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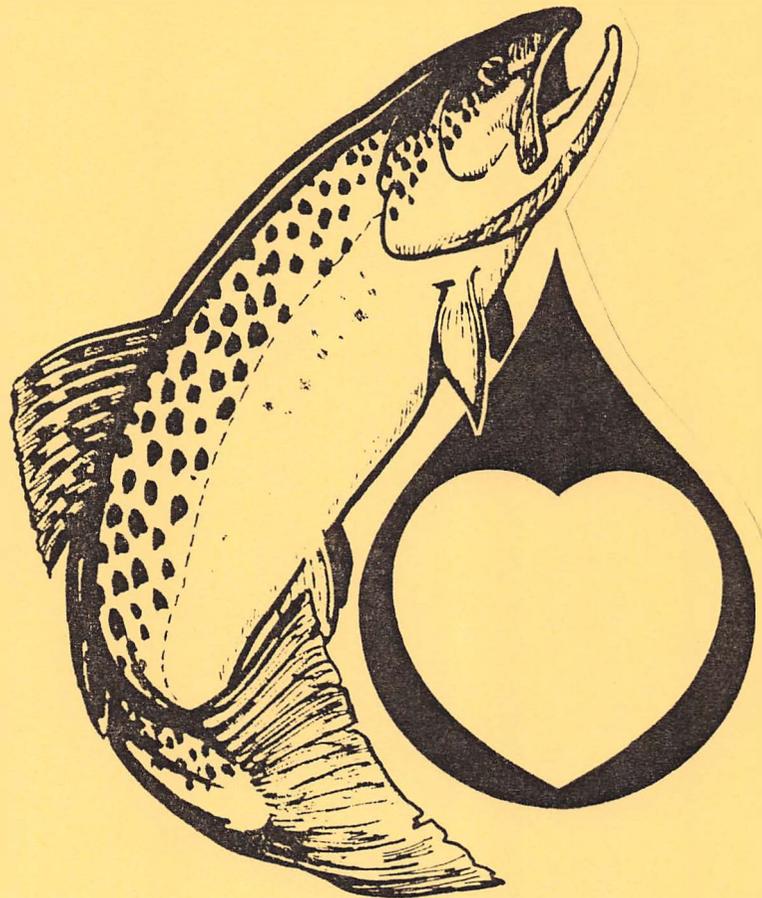
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Fish and Fish Oil in the Diet and its Effects on Certain Medical Conditions

A general discussion largely
in non-technical language

Maurice E. Stansby
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Chapter 1 - Introduction

In June 1990 a book, "Fish Oils in Nutrition" which I had edited was published. This book was written for scientists and was accordingly, for the most part, not readily understandable to readers with limited or no background in such topics as chemistry, nutrition or the like. In 1985 I had published a report, "Medical Effects of Fish or Fish Oil in the Diet." Although this report was written employing limited scientific wording, it would still be to some extent difficult for the average reader without scientific background to understand.

This present report has been assembled to bring up-to-date much of the material given in the 1985 report, yet on the whole, eliminating most of the more scientific wording so that it could be readily understandable to any readers. In some instances the text will make reference to the Appendix where some of the more scientific aspects have been enlarged upon.

The main portions of this article will not have references in the text listed at the end of each chapter. This is being done because, for the most part, the references are nearly all written from the standpoint that the reader can readily understand scientific terminology.

This article will have 7 sections (chapters). In addition to this Chapter 1, Introduction, the following chapters are included. Chapter 2 entitled "Chemical Aspects of Fish Oils" covers in simple language information on such aspects as the make-up of fish oils, the variation of the amount of fatty acids in fish oils made from the same species etc. Chapter 3 on nutritional effects of fish oils for humans brings what is known together covering both information known long ago up to recent findings.

The next section, Chapter 4, describes how the fatty acids of fish oils which result in beneficial medical effects also render them highly vulnerable to oxidation and how this ^a effects the oils. Chapter 5 deals with the manufacture of crude fish oils followed by processing to give refined oils which are suitable for human consumption. Chapter 6 relates to compounds sometimes found in fish oils other than triglycerides and phospholipids. Most of it relates to glyceryl ethers.

The last section, Chapter 7, designated as Appendix contains material with somewhat more scientific terminology for use by readers who can understand it. For example, in Chapter 2 there are listed many compounds with their odor or flavor which occur sometimes in fish oils. In the text in Chapter 2, the chemical names for these substances are not given; rather they are listed as substance #1, 2, 3, etc. For those readers who might be

interested in just which chemical compounds are involved the scientific name such as 1, 3, 5 trimethyl benzene or isobutanoic acid are given in the Appendix for each of the various substances which in the text of Chapter 2 had been listed only as numbers.

Chapter 2. Chemical Aspects of Fish Oils

I. Composition of Foods

Most foods contain water, protein sometimes carbohydrates, fat or oil, ash (minerals) and sometimes vitamins. Fish ordinarily contain water, protein, carbohydrates, fat or oil, minerals, and vitamins (see Appendix, page 86). The amount of carbohydrates in fish is quite small except in shellfish and crustaceans such as clams and shrimp where their amount often can be considerable sometimes up to a range of 3 to 4 percent. The amount of vitamins in percent is always extremely small yet from a nutritional standpoint it may be quite significant.

Usually the sum of the amount of oil and water in fish is equal to close to 80%. Thus, if the oil content is 10% the water content is about 70% or if the oil content is 1% the water content is usually about 79%. The components of fish which can give them the ability to decrease incidence of certain diseases such as those of the heart occur in the oil of the fish.

II. Oil or Fat in Fish

Whereas the fat in such foods as meat or poultry occurs as solid fat, that in fish is liquid at room temperature and is, therefore an oil. Hereafter we will use the term fish oil rather

than fat. The amount of oil in the edible portion of most species of fish can vary tremendously both from species to species and also from one sample of a species to another sample of the same species. Because of this high variation, before the chemical nature of the oil of fish is discussed, a detailed section is included here to describe this variation. Because of this wide variation, the content of oil in a sample of fish is ordinarily of greater importance from a nutritional standpoint than is the chemical nature of the oil.

III. Amount of Oil in Edible Portions of Fish

In samples of fish from different species, the oil content in edible portions very often varies from 0.3% to 14%, a difference of 35 times. This is a very conservative estimate. Actually in quite a few instances the variation can be greater. For example, it is not unusual for some lean species of fish like cod to have as little as 0.2% oil while fatter fish such as mackerel can, at peak of production, have as much as 20% oil. This is a difference of 100 times. In a few rare instances, for example, with siscowet trout caught in deep areas of Lake Superior the oil content of the edible flesh has been found to be as high as 70%. For very lean fish occasionally the oil content of the edible portion can be as low as 0.2%. This is a variation of 350 times.

The oil content of fish which undergo long spawning migrations vary with the period of the migration. The fish initially build up fairly high oil content in their flesh. When they begin a long spawning migration, they may not eat any food. Instead they use a portion of their excess body fat which occurs in the flesh. For example, pink salmon, which may begin the season with an oil content up to 11% or a little more, use up their fat as a source of calories during their spawning migration at the end of which their fat content may be only 3% or less. In the salmon canning industry, the fish are caught and canned ordinarily before any huge decline in oil content has taken place.

Table 2-1 shows average oil content and range of oil contents in the edible flesh of a number of common species of fish.

Table 2-1. Average and Ranges of Oil Content¹ of 17 Common Species of Fish.

Species	Range % of Oil Content	Average % of Oil Content ¹
Carp	1.0-12.0	5.0
Chub, lake	4.0-13.0	8.5
Cod, Atlantic	0.2-0.9	0.4
Flounder	0.3-3.4	1.4
Haddock	0.2-0.6	0.35
Mackerel, Atlantic	2.7-25	14.0
Ocean perch, Pacific	3.0-6.0	4.2
Perch, yellow	0.8-1.2	0.9
Pike, yellow	0.8-3.0	1.3
Rockfish (Sebastes)	1.2-4.3	2.5
Salmon, Chum	2.2-7.3	4.0
Salmon, Coho	3.3-11.2	7.0
Salmon, Pink	3.2-11.6	6.5
Salmon, Sockeye	7.8-13.7	11.3
Smelt, Marine	4.6-8.8	6.3
Smelt, Lake	1.5-3.3	2.4
Whitefish, Lake	4.7-18.8	9.6

¹ Average value based upon fish taken during the main industrial harvest season, although even then a fairly large range of fat content usually occurs.

The oil content of different parts of the edible flesh vary as shown in Table 2-2. Ordinarily the flesh taken from near the head has a higher oil content than is the case with that taken from near the tail.

Table 2-2. Variation in the Oil Content of Different Portions of the Edible Flesh of the Same Fish.

<u>Species</u>	<u>Section Analyzed</u>	<u>Oil %</u>
Red King Salmon	Near Head	20.2
Red King Salmon	Near Tail	11.1
White King Salmon	Near Head	15.1
White King Salmon	Near Tail	8.1
Yellowfin Tuna	Middle	3.2
Yellowfin Tuna	Near Tail	1.4

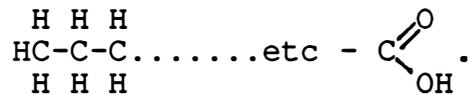
From what has been said, it is now obvious that the oil content of fish can vary over extremely wide ranges and that the amount present is not determined solely by the particular species of fish involved.

IV. Chemical Nature of Fatty Acids in Fish Oils - see also Appendix (Chapter 7, page 87).

Most of the oil in fish consists of triglycerides where three molecules (often 3 different molecules) of fatty acids are grouped together and are called triglycerides. A much smaller

proportion of the oil of most fish are attached to a molecule called phospholipids (and which contains phosphorous).

The fatty acids in any oil or fat are of two varieties, saturated or unsaturated. The fatty acids consist of a long chain of carbon atoms (designated as C) thus



Each carbon atom has attached to it hydrogen atoms (H) as shown and the last carbon atoms has the group $\text{C} \begin{array}{l} \text{=O} \\ \text{OH} \end{array}$ which makes it into an acid rather than some other compound. The saturated fatty acids have each carbon atom in the long chain attached to an adjacent carbon atom by a single bond. Two other types of fatty acids are the monoenes in which just two of the carbon atoms are connected by two rather than one bond thus $\text{C}=\text{C}$. This is known as a double bond. The third type of fatty acid contains two or more pairs of fatty acids connected by double bonds. These are known as polyunsaturated fatty acids. Vegetable oils contain mostly two or, at the most, three such double bonds within a molecule except that in a few cases a few tenths of 1% of fatty acids with four double bonds can occur. Fish oils, on the other hand, often contain five or six such double bonds within one molecule. Fish oils also, of course, contain saturated and monoene fatty acids.

Fatty acids vary in their nature in another way. This has to do with the location of the double bonds within the molecule. Ordinarily in counting the carbon atoms in an organic compound where these carbon atoms are arranged in a long chain, the number 1 carbon atom is the one where the group characteristic for the compound is located. In an acid this characteristic carbon atom would be the one making it an acid, i.e. the $\text{C} \begin{matrix} \text{=O} \\ \text{OH} \end{matrix}$ group. However when the nutritional properties of a fatty acid are involved, the carbon atoms are counted from the other, far end of the carbon chain. This far end is designated as the omega end (omega is the last letter in the Greek alphabet).

An omega-6 fatty acid would have the following arrangement:
 $\begin{matrix} \text{H} & \text{H} & \text{H} & \text{H} & \text{H} & \text{H} & \text{H} \\ \text{HC} & -\text{C} & -\text{C} & -\text{C} & -\text{C} & =\text{C} & \dots\dots\dots -\text{C} \begin{matrix} \text{=O} \\ \text{OH} \end{matrix} \end{matrix}$ and an omega-3 fatty acid as follows:

$\begin{matrix} \text{H} & \text{H} & \text{H} \\ \text{HC} & -\text{C} & -\text{C} & =\text{C} & \dots\dots\dots -\text{C} \begin{matrix} \text{=O} \\ \text{OH} \end{matrix} \end{matrix}$. Another system for naming such fatty acids is to substitute "n-" for omega. Thus, an omega-3 acid can be called a n-3 acid.

