Nutritional Composition, Quality and Spoilage Capacity of Orange Roughy *(Hoplostethus atlanticus)* from the North East Atlantic

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M.Sc. Thesis

By

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Declaration of Masters Thesis

I hereby declare that the work presented in this thesis is my own and that it has not been used to obtain a degree in this Institute of Technology or elsewhere.

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ABSTRACT

The nutritional composition of orange roughy (collected from the Northeast Atlantic near the Rockall Trough) was studied on a seasonal basis. In addition samples were aged and stability assessed.

Protein levels (16.68-16.21% $^{w}/_{w}$) were found to be slightly higher than those recorded for the New Zealand species of orange roughy and compared favourably with protein values for fish muscle in general. Statistically results show a significant seasonal variation with no variation from fish to fish or in the location within the fish.

Lipid content (3.6-4.5% $^{w}/_{w}$) was found to be much lower than that recorded for New Zealand. As with protein statistically results show a significant seasonal variation and no variation from fish to fish or in the location within the fish.

Moisture levels (77.3.79.6% $^{w}/_{w}$) compared favourably with values obtained from other studies. Again statistically results show a significant seasonal variation with no variation from fish to fish or within the fish.

Iodine values (74.63-79.54) indicate the likely presence of a high level of mono unsaturated fatty acids. Statistically results show no significant seasonal variation and no sample variation or variation within fish.

Thin layer chromatography of the extracted fat showed the major type to be wax esters with a much lower amount of triglycerides and smaller amounts of polar lipids, free sterols and free fatty acids.

Total fatty acid composition was found to be very similar to that recorded from other studies and showed that most of the oils extracted from the fish muscle contained a high percentage of mono unsaturates namely 16:1,18:1, 20:1 and 22:1 (85.63 - 91.14%)with 16:1 present in the smallest amounts and 18:1 the major one. The only saturated fatty

acids present in significant quantities were 14:0, 16:0 and 18:0, the total varied from a seasonal average high of 4.05 % to an average low of 2.27%.

The polyunsaturated fatty acids linoleic and arachidonic acid were present in small quantities varying in total from 0.89% to 1.50%. Docosapentaenoic acid (DPA) was found only in trace quantities in spring, autumn and winter samples and undetected in summer. Levels of Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) were also found in very low percentages and varied on a seasonal basis with average values ranging from 0.41% in summer to 1.03 % in autumn for EPA and from 1.44 % in summer to3.20 % in autumn for DHA. Again statistically results show a significant seasonal variation with no variation from fish to fish or location within the fish.

Levels of freshness were measured using the Thiobarbituric acid (TBA), Total volatile base nitrogen (TVB-N) and Trimethylamine (TMA) techniques. The quality of the fish upon arrival was excellent and well below legal/acceptable limits.TVB-N values ranged from 6.88-8.91 mg/100g and TMA values from 4.82-6.46 mg/100g Values for TBA ranged from 0.18-0.35 mg Malonaldehyde/kg fish. The summer values were higher than the other seasons. Seasonal variation was significant for all methods with no variation from fish to fish or within the fish.

Fish aged at +4°C in air did not exceed the TVBN limit of 35mg/100g until day 6 whereas the TVBN limit was extended to 8 days for fish aged at +4°C in vacuum. However the TMA limit of 12mg/100g was reached on day 4 for fish stored at +4°C in air and on day 5 for vacuum packed samples stored at +4°C. Fish stored at -5°C in air and vacuum packed did not reach the TVBN limit until day 61 but the TMA limit was reached on day 24 for fish stored at -5°C in air and was extended to 31 days for vacuum packed fish stored at-5°C.

Prolonged storage at -18°C caused some deterioration of the frozen fish muscle. Upon thawing the shelf life of fish stored for 12 months was much shorter than that stored for 6

months. This in turn deteriorated faster than fresh fish held at refridgeration temperature in air.

Orange roughy were found to be a good source of protein with moisture levels similar to that of other fish. They were of medium fat content but have a very poor content of the essential omega 3 and omega 6 fatty acids.

Orange roughy can be stored at -18°C but its subsequent refridgerated shelf life will be shorter than that of unfrozen orange roughy stored at refridgeration temperature.

Orange roughy are a very important part of the ecosystem. Their composition is less nutritionally beneficial than more readily available fish for human consumption and therefore should not be fished at all.

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1 INTRODUCTION

Pelagic Fish have contributed to our healthy diet for generations. More recently, how ever, the importance of polyunsaturated fats as part of a healthy diet has become widely known and populations are being encouraged to increase their dietary intake of fish. As traditional fisheries on the continental shelf declined, due to over fishing, distant/deep water fleets developed to exploit less accessible populations and species. The global expansion of fisheries uncovered deep-water habitats, such as seamounts, with substantial aggregations of benthopelagic fishes. **(Boehlert & Sasaki, G.W., 1988; Koslow, T., 1996)** Many of the dominant species in these environments, such as orange roughy, (*Hoplostethus Atlanticus)* were previously considered obscure and although markets have been developed on the continent they are still not commonly eaten in Ireland. Orange Roughy is predominantly fished on seamounts and deep plateaus at 700-1200m depth around New Zealand and Australia, smaller fisheries are found in the Northeast Atlantic and off Namibia in the Southeast Atlantic. **(Clarke, M., 1999, Koslow et al., 1997)** They are a slow growing fish which do not start to breed until they are 25-30 years old and are thought to live up to 150 years. Most fish caught are 30-50 cm long and weigh between 0.9 and 3.6 kg. **(http://www.starfish.govt.nz).** There is a great variety in fish size from region to region. Off Namibia, orange roughy are generally smaller than in other orange roughy fisheries, with a standard length of 20-32cm. Around Australia and New Zealand adults are typically 20-50cm long. Off Chile nearly all fish are 29-49cm long. Throughout the North-East Atlantic, orange roughy are considerably larger than in the southern hemisphere, with most fish 40-58cm. The largest recorded specimens are those from the Faroese catches, which can reach 62cm and a body mass of 7kg **(Branch, T, A., 2001).**

The ecological characteristics of these fish make them vulnerable to over-exploitation and slow to recovery from it **(Clark, M.R., 1995, Koslow et al., 1997).** They have a slow growth rate, high longevity, low fecundity and hence low productivity **(Clark et al., 1994).**

They feed opportunistically on fish such as viperfish and whiptails as well as crustaceans and amphipods. Because they aggregate densely, this results in catches of up to 50 tons

per minute **(Ross, D. M., & Smith, D. C., 1977).** Orange Roughy trawl fisheries have developed off Namibia, New Zealand, and the Southern Indian Ocean and off Chile. In most cases, stocks have rapidly been fished down to, and often below, the long term sustainable yield. The history of orange roughy fisheries in New Zealand and Australia tends to illustrate this, with rapid development and an equally rapid decline as stock size declines rapidly.

The first comprehensive analysis of the fecundity and reproductive maturity of the orange roughy, from a specific area in the Northwest Atlantic, was reported in 2006 (Minto, C & Nolan, C., 2006, http://www.ices.dk/aboutus/pressroom.asp). Specimens were collected on the Porcupine Bank in waters between 1400-1650 meters in depth. Sampling protocol was implemented to collect mature female fish between 300-540mm and their overies were studied. Total fecundity ranged between 20,352 and 244,578 oocytes per female with mean total fecundity estimated to be 33,376 oocytes per kg. Fecundity was not shown to decrease with age and macroscopic analysis showed that 50% of females were not mature until they reached 27.5 years and 37cm in length. Comparsion with stocks from the southern hemisphere indicate that orange roughy from the Northeast Atlantic mature at a larger size and generally have a higher fecundity than than those found in the southern hemisphere.

Orange roughy are synchronous spawners (Punkhurst, N.W.,1988), forming dense aggregations for spawning and feeding events. It is thought they migrate up to 200kg to spawn, as mature fish are widespread but spawning occurs in just a few specific areas (Francis, R & Clark, M. R., 1998). Time of spawning varies between areas, in the southern hemisphere, spawning occurs from June to August (Clark, M. R., 1995). While in the North Atlantic, near the Faroe Islands, the main spawning season is between late January and early March (Thomsen, B., 1998). For most stocks, spawning occurs annually though not all mature fish mature every year (Bell et al., 1992, Zeldis et al., 1997, Annala et al., 2001). Distribution of young (0+ and 1+ cohorts) is not well understood because young orange roughy are rarely encountered during trawling operations (Mace et al., 1990). In New Zealand large catches of juveniles have been found in only one area, at a depth of 800-900 meters, 150km east of the north Chatham Rise main spawning ground (Annala et al., 2001).

Orange Roughy are thought to be opportunistic predators, taking advantage of prey often associated with seamounts, such as prawns, squid and small fishes that migrate during the day (Koslow, J.A., & Bulman, C.M., 2002). Other prey items include amphipods, mysids and decapod crustaceans, depending on local abundance of these items. Availability of prey on and around seamounts may explain non-spawning aggregations observed on certain fishing grounds (Bulman, C.M., & Koslow, J.A., 1992).

Early deep-sea fishing exploration in the Northeast Atlantic was conducted by Russian trawlers primarily targeting Roundstone grenadier (*Corypaenoides rupestris*) and Greenland halibut (*Reinhardtius hipposylossoides*) (Lorance, P., & Dupouy, H.,2001). Word of large orange roughy catches in the Southern hemisphere motivated a search for this species in the Northeast Atlantic. In the late 1980s catches of many deep-water species, including orange roughy, increased (Charuau et al., 1995). French trawlers fishing in the area landed most of the areas catch in the 1990s, up to 15,000 tons in 1996 (Anon, 1998). The Faroese fleet has taken their catch mainly from the area north of the Azores, peaking at 1,300 tons in 1996. Infrequent catches were recorded by Spain, Ireland, the United Kingdom and Norway (Branch, T.A., 2001).

Commercial fishing for orange roughy requires heavy-duty trawl gear towed several kilometres behind and up to a kilometre below the vessel. This trawling gear has been implicated in the destruction of benthic communities, as well as decreasing overall faunal diversity (Probert et al 1997).

Probert et al., (1997) examined bycatch occurring in orange roughy trawls from the Chatham Rise fishing area of New Zealand and found the largest bycatch to be slow-growing corals, which they suggest may take a century to recover. Another study by Clark et al, (2000) on this area found declines in abundance of 10 of 18 bycatch species of fish and sharks from 1979 to 1997. A photographic survey of the continental slope south of Tasmania showed that trawl operations remove significant amounts of organisms. The average benthic biomass of dredge samples from unfished seamounts was 106% greater than heavily fished seamounts, and the number of species per sample was 46% greater than in trawled areas (Koslow, J.A., 1999). While bycatch of other fishes is often low, observed species landed with orange roughy catches around New Zealand include black oreo, smooth oreo. deepwater dogfish, slickheads, rattails and basketwork eels (Clark, M., & Tracey, D.M.,1993).

Orange roughy and other fisheries in the Northeast Atlantic are assessed and managed individually by countries, and as a collective through the North Atlantic Fisheries Organisation (NAFO). The International Council for the Exploration of the Sea (ICES) gives scientific council. The waters in this area are shared collectively among interested fishing countries including Canada, the U.S, the European Union, France, Iceland, Norway and Poland (NAFO 2002). Participating NAFO countries have established and agreed to the following regulatory measures:

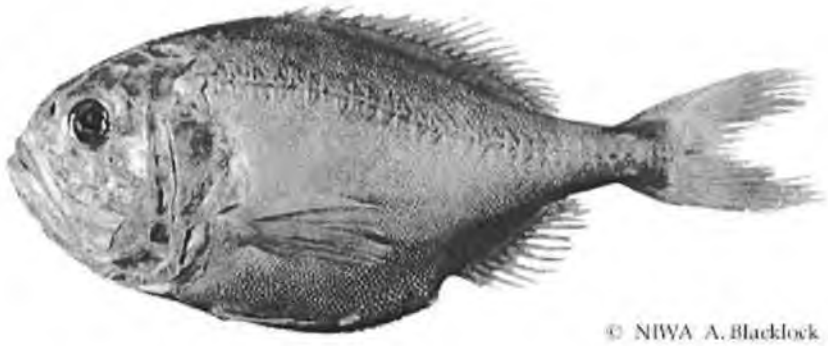
1. A reporting system of fishing vessels and air surveillance in the regulatory area.
2. Improved inspections and transparency of apparent infringements.
3. Reporting of catch on board fishing vessels and details of fishing.
4. Minimum fish size and processed length equivalent.
5. A pilot project for observers and satellite tracking.

These and other regulations became binding measures during 1994-95 (NAFO 2002).

The purpose of this project is to comprehensively analyse a significant number of orange roughy fish caught in the Northeast Atlantic, near the Porcupine Bank/ Rockall Trough. **(Figure 1)**. The protein, lipid and moisture content will be determined and analysed to see the type of variation that occurs from animal to animal, from head to tail and from season to season. The composition of the lipid will be assessed, in particular the fatty acid composition. The level of polyunsaturated fatty acids (PUFA): Eicosapentaenoic acid (EPA), Docosapentaenoic acid (DPA) and Docosahexaenoic acid (DHA) will be quantified and the results compared with previous data from the Southern Hemisphere. These PUFA are well known to contribute to the prevention of heart attacks which is one of the main reasons humans are being asked to increase their consumption of fish.

The aging/stability of the fish in the presence and absence of air at 4°C and -5 °C will also be investigated.

The overall study should give us a comprehensive understanding of the nutritional value of this particular fish and may indicate the types of food these fish live on and their benefit to mankind.



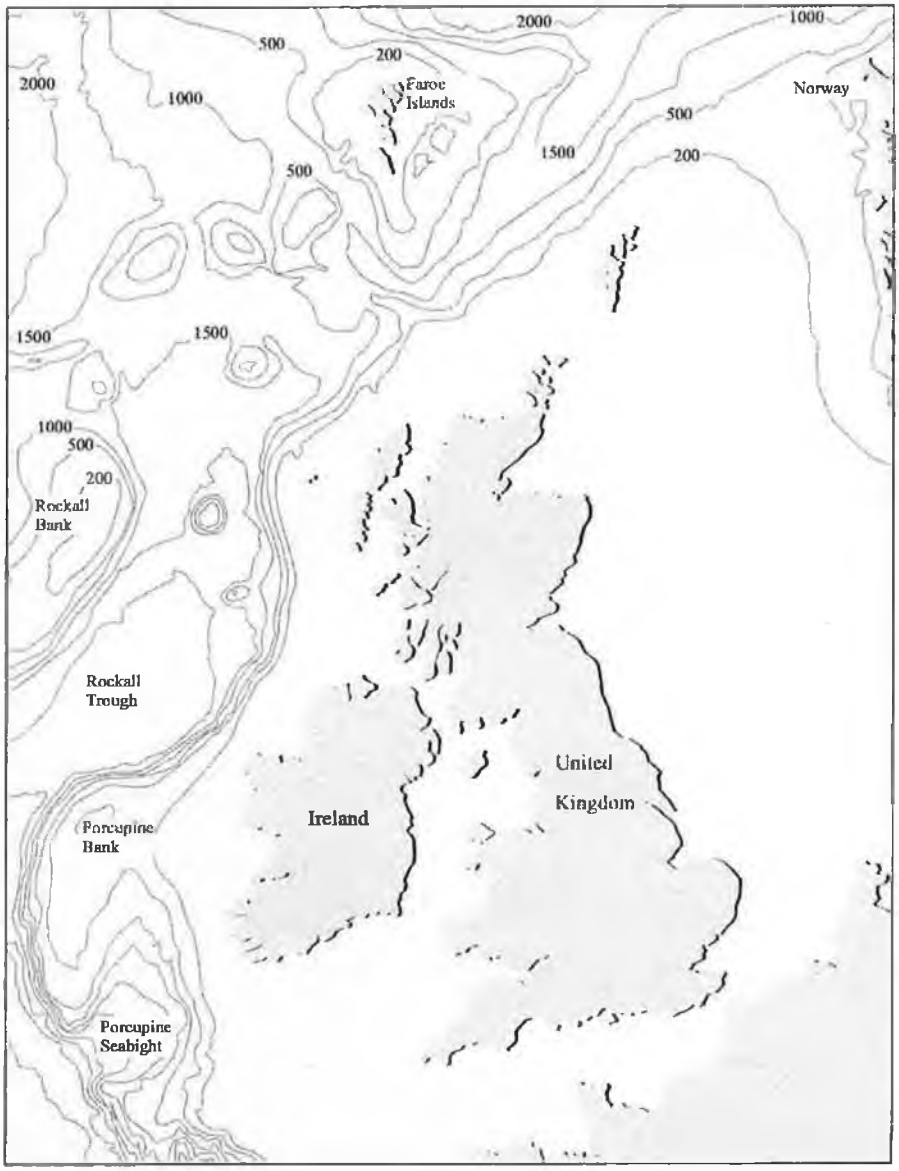


Fig.1.Orange Roughy and the Rockall Trough, Northeast Atlantic. (http://journal.nafo.int/archive22-33/J31/session1/gordon.pdf)

2 LITERATURE REVIEW

2:1 NUTRITIONAL COMPOSITION OF FISH

The main constituents of fish are water, protein and fat. However fish also contain carbohydrates, minerals and vitamins **(Love, R.M., 1982).** Water can account for up to 80% of the weight of a whitefish fillet, whereas the average water content of fatty fish is about 70% **(Mc Cance, M & Widdowson, R., 1991)**. The water in fresh fish muscle is tightly bound to the proteins in the structure in such a way that it is not easily expelled even under high pressure. After prolonged chilled or frozen storage, however, cells become ruptured and the proteins are less able to retain water, and some of it, containing dissolved substances, is lost as drip.

The amount of carbohydrate in white fish muscle is generally too small to be of any significance in the human diet. Fish muscle normally contains only traces of carbohydrates, in the form of sugars, sugar phosphates and glycogen. Some other tissues such as liver contain larger amounts of glycogen, and most molluscan shellfish also contain a fair amount of glycogen. In both white and fatty fish species, carbohydrate is not present. Some molluscs, however, contain up to 5% of the carbohydrate glycogen. **(Mc Cance, M. & Widdowson, R., 1991).**

Vitamins can be divided into two groups, those that are soluble in fat, such as vitamins A, D, E and K and those that are soluble in water, such as vitamins B and C. All the vitamins necessary for good health in humans are present to some extent in fish, but the amounts vary widely from species to species, and throughout the year. The vitamin content of individual fish of the same species, and even of different parts of the same fish, can also vary considerably. Often the parts of a fish not normally eaten, such as the liver and the gut, contain much greater quantities of oil-soluble vitamins than the flesh; the livers of cod and halibut for example contain almost all of the vitamins A & D present in those species. Water-soluble vitamins in fish, although present in the skin, the liver and gut, are more uniformly distributed, and the flesh usually contains more than half the total amount present in the fish **(Feng, K.J., 1989).**

The amount of protein in fish muscle is usually somewhere between 15 and 20%, but values lower than 15% or as high as 28% are occasionally recorded in some species **(Mc**

Cance, M. & Widdowson, R., 1991). Proteins are acquired from the diet by the body as a source of amino acids from which the body synthesises its own protein molecules required for tissue formation and repair. The essential amino acid content of fish is very high i.e. the fish protein is very digestable and the released amino acids are easily absorbed and rich in the eight essential amino acids **(Lake, B., Waterworth, M., 1980)**. All proteins including those from fish, are chains of chemical units linked together to make one long molecule. These units, of which there are about twenty types, are called amino acids and certain ones of them (eight in total) are essential in the human diet for the maintenance of good health. Two essential amino acids called lysine and methionine are generally found in high concentrations in fish proteins. Hence the reason fish is considered to be a good source of protein of high biological value. In contrast these amino acids are usually present in low concentrations in cereals like maize, rice, potatoes and/or wheat. Cereals, however, contain high concentrations of the non-essential amino acids and because of their abundance in the diet, are a good source of total protein precursors. Thus fish and cereal protein can supplement each other in the diet. Fish protein provides a good combination of amino acids which is highly suited to man's nutritional requirements and these results are similar to that provided by meat, milk and eggs.

In seafood, lipids are the second largest component after protein. Lipids are water insoluble, organic substances, which are extracted from tissues and cells by non-polar solvents,e.g.,chloroform and petroleum spirit. Several classes of lipids exist, each containing large non-polar hydrocarbon-like structures **(Lehninger, A.L., 1983)**. They serve two major functions, as structural components of membranes and as storage forms of metabolic fuel.

There are several classes of lipids, each of which has specific biological functions – the major ones being: Triglycerides, Phospholipids, Waxes, Sphingolipids, Sterols and their fatty acid esters.

Fatty acids are long-chain organic acids having from four to twenty four carbon atoms, with a single carboxyl group and a long, non polar hydrocarbon 'tail' which gives them their water insoluble nature **(Lehninger, A.L., 1983)**. They fill two major roles in the body: as the components of more complex membrane lipids and as the major component of stored fat in the form of triglycerides or wax esters.

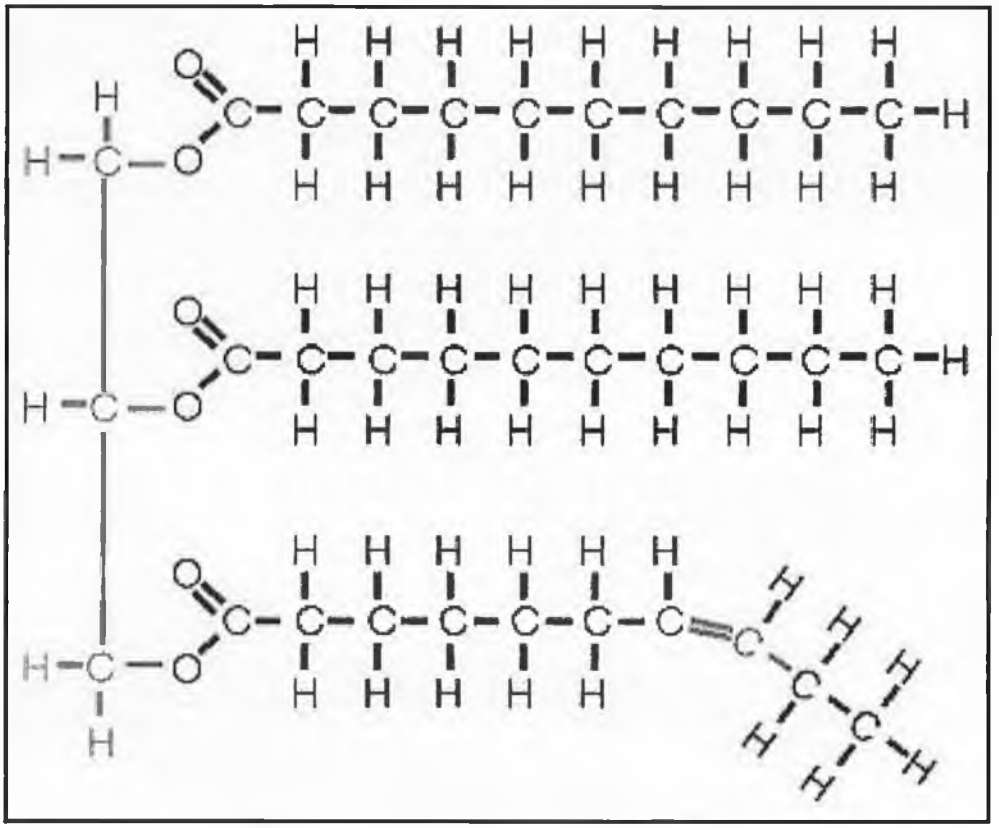


Figure 2. Structure of Fatty Acids in a triglyceride.

http://biology.clu.uc.edu/cources/biol04/lipida.htm

The terms saturated, monounsaturated and polyunsaturated refer to the number of hydrogens attached to the hydrocarbon tails and the number, if any, of double bonds between the carbons in the tail.

Those that contain no carbon-carbon double bonds are termed saturated fatty acids, those that contain one double bond are termed monounsaturated fatty acids and those containing more than one double bond are termed polyunsaturated fatty acids **(Figure 3)**.

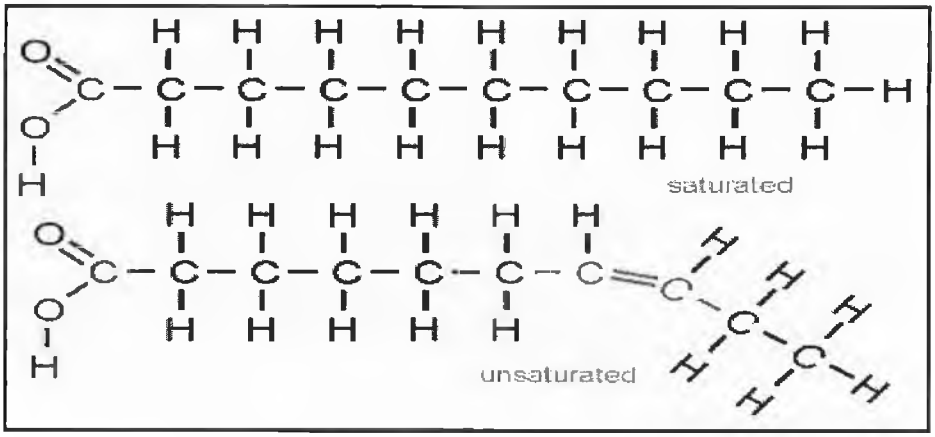


Figure 3. Saturated and Unsaturated Fatty Acids.

http://biology.clu.uc.edu/cources/biol04/lipida.htm

The numeric designations used for fatty acids come from the number of carbon atoms followed by the number of sites of unsaturation, e.g. palmitic acid is a sixteen carbon fatty acid with no unsaturation and is designated by 16:0. The site of unsaturation in a fatty acid is indicated by the symbol Δ and the number of the first carbon of the double bond – for example, palmitoleic acid is a sixteen carbon fatty acid with one site of unsaturation between carbons nine and ten and is designated by 16:1Δ9, from the carboxyl end, or n-7 from the methyl end.

In unsaturated fatty acids, there are two ways the pieces of the hydrocarbon tail can be arranged around a C=C double bond. The prefixes "cis" and "trans" describe the orientation of the hydrocarbon tails with respect to the double bond. "cis" means "on the same side" and "trans" means "across" or "on the other side" **(Figure 4).**

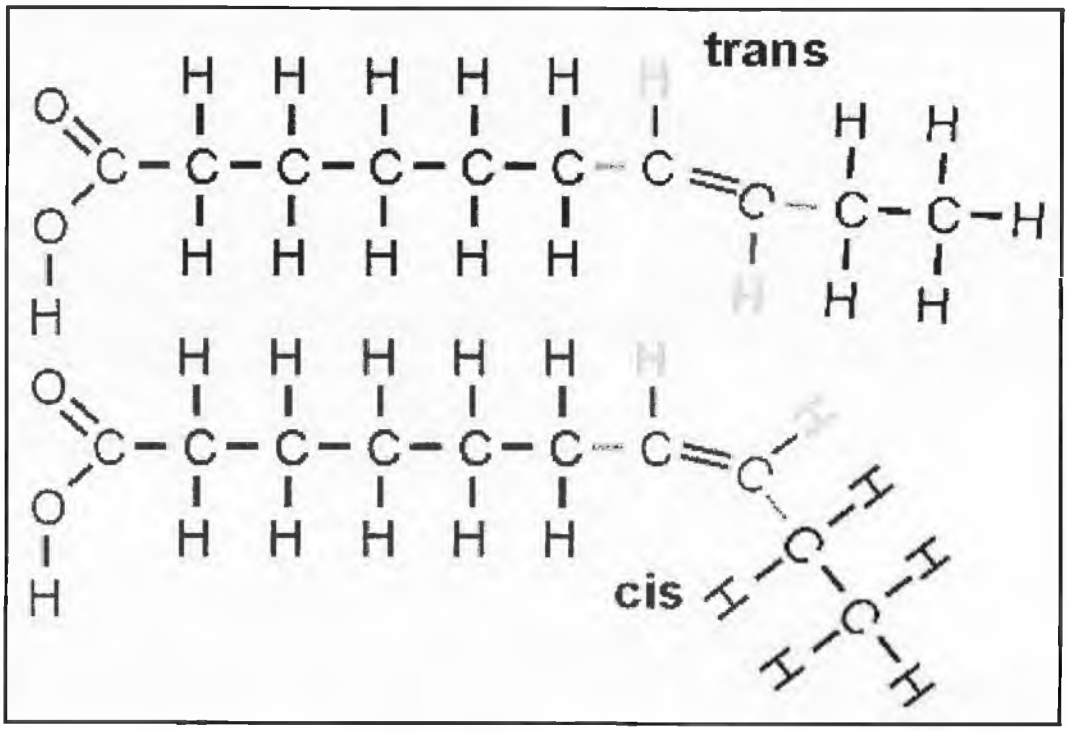


Figure 4. Trans and cis Fatty Acids.

http://biology.clu.uc.edu/cources/biol04/lipida.htm

Naturally occurring unsaturated vegetable oils have almost all cis bonds, but the use of these oils in frying causes some of the cis bonds to convert to trans bonds. If the oil is constantly reused, as in the case of oils used in fast food machines, more and more of the cis bonds are changed to trans, resulting in a build up of fatty acids with trans bonds. The reason this is of concern is that fatty acids with trans bonds are carcinogenic. The levels of trans fatty acid in highly processed lipid containing products such as margarines have been shown to be quite high. http://biology.clu.uc.edu/cources/biol04/lipida.htm

Free fatty acids can be bound or attached to other molecules such as in triglycerides or phospholipids. When they are not attached to other molecules, they are known as "free" fatty acids. They may also come from the breakdown of a triglyceride into its components: fatty acids and glycerol. Free fatty acids are an important source of fuel for many tissues since they can yield relatively large quantities of ATP. Many cell types can use either glucose or fatty acids for this purpose. Heart and skeletal muscle, for example, use fatty acids whereas the brain relies on glucose or ketone bodies produced by the liver

from fatty acid metabolism during starvation, or periods of low carbohydrate intake, as its fuel source.

The majority of body fatty acids are acquired in the diet and the body has the capacity to synthesise its lipids using fatty acid modifying enzymes. The two key exceptions to this are the polyunsaturated fatty acids known as linoleic and linolenic acid, containing unsaturated sites beyond carbon nine and ten. Because these two fatty acids cannot be synthesised from precursors in the body they must be provided in the diet, they are known as essential fatty acids. Plants are capable of synthesising linoleic and linolenic acids so humans acquire these fatty acids by consuming a variety of plants or by eating the meat of animals that have consumed these plant fats. These Essential Fatty Acids (EFAs) have been given the terms Omega-3 (ω3) and Omega-6 (ω6) to identify the location of the last double bonds. Since these fatty acids are polyunsaturated, the terms n-3 PUFAs and n-6 PUFAs are applied to omega-3 and omega-6 fatty acids respectively. Linoleic acid is an omega-6 fatty acid because it has a double bond six carbons away from the last "omega" carbon **(Figure 5).** Similarly, alpha – linolenic acid is an omega-3 fatty acid because it has a double bond three carbons away from the last "omega" carbon **(Figure 6).**

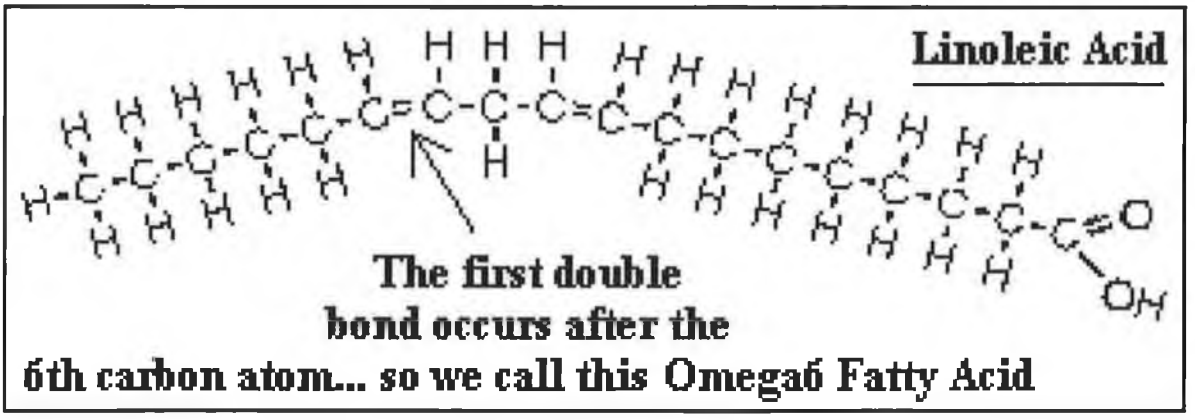


Figure 5. Structure of an omega-6 fatty acid.

http://www.cyberlipid.org/fa/acid0001.htm

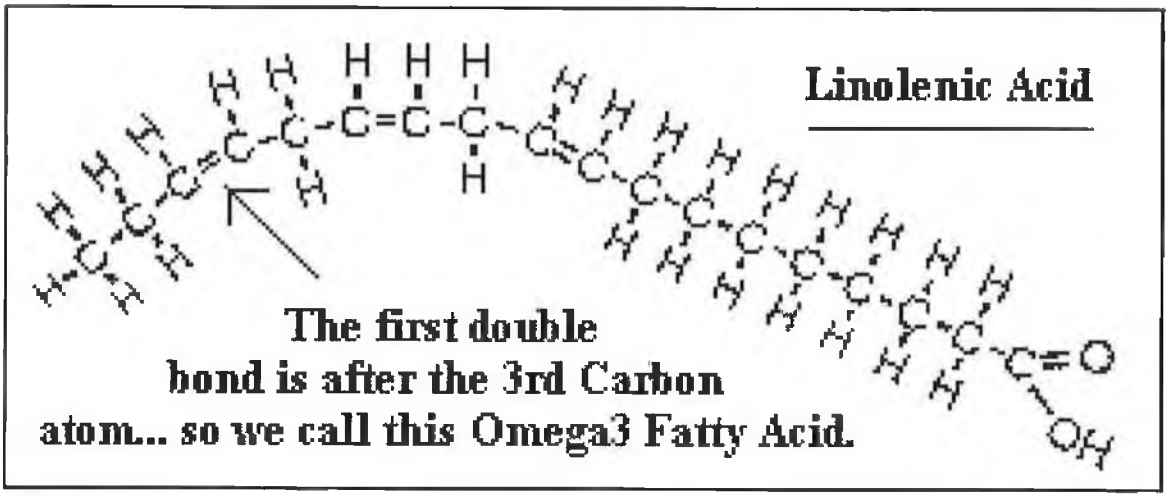


Figure 6. Structure of an omega-3 fatty acid.
http://www.cyberlipid.org/fa/acid0002.htm

Arachidonic acid C20:4(ω6), **(Figure7)**, and docosahexaenoic acid C22:6(ω3) are both crucial to the optimal development of the brain and eyes **(Neuringer et al,. 1988)**. The importance of arachidonic acid (AA) and docosahexaenoic acid (DHA) in infant nutrition is well established, and both substances are routinely added to infant formulae.
Excessive amounts of omega-6 polyunsaturated fatty acids and a very high omega-6 / omega-3 ratio have been linked with the occurrence of many diseases, including cardiovascular disease, cancer and inflammatory and autoimmune diseases **(Neuringer et al,. 1988)**.
The ratio of omega-6 to omega-3 in modern diets is approximately 15:1, whereas ratios of 2:1 to 4:1 have been associated with reduced mortality from cardiovascular disease, suppressed inflammation in patients with rheumatoid arthritis, and decreased risk of breast cancer (http://www.en.wikipedia.org).

