Lipid Changes in Muscle Tissue of Ray (*Raja clavata*) during Processing and Frozen Storage

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The object of the work was to analyze different lipid compounds (fatty acids and classes of lipids—phospholipids, triacylglycerides, free fatty acids, sterols, and sterol esters plus waxes) of ray muscle (*Raja clavata*) and to determine the possible changes originated in frozen storage at temperatures of −18 ± 1 and −40 ± 2 °C during 36 and 180 days, comparing the influence that the particle size (8 and 12 mm) of the mince has on the quality of the frozen ray muscle. On day 0, the main fatty acids detected, except 20:5n − 3, showed higher levels in the previously minced samples. The triacylglycerides were observed in the whole muscle, and the phospholipids represented the majority fraction. During the time in which the sample remained frozen, independent of the particle size and the freezing temperature, the levels increased in the storage period and the majority of the fatty acids. 22:5n − 3 and 22:6n − 3 experienced only slight variations. After 180 days and when minced ray muscle was used, higher quantities of polyunsaturated fatty acids and n − 3 HUFA were observed in the greater sized particle samples.

INTRODUCTION

Modification in lipids during processing and storage of sea fish is one of the main problems which cause deterioration in quality from chemical and sensorial points of view.

In sea fish, long-chain lipids, mainly polyunsaturated fatty acids, are abundant; during processing and freezing of the fish, these lipids undergo changes due to oxidation.

Some authors maintain that the changes which occur in lipids during frozen storage are the result of hydrolysis of the phospholipids which decompose and produce an increase in free fatty acids (Bligh et al., 1966; Kinumaki et al., 1970; Braddock and Dugan, 1972; Viswanathan and Gopakumar, 1985; Srikar et al., 1989; de Koning and Mol, 1990). Other authors think that the lipid deterioration must also be attributed to polyunsaturated fatty acid oxidation (Takama et al., 1967, 1971; Suzuki et al., 1985) or to neutral lipid hydrolysis (de Koning et al., 1987; de Koning and Mol, 1990). There is no bibliography on the chemical behavior of ray in cold storage.

One of the authors' technological objectives is the revaluation of the ray species, to improve its low consumption. On these lines research has been carried out (Pastoriza and Sampedro, 1991) to examine the ray's capacity for possible processing as a new commercial quality product, making the capture of this fish resource profitable.

In this work, the variations produced in ray lipids (*Raja clavata*) during processing and frozen storage have been studied to discover the changes that cause alterations in the quality of the fish.

MATERIALS AND METHODS

The ray (*R. clavata*) was caught in April 1990 off the Galician coast and kept in ice for less than 24 h before processing. In the pilot plant 80 kg of ray was used, and after removal of the wings, the fish were skinned manually.

For freezing three different groups were prepared: (a) blocks or ray wings; (b) blocks of minced ray wing muscle, particle size 8 mm; and (c) blocks of minced ray wing muscle, particle size 12 mm.

A mincer (MOBBA 3CV) was used to prepare the minced ray wings, utilizing different orifice caliber disks in each case. The weight of each block was 3 kg (35 × 25 × 3.5 cm). The blocks were packed in polythene bags and pressed to remove air bubbles. The freezing of samples was carried out at temperatures of −18 ± 1 and −40 ± 2 °C in ultralow temperature freezers (Forma Scientific) provided with a temperature recorder; the samples were kept in storage individually in cardboard boxes for 180 days at the given temperatures.

The muscle sample analysis was carried out before freezing (day 0) and after 36 and 180 days in the freezing cabinet at the forementioned temperatures. All samples underwent freeze-drying before the analytical lipid determination.

Lipids were extracted following a modification of the method of Bligh and Dyer (1959). Lipids were first extracted with chloroform–methanol (1:2), and after centrifugation, the sediment was extracted again with chloroform–methanol (2:1); both supernatants were then washed with chloroform–methanol–water (8:4:3) (Folch et al., 1957).

