Reliability of Fatty Acid Values Purporting to Represent Composition of Oil from Different Species of Fish[1]

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ABSTRACT

Data reveal a very large variation in fatty acid pattern of oil from the same species of fish even when each batch of oil represents many thousands of individual fish. The necessity for using samples involving a much larger number of fish for studies to determine fatty acid patterns for a given species is emphasized. Other sources of error are considered briefly.

INTRODUCTION

Various factors concerned with interpretation of the fatty acid content of fish of a particular species have been overlooked by recent investigators so that results reported in the literature may not be at all representative of the oil from the given species. In this paper these factors will be discussed, especially that of high variability of fatty acid content within a species, since this is usually the major cause of publication of ambiguous results.

This situation is quite analogous to what happened nearly a century ago with respect to publication of information on the fat or oil content of American food fish. During the 1880s the first investigation of the proximate composition of various American species of fish was carried out by Charles Woods at Wesleyan University in Connecticut (1). Although analytical methods employed for this work are compatible with present-day procedures, the sample size used for various species was totally inadequate to allow for the sometimes ten-fold variation in oil content among individual fish of the same species. As a result, data on the fat content of many species of fish were introduced into the literature which in many cases were grossly misleading. These results found their way into many nutritional tables which have been cited over many decades. Today a similar situation seems to be developing with respect to the fatty acid content of the oil of different fish species.

The idea that oils from the same species of fish have a constant fatty acid make-up probably stems from the use of fat constants, much used in past years to define the composition of various oils. During the late nineteenth century fat constants were worked out for most vegetable and marine oils. These constants (such as iodine number) were usually calculated to several places past the decimal point. Thus, it might be reported that some species of fish oil had an iodine number of 168.02. When anyone found a slightly different value, it was assumed the discrepancy was caused by inept analytical work by the person reporting a value deviating from the accepted figure. Although Lovern (2) reported in 1938 that there could be variations in iodine numbers and fatty acid make-up of some fish oils at different stages of spawning migration of the fish from which the oil was prepared, little attention was paid to this finding. Even after the perfection of gas chromatography permitted more rapid and accurate determination of the fatty acid composition of oils, many investigators have continued to report results of such analyses when the oil used in the analyses was prepared from only a few fish.

By far the largest amount of data discussed in this paper comes from results from analysis of commercial fish oils. Nevertheless, the paper is directed also toward investigations of fatty acid patterns in food fish for use for nutritional purposes. The only published papers where adequate sample size has been used are in the commercial fish oil field where often thousands or millions of fish have been used to comprise one sample. The results from such studies are used here to emphasize to those investigators who are planning studies on food fish the need to employ adequate sample size in their work.

Variability in Fatty Acid Pattern of Oil from Different Batches of Fish of the Same Species

In contrast to the generally accepted idea that oils from one species of fish vary only a little, if at all, in fatty acid pattern, there is actually a tremendous variation. Such variation occurs from fish to fish in the same catch, from lots of fish caught in the same general area at different times of the year, from lots of fish caught at the same time but in different geographical locations and even from fish caugnt in one geographical location at one date from one year to another. These variations in fatty acid content may be very large. In fact, no investigation has yet been published on the fatty acid pattern of oil from any species of fish which has involved samples sufficiently large to give an adequate idea as to average or ranges for fatty acid pattern for this species.

How, then, do we even know that such variability occurs? We know this because of the availability of sufficient analyses of commercial batches of both menhaden and herring oil to give positive evidence that very great variability occurs. Individual batches of commercial fish oil may each represent thousands, sometimes even millions, of fish, yet there are often very substantial differences in the fatty acid content from one such large batch of commercial oil to that of another.

Despite differences between commercial fish oil and oil in edible flesh, there is no reason to believe that the variability in fatty acid content of the oil present in the edible fish is not equally as great as is the case for oil extracted commercially. Accordingly, we are fortunate to have data on fatty acid variation in large batches of commercial oil to act as an indicator of what must also take place in variations of fatty acid content of the oil in edible flesh of the fish. The task of trying to carry out an investigation on variability of fatty acid content of oil in edible fish of a given species would probably so formidable, involving collecting and analyzing samples from thousands or millions of fish, as to discourage such an undertaking.

