POSITIONAL DISTRIBUTION OF FATTY ACIDS ON TRIACYLGLYCEROLS OF MENHADEN (*BREVOORTIA TYRANNIS*) AND SALMON (*SALMO SALAR*) OILS

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ABSTRACT

Pure triacylglycerols of both menhaden and salmon oils were separated on Florisil columns. The triacylglycerols were subjected to positional distribution analysis using a modified procedure involving reductive cleavage with a Grignard reagent. Cleaved fractions were separated on thin layer chromatography (TLC) plates using hexane-diethyl ether-formic acid (80:20:1, v/v/v) as the mobile phase. Separated fractions were derivatized and analyzed by gas chromatographic method to determine the component fatty acids. Results showed that salmon oil contained a greater percentage of PUFA at the Sn-2 position of the triacylglycerol as compared to menhaden oil. The PUFA of menhaden oil were randomly distributed in the triacylglycerol. In salmon oil, a greater percentage of 18:3 (n-3), 20:4 (n-3), 20:5 (n-3), 22:5 (n-3), and 22:6 (n-3) was in the Sn-2 position as compared to menhaden oil. However, a greater percentage of 16:4 (n-1), 20:4 (n-6) and 18:4 (n-3) was located in the Sn-2 position of menhaden oil compared to salmon oil.

INTRODUCTION

The oxidative stability and physical properties of oils are affected by their fatty acid composition, triacylglycerol composition, and position of the component fatty acids on the triacylglycerols (Neff *et al.* 1992; Neff and Mounts 1993; Warner *et al.* 1989). Numerous studies directed toward altering the fatty acid composition of oils in order to improve their stability have proven successful (Kimoto *et al.* 1994; Liu and White 1992; Mounts *et al.* 1988; Neff *et al.* 1994; O'Keefe *et al.* 1993; Prevôt 1990). These studies indicated that polyunsaturated fatty acids (PUFA)

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located at the β-position of triacylglycerols are more stable than those at α-positions (Lau *et al.* 1982; Raghuveer and Hammond 1967).

The positional distribution of fatty acids in triacylglycerols of both vegetable and marine oils has been determined (Anderson *et al.* 1967; Brockerhoff 1965; Brockerhoff 1967; Brockerhoff *et al.* 1968; Christie and Moore 1969; Yurkowski and Brockerhoff 1966). Mathematical models have been developed to estimate the distribution of some PUFA (Litchfield 1968; Litchfield 1973), and the percent distribution of fatty acids in triacylglycerols found in primary positions (Mattson and Volpenhein 1961). These studies involved the use of stereospecific enzymes to hydrolyze the fatty acids in the triacylglycerols. However, the use of stereospecific enzymes for the analysis of triacylglycerols of marine oils is difficult due to the resistance of long-chain polyunsaturated fatty acids to lipase hydrolysis (Bottino *et al.* 1967; Christie 1982). A second drawback is the lack of specificity of lipolytic enzymes to fatty acids of certain chain-length and compositions in the triacylglycerols (Ando *et al.* 1992; Jensen *et al.* 1964; Luddy *et al.* 1964). These drawbacks led to the development of several chemical hydrolysis methods (Ando *et al.* 1992; Brockerhoff 1967; Christie and Moore 1969; Yurkowski and Brockerhoff 1966).

Ackman (1989) has clearly shown the biosynthetic preferential placement of PUFA is Sn-2 position of fish oil, but factors influencing this preferential placement of PUFA in Sn-2 position have not been fully explained or understood. This study was undertaken to determine differences in the positional distribution of PUFA in triacylglycerols of menhaden and salmon oils.

MATERIALS AND METHODS

Materials

Salmon oil was donated by Body Products, Inc. (Van Nuys, CA), whereas menhaden oil was obtained from Zapata Proteins, Inc. (Reedville, VA). Bromoethane was purchased from Eastman Kodak Company (Rochester, NY). Other supplies and chemicals were purchased from Fisher Scientific (Norcross, GA) and included magnesium turnings, silica gel 60 (0.25μm, 20 × 20 cm) thin layer chromatography (TLC) plates, anhydrous diethyl ether, potassium bicarbonate, anhydrous sodium sulfate, and tetrahydrofuran. The 3M methanolic-HCl, used for transesterification, was purchased from Supelco Inc. (Bellefonte, PA) and reference fatty acid standards were obtained from Nu-Check Prep, Inc. (Elysian, MN).

