International Fishmeal & Oil Manufacturers Association

GUIDELINES FOR CHARACTERISING FOOD GRADE FISH OIL

by

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EXECUTIVE SUMMARY

To date there have been well over 8,000 publications dealing with fish oils and n-3 fatty acids. In many publications, the authors only report that fish oil was used. There is no indication of the species of fish, quality of the oil, whether anti-oxidants were added or the nature of the product.

After reviewing the scientific literature of expensive animal and clinical studies, it was apparent that publication of guidelines for fish oils used in these types of studies would be useful. Therefore IFOMA decided to compile a list of suggested guidelines as a way to harmonise the reporting of these characteristics.

Quality guidelines are listed for crude fish oils together with information on the various processing steps available to purify the fats and oils and to produce fractions.

It is hoped that these guidelines will provide future authors with the possibility of describing the fish oils or their fractions in more detail.

Edible fish oil

Guidelines for characterizing food-grade fish oil

Fish oils have been around for a very long time. It has been reported (1) that there are formal 800-year-old Nordic regulations regarding fishing and, in fact, the cod fishery in Scandinavia has been well-established for more than a millennium. Another report (2) goes back even farther and mentions sources in the Bible and in early Greek and Roman writings. The first known clinical investigation using cod liver oil was done by Dr. Samuel Kay at the Manchester Infirmary between 1752 and 1783 (3). He found that cod liver oil gave relief to people suffering from rheumatism. Other work indicated it was effective in curing night blindness. These were published in a British scientific journal in 1783.

It was not until the early 1900s that cod liver oil was shown to be effective in the treatment of rickets. The active ingredient in the oil was found to be vitamin D, and the use of this oil then moved from a curative agent to a preventative agent. Up through World War II, cod liver oil was a primary source of vitamins A and D. Then when scientists managed to produce these vitamins synthetically, the demand for cod liver oil dropped. People preferred vitamin tablets with no taste to that of liquid cod liver oil with its distinctive fishy flavor.

Another report (2) described studies in the United States, particularly the 19-year clinical trial conducted by Dr. Avery Nelson in Seattle. Dr. Nelson had heard about the effects of fish and cod liver oil consumption in Norway during World War II on the incidence of heart disease and decided to run similar tests in the United States. His studies began in 1953 with patients referred to him by other physicians. His patients were advised to eat fatty fish at least three times per week as a main course meal. His results showed 4.5 times more deaths among patients who did not adopt the diet of fish compared to those who did.

In 1979 a paper which described the role of n-3 fatty acids in the prevention of cardiovascular diseases was published (4). From that point forward, the interest in fish oils as a source of these fatty acids has been increasing. Fish oil triglycerides have been offered in liquid form, capsules, tablets, and powders as natural products, reflecting the composition of the fish species processed.

To date, there have been well over 8,000 publications dealing with fish oils and n-3 fatty acids. These are covered in four bibliographic searches conducted initially by the U.S. National Marine Fisheries Service Laboratory in Charleston, South Carolina (5), and then by the National Library of Medicine in Bethesda, Maryland (6–8). The recent searches deal primarily with the health and

This section was prepared by Anthony P. Bimbo as a consultant for the International Fishmeal and Oil Manufacturers Association, St. Albans, Hertfordshire, United Kingdom.