In the middle column of Table I are shown variations of fatty acid content of menhaden oil in which all samples were commercial batches of oil, each representative of

[1] Presented at the ISF-AOCS World Congress in New York, April 1980.

many thousands of fish (3,4). As will be noted, there are very wide ranges in values for the various fatty acids. In fact, of the fourteen different fatty acids, ten have the maximum values of the ranges more than double that of the minimum and, in some cases, the maximum value is up to nine times that of the minimum. The fact that seasonal variation in fatty acid content is of greater importance than is geographical variation is apparent from comparison of figures in the last column with those of the middle column. In the last column, all values were from menhaden oil composited throughout the season such that seasonal change was no longer a factor. In this case only two of the fourteen fatty acids had a range of as much as two times (as compared to the ten such samples in the middle column). In Table II are listed separately the fatty acid ranges of commercial herring oils obtained from herring caught in 1964 and 1965 in Alaska (5) and for herring caught near Halifax in 1966 (6). Again, as with menhaden, the fatty acid patterns extended over fairly large ranges for some of the fatty acids. The values for Alaskan and East Coast Canadian herring oil fatty acids are usually similar. Principal differences seem to be in the 20:5 fatty acids which are higher (11.4 to 15.2%) in the Alaskan-produced herring oil than in that produced from fish caught off Nova Scotia (range: 3.9 to 8.8%) and in the 22:1 fatty acids. For the latter fatty acid, the Nova Scotia-produced oil had a content ranging from 14.8 to 30.6% as compared to only 6.9 to 15.2% in the oil produced in Alaska.

TABLE I

Variation in Principal Fatty Acids of Menhaden Oil from One Large Batch to Another

Fatty acid	Overall variation[a]	Variation without seasonal variation as a factor[b]
14:0	6.7 - 16.3	8.7 - 10.6
16:0	19.6 - 24.0	20.1 - 21.2
16:1	11.2 - 17.9	11.2 - 13.9
18:0	2.4 - 3.4	3.0 - 3.4
18:1	10.7 - 23.4	10.8 - 12.1
18:2	0.9 - 1.7	1.0 - 1.7
18:3	0.4 - 3.7	1.3 - 3.7
18:4	0.8 - 3.6	1.9 - 3.6
20:1	1.1 - 2.7	1.1 - 2.0
20:4	0.6 - 2.3	1.7 - 2.3
20:5	10.2 - 14.1	11.8 - 14.1
22:1	0.2 - 1.0	0.2 - 0.7
22:5	1.1 - 2.5	1.9 - 2.5
22:6	3.3 - 10.6	5.6 - 10.6

[a]From data of Gruger (3) and of Bimbo (4).

[b]From data of Bimbo (4) only. In these analyses all samples were ones composited from oils collected throughout the season so that seasonal variation was no longer a factor.

TABLE II

Variations in Principal Herring Oil Fatty Acid Content in Large Batches of Oil from Fish Caught near Alaska and Nova Scotia

	Percentage ranges of total fatty acids	
Fatty acid	Alaska[a] fish caught in 1964 & 1965	Nova Scotia[b] fish caught in 1966
14:0	5.6 - 7.7	4.6 - 8.4
16:0	11.8 - 18.6	10.1 - 15.0
16:1	6.2 - 8.0	7.0 - 12.0
18:0	1.1 - 2.0	0.7 - 2.1
18:1	11.7 - 25.2	9.3 - 21.4
18:2	0.1 - 0.6	0.6 - 2.9
18:3	none	0.3 - 1.1
18:4	1.1 - 2.8	1.1 - 2.5
20:1	7.3 - 19.1	11.0 - 19.9
20:4	0.3 - 0.8	0.4 - 1.2
20:5	11.4 - 15.2	3.9 - 8.8
22:1	6.9 - 15.2	14.8 - 30.6
22:5	0.3 - 1.0	0.5 - 1.3
22:6	4.8 - 7.8	2.0 - 6.2
24:1	0.6 - 1.3	0.2 - 0.9
24:5	–	0.2 - 0.5

[a]From data of Robisch and Gruger (5).

[b]From data of Ackman and Eaton (6).

Most of the data in Table I were obtained in an exhaustive unpublished study of the fatty acid range of menhaden oil in large batches of this commercial oil collected at different plants on the Atlantic and Gulf of Mexico coast over a three-year period by Zepata Haynie Co. with analyses made at the laboratory of Robert Ackman. Through the courtesy of Anthony Bimbo, technical director of Zepata Haynie Co., I have been authorized to show these data.

