Polyunsaturated Fatty Acids in Tuna Phospholipids: Distribution in the *sn*-2 Location and Changes during Cooking

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Fatty acid composition with a special stress on polyunsaturated fatty acids (PUFA) was studied in the *sn*-2 location of tuna phospholipids (PL) in eight commercial species. Comparison with the *sn*-1 location and changes of both positions (*sn*-1 and *sn*-2) during cooking were also analyzed. When the *sn*-1 and *sn*-2 locations in albacore tuna were compared, some differences were noted. The *sn*-1 position showed higher contents in saturated (16:0 and 18:0) and 18:1ω7 fatty acids; in contrast, higher proportions in PUFA (22:6ω3, 20:5ω3, and 20:4ω6) and in 18:1ω9 were obtained in the *sn*-2 location. The comparative study of the *sn*-2 position of PL showed a similar pattern of distribution in the different tuna species. In all cases the main fatty acid was 22:6ω3, followed by 18:1ω9, 20:5ω3, 16:0, 20:4ω6, and 24:1ω9. A high ω3/ω6 ratio was found in all cases, although great differences were noted between the highest (bonito and yellowfin) and the lowest (little tunny and skipjack) values; ratios between the most abundant PUFA belonging to the ω3 and ω6 series (22:6ω3/20:4ω6 and 20:4ω6/20:5ω3) showed similar differences. The behavior of both positions (*sn*-1 and *sn*-2) in albacore PL was studied during cooking. As a result of the thermal treatment, some changes in the fatty acid composition were seen in both locations. The *sn*-2 position showed a decrease in 22:6ω3 and an increase in 18:1ω9. On the contrary, a decrease in 18:0 was obtained in the *sn*-1 position, along with an increase in 22:6ω3 and 20:4ω6.

Keywords: *Tuna fishes; phospholipids; sn-1 and sn-2 positions; PUFA, ω3-PUFA; thermal treatment*

INTRODUCTION

The principles that regulate the positional distribution of fatty acids in animal lipids are not well understood (Litchfield, 1972; Breckenridge, 1978; Takahashi et al., 1985). Many studies refer to the fatty acid distribution in triglycerides (TG) of marine animals. As a general rule, a typical structural pattern has been observed, showing that polyunsaturated fatty acids (PUFA) are preferentially located in the *sn*-2 position of glycerol (Brockerhoff and Hoyle, 1963; Brockerhoff et al., 1963; Dolev and Olcott, 1965; Aubourg et al., 1990; Sacchi et al., 1993).

Phospholipids (PL) are known to be made up by a large quantity of PUFA (Vaskowski, 1989). Previous experiences concerning the *sn*-1/*sn*-2 distribution of fatty acids in PL have proved that PUFA also tend to accumulate in the *sn*-2 position (Menzel and Olcott, 1964; Takahashi et al., 1978b; Medina and Sacchi, 1994), leading to a very high concentration of such fatty acids in this location. The little information available about changes occurring in both (*sn*-1/*sn*-2) PL positions during fish processing refers to frozen storage (Takahashi et al., 1978a; Ohshima et al., 1984).

Among PUFA, ω3 fatty acids consumption has shown a positive role in preventing certain human diseases (Carroll and Braden, 1986; Illingworth and Ullmann, 1990), and the ω3/ω6 ratio has also been of great interest in the study of fish health and nutritional needs (Watanabe, 1982; Weber, 1992). From a technological point of view, a great number of studies have proved the incidence of PUFA in food damage (oxidative and hydrolytic) during processing (Pigott and Tucker, 1987; Hsieh and Kinsella, 1989; Medina et al., 1994b); this subject has merited great attention in the case of marine products because of their higher content in PUFA (Ackman, 1989).

The present study is focused on the PUFA composition of the *sn*-2 location of tuna PL. First, a comparative study of both positions (*sn*-1 and *sn*-2) in albacore is carried out. Then, the fatty acid composition of the *sn*-2 location is compared in the white muscle of eight tuna species widely employed in the canning industry; special stress is given to ratios among the major PUFA (20:4ω6, 20:5ω3, and 22:6ω3) and PUFA groups ($\omega-3/\omega-6$). Finally, changes occurring in both positions during the thermal treatment (cooking) of albacore are studied.