Fatty acids are generally indicated by a C followed by the number of carbon atoms present; then a colon is followed by the number of double bonds present; if known, this is then followed by ω -3, ω -6, or whatever. Thus C20:5 ω -3 indicates a compound with 20 carbon atoms, 5 double bonds of the omega-3 type. It could also be designated as C20:5n-3.

V. Fatty Acid Content of Oils of Different Fish Species

Unlike the oil content of different fish species about which a great deal is known, very little is known about the fatty acid content of different species. This is not to say that there is little in the literature on this matter. The problem is that most investigators, unaware that there can be a considerable variation in fatty acid content of the same species taken at different places and at different times, have used totally inadequate number of fish samples to give any idea as to the range of values representative for the species. Thus the literature is full of many totally inaccurate values which are for the most part quite meaningless.

This situation has arisen because back in the late nineteenth century there was the belief that the oil of any given species of fish always had identical properties. In those days fatty acids were not measured. Rather various factors such as iodine numbers were employed. The iodine number is actually based upon the proportion of fatty acids relating to the degrees of saturation or unsaturation. In this early time period the iodine number would be determined of a sample of oil by some well established laboratory. It was determined to a high degree of accuracy and might be reported as 140.07. Actually today we know that iodine numbers of samples of oil of the same species can vary considerably. Thus, the oil with the iodine number 140.07

might vary from one sample to another by something like 130-155. In the 19th century if some other investigator used an oil of the species for which the value 140.07 had been determined, but they found some other value, it was assumed that this was due to inept analysis by the individual carrying out the test.

These erroneous ideas about the chemical make-up of fish oils of the same species carried over into the twentieth century. Thus often after it became customary to carry out fatty acid analyses, it was still believed that there was little or no difference in such results for any or all oil samples of a given species. Most initial analyses, therefore, were made on the oil using only a few fish usually all taken from the same location and at the same time. The current literature is full of such erroneous values for the fatty acid make-up of fish oils.

Actually there are only 3 fish oils for which there are sufficient fatty acid values available which are reasonably accurate. These are commercial menhaden oil, commercial herring oil and the oil of the food fish, mullet. For commercial menhaden oil, very good analytical results are available for samples of oil taken in quite a few locations on the Gulf and Atlantic Coasts and at different times over an 11 year period. The results of these analyses are shown in Table 2-3. The samples used for analysis in this table are each from large commercial tanks, each representative of millions of fish.

Table 2-3. Range of Fatty Acid Content in Large Batches of Commercial Menhaden Oil Taken Annually from 1977 Through 1988.

Fatty Acid	Range % of Total Fatty Acids	Ratio Highest to Lowest
C14:0	7.2-12.1	1.7
C15:0	0.4-2.3	5.8
C16:0	15.3-25.6	1.7
C16:1	9.3-15.8	1.7
C16:2	0.3-2.8	9.4
C16:3	0.9-3.5	3.9
C16:4	0.5-2.8	5.6
C17:0	0.2-3.0	15.0
C18:0	2.5-4.1	1.6
C18:1	8.3-13.8	1.7
C18:2	0.7-2.8	4.0
C18:3	0.8-2.3	2.9
C18:4	1.7-4.0	2.4
C20:0	0.1-0.6	6.0
C20:4	1.5-2.7	1.8
C20:5	11.1-16.3	1.5
C22:1	0.1-1.4	10.0
C22:5	1.3-3.8	2.9
C22:6	4.6-13.8	3.0

Source: Zapata-Haynie Co., Anthony Bimbo, laboratory director

It should be noted that there is a very large difference in results for each fatty acid as shown in the last column. Of the 20 fatty acids for which results are shown only 3 had a difference in values as little as 50%. Two fatty acids (C17:0 and C22:1 had variability of from 10 to 15 times. There was a variation of 2 1/2 times or more for 11 of the fatty acids listed. These results demonstrate quite clearly how inaccurate results can be. Most of the data in the literature are based upon so few samples that the results give no idea as to the actual range of fatty acids for the particular species.

Lest it be thought that these results are something that relate only to commercial menhaden oil, very similar variation occurs for commercial herring oil for which considerable data are available. Table 2.4 gives similar variation and the values are for herring caught on both the Atlantic and Pacific Coasts of North America.

Table 2-4. Range of Fatty Acid Composition of Commercial North American Herring Oils.

Fatty Acid	Range % of Total Fatty Acids
C14:0	4.6-8.4
C16:0	10.1-18.6
C16:1	6.2-12.0
C18:0	0.7-2.1
C18:1	9.3-25.2
C18:2	0.1-0.6
C18:3	0.0-1.1
C18:4	1.1-2.8
C20:1	7.3-19.9
C20:5	3.9-15.2
C22:1	6.9-30.6
C22:5	0.3-1.3
C22:6	2.0-7.8
C24:1	0.2-1.3
C24:5	0.0-0.5

The difference in amounts of a given fatty acid in the commercial herring oil was not quite so great as for commercial menhaden oil. This may have been due to the fact that there were far less samples in the herring oil analyses than was the case for the menhaden oil where samples were taken over an eleven year period. If the samples in table 2-4 where the minimum amount was 0 are ignored, then the three fatty acids C22:1, C18:2 and C24:1 had the greatest variation (5 to 6 times). C22:1 was also one of the fatty acids showing greatest variation in the analyses for menhaden oil.

Both menhaden and herring oil are ones made under commercial conditions whereby the oil was extracted by cooking and pressing. There is just one food fish, mullet, for which the oil was extracted by dissolving it with solvents. This species was studied not because of any general interest in the fatty acid content but rather because of the fact that mullet, unlike all other fish, sometimes contains high amounts of odd carbon fatty acids, e.g. C15 and C17. A study was carried out by taking monthly samples for a full-year at four different locations. The results of this study are shown in table 2-5. Not only the odd carbon chain fatty acids but also several others showed considerable variation. Thus, the C16:1 varied from 13.4 to 29.3 and the C22:5 from 1.3 to 3.6.

Table 2-5. Range of Fatty Acid Composition of Mullet Oil.

Fatty Acid	Range % of Total Fatty Acid ^{a/}
C14:0	4.6-11.5
C15:0	3.2-12.4
C15:1	0-1.2
C16:0	20.2-33.7
C16:1	13.4-29.3
C17:0	0-2.5
C17:1	1.7-8.2
C17:2	0.0-4.4
C18:0	1.8-5.4
C18:1	7.1-13.6
C18:2	0.7-2.7
C18:3	0.3-1.3
C18:4 ω 3	0.7-2.2
C18:4 ω 6	0.1-2.3
C19:1	0-2.5
C20:0	0.0-3.9
C20:3 ω 3	0.1-0.8
C20:3 ω 6	0.0-1.7
C20:4 ω 3	0.3-0.6
C20:4 ω 6	1.6-3.8
C20:5 ω 3	4.6-8.1
C22:3 ω 3	0.0-0.2
C22:4 ω 3	0.2-0.6
C22:5 ω 3	1.3-3.6
C22:5 ω 6	0.4-1.0
C22:6 ω 3	0.7-3.9

^{a/} Data from Deng et al. (1976).

It should be mentioned that the fatty acid content of commercial fish oil which is made by cooking and pressing the fish does not apply to oil made from the same species but extracted by solvents. For example the content of C22:6 fatty acid in commercial menhaden oil ranges from 4.6 to 13.8%. Yet in menhaden oil made by solvent extraction of the whole fish the content of C22:6 ranges from 11 to 19%. The reason for this difference probably relates to the fact that when the commercial oil is manufactured, a part of the oil remains behind in the unpressed fish which is later dried to make fish meal. Commercial menhaden fish meal contains around 10% oil of which a considerable portion is in the form of phospholipids which contain a higher proportion of C22:6 than occurs in menhaden oil in the form of triglycerides. These factors most likely account for the considerably higher proportion of C22:6 in the oil made by solvent extraction than is the case with the regular commercial menhaden oil.

VI. Fatty Acid Content of Salmon Oils

While there have been only three fish oils where sufficient fatty acid analyses have been made to establish the range of values which occur within a species, there are other ways of getting some information along this line. A good example is the situation regarding salmon oils. There are five salmon species occurring on the Pacific Coast. Unfortunately, none of these

have had anything like sufficient samples used when their fatty acid make-up has been determined. Sometimes we see in tables of fatty acid content something listed just as salmon oil with values for the amounts of different fatty acids present. This is meaningless because the salmon of the different species have quite different chemical make-up. Furthermore, salmon oil is ordinarily manufactured from neither the whole salmon nor from its edible portion but rather from the waste material remaining after the edible portion has been removed. Also, a listing of just "salmon" gives no indication of which species of salmon is involved.

In other cases fatty acid values are given according to different species of salmon such as king salmon or pink salmon, but an examination as to the source of this information leads to the conclusion that such a few individual fish were used to determine the fatty acid make-up that the values are probably meaningless.

Fortunately, we can get at some of the needed information in another quite different way. For many years the National Food Processors Co., held annual salmon cutting meetings in Seattle. They opened each year over 2,000 cans of salmon divided among the five different Pacific Coast species and made a careful examination of each can. One thing done was to measure the refractive index of the oil in each can. This gives a measure of

the chemical make of the oil. From this index the iodine number can be calculated. The iodine number is a measure of the type of fatty acids in the oil. The higher the iodine number the more of such fatty acids as C20:5 and C22:6 are present. These are also the fatty acids which make fish oils highly subject to reacting with oxygen to give disagreeable rancid flavors and odors.

The results of these tests made upon about 400 cans for each of the five species gave the following listings with the iodine number increasing from top of list to the bottom.

- 1 - chinook or king salmon
- 2 - sockeye or red salmon
- 3 - chum or keta salmon
- 4 - silver or coho salmon
- 5 - pink or humpback salmon

The groups are also proportional to the likelihood of the particular species after being frozen and stored under commercial conditions to oxidize and become rancid. Thus, the first group, chinook or king salmon, can be held in cold storage for a year or more with very little change in the oil and no apparent rancidity developing. The fish in the last group, pink or humpback salmon on the other hand, start to become rancid after just two or three weeks in cold storage and are quite inedible after only two or three months of storage. Consequently this species is never commercially frozen and stored.

A few fatty acid determinations upon salmon have been made but in each case the number of samples used have been quite inadequate. For example when the method for determining fatty acids known as gas chromatography had first been introduced, one laboratory tried it out using a number of different species of fish. They did not use very many individual fish for a given species, as few as five in most cases, only one in one instance. They included the following statement in their paper "No attempt was made to prepare composite samples representative of the particular species variations from one time of year to another or from one catching area to another. In this respect, the sampling was definitely inadequate to be representative of the species." Nevertheless very many of the fatty acid results for different species included in this paper were picked up and used in tables as being representative of the species.

VII. General Items Concerning Fish Oil Fatty Acids

In addition to the ordinary fatty acids in fish oil, it was first noted in 1974 that fish oils contain small quantities, sometimes up to 1% of furanoid fatty acids. These are ones which contain a ring structure in which four carbon atoms and one oxygen atom are arranged in the form of a ring. Investigations since 1974 have shown that there may be as many as eight different such furanoid structures. They occur to a greater

extent in fish oils from fish living in freshwater. Little or nothing is known about their function nor as to whether they serve any useful role in nutrition.

To the greatest extent fatty acids in fish are derived from food which the fish consume. Such food to a large extent consists of microscopic plant and animal organisms which float or drift in large numbers in both fresh and salt water. These plankton contain a wide variety of fatty acids which when consumed by fish may either be utilized without change, or altered by the fish to form in some cases one long chain of polyunsaturated fatty acids. While fish rely to a large extent upon availability of fatty acid in plankton or other food in the waters in which they live, many species have ability, for example, to build up long chain, highly polyunsaturated fatty acids from shorter chain, less polyunsaturated, in their food supply. Rainbow trout, for example, can readily convert C18:3 fatty acids to either C20:5 or C22:6 fatty acids.