Total lipids were determined according to the method of Marsh and Weinstein (1966) using tripalmitin as a standard. Fatty acids from total lipid were transesterified to methyl esters with methanolic hydrogen chloride as described by Christie (1982) and subsequently analyzed by gas–liquid chromatography as previously described (Fernández-Reiriz et al., 1989). Nonadecanoic acid was used as internal standard, and a response factor was calculated for each fatty acid to perform quantitative analysis.

Lipid classes were studied by thin-layer chromatography (TLC)–densitometry. Silica gel 60 W plates (Merck 16486) with a size of 20 × 20 cm and a layer thickness of 0.25 mm were used. Samples were applied on the plate with the help of automatic TLC sampler (Camag 27220). The chromatographic stain was made by following the technique described by Freeman and West (1966). The plates were stained with a 10% $CuSO_4$ solution in 0.85% H_3PO_4 by heating to 180 °C (Bitman and Wood, 1982). For the quantitative analysis, cholesterol palmitate, cholesterol, palmitic acid, and tripalmitin (Sigma) were used as standards for sterol esters plus waxes, sterols, free fatty acids, and triacylglycerides, respectively. For phospholipids a standard obtained from ray (*Raja radiata*) was used. The plates were scanned with a Shimadzu CS9000 desitometer, using a beam monochromatic 370 nm of 0.4 × 0.4 mm working in the zigzag mode, reading the whole spot, and with automatic autozero for baseline correction.

Ashes were determined for calcination at 550 °C during 24 h.

RESULTS

Table I gives the percentages (dry weight basis) of lipids and ashes in the ray subjected to the different

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Table I. Percent Lipids and Percent Ashes in Whole Ray Muscle and in Minced Ray Muscle[a]

		36 days		180 days	
	0 days	−18 °C	−40 °C	−18 °C	−40 °C
		Whole Muscle			
ash	3.69 (0.06)	6.09 (0.08)	6.82 (0.05)	7.06 (0.09)	6.08 (0.08)
lipids	3.55 (0.05)	3.40 (0.02)	3.21 (0.04)	3.77 (0.04)	3.96 (0.02)
		Minced Muscle (8 mm)			
ash	3.83 (0.07)	4.91 (0.08)	4.61 (0.05)	4.03 (0.09)	4.69 (0.06)
lipids	3.87 (0.04)	3.52 (0.05)	3.27 (0.05)	3.91 (0.02)	3.92 (0.05)
		Minced Muscle (12 mm)			
ash	3.97 (0.05)	4.61 (0.08)	4.67 (0.05)	4.49 (0.05)	4.53 (0.04)
lipids	3.95 (0.03)	3.62 (0.04)	3.96 (0.04)	4.88 (0.05)	3.77 (0.04)

[a] Standard deviations in parentheses, $n = 3$.

Table II. Fatty Acids and Fatty Acid Families in Whole Ray Muscle (Milligrams per Gram of Dry Weight)[a]

