Tuna Fatty Acids: I. Initial Studies on the Composition of the Light and Dark Meats of Bluefin Tuna *(Thunnus thynnus)* - Structural Isomers of the Monoenoic Fatty Acids

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Abstract

Bluefin tuna *(Thunnus thynnus)* is consumed in substantial amounts by humans. However, little has been reported on the fatty acid composition of bluefin body oil and on the isomeric structures of the unsaturated fatty acids. Because of the probable nutritional significance of unsaturated fatty acids, the present work was undertaken as an introductory study of the composition and structure of the fatty acids of tuna. The fatty acid composition of the light and dark meats from three bluefin tuna was determined by gas-liquid chromatography. A wide variety of saturated and polyunsaturated fatty acids were present in the oil from the meat of these specimens. The monoenoic fatty acid fraction, which comprises 34% of the total fatty acids, was isolated and the isomers determined. Isomers found were *cis*-9-hexadecenoic acid, *cis*-9-octadecenoic acid, *cis*-11-octadecenoic acid, *cis*-9-eicosenoic acid, *cis*-11-eicosenoic acid, *cis*-11-docosenoic acid, and *cis*-13-docosenoic acid.

Introduction

BLUEFIN TUNA is one of the species used in the domestic tuna-canning industry. The light meat from this species is consumed by humans, and the dark meat and by-products are used in the preparation of pet food and fish meal (1).

Bluefin tuna, as well as the other species of tuna, is rich in many nutritional factors, including protein and polyunsaturated fatty acids. The role of unsaturated fatty acids in human nutrition is being viewed with increasing interest, and the nutritional and therapeutic effects of these fatty acids on human physiology have been documented (2). Therefore, it is important to know the composition and structures of tuna fatty acids.

Igarashi and co-workers (3) have reported the approximate composition of the fatty acids of *Thunnus orientalis*. However, nothing has been reported on the structural isomers of the unsaturated fatty acids of tuna. Because of the probable nutritional significance of these unsaturated fatty acids, the present work was undertaken as an introduction to the study of composition and structures of the fatty acids in tuna oil.

This paper reports: (a) the fatty acid composition of the light and dark meats from three bluefin tuna and (b) the structural isomers of the C_{16}, C_{18}, C_{20}, and C_{22} monoenoic fatty acids. These monoenoic fatty acids comprise a large group of the unsaturated fatty acid fraction. Because the light meat is the only portion consumed by humans, the light and dark meats were analyzed separately; the total analysis of the combined meats was not performed.

Since the results reported here are for only three fish, they are not necessarily representative of the species. However, although comprehensive studies will be required to establish the fatty acid composition of bluefin tuna oil, the results reported show what fatty acids are present and indicate their relative amounts and distribution in the flesh.

Experimental

The triglycerides of the light and dark meat were obtained by solvent extraction and were converted to the methyl esters for analysis by gas-liquid chromatography (GLC). The monoenoic fatty acid fraction was concentrated, fractionated, and cleaved at the double bonds by acid-permanganate. The fragments were analyzed by analytical GLC, and the structures of the monoenes were determined.

Isolation and Preparation of Light and Dark Meat Samples. Three bluefin, weighing approximately 14 lb each, were caught off the southern California Coast and frozen immediately. They were then kept in this condition until processed. The frozen fish were sawed in half (sagittal section), and the dark meats were stripped from the right-hand sections and combined. The remaining light meat from the right-hand sections were also combined, and the oils were solvent extracted from the light and dark meats, using the method of Bligh and Dyer (4). Both samples of oil were then saponified by conventional methods, and the unsaponifiable substances were removed by extraction of the aqueous soaps with diethyl ether prior to acidification. The fatty acids of the light and dark meats were then converted to their methyl esters with diazomethane, using the procedure of Schlenk and Gellerman (5), and were analyzed by GLC.