The relatively high variability in the fatty acid content of large commercial batches of both menhaden and herring certainly emphasizes that when we rely on analyses of a few dozen specimens of other species often picked up in a single geographical area and not even always sampled over different seasons of the year, we will obtain results for fatty acid values only partially, if at all, typical for the particular species of fish. If, for example, even where over 1,000 menhaden had been composited and analyzed from a school of fish off Port Monmouth, NJ in May 1960 (from where a sample of commercial menhaden oil was obtained and analyzed by Gruger [3] at that time) a completely false impression of the content of the 18:1 fatty acid of 23.4% would have been obtained for menhaden as a whole since the average for menhaden oil of this fatty acid is not more than 12% (3,4). Yet the analyst reporting such a value might argue that his results were obtained on a sample size many times greater than is normally employed. If only a few fish, e.g., ten, had been used, the discrepancy could have been far greater. In the case of herring, if thousands of fish of this species had been composited and analyzed for 22:1 fatty acid, a quite erroneous value could have been obtained. For example, if these fish had been collected in Alaska on September 14, 1964, from where commercial herring oil was prepared and analyzed (5) a value of only 6.9% would have been reported. Yet the most typical fatty acid characteristic of herring oil is its very high content of long chain monoenes. The usual content of 22:1 fatty acids in herring ordinarily ranges up to 30% and values under 10% are uncommon. Again, had only a few herring been analyzed, as is commonly done, an even greater discrepancy could have occurred.

The reasons for the wide variation in fatty acid content of oils made from the same species of fish are undoubtedly related to the great importance of the fatty acid content of the feed available to fish from season to season or from year to year upon the fatty acid pattern in the flesh of these fish. By metabolic processes the fish can to some extent alter the particular fatty acids laid down in its flesh to something different from that of the fatty acid pattern of the feed. Nevertheless, when feed with abnormal fatty acid content is all that is available to the fish, the effect of the feed fatty acid pattern often overrides the ability of the fish to metabolize the fatty acids to a pattern usually considered characteristic for the species.

Present Status of Knowledge of Fatty Acid Content of Fish

Our current knowledge of the fatty acid content of com-

mercial menhaden oil is excellent. Aside from insufficient information on possible deviations in values over a period of years (adequate data are limited to values for oil produced in 1960, 1961, 1976, 1977 and 1978) there would seem to be nothing further that needs clarification. With respect to the fatty acid composition of herring oil, fairly good coverage has been made on the fatty acid composition of commercial oil produced from the North American Pacific coast and the Canadian Atlantic coast, but the knowledge is considerably less than exists for menhaden. For menhaden, the existing fishery is limited to the Atlantic coast, including the Gulf of Mexico waters adjacent to the United States. Herring occurs in areas other than adjacent to the United States and Canada, and, for many of the other locations, information on fatty acid content is limited. Furthermore, even for herring caught in North America, samples from only a few years' production have been covered. In the experience of the end users of Pacific coast commercial herring, there is considerable variation from year to year, so that considerably more work is needed before we can feel that our knowledge for even North American commercial herring oil fatty content is adequate.

When it comes to the fatty acid content of oil in the flesh of fish, there are no species for which adequate data are available. Even for herring and menhaden fatty acid patterns, reasons based on differences (between fatty acids in commercial oil and in the fish itself) concerned with both the extraction methods and the kinds of raw material used prevent extrapolation from commercial oil to oil in the flesh of the fish. As an example for commercial menhaden oil the 22:6 fatty acid content ranged from 3.3 to 8.7%, yet in a fairly well sampled study over the course of one year (1972) the content of the 22:6 fatty acid in the flesh of the menhaden taken in one locality ranged from 11.0 to 11.8% (7).

For other species of fish there are many scattered reports on a wide variety of species, yet in none of such cases has sufficient sampling been carried out to give more than an uncertain idea as to fatty acid patterns. If, over a long period of time, we could combine results of small individual investigations, knowledge might be built up so as to eventually obtain meaningful ranges of fatty acid patterns in various fish species. Even here, however, a difficulty arises. Many, if not most, investigators fail to give adequate information on the number of fish samples employed and whether a sample came from one fish, or, if more than one, how many. Complete information on geographical locations or seasons of the year, and where or when samples were procured, is often not supplied.

Information for some species sometimes is obtained, indicating some extremely different fatty acid pattern than that found in other species of fish. For example, in mullet there are often, but not always, a much greater proportion (up to ten times more) of odd chain length, e.g., C 17 fatty acids than occur in other species. Also in mullet there are much higher quantities of 16:1 fatty acids than in any other fish (8). Where for most species the 16:1 content usually is well below 10%, in mullet values between 20 and 30% are common. When such peculiarities occur, they may form a method for identifying the species even though the exact range of such values may be quite variable.

Other Sources of Error

Up to this point, major attention has been focused upon errors in reporting fatty acid content of fish oils caused by use of inadequate sample size. In this short section some other factors will be briefly discussed.