		36 days		180 days	
	0 days	−18 °C	−40 °C	−18 °C	−40 °C
14:0	0.08 (0.01)	0.12 (0.01)	0.10 (0.01)	0.33 (0.01)	0.35 (0.01)
16:0	0.40 (0.02)	2.03 (0.10)	2.01 (0.12)	4.99 (0.30)	6.09 (0.30)
16:1$n-9$	0.00 (0.00)	0.07 (0.01)	0.07 (0.01)	0.00 (0.00)	0.00 (0.00)
16:1$n-7$	0.09 (0.01)	0.12 (0.01)	0.11 (0.01)	0.37 (0.02)	0.40 (0.02)
17:0	0.09 (0.01)	0.20 (0.01)	0.11 (0.01)	0.00 (0.00)	0.00 (0.00)
17:1$n-7$	0.08 (0.01)	0.14 (0.01)	0.15 (0.01)	0.00 (0.00)	0.00 (0.00)
18:0	0.60 (0.03)	0.59 (0.03)	0.68 (0.04)	1.99 (0.10)	2.12 (0.11)
18:1$n-9$	0.80 (0.04)	0.97 (0.05)	0.90 (0.06)	1.85 (0.09)	2.46 (0.13)
18:1$n-7$	0.22 (0.02)	0.29 (0.02)	0.27 (0.02)	0.77 (0.05)	0.91 (0.04)
18:2$n-6$	0.11 (0.01)	0.08 (0.01)	0.09 (0.01)	0.28 (0.01)	0.27 (0.01)
18:3$n-6$	0.06 (0.01)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
20:1$n-9$	0.19 (0.02)	0.15 (0.01)	0.20 (0.01)	0.41 (0.03)	0.32 (0.02)
20:3$n-6$	0.09 (0.01)	0.21 (0.01)	0.19 (0.01)	0.39 (0.02)	0.41 (0.03)
20:4$n-6$	0.43 (0.02)	0.53 (0.02)	0.40 (0.02)	1.15 (0.06)	0.89 (0.04)
20:4$n-3$	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.09 (0.01)	0.12 (0.01)
20:5$n-3$	0.55 (0.03)	0.46 (0.02)	0.48 (0.03)	1.01 (0.05)	0.88 (0.04)
22:5$n-3$	0.29 (0.02)	0.41 (0.02)	0.35 (0.02)	0.25 (0.01)	0.27 (0.02)
22:6$n-3$	2.92 (0.15)	2.78 (0.18)	2.91 (0.16)	3.20 (0.17)	2.75 (0.16)
Σ satd	1.17 (0.04)	2.93 (0.11)	2.90 (0.12)	7.31 (0.14)	8.56 (0.32)
Σ monounsatd	1.37 (0.06)	1.74 (0.06)	1.69 (0.06)	3.40 (0.11)	4.08 (0.14)
Σ polyunsatd	4.44 (0.15)	4.47 (0.18)	4.41 (0.16)	6.37 (0.19)	5.59 (0.17)
$\Sigma n-3$	3.76 (0.15)	3.65 (0.18)	3.74 (0.16)	4.56 (0.18)	4.02 (0.17)
$\Sigma n-6$	0.68 (0.02)	0.82 (0.02)	0.67 (0.02)	1.82 (0.06)	1.57 (0.05)
$\Sigma n-7$	0.38 (0.02)	0.50 (0.02)	0.53 (0.02)	1.13 (0.05)	1.31 (0.04)
$\Sigma n-9$	0.99 (0.04)	1.20 (0.05)	1.16 (0.06)	2.27 (0.09)	2.77 (0.13)
$\Sigma n-3$ HUFA	3.76 (0.15)	3.64 (0.18)	3.74 (0.16)	4.46 (0.18)	3.90 (0.17)
$n-3/n-6$	5.52	4.45	5.58	2.50	2.56

[a] Standard deviations in parentheses, $n = 2$.

treatments, showing for lipids similar percentages in all cases (around 3.5%). As far as ashes are concerned, on day 0 similar percentages are observed (whether the sample is whole or minced), approximately 4%. During storage (36 and 180 days) slightly higher percentages are seen in the whole sample.

Fatty Acids. Tables II–IV represent the quantities (milligrams of fatty acid per gram of dry weight) of fatty acids and the different fatty acid families.

With reference to the individualized fatty acids, although the whole and the minced ray present similar profiles regarding the fatty acids detected (Tables II–IV) at the beginning of the experiment (day 0), differences exist according to whether the ray had been previously minced or not, with greater levels in the main fatty acids of the minced ray (except 20:5$n-3$) being observed.

During frozen storage time, a similar behavior in the evolution of the different fatty acids detected is observed, irrespective of whether the sample is whole or minced, the size of the minced particles (8 and 12 mm), and the freezing temperature (−18 and −40 °C). In 22:5$n-3$ and 22:6$n-3$, the quantities undergo only slight variation. In the rest of the fatty acids detected (Tables II–IV) the levels augment during the time that the sample remains frozen. Variations are observed regarding the size of the mince, showing that the levels of all of the fatty acids detected are lower when the particle size is 12 mm except 20:5$n-3$, 22:5$n-3$, and 22:6$n-3$ at both temperatures.

