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Zonal Distribution of Fatty Acids in Albacore (*Thunnus alalunga*) Triglycerides and Their Changes during Cooking

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The α–β distribution of fatty acids in albacore muscle triglycerides was studied. This analysis was achieved in cooked and uncooked samples in order to assert possible variations during processing. On the other hand, because of their different locations and also different exposures to steam during cooking, three zones of the muscle were considered. Great differences between the α- and β-compositions were observed in the three zones. Fatty acids like 16:0, 18:0, 18:1, 18:3, and 22:1 showed a higher content in the α-position, while 22:6 content was far bigger in β. Due to cooking it was observed that the composition at the β-location suffered more modifications than the α-one. Depending on the zone of the muscle, significant variations were obtained for the main fatty acids.

The first studies on the distribution of fatty acids of triglycerides (TG) of marine fish and invertebrates (Brockerhoff and Hoyle, 1963; Brockerhoff et al., 1963; Dolev and Olcott, 1965) have shown that this lipid class follows the structural pattern typical for most animal fats in which the polyunsaturated fatty acids (PUFA) are preferentially located in the β-position of glycerol. As an explanation of this distribution, it was suggested that the typical structure may be originated in the plankton and then be retained through the food chain. In this point, Brockerhoff et al. (1964) studied the fatty acid distribution in lipids of marine plankton and agreed with this explanation although they pointed out that this may not be the only factor responsible for the typical fatty acid pattern of marine fats.

The characterization of animal fats by fatty acid analysis using the "characteristic" fatty acids ratio has been reported by Litchfield (1972), Carisano and Riva (1976), and Doro (1977). However, three effects make it difficult to recognize the positional distribution of TG: First, dietary fats can alter the composition of animal depot fats (Bishop et al., 1976). Second, the positional distribution patterns are not the same for all kinds of animals. Third, the positional distribution may vary between different body tissues in the same animals.

More recent work in accord with the need for reliable methods to determine the animal species from which processed fish products are made have been published (Takahashi et al., 1978a,b). In them, relations between the main fatty acids of raw and frozen samples which belonging to the different location in triglycerides studied. Later on, Takahashi et al. (1985) developed a mathematical model for the prediction of molecular species in TG by HPLC.

The purpose of the present work was to study the α–β distribution in TG of albacore in order to establish the differences between the raw and cooked material. This species was chosen for its great importance in the Spanish canning industry. Since during cooking the different parts of albacore are not exposed the same way to steam, a zonal study of the muscle (back muscle, ventral

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Table I. Lipid Content[a] (Grams/100 g of Muscle) for the Raw and Cooked Samples[b]

sample	lipid content
raw BM	5.82 ± 0.15
cooked BM	4.92 ± 0.11
raw VM	4.32 ± 0.13
cooked VM	3.88 ± 0.10
raw BFM	18.54 ± 0.92
cooked BFM	13.99 ± 0.58

[a] Mean of three determinations ± standard deviation. [b] Key: back muscle = BM; ventral muscle = VM; belly flap muscle = BFM.

muscle, and belly flap muscle) was also undergone.

The results reported here are applicable only to the tuna samples analyzed, all of which were from fish taken at the same time and place. It must be recognized that albacore tuna taken at different locations and different times usually have great differences in lipid content and in fatty acid content of the lipids. Such a difference could change the distribution of fatty acids between α- and β-positions on the triglyceride molecule.

MATERIALS AND METHODS

Raw Material, Sampling, and Lipid Extraction. Albacore (*Thunnus alalunga*) used in this work was caught by a commercial tuna vessel round the point 43° N and 27° W during June 1985. The fish were kept in boxes and transported on ice for 10 days. After arrival to our laboratory, the fish were frozen at -40 °C and stored at -18 °C analysis.

Six individuals were employed, and three zones of the muscle, known in the commercial literature as back muscle (BM), ventral muscle (VM) and belly flap muscle (BFM), were used in this experiment.

The fish was processed in our pilot plant according to the following procedure: Whole eviscerated and beheaded fish were cooked (at 102–103 °C) with steam until a final backbone temperature of 65 °C; then, it was cooled at room temperature (14 °C) for about 5 h before sampling.