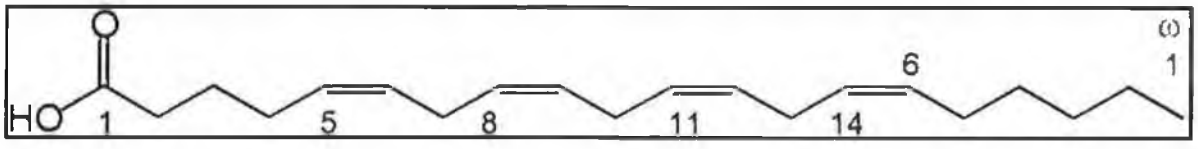


Figure 7 Structure of Arachidonic acid. http://en.wikipedia.org/wiki/Arachidonic_acid

Arachidonic acid (20:4 ω-6) is a major constituent of membrane lipids in humans and is the principal precursor by enzymatic action of hormone-like compounds known as eicosanoids including the prostaglandins.

The omega-3 (n-3) polyunsaturated fatty acids (PUFA) in fish lipids are of considerable dietary interest with respect to the prevention of circulatory ailments **(Goodnight et al., 1982, Kinsella, J.E., 1986)**. The two major n-3 PUFA from the fish species are eicosapentaenoic acid (EPA), C20:5n-3 and docosahexaenoic acid (DHA), C22:6n-3. Findings by **Monsen, E.R., (1985**) showed that marine finfish generally contained higher proportions of n-3 PUFA than freshwater fish. Monsen's studies showed that all freshwater species, with the exception of rainbow trout, were lower than 10% in n-3 PUFA. The highest proportions of n-3 PUFA in the marine finfish fatty acids were found in southern blue whiting (48.5%) and the lowest in orange roughy (0.9%). Lipid levels and fatty acid composition vary with species, sex, and age, season of the year, food availability, salinity and water temperature **(Monsen, E.R., 1985, Stansby, M. E., 1981)**. The oil content of orange roughy can be up to 18% of the total bodyweight, the majority of which is located in the swim bladder, head, frame and skin. Muscle from the orange roughy can contain up to 10% of the total lipid **(Busson et al., 1982).**

The muscle lipids from orange roughy in the Southern hemisphere are comprised predominantly of wax esters rich in monounsaturated fatty acids and alcohols **(Busson et al., 1982)**. In contrast, according to **Sargent et al., (1977)** the fatty acid moieties of marine wax esters are usually dominated by polyunsaturated fatty acids (dietary-derived) and the fatty alcohol components are invariably saturated or monounsaturated. **Sargent et al., (1977)** and **Sargent et al., (1983)** suggested that the monounsaturated alcohols were produced by de novo biosynthesis, either by chain elongation and desaturation of shorter chain moieties of dietary origin, or through direct dietary uptake in the form of the acid followed by reduction or as the alcohol.

Triglycerides

The most abundant lipids are the neutral fats or triglycerides. These are the most important storage form of chemical energy in animals and the major fuels for most organisms **(Lehninger, A.L., 1983)**. Adipose tissue is approximately 90% triglycerides.

Phospholipids

Phospholipids differ from triglycerides in that they possess one or more highly polar head groups in addition to their hydrocarbon tails and are the major components of membrane lipids. They serve primarily as structural elements of membranes and are never stored in large amounts. They contain phosphorus in the form of phosphoric acid groups and the major phospholipids found in membranes are the phosphoglycerides **(Figure 9),** which contain two fatty acid molecules esterified to the first and second hydroxyl groups of glycerol. The third hydroxyl group of glycerol forms an ester linkage with phosphoric acid. Phosphoglycerides also contain a second alcohol which is also esterified to the phosphoric acid. Thus the second alcohol group is located on the polar head of the phosphoglyceride molecule. There are several different types of phosphoglycerides which differ in their head alcohol group but they all contain two nonpolar tails and long chain fatty acids. The most abundant fatty acids found in phosphoglycerides have sixteen to eighteen carbons and usually one of the fatty acids is saturated and the other is unsaturated **(Lehninger, A.L., 1983)**. Phospholipids are usually more unsaturated (and have a higher iodine value) than triglycerides, store more of the PUFA and are more prone to deterioration. Their main role being in membrane structure, they do not show the variation that triglycerides do from season to season and diet to diet. They are essential to membrane structure. Herring, for example, in the North Atlantic Ocean, has a fat level of 23% after the seasonal period August/September. They use up the storage triglycerides, approximately 20%, over the winter and have a minimum fat level after spawning in the spring (March/April) of 3 – 4%. The common mackerel is similar. http://en.wikipedia.org/wiki/Phospholipid

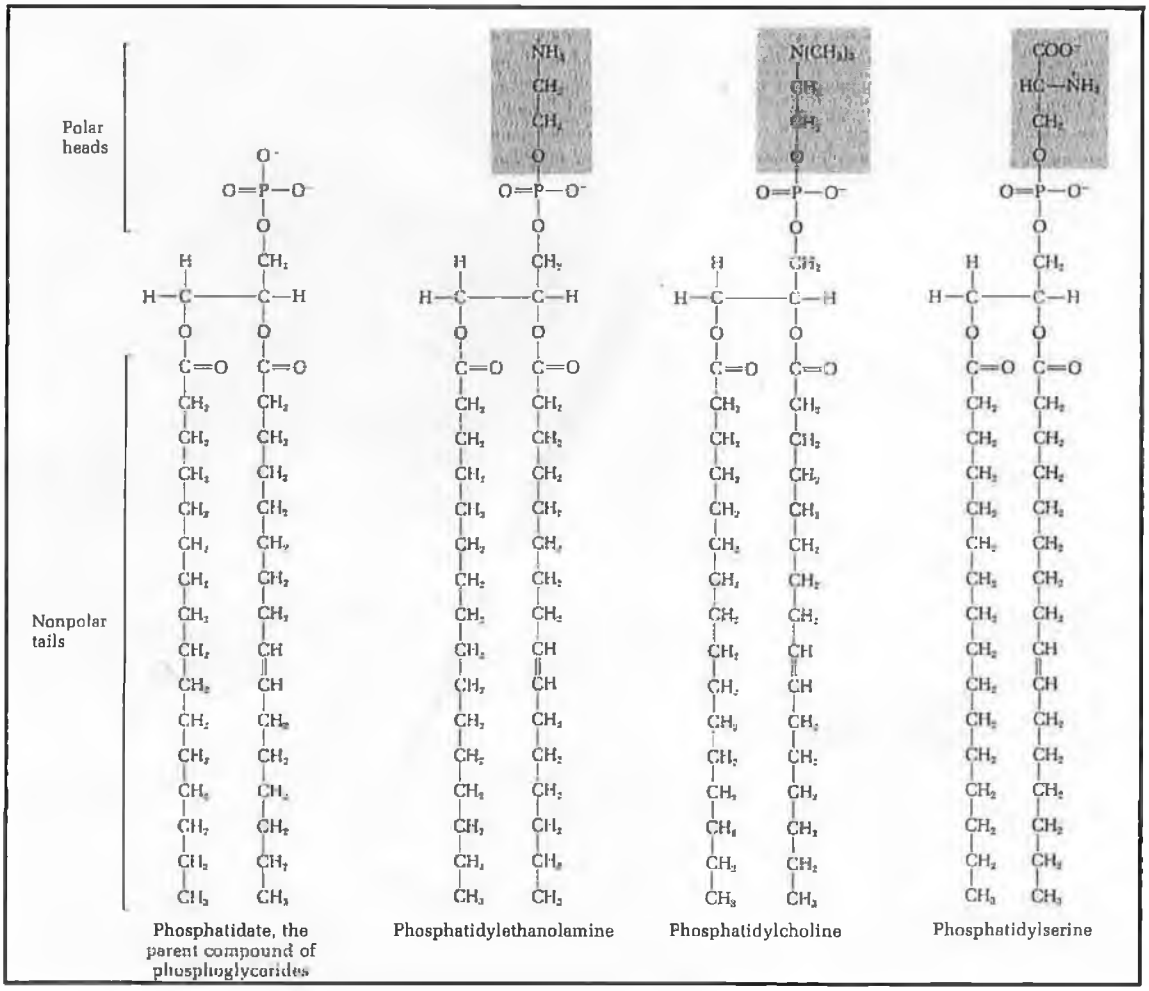


Figure 8. Structure of Phosphoglycerides. (Lehninger..A.L., 1983).

For a long time, phospholipids were merely regarded as building blocks for the biological membrane. But, in the mid 1970s, it was discovered that they participate in the transduction of biological signals across the membrane. An example of this is when the hormone glucagon/epinephrine is bound to its receptor on the plasma membrane of a liver cell, the binding sets off a cascade of reactions which result in enhanced breakdown of glycogen in the liver cell, thus producing more glucose.

Studies by **Ackman (1967)** have shown that there is an inverse relationship between N-3 fatty acid content and the total fat content of species. The phospholipids proportion of fish tissue stays more or less constant. The variation in the fat content is related to the triglycerides and fish with higher fat content contain a higher amount of triglycerides

than lean fish. Lipid can be regarded as an energy reserve when it forms more than 0.5 – 1% of the fresh muscle weight.

Waxes

Waxes are esters of long-chain saturated and unsaturated fatty acids, having from fourteen to as many as thirty-six carbon atoms, with long-chain alcohols, having from sixteen to twenty-two carbon atoms **(Figure 9)**.

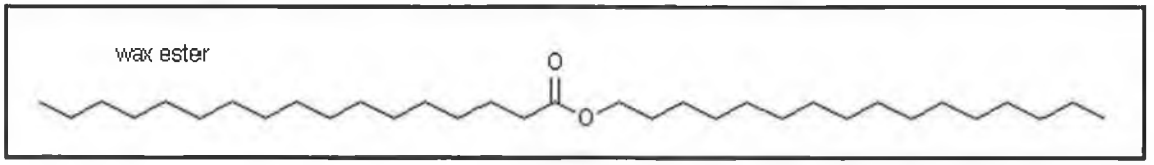


Figure 9. Structure of a wax ester

http://www.lipidlibrary.co.uk/Lipids/waxes/index.htm

Technologists use the term for a variety of commercial products of mineral, marine, plant and insect origin that contain fatty materials of various kinds. The nature of the lipid constituents as well as the chain-length and the degree of unsaturation and branching of the aliphatic chain varies greatly with the source of the waxy material. In vertebrates, waxes are secreted by skin glands as a protective coating, to keep the skin pliable, lubricated and waterproof. The leaves of many plants are coated with a protective layer of waxes. Waxes are also formed and used in very large amounts in marine life, especially in planktonic organisms, in which wax serves as the chief storage form of calorific fuel. The lipid components of planktonic organisms are the major metabolic fuels in the sea. Various studies carried out on the biochemical composition of planktonic organisms have given valuable information on the amount of lipid, the fatty acid composition and the different lipid components of zoo-and phytoplankton **(Corner, E.D.S., & Lowey, C.B., 1968, Chuecas, L., & Riley, J.P., 1969, Culkin, F., & Morris, R.J., 1969)**. The role of wax esters is not well understood at present but the finding of large amounts of wax in a tissue equivalent to the swimbladder suggests that one function might be to provide buoyancy for the fish, as the fish lives at depths of 1000m or more where the low dissolved gas concentration and the extreme pressures would make lipid

an ideal buoyancy agent **(Grigor et al., 1990)**. Research on the anatomy and feeding behaviour of sperm whales have shown that about 90% of the weight of the head is made up of the spermaceti organ which is located above the long upper jaw. This is a blubbery mass consisting of some muscles surrounded by oily connective tissue. This organ contains up to four tons of spermaceti oil which is a mixture of waxes containing an abundance of unsaturated fatty acids. It is liquid at the normal resting body temperature of the whale, about 37°C, but it begins to crystallise at about 31°C and becomes solid when the temperature drops several more degrees. The probable biological function of spermaceti oil has been deduced from studies of the anatomy and feeding behaviour of the sperm whale. Sperm whales feed almost excessively on squid in very deep water. In their feeding dives, they remain submerged for some fifty minutes and they require only ten minutes on the surface to replenish their oxygen supply and blow off CO_2. Their feeding dives can be from one thousand up to recorded depths of three thousand metres and they remain at these depths for up to 75% of their diving time. For a marine animal to remain at a given depth, it must have the same density as the water surrounding it, and to make this possible, some species have a swim bladder filled with air or nitrogen, others store body fat which has a lower density than seawater. But the sperm whale is capable of changing its buoyancy, keeping it neutral on tropical ocean surfaces as well as at great depths where the water is much colder and has a greater density. The key to the sperm whale's ability to change its buoyancy is the freezing point of spermaceti oil. When the temperature of liquid spermaceti oil is lowered several degrees during a deep dive, it crystallises and becomes more dense, thus changing the buoyancy of the whale to match the density of seawater at great depths **(Wittenberg et al., 1980)**. During the return to the surface, the congealed oil is warmed again and melted, decreasing its density to give it neutral buoyancy equal to that of the surface water. The triglycerides synthesised by the sperm whale contain fatty acids of the proper chain length and degree of unsaturation to give the spermaceti oil the ideal melting point, thus the animal can remain at great depths to feed with minimum expenditure of energy, without the constant swimming effort that would otherwise be required **(Lehninger, A.L., 1983)**. Wax esters may have a similar role in other deep sea fish such as orange roughy. Studies of orange roughy in the Southern Hemisphere have shown that several tissues contain large amounts of lipid, mainly as wax esters. These include a lipid rich swimbladder, the skin, the skeleton and a

fatty tissue located in the neurocranial cavity **(Buisson et al., 1982, Sargent et al., 1983)**. Studies have shown that each of these tissues can synthesise lipid and incorporate long chain fatty acids into lipids including wax esters **(Grigor et al., 1990)**. Metabolic studies suggest that there may be a turnover of wax esters and the wax esters may act as energy reserves as do triglycerides in other fish **(Phleger, C.F., 1988).** Alternatively, it has been suggested that they may have a role in determining the buoyancy of the fish **(Phleger, C.F., & Holt, R.B., 1973)**. Intraneurocranial fat deposits in orange roughy were found to account for 4% of the total lipid of the fish. The skull and the spine together were shown to comprise of about 8% of the total body lipid. The two major lipid containing fractions were the flesh, which includes the muscle and the skin, and the viscera, where the swimbladder probably accounts for at least 50% of the lipid **(Grigor et al., 1990)**. Similar to studies carried out on the sperm whale, observations suggest that the lipids of orange roughy do modify the buoyancy of the fish at the surface. There is a difference of 8°C between the temperature of the water at the surface and that at 1000m depth and the properties of the lipid change between these two temperatures. A number of other deep sea fish have been shown to have an oxygen filled swimbladder that is also rich in lipids **(Patton, S., & Thomas, A.J., 1971, Phleger, C.J., & Benson, A.A., 1971)**. The membrane lipids, which are composed primarily of cholesterol, phospholipid and protein, form a mass of bilayered membrane **(Phleger, C.J. & Holt, R.B., 1973, Josephson et al., 1975)**. This lipid may either facilitate oxygen filling at great pressures or provide a barrier to oxygen diffusion out of the swimbladder **(Scholander, P.F., 1954)**. It is possible that the wax esters in orange roughy might also function as an energy store. Wax esters figure prominently in the marine food chain being synthesised by the calanoid zooplankton where they appear to act as an energy store. Most animals consuming these calanoid copepods can also convert the alcohol to fatty acids and store triglycerides **(Sargent et al., 1977)**. Studies carried out on the occurrence of wax esters in the tissues of the orange roughy showed that the skin and skeleton has a lipid content of 20% or greater with the swim bladder containing over 60% lipid. The lipid content of the other tissues such as the muscle, liver, testes and roe were lower, ranging from 2 – 5% Tissues with the greatest lipid content had over 90% of their lipid as wax esters, whereas in the other tissues, the wax ester content was 20% or less of the total lipid. Of the tissues examined, the muscle was the only exception to this pattern where despite a lower lipid

content, the lipid was mainly wax esters. The lipids of the liver, roe and testes differed from those of other tissues in that they contained appreciable concentrations of triacylglycerides, nonesterified fatty acids, cholesterol and phospholipids. As nonesterified fatty acids do not occur in nature in high concentrations in normal tissues, the levels found in liver and to a lesser extent in roe, suggest that they may result from lipolysis during storage and thawing. Hydrolysis of lipids during storage of fish tissues had been reported previously **(Ackman, R.G., 1976, Grigor et al., 1990)**. Limited studies carried out on orange roughy caught in deep waters off the west coast of Britain have shown them to be similar to those caught in deep waters off New Zealand, in that both species have copious amounts of wax esters rich in 20:1 (n-9) and 22:1 (n-11) fatty alcohols and fatty acids **(Mori et al., 1978, Hayashi, K., & Takagi, T., 1980, Buisson et al., 1982)**. The head region of orange roughy caught in the North East Atlantic, has been found to contain the greatest percentage, about 40% of the total oil in the fish **(Buisson et al., 1982)**. This oil is likely to be derived from the cranial cavity and is also partly from the swim bladder which extends forwards towards the rear of the fish's cranium. This wax rich oil is particularly valuable due to it being a highly acceptable substitute for sperm whale oil **(Buisson et al., 1982)**.

Sphingolipids

Sphingolipids are the second largest class of membrane lipids and like phospholipids, they also have a polar head and two non-polar tails but they contain no glycerol. They are composed of one molecule of a long-chain fatty acid, one molecule of the long-chain amino alcohol sphingosine or one of its derivatives, with a polar head alcohol **(Figure 10)**.

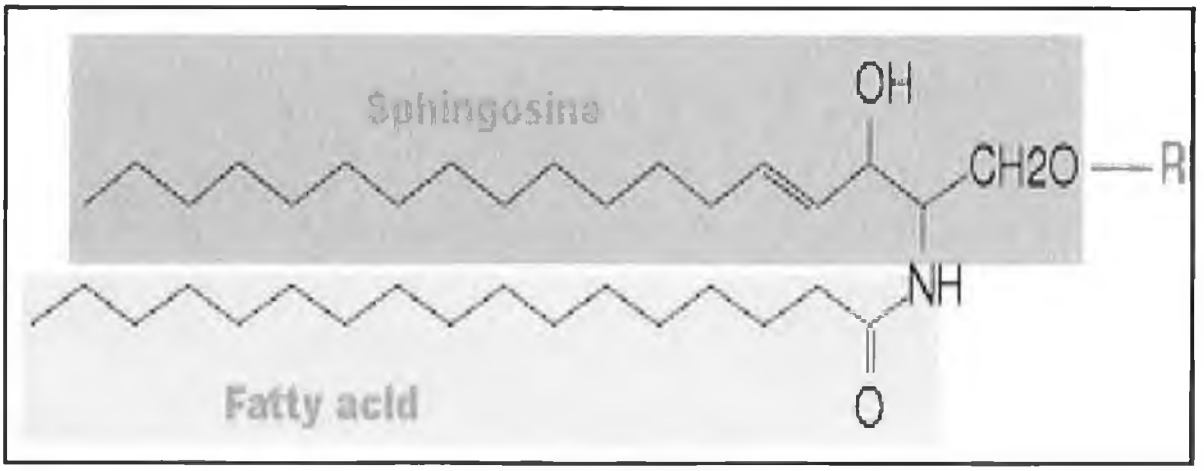


Figure 10. Different substitutes (R) give H- ceramide, phosphocholine-sphingomyelin, sugar(s) and glycosphingolipid(s).
http://en.wikipedia.org/wiki/Sphingolipid

There are three main types of sphingolipids: ceramides, sphingomyelins, and glycosphingolipids, which differ in the substituents on their head group. Ceramides are the simplest type of sphingolipid. They consist simply of a fatty acid chain attached through an amide linkage to sphingosine. Sphingomyelins have a phosphorylcholine or phosphorylethanolamine molecule esterified to the 1-hydroxy group of a ceramide. Glycosphingolipids are ceramides with one or more sugar residues joined in a β-glycosidic linkage at the 1-hydroxyl position. Sphingolipids are commonly believed to protect the cell surface against harmful environmental factors by forming a mechanically stable and chemically resistant outer layer on the plasma membrane lipid bilayer. They are also located throughout the body in nerve cell membranes and make up about 25% of the lipids in the myelin sheath which surrounds and insulates cells of the central nervous system.

Sterols

Sterols or steroid alcohols are a subgroup of steroids with a hydroxyl group in the 3-position of the A-ring. Chemically these sterols have the same basic structure as cholesterol **(Figure 11)**. They are amphipathic lipids synthesised from acetyl-coenzyme

A. The hydroxyl group on the A ring is polar and the rest of the aliphatic chain is non-polar.

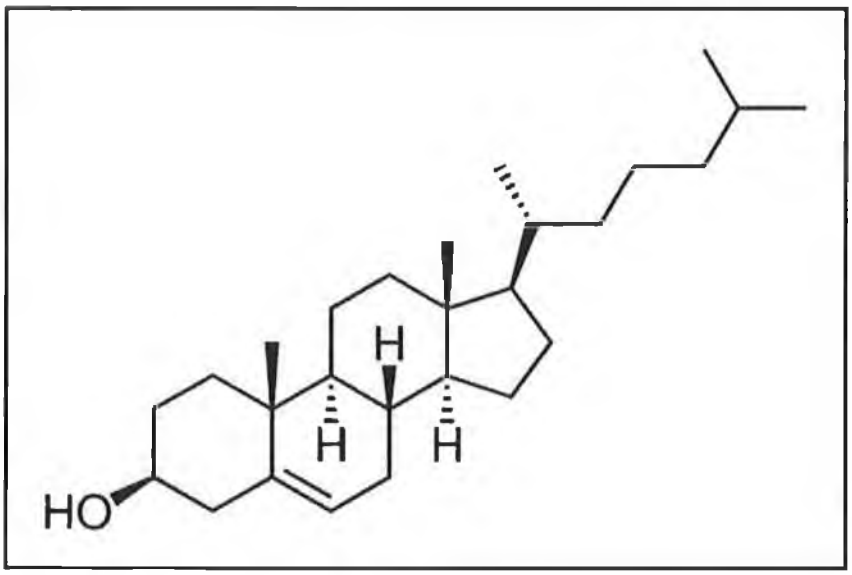


Figure 11. Structure of Cholesterol

http://en.wikipedia.org/wiki/Image:Cholesterol.svg

This hydroxyl group is often esterified with a fatty acid like, for example, cholesterol esters. The hydocarbon chain of the fatty-acid subsistent varies in length, usually from sixteen to twenty carbons and can be saturated or unsaturated. They commonly contain one or more double bonds in the ring structure and also have a variety of substituents attached to the rings. Sterols of plants are called phytosterols and the most common sterol in animals is called cholesterol. They play essential roles in the physiology of eukaryotic organisms. For example cholesterol forms part of the cellular membrane where it modulates the cell membrane's fluidity and function and serves as a secondary messenger in developmental signaling. Plant sterols are also known to block cholesterol absorption sites in the human intestine thus helping to reduce blood cholesterol in humans. http://en.wikipedia.org/wiki/Sterol

Cholesterol is required in the membranes of mammalian cells for normal cellular function, and is either synthesised in the endoplasmic reticulum, or derived from the diet, in which case it is delivered by the bloodstream in low-density lipoproteins (LDL). High density lipoprotein (HDL) transports cholesterol back to the liver for excretion. It has been established that blood cholesterol, particularly low density lipoprotein (LDL)

cholesterol, should be controlled in the body in order to minimise the risk of coronary heart disease. Excess LDL cholesterol in the diet can result in a build up on artery walls, resulting in the restricting of blood flow and elevating blood pressure, thereby increasing the risk of coronary heart disease which can lead to a heart attack or a stroke. It is desirable therefore that dietary intake of cholesterol be restricted, if necessary and/or protective factors known to lower blood LDL cholesterol be increased if possible.

2.2 ASSESSMENT OF NUTRITIONAL COMPOSITION

2.2.1 Moisture Content.

Water is the main constituent of fish tissue. The water content of fish muscle and the water holding capacity are important quality parameters influencing the texture, juiciness and overall impression of the fish product.

Gravity drip-loss refers to the natural exudates or drip that comes from fillets and seafood products. Excessive weight loss due to drip has been reported sporadically, but fish stored under traditional methods have drip levels ranging from 3 to 8% **(Boknaes et al., 2002)**. Drip and moisture loss is of economic significance, as it not only affects the weight of the final product (and thus the economic gain) but also the perceived freshness of the product. The ability of the fish to retain their intrinsic fluids is of major importance to their commercial value and consumer acceptance. Freezing can cause tissue damage, which results in drip during thawing. Moisture loss from the flesh of fish is due primarily to the reduction of the moisture binding capacity of the protein, when the acidity of the muscle is increased by lactic acid formation **(Boknaes et al., 2002).**

Faster freezing rates mean smaller ice crystal formation, so less damage occurs in the cell structure. Therefore, when a product is thawed, there is less drip. **Nilsson, K., & Ekstrand, B., (1994)** also found that the fast freezing of rainbow trout resulted in lower drip-loss than slow freezing. The fast frozen trout had a drip of 8.4% while the slow frozen trout had a drip of 9.9%.

Studies carried out on the nutritional composition of fresh fish have recorded moisture values of 68-82% **(Beklevik, G et al., 2005, Khan et al., 2006 & Perez., M.A., 1994)**. Also previous studies carried out on the moisture content of orange roughy by **Buisson et al., (1982)** have recorded average values of 75% $^{w}/_{w}$

The water content of the fish tissue can be determined by drying in an oven and measuring the loss in weight according to the AOAC official method.

2.2.2 Protein Content.

Determination of the protein content of fish can be carried out by the Kjeldahl procedure. This method of protein determination is the legally accepted method adopted by most governments (www.unitedstatesfoodanddrugadministration /protein analysis). This method is used for either solid or liquid samples where the protein present may be partially soluble and partially insoluble. Insoluble components rule out colorimetry. This technique involves digestion of the sample by heating with concentrated sulphuric acid, which results in the reduction of organic nitrogen to ammonia.

The ammonia is trapped in the digest as ammonium sulphate and is released by steam distillation in alkaline conditions. The liberated ammonia is distilled over into boric acid and trapped as ammonium borate. The distillate is then titrated to determine the nitrogen content of the sample. A conversion factor of 6.25 is then used to determine the protein content of the sample. **(Egan et al., 1981)**

The process can be shown as follows:

Protein $\xrightarrow{\text{Conc. } H_2SO_4 \text{ Digestion}}$ $(NH_4)_2SO_4$

$(NH_4)_2SO_4$ $\xrightarrow{NaOH}$ NH_4OH

Boric Acid + Indicator + NH_3 (pH 5) $\rightarrow$ **Ammonium borate/ Indicator** (pH >>5)

Back Titration

Ammonium borate/indicator + $H_2SO_4 \rightarrow (NH_4)_2SO_4$ +Boric Acid + Indicator
(pH>> 5) (pH ≈ 5)

The advantage of this method is that it can be applied to both insoluble and soluble proteins. The disadvantages of this method are that it is extremely slow, tedious and can be dangerous. The amount of protein in fish muscle has been recorded to be somewhere

between 15-20% **(Mc Cance & Widdowson, 1991)**. Orange roughy caught in waters off New Zealand were found to have protein averages of 12-13% **(James et al., 1986)**.

2.2.3 Lipid Content.

Fish can be classified as oily or non-oily. Non-oily, 'lean' fish contain 0.5-1.5% lipid in the muscle tissue which exists mainly as phospholipids and lipoproteins. Such fish store additional lipid as triglyceride in the liver and under the skin. The tissue of oily fish e.g., herring, may contain up to 25% lipid, which is distributed under the skin and throughout the fish tissue, mainly as triglycerides. **(Allen, J.C., & Hamiliton, R.I., 1995)**

Previous studies carried out on the lipid content of orange roughy have given values ranging from 5-20%, depending on which waters the fish were caught in and if the whole fish was analysed or the skinless fish muscle **(Buisson et al., 1982, Grigor et al., 1990, Sigurgislanōttir, S. U., & Pūlmadōttir, H., 1993, Mc Clutchie, S., & Ye, Z., 2000, & de Koning, A.J., 2005).**

Lipids are nutritionally important as a source of energy, essential fatty acids and fat-soluble vitamins.

Most methods of measuring the fat content of biological tissue samples depend on extracting the fat by dissolving it in a suitable solvent. When extracting lipids or fats from foods both the method as well as the solvents chosen to perform a complete, or close to complete, extraction are important. If these two elements are not taken into consideration, the extraction may not be complete, or the extract may contain a large quantity of undesired impurities. As lipids are relatively non-polar molecules, they can be pulled out of a sample using relatively non-polar solvents. With a non-polar solvent, only non-polar molecules in the sample dissolve while polar ones do not. Problems arise however, in cases where lipids are bound in animal or plant cell membranes. Animal and plant cell membranes are made up of molecules that have both polar and non polar regions such as sphingolipids and phospholipids. These molecules end up grouping together with their polar heads sticking outwards and non-polar tails inwards, making it difficult for non-polar solvents to interact with the non-polar tails and extract them. As these molecules are part non-polar and part polar, a solvent that presents some of these same characteristics is required. This is why a mixture of two solvents such as methanol and chloroform is used, as is the case of the Folch Extraction Method to extract the polar

mixture. **(Folch, J., Lees, M., & Stanley, G.H.S., 1957, Bligh, E.G., & Dyer, W.H., 1959)**

In order to determine the lipid content of a fish sample, the lipids present must first be extracted. Soxlet extraction with petroleum ether results in the extraction of free lipids only **(Bailey, A.J., 1983)**. Polar solvent mixtures (Chloroform:Methanol 2:1 v/v), Folch Extraction, must be used to obtain total lipid extraction. The Folch Extraction method for lipid extraction, which is the chosen extraction method for this study is a gravimetric method first used in 1959. It has the advantage that you do not need to dry the sample in advance.

After extraction, the lipid is obtained by evaporation of the solvent in a fume hood overnight. The methanol is polar enough to interact with the polar regions and help "pull apart" the cell membrane while also being non-polar enough to help in extracting non-polar fats these being soluble in chloroform.

2:2:4 Separation of Lipids into separate groups.

According to **Christie, W.W (1993,b)**, the total lipid extracted can be composed of a mixture of triglycerides, waxes, sterols and their esters, phospholipids, sphingolipids, glycolipids, terpenes, steroids, fat soluble vitamins (A,D,E etc) and in ratios that vary widely from species to species, tissue to tissue and organ to organ. Within each group the fatty acid components can be very unevenly dispersed and their roles can be dramatically different. Therefore, it may be desirable to separate the fat types, prior to assessing their nutritional quality.

The use of a silica gel column in a disposable Pasteur pipette is a simple procedure widely used for isolating groups of lipids on a small scale (0.5-1.0g of silica gel). The simplest lipids are eluted with chloroform or diethyl ether, acetone is used for the elution of glycolipid and methanol elutes phospholipid from the column. **(Christie, W.W., 1993,b)**.

Other studies suggest that a similar adsorption column chromatographic procedure using silica gel G as the adsorbent affords complete separation of all major lipid classes except diglycerides.(www.lipidlibrary.co.uk) It also has the advantage that the only pre-treatment required is adjustment of moisture content. This is a particularly useful method

for separating serum and tissue lipids. The lipid components of the whole serum are first extracted with chloroform/methanol (2:1 v/v).

Separation into individual fractions containing hydrocarbons (1), cholesterol ester (2), triglycerides (3), cholesterol (4), free fatty acids (5) and phospholipids (6) is achieved by elution at room temperature with five to ten column volumes of petroleum ether (1), five to ten column volumes of 6% diethyl ether in petroleum either (2), five to ten column volumes of 10% ethyl acetate in petroleum ether (3,4), 50 ml diethyl ether (5) and ten to twenty column volumes of methanol / acetic acid / water (8:1:1v/v). Lipids can then be identified and their purity confirmed by the use of Silica Gel G thin-layer adsorption chromatography. The chromatoplates can be developed with a mixture of either 5% diethyl ether in petroleum ether, for neutral lipids or chloroform / methanol / water (65:2:4v/v) for separating phospholipids and can be sprayed with 50% sulphuric acid and charred in an oven or on a hot plate to detect the organic molecules. The qualitative composition of the lipids in a total lipid extract can be assessed by direct Thin Layer adsorption chromatography on Silica Gel G in such a manner also, without using any column chromatography.

2:2:5 Fatty Acid Composition of the extracted lipids. (F.A.M.E. Production and assessment by G.C).

Fatty acids can occur in nature in the free (unesterified) state but they are most often found as esters which are linked to glycerol, cholesterol or long chain alcohols and as amides in sphingolipids.

The technique of gas chromatography has revolutionised the study of lipids by making it possible to determine their complete fatty acid composition in one step (**Christie, W.W., 1989**). For this purpose, the fatty acid components of lipids are converted to the simplest derivative usually methyl or ethyl esters. Several esterification and transesterification procedures exist. None is ideal, all have advantages and disadvantages, hence the multiplicity of procedures still in use.

Most commonly fatty acids are combined in more complex molecules such as triglycerides, cholesterol esters or waxes and must first of all be liberated into free fatty acids prior to esterification. This can be achieved by either saponification or acidic

hydrolysis and then derivatisation into fatty acid methyl esters (FAMES). FAMES may also be obtained directly by transesterification in an alcoholic KOH solution. Saponification involves the use of an alkaline reagent such as methanolic potassium hydroxide or potassium t-butoxide to produce a fatty acid soap as shown in **Figure 12.** Potassium t-butoxide in methanol is a much stronger alkali than KOH and cleaves more resistant ester linkages therefore it should achieve complete hydrolysis of the more resistant waxes into free fatty acid soaps which can then be extracted into heptane after acidification.