VIII. Summary of Main Topics in Chapter 2

The oil content of fish of different species can vary tremendously. Some species have oil content in the range of 0.3 to 1%; at the other extreme some species have oil content from 5% to 25% and in extreme rare cases the oil content can be 60% or

even more. The oil content in different parts of the same fish often vary. Usually the oil content in the edible flesh is considerably higher in the portion near the head than that near the tail.

The chemical nature of fish oil fatty acids has been described. Fatty acids consist of long chains of carbon atoms, usually from 14 to 22 carbon atoms per fatty acid. The carbon atoms are connected together in most cases by a single bond: C-C. In some cases two bonds (a double bond) occurs: C=C. All fatty acids have at one end of the long chain an acid group

C-C-C....C- $\begin{array}{c} \text{O} \\ \parallel \\ \text{C} \\ \backslash \\ \text{OH} \end{array}$. The $\begin{array}{c} \text{O} \\ \parallel \\ \text{C} \\ \backslash \\ \text{OH} \end{array}$ is the group which makes the compound a fatty acid. The positions of the double bonds within the long carbon chain make an important difference in the nature of the fatty acids. The position is counted from the carbon atoms at the extreme end away from the $\begin{array}{c} \text{O} \\ \parallel \\ \text{C} \\ \backslash \\ \text{OH} \end{array}$ acid group. Most fatty acids have the first double bond 6 carbon atoms from the extreme end, these are known as omega-6 fatty acids. In fish oils, quite a few of the fatty acids have their first double bond 3 carbons from the extreme end. These are called omega-3 fatty acids.

There are about 20 to 25 different fatty acids which occur to a minimum extent of 1% or more in most fish oils. These differ from each other as to number of carbon atoms per fatty acid, also number of double bonds, as well as position of the

double bonds within the fatty acid. It had originally been assumed that the fatty acid make-up of the oil of any one species was always the same or very nearly the same. Actually this is not the case and the fatty acid composition of the oil of a given species can vary to a large extent. Nevertheless many investigators are unaware of this situation and when they analyze for fatty acids in a new species, they use such a few individual fish that the resulting analysis is not at all the average fatty acid content for that species. The literature is full of many, many quite erroneous fatty acid tables purporting to be the content of different fish.

Actually only three fish oils have been studied sufficiently to enable us to know what the range of their fatty acids are. These three oils are commercial menhaden oil, commercial herring oil and the oil of the food fish, mullet.

The fatty acids in the oils of different species of fish originate to a considerable extent from the fatty acid content of the food in the waters where the fish live. To a large extent this food is plankton. The fish, however, have the ability to convert fatty acids taken in from the food to other fatty acids.

Chapter 3. Nutritional Value for Humans

I. Introduction

Recently, considerable research has been carried out which indicates that fish or fish oil in the diet may well be of value for treatment of a number of medical effects. The general impression seems to be that this idea is a new one. Actually some beliefs along this line have been held for centuries in the past, starting with a few mentions in early Roman and Greek sources and it has even been mentioned in the Bible. A quite good 10-year scientific investigation was carried out in a British hospital between about 1770 and 1780 on the value of treatment of arthritis by consumption of cod liver oil and a paper was published on these results in a British scientific journal in 1783.

It is true, however, that a very large amount of research along these lines has been carried out starting in the early 1950's up to the present time. The largest portion of such research has dealt with heart disease and this will be discussed first.

II. Research on Effects of Fish or Fish Oil in the Diet on Heart Disease

Probably the first indication that perhaps fish in the diet might result in reduction in incidence of heart disease was the fact that it was noted that during the period of years when Norway was occupied by the Germans during World War II the incidence of heart disease declined significantly. After the war was over the incidence of heart disease increased back to the level it had been before the German occupation of the country. One difference between these time periods was the fact that during the Norwegian occupation almost no meat at all was available. In its place, the inhabitants ate fish. This led to the belief by some that fish in the diet reduced likelihood that heart disease might develop.

In 1945 a Seattle physician, Dr. Averly Nelson, decided he would like to study the effect of diet on heart disease. In order to prepare himself to better undertake such an investigation he discontinued his practice as a physician for one year from 1945 to 1946 and enrolled in a course at the University of California at Los Angeles which involved studies in such fields as nutrition and biochemistry leading to a degree of Master of Medical Science. During the latter part of his study in Los Angeles, one of his professors spent several weeks in 1946 in Norway where he heard the theory that changes in the diet of

Norwegians when they ate a great deal of fish coincided with a period of time when the incidence of heart disease was considerably lower than it had been previously.] Dr. Nelson was greatly impressed by this idea that fish in the diet might well be a measure which could lower incidence of heart disease. In 1953, he began a 19 year study of the effect of diet on heart disease. He used patients referred to him by other physicians for most of this work.

When Dr. Nelson began his study it was well known that polyunsaturates in the diet lead to reduction of serum cholesterol levels which in turn reduced the chance of heart attacks. At that time, however, it was believed that this applied only to polyunsaturated fatty acids in vegetable oils. Vegetable oils contain many times as much polyunsaturated fatty acids as saturated ones. Often there are ten times as much or even more. With fish oils, on the other hand, the amount of saturated fats may be almost as much as that of the polyunsaturated ones. Therefore, in the early 1950's fish oils were never used for lowering serum cholesterol levels. However, in 1956 it was shown by Bronte Stewart that fish oils were considerably more effective than vegetable oils for lowering serum cholesterol levels.

In Dr. Nelson's experiment, patients were advised to eat fatty fish at least three times per week as a main course meal. After the evidence of Bronte Stewart's 1956 paper was published the use of fatty fish three times per week became a mandatory part of the diet. At the end of 19 years on a percentage basis 4 1/2 times as many deaths from heart attacks occurred for those patients who did not adopt the diet with 3 meals of fatty fish per week as was the case when such a diet was followed.

Another more recent test was conducted in Holland by a Dr. Kromhout and co-workers. A group of 872 individuals, none of whom had ever had a heart attack, were followed for 20 years from 1960 to 1980. About 20% of these individuals ate no fish at all. A portion of the remaining individuals ate on an average 45 or more grams of fish per day. Of those who ate no fish at all 2 1/2 times as many (on a percentage basis) died of a heart attack as was the case for those consuming 45 or more grams of fish per day.

The 19-year project of Dr. Averly Nelson in the United States and the Dutch 20-year study of Kromhout and coworkers are the only two such very long investigations ever carried out. That of Nelson where 4.5 times as many individuals not consuming fish died of a subsequent heart attack would appear to indicate a greater effect than the 2.5 times difference occurring in the Dutch study. However, this greater effect in the Nelson project

might well be due to the fact that all of Nelson's patients at the start of the investigation had had at least one previous heart attack whereas in the project of Kromhout none of the participants had had a heart attack previous to the start of that test. It would seem quite probable that patients subject to heart attacks would be more apt to suffer another more often than those who had never before had one.

In addition to the two long term studies of Nelson and Kromhout, a great deal of research has been carried out in other ways which indicates that fish or fish oil in the diet is likely to reduce risk of heart attacks. One such line of research deals with many short-term tests which shows that fish oils in the diet of animals and of humans reduce the level of cholesterol in the blood. At least five separate investigations showed clearly that the serum cholesterol levels of human subjects could be lowered when fish or fish oil occurred in the diet. Also, many investigations showed that oil from a large number of different species of fish were effective. Such tests demonstrated that oil from the following species of fish lower serum cholesterol levels: cod liver oil, halibut liver oil, dogfish liver oil, menhaden oil, tuna oil, salmon oil, sardine oil, pilchard oil, ocean perch oil, mullet oil and herring oil. The species of animals which were effected include rats, mice and chickens.

A great deal of research was carried out between 1955 and 1966 by Peifer, working at Hormel Institute under a contract from the Seattle laboratory of the U.S. Bureau of Commercial Fisheries. His work went much further than merely showing that various fish oils lowered serum cholesterol levels. For example, he showed that some of the omega-3 fatty acids in fish oil (e.g. C20:5 omega-3 and C22:6 omega-3) occurred at greatly increased levels in the heart of animals fed fish oils. The levels increased up to seven times as much as was the case when fish or fish oils were not present. Work of another investigator (Kingsbury) also has indicated that the level of omega-3 fatty acids increased in the hearts of human individuals who had consumed fish oil. This work of Peifer et al was carried out in the period of the late 1950's and 1960's.

Much later during the 1970's and 1980's a great deal of research has been or currently is being carried out on omega-3 fatty acids in fish oils in the diet and its relationship to heart disease. This originated when Danish workers noted the fact that individuals living in remote areas of Greenland very seldom had heart disease. Workers at the Aalborg Hospital at Aalborg, Denmark, primarily Drs. Dyerberg and Bang, decided to look into the cause of this situation. They sent medical statisticians to Greenland where it was confirmed that there was almost no heart disease in remote areas of Greenland and that the food consumed was almost all seafood. Research was then carried

out in Denmark which lead to the conclusion that the high omega-3 content of the diet of Eskimos was most likely the reason that the death rate from heart disease there was only a small fraction of what it was in Denmark or the United States.

A similar situation was found to be the case in Japan. Japanese fishermen and their families who live along the coast consume a diet which contains a far larger proportion of seafood than is the case with families living nearby in farming villages where only very small amounts of seafood are consumed. The death rate from heart disease in the fishing villages is far less than that of those in the farming villages.

In very recent times a tremendous amount of research on the effect of omega-3 fatty acid consumption on lowering incidence of heart disease has been carried out. Such findings have been established by research in Great Britain, the United States, Canada, Germany, Australia, the Netherlands and many other countries. The great extent of this evidence leaves no doubt that consumption of long carbon chain omega-3 fatty acids found in our food almost exclusively in the oil of fish can decrease the incidence of heart disease. Much of the current work deals with the mechanism of just how the omega-3 fatty acids bring about this effect. It appears that the omega-3 fatty acids do

not carry out their effect directly but rather change the nature of certain other compounds which are present which, in turn, slow down the coagulation of the blood in the arteries.

Some of the current research on omega-3 fatty acids and their effects on heart disease has been concerned with whether higher amounts of omega-3 fatty acids in the diet might have any detrimental effect (in addition to beneficial effect toward heart disease). Of course slowing down of blood coagulation might be considered a detrimental effect. However, the amount of fish in the usual diet is not nearly as great as the amount in the diet of the Greenland Eskimos. It has been shown that beneficial effects toward heart disease can still result and with only a very small increase in blood coagulation time, much less than that of eskimos whose diet was extremely high in seafood. Other research is continuing to see if any other detrimental effects of omega-3 fatty acids might occur, but to date no such effect has been established.

III. Use of fish oil for treatment of diseases other than those of the heart

A. Arthritis

The first scientific investigation of the use of a fish oil to treat a human disease started more than 200 years ago at the

Manchester Infirmary in England on the use of cod liver oil for patients suffering from arthritis. Around 1770 this hospital had been treating such patients by giving them doses of guaiacum. It was customary at that time to rub the patients joints with cod liver oil apparently based upon the idea that they were oiling squeaky joints. Such treatment never seemed to do much good. In 1772 an outpatient with arthritis who had been coming to the Manchester Infirmary suggested perhaps she should take the cod liver oil internally. The physicians, while believing this would do no good, did not object and the woman did so and quite soon improved greatly. The hospital attendees felt that the improvement was due not to any effect of the cod liver oil but rather to a change in the weather which often affected arthritis. About a year later the same woman returned to the hospital with far worse problems with arthritis than she had previously had and requested that she be allowed to take cod liver oil internally. When this was done her arthritis improved rapidly. As a result of this case, all arthritis patients at the Manchester Infirmary starting in 1772 were treated with cod liver oil taken internally. Over the next decade such treatment resulted in almost 100% improvement of the many patients involved. A paper describing these results was read at a meeting of the Royal Society of Physicians held in Paris in 1782 and published in 1783 in the London Medical Journal.