In the evolution of the different fatty acid families (Tables II–IV) it is observed that the quantities of saturated and monounsaturated fatty acids increase during the 180 days of freezing, but in the case of the mince, after 36 days, there is scarcely any difference with regard to the levels observed at the beginning of the experiment. The most noticeable differences are seen after 180 days.

As far as the polyunsaturated fatty acids are concerned, the quantities increase throughout the process, and it must be emphasized that the levels are greater in the minced samples than in the whole ones. Furthermore, between the minced samples the amounts of the said polyunsaturated fatty acids are higher in the 12-mm samples at both storage temperatures. With respect to the saturated and monounsaturated fatty acids, small differences are seen in relation to the particle size.

Class of Lipids. Table V corresponds to the different classes of lipids studied (milligrams per gram of dry

Table III. Fatty Acids and Fatty Acid Families in Minced (8 mm) Ray Muscle (Milligrams per Gram of Dry Weight)[a]

		36 days		180 days	
	0 days	−18 °C	−40 °C	−18 °C	−40 °C
14:0	0.10 (0.01)	0.05 (0.01)	0.12 (0.01)	0.33 (0.02)	0.40 (0.02)
16:0	2.07 (0.12)	1.59 (0.08)	2.33 (0.12)	7.39 (0.37)	7.72 (0.40)
16:1$n-9$	0.00 (0.00)	0.04 (0.01)	0.07 (0.01)	0.00 (0.00)	0.00 (0.00)
16:1$n-7$	0.08 (0.01)	0.07 (0.01)	0.05 (0.01)	0.43 (0.03)	0.42 (0.02)
17:0	0.10 (0.01)	0.05 (0.01)	0.11 (0.01)	0.00 (0.00)	0.00 (0.00)
17:1$n-7$	0.12 (0.01)	0.08 (0.01)	0.05 (0.01)	0.00 (0.00)	0.00 (0.00)
18:0	0.71 (0.03)	0.43 (0.02)	0.71 (0.03)	2.20 (0.10)	2.44 (0.13)
18:1$n-9$	0.87 (0.04)	0.67 (0.03)	0.99 (0.05)	2.86 (0.15)	3.04 (0.17)
18:1$n-7$	0.26 (0.02)	0.19 (0.02)	0.33 (0.02)	0.99 (0.05)	1.02 (0.05)
18:2$n-6$	0.09 (0.01)	0.08 (0.01)	0.11 (0.01)	0.30 (0.01)	0.46 (0.03)
18:3$n-6$	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
20:1$n-9$	0.20 (0.01)	0.15 (0.01)	0.14 (0.01)	0.46 (0.02)	0.40 (0.03)
20:3$n-6$	0.12 (0.01)	0.08 (0.01)	0.22 (0.01)	0.43 (0.02)	0.51 (0.02)
20:4$n-6$	0.41 (0.02)	0.11 (0.01)	0.53 (0.03)	0.95 (0.04)	0.97 (0.05)
20:4$n-3$	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.17 (0.01)	0.22 (0.01)
20:5$n-3$	0.31 (0.02)	0.27 (0.02)	0.47 (0.03)	0.94 (0.04)	1.05 (0.05)
22:5$n-3$	0.34 (0.02)	0.32 (0.02)	0.36 (0.02)	0.27 (0.02)	0.25 (0.01)
22:6$n-3$	3.34 (0.17)	2.62 (0.15)	3.57 (0.21)	3.11 (0.18)	2.47 (0.13)
Σ satd	2.99 (0.12)	2.12 (0.08)	3.26 (0.12)	9.92 (0.38)	10.56 (0.16)
Σ monounsatd	1.53 (0.04)	1.21 (0.04)	1.63 (0.05)	4.75 (0.16)	4.87 (0.18)
Σ polyunsatd	4.61 (0.14)	3.47 (0.15)	5.26 (0.21)	6.17 (0.19)	6.04 (0.15)
$\Sigma n-3$	3.99 (0.17)	3.21 (0.16)	4.40 (0.21)	4.49 (0.18)	3.99 (0.14)
$\Sigma n-6$	0.62 (0.02)	0.28 (0.02)	0.87 (0.03)	1.68 (0.04)	1.94 (0.06)
$\Sigma n-7$	0.46 (0.02)	0.34 (0.02)	0.42 (0.02)	1.43 (0.05)	1.43 (0.03)
$\Sigma n-9$	1.07 (0.04)	0.87 (0.03)	1.20 (0.05)	3.32 (0.15)	3.44 (0.17)
$\Sigma n-3$ HUFA	4.00 (0.17)	3.21 (0.15)	4.40 (0.21)	4.32 (0.18)	3.77 (0.14)
$n-3/n-6$	6.43	11.46	5.05	2.67	2.05