Lipids were extracted from the samples by the Bligh and Dyer method (1959). An aliquot of the extract was dried to constant weight by a nitrogen flow in order to obtain the lipid content.

Purification of Triglycerides. Lipid classes were separated by column chromatography on silica gel (Aubourg, 1987). TG were further purified by thin-layer chromatography on 20 × 20 cm plates coated with a layer (0.5 mm) of silica gel G (Merck) eluted in hexane–ethyl ether–acetic acid (70:30:1).

Triglyceride Hydrolysis by the Grignard Reagent. Random diglycerides (DG) were produced by the action of the Grignard reagent, ethylmagnesium bromide, on triglycerides according to the method recommended by Breckenridge (1978) and developed by Christie and Moore (1969). 1,2;2,3-DG and 1,3-DG were quickly isolated by preparative TLC on silicic acid impregnated with 5% (w/w) boric acid to prevent acyl migration.

Fatty Acid Analysis. Random diglycerides coming from the Grignard reaction, as well as initial triglycerides, were transesterified by using the BF_3 complex, in accord with Morrison and Smith (1964).

Then, fatty acid methyl esters were analyzed with a Varian Vista 6000 gas chromatograph equipped with a 30-m flexible capillary column SP-2330 (Supelco). An isothermal separation at 190 °C with injector temperature 220 °C and detector temperature 230 °C was carried out. Carrier gas used was N_2 flowing with a linear velocity of 18 cm/s. The individual fatty acid methyl esters were identified by comparing the retention times to those of standard ester mixtures including PUFA No. 1, marine source (Supelco), and by semilogarithmic plots of retention times against carbon chain lengths (Ackman, 1969).

RESULTS AND DISCUSSION

Table I shows the lipid content of the different samples employed in this triglyceride study. It is worth pointing out the high level of belly flap muscle in front of the two other zones, as well as the general decrease in lipid content due to cooking.

Table II. Content (%) of Fatty Acids (FA) in the α- and β-Positions in the Back Muscle (BM)[a]

raw BM			cooked BM	
α	β	FA	α	β
3.0	5.1	14:0	3.1	5.5
1.9	1.1	15:0	1.2	2.7
20.8	18.3	16:0	21.9	14.7
5.7	6.6	16:1	6.7	4.3
1.9	3.4	17:0	2.2	2.8
1.1	0.2	17:1	1.0	1.4
8.9	2.0	18:0	7.7	2.6
22.8	17.1	18:1	21.5	18.2
1.6	3.4	18:2	2.7	0.9
5.1	1.5	18:3	5.0	0.8
0.6	2.1	18:4	0.7	2.2
4.8	1.2	22:1	4.5	0.6
0.9	2.7	20:4	1.2	2.1
4.5	6.0	20:5	5.7	3.9
1.5	0.6	24:1	1.5	1.0
0.6	0.9	22:4	0.5	1.3
1.5	1.5	22:5	1.4	1.7
11.0	23.5	22:6	9.4	28.3

[a] Mean of three determinations; mean standard deviation ±0.4.

Table III. Content (%) of Fatty Acids (FA) in the α- and β-Positions in the Ventral Muscle (VM)[a]

raw VM			cooked VM	
α	β	FA	α	β
3.3	5.6	14:0	3.8	4.7
1.2	2.4	15:0	1.4	1.4
20.7	17.1	16:0	22.8	15.0
6.2	6.2	16:1	6.7	3.1
1.8	3.0	17:0	2.0	1.4
1.1	0.8	17:1	0.9	0.3
8.2	1.3	18:0	7.4	3.5
22.9	14.8	18:1	21.0	17.7
2.5	1.6	18:2	1.8	3.0
4.3	1.9	18:3	3.5	3.5
0.5	2.6	18:4	1.0	2.6
4.0	1.3	22:1	4.4	1.7
1.1	3.5	20:4	1.2	2.4
4.6	5.2	20:5	4.9	4.6
1.5	0.6	24:1	1.6	1.0
0.7	0.4	22:4	0.7	0.4
1.4	1.4	22:5	1.6	1.0
11.3	24.8	22:6	8.8	30.4

[a] Mean of three determinations; mean standard deviation ±0.3.