One problem encountered in their research was the very bad flavor of the cod liver oil. Today, cod liver oil has a somewhat unpleasant flavor, but it is made from fresh livers by merely cooking the livers to release the oil. In the 1700's cod liver oil was made by rotting the livers. This resulted in an exceedingly vile flavor. The Manchester Infirmary got around this problem by first changing the cod liver oil by addition of an alkali to a soap followed by treatment with peppermint resulting in a product not objected to by the patient. Immediately after swallowing the cod liver oil soap, lemon juice was taken which changed the soap back to cod liver oil. The Manchester hospital stated that this treatment could not be used at home because the conversion of the cod liver oil to a soap had to be carried out in a laboratory. This situation, however, probably accounts for the fact that this treatment never became well known and eventually was completely forgotten.

Since 1970, however several recent investigations (although the workers were quite unaware of the work carried out over 200 years ago) have shown that patients suffering from arthritis can be helped by including fish or fish oil in the diet. In a recent project published in 1987, for example, some patients were fed MAXEPA tablets (a source of omega-3 fatty acids) while others served as controls. The consumption of the omega-3 fatty acids clearly showed a decrease in arthritis symptoms.

For another inflammation disease, nephritis involving kidney inflammation, two different investigations using animals have been carried out. In each investigation patients have been benefitted by consumption of omega-3 fatty acids.

B. Multiple Sclerosis

Multiple sclerosis is a very difficult disease to investigate as to effects of fish or fish oil in the diets. This is because multiple sclerosis occurs only in humans, never in animals. With most diseases, initial research is carried out with animals and then any favorable effects extended to humans.

Nevertheless, starting in about 1950 several attempts to determine whether fish or fish oil in the diet could have any beneficial effects on this disease have been investigated. Probably the most extensive of such work has been carried out by R.L. Swank working mostly in Oregon. He has been investigating multiple sclerosis for over 40 years. In 1950 he and co-workers reported that statistics on multiple sclerosis in Norway showed that those inhabitants living inland when much meat but little fish was consumed had a much higher incidence of multiple sclerosis than those living on the coast where much fish was eaten. Swank has continued such work over the years. In 1988 he published results of a 35-year study involving more than 100

patients. The results indicated that there was less multiple sclerosis among those who ate considerable fish than for those who did not. The difference was, however, quite small.

Another investigation was carried out by two investigators in Illinois, Bernsohn and Stephanides. Most of their work dealt with comparing statistics on incidence of multiple sclerosis in different countries throughout the world. They had a hypothesis that even small traces of omega-3 fatty acids in the diet were sufficient to prevent the disease. They believed that individuals who developed multiple sclerosis probably had faulty enzyme systems for converting fatty acids to traces of omega-3's. They attempted to carry out experiments on two islands off the coast of Scotland. One island was inhabited by fishermen and there was much fish in their diet. The other island was occupied by farmers who ate little fish. There were more cases of multiple sclerosis among families living on the island where farming was the occupation. However, because the population of these islands was small, the number of cases of multiple sclerosis were so few that no statistically significant differences occurred; consequently the results of these tests were never published.

Recently (1989) a report of an investigation on treatment of multiple sclerosis with omega-3 fatty acids was published. This investigation involved 292 individuals half of which consumed

omega-3 fatty acids, the other none. As was the case in the investigation of Bernsohm and Stepanides made about thirty years earlier there was no difference at the 95% confidence level. However, after two years only 65 patients not consuming omega-3 fatty acids were improved as compared to 79 for those ingesting the omega-3 fatty acids. It would appear that although omega-3 fatty acids in the diet may have some small beneficial effect, the improvement for multiple sclerosis is not of any great extent if any at all.

C. Cancer

Work on looking into the effect on cancer of fish oil omega-3 fatty acids began in 1967. Since then, several investigations most using animals but some using humans have been conducted dealing with both breast cancer and colon cancer. Although the extent of such research has not been nearly as great as that for heart disease, there has been considerably more work on breast and colon cancer than on any disease other than that carried out dealing with the heart. It would appear from presently completed research on breast and colon cancer that probably the ingestion of fish oil omega-3 fatty acids has a beneficial effect. Considerable more such research is currently underway.

D. Other Diseases

There is no evidence that omega-3 fatty acids lower the incidence of strokes, but one investigation using animals would indicate that perhaps the degree of damage done to body tissues as a result of a stroke may be less when omega-3 fatty acids have been ingested. Several investigations indicate that certain skin diseases such as psoriasis may be alleviated by consumption of omega-3 fatty acids. There is some evidence that high blood pressure can be diminished by consumption of fish oil omega-3 fatty acids. Recently, some work has been carried out on effects of consumption of fish oil omega-3 fatty acids upon certain problems with the eye and also with the brain. Work in these areas is fairly new but considerable research is now underway along such lines.

VI. Current Extent of Research on Omega-3 Fatty Acids and Disease

Research on effects of omega-3 fatty acids and disease began in the 1970's at a slow pace. Over the years, there has been a rapid expansion in the number of such investigations. The extent of the present level of such work can be estimated by the number of investigations reported at the International Conference on the Health Effects of Omega-3 Polyunsaturated Fatty Acids in Seafoods held in March 1990 in Washington, D.C.

Table 3-1. Objectives of Investigations Underway in 1990 on Omega-3 Effects on Disease as Measured by Topics Reported at March 1990 International Conference on Health Effects of Omega-3 Fatty Acids in Seafoods.

<u>Topic</u>	<u>Number of Investigations</u>
Omega-3 and Heart Disease	35
Omega-3 Effects on non-Human Subjects	34
Omega-3 Alteration Mechanisms	18
Omega-3 and Cancer	10
General and Miscellaneous	10
Omega-3 and Blood Pressure	6
Omega-3 and Food Consumption	6
Omega-3 Effects on Eye and Brain	5
Omega-3 Effects on Serum Cholesterol Levels	5
Omega-3 Consumption Effects on the Elderly	4
Omega-3 Consumption Effects on Infants	4
Omega-3 Concentrates Preparation	3
Omega-3 Effects on Immune Response	3
Omega-3 Effects on Pregnancies	3
Omega-3 Effects on Skin Diseases	3
Omega-3 Effects on Miscellaneous Inflammation Diseases	2
Omega-3 and Microalgae	2
Omega-3 Effects on Arthritis	1
Omega-3 Effects on Diabetes	1
Omega-3 and Ether Linked Phospholipids	1
Omega-3 Effects on Malaria	1

At this meeting both full research papers and poster sessions were presented as follows:

Full research papers read	33
Number of poster sessions presented	<u>87</u>
Total	120

This total of 120 investigations are of course, not all of those underway in 1990. Undoubtedly there were a great many more which were being carried out but from which no individual was present at the meeting. It would seem likely that the total number of such investigations underway in 1990 exceeds 200.

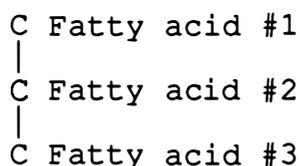
Table 3-1 lists the topics discussed at this March 1990 meeting of the International Conference on Health Effects of Omega-3 Fatty Acids in Seafoods. The topics are arranged in order of the number of investigations for each topic. The topic, omega-3 and heart disease still leads the list with 35 investigations. The second largest number of investigations (34) is effects of omega-3 fatty acids on non-human subjects. While many of these are tests using mice or rats, there are a great many also on effects upon, for example, rabbits, fish, monkeys, pigs and birds. A topic which is increasing in number of investigations deals with study of the various mechanisms whereby during ingestion of omega-3 fatty acids such acids are altered to produce other substances. There were 18 such investigations listed. The disease other than heart disease for which the greatest number of investigations were listed is cancer. Most of these dealt with breast cancer; there were a few on colon cancer. Another topic for which there is increasing interest is the effects of omega-3 fatty acids on the brain and the eye (5 investigations).

In this Chapter 3 there has been discussed effects of ordinary fish oil upon diseases of humans. This fish oil is largely in the form of triglycerides or phospholipids. There are other forms of fish lipids such as, for example glyceryl ethers which will be discussed in Chapter 6.

Chapter 4. Deterioration of Fish Oils and its Effects

I. Introduction

The oils in fish are extremely susceptible to deterioration both while still in the flesh of fish but especially after they have been extracted. The deterioration in most cases is caused by the much greater liability for oxygen from the air to combine with the fatty acids of which the oil is composed. For the most part fatty acids in fish occur as triglycerides wherein three fatty acids occur thus:



The three fatty acids are most frequently each different ones. This results when the fish oils oxidize in the development of disagreeable flavors described as fishy and rancid. As mentioned in Chapter 2, the fatty acids of fish oil contain up to six double bonds. The oxygen from air attacks these double bonds yielding components having disagreeable flavors usually described as fishy or rancid. Whereas the oils in other foods such as meats, poultry or vegetables contain in each molecule usually only 2 or at the most 3 such double bonds, in fish oil their fatty acids can have up to six such double bonds making the fish oils very highly polyunsaturated and therefore much more highly subject to development of disagreeable rancid flavors.

The rate at which oxygen from the air combines with the polyunsaturates of fish oil can be reduced by addition of antioxidants. However, this reduction is not nearly as great as is the case with antioxidants applied to other oils. With fish oils the only way to completely stop the oxidation is to store the fish or its oil in the complete absence of air. For extended storage of fish oils it is necessary to bubble an inert gas such as nitrogen through the fish oil in order to eliminate air dissolved in the oil.

In addition to any antioxidants that might be added to help stabilize a fish oil, all fish oils contain varying amounts of naturally occurring antioxidants. The most frequently occurring such natural antioxidant is tocopherol (vitamin E). The amount which occurs naturally in the oils from different species varies to a considerable extent. See Table 4-1.

Table 4-1. Tocopherol (Vitamin E) Content in the Oil of Flesh of Several Species of Fish

Species	Tocopherol Content (micrograms per gram of oil)
Sardine	40
Menhaden	70
Tuna	160
Herring	140
Whale	220
Sablefish	630

The stability against oxidation follows quite closely the increase in tocopherol content; thus sardine oil is one of the most unstable fish oils becoming rancid very rapidly when exposed to air. On the other hand, sablefish, although containing considerable oil in the flesh, is one of the most stable species known.

II. Flavors and Odors During Oxidation of Fish Oils

A. Flavor and Odor of Pure Unoxidized Oil

First to be considered is the relationship between flavor and odor of fish oil. In the case of extracted fish oil, while the flavor and the odor are closely related ordinarily the flavor is much more easy to assess than that of the odor. The odor is in most cases quite faint for completely unoxidized oil. The flavor, on the other hand, often can be assessed in two stages. First there is the initial flavor which is noted at once when the

oil is first inserted in the mouth. After a short time, the flavor often changes resulting in what is known as the aftertaste. This aftertaste is frequently more pronounced than the initial flavor.

The flavor of unoxidized fish oil ordinarily varies from that made from different species of fish. That is to say many species of fish have odors and flavors in their fresh unaltered stage which is characteristic for that species. For example salmon oils have such a characteristic flavor or odor. In most cases we do not as yet know just which chemical substances in the salmon oil, for example, are recognized as resulting in the flavor we associate with salmon. In a similar way the flavors of the oil in fresh fish of many other species such as mackerel, herring, and lake trout have flavors which are associated with the various species in their fresh state.

B. Flavors and Odors of Oxidized Oils

One of the earliest types of odors and flavors which develop upon oxidation of the oils in fish are a type designated as "fishy". These so-called fishy flavors and odors are of several varieties and some of them are shown in Table 4-2.