Isolation of Triacylglycerols

Pure triacylglycerols from each oil (1g) were separated on florisil columns as described by Christie (1992). The procedure involved the use of Pasteur pipettes

packed with florisil (2 cm), followed by elution of the triacylglycerols with a mixture of hexane-diethyl ether (95:5, v/v).

Partial Reductive Cleavage of Triacylglycerols

Triacylglycerols (40 mg) were dissolved in anhydrous diethyl ether (2 mL), and 1 mL of freshly prepared ethyl magnesium bromide (0.5 M) was added. Freshness of the ethyl magnesium bromide was essential for quicker cleavage. The substrates were partially cleaved over a 1 min period as described by Christie and Moore (1969). The products were extracted with 3 × 10 mL anhydrous diethyl ether. The extracts were washed with 5 mL aqueous (2%, w/v) potassium bicarbonate, followed by 5 mL of water to remove any artifacts, and dried over anhydrous sodium sulfate to remove any residual moisture. Solvent was removed under nitrogen at 30C in a rotary evaporator. The 2-monoacylglycerols were obtained by preparative TLC on boric acid impregnated silica gel 60 plates and developed in a hexane-diethyl ether-formic acid solvent system (80:20:2, v/v/v). Bands were visualized under UV light after spraying with 2, 7-dichlorofluorescein solution, and the corresponding bands that contained 2-monoacylglycerols were scraped into preweighed tubes and extracted with 3 × 5 mL of a chloroform-methanol solution (95:5, v/v). TLC plates were placed in a nitrogen-flushed desiccator before scraping the 2-monoacylglycerol bands into tubes. Solvents were evaporated under nitrogen at 30C and immediately capped. The maximum contamination of fatty acid composition of the 2-monoacyl-Sn-glycerols has under similar conditions been determined to be 2% when 1,3- dipalmitoyl 2-oleoyl-Sn-glycerol was used (Becker *et al.* 1993).

Methylation and GC Analysis of Methyl Esters

Triacylglycerols (50 mg) and the 2-monoacylglycerol (6-7 mg) obtained from preparative TLC were dissolved in tetrahydrofuran and transesterified overnight (80C) by the addition of 3M methanolic-HCl. Crude methyl esters were extracted into hexane, washed consecutively with aqueous solutions of sodium chloride (5%) and potassium bicarbonate (2%) and dried over anhydrous sodium sulfate. The hexane was evaporated at room temperature under nitrogen and the methyl esters re-dissolved in 400 μL isooctane. Samples were injected into a Hewlett Packard 5890 gas chromatograph (Avondale, PA) equipped with a 30m × 0.25mm i.d. DB 225 fused silica capillary column (J & W Scientific Co., Folsom, CA), a flame ionization detector, and an HP 3393A integrator. Column temperature was programmed from 180C to 230C at 2C/min with a final hold time of 2 min. Injector and detector temperatures were set at 250C and 275C, respectively. Helium was used as the carrier gas. Fatty acid methyl esters (FAME) were identified by comparison with authentic methyl ester standards (Nu-Chek Prep,

Elysian, MN) and a fatty acid internal standard (23:0). Individual fatty acid concentration was expressed as a mole percent of the total FAME.

RESULTS AND DISCUSSIONS

There are several factors that can affect the fatty acid composition and oxidative stability of fish oils. These include factors such as species and variety of fish, geographical location, processing methods and practices, among others. The positional factors could affect fatty acid composition of fish oils and their oxidative stability. The results obtained from this study, though not definitive for the species utilized, indicate that factors that affect fatty acid composition of fish oil also will influence their positional distribution in Sn-1, Sn-2 and Sn-3.

The positional distribution of fatty acids in triacylglycerols of both menhaden and salmon oils are shown in Table 1. The percent of each fatty acid in total triacylglycerols found in Sn-1 and Sn-3, and Sn-2 positions of menhaden and salmon oils were calculated using data from Table 1 as follows:

% in Sn-2 = [mole% Sn-2 fatty acid/mole % fatty acid in triacylglycerols $\times$ 3] $\times$100, and % in Sn-1 and 3 = 100 - % in Sn-2.