Tables II–IV show the content (%) of the fatty acids in the α- and β-positions for each of the samples analyzed.

Comparing the α- and β-contents in the raw samples, a significantly higher content in 16:0, 18:0, 18:1, 18:3, and 22:1 in the α-position was observed. At the same time, other fatty acids showed higher values in β: 14:0, 17:0, 18:4, 20:4, and 22:6 in back muscle and ventral muscle; 20:5 and 22:6 in belly flap muscle.

The big difference between the contents of 22:6 in both positions is in accord with an already commented theory (Brockerhoff et al., 1964; Menzel and Olcott, 1964). As a result, the finality of this location would be the protection from oxidation during the metabolic processes of the PUFA through the formation of the β-MG structure with a PUFA in this location.

Due to cooking, the higher differences are related to the more abundant fatty acids. It is worth pointing out that the composition of the β-location has suffered more significant modifications than the α-one.

Table IV. Content (%) of Fatty Acids (FA) in the α- and β-Positions in the Belly Flap Muscle (BFM)[a]

raw BFM			cooked BFM	
α	β	FA	α	β
3.9	4.8	14:0	4.2	4.2
1.5	1.2	15:0	1.3	2.8
22.9	16.9	16:0	23.2	12.4
6.0	7.8	16:1	6.9	5.1
2.2	1.9	17:0	2.6	1.7
1.2	0.6	17:1	1.1	0.2
8.0	1.4	18:0	7.2	3.6
21.7	13.6	18:1	20.9	16.7
2.7	0.6	18:2	2.5	1.6
4.4	2.6	18:3	4.0	2.2
1.0	1.6	18:4	1.2	1.2
3.8	2.0	22:1	3.7	2.8
1.8	0.6	20:4	1.7	0.5
3.7	9.7	20:5	4.3	7.3
1.5	0.6	24:1	1.5	0.9
0.3	1.0	22:4	0.3	1.2
1.1	1.7	22:5	1.0	2.2
7.1	31.4	22:6	7.2	33.3

[a] Mean of three determinations; mean standard deviation ±0.4.

Table V. Content[a] (%) of Saturated (ST), Monounsaturated (MU), and Polyunsaturated (PU) Fatty Acids in the α- and β-Positions for the Three Zones

	raw		cooked	
	α	β	α	β
		Back Muscle		
ST	36.5	29.9	36.2	28.3
MU	35.9	25.7	35.2	25.5
PU	25.8	41.6	26.6	41.2
		Ventral Muscle		
ST	35.4	29.4	37.4	26.0
MU	35.7	23.7	34.6	23.8
PU	26.4	41.4	23.5	47.9
		Belly Flap Muscle		
ST	38.5	26.2	38.5	24.7
MU	34.2	24.6	34.1	25.7
PU	22.1	49.2	22.2	49.5

[a] Mean of three determinations; mean standard deviation ±1.4.

In the back muscle and ventral muscle the 22:6 increased in the β-position with the opposite result in the α-one. In the ventral muscle and belly flap muscle an increase in 18:0 and 18:1 as well as a decrease in 16:0 were observed in the β-location. In the back muscle and belly flap muscle decreases in 16:1 and 20:5 in the β-position were obtained.

In Table V fatty acids are pooled together in saturated (ST), monounsaturated (MU), and polyunsaturated (PU) groups.

In all the samples, a very significant difference was obtained for the distribution of the PU fatty acids between the two locations: the β-contents were nearly the double the α-ones.

Comparing the composition of the three raw samples, significant differences were only found in the belly flap muscle: ST fatty acids had higher values in the α-locations and lower values in the β-ones while the opposite situation was found in the PU fatty acids.

Due to cooking, the only significant differences were obtained in the ventral muscle for the PU fatty acids, which increased in the β-location and decreased in the α-one.

As a conclusion, it can be mentioned that changes in individual fatty acids have been observed due to cooking although if they are considered by unsaturation groups, these changes are really smaller.

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Registry No. 16:0, 57-10-3; 18:0, 57-11-4; 18:1, 112-80-1; 18:3, 463-40-1; 22:1, 28929-01-3; 22:6ω3, 6217-54-5.

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