Table 4-2. Flavors and Odors Described as "Fishy"

<u>Description</u>	<u>Principal Source</u>	Predominance as flavors (F) or odors (O)	
		<u>Primary</u>	<u>Secondary</u>
Burnt	Fish oil and fish meals	O	F
Freshwater Fish	Freshly caught freshwater fish in skin or flesh	O	F
Green	Fish oils	F	O
Iodine-Like	Certain species such as sole especially after they have been feeding on some special particular types of food	F	O
Pure Oxidation Types	Fish oils	F	O
Sweet: An intense sweet odor generally considered quite unobjectable	Well iced fish	O	F

The term fishy odor is not exclusively used for odors in fish. For example butter may be described as fishy. The flavor described in this way is either identical or nearly so to that described in fish as fishy. Other foods which may at times be said to have fishy odors include poultry products. This occurs often when the poultry are fed fish at excessively high levels. Often the poultry are fed fish until shortly before slaughter and then fish are eliminated from the diet which diminishes the fishiness.

On the other hand the term "fishy" may be used to describe an entirely different type of flavor. Thus, in one instance, a panel carrying out storage tests on meat described a pork sample as fishy. When this same sample was examined by a panel working with fish, the pork sample was described not at all as fishy, but rather as having a mutton flavor.

The chemical compounds responsible for the fishy odors are quite numerous. For example in one study of crude menhaden oil 55 different chemical compounds were found. Some of these together with the description of their various odors are shown in Table 4-3.

Table 4-3. Some Compounds with their Quantity and Odor Found in a Crude Menhaden Oil

<u>Number</u> ^{\1}	<u>Odor Characteristic</u>	<u>Concentration Parts per Billion</u>
1	painty	130
2	cut grass, green	1380
3	greasy green, musty	930
4	sickly sweet, cooling	8530
5	waxy green, grassy	410
6	sharp green, oily	540
7	pesticide-like	20
8	pesticide-like	40
9	citrus, fatty, orange	240
10	sharp, green, greasy	140
11	pesticide-like	20
12	musty with citrus topnote	620
13	fatty floral	500
14	musty, waxy, floral	280
15	irritative, vinegar like	1380
16	vegetable green	1880
17	sweet, green fruity, fatty with citrus topnote	640
18	cherry almond, sweet fruity	60
19	fatty, waxy, musty	200
20	astringent, acidic	2410
21	sweaty, dirty sox	100
22	dirty sox	8110
23	parmesan cheese, dirty sox	330
24	sweaty, dirty sox	810
25	oxidized fish oil	70
26	medicinal disinfectant	110

^{\1} See Chapter 7, Table 4-3 for identity of compound

When oxidation of fish oils passes the range called "fishy" it enters the phase known as rancidity. Most all other oils also when oxidized pass through the zone of rancidity. Table 4-4 lists the various levels of rancidity based on flavor and odor.

Table 4-4. Levels of Rancidity Based on Flavor and Odor

<u>Degree</u>	<u>Flavor Description</u>	<u>Aftertaste Description</u>
Very slight	Barely distinguishable	Usually absent
Slight	Flavor weak but quite definite and somewhat objectionable to many	Slight
Moderate	Unpleasant to most	Lasting; undesirable to most
Pronounced	Undesirable to almost everyone and quite objectionable to many	Persistent, annoying
Extreme	Exceedingly disagreeable	Very disagreeable

In what has been discussed so far, the results leading to rancid properties have referred only to fish oil triglycerides. Most of the fish oil fatty acids occur as triglycerides yet an appreciable amount of the fatty acids occur as phospholipids (see Chapter 7, page 89). Those fatty acids occurring as phospholipids are subject to oxidation similar to what is found with fatty acids occurring as triglycerides. The fatty acids may require a longer period of oxidation when occurring as phospholipids than is the case with those present as triglycerides. Eventually, however, the fatty acids in phospholipids are released and then oxidize rapidly to yield rancid flavors and odors.

The most highly unsaturated fatty acids occur as phospholipids to a greater extent than is the case with other fatty acids combined as triglycerides. Thus after their release from the phospholipids, these highly polyunsaturated fatty acids yield more rancid products than those resulting from fatty acids-occurring as triglycerides, even though it may take longer for the oxidation to occur.

In preparation of products such as fish protein concentrate, removal of the triglycerides is generally carried out by extraction of the fish with fat solvents. Fat solvents which readily extract all of the triglycerides do not so readily extract all of the phospholipids. It is thus desirable to carry out such extraction over a longer period of time so as to remove not only the main fatty acids (those present as triglycerides) but especially those present as phospholipids, which, if left behind in the product, can result in considerable problems with rancidity.

C. Flavors and Odors from Using Spoiled Fish in Making Fish Oil

Up to this point the odors or flavors of fish oil resulting only from oxidation of the oil has been discussed. Another quite different source of undesirable odors and flavors of fish oil can occur from preparation of the oil from partially spoiled fish. If fresh fish are used as the raw material for manufacture of

fish oil none of this second type of odor or flavor results. Unfortunately, however, in some instances the fish to be used for manufacture of fish oil may not be entirely fresh. This can occur if the fish after catching are not adequately refrigerated while in transit from the fishing grounds to the fish oil manufacturing plant. This problem can be considerable where the fish are caught at a great distance from the manufacturing facility and especially where the temperature is quite high. Ordinarily icing of the fish aboard the fishing vessel will be sufficient to minimize or eliminate this problem. If, however, inadequate care is taken, the initial spoilage usually takes place resulting in the formation of nitrogen containing components either ammonia, trimethylamine or both. At more advanced stages of spoilage various sulfur compounds such as hydrogen sulfide will form and these sulfur compounds can lead to extremely unpleasant odors and flavors if present in the resulting oil. Fortunately at present day practices very seldom is such an advanced stage of spoilage reached whereby the level of sulfur compounds becomes much of a problem. If, however, sulfur compounds do occur in the oil, their level can generally be reduced by clay bleaching. The presence of ammonia or trimethylamine, on the other hand is more likely to occur.

Although there is a common idea that trimethylamine has a pronounced, characteristic fishy odor, this is only partially true. This odor of trimethylamine occurs only at a level between

1:1,500 and 1:8,000. At higher levels the odor of trimethylamine is identical to that of ammonia while at levels lower than 1:8,000 there is no detectable odor whatever. On the other hand ammonia when mixed with air to a level of 1:2,000 passes a fishy level quite similar to that of trimethylamine diluted to a level of 1:6,000. Thus, trimethylamine possesses the fishy flavor at only about 1/3 the level for ammonia.

These aspects of the fishy odors, however, are not quite as simple as these figures might indicate. The odor of the diluted trimethylamine and of ammonia although resembling to a considerable extent that of stale fish are not really exactly the same. If one compares the odor of the same amount of ammonia and trimethylamine present in a sample of fish having a given level of fishy odor, it does not correspond to the odor of that sample of fish. No matter at what level of trimethylamine and ammonia gas are noted, the fishy odor is not as great as is the case for the odor in a fish sample described as having a fishy odor. Another aspect showing that this relationship is not what it seems is the fact that the level of trimethylamine in canned salmon is sometimes very high without any fishy odor whatever being present.

An early investigation in 1936 by Daviess and Gill seemed to show that fishiness in fish oil might involve presence of substances other than merely trimethylamine and ammonia, but this

research has never been followed up. As the situation stands at present it would appear that oxidation of fish oils yielding trimethylamine and probably ammonia results in a fishy flavor but the mere presence of trimethylamine does not necessarily mean that fishy flavor is present in the fish. Furthermore, fish with the typical "fishy" odor may contain either little or no measurable ammonia or trimethylamine.

III. Measuring Levels of Flavor and Odor

A. Handling and Sampling Commercial Fish Oils

Owing to the great instability of fish oils caused by their extremely high content of polyunsaturated fatty acids, the oils are apt to rapidly deteriorate in composition during handling. This places upon the individual responsibility for paying careful attention to storage procedures for use during the handling and even during the subsequent analysis to make certain that no appreciable oxidation has taken place. Alteration can occur not only due to the presence of air in the space above the oil in containers but also even from oxygen dissolved in the oil.

Because of this instability, fish oils, during handling must be provided with an inert atmosphere for which nitrogen is most frequently employed. Carbon dioxide has some additional advantages but it is often unavailable in commercial quantities

in a pure enough state. Nitrogen is applied ordinarily by bubbling it through the fish oil in the container in which the oil will be stored. The inert gas should be bubbled through the oil at a rate so as to remove the air dissolved in the oil and, at the same time to provide some circulation of the oil itself. If the oil is to be stored for quite a long time, the inert gas should be bubbled through the oil for at least one hour.

The inert gas used must be free of any small amounts of oxygen. Nitrogen can be purchased specially treated to contain no more than 0.01% oxygen or the regular commercial grade can be heated over copper to remove any oxygen present. If a great deal of work is done using inert gas it is best to provide the purified gas piped to the workbenches.

Carbon dioxide has one advantage over nitrogen. Its density is greater than that of nitrogen. When sealing fish oil under an inert gas, it is very important that during the sealing process no air be introduced into the container at the point when it is sealed off or stoppered. Because carbon dioxide is heavier than air there is less likelihood of this happening than would be the case when nitrogen is employed.

Fish oil is manufactured in very large quantities. It is customary for such manufacturers to hold their oil in storage tanks ranging from 50,000 to 750,000 gallons or sometimes in

barges which may hold about 200,000 gallons or more. It is a very difficult problem to obtain samples representative of fish oil stored in such very large quantities. Special equipment is used in order to be certain that truly representative samples are being obtained. For this purpose a piece of equipment called a Bacon bomb sampler thief is usually employed. This thief ordinarily consists of a cylindrical tube of 16 ounce capacity. The tube is about 12 inches (305 mm) long and 2 3/4 inches (66 mm) outside diameter. The oil enters the bottom of the thief through an attached center tube. The thief, attached to a cord is lowered at a uniform rate down through the tank. The rate at which the oil enters the thief is controlled by adjusting a needle valve at the top. This varies the speed at which air enters the thief and, of course, this in turn varies the speed at which the oil enters the thief. The thief gives a representative sample from top to bottom of a cross-section of the tank. In use it is very important that it should not be completely filled by the time it reaches the bottom of the tank. This would prevent it from giving an accurate cross section sample.

When sampling oil at low temperatures, stearine may have formed. Stearine consists of some portion of the oil which is solid at the temperature at which the oil is stored. This problem can be overcome usually by stirring the oil for several hours, or, if this does not work, by heating it.

In sampling large lots of oil, 1 pint is usually considered representative for amounts up 10,000 gallons; for 200,000 gallons, such as might occur in a very large tank or a barge, 4 quarts is generally considered to be sufficient.

The use of the Bacon sampling thief is useful when it is desired to obtain oil samples representative throughout the tank. If samples representative of the tank of oil at different levels are desired, special sampling thieves are available.

B. Analytical Extraction of Oil from Fish Flesh

When fish oil which is contained in fish flesh is to be tested for odor or flavor, it must first be extracted from the fish flesh. This is generally done by grinding the fish flesh and then extracting the oil by use of some suitable solvent.

Grinding of the fish flesh prior to extraction of the oil is carried out using ordinarily a cutting type of grinder. The common kitchen meat grinder in which fish can be introduced over a spiral shaft forcing it through a plate with holes can be used. However, better grinding leading to a more homogenous sample can be obtained by use of a sausage grinder where flesh is cut by a rotating circular knife.

Ordinarily it is best to use bone-free flesh such as a fillet for extraction of the oil. If fish with bones are to be used, two alternatives are possible. The fish with bones can be ground in a hammer-type grinder. Alternatively the fish can be cooked under pressure. This will soften the bones sometimes making it possible to grind it in any of the cutting type grinders.

Once the fish has been ground, there are two situations that must be kept in mind while storing it prior to oil extraction. In the first place the ground fish will lose moisture rapidly unless stored carefully in a closed container. The second aspect relates to the fact that upon standing for any great length of time the moisture in the fish tends to separate out resulting in solids sitting in a pool of liquid. This condition occurs mostly with fish which have been frozen before they were ground. It is necessary, then, to stir the ground fish very thoroughly just before it is to have the oil removed.

The simplest method for extracting the oil from the minced fish flesh is to use a method known as the Bligh and Dyer procedure in which the fish is blended with a mixture of chloroform, methanol and water. Eventually the chloroform layer in which the fish oil is contained is heated to distill the chloroform leaving

behind the fish oil. Alternately the minced fish mixed with sand can be dried over concentrated sulfuric acid followed by extraction of the oil with a suitable solvent.