For the most part, most of the knowledge of the fatty acid pattern in menhaden oil comes from studies made on the commercially extracted oil. In this process the oil is removed from the fish by cooking, followed by separation of the oil in a mechanical press. In only one investigation involving solvent extraction has the fatty acid pattern been carried out in a thorough enough manner with use of any sort of sampling to give meaningful results. Dubrow, Hale and Bimbo (7) studied the fatty acid pattern of menhaden oil recovered during preparation of fish protein concentrate by an isopropanol extraction procedure. They employed numerous batches of menhaden collected at different seasons of the year at two sites. With one exception, the fatty acid pattern in these isopropanol prepared oils were within the ranges of patterns for commercial menhaden oils. The exception was for the most polyunsaturated fatty acid the 22:6 (docosohexaenoic acid). For this fatty acid the amounts present in the isopropanol extracted oils varied from 11.0 to 18.8. For the commercially produced menhaden oils, values for large batches varied for the 22:6 fatty acid from 3.3 to 10.6. These quite definite differences are probably due to a preferential resistance to extraction of the 22:6 fatty acid which occurs to a large extent as phospholipid (9) by the milder cooking method. It is generally found that the portion of the fish oil which is not recovered by cooking turns up in the fish meal, and this oil is usually more polyunsaturated than the main bulk of the oil (10). The 22:6 fatty acids as phospholipids may be bound loosely to protein of the fish and are thus not completely extracted during the conventional steam cooking process employed for manufacture of the commercial fish oil.

Another consideration which has also been previously mentioned is the fact that the fatty acid pattern of fish oils made from the whole fish (as is ordinarily used in making the commercial oil) will be different from that of the oil in the flesh of the fish. Thus, fatty acid patterns for commercial herring oil are not the same as for the oil residing in the flesh as it might be used for human consumption. We must thus be careful not to employ information on fatty acid composition of commercially produced fish oil for nutritional use if the fish is for human consumption.

Another quite different source of error involves misidentification of fatty acids in gas chromatographic analyses. When gas chromatography was first introduced, methodology was in a developmental stage. It was possible to get incomplete separation of peaks so as to blanket under one peak more than one fatty acid. Furthermore, there was not the present-day availability of standard fatty acids to check on retention times. It is likely that in some of the early investigations of fatty acids in fish oils that errors of these types have been involved and that thus some of the earlier papers have included such errors. Today with excellent availability of reference standards there is no excuse for introducing into the literature such errors. Where such errors still are made, they probably are caught by editorial referees before the papers are published. Nevertheless, this author has seen some recent unpublished tables of fatty acid patterns in use by commercial fish oil companies where it is quite obvious that such errors are present.

Recommendations for Future Investigations

An enormous amount of work would be necessary to pinpoint the fatty acid pattern variations for any particular species of fish with respect to area of catch, season of year, from one year to another and perhaps other factors. It is most unlikely that a single investigation would suffice. Rather it is more than likely that over a period of years, after many different investigations of fish of the same species had been studied, it would be possible to piece

together ranges of fatty acid patterns with respect to the various factors. Such integration of results, however, would be possible only if the various investigators most carefully described their sampling procedures so that comparisons would be possible from one study to another. Accordingly, the following recommendations are given to help make possible adequate comparisons of data from different investigators:

1. As far as possible, use composited fish samples for fatty acid analysis. Samples might be composited from fish caught at one time, from fish caught at different seasons of the year, from different years, and from fish caught in different geographical locations. Analyses should be made, not only on composited samples, but to include enough individual samples to show the extent of variability from fish to fish.

2. In order to carry out a really adequate program, in addition to using an adequate number of composited samples, an attempt should be made to determine just how many individual fish are required to provide an adequate composited sample. I have never come across even one published investigation where sampling has been carried out in a manner to permit statistical treatment of this problem. What is needed, of course, is to have analyses carried out, not only on composited samples, but also upon sufficient numbers of individual fish to be able to determine variability.

3. Regardless of how well it is possible to provide adequate sampling, a clear description should be provided as to when and from where the fish were obtained and how many fish comprised a lot or sample. Other pertinent information should be noted. For example, if recent major storms in an area where samples were obtained had caused possible depletion of the fishery or feed resource, notation to this effect should be included.

4. The method by which the oil upon which the fatty acids were analyzed was extracted from the fish should always be described.

5. Emphasis should be made in the text, even in the title of the paper, of the sample limitations. For example, a good title might read "Fatty Acid Patterns of Oil from Coho Salmon taken during August of 1978 and 1979 and Caught near Neah Bay, Washington." Use of such paper titles and with appropriate emphasis in the text will discourage readers from erroneously assuming that results of such papers can be used to represent the fatty acid pattern of all fish from the particular species involved.

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