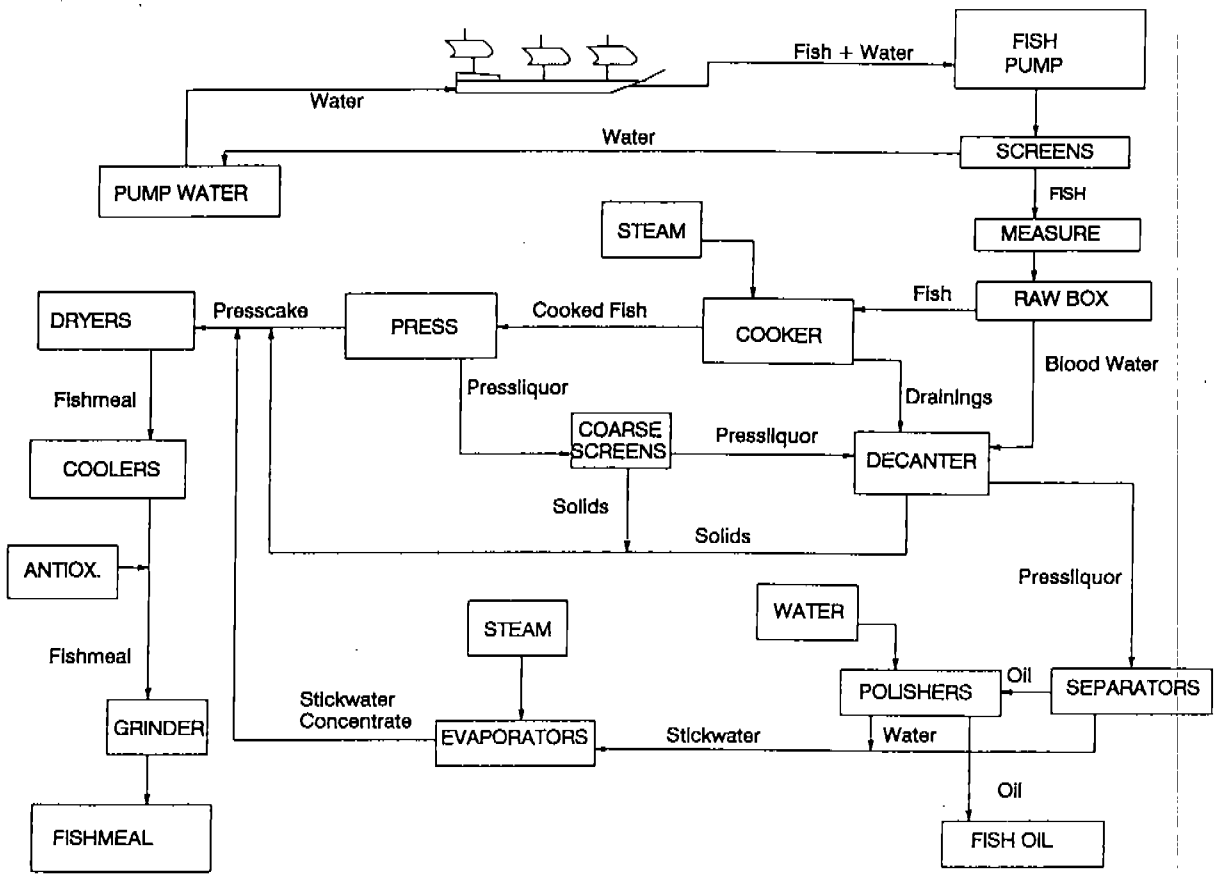

Figure 1. Standard fishmeal production process

medical aspects of fish oils and the n-3 fatty acids. In many publications, the author(s) only report that fish oil was used. There is no indication of the species of fish, quality of the oil, whether antioxidants were added, or the nature of the product. In cases where the product came through the Biomedical Test Materials Program (BTM) sponsored by the U.S. National Oceanic and Atmospheric Administration (NOAA)-National Marine Fisheries Service, U.S. National Institutes of Health (NIH), and the U.S. Alcohol, Drug Abuse and Mental Health Administration (ADAMH), the oils are well-characterized according to the quality-assurance screening outlined in U.S. Biomedical Test Materials Program Drug Master File (9).

After reviewing the literature concerned with animal and clinical studies, it was apparent that publication of guidelines for fish oils used in these types of studies would be useful. Because the characteristics of some of the oils used are not reported, the International Fishmeal and Oil Manufacturers Association (IFOMA) decided to compile a list of suggested guidelines as a way to harmonize the reporting of these characteristics. IFOMA represents over 20 countries engaged in the manufacture of fish oil and fishmeal. The IFOMA Scientific Committee has been very active in developing analytical methodology, specifications, and processing methods for fishmeal and oil for more than 26 years. Information was gathered from the Food and Agriculture Organization (FAO), the Codex Alimentarius Commission (CAC), the U.S. Biomedical Test Materials Program Drug Master File, the U.S. Pharmacopeia, the European Pharmacopeia, the GRAS (generally recognized as safe) affirmation submission for menhaden oil in the United States, and members of the scientific community. Input from all members of IFOMA's Scientific Committee and office staff is greatly appreciated. This document could not have been developed without their valuable comments and guidance.