Isolation of the Monoenoic Fatty Acids. The combined light and dark meats of the left-hand sections were extracted with mixed solvents, and the fatty acids were obtained as described above. By use of the procedure of Malins and Houle (6), the monoene fatty acid fraction was concentrated via urea complex fractionation and low temperature acetone precipitation. The resulting fatty acids were converted to their methyl esters, using methanol with sulfuric acid as the catalyst, and then fractionally distilled at reduced pressure with a spinning band column. These fractions of methyl esters were chiefly one chain length as shown by GLC.

The monoenoic methyl esters obtained from the fractional distillation were not of sufficient purity for oxidation studies. After fractionation by preparative GLC, these esters were obtained in 99–100% purity using 0.25-ml portions. Preparative GLC was carried out with a Beckman GC-2 Gas Chromatograph equipped with a 5′ × ⅜″ OD column packed with

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TABLE I
GLC Analysis of Light and Dark Meat of Three Samples of Bluefin Tuna

Fatty acid chain length: double bonds	Content of indicated fatty acid (area %)	
	Light meat	Dark meat
14:0	4.5	4.0
15:0	0.6	0.7
16:0	22.1	25.7
16:1	2.8	3.5
16:2	0.5	0.4
17:0	0.8	0.7
18:0	6.1	12.5
18:1	21.7	14.5
18:2	0.8	0.6
18:4	0.9	1.0
Unknown	tr	0.5
20:1	6.3	3.2
20:4	1.0	1.4
20:5	6.4	5.5
22:1	5.4	2.1
22:3	1.2	tr
22:4	1.1	1.0
22:5	1.4	1.8
22:6	17.1	21.0

40% precipitated DEGS (7) on Chromosorb (30/60 mesh). Helium carrier gas at a flow rate of 1200 ml/min was used, and the column was maintained at 224C.

Analytical Gas-Liquid Chromatography. Analytical GLC [2] of the methyl esters was conducted with a 10′ x ¼″ OD stainless steel column containing 21 g of 2.7% DEGS on acid washed and siliconized (8) Chromosorb W (60/80 mesh). For the analysis of the light and dark meat methyl esters, an argon flow rate of 30 ml/min was maintained, and the column was held at 174C.

Identification of the peaks appearing on the chromatogram was made: (a) by comparing logarithmic plots of relative retention time (methyl stearate = 1) against chain length or degree of unsaturation with plots prepared from known standards, (b) by comparing hydrogenated with non-hydrogenated samples, and (c) by comparing retention times of isolated esters with reference compounds. Results of the GLC analyses of the light and dark meats are presented in Table I.

Oxidative Cleavage. Oxidation of the monoenes was carried out by adaptation of the acetic acid-permanganate procedure of Haverkamp-Begemann et al. (9) as reported by Malins and Houle (6). The oxidation fragments were isolated by the method of Fulco and Mead (10), converted to the methyl esters with diazomethane in ether (5), and then analyzed by GLC.

Four major peaks were observed for each of the oxidized monoene fractions of C_{18}, C_{20}, and C_{22} while only two peaks were observed in the case of the C_{16} monoene fraction. In the latter case, it was shown by comparison with reference standards that one peak corresponded to a C_9 dicarboxylic acid and the other to a C_7 monocarboxylic acid. Thus it was evident that the C_{16} monoene fraction consisted of a single isomer. It was similarly shown that two isomers existed for each of the remaining monoene fractions. These data are presented in Table II.