MATERIALS AND METHODS

Raw Material, Sampling, Processing, and Preliminary Analyses. The following tuna fishes were studied (numbers of individuals employed are indicated): albacore (*Thunnus alalunga*) (6); little tunny (*Euthynnus alletteratus*) (5); skipjack (*Katsuwonus pelamis*) (3); frigate (*Auxis thazard*) (3); big eye tuna (*Thunnus obesus*) (5); yellowfin (*Thunnus albacares*) (5); bluefin (*Thunnus thynnus*) (4); and bonito (*Sarda sarda*) (3). The fish were purchased in a commercial center and were kept frozen at −20 °C prior to analysis. Individual fishes were analyzed separately to establish a statistical study.

Albacore (six individuals) was processed in our pilot plant according to the following procedure: whole eviscerated and beheaded fish was cooked (102−103 °C) with steam to a final backbone temperature of 65 °C; then it was cooled at room temperature (14 °C) during 5 h before sampling. Individual fishes were also considered separately.

The white muscle of each fish species was considered. Lipids were extracted from the fish flesh following the Bligh and Dyer (1959) method. Quantification of lipids was established according to the procedure of Herbes and Allen (1983). All organic solvents employed throughout the present work were of reagent grade (E. Merck).

Quantification and Purification of Phospholipids (PL). PL were quantified by measuring the organic phosphorus on total lipid extracts according to the Raheja et al. (1973)

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method based on a complex formation with ammonium molybdate (Merck).

Lipid extracts were subjected to thin layer chromatography (TLC) on 20 × 20 cm plates of silica gel 60W (0.8 mm) (Merck) and developed twice in the same direction with $CHCl_3$−CH_3OH−CH_3COOH (100:5:2 v/v/v). The PL fraction remained at the bottom of the plate and was recovered from the silica gel by elution with $CHCl_3$−CH_3OH (2:1, v/v).

Phospholipid Hydrolysis with Phospholipase A_2. A stock solution of phospholipase A_2 (1060 units; Sigma, from *Crotalus adamanteus* venom) in 20 mL of 1 M tris buffer (pH 7.5) containing calcium chloride (4 mM) was made up. Hydrolysis was carried out by adding 100 μL of this solution to phospholipids (2−5 mg) in diethyl ether (2 mL) (Robertson and Lands, 1962). The mixture was shaken vigorously for 2 h; then it was washed with a mixture of methanol (2 mL) and chloroform (4 mL) and then dried with anhydrous sodium sulfate. The solution was filtered and the reaction mixture was separated by TLC on 20 × 20 cm plates of silica gel 60W (0.25 mm) (Merck) developed with a solvent mixture of hexane−diethyl ether−acetic acid (50:50:1; v/v/v). The free fatty acids resulting from the phospholipase hydrolysis (*sn*-2 position) were extracted from the silica with chloroform; resulting *sn*-1 lysophospholipids were recovered from the bottom of the plate by elution with chloroform−methanol (2:1).

Completion of reaction (disappearance of diacylphosphoglycerides) was ensured by monitoring the reaction mixtures by TLC (Brockerhoff, 1975).

Methylation of Lipid Samples. Free fatty acids and lysophospholipids obtained from the enzymatic reaction were converted into fatty acid methyl esters (FAME) according to the Lepage and Roy (1986) method.

Gas Chromatography (GC) Analysis. FAME were analyzed by GC (Perkin-Elmer 8700 chromatograph) employing a fused silica capillary column SP-2330 (0.25 mm i.d. × 30 m, Supelco, Inc., Bellefonte, Pa), programmed from 145 to 190 °C at 1.0 °C/min and from 190 °C to 210 °C at 5.0 °C/min and then followed by a hold during 13.5 min at 210 °C. Nitrogen at 10 psig as carrier gas and a flame ionization detector at 250 °C were used. A programmed temperature vaporizer (PTV) injector was employed in the split mode (150:1) and heated from 45 to 275 °C at 15 °C/min (Medina et al., 1994a).

Peaks were identified by comparison of their retention times with standard mixtures (Supelco, PUFA 1 and 2; Larodan, Qualmix Fish). Peak areas were automatically integrated, and 19:0 fatty acid was used as an internal standard.

Gas Chromatography−Mass Spectroscopy (GC−MS) Analysis. GC−MS analysis of FAME was performed on a HP-5971A quadrupole mass spectrometer system operating in the electron impact ionization mode (70 eV). The injection and ion source temperatures were 280 °C. The column and oven temperatures were the same as for the GC analysis previously described. Helium was used as carrier gas at 10 psig. Mass spectra were scanned in the range *m*/*z* 40−450.