Production of crude fish oil

In most cases, fish oil is produced by the wet-rendering process which involves cooking the fish with steam to rupture the fat cells and pressing the cooked fish to separate the liquid fraction from the solid fraction. The solid fraction is then dried to produce fishmeal.

The liquid fraction contains minor amounts of suspended solid protein material, water, and oil. This liquid is processed through a series of screens and centrifuges first to remove the solids, then to separate the oil from the water, and finally to wash, or polish, the oil fraction. The crude oil is then stored for further processing. The water fraction is evaporated and added back to the solids fraction before drying. The process has been described in

great detail, and the reader is referred to the references for more information (10–12). Figure 1 shows a typical flow diagram for the production of fishmeal and crude fish oil. It is a generic process, generally suitable for use anywhere in the world.

Production of refined fish oil for food or pharmaceutical purposes

Crude fish oil, like other edible fats and oils, contains minor amounts of nontriglyceride substances. While some of these, such as tocopherols which protect the oil from oxidation, are considered beneficial to the stability of the oil, other impurities are objectionable because they render the oil dark-colored, cause it to foam or smoke, or are precipitated when the oil is heated in subsequent processing operations. Other impurities reduce acceptability because of the flavors and odors they produce in the oil or because they reduce the stability and shelf life of the foods to which the oil is added. Table 1 (13,14) characterizes the typical chemical and physical properties of crude fish oil. Since there are numerous species of fish processed for their oil and its composition can vary by season, area caught, and age of the fish, these are general characteristics for information purposes only.

Nontriglyceride substances in fish oil can be classified according to their effects (13):

Table 1
Crude fish oil quality guidelines and physical characteristics

Quality Guidelines	
Moisture and impurities, %	usual basis 0.5 up to 1% maximum
Free fatty acids, % oleic	range 1–7% but usually 2–5%
Peroxide value, meq/kg	3–20
Anisidine number	4–60
Totox value	10–60
Iodine value	
Capelin	95–160
Herring	115–160
Menhaden	120–200
Sardine	160–200
Anchovy	180–200
Jack mackerel	160–190
Sand Eel	150–190
Color, Gardner scale	up to 14
Iron, ppm	0.5–7.0
Copper, ppm	less than 0.3
Phosphorus, ppm	5–100
Physical characteristics	
Specific heat, cal/g	0.50–0.55
Heat of fusion, cal/g	about 54
Caloric value, cal/g	about 9,500
Slip melting point, °C	10–15
Flash point,°C	
As triglycerides	about 360
As fatty acid	about 220
Boiling point, °C	greater than 250
Specific gravity	
At 15°C	about 0.92
At 30°C	about 0.91
At 45°C	about 0.90
Viscosity, cp	
At 20°C	60–90
At 50°C	20–30
At 90°C	about 10

Sources: References 13, 16.

Table 2
Processing steps used to purify fats and oils

Technique	Purpose
Oil storage	Remove insoluble impurities
Degumming	Remove phospholipids, sugars, resins, proteinaceous compounds, trace metals, and other materials
Alkali refining	Remove free fatty acids, pigments, phospholipids, oil-insoluble material, water-soluble material, and trace metals
Water washing	Remove soaps
Drying	Remove moisture
Bleaching	Remove pigments, oxidation products, trace metals, sulfur compounds, and trace soaps
Deodorization	Remove free fatty acids, mono- and diglycerides, aldehydes, ketones, chlorinated hydrocarbons, and pigment decomposition products
Winterization	Remove higher-melting triglycerides, enhance unsaturated triglycerides
Vacuum stripping or thin-film distillation	Remove chlorinated hydrocarbons, fatty acids, oxidation products, and cholesterol

Sources: References 13, 14, 17–19.