C. Extraction of Oil from Fish Meal

In fish meal the oil sometimes occurs in a form in which most of it can not be extracted by fat solvents. This occurs especially with fish meals made from fish having highly unsaturated oil such as sardine. The best procedure, based upon the American Association of Analytical Chemists method, is first to extract the fish meal with acetone. This is followed by a procedure involving acid hydrolysis of the meal with hydrochloric acid. The so treated meal is then re-extracted with acetone. The acetone is then removed to leave the oil. This procedure is needed because, as fish meals are stored, their oil may react with the protein in the meal giving substances that can not be extracted until after acid hydrolysis releases the oil. As an example of the need for such a procedure, fish meal containing 12% oil may after storage, which binds the oil, appear to have an oil content of only 2% or even less unless the acid hydrolysis procedure is used.

D. Measurement of Flavors and Odors Using Chemical Tests

1. Chemical Tests for Oxidative (Rancidity) Odors and Flavors

There are a number of chemical tests which purport to give values which will indicate the degree of rancidity in fish oils. Among these are peroxide number, thiobarbituric acid value (TBA) and the measurement of the gain in weight of fish oil during oxidation. However, none of these tests will give values which are always consistent with the degree of rancidity. Of the various such tests, peroxide number has been used much more than any other. It measures the amount of peroxides which are the first substances formed from fish oil fatty acids oxidation before they break down into various end products which are the ones possessing the typical flavors making up rancidity. There are a number of procedures for determination of the peroxide number of an oil or fat. Probably the simple test devised by Wheeler in 1932 is as good as any of these tests and is the most easy to perform. It is based upon the fact that peroxides will release iodine from iodine salts such as potassium iodide. In the Wheeler peroxide value determination, 3 to 10 grams of oil are dissolved in a mixture of 40% chloroform and 60% acetic acid. When 1 milliliter of saturated potassium iodide is added, iodine is released. The reaction is stopped by adding water, and then the amount of iodine in the mixture is determined by reacting it

(from a buret) with a standardized solution of sodium thiosulfate. The sodium thiosulfate reacts to remove the iodine, and the amount of iodine can be calculated from which the peroxide value is obtained.

Although the measurement of the peroxide value is supposed to be a measure of the extent of rancid flavors and odors, it must always be remembered that this is not universally the case. For example, when fish oils are stored at different temperatures, the peroxide value occurring for some degree of rancidity may be entirely different from that if the same oil had been stored at some other temperature. Thus if one sample of oil from fish which had been stored unfrozen in ice had some given peroxide number, the peroxide number for the same flavor from the oil from fish, frozen and held say at 0° F probably would be entirely different. These same inconsistencies occur for whatever chemical test is used. For this reason results from chemical tests for rancidity of fish oil such as peroxide numbers must be frequently checked against the actual flavor.

2. Chemical Tests for Non-Rancidity Flavors and Odors

We know so little about the causes of flavors and odors in fish oils caused by changes other than that brought about by oxidation that it is difficult to understand just what substances would need to be determined. Probably the amount of

trimethylamine and of ammonia present would be the most useful based upon our present knowledge of the situation. The amounts of either trimethylamine or ammonia present in oils made from fish which had not been fresh when the oil was manufactured is so small that little can be done to develop such tests. One possibility, however, is to determine the total nitrogen in the oil. This would include both trimethylamine and ammonia. Some methods for determining trace amounts of total nitrogen in oil are available.

Nitrogen in oil is commonly determined by the Kjeldahl method which ordinarily is used for determination of, for example, the protein in fish. There is, however, a micro-Kjeldahl method for determination of trace amounts of nitrogen. It is based upon a colorimetric evaluation of very small traces of nitrogen. It is not certain, however, whether even this micro method for nitrogen in fish oils would be sensitive enough to measure the extremely small amounts of nitrogen present. Another method, the Dumas method for nitrogen determination might possibly be of greater sensitivity.

E. Sensory Examination Methods

1. Need for Such Methods

As we have seen there are a number of chemical tests available for giving an approximation as to the odors and flavors in fish. It should be emphasized that such chemical tests give far from reliable indication of the condition of the fish. They are useful only if the true condition of the fish based upon frequent checking of the odors and flavors are made. Such checking can only be carried out using sensory panels of individuals who are familiar with the odors and flavors of the oil of fish.

2. Sensory Panels

Flavor and odor of fish oils must be established by examination by individuals who have had experience with the odor and flavors and can reproducibly repeat ratings. The opinion of a single individual no matter how expert and experienced he may be is not satisfactory. All members of the panel should, however, be experts based upon having had long experience in examining fish oil for odor and flavor.

3. Judging Flavors of Fish Oils

The judging of fish oil flavors requires a panel with a minimum of at least four members. Preferably a somewhat larger panel is desirable, although not more than six individuals, if their experience is extensive would be sufficient. It is best not to attempt to evaluate a large number of samples at one time, with a limit of six such samples being maximum if most consistent results are to be obtained.

Oil samples can best be presented to each panel member on a 9 cm disposable Petri dish. Such containers, containing a large area, make it easy to observe both odor and flavor. Plastic spoons can have a small amount of the oil transferred from the Petri dish to the tip of the tongue. This keeps large amounts of oil from getting on the lips which might interfere with tasting of subsequent samples. The strong aftertaste common in fish oils can best be removed between samples by rinsing the mouth with apple juice. This is just strong enough in flavor to remove the flavor of the previous fish oil sample. Yet it does not, itself, have sufficient flavor as may be the case with other fruit juices to remain in the mouth so as to interfere with tasting later other fish oil samples.

4. Judging of Flavor of Oil in Fishery Products

Examination of the flavor of oil in such items as frozen, thawed fish or cooked fish can be carried out in much the same way as that just discussed for fish oil. There is one important factor, however, that must be considered when dealing with the oil in the fish flesh. Fish flesh usually consists of two types. One of these is the light colored flesh, the other the dark flesh. In most cases the oil in the dark flesh oxidizes much more rapidly than does that in the light flesh. With some species, almost all of the oxidation of the oil takes place in the dark flesh and virtually none in the light flesh. This means that if the panel member happens to taste only the light meat he may find almost no rancid flavor. One way of avoiding this would be to homogenize the flesh so that all the meat, light and dark are well mixed. This, however, reduces upon the amount of information obtained by the panel. Often the amount of dark flesh is such a small fraction of the total that such a blended sample might present only a very small trace of rancidity.

The much better approach is to separate the light meat from the dark meat. Then the panel would be presented each types of flesh, but separately so that complete information would be available of the flavor in each type of flesh. The same consideration would also, incidentally, apply if chemical tests were to be made upon the flesh. Here again it would be much

better to run any chemical tests such as peroxide values on the light flesh and then again on the dark flesh.

5. Induction Period Measurements

In the oxidation of any oils the rate of oxidation at the very beginning is relatively slow. With some types of oil this early oxidation is so slow that for a considerable period of time almost no evidence that oxidation is occurring at all is apparent. Then after a period of time called the induction period has elapsed, the rate suddenly increases and the oxidation proceeds rapidly so that in a short period of time the oil is completely rancid.

With fish oil this same situation occurs except that in the initial induction period the rate of oxidation is somewhat greater than in the case with other oils. Nevertheless at the end of the induction period, there is a sharp rise in rate of oxidation.

A measurement of the length of time that the induction period lasts will give some idea as to the stability of the oil against rancidity. Some fish oils have quite different lengths of their induction period than do others. In measuring the length of the induction period, it is often set up in such a way

that the oxidation is accelerated so that the result can be obtained in a much shorter period of time than would be the case if no such accelerating action were taken. Such acceleration is usually accomplished either by heating the oil or by bubbling air through it.

Another way of speeding up the oxidation rate is to increase the exposure of the oil to air. This can be accomplished by soaking filter paper with the oil and then storing the filter paper with its adhering oil at some constant temperature. This speeds up the oxidation rate tremendously. The rate under these conditions may be 100 times as great as if the oil had been held in a beaker exposed to air. The filter papers can be tasted during this oxidation period or they can be used to run chemical tests.

There often is a large difference in the rate at which oils from different species of fish oxidize. Thus by using such a method as the use of filter paper, the relative rate of oxidation of different fish oils can readily be determined.

IV. Summary of Chapter 4

Fish oils are highly sensitive to deterioration. This, to a large extent, is due to their high degree of polyunsaturation which permits oxidation to give undesirable flavors and odors.

Anti-oxidants are commonly added to oil in general to slow down oxidation but are less effective with fish oils. The only sure way to keep fish oils from oxidizing is to keep all air away. Even the amount of air dissolved in the fish oil itself must be eliminated. Fish oil through which oxygen free nitrogen has been bubbled will keep for years without oxidation.

Under ordinary conditions, fresh fish oils often have desirable flavors characteristic of the particular species. Initial change caused by oxidation yields a "fishy" flavor. It is caused by formation of many different substances. In addition to oxidized substances causing fishy or other undesirable flavors and odors, if the fish oil is made using partially spoiled fish, ammonia and trimethylamine may add further to fishy flavors. Most undesirable flavors, however come from rancidity.

Commercial fish oils in bulk are sampled by special instruments such as the Bacon bomb sampling thief.

The oil can be removed from fish flesh by ordinary solvent extraction but from fish meal a preliminary acid hydrolysis often must precede the solvent extraction.

Flavors and odors in fish oil can to a limited extent be measured by chemical means. The most common such method is peroxide number which increases with increasing oxidation and

rancidity. The method however must always be checked frequently by means of determining the actual flavor by a taste panel. Chemical tests, e.g. peroxide number, give widely varying values with differing methods or temperatures used in storing the oil or the flesh in which the oil is contained. It is, therefore, essential that an adequate sensory examination panel be available to measure flavors or odors under investigation. In some instances a measurement of the induction period (length of time before rapid oxidation of fish oil occurs) is helpful. The induction period may vary over a range of several times for different fish oils.

Chapter 5. Manufacture and Processing of Fish Oil

I. Introduction

It might appear to be unnecessary to include in a report such as this, where special emphasis is placed on nutritional aspects of fish oil, a chapter on manufacture and processing methods. Actually the manner in which fish oil is manufactured and processed determines to a considerable extent the properties including nutritional properties of the resulting fish oil. Although most fish oil is made by a procedure called the wet process, some is made by other methods which result in different properties of the oil. Especially, however, since fish oil manufacture gives only a crude oil containing many impurities, the means by which it is processed to produce a final product is very critical with respect to the usefulness of the oil for consumption for any nutritional purposes. Furthermore, in processing fish oil, it is sometimes possible to increase the proportion of nutrients such as omega-3 fatty acids.

This chapter will therefore contain three main subdivisions. The first discusses manufacturing methods for making a crude fish oil. The second topic deals with methods of processing the oil to give a purified product. The final short section deals with ways to concentrate or increase those aspects of the fish oil such as the content of important nutritional substances.

II. Manufacture of Crude Fish Oil

Almost all of the fish oil manufactured on a world-wide basis is prepared by means of the wet process. A very few other methods have been used and some of them also will be briefly mentioned.

A. Wet Process

1. Unloading

Fish to be made into fish oil (and meal) are unloaded at the manufacturing plant either by a dry or a wet method. In the dry method, the fish may be loaded on to a bucket elevator which conveys the fish from the vessel to the plant. Alternately the suction or a vacuum may be used to carry fish from the vessel to the plant. The other method commonly used, called wet unloading, involves adding fresh or seawater into the hold of the vessel. A vacuum pump then brings the mixture of fish and water into the plant after which the water is removed by screening.

In the wet-processing method after unloading, the following steps are employed: cooking, pressing, decanting, and polishing.

2. Cooking

The cooking is carried-out to coagulate the fish protein and to rupture the cell walls in order to release the oil. If the fish were not cooked, even after considerably pressure, little or no oil would be released. Ordinarily continuous indirect type of cookers are used. Such cookers are large cylinders covered by a steam heated jacket.