[a] Standard deviations in parentheses, $n = 2$.

Table IV. Fatty Acids and Fatty Acid Families in Minced (12 mm) Ray Muscle (Milligrams per Gram of Dry Weight)[a]

		36 days		180 days	
	0 days	−18 °C	−40 °C	−18 °C	−40 °C
14:0	0.07 (0.01)	0.10 (0.01)	0.06 (0.01)	0.32 (0.02)	0.27 (0.01)
16:0	2.16 (0.11)	1.95 (0.10)	1.91 (0.09)	6.69 (0.34)	6.00 (0.31)
16:1$n-9$	0.00 (0.00)	0.05 (0.01)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
16:1$n-7$	0.09 (0.01)	0.10 (0.01)	0.08 (0.01)	0.42 (0.03)	0.35 (0.02)
17:0	0.08 (0.01)	0.10 (0.01)	0.09 (0.01)	0.00 (0.00)	0.00 (0.00)
17:1$n-7$	0.09 (0.01)	0.10 (0.01)	0.15 (0.01)	0.00 (0.00)	0.00 (0.00)
18:0	0.64 (0.04)	0.65 (0.03)	0.58 (0.06)	1.99 (0.10)	1.99 (0.10)
18:1$n-9$	0.84 (0.04)	0.85 (0.05)	0.76 (0.04)	2.67 (0.13)	2.24 (0.12)
18:1$n-7$	0.27 (0.02)	0.25 (0.01)	0.23 (0.01)	0.95 (0.05)	0.80 (0.06)
18:2$n-6$	0.09 (0.01)	0.10 (0.01)	0.08 (0.01)	0.38 (0.02)	0.36 (0.02)
18:3$n-6$	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
20:1$n-9$	0.19 (0.01)	0.20 (0.01)	0.18 (0.01)	0.49 (0.03)	0.47 (0.03)
20:3$n-6$	0.14 (0.01)	0.15 (0.01)	0.10 (0.01)	0.39 (0.02)	0.40 (0.03)
20:4$n-6$	0.45 (0.03)	0.40 (0.02)	0.37 (0.01)	1.09 (0.05)	0.86 (0.05)
20:4$n-3$	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.15 (0.01)	0.11 (0.01)
20:5$n-3$	0.47 (0.03)	0.45 (0.02)	0.38 (0.01)	1.21 (0.04)	1.16 (0.06)
22:5$n-3$	0.36 (0.02)	0.35 (0.01)	0.32 (0.02)	0.41 (0.03)	0.38 (0.02)
22:6$n-3$	3.57 (0.20)	2.95 (0.14)	3.00 (0.12)	4.36 (0.25)	4.30 (0.26)
Σ satd	2.95 (0.12)	2.75 (0.10)	2.64 (0.11)	8.99 (0.34)	8.26 (0.31)
Σ monounsatd	1.47 (0.05)	1.55 (0.05)	1.41 (0.04)	4.52 (0.15)	3.85 (0.14)
Σ polyunsatd	5.07 (0.20)	4.40 (0.14)	4.24 (0.12)	7.99 (0.26)	7.57 (0.27)
$\Sigma n-3$	4.40 (0.20)	3.75 (0.14)	3.70 (0.12)	6.13 (0.25)	5.95 (0.27)
$\Sigma n-6$	0.67 (0.02)	0.65 (0.02)	0.55 (0.01)	1.86 (0.18)	1.62 (0.06)
$\Sigma n-7$	0.44 (0.02)	0.45 (0.01)	0.38 (0.01)	1.37 (0.06)	1.15 (0.06)
$\Sigma n-9$	1.03 (0.04)	1.05 (0.05)	0.94 (0.04)	3.16 (0.13)	2.70 (0.12)
$\Sigma n-3$ HUFA	4.40 (0.20)	3.75 (0.14)	3.70 (0.12)	5.98 (0.25)	5.84 (0.27)
$n-3/n-6$	6.56	5.76	6.72	3.29	3.67