Mass spectra of the different peaks were checked to confirm the FAME elucidation.

Statistical Analysis. Data obtained for the individual fatty acid compositions, fatty acids groups, and ratios among the main PUFA were subjected to the ANOVA one-way method, according to the method of Sokal and Rohlf (1981). Significance was declared at $P < 0.05$.

RESULTS AND DISCUSSION

Table 1 compiles the results obtained from the preliminary lipid analyses. The total lipid content shows great differences among the species studied; the highest values in total lipids were obtained in the case of albacore, followed by bonito; the remaining species showed very low levels. PL content in total lipids also showed great differences among the various samples. An inverse ratio between lipid content and PL proportion in total lipids was observed, according to the constancy of the PL fraction in muscle (Pearson et al., 1977; Henderson and Tocher, 1987). Bonito was the exception, since it showed a very low content of PL.

Table 1. Total Lipid and Phospholipid Contents[a] of the Different Tuna Fishes

		phospholipids	
tuna fish	total lipids (g/100 g of muscle)	g/100 g of lipids	g/100 g of muscle
albacore (raw)	7.16 ± 3.18	2.66 ± 1.09	0.20 ± 0.07
albacore (cooked)	4.61 ± 2.08	4.64 ± 1.79	0.22 ± 0.03
little tunny	1.11 ± 0.35	25.08 ± 8.78	0.27 ± 0.06
skipjack	0.83 ± 0.23	25.19 ± 3.58	0.21 ± 0.02
frigate	0.65 ± 0.20	25.83 ± 2.77	0.18 ± 0.06
big eye tuna	0.76 ± 0.31	41.18 ± 15.05	0.30 ± 0.04
yellowfin	0.57 ± 0.04	56.71 ± 1.43	0.34 ± 0.02
bluefin	0.95 ± 0.49	26.41 ± 9.35	0.24 ± 0.02
bonito	1.94 ± 0.19	3.06 ± 0.51	0.06 ± 0.01

[a] Mean value ± standard deviation.

Table 2. Fatty Acid (FA) Composition (Percent)[a] of *sn*-1 and *sn*-2 Positions in Raw and Cooked Albacore

	raw		cooked	
FA	*sn*-1	*sn*-2	*sn*-1	*sn*-2
14:0	1.5 ± 0.5	1.3 ± 0.3	1.6 ± 0.2	1.9 ± 0.6
15:0	1.0 ± 0.3	0.6 ± 0.4	0.9 ± 0.2	0.9 ± 0.1
16:0	20.8 ± 1.8b	6.7 ± 0.9a	22.3 ± 4.4b	7.9 ± 0.8a
16:1ω11	2.1 ± 0.3	0.5 ± 0.1	1.0 ± 0.5	1.4 ± 0.2
16:1ω7	1.1 ± 0.4	1.2 ± 0.3	1.2 ± 0.2	0.6 ± 0.1
16:1ω5	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.5 ± 0.2
17:0	2.2 ± 0.1	1.1 ± 0.1	2.2 ± 0.2	0.9 ± 0.3
18:0	43.1 ± 2.4c	2.1 ± 0.6a	34.2 ± 3.3b	2.2 ± 0.6a
18:1ω9	7.7 ± 0.4a	13.7 ± 1.1b	7.8 ± 3.1a	20.2 ± 4.6c
18:1ω7	3.9 ± 0.9c	2.2 ± 0.1ab	3.2 ± 1.1bc	2.3 ± 0.2a
18:2ω6	1.5 ± 0.3	0.9 ± 0.1	1.1 ± 0.3	1.0 ± 0.1
18:3ω3	0.3 ± 0.1	0.4 ± 0.1	0.6 ± 0.1	0.5 ± 0.3
20:1ω9	0.8 ± 0.2	0.4 ± 0.2	0.8 ± 0.3	0.4 ± 0.2
20:4ω6	1.3 ± 0.3a	7.1 ± 0.5c	2.1 ± 0.3b	7.3 ± 0.4c
20:4ω3	0.6 ± 0.2	0.6 ± 0.2	0.5 ± 0.1	0.2 ± 0.1
20:5ω3	1.2 ± 0.1a	9.3 ± 0.9b	2.0 ± 0.4a	8.2 ± 1.1b
24:0	0.5 ± 0.2	0.7 ± 0.3	0.6 ± 0.1	0.7 ± 0.3
22:4ω6	0.5 ± 0.1	1.4 ± 0.2	0.5 ± 0.2	0.9 ± 0.2
24:1ω9	0.4 ± 0.1	0.9 ± 0.3	0.6 ± 0.1	1.2 ± 0.1
22:5ω3	0.4 ± 0.2	0.8 ± 0.1	0.5 ± 0.1	0.8 ± 0.2
22:6ω3	9.3 ± 1.2a	47.3 ± 1.9d	14.7 ± 1.1b	39.4 ± 2.2c