TABLE II
Structure of Monoenoic Fatty Acids of Bluefin Tuna Analysis of Oxidative Cleavage Products

Chain length	Cleavage product		Fatty acid	Approx relative amt of isomer	Percentage in original oil
	Monocarboxylic acid	Dicarboxylic acid			
C_{18}	Heptanoic	Adipic	*cis*-9-hexadecenoic	....	3
C_{18}	Pelargonic	Adipic	*cis*-9-octadecenoic	5	17.4
	Heptanoic	Undecanedioic	*cis*-11-octadecenoic	1	3.6
C_{20}	Undecanoic	Adipic	*cis*-9-eicosenoic	1	3.4
	Pelargonic	Undecanedioic	*cis*-11-eicosenoic	1	2.6
C_{22}	Undecanoic	Undecanedioic	*cis*-11-docosenoic	14	4.1
	Pelargonic	Brassylic	*cis*-13-docosenoic	1	0.3

[2] RESCO (Research Specialties Co., Richmond, Calif.) Model 600 series with an Argon Detector operated at 800 VDC.

The calculations of the amount of each isomer present were based on the GLC for the monocarboxylic oxidation fragments. The structural isomers and the relative amounts of the monoenoic fatty acids present in the bluefin samples are presented in Table II. Infrared analysis of the starting materials and of the isolated monoenes showed that double bonds were in the *cis* configuration.

Results and Discussion

The fatty acid composition of the light and dark meats is shown in Table I. Although the role of polyunsaturation in the human diet is not completely understood, unsaturated fatty acids other than essential fatty acids have been shown to play a part in physiological processes (2).

Tuna oil contains the monoenoic fatty acid, oleic acid (17%), and a wide variety of other unsaturated fatty acids. Approximately two-thirds of the fatty acids in the light meat and three-fourths of the fatty acids in the dark meat are composed of four fatty acids—16:0, 18:0, 18:1, and 22:6 (Table I). About one-fourth of the fatty acids are 20:5 and 22:6.

Although the acid-permanganate oxidation procedure gives rise to slight over-oxidation, the formation of side products was low and did not complicate the oxidation picture. The monoenoic fatty acids of the combined light and dark meats (Table II) comprise 34% of the total fatty acids and 59% of the unsaturated fatty acids. Although the bluefin tuna oil was not exceptionally rich in C_{20} and C_{22} monoenes, it did contain monoenes whose structures are of interest. The identification of *cis*-11-octadecenoic acid in bluefin tuna (Table II) indicates a more widespread occurrence of this acid in marine animals than has previously been reported (11). Adachi (12), who worked with cuttlefish oil, and Stoffel and Ahrens (13), who worked with wet-processed menhaden oil, did not find the 11-octadecenoic acid. Malins and Houle (6), however, found that this isomer and the others listed in Table II were present in the liver oil of dogfish.

Large losses often occurred when attempts were made to trap monoenoic methyl ester effluents from preparative GLC columns. These losses, which were

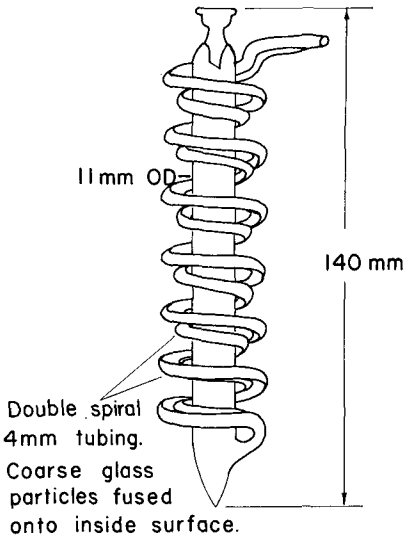

FIG. 1. Improved trap for collecting methyl esters of high molecular weight from preparative GLC columns.

due to a fog or mist issuing from commercial traps, may be caused by the high flow rate of carrier gas blowing out small particles of solidified esters. Commercial GC-2 traps were modified, and several other designs were tried, but they did not prove entirely successful. The author designed and constructed a new trap that overcame previous difficulties. This trap, shown in Figure 1, has two exit tubes with roughened internal surfaces. This system permitted up to 87% of the injected sample to be recovered as a pure material.