The calculated percentages are illustrated in Tables 2 and 3. The results clearly demonstrate differences in the positional distribution of the fatty acids. In menhaden oil, the unsaturated fatty acids were almost evenly distributed among the three positions of the triacylglycerols, except for 16:4 (n-1), 20:4 (n-3), and 21:5 (n-3) (Table 3) which were mostly located in the Sn-2 position, and 16:2 (n-4), 18:3 (n-6), 18:4 (n-3), 20:5 (n-3), and 22:6 (n-3) which were abundant in the Sn-1 and 3 positions (Table 3). Nine out of the thirteen PUFA detected in menhaden oil were present at > 70% in the Sn-1 and Sn-3 positions (Table 3). For salmon oil, the PUFA were mostly distributed in the Sn-2 position of the triacylglycerols. With the exception of 16:3 (n-4), 16:4 (n-1), 18:4 (n-3), and 20:4 (n-6), the PUFA were mainly located in the Sn-2 position of salmon oil. Unlike menhaden oil, four out of ten detected PUFA in salmon oil were located at > 70% composition in Sn-1 and Sn-3 positions (Table 3). The saturated fatty acids (14:0, 15:0, 16:0, 17:0) were mostly located in the Sn-1 and Sn-3 positions of salmon oil triacylglycerols (Table 2). This is in agreement with reported trends for most marine oils (Ando *et al.* 1992; Kimoto *et al.* 1994; Myher *et al.* 1990; Ota *et al.* 1994; Takagi and Ando 1991).

A comparison (Tables 2 and 3) of the percent distribution of PUFA in the triacylglycerols of both oils has shown that a greater percentage of the PUFA in the salmon oil triacylglycerols was esterified preferentially at the Sn-2 position compared to menhaden oil. This preferential positioning of PUFA at the Sn-2 position in salmon oil might possibly account for it being more stable than menhaden oil when both oils are subjected to thermal oxidation. There was also preferential positioning of certain isomers within the triacylglycerols of salmon oil.

TABLE 1.
POSITIONAL DISTRIBUTION OF FATTY ACIDS IN MENHADEN AND SALMON OIL TRIACYLGLYCEROLS (TAG, MOLE %)