[a] Mean value ± standard deviation. Values in the same row followed by different letters are significantly different ($P < 0.05$).

Comparative Study of the *sn*-1 and *sn*-2 Positions in Albacore. The compositions of the *sn*-1 and *sn*-2 positions in raw albacore are shown in Table 2. Both locations showed different fatty acid compositions. The *sn*-2 position showed a higher proportion in PUFA (22:6ω3, 20:5ω3, and 20:4ω6) and in 18:1ω9. In contrast, higher contents in saturated (ST) (16:0 and 18:0) and in 18:1ω7 fatty acids were obtained in the case of the *sn*-1 position.

These results agree with previous experiences concerning PL of marine species (Menzel and Olcott, 1964; Takahashi et al., 1978b; Medina and Sacchi, 1994); a general theory concerning a PUFA retention in the *sn*-2 position of all kinds of glyceride lipids has been proposed as occurring to protect PUFA from oxidative cleavages during metabolic processes (Brockerhoff et al., 1964, 1967).

In previous papers (Aubourg et al., 1990; Sacchi et al., 1993) the *sn*-1,3 and *sn*-2 positions of albacore triglycerides (TG) were studied. A PUFA retention in the *sn*-2 location was also observed, although PUFA proportions were smaller than in the present experience with PL; total ST fatty acids in TG showed a similar distribution between both kinds of positions (*sn*-1, 3 and

Table 3. Fatty Acid (FA) Composition (Percent)[a] of the *sn*-2 Position in Tuna[b] Phospholipids

FA	LT	SK	FR	BE	YF	BF	BO
14:0	2.4 ± 0.5	2.1 ± 0.3	0.7 ± 0.2	0.4 ± 0.2	0.7 ± 0.1	1.1 ± 0.2	1.6 ± 0.1
15:0	1.2 ± 0.2	1.2 ± 0.1	0.5 ± 0.0	0.4 ± 0.1	0.7 ± 0.1	0.3 ± 0.0	1.2 ± 0.1
16:0	10.8 ± 1.6	8.1 ± 0.8	5.4 ± 0.1	3.9 ± 1.1	8.6 ± 3.0	4.0 ± 0.5	8.4 ± 0.5
16:1ω11	2.2 ± 0.6	2.4 ± 0.5	0.8 ± 0.2	0.5 ± 0.2	1.2 ± 0.3	0.5 ± 0.1	2.3 ± 0.1
16:1ω7	1.1 ± 0.2	0.6 ± 0.1	0.6 ± 0.0	0.4 ± 0.0	0.5 ± 0.1	0.0 ± 0.1	0.5 ± 0.0
16:1ω5	0.3 ± 0.0	0.3 ± 0.1	0.2 ± 0.0	0.4 ± 0.1	0.3 ± 0.0	0.4 ± 0.1	0.3 ± 0.0
17:0	0.7 ± 0.1	1.0 ± 0.1	0.5 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.7 ± 0.2	0.6 ± 0.1
18:0	4.5 ± 1.2	3.8 ± 0.3	1.3 ± 0.0	0.8 ± 0.2	2.2 ± 0.9	1.1 ± 0.1	2.4 ± 0.2
18:1ω9	14.6 ± 1.4	9.6 ± 0.2	13.0 ± 0.2	11.3 ± 1.4	12.3 ± 1.2	15.6 ± 4.0	11.1 ± 0.2
18:1ω7	2.4 ± 0.5	1.1 ± 0.1	1.6 ± 0.2	1.2 ± 0.2	1.2 ± 0.0	0.9 ± 0.1	1.4 ± 0.1
18:2ω6	1.1 ± 0.4	0.8 ± 0.1	0.5 ± 0.0	0.4 ± 0.1	0.6 ± 0.2	0.4 ± 0.1	0.9 ± 0.0
18:3ω3	0.6 ± 0.1	0.4 ± 0.1	0.2 ± 0.0	0.4 ± 0.1	0.2 ± 0.0	0.4 ± 0.1	0.4 ± 0.0
20:1ω9	0.6 ± 0.2	0.4 ± 0.1	0.2 ± 0.0	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
20:4ω6	8.4 ± 0.6	8.7 ± 0.4	8.3 ± 0.2	8.9 ± 0.2	6.6 ± 0.7	8.1 ± 0.4	4.3 ± 0.2
20:4ω3	0.3 ± 0.1	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
20:5ω3	4.5 ± 1.1	4.9 ± 0.4	8.2 ± 0.3	5.1 ± 0.6	5.6 ± 0.4	6.5 ± 0.5	8.4 ± 0.3
24:0	1.4 ± 0.3	1.3 ± 0. 2	0.4 ± 0.0	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.9 ± 0.1
22:4ω6	0.6 ± 0.1	1.0 ± 0.3	0.5 ± 0.0	0.7 ± 0.1	0.3 ± 0.0	0.5 ± 0.1	0.6 ± 0.1
24:1ω9	2.7 ± 0.3	5.0 ± 0.4	4.5 ± 0.1	4.4 ± 0.3	3.3 ± 0.4	3.3 ± 0.6	2.0 ± 0.1
22:5ω3	1.5 ± 0.2	1.4 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	1.1 ± 0.1
22:6ω3	37.7 ± 3.7	46.0 ± 2.7	51.4 ± 0.3	58.1 ± 2.7	53.1 ± 5.3	53.7 ± 3.8	50.5 ± 0.4