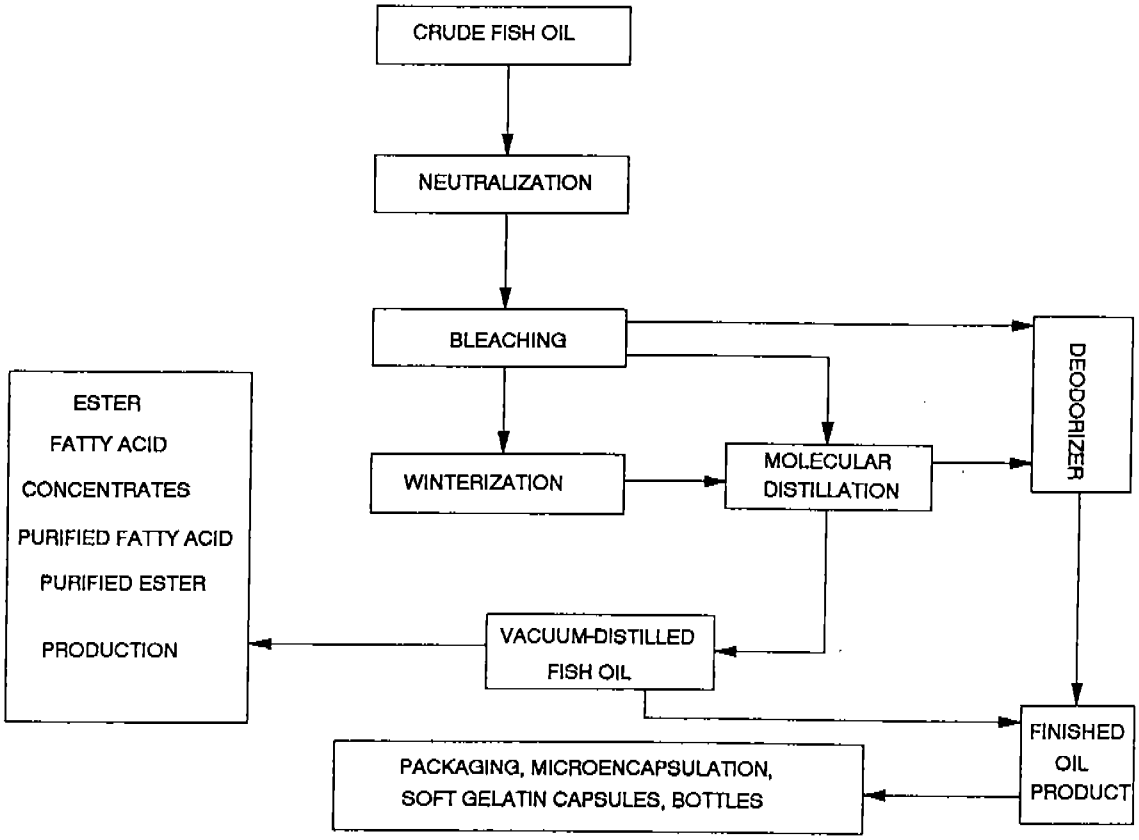

Figure 2. Production of pharmaceutical-grade fish oil

(a) Hydrolytic—moisture, insoluble impurities, proteinaceous compounds, free fatty acids, phospholipids, and soap;

(b) Oxidative—trace metals, oxidation products, pigments, and phospholipids;

(c) Catalyst poisons (substances which inhibit the hydrogenation reaction)—phospholipids, oxidation products, and compounds containing nitrogen, sulfur, and halogens;

(d) Miscellaneous—hydrocarbons, terpenes, resins, sterols, waxes, trace metals, and sugars, whose effect is less well-known but which can be classified as contaminants and also may have an effect on the final flavor of the oil.

One paper (15) that described the origins of off-flavors in fish oils divided them into three categories: (a) fishy off-flavor—caused by residues of nitrogen-containing substances, bio-

Table 3
Additional processing steps for purification of fractions

Step	Purpose
Interesterification	Rearrangement of triglycerides to a more random distribution
Hydrolysis or esterification	Splitting triglycerides, producing fatty acids or esters
Urea complexing	Low-temperature crystallization of the urea complex removes saturates and monounsaturates
Molecular distillation	Concentrating esters or fatty acids
Supercritical fluid extraction (SCF)	Purification of esters or fatty acids to produce 85%+ pure compounds and to remove cholesterol
Preparative high-performance liquid chromatography (HPLC)	Purification of esters or fatty acids to produce 95%+ pure compounds

Sources: References 12, 14–16, 19.

genic amines and their oxides, and protein degradation products; (b) putrified fish off-flavor—caused by residues of sulfur-containing substances such as mercaptans and sulfides; and (c) cod liver oil-type off-flavor—caused by oxidation products from the n-3 fatty acids.

To make fish oils more acceptable for food use, all of these substances must be removed from the oil.