[a] Standard deviations in parentheses, $n = 2$.

weight). At the beginning of the experiment (day 0) the phospholipids represent the majority fraction in all cases. In the rest of the lipids studied, their levels varing according to whether the sample had been previously minced or not.

So, in the whole sample, the amount of phospholipids is followed by that of sterols, free fatty acids, triacylglycerides, and, in continuation and in lesser proportions, the sterol esters plus waxes. The minced sample shows similar sterol and free fatty acid levels followed by sterol esters plus waxes, which are the only lipids that have different quantities according to the mince particle size.

During the time that the ray remains frozen, it is

Table V. Classes of Lipids (Milligrams per Gram of Dry Weight)[a]

	0 days	36 days		180 days	
		−18 °C	−40 °C	−18 °C	−40 °C
		Whole Muscle			
PL	15.80 (0.80)	12.62 (0.65)	12.05 (0.80)	13.44 (0.70)	15.35 (0.80)
St + W	0.12 (0.01)	0.00 (0.00)	2.57 (0.13)	0.00 (0.00)	2.73 (0.15)
TR	1.31 (0.07)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
FA	1.41 (0.07)	2.69 (0.12)	1.74 (0.10)	4.07 (0.20)	2.59 (0.13)
St	2.39 (0.13)	3.10 (0.17)	4.13 (0.22)	4.72 (0.30)	5.42 (0.28)
		Minced Muscle (8 mm)			
PL	14.68 (0.80)	11.18 (0.60)	9.73 (0.50)	8.20 (0.50)	16.11 (0.90)
St + W	0.67 (0.05)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	3.24 (0.18)
TR	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
FA	3.48 (0.18)	8.62 (0.50)	4.11 (0.21)	16.97 (0.92)	7.64 (0.40)
St	3.19 (0.15)	2.94 (0.15)	2.81 (0.14)	4.11 (0.21)	3.89 (0.20)
		Minced Muscle (12 mm)			
PL	17.28 (0.80)	10.81 (0.70)	11.05 (0.60)	11.94 (0.60)	15.09 (0.87)
St + W	2.43 (0.13)	2.66 (0.15)	0.00 (0.00)	2.80 (0.15)	0.00 (0.00)
TR	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
FA	3.34 (0.16)	5.59 (0.33)	3.63 (0.20)	17.74 (0.90)	6.92 (0.35)
St	2.82 (0.13)	2.93 (0.18)	3.08 (0.16)	3.36 (0.17)	3.09 (0.16)

[a] Standard deviations in parentheses, $n = 2$. PL, phospholipids; St + W, sterol esters plus waxes; TR, triacylglycerides; FA, fatty acids; St, sterols.

observed that in the whole sample phospholipid and triacylglyceride levels decrease and free fatty acid and sterol levels increase at both temperatures. However, the sterol esters plus waxes levels decrease at −18 °C but increase at −40 °C. The quantities that appear after 36 days of freezing are maintained practically without change at 180 days.

