently extracted from orange roughy oil and used in such industrial applications in New Zealand, there is great potential for this to be done.

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[1] Any of various polysulphates produced by reacting the olefin with sulfur or sulfur monochloride.

Table 7 - Properties of refined and deodorised Orange Roughy, Jojoba, and Rapeseed oils compared with a typical hydraulic oil

	Orange roughy	**Jojoba**	**Rapeseed**	**Hydraulic**
Kinematic Viscosity / mm^{2}s^{-1}				
at: 20°C	39.24		78.43	80.9
25°C	32.55	58.4		
40°C	20.04			30.64
60°C	11.84			
80°C	7.79			
100°C	5.52		8.12	5.33
Viscosity index	240	232	214	107
Density / kgL^{-1} at 20°C	0.8683	0.863	0.917	0.866
15°C	0.8711			
Pour point / °C	9	10	-21	-24
Cleveland open cup / °C				
Smoke point	222	195	270	190
Flash point	285	295	324	211
Fire point	320	338	360	239
Fourball extreme pressure test (10 second runs)				
Mean hertz load / kg		22	21.2	23.38
Initial seizure load / kg	63		63	100
Weld load / kg	141	160	141	178

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