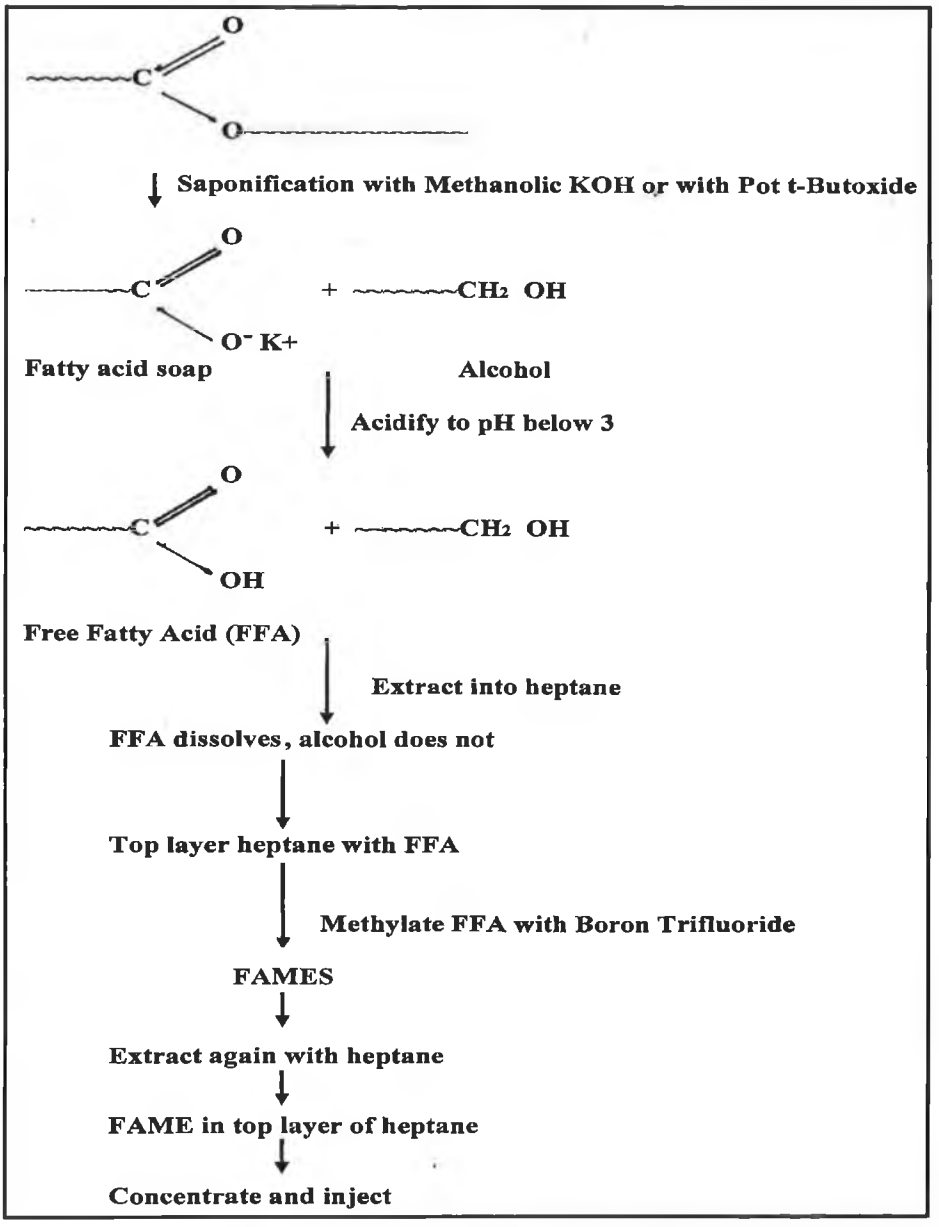


Figure 12. Production of FAMES

Boron Triflouride is commonly used to produce FAMES as it is one of the few such reagents that can be obtained commercially. The reaction can be completed in two minutes under reflux. However, as the reagent has a limited shelf life at room temperature and there is some evidence that artefact formation is likely with aged reagents it is recommended that boron trifluoride be stored under refrigeration **(Christie, W.W., 1993b)**.

Transesterification is the process of exchanging the alkoxy group of an ester compound with another alcohol typically methanol. Carboxylic acids i.e. free fatty acids can be esterified by alcohols in the presence of a suitable acidic catalyst, such as methanolic hydrogen chloride as shown in **Figure 13 (Christie, W.W., 1993b)**.

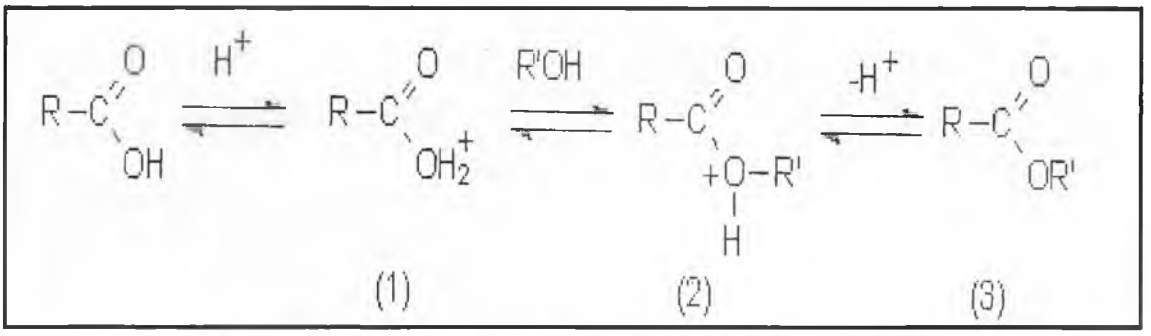


Figure 13. Acid-catalysed esterification of Fatty Acids

The initial step is protonation of the acid to give an oxonium ion (1) which can undergo an exchange reaction with an alcohol to give the intermediate (2). This in turn, can loose a proton to become an ester (3). Each step in the process is reversible but in the presence of a large excess of the alcohol (R'OH), the equilibrium point of the reaction is displaced so that esterification can proceed to completion. The presence of water would prevent esterification as it is a stronger electron donor than alcohol and therefore would prevent the formation of the intermediate. The preferred conditions for acid catalysed esterification is the presence of a large excess of the appropriate alcohol and the absence of water.

A typical esterification procedure, and the one used finally in this study, uses methanolic hydrogen chloride which is prepared by adding acetyl chloride to a large excess of dry methanol as shown in **Figure 14**. **(Christie, W.W., 1993,b).**

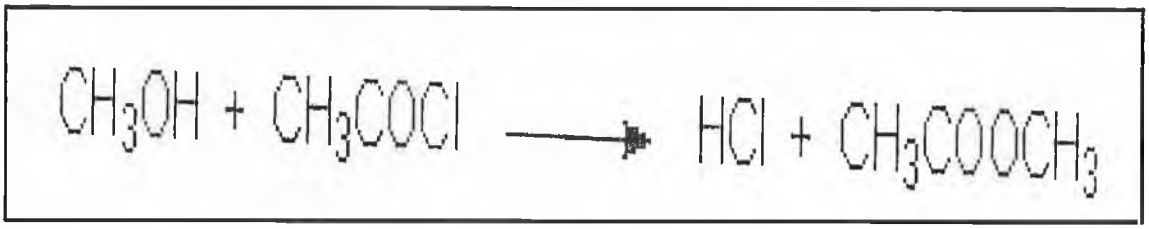


Figure 14. Preparation of methanolic hydrogen chloride via acetyl chloride.

Although methyl acetate is formed as a by product, it does not interfere with the reaction. The lipid sample must be dissolved in at least one hundred fold molar excess of this reagent to ensure the production of FAMES goes to completion.

Previous studies carried out on the fatty acid composition of orange roughy have shown that the fatty acid composition varies depending on where the fish were caught and the extraction techniques used (Table 1A). **(Bakes et al., 1995, Murray et al., 1983, Sargent et al., 1983, Sigurgisladōttir, S., & Palmadōttir, H., 1993 & Vliey, P., & Body, D.R., 1988)**. These results show that monounsaturated fatty acids account for most of the fatty acids present with the monounsaturates 18:1, 20:1 and 22:1 present in the highest concentration. Notably there are very low percentages of the nutritionally valuable PUFAs present. In fact in all, but one, of the studies the fatty acid Docosapentaenoic acid (DPA) was undetected and Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) were detected in very small amounts (Table 1A). In contrast common fish, for example herring and sardines contain relatively high concentrations of these PUFA (Table 1B) and therefore they are much more valuable nutritionally than previously tested orange roughy **(Patterson, H.B.W., 1994, Sigurgisladōttir,S., & Palmadōttir, H., 1993 & Ziatanos,S., & Laskaridis, K.,2006).**

Table 1A AVERAGE PERCENTAGE COMPOSITION OF FATTY ACIDS IN ORANGE ROUGHY FROM 5 COMPARATIVE STUDIES

	Murray et al 1983 New Zealand	Sargent et al 1983 West Britain	Vlieg & Body 1988 New Zealand	Sigurgisladottir & Palmadottir 1993 Icelandic Waters	Bakes et al 1995 Tasmania	Bakes et al 1995 Nt. Atlantic
14:0	1.4	0.8	1.3	1.0	ND	1.5
15:0	ND	ND	0.2	ND	ND	ND
16:0	3.6	1.3	1.8	2.7	2.8	2.1
17:0	ND	ND	0.1	ND	ND	ND
18:0	1.3	0.4	4.6	1.0	ND	ND
Σ Saturates	6.3	2.5	8.0	3.7	2.8	3.6
14:1	0.5	ND	1.3	ND	ND	ND
15:1	ND	ND	Tr	ND	ND	ND
16:1	11.0	8.8	9.0	7.2	8.4	6.5
17:1	ND	ND	0.6	ND	ND	ND
18:1	51.7	48.0	43.7	34.1	61.9	32.8
20:1	18.4	21.8	26.0	26.8	17.2	22.1
22:1	7.8	16.5	2.1	13.6	9.1	32.8
Σ Monounsaturates	89.4	95.1	82.7	81.7	96.6	94.2
18:2	NA	NA	Tr	1.3	ND	ND
18:3	NA	NA	ND	0.7	ND	ND
20:4	NA	NA	ND	0.3	ND	ND
20:5	NA	NA	0.2	1.0	0.7	0.7
22:5	NA	NA	Tr	ND	ND	ND
22:6	NA	NA	0.7	2.3	ND	1.6
Σ PUFA			0.9	5.6	0.7	2.3

ND: Not Detected; Tr: Trace; NA: Data not Available

Table 1B AVERAGE PERCENTAGE COMPOSITION OF FATTY ACIDS IN OILS OF COMMON FISH

	HERRING Patterson 1994	Cod Liver Oil Sigurgisladottir & Palmadottir 1993	SARDINES Ziatanos&Laskaridis 2006	SALMON Sigurgisladottir & Palmadottir 1993
14:0	6.0	3.5	7.03	5.3
15:0	0.4	0.5	0.86	
16:0	11.0	10.4	23.22	13.6
17:0	0.3	0.1		
18:0	1.2	1.2	3.37	2.4
Σ Saturates	18.9	15.7	34.48	21.3
16:1	7.0		6.09	7.6
17:1	0.3			
18:1	10.0	19.6	9.38	22.9
20:1	13.0	14.6	1.21	11.4
22:1	21.0	13.3	0.82	7.3
Σ Monounsaturates	51.0	47.5	17.5	49.2
18:2	1.0	0.8	1.52	3.8
18:3	2.0	0.2	1.96	0.9
20:4	1.7	1.7	1.2	1.2
20:5	7.4	5.0	10.67	3.8
22:5	0.8	2.0	1.11	1.4
22:6	5.7	10.6	20.83	5.1
Σ PUFA	18.6	19.3	37.29	16.2

2:2:6 Assessment of the degree of unsaturation of lipid

The iodine number of a fat is defined as the number of grams of iodine, which will react, with the unsaturated linkages of the fatty acids, in 100g of the fat. Where there is a double bond in a sample, iodine will add across it. Thus, the iodine value is a measure of the degree of unsaturation in the sample.

Previous studies carried out on the extracted fats/oils of orange roughy have shown them to have a relatively low degree of unsaturation **(Bakes et al., 1995, Buisson et al., 1982, Sargent et al., 1883). Buisson et al., (1982)** reported these values to be between 73-89.

An excess of Wijs reagent is allowed to react with a known weight of fat in chloroform for a definite period and the iodine in the Wijs reagent will add across the double bonds. After the addition reaction is complete, the amount of unused iodine is estimated by titration with a standard solution of sodium thiosulphate. The same procedure is carried out on a blank and by subtraction, the amount of iodine which has added across the double bonds in the sample, can be measured. The reaction mixture must be kept in the dark and the titration carried out as quickly as possible since potassium iodide (KI) is oxidised by light to iodine. Starch (1%) is used as the indicator. This is known as an Iodometric titration.

The principle of the test is as follows:

Iodine is contained in the Wijs reagent and is made up in glacial acetic acid and a small quantity of carbon tetrachloride or chloroform.

i. Wijs reagent is added to a control/blank and to the test samples
ii. After 20-30 minutes, a period in which the iodine is guaranteed to fully react with the unsaturated fatty acids, an excess of potassium iodide (10%) and 50cm^3 of distilled water are added to all bottles
iii. The amount of un-reacted iodine left in the bottles dissolves in the water as I_3^- and is found by titration with standardised sodium thiosulphate using starch as an indicator.

The 10% KI and 50cm^3 of distilled water are added to the bottles to extract the un-reacted iodine from the organic phase into the aqueous phase.

I_2 (Wijs) + I^- (KI)	→	**I_3^- (soluble in water)**

The I_3^- has the same concentration as I_2 and this is titrated with 0.1 M Sodium thiosulphate

$I_3^- + 2Na_2S_2O_3 + K^+$	→	**$2NaI + Na_2^+S_4O_6^=$**

The Iodine value is a measure of the unsaturation so high values are expected in fish oils as they are rich in polyunsaturated fatty acids such as EPA and DHA (e.g. sardines). It can be used commercially to identify adulteration of oils with cheaper substitutes.

2.3 Spoilage of Fish

2.3.1. Bacterial spoilage

Like mammalian meats, fish spoil through the combined effects of chemical reactions, continuing activity of endogenous degradative enzymes and bacterial growth. Bacteria also produce significant quantities of proteolytic, lipolytic, oxidative and other enzymes, all of which also contribute significantly to the spoilage of the fish. Different bacteria have widely different enzymatic content and therefore give rise to very different types of spoilage. Fresh fish and other seafood products are highly susceptible to spoilage by post-mortem microbial growth and constituents (e.g. enzymes) resulting from microbial growth. Their low body temperature (after death) provides a natural environment for psychrotrophic spoilage microflora **(Davis, H.K., 1993)**.

It is generally recognised that the predominant spoilage organisms belong to the genus *Pseudomonas.* **Shewan, J.M.,(1971)** reviewed the literature on the microbiology of fish and fish products between 1930 and 1970 and concluded that the initial flora of fresh North Sea fish consisted mainly of *Moraxella, Arthrobacter*, *Pseudomonas, Flavobacterium/ Cytophaga, Acinetobacter* and *Micrococcus* but *Pseudomonas* became successively dominant during prolonged storage usually when fish was stored on ice between days 0 and 15.

Despite diverse initial microbial populations most bacterial spoilage in air results from Gram-negative psychrophilic organisms, with Pseudomonas species and *Alteromonas* species dominant. Once fish are filleted or made into products, spoilage accelerates, mainly due to the fact that more of the body surface is exposed to the air (aerobic spoilage) and cross contamination of the internal body surface occurs during fillet or mince preparation **(Shewan, J.M., 1971)**.

The effect of microbial activity on fresh seafood is the eventual breakdown of proteins as the tissues of the fish are colonised by successive bacterial strains. The rate of decomposition is influenced by the initial number and types of bacteria and storage conditions, such as temperature, humidity and gaseous atmosphere **(Emborg et al., 2002).** Fish initially contain significant levels of trimethylamine oxide, which has an osmoregulatory function in the live animal. Both *Pseudomonas* and *Alteromonas* species reduce trimethylamine oxide (TMAO) to trimethylamine (TMA), a fishy-smelling volatile base **(Shewan J.M., 1971)**. Ammonia is another undesirable volatile base that is produced by both of these species as a degradative product of non-protein-nitrogen (NPN), such as amino acids and creatine. Total volatile base

nitrogen (TVBN) is a comprehensive nitrogen value that includes all volatile nitrogen molecules including both TMA and ammonia **(Villemure et al., 1986)**. Ammonia (NH_3) is the dominant contributor in meat while TMA is often the major base in fish deterioration.

Microorganisms are found on all the outer surfaces (skin and gills) and in the intestines of live and newly caught fish. The total number of organisms vary enormously ranging in the order of 10^2-10^7 cfu (colony forming units)/cm^2 on the skin surface. The gills and the intestines both contain between 10^3 and 10^9 cfu/g **(Shewan, J.M., 1971).** The first stage of fish spoilage is dominated by endogenous enzymes, whilst the bacterial flora of the gut cavity, gills and skin acclimatise and adapt to the changing environmental conditions **(Church, I. J., & Parsons, A.L., 1995)**.

Specific "spoiler" types of bacteria produce S-containing and other odorous compounds. Volatile sulphur-compounds are typical components of spoiling fish and most bacteria identified as specific spoilage bacteria produce one of several volatile sulphides. *S. Putrefaciens* and some *Vibrionaceae* produce H_2S (Hydrogen sulphide) from the sulphur containing amino-acid L-cysteine **(Gram et al., 1987)**.

The composition of the microflora changes quite dramatically during storage. Thus, under aerobically iced storage, the flora is composed almost exclusively of *Pseudomos spp* and *S. putrefaciens* after 1-2 weeks. This is believed to be due to their relatively short generation time at chill temperatures **(Morita, R.Y., 1975, Devaraju, A. N., & Setty, T.M.R., 1985)**. *Pseudomonas* are psychrotrophic and are ideally suited to refrigeration temperatures. They produce off-odours and off-flavours by producing compounds such as dimethyl disulphide, dimethyl trisulphide, trimethylamine, dimethylamine, butyrate and hexanoate **(Miller et al., 1973, a, b)**. However, bacterial action also produced lower chain fatty acids (C_2-C_3) from carbohydrates, aldehydes and ketones from lipids, ammonia, amines and biogenic polyamines from amino acids and volatile sulphides from sulphur-containing amino acids **(Davis, H.K., 1993)**. Spoilage can be followed by microbial growth measurements or the detection of any one or more of these spoilage products.

2:3:2 LIPID OXIDATION.

In fatty fish, rancidity can be a problem, which can dramatically shorten the remaining shelf-life of the product. Lipids in most fatty fish, for example in the Atlantic mackerel (*Scomber scombrus*) and the Atlantic salmon (*Salmo salar*), readily undergo oxidation on exposure to the air, even in frozen storage. In fatty fish, depot fat occurs as extracellular globules in the muscle and mesentery. A variable but high proportion of the total lipids are neutral lipids (triglycerides) with substantial contents of unsaturated C-20 and C-22 fatty acids **(Flo et al., 1970)**. These acids are among the constituents of fatty fish, in free or combined forms, most susceptible to autoxidation. The oxidation products of these unsaturated fatty compounds and a number of minor lipid compounds contribute to the natural aromas, flavours and colour substances which make fish and fishery products attractive in minor proportions, but aesthetically objectionable if present in excess **(Ackman, R.G., 1967)**.

The polyunsaturated fatty acids found in fatty fish make them particularly susceptible to lipid degradation, which can severely reduce the shelf life of the product. Lipid oxidation in fish is one of the more important factors responsible for quality loss in refrigerated and frozen storage. The sequence of reactions occurring during the autocatalytic mechanism of fat oxidation can be seen in **Figure 15.** Lipid oxidation is initiated by abstraction of a hydrogen atom from the central carbon of the pentadiene structure found in most fatty acid acyl chains containing more than one double bond.

$$-CH=CH-CH_2-CH=CH- \rightarrow CH=CH-\underset{\bullet}{C}H-CH=CH- + H\bullet$$

Lipid radicals (L·) react quickly with oxygen molecules to produce highly reactive peroxy radicals (LOO·) which again may abstract a hydrogen from another acyl chain resulting in a lipid hydroperoxide (LOOH) and a new radical. This propagation continues until one of the radicals is removed by reaction with another radical or with an antioxidant (AH) whose resulting radical (A·) is much less reactive.

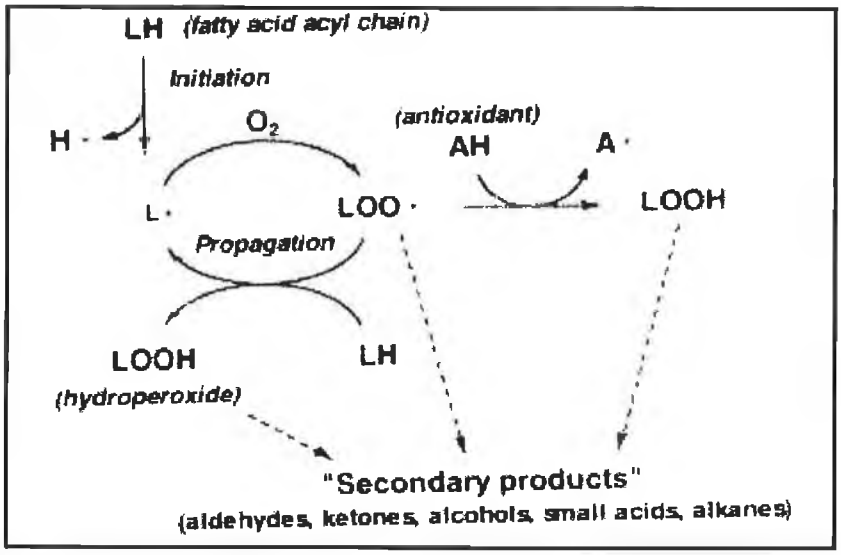


Figure 15. Autocatalytic mechanism for fat oxidation.

The hydroperoxides produced are broken down by a free radical chain reaction, which can be catalysed by heavy metal ions, to secondary autoxidation products of shorter carbon chain-length. Metal ions are very important in the first step of lipid autoxidation (the initiation process) in catalysing the formation of reactive oxygen species as for example hydroxyl radical (OH·). This radical immediately reacts with lipids or other molecules at the site where it is generated. The high reactivity may explain why free fatty acids have been found to be more susceptible to oxidation than the corresponding bound ones. Living cells possess several protection mechanisms against the production of lipid oxidation products. An enzyme, glutathione peroxidase, exists which reduces hydroperoxides in the cellular membranes to the hydroxyl-compounds. This reaction demands a supply of reduced glutathione and will therefore cease post mortem when the cell is depleted of that substance. The membranes also contain the phenolic compound ά-tocopherol (Vitamin E) which is considered the most important natural antioxidant present in fish.

Tocopherol can donate a hydrogen atom to the free radicals L· or LOO· functioning as the molecule AH in **Figure 15**. It is generally assumed that the resulting tocopheryl radical reacts with ascorbic acid (Vitamin C) at the lipid/water interface regenerating the tocopherol molecule. Other compounds, for example the carotenoids, may also function as natural antioxidants.

Autoxidation involves the chemical breakdown of fat in the presence of oxygen/air without any additions.

Lipid hydrolysis can also occur in fatty fish species impacting adversely on shelf life. During storage, a considerable amount of free fatty acids (FFA) appear more so in ungutted than in gutted fish, probably because of the involvement of digestive enzymes. Triglyceride in the depot fat is cleaved by triglyceride lipase **(TL in Figure 16)** originating from the digestive tract or excreted by certain microorganisms. Cellular lipases may also play a minor role **(Jiang, Y., & Noh, S.K., 2001)**.

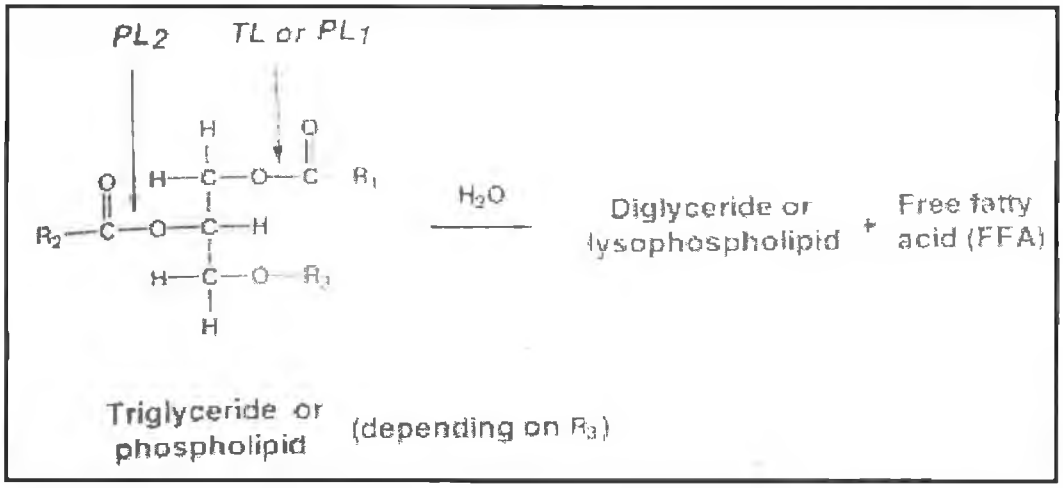


Figure 16. Primary hydrolytic reactions of triglycerides and phospholipids.Enzymes: PL1 & PL2 phospholipases; TL, triglyceride lipase.

In lean fish, for example Atlantic cod and whiting, production of free fatty acids also occurs even at low temperatures. The enzymes responsible are believed to be cellular phospholipases – in particular phospholipase A2 (PL2 in Figure 16) – although a correlation between the activity of these enzymes and the rate of appearance of FFA has as yet not been firmly established. **(Jiang, Y., & Noh, S.K., 2001)**. The fatty acids bound to phospholipids at glycerol-carbon atom 2 are largely polyunsaturated, and hydrolysis therefore often leads to increased oxidation as the liberated fatty acid is now more prone to oxidation. Furthermore, the fatty acids themselves may cause a "soapy" off-flavour.

2.3.3 Protein degradation

Protein degradation is another factor which can be used to determine the extent of spoilage in fish. Volatile amines are produced as a result of bacterial action on proteins and contribute to the "off flavours" and odours associated with spoilage. **(Egan, H., 1981)**. The total volatile base nitrogen (TVBN) content of fish tissue increases following death and much work has been carried out using these parameters to assess the quality and acceptability of fish. The total volatile base nitrogen (TVBN) consists of ammonia, dimethylamine, trimethylamine (TMA) and monomethylamine **(Villemure et al., 1986)**. It is assessed by distillation of the volatile bases over into boric acid and back titration with standardised acid. The ammonia (degradation of protein) can be separated from the trimethylamine (degradation of trimethylamineoxide, which is in particularly high concentration in deep sea fish) by modifying the procedure to trap the primary amines with formaldehyde and quantifying the TMA by distillation and titration. Subtraction of TMA from TVBN yields more or less the ammonia content.

2:4 MEASUREMENT OF Freshness/Rancidity

2.4.1 Lipid Oxidation.

Oxidative rancidity of fats and oils in foods results in a decreased quality and decreased nutritive value of the food. This is particularly relevant with foods such as fish, which contain high concentrations of polyunsaturated fatty acids **(Ackman, R.G., 1967)**. Autoxidation occurs when the double bonds of unsaturated fats become oxidised, resulting in the formation of hydroperoxides (ROOH). The higher the degree of unsaturation the more liable a fat is to oxidation. When the resulting peroxides reach a certain concentration, complex chemical changes occur, resulting in the formation of volatile compounds **(Egan et al., 1981)**. The acceptability of a food product depends on the extent to which deterioration has occurred. There are several experimental techniques available for assessing the extent of oxidation but there is no ideal chemical method which relates perfectly with changes in the organoleptic properties of oxidised lipids throughout the whole course of oxidation. Different methods give information about particular stages of autoxidation and the method of choice depends on a number of factors including the nature of the oxidised sample, the type of information required, the time available

and the test conditions. The ultimate criteria for the suitability of any test is its agreement with the sensory perception of rancid flavours and odours. Chemical tests available to quantify oxidative rancidity include 1. "Peroxide Value," Iodometric methods, of which there are several variations and 2.The 2-Thiobarbituric Acid Test (TBA) of which there are also many variations.

2.4.1.1 Peroxide Value

The primary products of lipid oxidation are hydroperoxides which are generally referred to as peroxides. Therefore, concentrations of peroxides can be used as a measure of the extent of oxidation. This method, however, is limited due to the transitory nature of the peroxides as they are intermediate products in the formation of carbonyl and hydroxyl compounds. Tests have shown that peroxide value passes through a maximum and is very sensitive to temperature changes. Because of the fact that the results and suitability of the peroxide test depends very much on experimental conditions and the reducing agent involved the method used in the determination should also be specified in detail.

Although the peroxide value is a common measurement of lipid oxidation, its use is limited to the initial stages of oxidation. However, good correlations have been reported between the official peroxide method and organoleptic flavour scores for various commercial fats such as lard, hydrogenated soybean oil and corn oil **(Fioriti et al., 1974)**. It is still a useful method for following the progress of oxidative rancidity. Peroxides can disappear in the later stages of deterioration, this can result in failure to detect the deterioration. It is a very popular method but it can only be carried out on purified fats extracted prior to analysis.

2.4.1.2 Iodometric Methods

Iodometric methods are based on the measurement of the iodine produced from potassium iodide by the peroxides present in a known weight of the oil. The American Oil Chemists Society (AOAC) recommends the use of ASTM method D1959-97, also known as the Wijs method, for determination of Iodine Value. The two principle sources of error in these methods are (1) the absorption of iodine at unsaturated bonds of the fatty material and (2) the liberation of iodine from potassium iodide by oxygen present in the solution to be titrated.

Another colorimetric method based on the oxidation of ferrous to ferric iron by the liberated iodine and the determination of the latter as ferric thiocyanate has been found suitable for the estimation of fat peroxides in milk powder and peroxide values determined by this method are considerably higher than by iodometric procedures. This method is very satisfactory for routine control purposes in such products as milk, cream, condensed and dried milk products as it is a more sensitive test than the titration methods and has excellent reproducibility. **(Gray, J.I., 1978)**.

2.4.1.3 The 2-Thiobarbituric acid Test. (TBA)

The 2-Thiobarbituric acid (TBA) test for the measurement of lipid oxidation, which was developed over forty years ago, is widely used for the measurement of the oxidative state of biological samples and food materials. A great advantage of this procedure is that it does not require prior extraction of the oil and can be used on compound samples. In 1944, Kohn & Liversedge observed that animal tissues which had been incubated aerobically with TBA, produced a pink colour which was the result of a complex formed from oxidation products of unsaturated fatty compounds and 2-thiobarbituric acid **(Bernheim et al., 1948)**. Although the chemistry of the reaction is still not fully understood, it is thought that malonylaldehyde, a final product of lipid oxidation is the major TBA reactive substance **(Yu, T.C., & Sinnhuber, R.O., 1957, Sinnhuber, R.O., & Yu, T.C., 1958, Tarladgis et al., 1960, 1962).**
The TBA reaction measures the total malonylaldehyde present in free forms under the conditions of the reaction. **Bird, R.P, & Draper, H.H., (1984)** reported that the reaction is thought to involve one molecule of malonylaldehyde reacting with two molecules of TBA with the elimination of two molecules of water **(Figure 17)** to yield a pink pigment which has an absorbance maximum at 532mn.

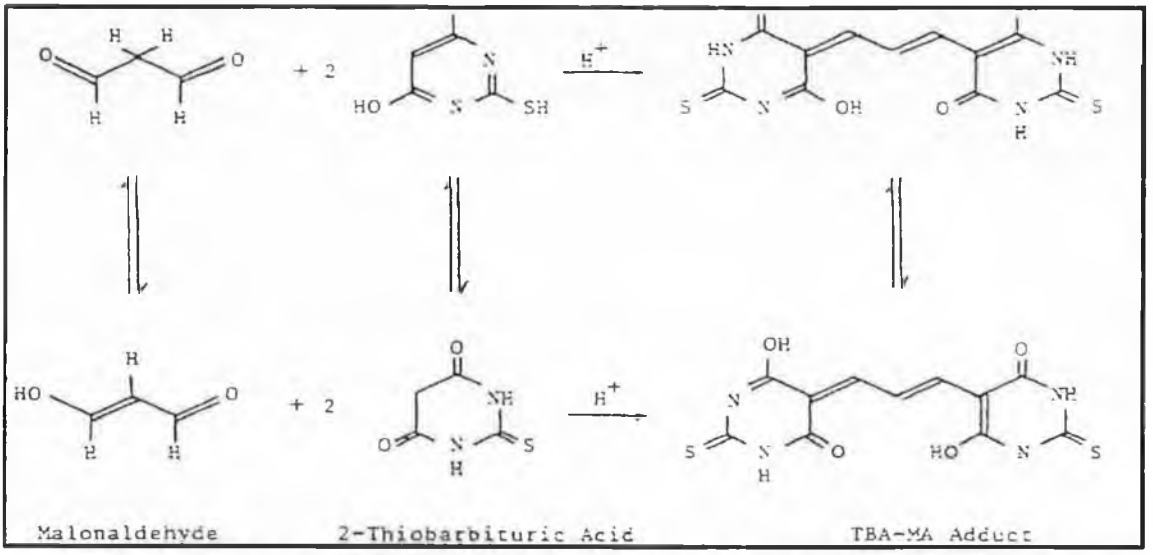


Figure 17. Adduct formation between Malonylaldehyde and the 2-Thiobarbituric Acid Reagent

Colour development is therefore usually by measurement of absorbance of the pink chromagen at 530 – 537mn **(Melton et al., 1981).** The various TBA test procedures which have been used on food products can be divided into four main types.

i. Test on the whole sample (a compound sample)
ii. Test on an aqueous or acidic extract of the sample
iii. Test on a steam distillate of the homogenised sample
iv. Test on extracted lipid from the sample

The whole sample method is reported to be quantitative but it's very time consuming and involves many solvent extractions **(Sinnhuber, R.J., & Yu, T.C., 1977). Witte et al., (1970)** claimed that the solvent extraction method is easier to use than the distillation method, uses less equipment and heating is not essential. **Pikul et al., (1989)** found solvent extraction methods to be faster and easier to perform than distillation methods and recommended solvent extraction procedures for use where a large number of samples need to be analysed rapidly.
The distillation method has been found to give lower recoveries compared to the solvent extraction method **(Siu. G.M., & Draper, H, H., 1978, Williams et al., 1983** and **Salih et al., 1987))** but is considered more sensitive and therefore more suitable for low fat samples or the early stages of rancidity.

Pikul et al., (1989) were of the opinion that the extracted fat method gave TBA numbers higher than the distillation method for replicate chicken meat samples. They recommended the lipid extraction procedure was particularly appropriate when the susceptibility to oxidation of different kinds of lipids or individual lipid components for example phospholipids, was studied. This method expresses lipid oxidation in mg of malonylaldehyde per kg of lipid as opposed to the more usual mg of malonylaldehyde per kg of sample.