Fish oil storage

The refining of an oil starts in the crude oil storage tanks because both quality and yields are affected by storage conditions (13). Particular areas of concern are free fatty acid (FFA) increases, oxidation, color setting, and contamination by insoluble impurities. Oxidation can be reduced by extending intake pipelines to the bottom of the storage tanks, and eliminating contact with iron, copper and copper alloys, all of which are prooxidants. The tank should be equipped with a sump at its lowest point so that moisture and other insoluble impurities can be drained off thus preventing FFA increases. Since some fish oils contain large quantities of high-melting triglycerides (stearines), the tanks should be equipped with side-mounted agitators and hot-water heating coils so that a homogeneous product is obtained. Mixing, however, may cause air entrainment which will lead to further oxidation of the oil. Air entrainment can also be caused by thermal convection which can be reduced by insulating the storage tanks.

Refining steps in the production of pharmaceutical-grade oils

Interest in the cholesterol content of foods and possible environmental contamination of fats and oils, including fish oils, has focused attention on processing steps that might be used to remove objectionable compounds from fish oils and other fats and oils.

The processing steps used to purify fats and oils and the impurities reduced or removed by them appear in Table 2 (16–20). Figure 2 combines these processing steps into a flow diagram outlining the production of several purified fish oil products.

Table 4
Typical[a] fatty acids in some commercially available marine oils, as % of the fatty acids

	Anchovy	Jack mackerel	Menhaden	Sardine/ pilchard	Capelin	Herring	Mackerel	Norway pout	Sand eel	Sprat	Tuna
C14:	9	8	9	8	7	7	8	5	7	—	3
C15:0	1	1	1	1	—	—	—	—	1	—	1
C16:0	17	18	19	18	10	17	14	12	13	17	22
C16:1	13	8	12	10	10	6	7	4	5	7	3
C17:0	1	1	1	1	—	—	—	—	—	—	1
C18:0	3	3	3	3	1	2	2	3	2	2	6
C18:1	10	16	11	13	14	14	13	10	7	16	21
C18:2	1	1	1	1	1	1	1	1	2	2	1
C18:3	1	1	1	1	1	2	1	1	1	2	1
C18:4	2	2	3	3	3	3	4	3	5	—	1
C20:1	1	2	1	4	17	15	12	13	12	10	1
C22:1	1	1	—	3	15	19	15	17	18	14	3
C20:5	22	13	14	16	8	6	7	9	11	6	6
C22:5	2	2	2	2	—	1	1	1	1	1	2
C22:6	9	15	8	9	6	6	8	14	11	9	22
Others[b]	7	8	14	7	7	1	7	7	4	14	6

[a] The fatty acid composition of fish oil can vary by season, area of the catch, food that the fish were consuming, sexual maturity of the fish, and age of the fish. These data reflect some general fatty acid profiles that should only be used to screen oils for possible use. In all cases, an updated fatty acid profile on the batch of oil to be used should be either supplied with the oil or performed by the researcher.

[b] Other fatty acids: C16:2, C16:3, C16:4, and C20:4.

Sources: Reference 13; Zaldivar (personal communication); Cornejo (personal communication).