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Tuna Fatty Acids: II. Investigations of the Composition of Raw and Processed Domestic Tuna

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Abstract

Oils from raw, precooked, and canned tuna were analyzed by gas-liquid chromatography. The analyses showed the presence in tuna oil of 20 fatty acids having chain lengths ranging from 14 to 22 carbon atoms and containing 0 to 6 double bonds. The polyunsaturated fatty acids underwent little change during processing from the raw to the canned product.

Introduction

YELLOWFIN *(Neothunnus macropterus)*, skipjack *(Katsuwonus pelamis)*, albacore *(Thunnus germo)*, and bluefin *(Thunnus thynnus)* are the principle species used in the domestic tuna industry. The production of canned tuna has increased at such a rate that tuna is now the most important canned seafood of the U. S. (1). Most tuna is packed in vegetable oil, but some is packed in an oil-free water broth.

Tuna by-products—which include dark meat, scraps, and precook oils—are also used extensively. The dark meat is used in the preparation of pet foods; the scraps and other refuse, in the production of meals and fish solubles for poultry feed; and the oil, in the manufacture of such products as paint.

Tuna light meat is the portion of the fish that is processed as food for humans. Although this high-protein food is canned in large quantity, relatively little is known about the fatty acid composition of the flesh oil. Even less is known about the structures of the isomers of the constituent unsaturated fatty acids. [In a recent investigation of bluefin fatty acids, the author isolated and determined the structural isomers of the monoenoic fatty acids from this fish. (In press.)] Igarashi and co-workers (2–4) have reported the composition of many of the phospholipids of "Kuromaguro" [oriental or black tuna *(Thunnus orientalis)*], and Katada (5,6) has recently published analyses of the sphingolipids of black tuna. (The true identity of black tuna is still undecided. Some ichthyologists believe it to be similar to *Thunnus thynnus:* others believe it to be a separate species.)

[1] Fish and Wildlife Service, U. S. Department of the Interior.

Knowledge of the fatty acid composition is desirable for an important fish such as tuna, not only because of the value of the fish as a foodstuff, but also because of possible new industrial applications of the oil. In light of recent dietary and medical emphasis on the role of fatty acids in human physiology (7,8), it is important to know the fatty acid composition and the structures of the isomers of the fatty acids of this group of fish. Since the treatment that the oil in the flesh undergoes during processing may influence flavor and quality of the oil, it is also desirable to determine whether processing destroys unsaturation or otherwise alters the oil.

The objectives of the present study therefore were: 1.) To determine what fatty acids are present and their relative amounts in the oils from the light and dark flesh of albacore, bluefin, yellowfin, and skipjack tuna. 2.) To study the effects of processing on the oils in regard to (a) destruction of unsaturation and (b) differential extraction of fatty acids. 3.) To determine to what extent tuna oils contribute to the fatty acids in (a) the drained oil and (b) the residual oil in a vegetable oil pack of tuna.

Experimental Work

The study was made with samples of two kinds: laboratory and commercial (Fig. 1).

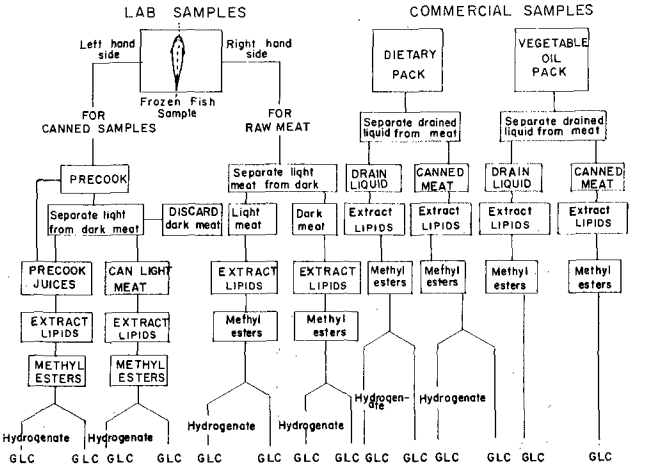

FIG. 1. Composition studies of raw and processed domestic tuna.