[a] Mean value ± standard deviation. [b] Tuna species: little tunny, LT; skipjack, SK; frigate, FR; big eye tuna, BE; yellowfin, YF; bluefin, BF; and bonito, BO.

sn-2) than in the present PL results. However, a different distribution was observed for monounsaturated (MU) fatty acids; in the case of TG this fatty acid group was mainly placed in the *sn*-1,3 position, while in the present PL results, MU fatty acids are preferentially located in the *sn*-2 position.

It is concluded that PUFA are predominantly located in the *sn*-2 position. From a quantitative point of view the difference between both locations is even greater if we consider that the *sn*-1 position in PL is partially occupied by a non-acyl fatty chain, such as an alkyl or alkenyl one (Sargent, 1989; Medina et al., 1993).

Composition of the *sn*-2 Position in Tuna Samples. The comparative fatty acid composition of the *sn*-2 location in PL of the eight raw tuna fishes can be seen in Tables 2 (raw albacore) and Table 3. A similar pattern of distribution between all of them was noted. In all cases the most abundant fatty acid was 22:6ω3, followed by 18:1ω9, 20:5ω3, 16:0, 20:4ω6, and 24:1ω9. Minor fatty acids observed were 16:1ω5, 18:3ω3, 20:1ω9, and 20:4ω3.

A high content in unsaturated fatty acids (PUFA, mainly) was obtained, in accordance with the formerly mentioned albacore *sn*-1/*sn*-2 distribution and with the above-mentioned theory about preserving this kind of fatty acid. Previous experience with tuna, salmon, and meaden showed that 91−99% of the fatty acids in the *sn*-2 position were unsaturated (Menzel and Olcott, 1964).

Because of the great interest in the different kinds of PUFA, ratios between the main polyunsaturated groups and between the major individual PUFA were studied (Tables 4 and 5) and compared throughout the different tuna species.

Table 4 shows the ω3− and ω6−PUFA contents. The ω3/ω6 ratio showed some significant differences, especially between extreme values such as little tunny and skipjack (the lowest) and yellowfin and bonito (the highest). Species were found to be divided into four different groups. Several external factors (such as nutrition and water temperature) have proved to alter this ratio, although some limitations have been observed during the incorporation of ω3−PUFA by means of experimental diets (Bell et al., 1985; Venkatraman et al., 1992).