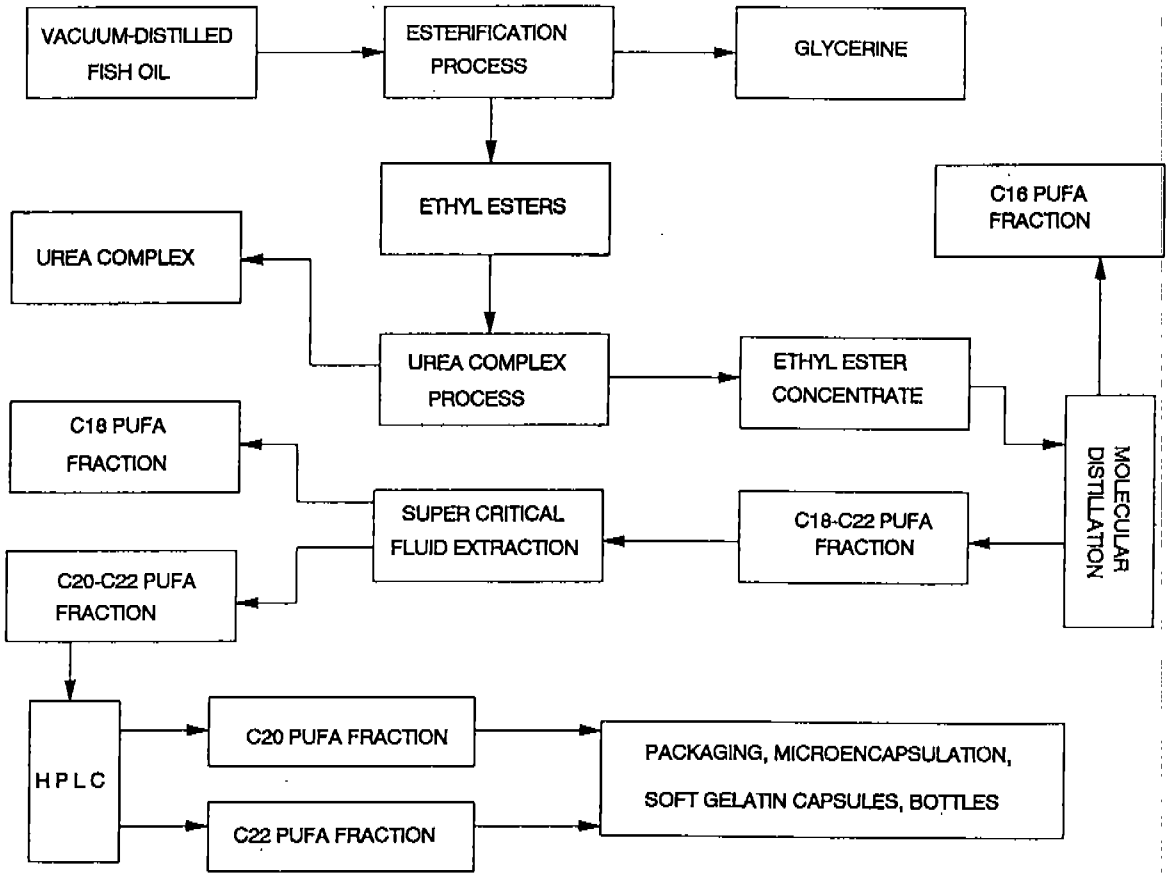

Figure 3. Production of pharmaceutical-grade fish oil fractions

Production of fish oil derivatives and fractions

A number of fish oil derivatives are on the market today. They have been offered as interesterified glycerides, resynthesized triglycerides, mixed methyl or ethyl esters, mixed fatty acids, as concentrates of n-3 fatty acids up to 85%, and finally as pure compounds of 95%+ purity in ester or fatty acid form. The production of these derivatives requires further processing steps, some of which are shown in Table 3 (17,18,20–22). Figure 3 combines the processing steps to manufacture these derivatives and concentrates in a flow diagram showing the production of several of these products on the international market today.

Characteristics of fish oils

Researchers should attempt to characterize the source of the oil as fully as possible before embarking upon expensive research studies. The species of fish from which the oil was manufactured is very important and should be supplied by the manufacturer because the fish oil composition will vary by species, age of the fish, and season of the catch. Table 4 (14) gives typical fatty-acid profiles for a number of fish oils of commerce. The researcher should use this table as a "snapshot" of what the variations are between species so that he or she doesn't obtain an oil high in monoene fatty acids when what is preferred is an oil high in polyunsaturates. On the other hand, because the composition of the oil within the species can vary by season, area caught, what the fish were eating, sexual maturity and age of the fish, the researcher should either analyze the oil personally or request an actual analysis of the lot from the supplier of the oil.

The additional information that is suggested for fish oils to be used in animal and clinical studies is listed in Table 5 along with the official methods of analysis that can be used to determine these variables (23–33). The BTM Program formally began in 1986. The processing facility is located at the U.S. National Marine Fisheries Service Laboratory in Charleston, South Carolina, and has been the main supplier of quality-assured and quality-controlled test materials to researchers involved in the study of the role of n-3 fatty acids in health and disease states (33). Initially set up for U.S. researchers, the labs' oils (prepared from menhaden) are now used globally. In addition to the variables listed in Table 5, the BTM Program also tests its finished products for the quality variables listed in Table 6 (27). Other variables are listed in Table 6 (23–30,32,34) that might be of interest in fully characterizing the oil. Table 7 lists the various quality variables, the potential problem areas or disadvantages because of