Fatty Acids	Menhaden Oil			Salmon Oil		
	Total TAG	Sn-2 Position	Sn-1+ Sn-3 Positions	Total TAG	Sn-2 Position	Sn-1+ Sn-3 Positions
14:0	9.30 ± 0.46	8.96 ± 0.51	9.47 ± 0.38	5.88 ± 0.38	3.93 ± 0.48	6.86 ± 0.31
14:1 n-5	0.23 ± 0.00	0.26 ± 0.02	0.22 ± 0.01	ND	ND	ND
15:0	0.56 ± 0.06	0.52 ± 0.00	0.58 ± 0.03	0.51 ± 0.03	0.29 ± 0.02	0.62 ± 0.08
16:0	16.44 ± 0.27	17.10 ± 0.26	16.11 ± 0.31	16.31± 0.95	13.36 ± 0.50	17.79 ± 0.38
16:1 n-9	ND	ND	ND	5.40 ± 0.28	4.47 ± 0.69	5.87 ± 0.43
16:1 n-7	12.62 ± 0.19	12.35 ± 0.23	12.76 ± 0.16	ND	ND	ND
16:1 n-5	0.29 ± 0.00	0.31 ± 0.01	0.28 ± 0.03	ND	ND	ND
16:2 n-4	0.52 ±0.39	0.42 ± 0.02	0.57 ± 0.02	ND	ND	ND
16:3 n-4	2.00 ±0.05	1.92 ± 0.06	2.04 ± 0.04	0.46 ± 0.05	0.29 ± 0.02	0.55 ± 0.03
16:4 n-1	0.40 ± 0.27	0.71 ± 0.03	0.25 ± 0.00	0.89 ± 0.08	0.68 ± 0.06	1.00 ± 0.04
17:0	3.48 ± 0.06	3.25 ± 0.11	3.60 ± 0.10	0.50 ± 0.07	0.31 ± 0.02	0.67 ± 0.01
17:1	1.18 ± 0.17	1.16 ± 0.09	1.19 ± 0.07	ND	ND	ND
18:0	2.89 ± 0.07	2.99 ± 0.03	2.84 ± 0.05	2.96 ± 0.14	2.89 ± 0.09	3.00 ± 0.05
18:1 n-9	6.77 ± 0.19	6.69 ± 0.07	6.81 ± 0.10	18.67 ± 0.93	17.97 ± 1.69	19.02 ± 0.89
18:1 n-7	3.68 ± 0.04	3.55 ± 0.06	3.75 ± 0.04	3.05 ± 0.14	2.72 ± 0.05	3.22 ± 0.03
18:2 n-6	1.42 ± 0.05	1.27 ± 0.02	1.50 ± 0.01	1.62 ± 0.04	1.55 ± 0.10	1.66 ± 0.04
18:3 n-6	0.91 ± 0.06	0.50 ± 0.00	1.12 ± 0.03	ND	ND	ND
18:3 n-3	1.01 ± 0.04	0.89 ± 0.02	1.07 ± 0.01	0.96 ± 0.10	1.05 ± 0.03	0.92 ± 0.01
18:4 n-3	3.06 ± 0.08	2.38 ± 0.05	3.40 ± 0.04	1.91 ± 0.37	2.60 ± 0.06	1.57 ± 0.02
19:0	1.01 ± 0.04	0.94 ± 0.01	1.05 ± 0.02	ND	ND	ND
20:1 n-11	ND	ND	ND	7.76 ± 0.53	8.39 ± 0.50	7.45 ± 0.17
20:1 n-9	1.08 ± 0.10	1.11 ± 0.08	1.07 ± 0.06	2.26 ± 1.28	3.08 ± 0.30	1.85 ± 0.07
20:4 n-3	1.26 ± 0.16	1.38 ± 0.12	1.20 ± 0.00	1.03 ± 0.12	1.47 ± 0.03	0.81 ± 0.02
20:4 n-6	1.03 ± 0.05	0.88 ± 0.07	1.11 ± 0.01	0.45 ± 0.25	0.17 ± 0.01	0.59 ± 0.01
20:5 n-3	17.27 ± 0.47	14.23 ± 0.39	18.79 ± 0.42	7.50 ± 1.48	8.37 ± 0.15	7.07 ± 0.06
21:5 n-3	0.63 ± 0.42	0.88 ± 0.22	0.51 ± 0.05	ND	ND	ND
22:1 n-11	ND	ND	ND	9.08 ± 0.83	9.94 ± 0.25	8.65 ± 0.30
22:5 n-3	3.14 ± 0.07	2.81 ± 0.05	3.31 ± 0.03	1.48 ± 0.27	1.88 ± 0.02	1.28 ± 0.08
22:6 n-3	8.23 ± 0.18	6.54 ± 0.21	9.08 ± 0.14	8.33 ± 1.67	9.20 ± 0.20	7.90 ± 0.16

ND = Not detected
Each row value is a mean of four determinations.

TABLE 2.
PERCENT SATURATED AND MONOUNSATURATED FATTY ACIDS OF TOTAL TRIACYLGLYCEROLS LOCATED ON SN-1 AND SN-3, AND SN-2 POSITIONS OF MENHADEN AND SALMON OILS TRIACYLGLYCEROLS

Fatty Acids	Menhaden Oil		Salmon Oil	
	Sn-2 Position[§]	Sn-1+ Sn-3 Positions[†]	Sn-2 Position[§]	Sn-1+ Sn-3 Positions[†]
14:0	32.11	67.89	22.28	77.72*
15:0	30.95	69.05	18.95	81.05*
16:0	34.67	65.33	27.30	72.70*
17:0	31.13	68.87	18.79	81.21*
18:0	34.49	65.51	32.55	67.45
19:0	31.02	68.98	ND	ND
14:1 n-5	37.68*	62.32	ND	ND
16:1 n-9	ND	ND	27.59	72.41*
16:1 n-7	32.62	67.38	ND	ND
16:1 n-5	35.63	64.37	ND	ND
17:1	32.77	67.23	ND	ND
18:1 n-9	32.94	67.06	32.08	67.92
18:1 n-7	32.16	67.84	29.73	70.27*
20:1 n-11	ND	ND	36.04*	63.96
20:1 n-9	34.26	65.74	45.43*	54.57
22:1 n-11	ND	ND	36.49*	63.51

[§] = [mole % *sn*-2 fatty acid/mole % fatty acid in triacylglycerols × 3] × 100.
[†] = 100 - product of [§]. * = Indicates predominance in given position. ND = Not detected.
Each row value is a mean of four determinations.