3. Pressing

Pressing of the cooked fish can be carried out in either continuous, single-screw presses or double screw presses. The latter type of press is more generally applicable covering various types of fish. The pressing operation separates the liquid phase which contains the oil from the solid phase from which fish meal is made. The liquid phase is first freed of bone and pieces of fish by passing through a vibrating screen.

4. Decanting and Separating

The liquid phase is next further separated from suspended particles by passing it through a decanter consisting of a cylinder-shaped conveyor which rotates inside a cylindrical bowl. Finally, the liquid from the decanter goes through a separator which yields the fish oil, by this time free of any fish solids.

5. Polishing

The final stage consists of washing the oil from the separators thoroughly and then separating the water from the oil. This is the crude fish oil which can either be sold as such or passed on to processing operations.

B. Other Methods of Manufacturing Fish Oil

Several alternate procedures have been used to produce fish oil and fish meal. Most of these methods are ones aimed at production of fish meal and if any fish oil is produced, the oil generally is of inferior quality. For example, the dry rendering process involves cooking and drying in a single step followed by extraction of oil. A patented dry rendering operation, the Carver-Greenfield method was used for many years producing a high grade fish meal but the fish oil had a very dark color and was of quite inferior quality. The same considerations occur for other alternate methods including use of hydrolysis with enzymes, and the method for silage production.

One method, however, solvent extraction used to produce fish protein concentrate, results in a oil of superior quality from a nutritional standpoint. When fish oil is made by the usual wet process, a considerable portion of the oil remains in the meal. It has been noted that the omega-3 fatty acid content of the oil

in the meal especially that of docosahexaenoic acid (DHA) is considerably higher perhaps twice as high in the oil remaining in the fish meal as it is in the oil in the original fish or in the oil made by the solvent extraction process.

The present difficulty in this situation is the fact that there are almost no markets for fish protein concentrate. In the past a great effort especially in the United States, was made first to learn how to manufacture fish protein concentrate and then to try to build up a demand for it. Although good methods involving solvent extraction for making fish protein concentrate were developed, no success was achieved in developing an adequate market for it. Should, in the future, there be a demand for production of fish protein concentrate, there would then be also a considerable demand for the resulting oil because of its superior quality.

III. Processing of Fish Oil

The method of manufacture of fish oil as discussed in the previous section results in production of a crude oil containing many impurities. While some applications for industrial oils might find this crude oil to be satisfactory, most potential uses would make it necessary to process the oil to improve upon its quality. This is especially the case with potential uses whereby

the oil would be used in some human food applications. In this section the various methods for refining crude fish oil will be discussed.

A. Alkali Refining

In refining oils, in general, the preliminary steps involve first degumming then alkali refining. The degumming is always carried out in refining vegetable oils but may be omitted for fish and sometimes other animal oils. In Europe fish oils, however, usually include a partial degumming step before alkali refining by being treated with phosphoric acid.

Alkali refining is carried out by adding sodium hydroxide (caustic soda) solution to the oil and then heating the mixture while it is being agitated to a temperature of about 180°F. This procedure accomplishes several objectives including neutralization of free fatty acids, producing soaps, coagulation of any gums present in the oil and improving the color of the oil. The alkali refining can be carried out either as a continuous or batch operation. After heating the oil at 180°F with the alkali, the oil is separated and then heated to 190°F with water, the amount of water being about 15% of that of the oil. The

mixture is then stirred vigorously. The oil and water is then separated. Sometimes a second washing of the oil with water is carried out and finally the oil is dried in a vacuum dryer which eliminates nearly all of the water.

During the alkali refining process the soaps formed from the free fatty acids in the oil are separated usually in a centrifuge. These soaps called acidulated soapstock are then sold to either feed or soap manufacturers.

B. Bleaching

The bleaching process applied to the alkali refined oil removes impurities which otherwise would cause the oil to have undesirable dark color, and poor flavor and which also might lead to undesirable instability against oxidation. The bleaching takes place by the action of an activated clay added to the oil usually at a level of between 1 and 4% clay. Three alternate procedures are available: batch atmospheric, batch vacuum, or continuous vacuum bleaching.

In batch atmospheric bleaching, the operation takes place in open kettles which are provided with agitators to keep the clay in continuous contact evenly throughout the oil and with heaters. About 60,000 lbs of oil is heated to 160°F and heating and mixing continued for about one half hour and the oil is then filtered

from the clay. Where insufficient bleaching has occurred, the filtered oil is returned to the kettle for repeated agitation followed by filtering.

In batch vacuum bleaching the operation takes place within closed vessels of around 35,000 lb capacity, and with heating coils and agitators. The operation is carried out at from 26 to 28 inches of vacuum. At one opening into the vessel, oil is sprayed in while at another opening clay is pulled into the vessel. After about 15 minutes at 165°F of bleaching the temperature is lowered to 160°F and filtered under vacuum and further cooled before the oil leaves the evacuated chamber. This batch vacuum method has several advantages over the batch atmospheric method. Oil has much less exposure to air. The bleaching time is less which further reduces chance for oxidation and the color of the oil is superior to that obtained by atmospheric bleaching.

Continuous vacuum bleaching offers the most efficient procedure. The oil is in contact with bleaching clay for the shortest period and the temperature during the bleaching is lowest. Furthermore the oil is best protected by this procedure against oxidation.

C. Winterization

Winterization is carried out primarily to remove those fatty acids which would be solid at room temperature (primarily stearine) but it also removes other unwanted material such as waxes and other constituents which are not triglycerides. The winterization process unfortunately removes not only these unwanted components but also removes some which are important from a nutritional standpoint. For example in the stearine which has been removed from menhaden oil by winterization there are 11% EPA (eicosapentaenoic acid) and 7% DHA (docosaheptaenoic acid). The amounts of these omega 3 fatty acids lost as a result of winterization is not large as compared to the amounts in the winterized oil itself. Thus in winterized menhaden oil there are still 15% EPA and 11% DHA, but the amounts would have been a little higher if a small part of the total ω -3 fatty acids had not been lost in the stearine.

Up until recently winterization of fish oils has been carried out only by the method called dry fractionation. Fish oil is held in refrigerated rooms or tanks in which the refrigeration is applied very slowly so that the oil is cooled to about 55°F and held for several hours. Then the temperature is further reduced to 45°F and the oil held for nearly a day longer. The oil is then allowed to stand for several days and finally is

filtered slowly sometimes taking another day or two using plate and frame filter presses. This entire process may take nearly a week to complete.

The dry fractionation method still remains the principal procedure for winterizing fish oil. Several somewhat newer procedures based on solvent crystallization have been used more recently. One such method, for example is the solexol process.

D. Deodorization

There are at least four different types of deodorizers. These are (1) batch deodorizers, (2) semicontinuous deodorizers, (3) continuous deodorizers, and (4) continuous thin film deodorizers. Rather than to describe each only the first (and simplest) and the last will be considered.

The batch deodorizer which is the one used in most cases, is surrounded by a vertical cylinder which is insulated and has cone heads. Within are coils for heating and cooling of the fish oil. The deodorizers range in size from a capacity of five to 20 tons of oil. Steam enters the bottom of the cylinder through a perforated pipe. The dimensions of this equipment are about 11 feet in diameter and 24 feet high.

The most complicated deodorizer of the continuous thin-film type can be represented by the Cambrian Campro equipment. Because deodorization takes place from thin films of oil, lower temperatures can be used, greatly minimizing the potential problems of oxidation.

E. Hydrogenation

The largest use for fish oils is for hydrogenation to produce a hardened fat which can be added to margarines. Fish oils are ordinarily hydrogenated after the refining steps of alkali refining bleaching and deodorization have taken place. This is done because trace amounts of various components in the original fish oil would, if still present, poison the catalyst used to bring about the hydrogenation.

Hydrogenation involves adding hydrogen to various double bonds in the fatty acid molecules. The product eventually produced for adding to margarine is not a completely hydrogenated product; some but not all of the double bonds are hydrogenated. The process of hydrogenation is stopped at the point where the texture of the product is comparable to that of the balance of the margarine to which the fish oil will be added so that when the partially hydrogenated fish oil is incorporated in the margarine it will be neither too hard nor too soft.

The hydrogenation process used for partially hydrogenating fish oil is a simple one. Hydrogen will not react with unsaturated oils unless a catalyst is present which stimulates the reaction without entering into it. For hydrogenation of fish oils the metal, nickel, is ordinarily used. The fish oil, in large tanks is agitated with the added nickel and hydrogen introduced under pressure within the hydrogenating vessel. The temperature within the vessel is maintained somewhere between 170°C to a little over 200°C. Different hydrogenation plants operate at somewhat different temperatures. The level of the nickel catalyst added is between 0.05 and 0.1%. After the desired reaction has reached the proper degree of partial hydrogenation, the contents of the vessel are cooled rapidly. In order to remove the catalyst the oil is filtered.

After hydrogenation the deodorization step described in the previous section is carried out.

IV. Preparation of High Concentrates of Omega-3 Fatty Acids

A special type of product is needed for research in which very highly purified concentrates of such fish oil omega-3 fatty acids as EPA or DHA are required (for example, for use for the cooperative program between National Marine Fisheries Service and National Institute of Health). Such a product can be prepared by one of several different procedures. The best such method will be described briefly here.

Refined fish oil as described above under IIIB (Bleaching) and IIIC (Winterization) are the starting material for preparation of such a high concentrate of omega-3 fatty acids. They are first converted from fish oil into fish oil ethyl esters. The fish oil is first molecularly distilled and then heated with glycerol and catalyst (zinc dust). It is next cooled and extracted with petroleum ether and then the solution washed successively with water, then hydrochloric acid, then sodium bicarbonate, then saturated sodium chloride solution and finally again with water. The petroleum ether is then evaporated giving a light, yellow powder which consists of ethyl esters of the fish oil fatty acids. These are next treated by urea reaction which results in a concentration of long chain omega-3 fatty acids. The use of this urea process can increase, for example, the amount of EPA (the C20 omega-3 fatty acid) to about 40%.

In order to obtain a much greater concentration, in the past, a number of methods such as fractional vacuum distillation or several forms of chromatography have been employed. These older methods have all had disadvantages mostly either that such high temperatures were needed that the highly fragile long-chain omega-3 fatty acids were partially destroyed or that toxic chemicals, very difficult to separate from the omega-3 fatty acids, had to be used. Much of the research involving production of very highly concentrated omega-3 fatty acids within National Marine Fisheries Service was being carried out at that agency's

laboratory in Seattle. One new method for concentrating omega-3 fatty acids at that laboratory was the use of supercritical fluid carbon dioxide (see Appendix, page 90). Such a procedure could be carried out at low temperatures and, of course, carbon dioxide had no toxic effects whatever. Such a procedure which had been used in the past for other separation methods appeared to be ideal for use in separation or concentration of highly unstable, long chain omega-3 fatty acids. Results of work along this line at Seattle, lead to the issuing of two Public Service patents, the first, U.S. Patent #4,675,132 dated June 23, 1987 entitled Polyunsaturated Fatty Acids from Fish Oils by Virginia F. Stout and John Spinelli, the other, U.S. Patent #4,692,280 dated September 8, 1987 entitled Purification of Fish Oils by John Spinelli, Virginia F. Stout and William B. Nilsson. Both of these patents dealt with use of supercritical carbon dioxide in purifying or concentrating fish oils. In addition to these two patents, a paper on fractionation of menhaden oil ethyl esters using supercritical fluid carbon dioxide was published in Journal of American Oil Chemists Society in January 1988.

When the method described above was used to concentrate omega-3 long chain fatty acids in menhaden oil it was possible to concentrate either EPA C20:5 omega-3) or DHA (C22:6 omega-3) in purities exceeding 90%.

Chapter 6. Glyceryl Ethers and Other Minor Components

Chapter 3 on nutritional properties for humans dealt with the two main components of fish lipids, triglycerides and phospholipids. There are other components which have in some cases nutritional value. Of these, glyceryl ethers are perhaps the most important.