Sinnhuber, R.O., & Yu, T.C., (1977) provided a comprehensive list of food applications on which the various TBA test procedures have been applied. Although tests on the whole sample or the extracted fat may be appropriate for some samples, the distillation method has the advantage that it can be applied to any foodstuff and is both rapid and reproducible **(Sidwell et al., 1955)**. The fact that the TBARS (TBA Reactive Substance) is obtained in clear aqueous solution, so that the pink reaction product can be measured accurately is a major advantage **(Tarladgis et al., 1964, Bird, R.P. & Draper, H.H., 1984).** Distillation also reduces interference, commonly seen in the whole sample method and extracted fat procedures, by compounds which may be present in a food sample **(Sinnhuber, R.O., & Yu, T.C., 1977).** The main disadvantage of the distillation method is that distillation is an empirical procedure requiring the collection of a specified volume of distillate. The time taken to distil can vary from laboratory to laboratory depending on the experimental conditions (heating mantle used, actual pH of the distillate, actual temperature in use) and they have an effect on the numerical value achieved.

Several modifications have been proposed to the original TBA procedures, the most common of these is the addition of antioxidants to the sample in order to prevent oxidation during the process **(Sinnhuber, R.O., & Yu, T.C., 1958)**. Various antioxidants have been added during the solvent extraction method and the distillation method prior to sample blending or after blending. **(Ang, C.Y.W., 1988, Crackel et al., 1988, Ke et al., 1984, Moerck, K.E., & Ball, H.R., 1974, Pikul et al., 1983, Rhee, K.S., & Ziprin,Y.A., 1981, Rhee, K.S., 1978, Yamauchi et al., 1982, & Yu, T.C., & Sinnhuber, R.O., 1967). Pikul et al., (1983)** reported that the antioxidant butylated hydroxytoluene (BHT) has no effect on the binding of malonylaldehyde to TBA at levels appropriate to the test but at higher levels, a slight positive effect was noted. **Rhee, K.S., (1978)** noted that phenolic antioxidants, including BHT, could increase TBA number as they increase the decomposition of lipid peroxides. He also noted that antioxidants

had no significant effect on pork, beef or chicken samples but did on fish samples. This was attributed to the differing fatty acid composition of the fish samples.

Other modifications include the use of chill blending **(Rhee, K.S., 1978)** and flushing of the sample flasks with nitrogen both of which are further attempts to prevent oxidation during the test. **Salih et al., (1987)** found that blending can accelerate lipid oxidation of poultry meat samples unless an antioxidant is used. **Yu, T.C., & Sinnhuber, R.O., (1967)** used silicone coated tubes in the extraction method to prevent a thin oil forming on the sides of the tubes as this was believed to be a major cause of inconsistent results, possibly because the oil sample was not in contact with the reagents or because, as a thin film, the oil would be subject to further oxidation.

Rhee, K.S., & Watts, B.M., (1966) modified the distillation method of **Tarladgis et al., (1960)** for use with raw plant tissues by adding acid during blending in order to inactivate lipoxidase to prevent its lipid oxidation potential. This method has been applied successfully to frozen vegetables and defatted soya flours **(Ree, K.S., & Watts, B.M., 1966)**. **Hoyland, D.V., & Taylor, A.S., (1989)** developed a rapid distillation time compared to conventional distillation methods using a commercial steam generator of the type used with protein quantification by Kjeldahl (Tecator). They reported satisfactory results for both standard solutions and a variety of food samples although recoveries were lower than with the conventional distillation methods. **Hung, S.S.O., & Shinger, S.J., (1981)** assessed a variety of chemical methods including a solvent extraction TBA method, to measure the oxidative quality of salmon oil, soybean oil, canola oil and canola soap stocks which had been oxidised in different ways. For oils oxidised at room temperature, peroxide value was shown to be the most sensitive method of oxidation measurement. Measurement of oxidation in oils which were highly oxidised could be achieved by any of the methods tested. The lack of sensitivity of the TBA test in parts of this experiment was related to the fact that TBA was more suitable for measuring oxidation in oils with fatty acids containing three or more double bonds **(Gray, J.I., 1978)**. Contradictory to these reports, **Turner et al., (1954)** had found that peroxide values showed considerably more variation and were much less reliable than TBA values. **Biggs, D.A., & Bryant, L.R., (1953)** reported that the TBA test was capable of detecting levels of oxidation below the organoleptic thresholds for off-flavours in butter, cheese and whole milk powder. **Green, B.E., & Cumuze, T.H., (1981)** claimed that in muscle foods, TBA values were highly correlated with sensory scores of oxidised flavour but **Fioriti et al., (1974)** found that a good correlation was only obtained for

lard and not for other fats. **Melton, S.L., (1983)** stated that fatty acids with three or more double bonds must be present for TBA number to be correlated with oxidised flavour and **Gray, J.I., (1978)** added that TBA values and change in flavours would have to be established for a given oil before TBA value could be used as an index of flavour. Other TBA procedures have also been used to determine the malonylaldehyde content in foods. These include physical and chemical methods such as those used by **Kwon, T., & Watts, B.M., (1963)** where the malonylaldehyde content in the steam distillate was measured using a method based on the pH dependence of the UV absorption spectrum of malonylaldehyde. Although the method was reported to be similar, more rapid and more specific for malonylalehyde than the TBA test, sensitivity of the test was only forty percent of the traditional TBA test as described by **Tarladgis et al., (1960).**

Several high pressure liquid chromatographic (HPLC) methods have been developed for the determination of trace levels of malonylaldehyde **(Esterbauer, H. & Slater, T.F., 1981). Lanuda et al., (1981)** used one such method to determine levels of malonylaldehyde in a steam distillate and reported a linear relationship between TBA number and HPLC results. Other HPLC methods, which do not require prior isolation by distillation found the HPLC method to be far more sensitive, accurate and specific for the detection of free malonylaldehyde than a solvent extraction TBA method for rat liver, beef, pork and chicken samples. **Bull, A.W., & Marnett, L.J., (1985)** developed an ion-pair HPLC method which reportedly avoids the interference found in other HPLC methods. The method is direct, no thiobarbiturate chromogen formation is required. Detection is accomplished by monitoring absorbance at 267 nm. **Williams et al, (1983)** suggested that a "standard" sample should be devised so that laboratories using any of the TBA methods could report results in terms of a standard. **Siu, G.M., & Draper, H.H., (1978)** and **Crackel et al., (1988)** recommend that recovery values for malonylaldehyde should be determined and standard curves prepared for calculation of the appropriate TBA conversion factor. Studies have shown that the formation of malonylaldehyde from the acid hydrolysis of 1,1,3,3 tetramethoxypropane (TEP) to yield one mole of malonylaldehyde from one mol of TEP is stoichiometric **(Tarladgis et al., 1964).**

During the course of this study it is proposed to use a steam distillation method on an acidified sample as developed by Tarladgis to determine TBA values. TBA reactive substances, believed to be mainly malonaldehyde, will be separated from the food matrix by acidification and a distillation process **(Ke et al., 1984).** Butylated hydroxytoluene (BHT) will be added to the

homogenates to help prevent autoxidation during the analytical procedure. The distillate will then be heated with thiobarbituric acid and the color developed compared to that of a standard tetra-ethoxy propane (TEP) solution which has been treated in the same way. The procedure will be taken further and quantified by using TEP to obtain the molar absorptivity of the pink chromogen under the conditions of the assay and the technique of standard addition will be employed to allow for accurate measurement of recoveries.

2.4.2 Protein Degradation.

Ammonia is the final degradation product of protein decay. Dimethylamine has been shown to be present in fresh fish in trace amounts ~0.2mg TMA – N /100g weight **(Oehlenschläger, J., 1997)**. Oehlenschläger states that development of TMA in fish tissue is dependent on the presence of the enzyme TMAOase. Protein degradation gives ammonia as an end product due to the splitting of amino acids. This ammonia is observed only at a fairly advanced stage of spoilage and is not a useful spoilage indicator in the early stages of degradation.

TMAO present in fish tissue is reduced to TMA after death, resulting in a decrease of TMAO levels as the fish ages and undergoes degradation. In studies carried out by J. Oehlenschläger, TMA levels in fish tissue continued to increase even after all TMAO was broken down, indicating TMAO is not the only source of TMA. In the same studies, an increase in TMA levels was observed, correlating with the onset of microbial action in the fish **(Oehlenschläger, J., 1997)**. Determination of the TMA levels in fish can therefore be used as an indication of fish spoilage.

TVBN determination involves the deproteinization of the sample by homogenisation with trichloroacetic acid to produce a filtrate, which is then separated by centrifugation. The filtrate is then assessed by steam distillation of the liberated volatile bases over into boric acid and back titration of the distillate with standardised acid to the original pH (5.0).

A modification of this technique can be used to determine TMA on its own. The addition of formaldehyde at alkaline pH prior to steam distillation results in the blocking of primary and secondary amines as follows: **(Figure 18).**

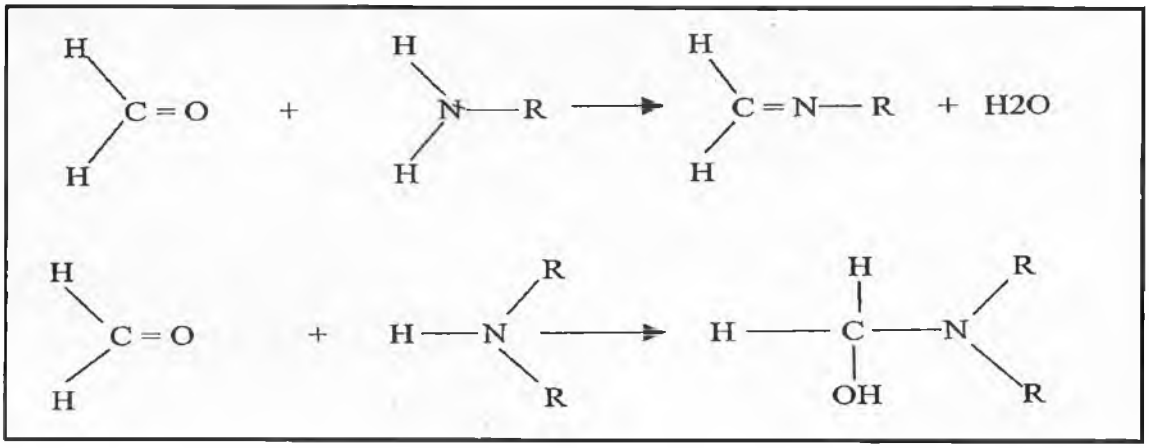


Figure 18. Blocking of primary and secondary amines

The products of this reaction are non-volatile and do not interfere, allowing the TMA present in the sample to be determined on its own **(Malle, P., & Tao, S.H., 1987)**. Subtraction of the TMA answer from the TVBN value yields the ammonia (a quantification of the deterioration of the protein independently)

2.5 Proposed Research

It is proposed to first determine the nutritional composition of orange roughy caught in the North East Atlantic. The protein, lipid and moisture content of the fish will be quantified. Protein content will be determined using the Kjeldahl method; a total batch extraction procedure will be used to extract the entire lipid while moisture content will be measured by weight difference after samples have been dried in an air-dried oven. It is also intended to identify the fatty acids present in each fat extract and their relative amounts as their importance, as part of a healthy diet, is highlighted more and more in today's society. Particular emphasis will be placed on assessing the relative amounts of EPA (C20:5) Eicosopentaenoic acid, DPA (C22:5) Docosapentanoic acid and DHA (C22:6) Docosahexcaenoic acid present. The fatty acid methyl esters (FAMES) will first have to be extracted from the lipid by saponification followed by methylation of the free fatty acids (FFA) or by transesterification with methanolic acid. After extraction, concentration and purification, the FFA samples will then be injected onto a GC machine for identification. Identification will be achieved by first identifying the retention times of known single standards and combinations of standards of fatty acids and comparing these to the retention times of the extracted fatty acids isolated from the lipid of the orange roughy. Quantification will be by peak area measurement.

The iodine value will be used to obtain a broad indication of unsaturation and should correlate with the FAME composition.

The degree of freshness of the initial samples will be determined by measuring protein degradation and lipid oxidation.

Protein degradation will be measured using the Total Volatile Base Nitrogen Test (TVBN) as well as a modification of the test, the Trimethylamine Test (TMA) to assess the levels of trimethylamine present in the fresh samples (a measure of TMAO breakdown and the fishy smell so characteristic of stale fish). The difference between the TVBN values and the TMA values yields more or less the ammonia content.

Lipid oxidation will be evaluated using the 2-Thiobarbituric Acid Test (TBA), to assess levels of malonaldehyde present in the compound fish.

Marine species generate highly perishable food products whose quality and freshness rapidly decreases post-mortem due to a wide variety of microbial and biochemical degradation mechanisms that limit their commercial shelf life **(Aubourg et al., 2007).**

Hence it is also important to examine the shelf life of these fish under typical storage conditions in air and vacuum packed. It is well known that biological samples can deteriorate faster when held at "elevated" frozen temperatures as a consequence stability at +4 °C and -5 °C will be assessed. Stability at freezer temperature will be investigated by ageing after six and twelve month periods.

3. MATERIALS AND METHODS

3.1 INTRODUCTION

Most deepwater fish are long-lived, slow-growing, have a low reproductive capacity and are adapted to live in an ecosystem of low energy turnover in which major environmental changes occur infrequently. Deepwater fishery resources are, therefore, highly vulnerable to exploitation. and deepwater habitats are sensitive and in need of protection **(Merrett, N. R., & Haedrich, R.L., 1997, Koslow et al., 2000, Anon, 2001)**. Experience in the South Pacific and elsewhere has shown that deepwater fish stocks can be depleted quickly and that recovery can be slow **(Koslow et al., 2000, Anon., 2001)**. In most cases, reliable information on stock status and fisheries production potential has lagged considerably behind exploitation **(Large et al., 2003)**. In the Northeast Atlantic this concern has been exacerbated by the fact that until 2003 most fisheries were completely unregulated. Knowledge of the biology and ecology of deepwater resources and communities remain limited. Major international projects funded by the European Union or Nordic Council focusing on biology and ecology were conducted in the 1990s **(e.g. Magnusson et al., 1997, Anon, 2000, Gordon, J.D.M., 2001, Menezes et al. 2001)**, but research activity has since declined and now depends heavily on scarce national funding. An Irish fishery for orange roughy commenced in 2000 and landing peaked at about 5,000 tonnes in 2002, but because of concerns about unsustainable fishing, the fishery was largely closed from 2005. The Marine Research Technology Development and Innovation Fund (RTDI) was launched in 2002 and this funding has resulted in the initiation of many studies including studies on current fish stock assessment and management areas for the waters around Ireland.

3.2 SAMPLE PROCUREMENT AND PREPARATION

All the fish analysed were caught off the Porcupine Bank/Rockall Trough. The spring samples were kindly supplied by the Marine Institute. They were landed in their frozen state and, upon arrival to the laboratory, the fish were thawed at 4°C, for three days, after which they were weighed and measured. The summer, autumn and winter samples were very kindly provided by deep sea fishing trawlers. All of these samples were stored on board ship on ice and delivered to the laboratory on ice. The sex of some of the fish was not determined. The size, weight and sex of each fish is given in Table 4.1 page

After weighing and measuring, the inner organs of each fish were removed and the heart, liver and swim bladder of each fish was vacuum packed and frozen at -18°C for any future studies. Each fish was then filleted and each mirror fillet divided into four approximately equal sectors and labelled A1 to A4 and B1 to B4 respectively from head to tail. All sections were vacuum packed, using a Euromatic Midy vacuum packer and frozen at -18°C until analysis began. Analysis of all samples began within one week of storage.

Nutritional evaluation, structural analysis and shelf life tests were carried out for each of the four seasons.

3.3 Quantitative Analysis

All chemicals were of general reagent grade, purchased from Sigma, except for specialist chemicals details of which are given in the relevant method.

3.3.1 Moisture Content (%)

Samples of thawed fish fillet (5g) were dried at 100°C for five hours in an air-dried oven to a constant weight. Moisture content was determined by weight difference and was expressed as a percentage ($^{w}/_{W}$) of the original thawed mass. Measurements were made in duplicate for each sample. **(AOAC., 1990)**

3.3.2 Protein Content (%)

The Kjeldahl method, developed in 1883, was used to determine protein using a Tecator Kjeltec digestion and distillation unit and the company's recommended procedure. 1g of fish sample was weighed into a digestion flask, in duplicate, and digested by heating at 470° C for about 40 minutes in the presence of sulphuric acid and the catalyst selenium, causing the release of the nitrogen present and converting it into ammonia. Digestion was continued until fully complete. After digestion, the ammonium sulphate was converted into ammonia gas by the addition of excess sodium hydroxide (40cm^3 of 40 % $^{w}/_{v}$). Excess boric acid (4%) in the receiving flask binds the ammonia gas as ammonium borate. The nitrogen content was then estimated by titration of the ammonium borate with standardised 0.05M sulphuric acid using a solution of bromocresol green / methyl red as the indicator. The concentration of hydrogen ions (moles) needed to reach the end point is equivalent to the concentration of nitrogen in the original sample. Because the Kjeldahl method does not measure the protein content directly a conversion factor (6.25) is needed to convert the measured nitrogen concentration to a protein concentration. The 6.25 conversion factor is equivalent to 0.16g nitrogen per gram of protein.

3.3.3 Lipid Content (%)

Lipid was extracted using the Folch extraction procedure. A known weight of tissue (25g) was homogenised with chloroform/methanol (2:1 $^{v}/_{v}$ in a ratio of 1:4). The homogenate was filtered and centrifuged @ 5000rpm to recover the liquid phase. The extract was then washed with one fourth volume of sodium chloride solution (0.85%) and centrifuged again to separate the two phases. After this centrifugation and siphoning off of the upper phase the lower chloroform phase, containing the lipids, was evaporated overnight in a fume hood. Lipid content was determined by weight difference of the receiving beaker and expressed as a percentage ($^{w}/_{v}$) of the original mass. **(Folch et al., 1957, Bligh,E.G., Dyer,W.J., 1959)**.

3.3.4 Iodine Value

Iodine value, which is a measure of the total number of double bonds present in the fat, is expressed in terms of "number of grams of iodine that will react with the double bonds in 100 grams of fat/oil.

The method used for determination of iodine value was the Wijs method, the method recommended by the **American Oil Chemists Society (1989).** The method involves the addition of Wijs solution (10cm^3) and 5cm^3 of chloroform to a suitable weight of sample, (0.03 to 0.05g, in this case). The solution was then allowed to stand in the dark for thirty minutes before the addition of 10 cm^3 of 10% KI and 50 cm^3 of distilled water. The remaining iodine was titrated with standardised sodium thiosulphate (0.1M) using a 1% starch solution as the indicator. All samples were tested in duplicate and duplicate blanks were also used.

3.3.5 Fatty Acid Methyl Ester Production (F.A.M.E.S)

As discussed in the literature review, several esterification and transesterification procedures exist for the conversion of the fatty acid components of lipids to the simplest derivatives of either methyl or ethyl esters. As none of these are ideal, all have advantages and disadvantages; it was decided to investigate a variation of three methods during the course of this study before deciding which would be the most suitable method to meet our needs and accurately determine the as yet unknown lipid composition of orange roughy.

(a). The first method involved the use of 14% ($^{w}/_{v}$) of boron trifluoride in methanol to produce the F.A.M.E.S: 20mg of the extracted lipid was transferred to a 50cm³ round bottom flask to which 50 µl of 23:0 FAME (0.100g/10 cm³ of hexane), as the internal standard, had already been added and evapourated to dryness. Methanolic NaOH (6cm³ of 0.5M) and pumice was then added and boiled under reflux using a condenser and a heating mantle for ten minutes (addition of the internal standard 23:0 allows for checking of retention times when running the gas chromatography and enables quantification when desired). 7cm³ of 14% boron trifluoride in methanol was then added and boiled for a further two minutes. After cooling, 5cm³ of heptane was added and the top layer was washed with a 10% Na Cl solution. The top (heptane) layer was then transferred to a glass centrifuge tube and centrifuged at 5000rpm for ten minutes to ensure complete recovery. A few grains of anhydrous sodium sulphate were then added to the heptane layer. The clean top layer was then transferred to a 10cm³ dry beaker and evaporated to dryness. This residue was re-dissolved in 1cm³ of heptane and analysed by thin layer adsorption chromatography on silica gel G to confirm the presence of F.A.M.E.S. and the absence of intact/undegraded waxes and triglycerides.

(b). The second method chosen was the Butoxide method, **(Bottino, N.R., 1975)**, where 20mg of the extracted fish lipid and 500µg of the 23:0 internal standard were saponified with 6cm³of a 10% $^{w}/_{v}$ solution of potassium t-butoxide in methanol by refluxing for thirty minutes. A further 6cm³ of a 10% $^{(w}/_{v)}$ aqueous solution of potassium hydroxide was then added and the solution further refluxed for another thirty minutes. This solution was then acidified to pH below three and the free fatty acids were then extracted by the addition of 5cm³ of heptane. The bottom layer was again extracted with another 5cm³ of heptane. The two heptane layers were combined and centrifuged for ten minutes at 5000rpm to ensure complete removal of the bottom layer from the top heptane layer. A few grains of anhydrous sodium sulphate were then added to this heptane layer. The dry top layer was then transferred to a 10cm³ dry beaker and evaporated to dryness. The residue was re-dissolved in heptane and analysed by thin layer adsorption chromatography on silica gel G to confirm the presence of F.A.M.E.S and the absence of intact/undegraded fats.

(c).The third method chosen was the use of methanolic hydrochloric acid (HCl) in a

transesterification/esterification step. Methanolic hydrochloric acid was produced by the addition of 5cm³ of acetyl chloride to 50cm³ of dry methanol drop by drop in a fume hood. 30mg of extracted lipid, 500µg of 23:0 internal standard and 3cm³ of methanolic HCl were all added to three screw capped teflon lined tubes. Between 0.1 and 0.3cm³ of 9:1 ($^{v}/_{v}$) heptane/diethyl ether was then added to help dissolve the lipid. The tubes containing the mixture were then flushed with nitrogen to remove any air and placed in a 90°C water bath for 1½, 4 and 8 hours respectively. The teflon caps were tightened after approximately five minutes in the water bath and the tubes were vortexed at regular intervals. 1cm³ of water and 1cm³ of heptane was then added to each tube and the top layer, containing the F.A.M.E.S. recovered. These extracts were then analysed by thin layer adsorption chromatography on silica gel G as before.

3.3.6 The use of Silicic acid columns for the removal of residual alcohols after FAMES extraction using the methanolic HCl procedure.

Because the presence of residual alcohols results in "false" peaks from the capillary GC chromatographic readouts towards the end of the elution profile interfering/coeluting close to DHA and C24:0 and also the fact that these alcohols can shorten the life of a relatively expensive column, it was considered important to remove these alcohols before injection. Disposable silicic acid columns were prepared by packing 0.5g of silicic acid (SIGMA, Cat no 306 363) into a standard glass, disposable pasteur pipette with a cotton plug and pre-treated with 100% ($^{w}/_{v}$) hexane (10cm^3). After application of the FAMES sample in 2cm^3 of heptane, the column was washed with 10% ($^{w}/_{v}$) ether in hexane (10cm^3) and the eluent containing the FAMES collected in a beaker. The alcohols were retained on the column. Other solvent eluent mixtures (1, 4, 8 and 25% $^{(w}/_{v)}$ ether/hexane, 35:1 and 80:20 $^{(v}/_{v)}$ ether/hexane and 9:1 and 35:1($^{w}/_{v}$) dichloromethane/ether were also tried but the 10% $^{(w}/_{v)}$ ether/hexane was found to give the most satisfactory result.

Effectiveness of the separation procedure was checked by the use of thin layer adsorption chromatography on Silica Gel G.

3.3.7 Use of TLC to assess lipid composition of fat and effectiveness of FAMES production and purification.

Thin layer adsorption chromatography is based on the separation of a mixture of components as they migrate, with the help of a suitable solvent, through a thin layer of absorbent material. (www.sfu.ca/bise/bise429/TLC.html)

Commercially prepared glass silica gel G plates (Merck Cat No 1.05721) and a solvent system of hexane/diethyl ether/acetic acid 80:20:1 ($^{v}/_{v}$) was used throughout this study to assess the lipid composition and the FAMES extractions produced.

In each case, silica gel G plates were spotted, three times, using a 10µl capillary pipette. After each spot was applied, it was dried with a hairdryer. This resulted in having loaded approximately 10µl of each sample onto the TLC plate. Standards used were cholesterol, cholesteryl oleate, triolein, oleic acid, phytol, phosphatidyl choline and the wax esters oleyl oleate and arachidyl oleate. After loading, plates were transferred to a chromatography tank which had been pre- equilibrated with the solvent system and allowed to run for approximately forty minutes until the solvent front had reached 1cm from the top of the plate. After removal from the tank, the solvent front was marked and the solvent dried off with a hairdryer in a fume hood. They were then sprayed with a 20% ($^{w}/_{v}$) solution of sulphuric acid and placed in a 120°C hot air oven for approximately one hour to char the carbon containing compounds.

3.3.8 The use of Gas Chromatography (GC) for Fatty Acid Analysis

Gas chromatography was discovered in the mid 1950's and has been widely used since for the analysis of fatty acids. Flame Ionisation Detection (FID) is most commonly used for the detection of separated FAMES.

The principle of the technique involves the separation of the volatile organic components in a mixture on a polar column and their detection, as they exit the GC column, by a specific detector. The choice of detector depends on the separation and application. In our case, the choice was a flame-ionisation detector (FID), set at 250°C, which is highly sensitive to a large range of carbon containing compounds but has the disadvantage of destroying the sample. The following are the experimental conditions employed throughout:

Instrument:	GC 17 A SHIMADZU
Column:	BP 70 CAPILLARY, SGE
Printer:	SHIMADZU CR8A CHROMATOPAC
Column Conditions: Gas Flow Rate	 Air – 51 psi Helium – 53.5 psi Hydrogen – 47 psi
Injector Temperature	250°C
FID Detector Temperature	250°
*Split Ratio	50:1
*Injection Volume	1 µl

Programme Cycle was as follows:

Temperature	**Duration**
150°C	1 minute
150°C - 190°C @ 3°C/min	12 minutes
190°C - 220°C @ 10°C/min	3 minutes
220°C Hold	10 minutes

Run completed in 35 mins.

The parameters set for the Shimadzu C-R8A chromatopac were as follows:

Width	5
Slope	120
Drift	0
Minimum Area	100
T. DBL	999.35
Stop Time	35 minutes

*Split ratio of 1:5, 1:25, 1:30, 1:60 and 1:100 had also been tried but 1:50 gave the most satisfactory results. Using a split ratio typically yields higher recoveries of the lower molecular weight species such problems with the injecting technique were satisfactorily solved by drawing up 1µl of air into the micro syringe after the 1µl of sample and then injecting quickly through the

rubber septum. Proof was obtained/confirmed by the use of two commercially available standards- Supelco No 1899 (containing 20% each of 5 saturated methyl esters) and Supelco No 1894 (containing 25% each of 4 monounsaturated methyl esters).

The composition of the extracted FAMES was identified by the use of well known individual single standards of saturated and unsaturated methyl esters as well as a marine standard. A reference sample of C23:0 was also used as an internal standard to check peak retention times and quantitative recovery, if desirable.

Accuracy of the fat extraction technique and preparation of the FAMES was checked by preparing the FAMES from the same lipid sample five times and then injecting these and determining mean, standard deviation and coefficient of variation (Tables 8.1, 8.2).

Accuracy of the injection technique was checked by repeatedly injecting the same processed sample ten times and finding the mean, standard deviation and coefficient of variation (Table 8).

3.4 Quantitative Analysis of Deterioration

3.4.1 Determination of Thiobarbituric Acid (TBA) reactive substances present in fish tissue.

As outlined in the literature review, the TBA test measures the total malonylaldehyde (MDA) present under the conditions of the reaction.

Malonylaldehyde present in the fish samples were measured by first homogenising the sample in an acidic medium and distilling the volatile malonylaldehyde over by the use of reflux distillation. The compounds in the distillate were then measured by spectrophotometric measurement at 530 nm (λ max) after the reaction with 2-thiobarbituric acid.

Because test conditions vary from laboratory to laboratory and depends on reagent quality and equipment used, the percentage recovery of MDA for any specific test conditions must first be determined by preparation of a standard curve and production of a conversion factor to convert absorbance to mmoles (or mg) MDA per kg sample of fat. Legal limits of 14mg of malonylaldehyde/100g of muscle was set by **Connell, J.J.,(1975)** in **(Simeonidou et al.,1998)**

TBA Test Procedure

10g samples of frozen fish tissue were transferred to a Waring blender, 100mg each of propyl gallate and disodium EDTA were also added and blended for two minutes with 50cm^3 of

distilled water. The homogenate was transferred to a 500cm³ round bottom Kjeldahl flask with a ground glass connection by washing with a further 45 cm³ of distilled water and 2.5cm³ of 4M HCl was also added (4M HCl was prepared by pipetting 172cm³ of HCl to a 500cm³ volumetric flask and diluting to volume) as well as approximately 3 drops of antifoam. Distillation was started immediately and exactly 50cm³ of distillate was collected in a graduated cylinder within 25 minutes. 5cm³ samples each of TBARS distillate and 5cm³ of TBA reagent were pipetted, in duplicate, into screw cap teflon lined tubes and heated at 100°C for 35 mins. Duplicate blank samples of 5cm³ of distilled water and 5cm³ of TBA reagent were run simultaneously. After cooling, absorbance was read at 532 nm (λ max) and converted to mmoles using the standard graph. The TBARS values were expressed as mg malonylaldehyde per kg of fish.

Preparation of TEP Standard Solution

0.220g of 1,1,3,3-Tetraethoxypropane (TEP) was weighed out and diluted to 100 cm³ in a volumetric flask to produce a $1x10^{-4}$ M stock solution.

Preparation of TBA Solution

0.1142g of 2-Thiobarbituric Acid (TBA) was weighed out and diluted in 45 cm³ of glacial acetic acid and 5cm³ of distilled water on a magnetic stirrer until dissolved and transferred to a 50cm³ volumetric acid and made up to the mark.

Recoveries of the MDA on distillation are not necessarily completely stoichiometric because of the volatile nature of the aldehyde. The length of the distillation time can have a profound effect upon recoveries and in addition slow distillation/heating of the acidic homogenate can increase the production of the aldehyde in samples. To obtain an accurate assessment of the recovery, known quantities of 1,1,3,3-TEP (0-4 μmoles), at various concentrations, were added to a 10g sample of a typical fresh fish. and the recovery of total MDA in the distillates quantified colorimetrically. This is known as standard addition. The digestion and distillation was performed under the conditions previously detailed (incl. EDTA, Propyl Gallate, HCl & antifoam).

1 mole of 1,1,3,3-Tetraethoxypropane breaks down into 1 mole of malonylaldehyde upon heating. The former is not volatile, the latter is.

A standard solution of $1x10^{-7}$ M TEP was used to set up a standard curve for the spectrophotometric assay as follows:

Tube No	Vol (cm^3) 1,1,3,3-TEP	Vol (cm^3) H_2O
1 (A,B)	0	5.00
2 (A,B)	0.15	4.85
3 (A,B)	0.30	4.70
4 (A,B)	0.60	4.40
5 (A,B)	0.80	4.20
6 (A,B)	1.00	4.00
7 (A,B)	1.50	3.50
8 (A,B)	2.00	3.00

$5cm^3$ of TBA reagent solution was added to each teflon lined tube. The tubes were well mixed and boiled at 100°C for 35 minutes. After cooling the absorbances of the solutions were determined at 532 nm (λ max). These readings were plotted against concentration of TEP to establish a molar absorptivity for the colorimetric assay. As the standard addition proved that the slope of the line did not alter upon prior digestion/distillation with 10g of fish, i.e. complete recovery was obtained with no inhibition/activation of colour formation from the fish, the same molar absorptivity could be used to convert the absorbance of the routine lipid oxidation assays to mg MDA/kg fish. A comparison of the slope of the standard curve in the presence and absence of sample enables one to determine the recovery of MDA under the conditions used.

3.4.2 Determination of Total Volatile Base Nitrogen (TVB-N) and Trimethylamine (TMA).

Fish is a highly perishable food product, quality deterioration occurs rapidly during handling and storage which limits its shelf life. Shelf life can be defined as the length of time the product remains fit and safe to eat when stored under defined conditions. The upper limits of acceptability for TVB-N are set at 35mg/100g and 12mg/100g for TMA **(Ruiz-Capillus,C., & Moral, A., 2001).** Throughout this study, for the purpose of determining shelf life, samples were tested for their quality upon arrival and stored at 4°C, -5 °C and -18°C in air and under vacuum and quality measured over time. As enzymatic and chemical reactions are responsible

for initial loss of freshness, the indices chosen in this study to assess the level of these changes over time were total volatile base nitrogen (TVB-N) and trimethylamine (TMA) content.
Total volatile base nitrogen was measured using the method of **Malle & Tao, (1986).** Samples were prepared by homogenising 100g of fish with 200cm³ of 7.5% ($^{w}/_{v}$) aqueous trichloroacetic acid (TCA) solution in a laboratory blender for 2 minutes. The homogenate was centrifuged at 7000 rpm for 10 minutes and the supernatant retained. 25 cm³ of the supernatant and 5 cm³ of 10% ($^{w}/_{v}$) aqueous NaOH solution was transferred into a Kjeldahl distillation tube and steam distillation was performed on the Tecator Kjeltec Distillation unit. The distillate (100 cm³) was received into an aqueous boric acid solution 4% ($^{w}/_{v}$) containing methyl red/bromocresol green indicator and was titrated against 0.05M sulphuric acid solution. The quantity of TVB-N was determined from the volume of 0.05M sulphuric acid used for reneutralisation back to the end point (pH 5.0).

The same experimental procedure was used for the TMA measurement. The only difference was the addition of 20 cm³, 35% ($^{w}/_{v}$), formaldehyde to the distillation tube to block the primary and secondary amines prior to distillation, thereby leaving only the tertiary amines free/volatile and able to distil over. Again the number of moles of TMA present was quantified by titration of the distillate against 0.05M sulphuric acid. Both TVBN and TMA results were expressed in mg nitrogen/100g sample.

3.5. Ageing Studies

The parameters used to assess the aging/stability of the fish in the presence and absence of air at + 4 °C and -5 °C were the Total Volatile Base Nitrogen (TVB-N) and Trimethylamine (TMA) as well as the TBA test.
Fish for these studies was first thawed from -18 °C in vacuum at refridgeration temperature then placed at the appropriate temperature and tested on days 0, 3, 5, 8,15 and 17 for the 4 °C storage temperature and on days 0, 4, 9, 18, 24 and 65 in the case of the -5 °C storage.
After storage of the vacuum packed fresh fish for six and twelve months respectively the fish was aged at refridgeration temperature in the presence and absence of air.