In the minced ray, it is seen that the phospholipids decrease but only if the ray was frozen at −18 °C. If the freezing temperature was −40 °C, the levels decrease after 36 days but increase after 180 days. The sterol levels remain practically constant, and the free fatty acids augment in all cases but more noticeably at a temperature of −18 °C. In the case of the triacylglycerides their presence is not observed in the minced samples. However, the sterol esters plus waxes decrease in the 8-mm minced ray when the freezing temperature is −18 °C; on the contrary, if the temperature is −40 °C, the levels increase. In the 12-mm minced ray, the quantities of the said lipids remain constant if the temperature is −18 °C but decrease at −40 °C.

DISCUSSION

On day 0, the main fatty acids detected except 20:5n − 3 show higher levels in the previously minced samples.

During the frozen storage time the fatty acids undergo a similar evolution in the whole or minced samples at the different temperatures. 22:5n − 3 and 22:6n − 3 undergo only slight variation, and in the rest of the fatty acids (Table II–IV) the levels are augmented during the storage period, maintaining higher levels after 180 days in the minced samples, and within these, the levels are superior for the smaller particle mince (8 mm).

After 180 days of storage and when minced ray muscle is used, higher polyunsaturated fatty acid and n − 3 HUFA quantities are seen in the larger size particle samples (12 mm). With respect to the saturated and monounsaturated fatty acids, small differences are observed in relation to particle size.

In the 8-mm minced sample, the decrease noted in the most polyunsaturated fatty acids and n − 3 HUFA (22:5n − 3 and 22:6n − 3) after 180 days of freezing is probably due to oxidation, since a smaller particle size produces a larger oxidizable surface, an effect that can be avoided by using an antioxidant.

The study of the different classes of lipids corroborates the tendencies shown by the total fatty acids, so at the beginning of the experiment (day 0) it is seen that the minced samples lack triacylglycerides and present greater quantities of free fatty acids than the whole ray, which leads us to suppose that during the processing of the raw material hydrolysis of the triacylglycerides could occur, shown by the said variation.

If the ray is frozen whole, after 36 days, the phospholipids, triacylglycerides, and sterol esters plus waxes decrease, but the sterols and free fatty acids increase in the same way that the total fatty acids augment. After 180 days, if the temperature is −18 °C, the previous tendency continues, but if the temperature is −40 °C, the phospholipid quantities increase slightly. Regarding the sterols, it must be mentioned that the levels remain practically unchanged throughout the process.

This could be the result of phospholipid hydrolysis at −18 °C producing an increase in free fatty acids, a process that is curbed at −40 °C. These observations were made by other authors in different species of fish (Bligh and Scott, 1966; Kinumaki et al., 1970; Viswanathan and Gopakumar, 1985; de Koning et al., 1987; Srikar et al., 1989; de Koning and Mol, 1990). So, Braddock and Dugan (1972) have ascertained that increases in free fatty acid content are primarily due to hydrolysis of the phospholipids catalyzed by phospholipases in the muscle and release of free fatty acids.

de Koning and Mol (1990), working with hake frozen at several temperatures, found that at temperatures of −5 and −18 °C samples showed an initial rapid surge of free fatty acid formation with a concomitant decrease in P content, followed by a second phase of shower-free fatty generation and loss of lipid P. At −40 °C only one phase of free fatty acid generation and loss of lipid P was observed, and the authors have observed a parallel evolution of the free fatty acid. Tressler (1950) and Le Gall (1950) say that to avoid enzyme action temperatures of around −40 to −30 °C are necessary. However, it is known that enzyme activity can exist below −40 °C.

With regard to the minced ray, it is also seen that when it is frozen at a lower temperature (40 °C), the phospholipid hydrolysis process begun at 36 days is apparently curbed.

The changes produced during the processing and storage

of the frozen products, which cause alterations in the fish quality, could be obviated by applying specific inhibiters which check the possible chemical modifications or using adequate equipment (vacuum mincer, homogenizer, and refrigerator).

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