Table 5
Information required about oils used in animal and clinical trials

Name of the proprietary product being used	This should be supplied by the manufacturer.
Species of fish used	This should be supplied by the manufacturer.
Processing steps performed on the oil	As described in Tables 2 and 3
Type of packaging	Self-explanatory
Color	AOCS Cc 13a-43; 13b-45 or Td 1a-64
Iodine value	AOCS Cd 1d-92 (modified solvent), ISO 3961:1996.
Free fatty acids	AOCS Ca 5a-40, ISO 660:1996, IUPAC 2.201
Fatty acid composition	AOCS Ce 1b-89, AOAC 963.22, ISO 5508:1990
Peroxide value	AOCS Cd 8b-90 (modified solvent), IUPAC 2.501, ISO 3960
p-Anisidine number	AOCS Cd 18-90, IUPAC 2.504, ISO 6885:1988. Totox value. Calculated as 2 x peroxide value + anisidine number
Vitamins A and D	European Pharmacopeia Commission PA/PH/Exp. 3/T (94) 71, Com,3R 1996
Vitamin E	ISO/FDIS 9936
Oxidative stability	AOCS Cd 12b-92 OSI, ISO6886:1996(E) Rancimat
Type and level of antioxidants	Consult the manufacturer of the oil.
Cholesterol and cholesterol esters	AOAC 970.51
Iron	AOCS Ca 15-75 or CAC/RM 14-1969
Lead	AOCS Ca 18c-91, AOAC 994.02, or BTM 5.4
Arsenic	AOAC 986.15 and 957.22 or BTM 5.4
Pesticides	AOAC 983.21, EPA Methods Sec. 9a-c

Sources: References 23–28, 31–33.

them, and the current CAC limitations as outlined in the Codex General Standard for Fats and Oils Not Covered by an Individual Standard (26). This particular Codex Standard, while not specific for fish oil, is the only international standard available at this time and distinguishes between virgin and nonvirgin oils. Several monographs that deal with fish oils in general are in the process of development (in the United States and in Europe), but none has been published so far. When in doubt about particular quality characteristics of individual marine oils,

Table 6
Additional variables determined on oils processed for the National Marine Fisheries Service/National Institutes of Health Biomedical Test Materials (BTM) Program and other additional information

Lipid class profile	BTM 1.0
Moisture	AOCS Ca-2b-38, IUPAC 2.601, ISO 662:1980
Specific gravity	ISO 6883:1995, CAC/RM 9
Saponification value	ISO 3657:1988, IUPAC 2.202
Unsaponifiable matter	ISO 3596-1:1988, IUPAC 2.401, AOCS Ca-6b 53
Soap	BS 684 Sec. 2.5
Insoluble impurities	ISO 663:1995, IUPAC 2.604, AOCS Ca 3a-46
Sensory attributes	AOCS Cg 2-83, BTM 7.0
trans Fatty acids	AOCS Cd 14-95, Ce 1c-89, AOAC 965.34, ISO/CD 15304
Mercury	EPA Method 245.1, 206.3, 270.3
Selenium	BTM 5.4
Cadmium	BTM 5.4
Copper	AOAC 990.05, AOCS Ca 18-79, AOCS Ca 15-75, BTM 5.4, IUPAC 2.631, ISO 8294:1994
Nickel	AOCS Ca 15-75 or BTM 5.4
Coliform bacteria and *E. coli*	AOAC 966.24, FDA/BAM 7 Ed
Salmonella	AOAC 967.25 – 967.27, ISO 6579:1993
Enterobacteriaceae	ISO 7402:1985

Sources: References 23–27, 29, 30, 32, 34.

Table 7
Quality guidelines and potential problem areas or disadvantages of various parameters