3.6 Statistical Analysis (http://www.itl.nist.gov/div898/handbook/prc/section4/prc437.htm)

The 2-way ANOVA is used to analyse the data for the effect of season and location on various biochemical markers as follows: If i is the season, j the location and k the replicate for i = spring, summer, autumn, winter; j = A1, A2, A3, A4; k = 1, 2, 3, 4 then the marker value for each such combination is

$$y(ijk) = \mu + ssn(i) + loc(j) + snlc(ij) + e(ijk)$$

where μ = average value over all seasons and locations

ssn(i) = effect of season i; loc(j) = effect of location j;

snlc(ij) = interaction effect of season i and location j

e(ijk) = random effect of replicate k for season i and location j

The total sum of squares is correspondingly partitioned as:

$$SS(total) = SS(ssn) + SS(loc) + SS(snlc) + SS(e)$$

For reference, the formulas for the sums of squares are:

$$SS(ssn) = 16\sum_i (y_{i..} - y_{...})^2 \; ; \; SS(loc) = 16\sum_j (y_{.j.} - y_{...})^2$$

$$SS(snlc) = 4\sum_i \sum_j (y_{ij.} - y_{i..} - y_{.j.} + y_{...})^2 \; ; \quad SS(e) = \sum_i \sum_j \sum_k (y_{ijk} - y_{ij.})^2$$

$$SS(total) = \sum_i \sum_j \sum_k (y_{ijk} - y_{...})^2$$

where, using the dot subscript to indicate the average over the corresponding omitted subscript/index, we have

$y_{...}$ = average over all seasons, locations and replicates = $\frac{1}{64}\sum_i \sum_j \sum_k y(ijk)$

$y_{i..}$ = average over all locations and replicates for season i = $\frac{1}{16}\sum_j \sum_k y(ijk)$

$y_{.j.}$ = average over all seasons and replicates for location j = $\frac{1}{16}\sum_i \sum_k y(ijk)$

$y_{ij.}$ = average over replicates for season i and location j = $\frac{1}{4}\sum_k y(ijk)$

The resulting ANOVA table is

Source	**SS**	**df**	**MS**	**F**	**df**
Season	SS(ssn)	(4-1)=3	SS(ssn)/3	MS(ssn)/MS(e)	(3,48)
Location	SS(loc)	(4-1)=3	SS(loc)/3	MS(loc)/MS(e)	(3,48)
Interaction	SS(snlc)	(4-1)(4-1)=9	SS(snlc)/9	MS(snlc)/MS(e)	(9,48)
Error	SS(e)	4×4×4–4×4=48	SS(e)/48		
Total	SS(total)	4×4×4–1=63			

4 RESULTS

4.1 Size, Weight & Sex of Fish Tested.

Table 2:

Spring Samples – Landed Feb 21st 2005

Fish	Size (cm)	Weight (kg)	Sex
1	53	2.4	Female
2	52	2.2	Male
3	60	2.65	Female
4	51	2.25	Male

Summer Samples – Landed June 15th 2005

Fish	Size (cm)	Weight (kg)	Sex
1	61	3.45	Male
2	58	3.22	Male
3	56	2.87	Not determined
4	62	3.67	Not determined

Autumn Samples – Landed September 12th 2005

Fish	Size (cm)	Weight (kg)	Sex
2	56	2.20	Not determined
1	57	3.05	Not determined
3	60	3.30	Not determined
4	58	2.95	Not determined

Winter Samples – Landed December 18th 2005

Fish	Size (cm)	Weight (kg)	Sex
1	60	3.25	Female
2	56	2.95	Not determined
3	45	1.65	Female
4	61	3.15	Female

4.2 Nutritional Composition of Orange Roughy

Table 3 Moisture Content (% w/w)

	Spring	Summer	Autumn	Winter
Sample 1 A1	80.03	74.54	78.09	79.38
Sample 1 A2	81.15	72.25	77.83	78.17
Sample 1 A3	79.78	77.11	77.84	78.84
Sample 1 A4	79.65	80.79	78.01	78.93
Sample 2 A1	79.56	77.86	79.26	78.19
Sample 2 A2	78.99	77.93	78.76	78.83
Sample 2 A3	80.15	77.27	78.16	78.78
Sample 2 A4	80.23	78.86	78.57	77.95
Sample 3 A1	77.25	78.24	76.5	79.27
Sample 3 A2	77.38	77.21	76.33	78.96
Sample 3 A3	81.43	77.54	76.97	78.94
Sample 3 A4	81.62	80.23	76.37	79.22
Sample 4 A1	80.54	78.24	76.83	79.19
Sample 4 A2	81.12	78.13	76.56	79.17
Sample 4 A3	78.79	77.83	75.09	79.03
Sample 4 A4	76.49	79.05	75.91	78.81

Source	SS	df	MS	F	P	Effect
Season	54.415	3	18.13821	9.521733	**4.8098E-05**	**YES**
Location	4.586	3	1.52859	0.80244	0.49866987	**NO**
Interaction	22.269	9	2.474367	1.29893	0.26238123	**NO**
Error	91.437	48	1.904927			
Total	172.706	63				

Table 4 Protein Content (% w/w)

	Spring	Summer	Autumn	Winter
Sample 1 A1	17.38	16.67	16.97	15.77
Sample 1 A2	15.37	16.87	16.50	15.89
Sample 1 A3	16.25	16.58	16.71	15.87
Sample 1 A4	15.41	16.68	16.89	15.95
Sample 2 A1	16.71	16.24	15.73	15.85
Sample 2 A2	17.10	17.53	16.41	15.94
Sample 2 A3	16.97	16.67	16.19	15.95
Sample 2 A4	16.88	16.44	15.80	18.88
Sample 3 A1	16.88	17.04	16.23	15.96
Sample 3 A2	16.76	17.42	16.38	15.97
Sample 3 A3	16.96	16.31	16.42	15.89
Sample 3 A4	16.91	17.01	16.47	15.96
Sample 4 A1	16.80	16.13	16.05	15.95
Sample 4 A2	16.74	16.07	16.03	16.06
Sample 4 A3	16.93	15.91	16.06	16.01
Sample 4 A4	16.77	16.15	16.07	16.04

Source	SS	df	MS	F	P	Effect
Season	3.242	3	1.080614	3.501246	0.02234019	**YES**
Location	0.236	3	0.078681	0.25493	0.85744144	**NO**
Interaction	3.028	9	0.336464	1.090161	0.38755875	**NO**
Error	14.815	48	0.308637			
Total	21.321	63				

Table 5 Lipid Content (% w/w)

	Spring	Summer	Autumn	Winter
Sample 1 A1	1.98	3.83	2.84	4.13
Sample 1 A2	2.22	3.88	5.74	4.15
Sample 1 A3	3.07	3.36	4.04	4.14
Sample 1 A4	3.87	3.39	4.57	4.15
Sample 2 A1	2.62	3.02	2.80	4.79
Sample 2 A2	2.45	3.57	4.31	5.18
Sample 2 A3	4.95	3.90	4.73	4.85
Sample 2 A4	3.64	3.80	4.72	4.80
Sample 3 A1	5.15	4.09	2.79	4.43
Sample 3 A2	4.41	4.06	4.69	4.61
Sample 3 A3	5.26	3.91	4.37	4.84
Sample 3 A4	4.97	3.09	4.73	4.78
Sample 4 A1	4.12	3.30	6.07	3.38
Sample 4 A2	3.22	3.34	5.67	4.57
Sample 4 A3	4.13	3.59	5.64	4.37
Sample 4 A4	4.51	3.40	5.59	4.41

Source	SS	df	MS	F	P	Effect
Season	11.587	3	3.862335	6.520269	**0.00086531**	**YES**
Location	3.742	3	1.247485	2.105964	0.11180564	**NO**
Interaction	6.721	9	0.746765	1.260664	0.28255061	**NO**
Error	28.433	48	0.592358			
Total	50.484	63				

Table 6 Assessment of Unsaturation of Lipid / Iodine Value (g/100g)

	Spring	Summer	Autumn	Winter
Sample 1 A1	80.80	77.09	87.38	80.02
Sample 1 A2	82.78	80.97	84.98	76.65
Sample 1 A3	85.41	74.54	85.16	76.97
Sample 1 A4	79.47	78.99	84.27	80.19
Sample 2 A1	76.38	86.74	71.73	82.04
Sample 2 A2	78.48	83.34	72.66	76.37
Sample 2 A3	78.55	81.52	71.80	78.42
Sample 2 A4	82.62	81.06	70.23	76.46
Sample 3 A1	71.51	59.09	76.46	81.52
Sample 3 A2	77.08	61.27	75.56	78.68
Sample 3 A3	74.08	70.44	74.00	78.52
Sample 3 A4	73.85	62.52	75.48	81.21
Sample 4 A1	85.76	76.96	77.01	78.95
Sample 4 A2	81.28	71.66	81.90	75.66
Sample 4 A3	81.35	74.03	79.87	76.13
Sample 4 A4	83.16	73.81	80.13	76.05

Source	SS	df	MS	F	P	Effect
Season	213.673	3	71.22435	2.001706	0.12627593	**YES**
Location	4.373	3	1.457802	0.04097	0.98880657	**NO**
Interaction	39.767	9	4.418588	0.124181	0.99888386	**NO**
Error	1707.928	48	35.58183			
Total	1965.741	63				

4.3 Fat Composition of Orange Roughy Oil by Thin Layer Adsorption Chromatography

Table 7

Standards	R_F Value
Cholesterol	0.10
Cholesteryl Oleate	0.69
Triolein (Triglyceride)	0.40
Oleic Acid (Fatty acid)	0.19
Phosphatidylcholine	0.00
Oleyl oleate	0.60
Oleic acid arachidyl ester	0.68
Sample of Oil	R_F Value
Wax/Steryl esters	0.70 (Most common)
Triglyceride	0.39 (Second most common)
Polar Lipid	0.00
Sterols, Free fatty acids, Mono & di glycerides	0-0.17, 0.19, 0.20 (Small quantities)

4.4 Reproducibility of the Experimental Process for Fatty Acid Determination of Orange Roughy Oil

Table 8.1 Reproducibility of Extraction Method

% Lipid Extracted	Extraction 5	Extraction 4	Extraction 3	Extraction 2	Extraction 1		Average	Std. Dev.	% Coefficient of Variation
	3.30	3.59	3.34	3.40	3.36		3.398	0.1132	3.3321

Table 8.2 Reproducibility of Preparation of FAMES

Identification	Extraction 5	Extraction 4	Extraction 3	Extraction 2	Extraction 1	Average	Std. Dev.	% Coefficient of Variation
Myristic Acid (14:0)	0.33	0.447	0.42	0.44	0.44	0.415	0.0488	11.7461
Palmitic Acid (16:0)	1.71	1.74	1.72	1.71	1.73	1.722	0.0130	0.7572
Stearic Acid (18:0)	0.71	0.73	0.72	0.71	0.71	0.716	0.0089	1.2492
Palmitoleic Acid (16:1)	5.86	5.84	5.88	6	6.04	5.924	0.0899	1.5174
Vaccenic Acid (18:1 n-7)	5.29	5.21	5.22	5.24	5.27	5.246	0.0336	0.6408
Oleic Acid (18:1 n-9)	34.66	34.12	34.26	34.52	34.55	34.422	0.2237	0.6497
Gondoic Acid (20:1 n-9)	25.38	24.84	25.25	25.14	25.08	25.138	0.2020	0.8037
Gadoleic Acid (20:1 n-11)	0.95	0.93	0.95	0.94	0.93	0.940	0.0100	1.0638
Erucic Acid (22:1 n-9)	1.55	2.49	1.63	1.59	1.58	1.768	0.4046	22.8859
Cetoleic Acid (22:1 n-11)	18.26	18.07	18.13	17.93	17.87	18.052	0.1563	0.8657
Linoleic Acid (18:2 n-6)	0.88	0.84	0.85	0.85	0.87	0.858	0.0164	1.9151
Arachidonic Acid (20:4 n-6)	0.62	0.61	0.62	0.62	0.61	0.616	0.0055	0.8892
Docosapentaenoic Acid (22:5)	0.23	0.23	0.24	0.24	0.23	0.234	0.0055	2.3407
Eicosapentaenoic Acid (20:5)	0.61	0.64	0.59	0.61	0.61	0.612	0.0179	2.9230
Docosahexaenoic (22:6)	2.22	2.35	2.21	2.24	2.23	2.250	0.0570	2.5337

Table 9 Reproducibility of Injection Technique

identification	Inj 1	Inj 2	Inj 3	Inj 4	Inj 5	Inj 6	Inj 7	Inj 8	inj 9	Inj 10	Average	Std. Dev.	% Coefficient of Variation
Myristic Acid (14:0)	0.43	0.42	0.44	0.42	0.42	0.42	0.42	0.43	0.42	0.41	0.423	0.0082	1.9463
Palmitic Acid (16:0)	1.68	1.68	1.71	1.66	1.66	1.67	1.68	1.68	1.65	1.65	1.672	0.0181	1.0846
Stearic Acid (18:0)	0.70	0.70	0.71	0.70	0.69	0.70	0.70	0.71	0.69	0.69	0.699	0.0074	1.0556
Palmitoleic Acid (16:1)	5.95	5.95	6.07	5.90	5.92	5.96	5.96	6.00	5.86	5.82	5.939	0.0702	1.1812
Vaccenic Acid (18:1 n-7)	5.13	5.22	5.20	5.05	5.17	5.08	5.15	5.10	5.04	4.99	5.113	0.0742	1.4521
Oleic Acid (18:1 n-9)	34.32	34.33	34.50	33.91	34.08	34.22	34.11	34.29	33.96	33.70	34.142	0.2381	0.6975
Gondoic Acid (20:1 n-9)	24.35	24.58	24.44	24.02	24.41	24.23	24.23	24.15	24.25	24.06	24.272	0.1750	0.7209
Gadoleic Acid (20:1 n-11)	0.90	0.91	0.91	0.89	0.90	0.91	0.86	0.91	0.90	0.86	0.895	0.0196	2.1876
Erucic Acid (22:1 n-9)	1.27	1.27	1.26	1.25	1.25	1.27	1.26	1.26	1.27	1.25	1.261	0.0088	0.6944
Cetoleic Acid (22:1 n-11)	18.26	17.06	18.23	17.96	18.35	18.14	16.74	16.63	18.31	18.12	17.780	0.6863	3.8598
Linoleic Acid (18:2 n-6)	0.87	0.86	0.87	0.85	0.85	0.87	0.86	0.86	0.86	0.85	0.860	0.0082	0.9494
Arachidlonic Acid (20:4 n-6)	0.35	0.35	0.37	0.35	0.36	0.34	0.36	0.36	0.35	0.35	0.353	0.0085	2.3973
Docosapentaenoic Acid (22:5)	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.20	0.200		0.0000
Eicosapentaenoic Acid (20:5)	0.62	0.61	0.63	0.63	0.63	0.70	0.70	0.69	0.76	0.67	0.664	0.0472	7.1191
Docosahexaenoic Acid (22:6)	2.22	2.26	2.25	2.22	2.27	2.24	2.25	2.23	2.27	2.24	2.245	0.0184	0.8200

4.5 Fatty Acid Content Of Orange Roughy Oil

Table 10.1 % * Myristic Acid (14:0) in Orange Roughy Oil

	Spring	Summer	Autumn	Winter
Sample 1 A1	0.48	0.35	0.80	0.82
Sample 1 A2	0.43	0.36	Tr	0.83
Sample 1 A3	0.44	Tr	0.81	0.83
Sample 1 A4	0.43	0.35	Tr	0.82
Sample 2 A1	0.48	0.43	0.75	0.73
Sample 2 A2	0.45	0.36	0.76	0.82
Sample 2 A3	Tr	Tr	0.75	0.73
Sample 2 A4	0.46	0.35	0.76	0.82
Sample 3 A1	0.38	0.36	0.65	0.78
Sample 3 A2	0.37	0.39	0.62	0.69
Sample 3 A3	0.34	Tr	0.62	0.67
Sample 3 A4	0.76	0.40	0.60	0.64
Sample 4 A1	0.53	0.51	0.66	0.45
Sample 4 A2	0.56	0.59	0.66	0.61
Sample 4 A3	0.72	Tr	0.68	0.50
Sample 4 A4	0.57	0.58	0.65	0.50

Source	SS	df	MS	F	P	Effect
Season	1.393	3	0.464438	15.44746	3.63E-07	**YES**
Location	0.149	3	0.049613	1.65014	0.190263175	**NO**
Interaction	0.625	9	0.069478	2.310871	0.030012508	**YES**
Error	1.443	48	0.030066			
Total	**3.611**	**63**				

* % of Total Fatty Acids

Table 10.2 % * Palmitic Acid (16:0) in Orange Roughy Oil

	Spring	Summer	Autumn	Winter
Sample 1 A1	3.87	1.22	3.25	2.06
Sample 1 A2	2.00	1.30	3.14	2.17
Sample 1 A3	1.47	1.26	3.28	2.09
Sample 1 A4	1.34	1.28	3.11	2.08
Sample 2 A1	1.32	1.69	2.10	2.14
Sample 2 A2	1.22	1.23	2.01	3.08
Sample 2 A3	3.10	1.66	2.11	2.18
Sample 2 A4	3.03	1.22	2.02	3.09
Sample 3 A1	1.85	1.28	1.90	3.43
Sample 3 A2	1.72	1.68	1.78	2.68
Sample 3 A3	1.68	1.39	1.83	2.64
Sample 3 A4	1.86	1.74	1.74	2.52
Sample 4 A1	2.34	1.32	1.86	2.55
Sample 4 A2	2.99	1.69	1.86	2.57
Sample 4 A3	3.09	1.38	1.93	2.41
Sample 4 A4	1.97	1.67	1.86	2.10

Source	SS	df	MS	F	P	Effect
Season	9.784	3	3.261331	9.078915	7.22E-05	**YES**
Location	0.08	3	0.026718	0.074378	0.973470135	**NO**
Interaction	0.605	9	0.067231	0.187157	0.994561213	**NO**
Error	17.243	48	0.35922			
Total	27.712	63				

* % of Total Fatty Acids

Table 10.3 % * Stearic Acid (18:0) in Orange Roughy Oil

	Spring	Summer	Autumn	Winter
Sample 1 A1	1.31	0.58	1.09	0.78
Sample 1 A2	0.89	0.83	1.09	0.80
Sample 1 A3	0.70	0.57	1.09	0.81
Sample 1 A4	0.69	0.59	1.07	0.78
Sample 2 A1	0.72	0.72	0.79	0.75
Sample 2 A2	0.69	0.76	0.76	1.41
Sample 2 A3	1.01	0.71	0.76	0.76
Sample 2 A4	1.00	0.56	0.76	1.42
Sample 3 A1	0.74	0.57	0.43	0.86
Sample 3 A2	0.70	0.81	0.54	0.74
Sample 3 A3	0.69	tr	0.54	0.70
Sample 3 A4	0.74	1.04	0.54	0.69
Sample 4 A1	0.71	0.43	0.60	0.91
Sample 4 A2	0.87	tr	0.59	0.79
Sample 4 A3	0.93	tr	0.62	0.79
Sample 4 A4	0.62	0.53	0.59	0.80

Source	SS	df	MS	F	P	Effect
Season	0.939	3	0.313154	4.994235	0.004280566	**YES**
Location	0.118	3	0.039363	0.62776	0.600617159	**NO**
Interaction	0.281	9	0.031189	0.497406	0.868942774	**NO**
Error	3.01	48	0.062703			
Total	4.348	63				

* % of Total Fatty Acids

Table 10.4 % * Palmitoleic Acid (16:1) in Orange Roughy Oil

	Spring	Summer	Autumn	Winter
Sample 1 A1	6.20	4.60	7.20	7.11
Sample 1 A2	5.83	4.61	6.68	7.47
Sample 1 A3	5.56	4.89	7.31	7.60
Sample 1 A4	5.34	4.54	6.84	7.56
Sample 2 A1	5.50	4.83	6.22	7.62
Sample 2 A2	5.27	4.63	6.22	8.27
Sample 2 A3	5.57	4.68	6.23	7.73
Sample 2 A4	5.51	4.59	6.25	8.36
Sample 3 A1	5.56	4.64	7.83	8.31
Sample 3 A2	4.87	4.55	7.54	7.55
Sample 3 A3	4.73	4.94	7.49	7.13
Sample 3 A4	5.07	4.69	7.36	7.04
Sample 4 A1	6.53	4.73	7.08	5.74
Sample 4 A2	6.57	5.39	7.36	6.31
Sample 4 A3	6.33	4.84	7.27	5.94
Sample 4 A4	6.75	5.33	7.35	5.02

Source	SS	df	MS	F	P	Effect
Season	61.945	3	20.6484	41.08785	2.65E-13	**YES**
Location	0.162	3	0.05398	0.107413	0.955360934	**NO**
Interaction	0.659	9	0.073245	0.145748	0.997903855	**NO**
Error	24.122	48	0.502543			
Total	**86.888**	**63**				

* % of Total Fatty Acids

Table 10.5 % * Vaccenic Acid 18:1 (n-7) in Orange Roughy Oil

	Spring	Summer	Autumn	Winter
Sample 1 A1	4.45	3.87	5.23	4.96
Sample 1 A2	4.91	3.92	5.35	4.89
Sample 1 A3	4.64	4.28	5.19	5.14
Sample 1 A4	4.59	3.86	5.37	4.94
Sample 2 A1	4.83	4.09	4.77	4.87
Sample 2 A2	4.73	3.84	4.81	5.00
Sample 2 A3	4.21	4.13	4.80	4.97
Sample 2 A4	4.10	3.82	4.86	5.01
Sample 3 A1	4.11	3.78	5.56	5.59
Sample 3 A2	4.25	3.22	5.89	5.34
Sample 3 A3	3.92	4.12	5.55	5.20
Sample 3 A4	4.19	3.91	5.42	5.15
Sample 4 A1	4.70	3.87	5.28	5.03
Sample 4 A2	4.73	4.34	5.45	5.11
Sample 4 A3	5.49	4.16	5.43	5.05
Sample 4 A4	4.65	4.31	5.46	4.98

Source	SS	df	MS	F	P	Effect
Season	16.556	3	5.518593	52.98459	2.91E-15	**YES**
Location	0.106	3	0.035293	0.338854	0.797303747	**NO**
Interaction	0.39	9	0.043288	0.415609	0.920494181	**NO**
Error	4.999	48	0.104155			
Total	**22.051**	**63**				

* % of Total Fatty Acids

Table 10.6 % * Oleic Acid 18:1 (n-9) in Orange Roughy Oil

	Spring	Summer	Autumn	Winter
Sample 1 A1	25.71	24.58	32.33	35.33
Sample 1 A2	32.35	23.92	33.05	34.72
Sample 1 A3	31.12	25.18	32.34	36.14
Sample 1 A4	31.21	24.45	33.07	35.07
Sample 2 A1	32.24	25.73	29.95	34.99
Sample 2 A2	31.97	24.73	30.06	35.69
Sample 2 A3	26.02	25.83	29.96	35.49
Sample 2 A4	25.68	24.50	30.16	35.76
Sample 3 A1	26.12	24.32	33.47	33.08
Sample 3 A2	25.19	24.37	33.11	31.98
Sample 3 A3	24.84	26.12	32.79	31.15
Sample 3 A4	26.19	24.93	32.39	31.01
Sample 4 A1	32.94	22.64	31.79	32.80
Sample 4 A2	32.11	24.49	32.73	33.00
Sample 4 A3	39.24	23.81	32.61	33.58
Sample 4 A4	32.00	24.35	32.73	32.70

Source	SS	df	MS	F	P	Effect
Season	774.396	3	258.132	39.05828	6.27E-13	**YES**
Location	4.079	3	1.359743	0.205744	0.891925	**NO**
Interaction	6.598	9	0.733111	0.110928	0.99929	**NO**
Error	317.227	48	6.608894			
Total	1102.3	63				

* % of Total Fatty Acids

Table 10.7 % * Gondoic Acid 20:1 (n-9) in Orange Roughy Oil

	Spring	Summer	Autumn	Winter
Sample 1 A1	21.77	27.52	14.69	0.46
Sample 1 A2	25.16	27.00	15.74	0.56
Sample 1 A3	25.93	27.83	14.82	0.49
Sample 1 A4	26.19	27.66	15.35	0.51
Sample 2 A1	27.11	28.89	21.97	0.50
Sample 2 A2	27.66	27.44	22.08	0.67
Sample 2 A3	25.73	29.01	21.94	0.52
Sample 2 A4	25.50	27.47	22.15	0.67
Sample 3 A1	27.35	27.15	21.74	0.54
Sample 3 A2	27.03	27.11	22.02	0.48
Sample 3 A3	27.08	20.72	21.70	0.40
Sample 3 A4	28.31	27.34	21.56	0.37
Sample 4 A1	21.87	30.13	21.79	0.52
Sample 4 A2	20.79	30.76	21.75	0.55
Sample 4 A3	26.28	30.81	22.28	0.30
Sample 4 A4	21.98	30.68	21.71	0.00

Source	SS	df	MS	F	P	Effect
Season	7434.693	3	2478.231077	397.6379468	6.93E-34	**YES**
Location	0.423	3	0.140959896	0.022617344	0.995328166	**NO**
Interaction	10.477	9	1.164065451	0.186777012	0.994602318	**NO**
Error	299.154	48	6.232380729			
Total	7744.747	63				

* % of Total Fatty Acids

Table 10.8 % * Gadoleic Acid 20:1 (n-11) in Orange Roughy Oil

	Spring	Summer	Autumn	Winter
Sample 1 A1	0.85	1.08	0.82	0.88
Sample 1 A2	0.99	1.00	1.54	0.86
Sample 1 A3	1.01	1.15	0.79	0.91
Sample 1 A4	0.99	1.10	1.93	0.84
Sample 2 A1	1.06	1.12	0.87	0.85
Sample 2 A2	1.09	1.06	0.89	0.80
Sample 2 A3	1.06	1.66	0.86	0.86
Sample 2 A4	1.04	1.04	0.88	0.80
Sample 3 A1	1.09	1.07	0.79	0.73
Sample 3 A2	1.10	1.07	0.80	0.74
Sample 3 A3	1.08	1.34	0.79	0.76
Sample 3 A4	1.26	1.06	0.79	0.76
Sample 4 A1	0.90	1.24	0.81	0.79
Sample 4 A2	0.86	1.14	0.80	0.74
Sample 4 A3	1.04	1.14	0.83	0.79
Sample 4 A4	0.93	1.13	0.81	0.86

Source	SS	df	MS	F	P	Effect
Season	0.979	3	0.326261	9.191962	6.51E-05	**YES**
Location	0.063	3	0.021027	0.592421	0.622994	**NO**
Interaction	0.363	9	0.040282	1.134901	0.357587	**NO**
Error	1.704	48	0.035494			
Total	3.108	63				

* % of Total Fatty Acids

Table 10.9 % * Erucic Acid 22:1 (n-9) in Orange Roughy Oil

	Spring	Summer	Autumn	Winter
Sample 1 A1	0.43	0	1.59	1.51
Sample 1 A2	0.46	0	1.72	1.62
Sample 1 A3	0.26	0	1.48	1.69
Sample 1 A4	0.36	0	1.75	1.61
Sample 2 A1	1.77	0	1.79	1.50
Sample 2 A2	1.88	0	1.84	1.42
Sample 2 A3	1.82	0	1.74	1.57
Sample 2 A4	1.78	0	1.82	1.46
Sample 3 A1	0.48	0	1.63	1.33
Sample 3 A2	0.46	0	1.68	1.53
Sample 3 A3	0.47	0	1.64	1.58
Sample 3 A4	0.93	0	1.63	1.47
Sample 4 A1	tr	0	0.90	1.22
Sample 4 A2	tr	0	1.66	1.40
Sample 4 A3	tr	2.24	1.72	1.27
Sample 4 A4	tr	2.30	1.61	1.40

Source	SS	df	MS	F	P	Effect
Season	17.982	3	5.99405	19.04825714	2.82E-08	**YES**
Location	0.691	3	0.230212741	0.731584068	0.5382522	**NO**
Interaction	0.928	9	0.103100222	0.327638165	0.961636094	**NO**
Error	15.105	48	0.314677083			
Total	34.705	63				

* % of Total Fatty Acids

Table 10.10 % * Cetoleic Acid 22:1 (n-11) in Orange Roughy Oil

	Spring	Summer	Autumn	Winter
Sample 1 A1	22.26	28.84	17.10	16.57
Sample 1 A2	18.68	29.40	19.17	16.25
Sample 1 A3	22.48	28.79	17.11	16.60
Sample 1 A4	22.35	29.17	19.18	17.49
Sample 2 A1	21.22	29.54	20.96	15.94
Sample 2 A2	22.48	28.50	21.03	14.26
Sample 2 A3	23.92	29.91	20.99	16.15
Sample 2 A4	23.71	28.59	21.34	14.16
Sample 3 A1	27.80	28.10	18.42	15.28
Sample 3 A2	28.46	28.05	19.04	17.01
Sample 3 A3	28.69	27.78	18.68	17.27
Sample 3 A4	25.86	27.96	18.65	17.33
Sample 4 A1	23.88	27.75	21.14	14.83
Sample 4 A2	22.58	26.17	18.54	15.54
Sample 4 A3	22.94	27.06	19.80	15.17
Sample 4 A4	25.34	26.20	18.60	16.80

Source	SS	df	MS	F	P	Effect
Season	1360.337	3	453.4458	130.3926	4.48E-23	**YES**
Location	2.624	3	0.87471	0.251531	0.859853767	**NO**
Interaction	5.475	9	0.608377	0.174944	0.995777863	**NO**
Error	166.922	48	3.477544			
Total	1535.359	63				

* % of Total Fatty Acids

Table 10.11 % * Linoleic Acid 18:2 (n-6) in Orange Roughy Oil

	Spring	Summer	Autumn	Winter
Sample 1 A1	0.87	0.63	1.04	0.88
Sample 1 A2	0.87	0.63	1.03	0.87
Sample 1 A3	0.77	0.64	1.03	0.89
Sample 1 A4	0.75	0.60	1.01	0.88
Sample 2 A1	0.75	0.61	0.88	0.96
Sample 2 A2	0.70	0.65	0.88	1.06
Sample 2 A3	0.79	0.59	0.87	0.96
Sample 2 A4	0.76	0.63	0.87	1.03
Sample 3 A1	0.70	0.64	1.05	1.18
Sample 3 A2	0.67	0.63	1.04	1.07
Sample 3 A3	0.65	0.64	1.04	1.00
Sample 3 A4	0.60	0.64	1.02	1.01
Sample 4 A1	0.81	0.76	0.98	0.86
Sample 4 A2	0.92	0.85	1.00	0.92
Sample 4 A3	1.08	0.81	0.98	0.86
Sample 4 A4	0.85	0.84	1.01	0.85

Source	SS	df	MS	F	P	Effect
Season	1.024	3	0.34121	32.36779	1.37E-11	**YES**
Location	0.006	3	0.002035	0.193083	0.900604	**NO**
Interaction	0.017	9	0.00189	0.179249	0.995373	**NO**
Error	0.506	48	0.010542			
Total	1.553	63				

* % of Total Fatty Acids

Table 10.12 % * Arachidonic Acid 20:4 (n-6) in Orange Roughy Oil

	Spring	Summer	Autumn	Winter
Sample 1 A1	0.46	0.41	0.85	0.37
Sample 1 A2	0.48	0	0	0.41
Sample 1 A3	0.34	0	0.83	0
Sample 1 A4	0.31	0.43	0	0.21
Sample 2 A1	0	0	0	0.48
Sample 2 A2	0	0.47	0	0.64
Sample 2 A3	0.67	0	0	0.50
Sample 2 A4	0.66	0.36	0	0.64
Sample 3 A1	0.42	0.63	0	0.47
Sample 3 A2	0.38	0.39	0.40	0.38
Sample 3 A3	0.39	0.26	0.39	0.70
Sample 3 A4	0	0.36	0.39	0.79
Sample 4 A1	0.30	0.27	0.33	0.86
Sample 4 A2	0.53	0	0.36	0.74
Sample 4 A3	0.59	0	0	0.61
Sample 4 A4	0.31	0	0.37	0.86

Source	SS	df	MS	F	P	Effect
Season	1.018	3	0.33925	5.122768	0.003728	**YES**
Location	0.019	3	0.006446	0.097334	0.96113	**NO**
Interaction	0.348	9	0.038718	0.584653	0.803036	**NO**
Error	3.179	48	0.066224			
Total	4.564	63				

* % of Total Fatty Acids

Table 10.13 % * Docosapentaenoic Acid 22:5 (n-3) in Orange Roughy Oil

	Spring	Summer	Autumn	Winter
Sample 1 A1	0.32	0	0	0
Sample 1 A2	0	0	0	0
Sample 1 A3	0	0	tr	0
Sample 1 A4	0	0	0	0
Sample 2 A1	0	0	tr	0
Sample 2 A2	0	0	0	0
Sample 2 A3	0	0	tr	tr
Sample 2 A4	0	0	0.74	tr
Sample 3 A1	0	0	tr	tr
Sample 3 A2	0	0	tr	0.34
Sample 3 A3	tr	0	tr	tr
Sample 3 A4	0	0	tr	tr
Sample 4 A1	0	0	tr	tr
Sample 4 A2	tr	0	tr	0.63
Sample 4 A3	0.23	0	0	0.24
Sample 4 A4	0	0	tr	tr

Source	SS	df	MS	F	P	Effect
Season	0.047	3	0.015664	0.887484	0.454365	YES
Location	0.016	3	0.005206	0.294995	0.828813	NO
Interaction	0.265	9	0.029445	1.668311	0.123106	NO
Error	0.847	48	0.017649			
Total	1.175	63				

* % of Total Fatty Acids

Table 10.14 % * Eicosapentaenoic Acid 20:5 (n-3) in Orange Roughy Oil

	Spring	Summer	Autumn	Winter
Sample 1 A1	0.38	0.62	1.30	0.54
Sample 1 A2	0.84	0.61	1.34	0.45
Sample 1 A3	0.57	0.63	1.29	0.39
Sample 1 A4	0.49	0.59	1.31	0.53
Sample 2 A1	0.42	tr	1.05	0.73
Sample 2 A2	tr	0.6	0.92	0.48
Sample 2 A3	0.90	tr	1.07	0.62
Sample 2 A4	0.88	0.63	1.04	0.79
Sample 3 A1	0.55	0.63	0.89	1.54
Sample 3 A2	0.48	0.58	0.88	1.33
Sample 3 A3	0.59	0.54	0.88	1.11
Sample 3 A4	tr	0.60	0.84	1.04
Sample 4 A1	0.53	0.58	0.91	0.88
Sample 4 A2	0.96	tr	0.92	0.98
Sample 4 A3	1.27	tr	0.93	0.98
Sample 4 A4	0.69	tr	0.88	0.89