Table 4. Content (Percent)[a] of ω3- and ω6-PUFA, and ω3/ω6 Ratio Value of the *sn*-2 Position in Tuna Phospholipids

tuna fish	ω3-PUFA	ω6-PUFA	ω3/ω6 ratio
albacore	58.3 ± 2.2bc	9.3 ± 0.5cde	6.3 ± 0.4b
little tunny	44.5 ± 4.9a	10.2 ± 0.3def	4.4 ± 0.4a
skipjack	52.8 ± 2.5b	10.5 ± 0.4f	5.0 ± 0.4a
frigate	61.0 ± 0.5cd	9.3 ± 0.1cd	6.6 ± 0.2b
big eye tuna	65.0 ± 3.0d	10.1 ± 0.2def	6.5 ± 0.2b
yellowfin	60.0 ± 5.3cd	7.5 ± 0.9b	8.1 ± 1.5c
bluefin	61.8 ± 4.3cd	9.0 ± 0.5c	6.9 ± 0.4b
bonito	60.4 ± 0.7bcd	5.8 ± 0.3a	10.4 ± 0.4d

[a] Mean value ± standard deviation. Values in the same column followed by different letters are significantly different ($P < 0.05$).

Table 5. Ratios[a] between the Proportions of the Major PUFA in the *sn*-2 Location of Tuna Phospholipids

tuna fish	20:4ω6/20:5ω3	22:6ω3/20:4ω6	22:6ω3/20:5ω3
albacore	0.77 ± 0.09a	6.70 ± 0.43b	5.12 ± 0.50a
little tunny	1.97 ± 0.40c	4.46 ± 0.18a	8.74 ± 1.67bc
skipjack	1.79 ± 0.14c	5.29 ± 0.39ab	9.52 ± 1.30cd
frigate	1.02 ± 0.05ab	6.16 ± 0.16b	6.29 ± 0.28ab
big eye tuna	1.77 ± 0.14c	6.50 ± 0.22b	11.49 ± 1.13d
yellowfin	1.23 ± 0.15b	8.19 ± 1.42c	9.93 ± 1.14cd
bluefin	1.25 ± 0.09bc	6.64 ± 0.32b	8.26 ± 0.25bc
bonito	0.52 ± 0.01a	11.68 ± 0.48d	6.01 ± 0.15ab

[a] Mean value ± standard deviation. Values in the same column followed by different letters are significantly different ($P < 0.05$).

Ratios between the most abundant individual PUFA were calculated (Table 5). Different groups were obtained when fatty acids of different biosynthesis pathways (ω3 and ω6 routes) were considered. The 22:6ω3/20:4ω6 ratio showed the same groups as the ω3/ω6 ratio. Interesting different groups were also observed for the 20:4ω6/20:5ω3 ratio; this ratio has been seen to differ among salmonid species (Henderson and Tocher, 1987). For the 22:6ω3/20:5ω3 ratio, the species were not found to be segregated into defined groups, although significant differences were noted in the case of extreme values.

It is concluded that there is a similar PUFA distribution among the different tuna species. However, some differences in the fatty acids ratios have been observed,

which could be explained as a result of external factors and internal or metabolic needs.

Changes in the *sn*-1/*sn*-2 Composition of Albacore after Thermal Treatment. Cooking led to a decrease in the lipid content of the muscle and an increase in the proportion of PL in total lipids; the PL content in the muscle remained quite constant (Table 1). The preferential loss of depot lipids (TG, principally) during cooking was noted previously in different zones of albacore muscle (Gallardo et al., 1989).

Table 2 shows a comparison between the fatty acid compositions of both PL positions before and after the cooking of albacore muscle. Some significant changes were observed; the behaviors of both locations were different. The *sn*-2 position showed a decrease in the 22:6ω3 proportion. This result agrees with the preferential thermal (cooking and sterilization) hydrolysis which has been observed for PUFA in the *sn*-2 position related to all kinds of glyceride lipids (Medina et al., 1994b). In contrast, an increase in the proportion of 18:1ω9 was observed.

With reference to the *sn*-1 position, some differences were noted after cooking. The most abundant fatty acid (18:0) showed a decrease in its proportion, while the proportions of some PUFA (20:4ω6 and 22:6ω3) increased. Apparently, this result does not agree with the fact that PUFA are more unstable to heat than ST. No previous information is available about the behavior of the *sn*-1 location of PL during a thermal treatment. However, during vegetable oil (TG, principally) processing, 1,2-DG present in fresh virgin oil have been shown to be converted into the 1,3-DG form when refined and/or stored for some time (Leone et al., 1988; Amelotti et al., 1989).

As a result, it is concluded that a more accurate study of the changes occurring in PL during thermal processing is obtained when analyzing both positions separately. Additional experiences (labeled fatty acids, and so on) are recommended to ascertain whether there is any interaction between the compositions of both positions during cooking.

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