For example, 20:1 (n-9) and 20:4 (n-3) in salmon oil (Table 2 and 3) were located preferentially in the Sn-2 position compared to their isomers whose double bonds were further away from the methyl group. This trend was observed in other published studies (Ando *et al.* 1992; Takagi and Ando 1991). It appears that the fatty acids are arranged and distributed in a manner that is most thermodynamically stable for the fish to adapt in their native environment. This property is influenced by the environment (geographical location and temperature), fatty acid composition, and species of fish (Joseph 1985; Bimbo 1989).

The composition of 20:1(n-9) isomer was predominant in Sn-2 position of salmon oil compared to menhaden oil, whereas 20:1 (n-11) and 22:1 (n-11) were not detected in menhaden oil. Interestingly, Ando *et al.* (1992) showed that the positional distribution of longer chain PUFA (20:5, 22:5, 22:6) in fish oil

TABLE 3.
PERCENT PUFA DISTRIBUTION ON TRIACYLGLYCEROLS OF TOTAL TRIACYLGLYCEROLS LOCATED ON SN-1 PLUS SN-3, AND SN-2 POSITIONS OF MENHADEN AND SALMON OILS

Fatty Acid	Menhaden Oil		Salmon Oil	
	Sn-2 Position§	Sn-1 + Sn- 3 Positions†	Sn-2 Position§	Sn-1 + Sn-3 Positions†
16:2 (n-4)	26.92	73.08*	ND	ND
16:3 (n-4)	32.00	68.00	21.01	78.99*
16:4 (n-1)	59.17*	40.83	25.47	74.53*
18:2 (n-6)	29.81	70.19*	31.89	68.11
18:3 (n-3)	29.37	70.63*	36.46*	63.54
18:3 (n-6)	18.32	81.68*	ND	ND
18:4 (n-3)	25.93	74.07*	12.59	87.41*
20:4 (n-3)	36.51*	63.49	47.57*	52.43
20:4 (n-6)	28.48	71.52*	12.59	87.41*
20:5 (n-3)	27.47	72.53*	37.20*	62.80
21.5 (n-3)	46.56*	52.44	ND	ND
22:5 (n-3)	29.83	70.17*	42.34*	57.66
22:6 (n-3)	26.49	73.51*	36.81*	63.19

§ = [mole % *sn*-2 fatty acid/mole % fatty acid in triacylglycerols × 3] × 100.
† = 100 - product of§. * = Indicates predominance in given position. ND = Not detected.
Each row value is a mean of four determinations.

triacylglycerols is influenced by the amounts of 20:1 and 22:1 present in the oil. According to their report, the presence of both 20:1 and 22:1 fatty acids favor positioning of PUFA in the Sn-2 position. The findings from this study appear to support their assumption, but further studies are needed to confirm this postulate. The results obtained in this study for menhaden oil do not agree with other published data which show that PUFA were located in the Sn-2 position of the triacylglycerol (Ando *et al.* 1992; Myher *et al.* 1990). The differences could be attributed to various factors such as differences in methodologies (Kimoto *et al.* 1994), source of the oil (Ando *et al.* 1992; Ota *et al.* 1994), or seasonal variation in the fatty acid profiles of menhaden oil (Joseph 1985; Bimbo 1989). Methods involving the use of enzymes and mathematical models for estimation of positional distribution of fatty acids in marine oils are not very accurate. The inaccuracy is due to the inefficient hydrolysis of lipids containing long chain fatty acids by both

phospholipase A_2 and lipases. Besides, mathematical models work under the assumption (Litchfield 1968; Litchfield 1973) that PUFA of marine oil sources, especially 22:6 (n-3) and 22:5 (n-3), are preferentially distributed in the Sn-2 position.

The results of stereospecific analysis obtained from this study show that there are differences in positional distribution of fatty acids in triacylglycerols of salmon and menhaden oils. It also shows that factors such as preferential positioning of isomers and species of fish influence positional distribution of fatty acids in fish oil triacylglycerols. The preferential positioning of some isomers in the Sn-2 position and the influence of elevated levels of 20:1 and 22:1 on positional distribution need further investigation. Knowledge from such a study will have a significant impact on the aquaculture industry in particular, and the oil industry as a whole. It may be possible through diet to manipulate the fatty acid composition of cultured fish and in turn improve the oxidative stability of their oils.

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