Source	SS	df	MS	F	P	Effect
Season	3.461	3	1.153802	12.14934	4.89E-06	**YES**
Location	0.011	3	0.003735	0.039328	0.989457	**NO**
Interaction	0.434	9	0.04825	0.508066	0.861418	**NO**
Error	4.558	48	0.094968			
Total	8.465	63				

* % of Total Fatty Acids

Table 10.15 % * Docosahexaenoic Acid 22:6 (n-3) in Orange Roughy Oil

	Spring	Summer	Autumn	Winter
Sample 1 A1	1.00	1.66	5.19	1.76
Sample 1 A2	2.89	1.53	5.15	1.81
Sample 1 A3	1.75	1.75	5.23	1.79
Sample 1 A4	1.44	1.54	5.02	1.85
Sample 2 A1	1.10	0.87	3.38	2.25
Sample 2 A2	1.71	1.71	3.26	2.89
Sample 2 A3	2.98	0.84	3.40	2.27
Sample 2 A4	2.91	1.71	2.99	2.85
Sample 3 A1	1.60	1.71	2.06	4.87
Sample 3 A2	1.56	1.69	2.03	3.85
Sample 3 A3	1.58	1.72	2.08	3.45
Sample 3 A4	1.88	1.68	1.97	3.18
Sample 4 A1	1.28	1.16	2.39	3.34
Sample 4 A2	1.52	1.17	2.27	3.47
Sample 4 A3	1.36	1.20	2.46	3.15
Sample 4 A4	1.76	1.15	2.26	3.59

Source	SS	df	MS	F	P	Effect
Season	34.772	3	11.59074	13.63247	1.47E-06	YES
Location	0.286	3	0.095446	0.112259	0.952522	NO
Interaction	1.794	9	0.199375	0.234495	0.987639	NO
Error	40.811	48	0.85023			
Total	77.664	63				

* % of Total Fatty Acids

Table 11 Overall Fatty Acid Composition of the Fish Oil

% Fatty Acids Averaged For Each Season

FATTY ACIDS	SPRING	SUMMER	AUTUMN	WINTER	ANNUAL AVERAGE
14:0 Myristic	0.46	0.31	0.61	0.70	0.52
16:0 Palmitic	2.18	1.44	2.24	2.49	2.09
18:0 Stearic	0.81	0.54	0.74	0.86	0.74
Σ Saturates	3.45	2.29	3.59	4.05	3.35
16:1 Palmitoleic	5.70	4.78	7.01	7.17	6.17
18:1 (n-7) Vaccenic	4.53	3.97	5.28	5.08	4.72
18:1 (n-9) Oleic	29.68	24.62	32.03	33.91	30.06
20:1 (n-9) Gondoic	25.36	27.97	20.21	20.78	23.58
20:1 (n-11) Gadoleic	1.02	1.15	0.94	0.81	0.98
22:1 (n-9) Erucic	0.93	0.28	1.64	1.47	1.08
22:1 (n-11) Cetoleic	23.92	28.24	19.36	16.41	21.98
Σ Monounsaturates	91.14	91.01	86.47	85.63	88.56
18:2 (n-6) Linoleic	0.78	0.67	0.98	0.96	0.85
20:4 (n-6) Arachidonic	0.37	0.22	0.25	0.54	0.35
22:5 Docosapentaenoic	0.03	0.00	0.05	0.08	0.04
20:5 Eicosapentaenoic	0.60	0.41	1.03	0.83	0.72
22:6 Docosahexaenoic	1.77	1.44	3.20	2.90	2.33
Σ Poly Unsaturates	3.55	2.74	5.51	5.31	4.28

4.6 Quality / Freshness of Fish when Landed

Table 12 Freshness of Fish (Lipid Oxidation), TBA (mg/kg fish)

	Spring	Summer	Autumn	Winter
Sample 1 A1	0.2990	0.4362	0.1861	0.2032
Sample 1 A2	0.2443	0.5874	0.1977	0.2268
Sample 1 A3	0.1337	0.7154	0.1803	0.1861
Sample 1 A4	0.2268	0.6979	0.1687	0.2733
Sample 2 A1	0.4304	0.2966	0.2036	0.2908
Sample 2 A2	0.3548	0.2501	0.1977	0.2209
Sample 2 A3	0.3548	0.2734	0.2036	0.2209
Sample 2 A4	0.1079	0.3664	0.2152	0.1919
Sample 3 A1	0.2030	0.3257	0.1687	0.2501
Sample 3 A2	0.1580	0.1977	0.2093	0.1977
Sample 3 A3	0.2570	0.2443	0.1803	0.2443
Sample 3 A4	0.4180	0.2094	0.1745	0.1919
Sample 4 A1	0.2560	0.2443	0.1279	0.1861
Sample 4 A2	0.2670	0.2675	0.1683	0.2268
Sample 4 A3	0.2330	0.2849	0.1396	0.2035
Sample 4 A4	0.2730	0.2210	0.1338	0.2443

Source	SS	df	MS	F	P	Effect
Season	0.260	3	0.086567	7.341254	0.00038003	**YES**
Location	0.001	3	0.00027	0.022862	0.99525337	**NO**
Interaction	0.018	9	0.002002	0.169789	0.99622996	**NO**
Error	0.566	48	0.011792			
Total	0.8445	63				

Table 13 Freshness of Fish (Protein & TMAO Deterioration TVBN (mg/100g fish)

	Spring	Summer	Autumn	Winter
Sample 1 A1	6.49	8.76	6.58	8.13
Sample 1 A2	6.73	8.60	5.80	7.99
Sample 1 A3	6.26	8.92	6.11	8.28
Sample 1 A4	6.87	8.61	6.27	7.81
Sample 2 A1	8.44	8.14	6.74	7.52
Sample 2 A2	8.61	7.82	6.27	7.37
Sample 2 A3	8.30	8.12	6.89	7.83
Sample 2 A4	7.84	8.76	6.27	7.52
Sample 3 A1	8.77	9.87	6.11	8.46
Sample 3 A2	8.78	9.09	6.58	8.31
Sample 3 A3	8.78	9.25	5.95	8.46
Sample 3 A4	8.45	9.38	6.27	8.31
Sample 4 A1	8.46	9.51	8.78	7.19
Sample 4 A2	8.46	9.22	8.46	7.05
Sample 4 A3	8.77	9.35	8.46	7.35
Sample 4 A4	8.46	9.21	8.46	7.20

Source	SS	df	MS	F	P	Effect
Season	33.664	3	11.22118	15.16	4.5121E-07	**YES**
Location	0.309	3	0.10291	0.139	0.93616997	**NO**
Interaction	0.543	9	0.06037	0.0816	0.99979841	**NO**
Error	35.530	48	0.740203			
Total	70.045	63				

Table 14 Freshness of Fish (TMAO Deterioration), TMA (mg/100g fish

	Spring	Summer	Autumn	Winter
Sample 1 A1	6.43	5.93	4.43	5.70
Sample 1 A2	6.77	6.28	4.08	5.60
Sample 1 A3	6.52	6.41	4.31	5.81
Sample 1 A4	7.07	5.94	4.43	5.58
Sample 2 A1	6.05	5.59	4.90	5.36
Sample 2 A2	6.06	5.35	4.32	5.25
Sample 2 A3	6.05	5.81	4.78	5.48
Sample 2 A4	5.59	6.29	4.31	5.25
Sample 3 A1	6.17	7.34	4.32	5.94
Sample 3 A2	6.18	7.11	4.55	5.83
Sample 3 A3	6.29	6.70	3.96	5.94
Sample 3 A4	6.29	6.98	4.32	5.83
Sample 4 A1	6.06	6.96	6.18	4.89
Sample 4 A2	6.06	6.97	6.06	4.90
Sample 4 A3	6.29	6.84	6.06	5.00
Sample 4 A4	6.29	6.85	6.06	4.78

Source	SS	df	MS	F	P	Effect
Season	27.630	3	9.209952	23.83274	1.3695E-09	**YES**
Location	0.033	3	0.010902	0.02821	0.99352673	**NO**
Interaction	0.226	9	0.02507	0.064873	0.99992271	**NO**
Error	18.549	48	0.386441			
Total	46.437	63				

4.7 Ageing of Fish at +4°C & -5°C

Table 15.1 Spring Samples

+4°C in AIR

	TBA	TVBN	TMA
	mg MDA/kg fish	mg/100g fish	mg/100g fish
DAY 0	0.54	5.16	4.09
DAY3	0.73	8.75	7.51
DAY5	1.19	30.09	28.16
DAY8	2.97	69.21	63.82
DAY15	4.36	170.20	162.31
DAY17	3.15	195.63	190.07

+4°C in VACUUM

	TBA	TVBN	TMA
	mg MDA/kg fish	mg/100g fish	mg/100g fish
DAY 0	0.21	5.00	3.93
DAY3	0.46	7.21	6.36
DAY5	0.50	11.69	10.39
DAY8	1.73	46.60	41.79
DAY15	1.91	145.36	140.00
DAY17	0.91	157.37	151.02

-5°C in AIR

	TBA	TVBN	TMA
	mg MDA/kg fish	mg/100g fish	mg/100g fish
DAY 0	0.47	4.98	3.75
DAY4	0.62	6.95	5.85
DAY9	0.63	8.21	7.17
DAY18	0.93	12.38	11.26
DAY24	1.11	15.15	13.43
DAY65	1.37	34.50	32.67

-5°C in VACUUM

	TBA	TVBN	TMA
	mg MDA/kg fish	mg/100g fish	mg/100g fish
DAY 0	0.19	2.02	1.00
DAY4	0.38	4.19	2.92
DAY9	0.39	6.57	4.94
DAY18	0.58	12.50	11.30
DAY24	0.84	14.87	13.67
DAY65	0.94	30.47	28.91

Table 15.2 Summer Samples

+4°C in AIR

	TBA	TVBN	TMA
	mg MDA/kg fish	mg/100g fish	mg/100g fish
DAY 0	0.65	6.78	5.62
DAY3	0.89	9.78	8.30
DAY5	1.98	35.71	33.66
DAY8	3.89	72.11	53.19
DAY15	5.00	185.99	183.33
DAY17	4.67	201.45	198.79

+4°C in VACUUM

	TBA	TVBN	TMA
	mg MDA/kg fish	mg/100g fish	mg/100g fish
DAY 0	0.37	5.13	3.42
DAY3	0.59	7.85	6.72
DAY5	0.76	12.81	11.53
DAY8	2.75	56.79	54.59
DAY15	3.53	156.78	154.65
DAY17	3.12	172.94	171.19

-5°C in AIR

	TBA	TVBN	TMA
	mg MDA/kg fish	mg/100g fish	mg/100g fish
DAY 0	0.41	5.23	4.41
DAY4	0.64	7.70	6.26
DAY9	0.67	9.01	8.17
DAY18	1.11	14.42	13.15
DAY24	1.34	16.93	15.66
DAY65	1.98	37.82	35.56

-5°C in VACUUM

	TBA	TVBN	TMA
	mg MDA/kg fish	mg/100g fish	mg/100g fish
DAY 0	0.25	3.17	1.92
DAY4	0.43	5.76	4.45
DAY9	0.46	7.23	6.12
DAY18	0.73	13.17	12.03
DAY24	0.92	18.36	17.19
DAY65	1.10	32.80	31.84

Table 15.3 Autumn Samples

+4°C in AIR

	TBA	TVBN	TMA
	mg MDA/kg fish	mg/100g fish	mg/100g fish
DAY 0	0.50	5.01	4.52
DAY3	0.70	7.95	6.18
DAY5	0.86	23.52	21.26
DAY8	2.16	56.54	54.52
DAY15	4.13	153.54	150.12
DAY17	5.91	172.45	171.23

-5°C in AIR

	TBA	TVBN	TMA
	mg MDA/kg fish	mg/100g fish	mg/100g
DAY 0	0.21	3.21	3.00
DAY4	0.36	5.45	4.26
DAY9	0.46	7.21	6.02
DAY18	0.69	11.52	10.18
DAY24	0.93	14.54	13.12
DAY65	1.91	38.45	37.23

+4°C in VACUUM

	TBA	TVBN	TMA
	mg MDA/kg fish	mg/100g fish	mg/100g fish
DAY 0	0.40	4.01	3.89
DAY3	0.40	6.31	5.62
DAY5	0.44	11.61	10.31
DAY8	1.19	35.18	33.02
DAY15	1.91	100.26	99.01
DAY17	2.00	158.92	156.15

-5°C in VACUUM

	TBA	TVBN	TMA
	mg MDA/kg fish	mg/100g fish	mg/100g fish
DAY 0	0.10	2.27	1.99
DAY4	0.22	4.61	3.87
DAY9	0.39	5.18	4.02
DAY18	0.46	9.15	8.02
DAY24	0.61	12.26	11.15
DAY65	0.80	26.92	25.15

Table 15.4 Winter Samples

+4°C in AIR

	TBA	TVBN	TMA
	mg MDA/kg fish	mg/100g fish	mg/100g fish
DAY 0	0.45	4.83	3.72
DAY3	0.64	6.49	5.33
DAY5	0.73	19.74	18.18
DAY8	1.98	46.62	45.21
DAY15	3.96	148.94	147.12
DAY17	4.55	165.37	163.66

+4°C in VACUUM

	TBA	TVBN	TMA
	mg MDA/kg fish	mg/100g fish	mg/100g fish
DAY 0	0.19	3.79	3.10
DAY3	0.39	5.79	4.28
DAY5	0.43	10.37	9.08
DAY8	1.05	32.86	31.10
DAY15	1.77	99.55	98.00
DAY17	1.85	124.19	122.77

-5°C in AIR

	TBA	TVBN	TMA
	mg MDA/kg fish	mg/100g fish	mg/100g fish
DAY 0	0.19	2.80	1.82
DAY4	0.30	4.06	3.00
DAY9	0.40	4.99	3.12
DAY18	0.60	10.30	9.04
DAY24	0.85	15.70	14.00
DAY65	1.87	31.29	29.05

-5°C in VACUUM

	TBA	TVBN	TMA
	mg MDA/kg fish	mg/100g fish	mg/100g fish
DAY 0	0.10	1.98	1.00
DAY4	0.21	3.27	2.19
DAY9	0.30	4.40	3.06
DAY18	0.42	8.97	7.04
DAY24	0.60	11.10	9.32
DAY65	0.79	24.92	23.12

Table 16 AGEING of ORANGE ROUGHY (AFTER 6 MONTHS STORAGE @ -18°C)

(a)

+4°C in AIR

	TBA	TVBN	TMA
	mg MDA/kg fish	mg/100g fish	mg/100g fish
DAY 0	0.61	5.13	4.00
DAY3	1.00	11.59	9.48
DAY5	2.20	46.89	42.00
DAY8	4.76	83.22	81.16
DAY15	6.53	221.45	219.45
DAY17	7.88	245.31	243.37

+4°C in VACUUM

	TBA	TVBN	TMA
	mg MDA/kg fish	mg/100g fish	mg/100g fish
DAY 0	0.41	4.09	2.85
DAY3	0.73	8.74	6.25
DAY5	1.47	35.77	33.43
DAY8	4.24	79.17	77.15
DAY15	5.62	197.07	195.00
DAY17	6.90	227.53	225.78

AGEING of ORANGE ROUGHY (AFTER 12 MONTHS STORAGE @ -18°C)

(b)

+4°C in AIR

	TBA	TVBN	TMA
	mg MDA/kg fish	mg/100g fish	mg/100g fish
DAY 0	0.90	6.91	5.27
DAY3	1.63	13.75	11.51
DAY5	2.91	58.63	57.16
DAY8	5.12	86.15	84.85
DAY15	7.13	235.16	234.00
DAY17	8.96	257.12	255.51

+4°C in VACUUM

	TBA	TVBN	TMA
	mg MDA/kg fish	mg/100g fish	mg/100g fish
DAY 0	0.73	4.92	3.11
DAY3	1.43	9.00	7.10
DAY5	2.56	45.13	41.00
DAY8	4.95	88.32	86.55
DAY15	5.68	219.54	217.94
DAY17	6.91	229.59	227.67

Table 17 Effect of Storage @ -18°C on Freshness of Orange Roughy Fillets

Storage Time	TVBN (mg/100g)	TMA (mg/100g)	TBA (mg MDA/kg)
0 Months	4.01	3.52	0.50
6 Months	4.13	3.59	0.91
12 Months	4.91	3.27	1.90

Table 18 Effect of Storage @ -18°C on Shelf Life of Refridgerated Fillets

TMA +4°C in Air (mg/100g fish)

Day	Fresh	After 6 Months	After 12 Months
0	4.52	4.00	5.27
3	6.98	9.48	11.52
5	21.26	42.00	57.16
8	54.52	81.16	84.85
15	150.12	219.49	234.00
17	171.23	243.37	255.51

TMA +4°C in Vacuum (mg/100g fish)

Day	Fresh	After 6 Months	After 12 Months
0	3.89	2.85	3.11
3	5.62	6.25	7.10
5	10.31	33.43	41.00
8	33.02	77.15	86.55
15	99.01	195.00	217.94
17	156.15	225.78	227.67

TMA -5°C in Air (mg/100g fish)

Day	Fresh	After 6 Months	After 12 Months
0	3.00	4.00	5.27
4	4.26	9.48	11.51
9	6.02	42.00	57.16
18	10.18	81.16	84.85
24	13.12	219.45	234.00
65	37.23	243.37	255.51

TMA -5°C in Vacuum (mg/100g fish)

Day	Fresh	After 6 Months	After 12 Months
0	1.99	2.85	3.11
4	3.87	6.25	7.10
9	4.02	33.43	41.00
18	8.02	77.15	86.55
24	11.15	195.00	217.94
65	25.15	225.78	227.67

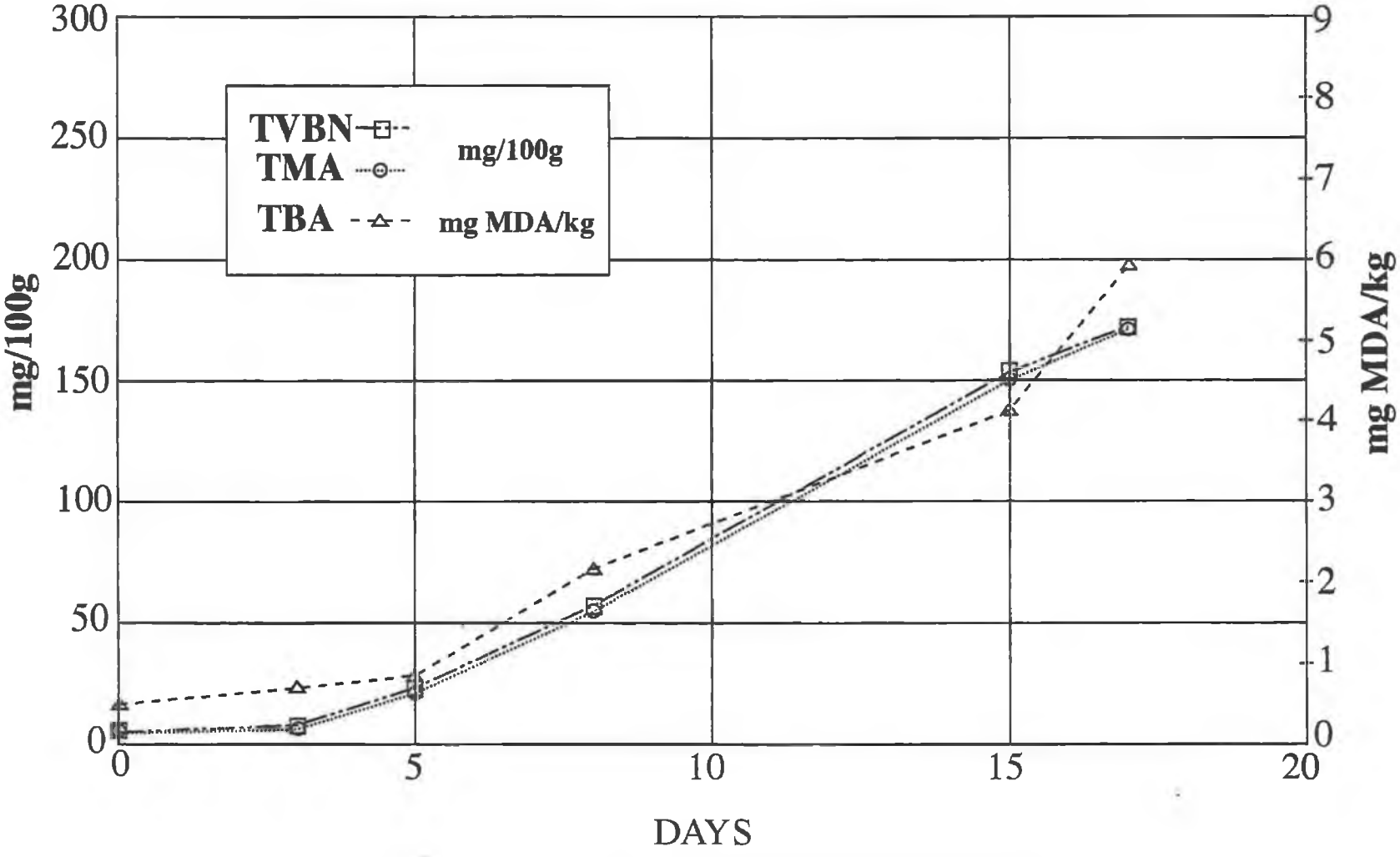


Fig. 19 **The protein and TMAO deterioration and lipid oxidation of fresh/frozen Orange Roughy in the presence of air at +4°C**

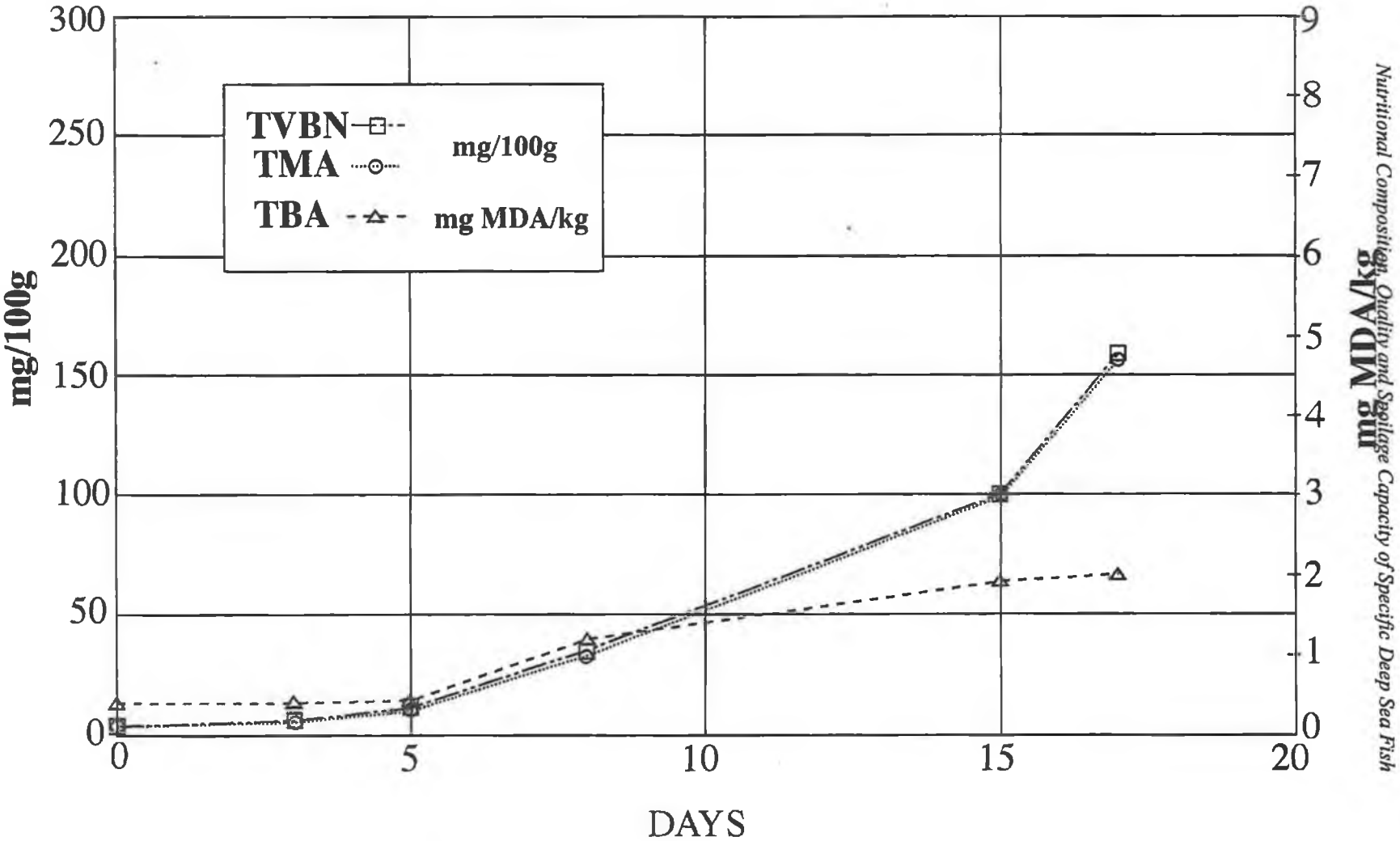


Fig. 20 **The protein and TMAO deterioration and lipid oxidation of fresh/frozen Orange Roughy in the absence of air at +4°C**

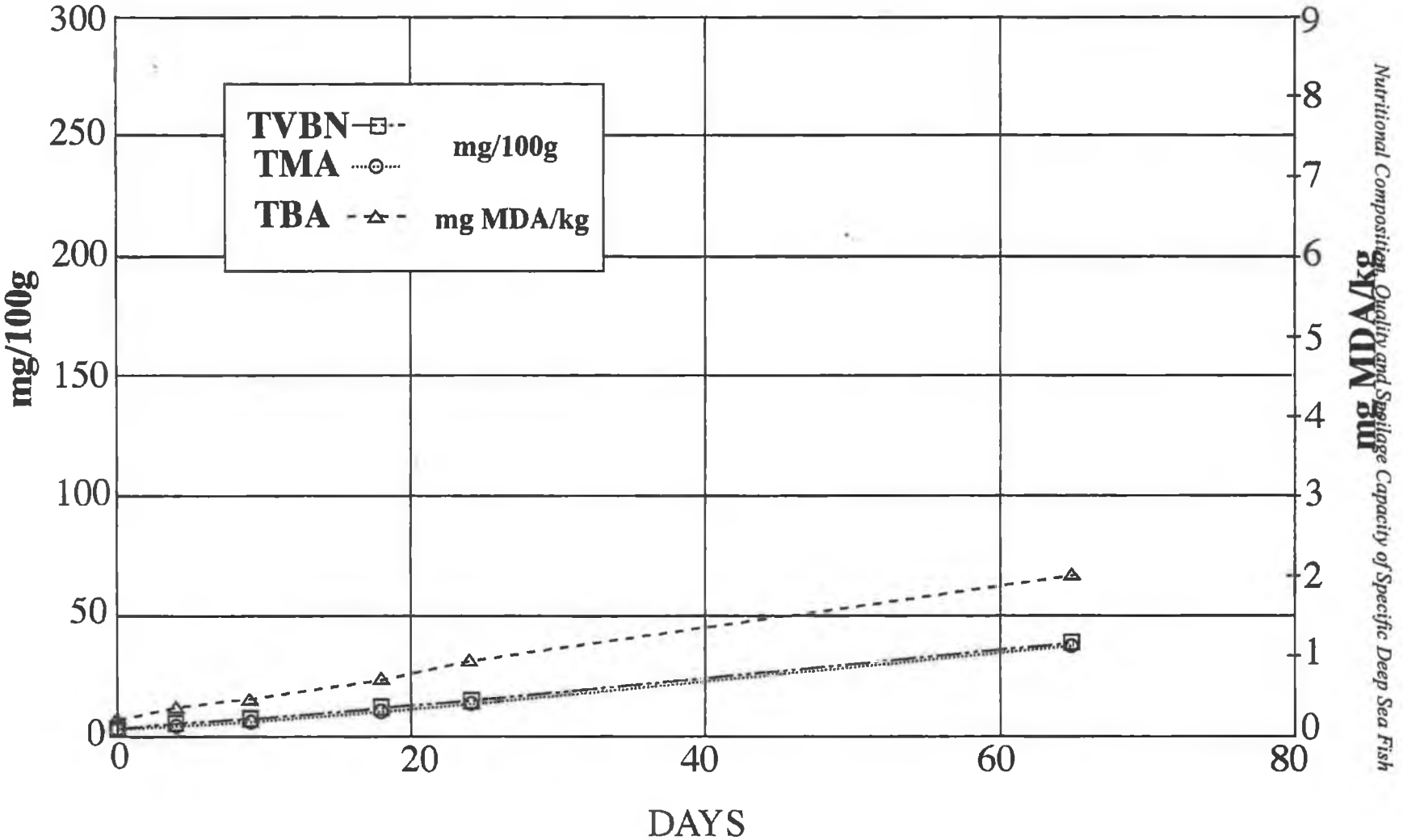


Fig. 21 **The protein and TMAO deterioration and lipid oxidation of fresh/frozen Orange Roughy in the presence of air at -5°C**

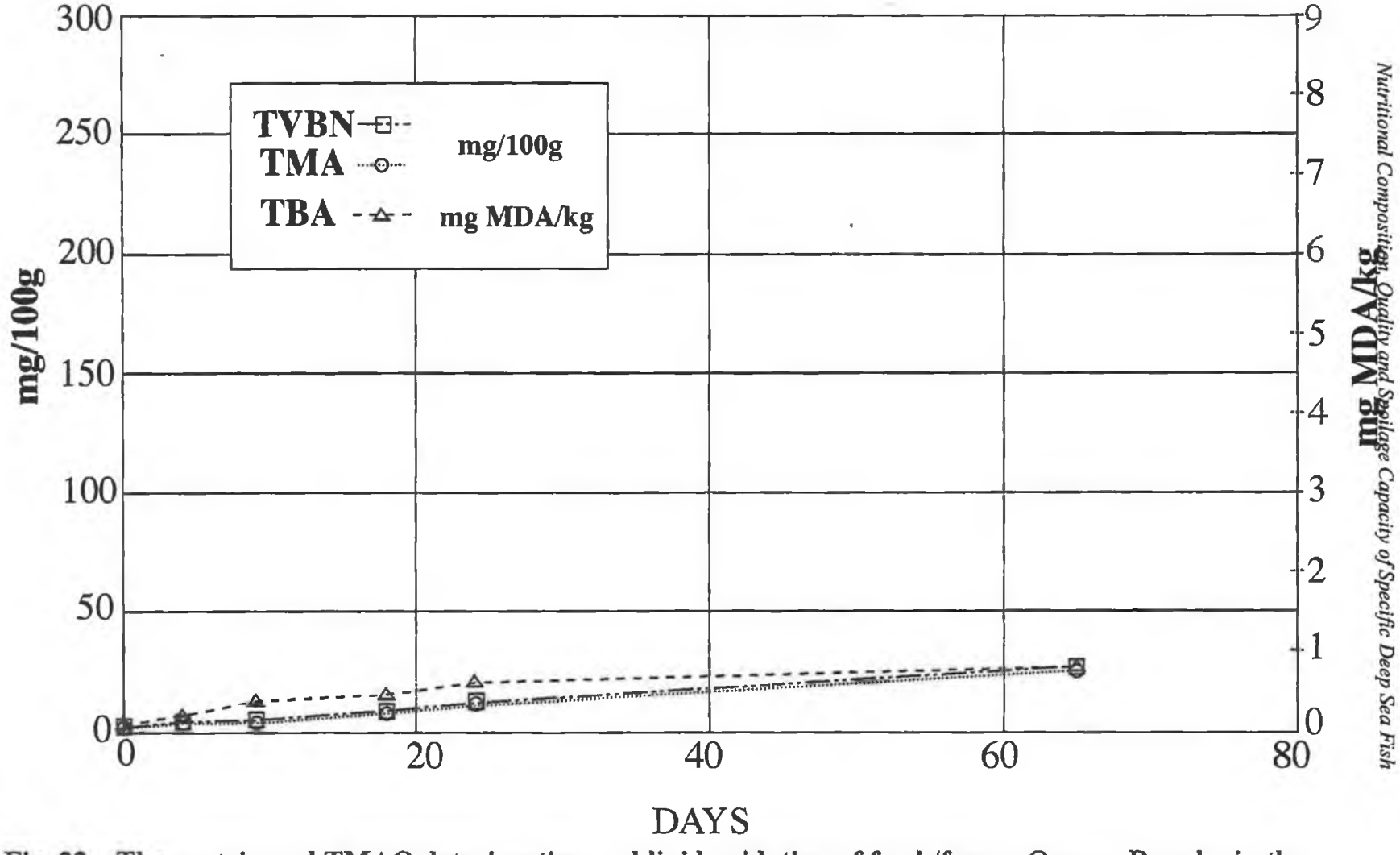


Fig. 22 **The protein and TMAO deterioration and lipid oxidation of fresh/frozen Orange Roughy in the absence of air at -5°C**