Quality unit	Disadvantage or potential problem area	Codex specification (CAC/RS 19-1981 Rev. 1 1989)
Color	Dark-colored oils may be crude and contain contaminants normally removed by refining, or the color may indicate overheating during refining.	No standard
Iodine value (IV)	IV varies with the species of fish. In general, IV is a measure of the unsaturation in oils. High IV oils are generally more susceptible to oxidation.	No standard
Acid value[a]	High acid value crude oils may indicate poor-quality fish were processed or the oil deteriorated in storage.	0.6 mg KOH/g fat max refined oils; 4 mg KOH/g fat max virgin oils; 4 mg KOH/g fat max cold-pressed oils
Peroxide value (PV)	Peroxide value is the primary measure of rancidity (oxidation) in an oil or fat. It reflects recent oxidation.	10 meq/kg fat max virgin and cold-pressed oils; 5 meq/kg fat max other oils
p-Anisidine number (AN)	The anisidine number also measures products of oxidation; however, it reflects oxidation that has taken place in the past.	No standard
Totox value	A relationship between peroxide value and anisidine number that is used to measure the rancidity level of fats and oils. It is defined as (2 x PV) + AN. It reflects total oxidation to date.	No standard
Moisture	Considered an impurity. High levels of moisture in an oil can lead to deterioration in storage.	0.20% max
Soap	Soap can be formed when moisture is present in the crude oil and reacts with the free fatty acids and a catalyst (alkali ion), or it can result from incomplete removal of soap from washed refined oil.	0.005% max
Insoluble impurities	These substances, including traces of protein, dirt, rust and other materials, tend to precipitate out of the oil during storage. Depending on the substance, they can reduce the stability of the oil.	0.05% max
Unsaponifiable matter	These constitute sterols, hydrocarbons, glyceryl ethers, and fatty alcohols. There may also be traces of pigments, vitamins, and oxidized oil. Unsaponifiables vary with the species of fish.	No standard
Organochlorine, organophosphorus pesticides and other chlorinated hydrocarbons	There are numerous compounds in this group. Generally, the pesticide content of the oil reflects the environmental conditions in the area where the fish are caught. The level of these compounds in the oils must be within the regulatory limits of the locality involved.	No standard
Total cholesterol	Cholesterol is a major part of the unsaponifiable fraction of fish oils. Generally, it is not removed except by vacuum stripping of the oil.	No standard
Iron	Iron is considered a prooxidant in fish oil and is removed by degumming and refining.	1.5 mg/kg max refined oil; 5 mg/kg max virgin oil; 5 mg/kg max cold-pressed oil
Copper	Copper is considered a prooxidant in fish oil and is removed by degumming and refining.	0.1 mg/kg max refined oil; 0.4 mg/kg max virgin oil; 0.4 mg/kg max cold-pressed oil
Arsenic	A heavy metal, naturally occurring in sea water. It is removed by the refining process.	0.1 mg/kg max
Lead	A heavy metal removed by the refining process.	0.1 mg/kg max
Mercury	A heavy metal removed by the refining process.	No standard
Selenium	A heavy metal removed by the refining process.	No standard
Cadmium	A heavy metal removed by the refining process.	No standard
Hygiene	Microbiological contamination by enterobacteria, salmonella, coliforms, or *Escherichia coli* would be an indication of the sanitary conditions under which the oil was manufactured.	CAC/RCP 1-1969, Rev. 2 1985 limits
Oil-soluble vitamins	Normally part of the unsaponifiable fraction of the oil. High vitamin A and/or D would indicate that the oil is a liver oil rather than a body oil.	No standard

[a] Acid value is defined as two times the free fatty acid content of the oil.

CAC defines edible fats and oils as foodstuffs composed of glycerides of fatty acids. They are of vegetable, animal, or marine origin. They may contain small amounts of other lipids such as phosphatides, unsaponifiables, or free fatty acids naturally present in the fat or oil. CAC defines virgin fats and oils as edible fats and oils obtained without altering the oil, by mechanical procedures and the application of heat only. They may be purified by washing with water, settling, filtering, and centrifuging only. CAC defines cold-pressed fats and oils as edible vegetable fats and oils obtained without altering the oils, by mechanical procedures without the application of heat. They may have been purified by washing with water, settling, filtering, and centrifuging only (Reference 35).

Source: *FAO/WHO/CAC/RS 19-1981 Rev. 1, 1989.*

researchers are strongly urged to discuss their concerns with the supplier of the oil before embarking on a long and expensive clinical study.

Summary

Fish oils have been used in human nutrition for a very long time. Medical studies related to the possible health benefits of fish oils were reported as early as the mid-1700s. The composition of fish oils produced around the world is diverse. Researchers must begin to characterize the product that they are using in their research, and a list of the types of information needed is outlined in this paper. It is simply not acceptable to record that the very expensive, time-consuming study used fish oil. Much of the information that is needed will require close collaboration between the researcher and the supplier of the oil, so it is very important to know the original source of the oil.

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