Glyceryl ethers closely resemble in their chemical nature the triglycerides. They differ in that at one point in the molecule there is an ether linkage (see Appendix, page 91). Many years ago it was believed that glyceryl ethers had several medical properties. Some of these properties have now been shown to be nonexistent. At least one such property, however, still may be of importance.

The presence of glyceryl ethers in fish was first reported in 1922. They occur to the greatest extent in the liver oils of shark, but also occur in the liver or flesh of many other species to a significant extent. Beginning in the 1940's considerable belief was held that the glyceryl ethers when applied externally were very effective in healing of burns or wounds. These ideas were strengthened by publication in 1958 by a paper of Bodman and Maisin. These workers had obtained evidence that during development in the foetus before birth, the presence of glyceryl ethers was important in development of the other lipids in the

human tissues during regeneration of new cells before any triglycerides could form. These authors did not suggest that application of glyceryl ethers externally would heal burns; in fact their results indicated that such application slowed down any healing. Somehow this aspect was overlooked by readers who were employed by manufacturers of ointments. This resulted in the sale to the public of ointments for healing of burns and wounds of products containing glyceryl ethers.

In 1966 workers at the U.S. Bureau of Fisheries in Seattle, doubting that this use of glyceryl ethers would effect rapid healing of wounds or burns began a cooperative program with the Mayo Clinic to look into this matter. Hairless mice under three months age were used. Some were given burns under standard identical conditions. Others were given standard wounds. They were then tested daily with 4 different treatments using

- 1) mineral oil
- 2) menhaden oil
- 3) menhaden oil containing 13% glyceryl ethers or
- 4) no treatment at all

Healing time was identical for the mice treated with each of the first three substances. When no treatment at all was used (item 4 above) the healing time was sometimes very slightly longer. These results show conclusively that glyceryl ethers applied externally have no effect to speed up healing time when

applied to burns or wounds. This is not surprising since officials at the Mayo Clinic stated that no substance whatever is known which would produce such an effect.

Glycerol ethers from fish oils have been claimed to have other effects. In most cases, however, just as many investigations for a given effect found no effect as those who found that an effect existed. Thus, one group of investigators in 1957 reported that cattle suffering from bracken poisoning from eating a type of fern resulting in bone marrow damage could be alleviated by consumption of glyceryl ethers, yet a similar study made in 1964 at another laboratory claimed no effect at all for use of glyceryl ethers. Likewise, in the case of treatment of carcinoma one investigation made in 1964 claimed a marked depression when glycerol ethers were fed, yet another study made in 1966 by another group of workers found no such results at all.

The best documented use of glycerol ethers is their use when taken internally to raise back to normal the white cell count in the blood which is often diminished in patients undergoing radiation treatment. At the Radiumhemmet Laboratory in Sweden, based upon some earlier work during the 1940's by other workers, a long investigation upon this effect was carried out by Astrid Brohult and co-workers. Starting in 1953 such alkoxyglycerides were fed to patients undergoing irradiation therapy at Radiumhemmet. A very long-term study was carried out from 1953

to 1966 the results of which were published by Astrid Brohult in a 93 page report. This work showed that of the glyceryl ethers used, selachyl alcohol was the most effective, considerably more effective than batyl alcohol which, however, to a lesser extent was effective. Work was carried out partly with animals such as mice, but also, of course, with human subjects. After publication of the 93 page report in 1963, research continued well in the 1970's.

About the only other type of lipids from fish that have sometimes been used is squalene. Squalene is a hydrocarbon. It occurs sometimes in the surface lipids of skin. For this reason squalene, or more often its hydrogenated, related compound (which is more stable) squalene is sometimes contained in cosmetics such as skin cream.

Chapter 7 - Appendix

The purpose of this Appendix is to furnish a little more scientific information for those readers who might be interested. In a few instances the information might be helpful to those readers who are not certain whether they understand what they have read in the main text. In most instances, however, the material in this Appendix will be useful largely for readers who had easily been able to understand what they had just read in the main text but who also would like a little more detail of a scientific nature. The general information in this Appendix is arranged in the order in which the material in this report appears.

Chapter 2, page 4. Additional information on general chemical nature especially with regard to fish.

The protein content of fish and shellfish is similar to that of beef. Whether or not protein of a given food is of high quality depends to a considerable extent upon the content of essential amino acids which are histidine, lysine, tryptophane, and cystine. Comparing the average content of these amino acids of 28 species of fish and shellfish with that in beef round steak shows that fish are slightly lower, on the averages, in histidine and lysine, higher in tryptophane and slightly higher in cystine.

So far as mineral content of fish and shellfish are concerned the contents of magnesium, phosphorous, iron and copper are similar to that of meats such as beef. Their content of iodine, although quite low is actually higher in fish than in any other food. The content of calcium in canned fish in which the bone remains in the can (such as canned salmon) have a considerably higher content of calcium than most other foods. Oysters contain a high content of both iron and copper. Arsenic occurs in fish in higher amounts than in most other foods. The arsenic occurring in fish, however, is in an organic form whereby it is not absorbed to any great extent during digestion.

Chapter 2, page 8--Chemical Nature of Fatty Acids in Fish Oils. The chemical nature of most components of fish such as protein and mineral content varies to only a small extent from that of the components of other foods such as meat or poultry. The one exception is the fatty acids which differ to a considerable extent in fish as compared to other foods.

Fatty acids in foods consist of long chains of carbon atoms connected together in most cases by a single bond but in some cases by two bonds. At one end of the long chain of fatty acid is the carbon atom which has connected to it both an oxygen atom and an OH group, thus $\text{C} \begin{array}{l} \text{=O} \\ \text{---} \\ \text{OH} \end{array}$. The presence of this $\text{C} \begin{array}{l} \text{=O} \\ \text{---} \\ \text{OH} \end{array}$ group is what makes it a fatty acid. The simplest fatty acid has only one carbon atom and it has the formula $\text{H}-\text{C} \begin{array}{l} \text{=O} \\ \text{---} \\ \text{OH} \end{array}$. The other fatty acids

in foods sometimes have up to 22 total carbon atoms arranged in a chain $\begin{array}{ccccccc} & \text{OH} & \text{H} & \text{H} & \text{H} & \text{H} & \\ & | & | & | & | & | & \\ \text{O} & =\text{C} & -\text{C} & -\text{C} & -\text{C} & -\text{C} & \\ & | & | & | & | & | & \\ & \text{H} & \text{H} & \text{H} & \text{H} & \text{H} & \end{array}$, etc. Most of the carbon atoms in this chain

are connected together by a single bond. Occasionally in a fatty acid, two adjacent carbon atoms are connected by 2 bonds $\begin{array}{ccc} & \text{H} & \text{H} \\ & | & | \\ & \text{C} & =\text{C} \\ & | & | \\ & \text{H} & \text{H} \end{array}$. Fatty acids in foods other than fish have carbon chains of up to 18 carbon atoms, and with a few of 20 carbon atoms.

The fatty acids in fish range ordinarily in carbon chain length from C_{14} to C_{22} . There are generally a considerable quantity of C_{20} and C_{22} fatty acids in the oils of fish, considerably more than occur in other oils or fats. Furthermore in the fish oils with five or six double bonds per molecule, these five or six double bonded fatty acids have their double bonds start at the third carbon atom from the end of the molecule farthest from the $\begin{array}{c} \text{O} \\ // \\ \text{C} \\ \backslash \\ \text{H} \end{array}$ end. Such fatty acids are called omega-3 fatty acids or sometimes n-3 fatty acids. In almost all of the fatty acids in the oil of or fat from sources other than fish, the first fatty acid at the far or omega end of the molecule occurs at the carbon atom 6 carbons removed from the end. Such fatty acids are called omega-6 fatty acids. It is because of the relatively tremendous amounts of omega-3 fatty acids of carbon chain length C_{20} or C_{22} in fish as compared to virtually none in the fats or oils of non-fish origin that fish oils possess nutritional properties related to negative effects upon heart and other diseases where other fats or oils do not have any such properties.

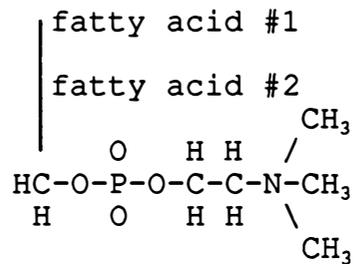
Chapter 4. Table 4-3. Identification of chemical compounds listed only by number.

<u>Number</u>	<u>Chemical identification</u>
1	(E)-but-2-enal
2	hexanal
3	(E)-pent-2-enal
4	heptan-3-one
5	heptanal
6	(E)-hex-2-enal
7	1,3,5-trimethylbenzene
8	1,2,4-trimethylbenzene
9	octanal
10	(E)-hept-2-enal
11	1,2,3-trimethylbenzene
12	nonan-2-one
13	nonanal
14	(E)-oct-2-enal
15	acetic acid
16	hepta-2,4-dienal
17	decanal
18	benzaldehyde
19	nonenal
20	propanoic acid
21	isobutanoic acid
22	butanoic acid
23	pentanoic acid
24	hexanoic acid
25	decatrional
26	phenol

Prefix E denotes a trans isomer and prefix 2 denotes a cis isomer.

Chapter 4, page 48, phospholipids. There are several different phospholipids. The largest amounts in fish are lecithins and phosphatidyl ethanolamines. The formula for phospholipids resembles to some extent that for triglycerides. There are usually 2 fatty acids followed by a third grouping which contains the phosphorous and sometimes a nitrogen grouping, thus:

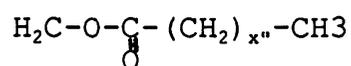
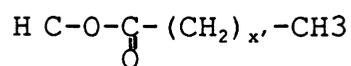
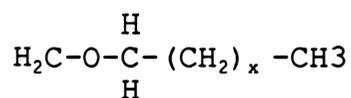
Lysolecithin



Chapter 5, page 81, line 1, Supercritical fluid carbon dioxide. Fatty acids of fish oils have been concentrated recently by means of use of supercritical fluid carbon dioxide which is carbon dioxide under high pressure above its critical temperature of 31°F. Before use of the supercritical fluid carbon dioxide method, however, ordinarily many of the fatty acids of low degree of unsaturation are eliminated usually making use of the urea complexing procedure. In the initial step the super critical fluid carbon dioxide process separates deleterious and undesirable impurities from the fish oil component polyunsaturated triglycerides. The purification resulting in concentration to high levels. For example, C20:5 or C22:6 fatty acids are produced by passing oil through a series of runs in which the pressure and temperature is varied. For instance on the first passing through the pressure might be 2,500 pounds per square inch and the temperature 74°C. In successive runs through the pressure and temperature could be altered and a total of 5 to 10 runs carried out. The samples collected in the last run would have the highest proportion of C20:5 or C22:6 fatty acid content.

More details are given in an article by Nilsson, et. al (in the Journal of the American Oil Chemists Society, volume 65, pages 109-117).

Chapter 6, page 82, glyceryl ethers. The formula for glyceryl ethers such as occur in fish, especially in the liver oils is as follows:



Where x , x' , and x'' represents varying long chain lengths.

Considerable information on glyceryl ethers giving much greater details has been published by Malins and Varanasi in the book "Ether Lipids - Chemistry and Biology edited by Snyder, pages 297-312.

1. The first part of the document is a letter from the author to the editor of the journal. The letter discusses the author's motivation for writing the paper and the importance of the research.

2. The second part of the document is the abstract of the paper. It provides a concise summary of the research objectives, methods, results, and conclusions.

3. The third part of the document is the introduction. It sets the context for the research, reviews the relevant literature, and states the research objectives.

4. The fourth part of the document is the methodology. It describes the research design, data collection methods, and the statistical analysis used in the study.

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7. The seventh part of the document is the conclusion. It summarizes the main findings of the study and provides recommendations for future research.

8. The eighth part of the document is the references. It lists the sources of information used in the study, including books, journal articles, and other relevant literature.

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