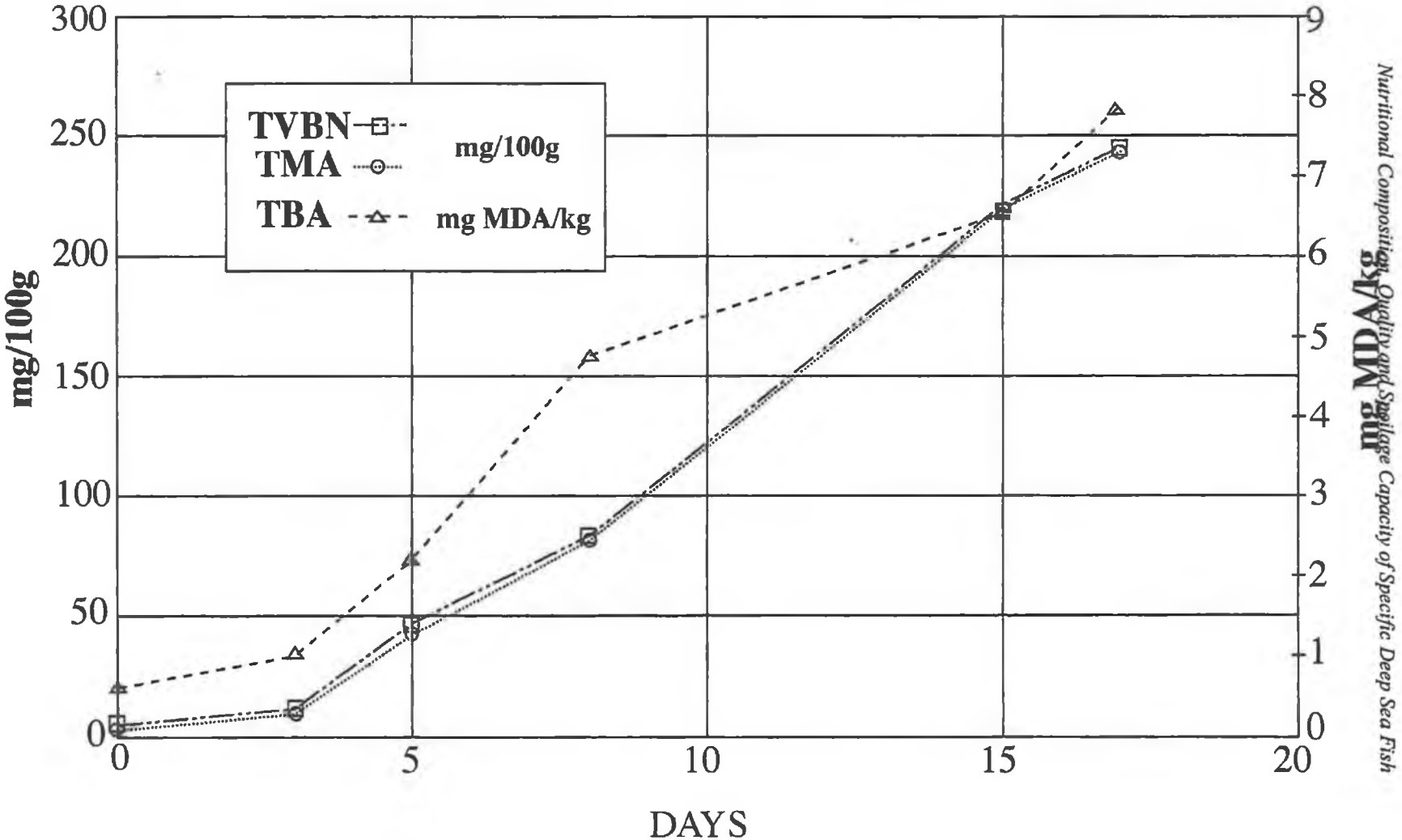


Fig. 23 **The protein and TMAO deterioration and lipid oxidation in Orange Roughy, pre-stored at -18°C for 6 months, in the presence of air at +4°C.**

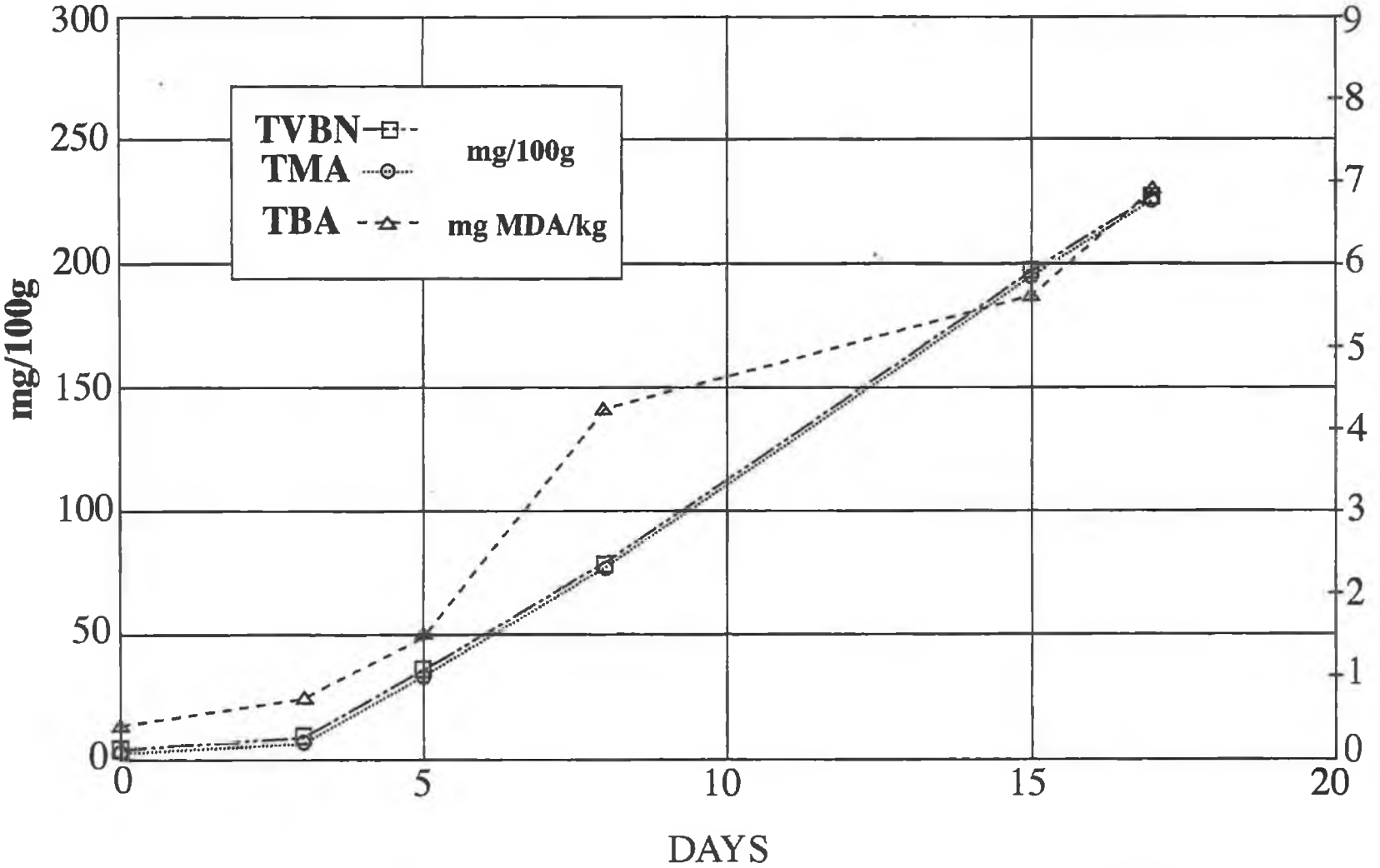


Fig. 24 **The protein and TMAO deterioration and lipid oxidation in Orange Roughy, pre-stored at -18°C for 6 months, in the absence of air at +4°C**

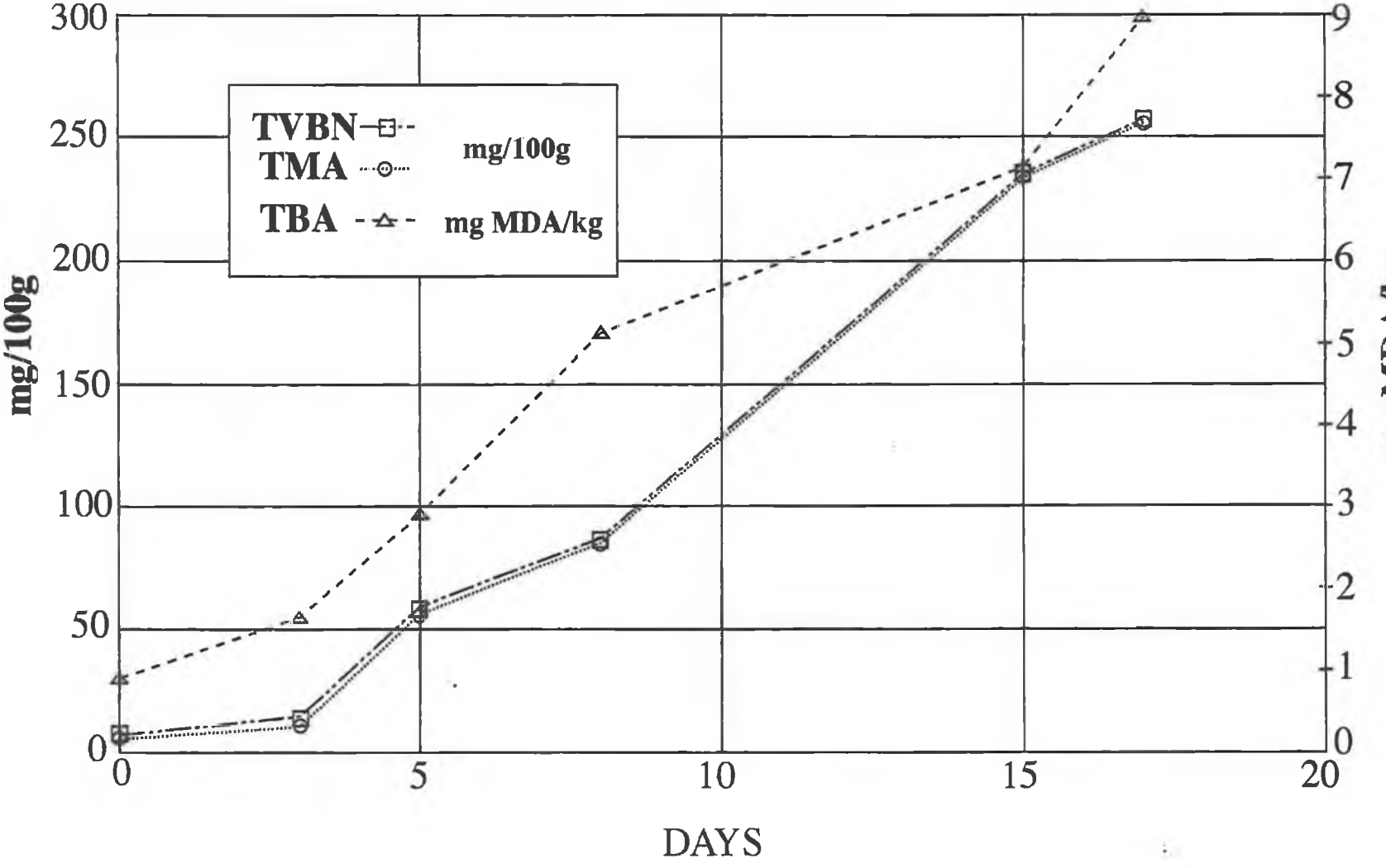


Fig. 25 **The protein and TMAO deterioration and lipid oxidation in Orange Roughy, pre-stored at -18°C for 12 months, in the presence of air at +4°C**

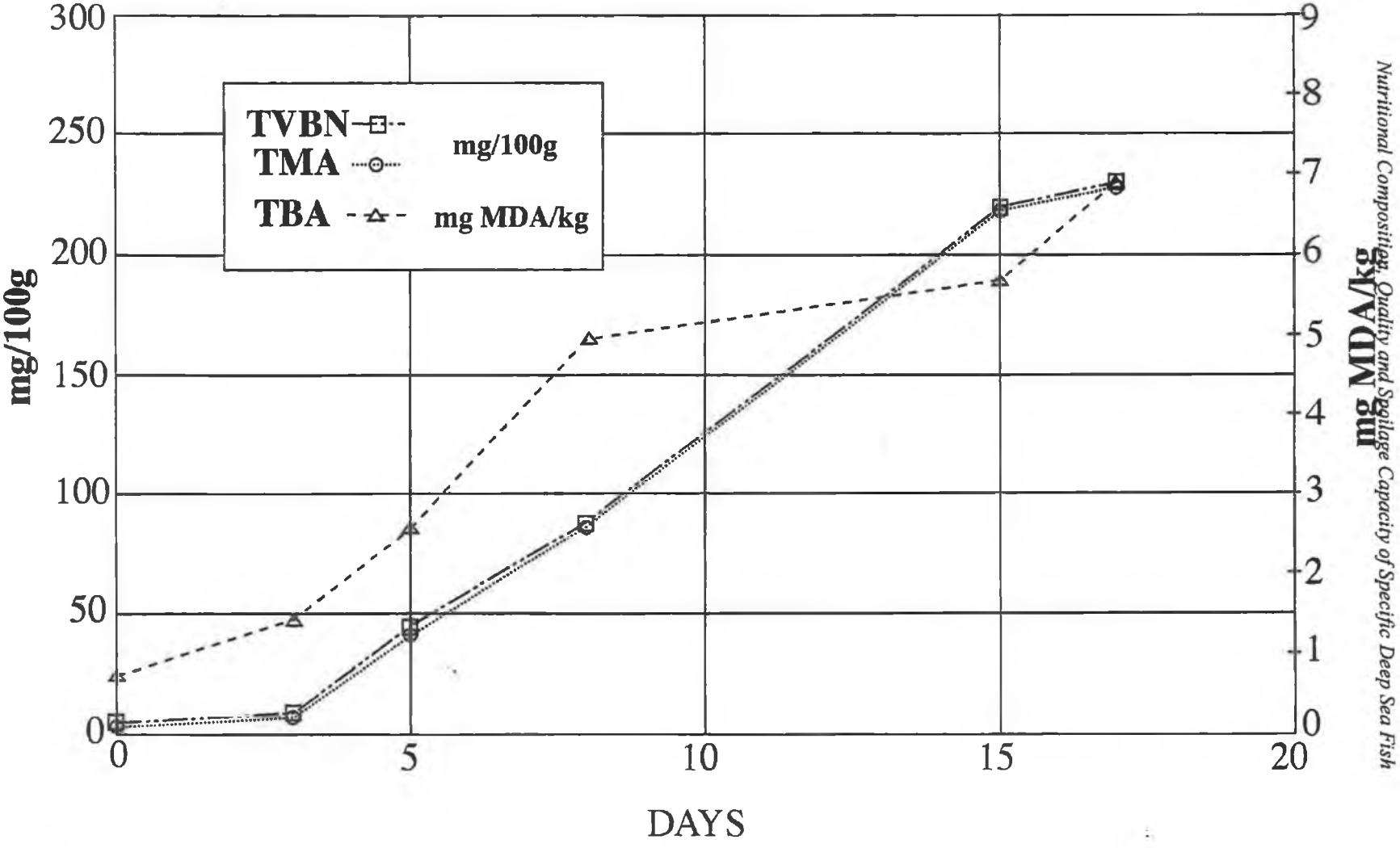


Fig. 26 **The protein and TMAO deterioration and lipid oxidation in Orange Roughy, pre-stored at -18°C for 12 months, in the absence of air at +4°C**

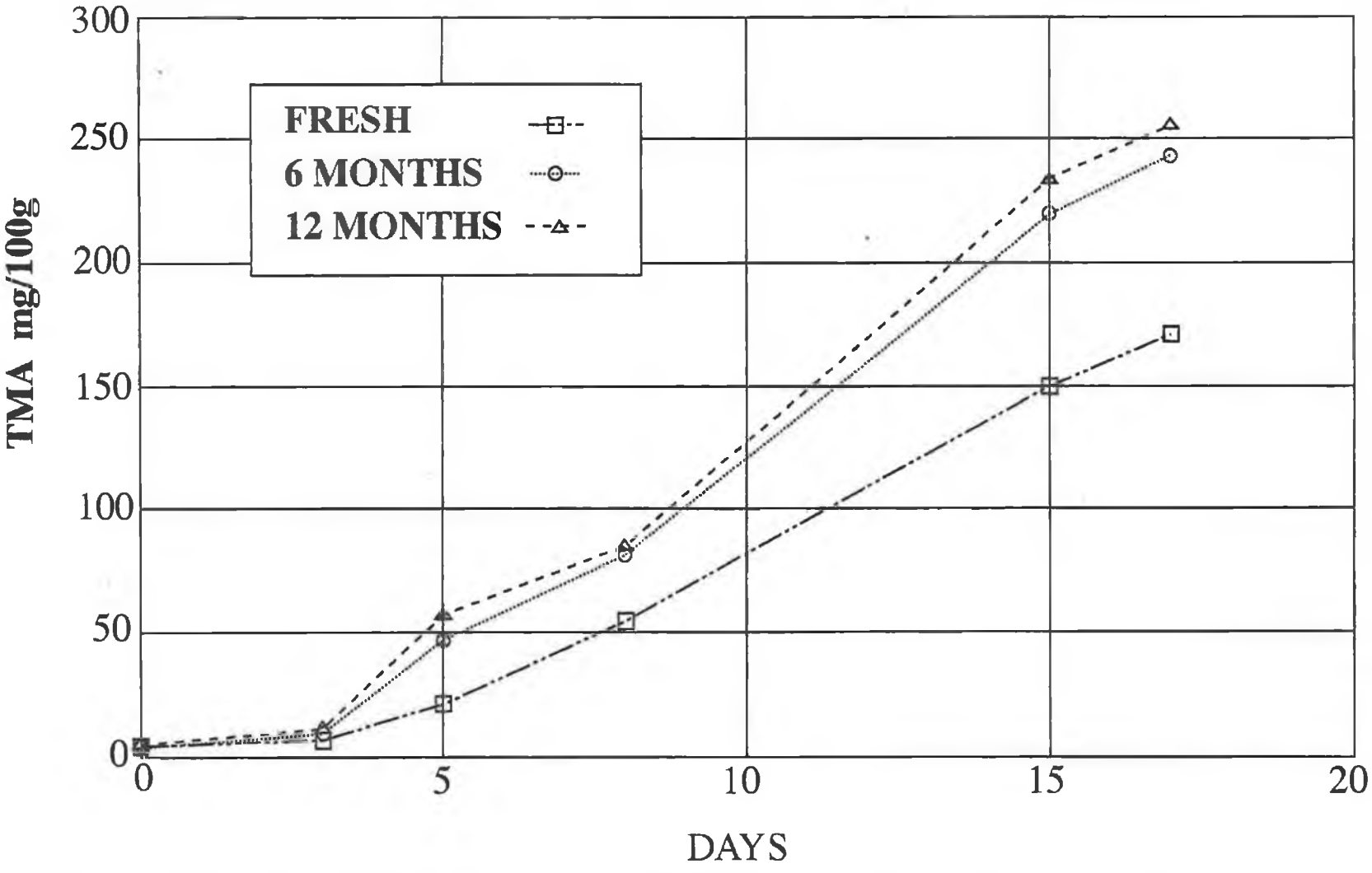


Fig. 27 **Effect of long-term storage at -18°C and subsequent shelf-life of freeze/thawed Orange Roughy fillets in the presence of air at +4 °C**

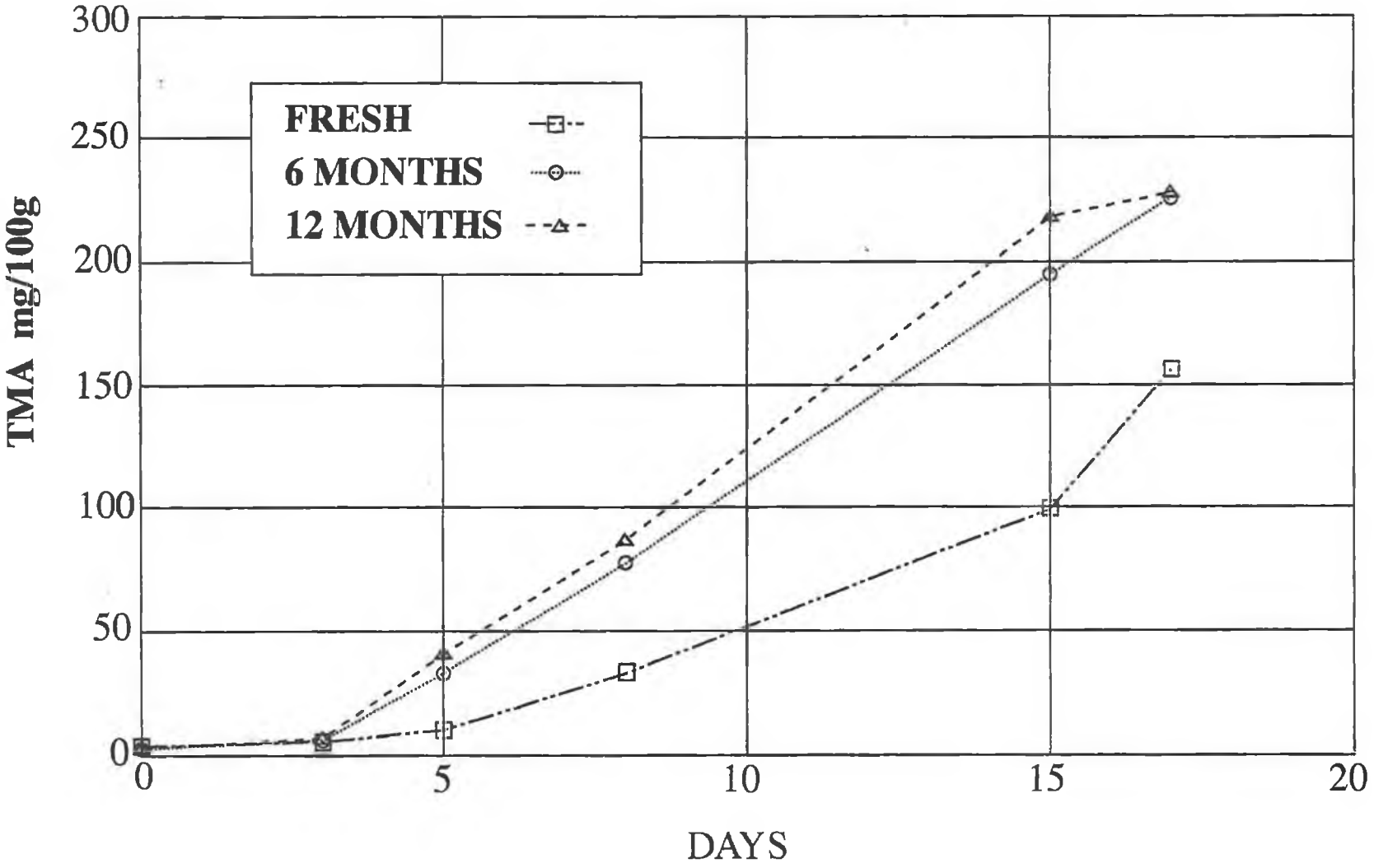


Fig. 28 **Effect of long-term storage at -18°C and subsequent shelf-life of freeze/thawed Orange Roughy fillets in the absence of air at +4 °C**

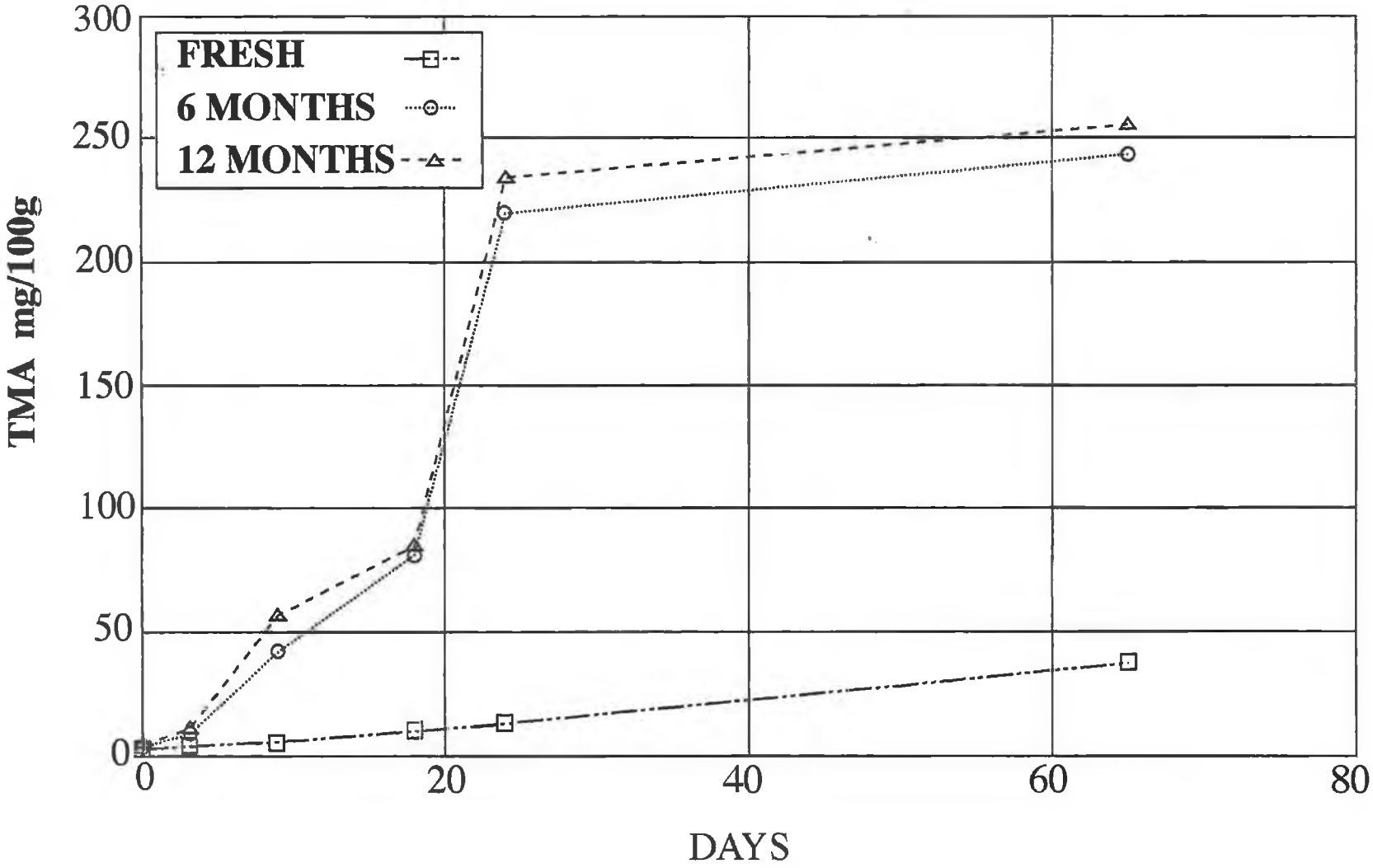


Fig. 29 **Effect of long-term storage at -18°C and subsequent shelf-life of freeze/thawed Orange Roughy fillets in the presence of air at -5°C**

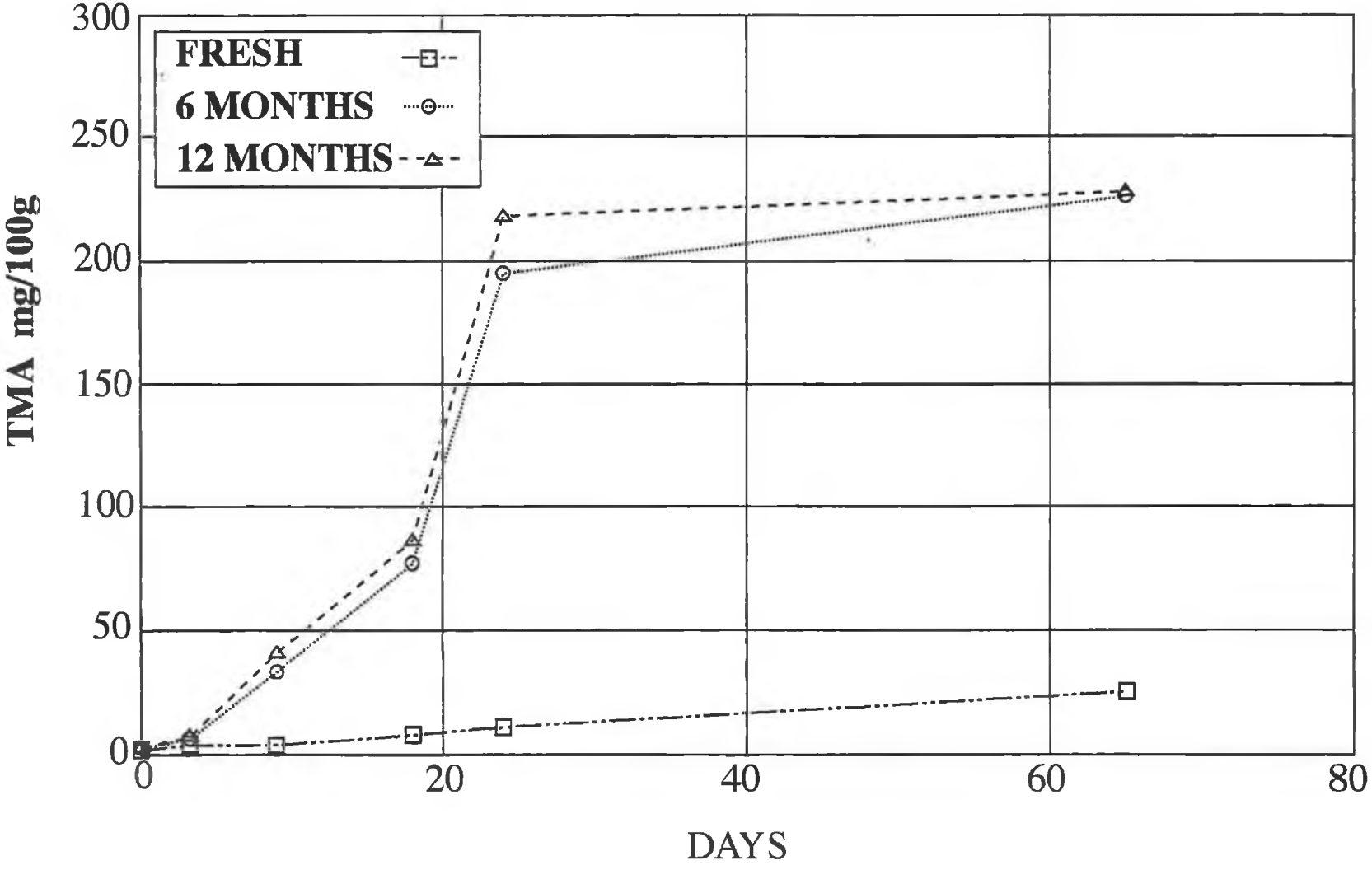


Fig. 30 **Effect of long-term storage at -18°C and subsequent shelf-life of freeze/thawed Orange Roughy fillets in the absence of air at -5°C**

5. DISCUSSION

Four specimens were oobtained for each season. Spring specimens ranged in length from 45 – 60cm and in weight from 2.25 - 2.65kgs, summer specimens ranged from 56 – 62cm and 2.8 – 3.7kgs, autumn specimens ranged from 50 – 58cm and 2.6-3.1kgs and winter specimens ranged from 45 – 61cm and from 1.65 – 3.25kgs in weight (Table 2). These measurements and weights correspond to mature specimens as values recorded for orange roughy caught in the North-East Atlantic have ranged in size from 40-58cm **(Branch, T.A., 2001)**. The specimens which were 61-62cm long are typical of the lengths recorded for orange roughy from the Faroese catches but the body masses are much smaller. The largest body mass recorded in this study was 3.66kg for an orange roughy which was 62cm long. Where as orange roughy caught from the Faroese had recorded body mass of up to 7kg for fish of the same length. **(Branch, T.A., 2001)**.

5.1 Moisture Content

The moisture content of fish fillets was at a high of 79.64%$^{w}/_{w}$ in the spring samples and the lowest of 77.32% $^{w}/_{w}$ was obtained for the autumn samples. Average values for summer and winter were 77.69%$^{w}/_{w}$ and 78.85%$^{w}/_{w}$ respectively. Statistically results show a significant seasonal variation with no variation from fish to fish or in the location within the fish (Table 3). According to **Love, R.M., (1997)** over 80% of the variation in lipid content is inversely proportional to moisture content. Values found for lipid and moisture content in autumn reflect this as lipid values were highest at 4.5% $^{w}/_{w}$ and the lowest moisture levels were recorded at 77.32% $^{w}/_{w}$.

Studies carried out on fillets of New Zealand species of orange roughy recorded an average value of 75.8% $^{w}/_{w}$ for moisture content **(Buisson, et al., 1982).** These results are similar to the figures recorded in this study (Table 3). Other studies published on the nutritional composition of fish give a value of 81.12% moisture content for fish in general **(Khan et al., 2006). Perez, M.A.(1994)** carried out studies on some Alaskan fishes and squid and moisture values ranging from 68.3% for Pacific herring to 79.4% for Pacific cod were obtained. Also studies carried out on Sea Bass showed initial moisture levels to be 77.38% **(Beklevik, G et al., 2005).**

5.2 Protein content

Protein analysis was carried out on the fish fillet as opposed to the whole fish. Studies carried out on New Zealand orange roughy gave results of protein averages of 12.2%$^{w}/_{w}$ for the whole fish and higher values of 13.3% $^{w}/_{w}$ for the fillet. **(James et al., 1986).**

In contrast, the orange roughy fillets in this study, had a protein content ranging from lower averages of 16.12%$^{w}/_{w}$ for fish caught in winter to the highest protein average of 16.68% $^{w}/_{w}$ for fish landed in spring. Average values for summer and autumn were 16.61% $^{w}/_{w}$ and 16.31%$^{w}/_{w}$ respectively (Table 4).

Other deep sea fish such as Black Oreo and Smooth Oreo have protein contents of 15.2%$^{w}/_{w}$ and 13.7%$^{w}/_{w}$ respectively for the whole fish **(James et al., 1986)**. These results are similar to protein values for in-shore mid-water fish such as Jack Mackerel, Red Cod, Hoki and Southern Blue Whiting which gave values of 22.9%$^{w}/_{w}$ 15.5%$^{w}/_{w}$, 20.8% $^{w}/_{w}$ and 15% $^{w}/_{w}$ respectively. **(James et al., 1986).**

Statistically results show a significant seasonal variation with no variation from fish to fish or in the location within the fish. (Table 4)

Studies carried out by **Mc Cane & Widdowson, (1991)** found that the amount of protein in fish muscle is usually somewhere between 15- 20% $^{w}/_{w}$ but values lower than 15%$^{w}/_{w}$ or as high as 28% $^{w}/_{w}$ are occasionally recorded in some species.

We can therefore conclude that the protein values recorded for the orange roughy analysed during this study, although significantly higher than those recorded for the New Zealand species of orange roughy, **(James et al., 1986)** compare very favourably with protein values for fish muscle in general and may be of a higher nutritional value than the New Zealand species. This would depend on the amino acid composition which was not looked at in this study.

5.3 LIPID CONTENT

Lipid values throughout this study ranged from a high of 4.58% $^{w}/_{w}$ for autumn samples to a low of 3.6% $^{w}/_{w}$ for summer samples with average values of 3.79% $^{w}/_{w}$ and 4.47% $^{w}/_{w}$ for spring and winter samples respectively (Table 5). These results are similar the value of 5.46% total lipid found in studies on skinned orange roughy **(de Koning A.J., 2005)** but are significantly lower than the figure of 9.9 $^{w}/_{w}$ recorded for the lipid content of the fish muscle of New Zealand species of orange roughy **(Buisson, et al., 1982)**. Values of between 18-20% $^{w}/_{w}$ for the whole fish have also been recorded **(Grigor et al., 1990, Mc Clutchie, S. and Ye, Z., 2000)**. Further studies of Icelandic species of Orange Roughy recorded values of 7.5% $^{w}/_{w}$ for lipid content **(Sigurgislanōttir, S. U., & Palmadōttir H., 1993).** As only four samples were analysed, for each season, during this study further studies on a larger scale would be desirable. However the statistics show very little variation between season or location within fish or from fish to fish(Table 5). It would also be desirable to undertake further studies to determine why there is such a variation in lipid values found between different researchers, as discussed above. Results can vary with the extraction technique chosen. Solvents such as hexane or petroleum spirit can yield low results because they only extract the triglycerides and wax esters. The chloroform/methanol method (Folch extraction) used in this work should however have yielded the total fat content with minimum/no carryover of non fat constituents. The high results of **Buisson et al., 1982, Grigor et al., 1990, Mc Clatchie, S., & Ye, Z., 2000** and **Sigurgislanōttir, S. U., & Palmadōttir, H.,1993** could be explained by the fact that whole fish were analysed in these studies. Skin of orange roughy has been reported to have an oil content of 43.9% $^{w}/_{w,}$ the head 20.2% $^{w}/_{w}$ the gut 24.1% $^{w}/_{w}$ and the frame 21.2% $^{w}/_{w}$ **(Buisson, et al., 1982)**. More likely the samples analysed in the other studies were taken at the end of the summer/autumn season when fat levels are known to be at their highest. In any case when you compare like with like, (skinned fillets) the findings in this study agree with the published results of **de Koning (2005)**.

5.4 IODINE VALUE

As iodine value reflects the degree of unsaturation of an oil and the oils/fatty acids extracted from orange roughy elsewhere have been shown to have a relatively low degree of unsaturation, **(Bakes et al., 1995 Buisson et al., 1982, Sargent et al., 1883)**, it was expected that the iodine values obtained here should reflect this. Previous studies have reported these values to be between 73-89 **(Buisson et al., 1982)** which is very much in line with the average values for each season obtained in this work. Spring had the highest value of 79.54 with summer giving the lowest average of 74.63. Autumn and winter gave very similar values of 78.04 and 78.37 respectively (Table 6). These results correlate with the FAME composition which shows over 85%$^{w}/_{w}$ of the extracted oil to be composed of the unsaturated fatty acids 16:1, 18:1, 20:1 and 22:1 (Table 11). It also agrees with the work of others (Table 1A, Introduction) which indicates significant quantities of monounsaturated fatty acids and little polyunsaturated fatty acids.
The fat content of fish oils varies from species to species. For example"lean" fish such as cod has a lipid content of less than 1% and cod liver oil has an iodine value of 162.6 **(Ackman, 1980)**. The majority of the lipids are polar (phosphatidyl choline, sphingomyelin and phosphatidyl serine) with a low percentage of the neutral lipids such as triglycerides, cholesterol esters and free fatty acids present **(Patterson, H.B.W., 1994)**
Other fish such as herring and sardines also have relatively high percentages of the unsaturated fatty acids 16:1, 18:1, 20:1 and 22:1. According to **Patterson, H.B.W. (1994)** herrings have values of 7.0% for 16:1, 10.0% for 18:1 and13.0% for 20:1 (Table 1B) and an iodine value of between 108-155. **Ziatanos, S., & Laskaridis, K., (2006)** have quoted values for sardines of 6.09% (16:1), 9.38% (18:1) and 0.82% (22:1), (Table 1B) and an iodine value of 185. All these iodine values are much higher than the results obtained in this project. Sardines and herring also had significant levels of polyunsaturated fatty acids. For example Table 1B (Introduction) shows sardine oil to contain 10.64 % (20:5), 1.11% (22:5) and 20.83% (22:6) **(Ziatanos, S., & Laskaridis, K., 2006)**. The average iodine value, of 77.64, obtained for orange roughy in this study is similar to the values of between 73-89 obtained by **Buisson et al., (1982)** for orange roughy oil.

Jojoba oil has been described as the liquid oil produced in the seed of the jojoba plant. This oil is a straight chain wax ester, 36-46 carbon atoms in length and has an iodine value of 80-84 **(Naqvi & Ting, 1990).** The oil itself is a substitute industrially for the wax ester rich oil of the sperm whale *Physeter catadon* **(Buisson et al., 1982).** The wax esters of both the orange roughy and the jojoba plant are rich in the unsaturates 18:1, 20:1 and 22:1 **(Mori et al., 1978, Hayashi & Takagi, 1980 & Buisson et al., 1982)** and findings in this study suggest orange roughy oils are similar. It was thought that the oil from orange roughy could be used as a replacement for jojoba oil and the whale oil industry but this is not now a commercially viable option as orange roughy are in danger of becoming extinct due to over fishing.

5.5 Oil Composition (Thin layer adsorption chromatography)

The qualitative composition of the fish oil was determined using adsorption chromatography on thin layer silica gel G plates. The extracted orange roughy oil was predominantly wax/steryl esters with a smaller amount of triglycerides and much smaller amounts of polar lipids, sterols free fatty acids and mono and diglycerides. No cholesterol esters were present (Table 7). Previous studies have indicated that the muscle and swimbladder lipid from orange roughy was comprised almost entirely of wax ester (98-100%) and the muscle of North Atlantic male orange roughy contained higher levels of wax ester than their Australian counterparts (270 vs. 85 mg/g) **(Bakes et al., 1995).** In contrast the wax ester content of the female orange roughy muscle was similar in specimens from the two regions (45 vs. 35 mg/g) **(Bakes et al., 1995).** It was not possible to conduct a quantitative assessment of the oil composition here as neither a densitometer nor a commercial applicator nor an Iatroscan machine was available.

5.6 Fatty Acid Methyl Ester (FAMES): recovery and purification

Three methods for complete Fatty Acid Methyl Ester (FAMES) production from the extracted lipid were investigated before a final method was chosen.

In the first method the fatty acids were saponified with sodium hydroxide and then methylated with boron triflouride-methanol reagent **(Crackel et al.,1988).** This method proved to be labour

intensive, slow and, more importantly, subsequent analysis by thin layer chromatography demonstrated that much of the wax esters were not converted into FAMES and alcohols by this technique. Several workers such as **Bottino (1975)** have stated that wax esters are much more resistant to hydrolysis than the triglycerides. The fatty acid composition of an incomplete hydrolysis was very different to that of completely hydrolysed and analysed oil. This indicated that the incorporation of fatty acids into different lipid types (waxes/triglycerides) is not random and preliminary work confirmed that oleic acid is selectively incorporated into wax esters in the orange roughy from the Rockall Trough.

The second method developed here was based on the method recommended by **Bottino, (1975)** involving the use of potassium t-butoxide in methanol. This method was also labour intensive and although subsequent analysis of the FAMES by thin layer chromatography showed that the method did satisfactorily break the wax-ester linkage, a high percentage of alcohols came out with the longer more unsaturaterd FAMES (e.g. docosahexaenoic acid) on the GC column.

The third, and final method, developed and adopted, used methanolic HCl, as described by **Christie (1993)** and involves acid-catalysed transesterification to prepare the FAMES. The commonest and mildest reagent used for this purpose is anhydrous hydrogen chloride in methanol. This was prepared by the method described by **Christie (1993)**. Yields of FAMES are influenced by a number of experimental factors including molar ratio between methanol and the lipid, the reaction temperature and time **(Freedman et al., 1984, MacGee & Williams, 1981)**. As previous workers such as **Christie (1993)** suggest, to ensure the reaction went to completion the molar ratio of methanol to lipid was at least one hundred throughout this study. This method was not as labor intensive as the others investigated and several samples could be worked on simultaneously. Analysis by thin layer chromatography showed that this method did produce a single spot of FAMES on the chromatogram. Subsequent use of mini silicic acid columns proved to be most satisfactory for the removal of all the residual alcohols by binding them to the stationary phase while the FAMES came straight through unbound. This is a similar method to that proposed by **Christie (1993)**. In contrast **Bottino, (1975)** removed the interfering alcohols by thin layer chromatography and recovered the FAMES by scraping off and extracting the relevant band. This is a far more work intensive and less reliable procedure than the silicic acid mini columns.

5.7 Reproducibility of the Extraction Technique and preparation of FAMES

The reproducibility of the extraction technique and preparation of FAMES was checked by extracting the oil from the same sample five times and preparing the FAMES from each extraction. The fatty acid composition of each sample was then assessed by gas chromatography (Tables 8.1,8.2). Reproducibility of the extraction techniques was very acceptable with a standard deviation of less than 0.115%, for the percentage lipid extracted, corresponding to a coefficient of variation of 3.3%. and a recovery of each of the thirteen commonest fatty acids of below 0.224 standard deviation and below 3% coefficient of variation. A high % coefficient of variation occurred mostly in the case of the fatty acids which were closest to the detection limit on the GC column. Major fatty acids present had a coefficient of variation of below 1.5% (Table 8.2).

5.8 Reproducibility of the Injection Technique

Before any of the prepared FAME samples were analysed by gas chromatography the final injection technique was first developed to ensure reproducibility of results. Two commercially available standards were first injected. The first standard Supelco (1899) contained 20% each of five saturated methyl esters and, as expected, five peaks of the same area were produced after their injection on the GC column. The second standard Supelco (1894) contained 25% each of four monounsaturated methyl esters and their subsequent injection on the GC column also resulted in the formation of four peaks of equal area. Repeated injection of the FAMES extracted from the same orange roughy oil sample (fish 4, winter sample) gave low standard deviations of 0.0074-0.6863 % and a % coefficient of variation of below 1.5% except for fatty acids myristic acid, gadoleic acid, cetoleic acid and arachidonic acid. All of these were below 4% and, with the exception of cetoleic acid, were present in very small quantities. Cetoleic acid was present in high concentration (17.78%) and appeared more variable than expected with a % coefficient of variation of 3.86% (Table 9). Incomplete resolution of the n-13, n-11, n-9 and n-7 isomers and a very busy chromatogram in this region may account for this.

5.9 Fatty Acid Composition of the extracted Oil of Orange Roughy

In the work carried out here it was found that average levels of 79-87% were recorded for the main monounsaturates 18:1, 20:1 and 22:1, (Table 11). Previous workers have documented that the monounsaturated fatty acids 18:1, 20:1 and 22:1 account for 70-85% of the fatty acids in orange roughy (Table 1A) **(Bakes et al., 1995, Murray et al., 1983, Sargent et al., 1983, Sigurgisladōttir, S. & Palmadōttir, H., 1993 & Vliey, P., & Body, D.R., 1988).** Results from this study here agree with these previous findings as they show the dominant lipid (by TLC) to be wax esters and the total monounsaturated fatty acids to account for 88.56% (Table 11). The abundance of wax esters in deep-water teleost fish was also documented by **Nevenzel, J.C., (1970). Kaufmann, H.P., & Gottschalk, E., (1954) & Mori et al., (1978)** reported large quantities of wax esters in the flesh of a berycoid caught at 1000m depth off New Zealand and identified as *H.Gilchristi*. In a later publication, **Hayashi, K., & Takagi, T., (1980)** demonstrated that *H. Atlanticus* (orange roughy) caught in the same region was rich in wax esters with the same composition as those in *H. Gilchristi* and stated that the latter fish was the same species as *H. Atlanticus*, agreeing with earlier findings of **Woods & Sonoda, (1973).** The work carried out by **Sargent et al., (1983)** on the lipids of orange roughy from deep water to the west of Britain showed that 79.8% $^{w}/_{w}$ of the lipid present in the muscle tissue consisted of wax esters. Their study also confirmed that these wax esters were rich in 20:1 (n-9) and 22:1 (n-11/9) fatty alcohols. These fatty alcohols had mainly been associated with the wax esters of zooplankton from near the surface and especially those from high latitudes. Statistical analysis of the work carried out here showed that there was a significant seasonal effect on the levels present (Table 10.1-10.15). Levels of 16:1 went from a high of 7.17% in winter to a low of 4.78% in summer with levels of 5.7% and 7.01% recorded for spring and autumn respectively (Table 11). Values for 18:1(n-9/7) went from a high of 38.99% in the winter to a low of 28.59% in summer with values of 34.21% and 35.31% for spring and autumn respectively (Table11). Notably, concentrations of 18:1(n-9):18:1(n-7) were present in a ratio of approximately 5:1. Values for 20:1 (n-11/9) went from their highest concentration of 29.12% in summer to their lowest value of 21.15% in autumn with values of 21.59% and 26.38% recorded in winter and spring respectively (Table 11). 20:1(n-9) and 20:1(n-11) were found to be present in a ratio of

approximately 20:1. Relative concentrations of 22:1(n-9) and 22:1(n-11) were also present in approximately the same 20:1 ratio as 20:1(n-9/11) but in the reverse order (table 11). Values for 22:1 (n-11/9) showed the greatest seasonal variation. The highest value of 28.52% was recorded in summer and the lowest value of 17.88% was recorded in winter. Values of 24.85% and 21.0% were recorded for spring and autumn respectively. Overall the sum of the four mono unsaturated fatty acids went from the highest value of 91.14% in spring to the lowest value of 84.47% in autumn. Values of 85.63% and 91.01% were recorded in winter and summer respectively (Table11). The fatty acids 20:1 and 22:1 were present in an approximate ratio of 1:1 for all seasons. Studies carried out by **Ackman, R.G., (1976)** observed that 44.9% of the fatty acids in wax esters in the copepods of North Atlantic were 22:1 and in almost all analysis of copepod alcohols where 22:1 alcohol is important it was roughly twice the percentage of 20:1 alcohol (Capelin eat mostly copepods and similar small crustaceae) **(Lambertsen,S.G. & Myklestad ,H., 1972). Lambertsen & Myklestad (1972)** also observed that the 20:1 and 22:1 fatty acids of capelin oils were present in a 1:1 ratio so it was concluded from these proportions that the capelin converts all of the 20:1 alcohol to fatty acid on absorption and probably only part of the 22:1 alcohol to acid, with the rest either being shunted off to total breakdown and resynthesised to other fatty acids. As orange roughy feed on copepods and capelin **(Ross, D. M., & Smith, D. C., 1977)** this would explain why the fatty acids 22:1 and 20:1 were present in a 1:1 ratio. Statistical analysis showed the seasonal variation to be a significant factor in the work presented here but there were insignificant differences between individual fish or parts of the fish. Previous workers for example **Lee, (1975)**, have also found that the 20:1 and 22:1 fatty acids are together present in very large amounts in calanoid copepods as fatty alcohols but the alcohols were not looked at during the course of this study. The fact that erucic acid (22:1n-9) was detected in relatively low amounts, highest levels of 1.64% were recorded in autumn and lowest levels of 0.28% recorded in summer with levels of 0.93% and 1.47% recorded in spring and winter respectively, is good from a dietary point of view as potential safety concerns have been associated with high dietary exposure to erucic acid. Studies carried out on animals have shown that high exposure to erucic acid results in a condition known as myocardial lipidosis which is reported to reduce the contractile force of heart muscle. So it seems reasonable to expect that humans would also be susceptible to myocardial lipidosis following exposure to high

levels of erucic acid **(Kramer et al., 1982).** Mustard oil is composed mostly of the fatty acids oleic acid, linoleic acid and erucic acid. Due to its high content of erucic acid, which is considered noxious, mustard oil is not considered suitable for human consumption in the United States, Canada and the European union (http://en.wikipedia.org/wiki/Mustard_oil).

Common fish oils such as cod, herring and salmon also contain relatively high levels of the monounsaturates 18:1, 20:1 and 22:1 (47.5%, 51% and 49.2% respectively). Whereas the levels of these monounsaturates present in sardine oil is much lower at 17.5% (Table1B) **(Ziatanos & Laskaridis, 2006).** The saturated fatty acids identified (14:0, 16:0 and 18:0) were present in relatively low amounts with 16:0 recorded in the highest concentration of the three (Table 11). Highest values of 3.59% were recorded in autumn and lowest values of 2.29% were recorded in summer. Values of 3.35% and 3.45% were recorded for winter and spring respectively. These figures agree with the previously published work of others Table 1A (Introduction) with the exception of **Murray et al. (1983) and Vlieg & Body, (1988**) who both carried out studies on orange roughy in New Zealand waters and obtained higher values of 6.3% and 8% respectively for these saturates. Common fish oils such as cod, herring, sardine and salmon contain higher levels of these saturates (Table 1B). The fact that orange roughy oil is low in saturates is good from a dietary point of view as the consumption of foods rich in saturated fatty acids can lead to health problems such as high cholesterol. Again statistical analysis showed the seasonal variation to be a significant factor but there were insignificant differences between individual fish or parts of the fish.

The n-3 (ω) poly unsaturated fatty acids, which are considered to be of great nutritional importance, were found to be present in very low amounts. Docosapentaenoic acid (DPA) was found only in trace amounts in spring, autumn and winter samples and was absent in summer. Eicosapentaenoic acid (EPA) was present at the highest level of 1.03% in autumn and at the lowest level of 0.35% in summer with levels of 0.59% and 0.83% for spring and winter respectively. Docosahexaenoic acid (DHA) was also present in low concentrations with the highest level of 3.2% recorded in autumn and the lowest value 1.44% recorded in summer. Levels of 1.75% and 2.90% were recorded respectively in spring and winter (Table11). These results show that there is a seasonal effect on the levels of PUFA fatty acids present in the muscle of orange roughy and correlate well with results obtained from studies by

Sigurgisladōttir, S., & Palmadōttir, H., (1993). As can be seen in Table 1A (Introduction) these workers obtained values of 1% and 2.3% respectively for eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the muscle of this fish. The presence of docosapentaenoic acid (DPA) was not detected in the 1993 study of **Sigurgisladōttir, S., & Palmadōttir, H.** which may suggest that the study was carried out in summer. Also, as can be seen from Table1A, earlier studies carried out by **Murray et al., (1983)** and **Sargent et al., (1983)** on the muscle of orange roughy from the waters off New Zealand and West Britain respectively did not detect the presence of poly unsaturated fatty acids. Later studies did not agree with these findings as they did record the presence of small quantities of poly unsaturated fatty acids. Findings from this study agree with those of **Bakes et al., (1995)**, Table 1A, in so far as they record the same value of 0.7% for the level of eicosapentaenoic acid (EPA) present and similar values for the amounts of docosahexaenoic acid (DHA) present, but differ in that very small quantities of docosapentaenoic acid (DPA), (0.04%) were detected in this study and not in the study of **Bakes et al., (1995)**. An explanation for this may again be that our studies were carried out on a seasonal basis and, as can be seen from Table 11, docosapentaenoic acid (DPA) was not detected in summer so it may have been that the fish studied by **Bakes et al., (1995)** were captured in summer. Low levels of linoleic acid (18:2n-6), an essential fatty acid used in the biosynthesis of arachidonic acid (20:4) which in turn is the precursor for prostaglandins, was also detected in this study. Levels ranged from the highest value of 0.98% in autumn to the lowest value of 0.67% in summer with values of 0.78% and 0.96% recorded in spring and winter respectively. The average level of 0.85% compares closest to the levels of 1.3%, for linoleic acid, recorded by **Sigurgisladōttir, S., & Palmadōttir, H. (1993),** Table 1A. **Vlieg & Body, (1988)** recorded its presence only in trace amounts and its presence was not detected by other workers (Table 1A). Arachidonic acid (20:4n-6) which is a precursor in the production of eicosanoids was also found in low levels in this study. Levels were highest in winter at 0.54% and lowest in summer at 0.22% with levels of 0.25% and 0.37% recorded in autumn and spring respectively. An overall average value of 0.35% for levels of arachidonic acid compares very favourably with the value of 0.3% recorded in the studies of **Sigurgisladōttir,S., & Palmadōttir, H. (1993)** for the levels of this fatty acid present in the oils of orange roughy caught in Icelandic waters. None of the other works presented in Table 1A recorded the

presence of arachidonic acid. Arachidonic acid has been shown to be present, in relatively low levels, in the oils of common fish (Table 1B).

The extraction of the lipid, for all studies, was carried out using either the Bligh and Dyer or the Folch extraction method and the fatty acids present were detected by gas liquid chromatography. An explanation for the fact that the presence of poly unsaturated fatty acids was not detected in the earlier studies may be that the methods by which samples of wax esters and triglycerides were prepared and converted to methyl esters caused destruction of these poly unsaturated fatty acids or they were selectively concentrated in the wax esters and these were incompletely hydrolysed by these workers.

From a nutritional point of view (in particular ω3-poly unsaturated fatty acids) orange roughy would not be considered to be of particular importance. Other deep water fish such as the oreos contain much higher concentrations of these fatty acids, values of generally above 10% for these fatty acids have been recorded **(Bakes et al., 1995)**. Most of the interest in exploiting the orange roughy had stemmed from the value of its wax-ester rich oil. This was considered to be an excellent substitute for, the commercially valuable, wax esters from the seeds of the desert jojoba plant *Simmondsia Chinensis*. Jojoba oil itself was used as a substitute for the wax-rich oil of the sperm whale *Physeter Catadon* **(Buisson et al., 1982).**

Common fish such as herring and sardines for example (Table 1B) have been shown to have much higher concentrations of the nutritionally valuable PUFAs docosapentaenoic acid (DPA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) **(Jensen et al., 2007, Shirai et al., 2002).** Also, given the fact that these fish are much more easily obtained than orange roughy, it makes them more valuable commercially. Studies by **Jensen et al., (2007) and Shirai et al., (2002)** have also shown that the concentrations of these fatty acids are seasonally dependant. They suggest that the reason for this might be related to variation in the fatty acid composition of the calanoid copepods on which the herring feed. **Shirai et al., (2002)** also notes that the characteristics of the fatty acids isolated from sardines in July were similar to those from plankton in the same season.

5.10 Freshness of Fish

5.10.1 Lipid Oxidation

The 2-Thiobarbituric Acid Test (TBA) was the method chosen to assess the extent of lipid oxidation of the orange roughy samples. Levels of malonylaldehyde (MDA) present in the compound fish were determined. The TBA reaction measures the total malonylaldehyde present in free form under the conditions of the TBA reaction **(Bird, R.P., & Draper, H.H., 1984)**. The criteria used to report results depend on the test conditions. Some results are expressed in mg/g fish, others use mg/g oil while others use m moles or absorbance per a specified weight of fish or oil so results are not directly comparable. **Connell, J.J. (1975)** has set the legal limit at 14mg malonaldehyde/100g of muscle for cod **(Simeonidou et al., 1998)**. As fish muscle was used in the work carried out in this study these are the limits chosen to interpret the results of lipid oxidation of the orange roughy muscle. All the fish samples tested were within very acceptable limits (Table 12). Summer samples gave the highest average level of 0.35mg malonylaldehyde/100g fish. This would be expected as higher summer temperatures would naturally make it harder to keep fish in a fresh state. Levels were lowest for autumn at 0.18mg malonylaldehyde /100g fish. Winter and spring levels were recorded at 0.22 and 0.26mg malonylaldehyde/100g fish respectively. Statistically results show a significant seasonal variation with no variation from fish to fish or in the location within the fish.

5.10.2 Protein and Trimethylamine-N-Oxide Deterioration (TVB-N)

The TVB-N method is one of the most commonly used methods for assessing the level of production of total volatile amines as a result of bacterial action on proteins. These amines contribute to the "off flavours" and odours associated with fish spoilage **(Egan, H., 1981)**. The upper limits of acceptability for TVB-N is regulated according to Directive (95/149/EEC) and is set at 35mg/100g for fish. **(Ruiz-Capillus, C., & Moral, A., 2001)**. All fish samples tested were well within acceptable limits reflecting the degree of freshness of the fish when landed (Table 13). As was the case with the TBA test, summer samples gave the highest average level of 8.91mg/100g. This would be expected as higher summer temperatures would naturally make it harder to keep fish in a fresh state. Autumn levels were lowest at 6.88mg/100g while levels

for winter and spring were 7.80 and 8.03mg/100g respectively. Again statistically results show a significant seasonal variation with no variation from fish to fish or in the location within the fish. Overall the fish was extremely fresh, significantly better than what would be expected for sale directly to the public in a fish market or supermarket.

5.10.3 Trimethylamine-N-Oxide Deterioration (TMA-N)

Directive (91/493/EEC) sets the legal limit for TMA-N at 12mg/100g in fresh fish **(Ruiz-Capillus, C., & Moral, A., 2001).** Research carried out by **Baixas-Nogueras et al., (2003)** on the suitability of volatile amines as freshness indices concluded that TVB-N was better as a spoilage index than a freshness one and TMA-N appeared to be a good index of quality. Results from the work reported here on TMA-N levels, for all seasons, were shown to be well within legal limits and reflect the quality of the fish landed (Table 14). Summer gave the highest average levels at 6.26mg/100g with lowest levels of 4.82mg/100g recorded for autumn. Levels for winter and spring were 5.54 and 6.26mg/100g respectively. The fact that both TVB-N and TMA levels were low when the fish were landed ashore means that these catches had been handled and stored properly after capture. The difference between the TVBN and TMA levels reflects the deterioration of the protein (ammonia). As can be seen from Tables 13 and 14 insignificant levels of ammonia were present, early spoilage is basically due to TMAO degradation. This supports previous studies by **Baixas-Nogueras et al., (2003)** that TVBN is a better measure/ indicator of quality and TMA of freshness.

5.11 AGEING/ STABILITY ANALYSIS

Fish shelf life is determined by the intensity of enzymatic reactions and by the number and type of micro-organisms affecting the products perishability. Other determining characteristics are the temperature during capture, the storage temperature, delay in refrigeration and variation in storage temperature **(Leistner, L., 1992).** All samples analysed were found to be extremely fresh on arrival to the laboratory. Results for the spring samples were similar to those found for the other seasons, although the spring samples were frozen on board ship and thawed upon arrival to the laboratory. The summer, autumn and winter samples were stored on ice, on board

ship, and were kept on ice until they arrived at the laboratory. Samples were stored and subsequently analysed for TBA reactive substances, total volatile base nitrogen (TVB-N) and trimethylamine (TMA) after specific periods (3, 5, 8, 15, and 17 days) at +4°C in air and while vacuum packed. Other samples were stored at -5°C in air and in vacuum packs for up to sixty five days and similarily analysed after specific periods of time (4, 9, 18, 24 and 65 days). The subsequent shelf life, at +4°C in air or vacuum packed, of samples which had been pre-stored in vacuum packs at -18 °C for six and twelve month periods was also determined.

Because the results obtained for these ageing experiments were nearly identical for all the samples tested over the four seasons (Tables 15.1-15.4) it was decided to graph and discuss only one season. Results for the autumn samples are shown graphically in Figures 19-22. The TBA levels obtained in this study for ageing/stability were not a good indication of the freshness of the fish. None of the samples tested exceeded or came close to the legal levels of 14mg malonaldehyde/100g fish **(Simeonidou.et al., 1998)** at any stage. The highest levels of TBA reached, for autumn samples, was 5.91mg malonylaldehyde/100g fish for samples aged at 4°C in air after seventeen days (Table 15.3, Figure 19). The very low TBA results in this study suggests that either lipid oxidation during the storage period studied is still at the induction stage **(Labuza, T.P., 1971)** or that either oxidation of the fat did not contribute very significantly to deterioration or/and the aldehydes produced were rapidly metabolised by the complex tissue. The low percentage total lipid would also reflect the low TBA as the results are expressed per 100g of fish not oil. As indicated by **Simeonidou et al., (1998)**, when studying the correlation between biochemical and sensory quality indices in hake, lipid deterioration would need to be correlated with sensory analysis (inspection and tasting) to get an accurate indication of when the fish reach their shelf life.

The most commonly used biochemical methods for assessing spoilage are TVBN and TMA **(Ruiz-Capillas, C., & Moral, A., 2001)**. There was a very high correlation between the TVBN and the TMA indices, (Figures 19-22), indicating that virtually all the TVBN is TMA and showing that TMAO is deteriorating primarily ,on ageing, rather than protein. Samples aged at +4°C in air did not exceed the TVBN legal limit of 35mg/100g until six days had elapsed with the exception of the summer sample which gave a value of 35.71mg/100g on day five, (Table 15.2), slightly above the legal limit. However the TMA upper legal limit of 12mg/100g was

reached for all the samples tested after three days. The development of TMA in fish tissue is dependent on the presence of the enzyme TMAOase. Protein degradation gives ammonia as an end product due to the splitting of amino acids. This ammonia is observed only at a fairly advanced stage of spoilage and is not as useful a spoilage indicator in the early stages of degradation. TMAO present in fish tissue is reduced to TMA after death, resulting in a decrease of TMAO levels as the fish ages and undergoes degradation **(J. Oehlenschläger, 1997)**. The upper limit for TMA is significantly less (12mg/100g) than for TVBN (35mg/100g) due presumably to its more "fishy" volatile odour and hence is reached faster in fish spoilage. In studies carried out by **Oehlenschläger, (1997)** TMA levels in fish tissue continued to increase even after all TMAO was broken down, indicating TMAO is not the only source of TMA. In the same studies an increase in TMA levels was observed correlating with the onset of microbial action in the fish. Determination of the TMA levels in fish can therefore be used as an indication of fish spoilage and is considered a better indicator of fish quality than TVBN. The results presented in this work would agree with this.

The shelf life of vacuum packed samples held at +4°C did not reach the TMA limit until day five (Table 15.3, Figure 20). Vacuum packing does prolong shelf life of fresh orange roughy fillets by one day. This is achieved by lowering the amount of oxygen which slows down the growth of aerobes and the speed of oxidation reactions **(Church, I. J., & Parsons, A. L., 1995).** A comparison of Figures 19 and 20 also show that the rate of lipid oxidation is dramatically reduced upon vacuum packing.

The shelf life of freeze-thawed fresh fillets can be extended significantly to twenty four days by storing at -5°C in air or to 31 days for vacuum packed fillets stored at -5°C (Figures 21- 22, Table 15.3) as assessed by TMA. Vacuum packing had a significant roll in slowing down the rate of deterioration at -5°C, (Figure 22, Table 15.3), but had a much less significant roll in slowing down the rate of deterioration at +4°C, (Figure 21, Table 15.3). Where the TVBN value is used as a means of assessing freshness the shelf life of freeze-thawed fillets is extended to 61 days by storing them at -5°C in air or vacuum (Figures 21-22) as opposed to 6 days for fillets stored at +4°C in air and 8 days for vacuum packed fillets stored at +4°C (Figures 19-20). In summary the shelf life of orange roughy fillets is dependant on storage temperature and storage conditions and it is advantageous to vacuum pack in order to further prolong the shelf life.

In all circumstances the TMA test is the most suitable for assessing storage/shelf life of fish. Frozen storage is a commonly used method for preserving fish. However the texture, flavour and colour of fish flesh change during long term frozen storage. These marked, undesirable, deteriorative changes in the quality of fish flesh take place due to a variety of biochemical reactions caused by hydrolysis, polymerisation, deamination, decarboxylation and oxidative process occurring in lipids and proteins after prolonged frozen storage **(Blinski et al., 1978, Grosch, W., 1987, De Koning, A. J., & Mol, T.H., 1991, Sylvia et al., 1995, Reynolds et al., 2002)**. Studies carried out by **Saeed, S., & Howell, N.K., (2001)** on the effect of lipid oxidation and frozen storage on muscle proteins of Atlantic Mackerel showed an increase in lipid oxidation products with storage time as well as deterioration of protein structure and functionality **(Saeed, S., & Howell, N. K., 2001).** Results obtained in this study on the effect of long term storage at -18°C and subsequent shelf life of orange roughy agree with these earlier findings. Storage in a freezer in vacuum packs over a twelve month period showed that no deterioration of protein or TMAO occurred at this low temperature (proteases and TMAOase were not active). However some oxidation of the fish lipid did occur (Table 17).

Samples which had been pre-frozen at -18°C for six months and others for twelve months were subsequently aged at +4°C and -5°C. Results from these findings are given in Tables 16 and 18. As can be seen in Figures 27-30 when the TMA production rate is assessed as a function of time the longer the pre frozen time the faster the fish subsequently ages upon thawing and storage in air or vacuum at +4°C or -5°C. The same is true for TVBN production. The aerobic refridgerated shelf life of the fish was reduced by half a day, from four days to three and a half days for fish which had been stored for six months at -18°C (Table 16 (a).Figure 23) and by one day, from four days to three days for fish which had been stored for twelve months at -18°C (Table 16, Figure 25). The shelf life of vacuum packed fish was reduced by one and a half days, from five and a half to four days, after six months storage at -18°C (Table 16, Figure 24) and by two days, from five and a half to three and a half days after twelve months storage at -18°C (Table 16 (b), Figure 26).

Conclusions

The nutritional composition of orange roughy is different to that of pelagic and fresh water fish.

The moisture content was typical of most fish averaging between 77-80% and was found to be inversely proportional to the lipid content.

Protein levels, which are genetically determined, averaged around 16%, irrespective of fish size, sex, season, sample procurement or location within the fillet. This is typical of the protein values for most fish such as cod, mackerel or herring for example. Therefore concluding that orange roughy is no more beneficial than more readily available fish for human consumption. The fat content, which is a measure of calorific content, was higher than lean fish and lower than oily fish. Thin layer adsorption chromatography showed that the extracted oil was predominantly wax/steryl esters. It was found to be a very poor source of the nutritionally valuable n-3 (ω), n-6 (ω) poly unsaturated fatty acids. Therefore orange roughy could not be considered to be a nutritionally valuable fish.

Results on the freshness of the samples upon arrival to the laboratory were similar for all seasons and indicated that pre-frozen fish (Spring, Marine Institute) and fish stored on ice (Summer, Autumn and Winter) were of superb freshness. This would indicate that both methods of storage were acceptable allowing no deterioration/degradation of the protein, TMAO or oxidation of the lipid prior to arrival. If these values were elevated, for any season, it would indicate that there was proetolytic/ lipid deterioration as a result of enzymatic activity and/or microbiological growth after the fish were caught. Such results would have indicated poor storage management of the catch prior to landing.

Results from the ageing studies concluded that fresh fish fillets stored at 4°C stay fresher for longer than fillets which were first frozen and subsequently stored at 4°C. Frozen storage does however prolong the edible shelf life up to/ longer than twelve months. Some deterioration does occur over the frozen period as the results of this study show that the longer the frozen time the shorter the subsequent shelf life of the thawed fillets.

6 RECOMMENDATIONS

Previous researchers have elucidated much about the orange roughy life history and little about its nutritional composition. In this project limited numbers of fish were extensively analysed biochemically. They covered all four seasons. Results indicated a nutritional composition very similar to most common fish with no beneficial ω3/ ω6 fatty acids, no observable difference between males/females or seasonally. The work strongly suggests the fish not be caught/consumed. It makes no environmental, nutritional or economic sense to disturb this finely balanced ecosystem.

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