DIFFERENTIAL LIPID DAMAGE IN VARIOUS MUSCLE ZONES OF FROZEN HAKE (*Merluccius merluccius*)

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SUMMARY

A comparison of the lipid damage produced in different hake zones was carried out during the frozen storage at -11°C and -18°C. Three light muscle zones and the dark muscle were considered. Lipid oxidation (conjugated dienes; thiobarbituric acid index, TBA-i; fluorescence formation) and hydrolysis (free fatty acids, FFA) were determined. The most predominant lipid damage in all zones was hydrolysis, reaching at the end of the storage values round 40% (for the light muscle zones) and 12% (for the dark muscle) of total lipids at -11°C. Significant ($p<0.05$) correlation values ($r = 0.67$-0.85) relationships between the frozen storage time and the FFA content were obtained for the four muscle zones at both temperatures. A comparison of regression lines slopes in the different zones showed that a lower ($p<0.05$) lipolitic activity was produced in the dark muscle compared to the three light zones at both temperatures. A low lipid oxidation development was produced in the three light muscle parts so that no significant differences between themselves could be assessed. However, the dark muscle showed a higher oxidation development (TBA-i and fluorescence formation) as a result of a higher lipid content and the presence of prooxidant constituents.

Key Words: Frozen storage, hake, lipid damage, muscle zones, quality

Running Title: Differential lipid damage in frozen hake

INTRODUCTION

During the frozen storage of fish, quality may decline as a result of several factors. One of the most important concerns the oxidation of highly unsaturated lipids present in fish species which leads to the production of off-flavours and odours and shorten the shelf life of fish products (1, 2).

Lipid damage studies during the frozen storage have been mostly focused in fatty fish species (3-5). In the case of lean fish such as gadoid species, quality loss has been mainly associated to formaldehyde (FA) formation; however, lipid hydrolysis and oxidation have shown to occur and become an important factor of fish acceptance (6, 7), as influencing protein denaturation, texture changes and functionality loss (8-10). Indeed, the relative influence of FA and lipid damage products in quality loss has been evaluated during the frozen storage of lean fish species (11, 12).

Fish constituents have not shown to be homogeneously distributed along the body of a fish (13, 14). Zonal differences such as lipid content, proximity to dark muscle and viscera, external or internal location and presence of non-lipid material can develop a differential lipid damage pattern. Some experiments related to fatty fish have reported notorious differences concerning the lipid damages of different zones during processing (3, 15-17).

In the case of lean fish species, some investigation has been carried out related to differences on lipid composition of the light and dark muscles (18). However, no information is available concerning the behaviour of different muscle parts as a result of processing and storage. The present work provides a comparison of lipid damages measured in four different zones of hake muscle during the frozen storage.

MATERIALS AND METHODS

Raw material, freezing, frozen storage and sampling

Fresh hake (*Merluccius merluccius*) was obtained in a local market. Upon arrival in our laboratory, individual fish were eviscerated, frozen at -40°C and then distributed into two storage conditions: -18°C and -11°C. At each storage temperature, hake was divided into three batches that were analysed separately along the whole experiment. Individual hakes of each batch were taken for subsequent analysis at the following storage times: 0, 1, 3, 6 and 12 months.

Four different muscle parts were considered and sampled separately in the present study (Figure 1): the dorsal (D) zone (placed upon most viscera and being a great proportion of fish flesh), the tail (T) zone (located far away from viscera, with a high collagen content and close proximity to dark muscle), anterodorsal (AD) zone (placed just upon the kidney, where a great FA content might be produced) and dark or red (R) muscle (with a higher lipid content and presence of prooxidants).

Water and lipid contents

Water content was determined by weight difference of the homogenised muscle (1-2 g) before and after 24 hours at 105°C; results were calculated as grams of water / 100 g of muscle. Lipids were extracted by the Bligh and Dyer (19) method; results were calculated as grams of lipids / 100 g of muscle.

Determination of lipid damages

Conjugated dienes (CD) formation was measured at 233 nm (20). Results are expressed according to the following formula: $CD = B \times V / w$, where B is the

absorbance reading at 233 nm, V denotes the volume (mL) of the sample and w is the mass (mg) of the lipid sample.

The thiobarbituric acid index (TBA-i) (mg malondialdehyde / kg sample) was determined according to Vyncke (21).

Free fatty acids (FFA) content was determined by the Lowry and Tinsley (22) method based on complex formation with cupric acetate-pyridine. Results are expressed as grams of FFA / 100 g lipids.

Fluorescence analysis

A Perkin-Elmer LS 3B fluorescence spectrophotometer was employed. Fluorescence formation was studied at 393/463 nm and 327/415 nm according to previous experiences (23-25). The relative fluorescence (RF) was calculated as: RF = F / F_{st}, where F is the sample fluorescence at each excitation/emission maximum, and F_{st} is the corresponding fluorescence intensity of a quinine sulphate solution (1 μg/mL in 0.05 M H_2SO_4). The fluorescence shift (δF) was calculated as the ratio between both RF values: $\delta F = RF_{393/463\ nm} / RF_{327/415\ nm}$ and was studied in the lipid extract resulting from the Bligh and Dyer (19) extraction.

Statistical analysis

The *Statistica* package (26) was employed. Data from the different lipid damage measurements were subjected to the ANOVA one-way method and correlation analysis ($p < 0.05$). Regression analysis and slope comparison of FFA in the four muscle zones were carried out according to Glantz and Slinker (27).

RESULTS

Water contents ranged between 79% and 82% in the three light muscle zones. The dark muscle showed a lower proportion that was included in the range 69-74%. No differences in the water content were obtained as a result of the time/temperature conditions during the frozen storage in any of the four zones.

No differences in the lipid content were also obtained in any of the four zones as a result of the frozen storage. Lipid contents ranged between 7% and 12% in the dark muscle and between 0.5% and 1.1% in the three light muscle zones, according to a known inverse relationship between lipid and water contents (3, 15). No differences could be inferred in the lipid content between the three light zones. In the case of fatty fish species, differences in lipid content have been shown between light muscle zones (16, 17, 28).

Lipid oxidation

At the level of primary oxidation, little significant differences were obtained between the three white muscle zones along the different time/temperature conditions (Table 1). It could be assumed that conjugated dienes (CD) formation was the same in all of them. However, the dark muscle showed in all cases lower levels than the other three zones.

If we consider the time/temperature CD evolution in each zone, a general increase in the four zones is observed at month 1 at -11°C; then, at this temperature, a decrease is observed at month 3, followed by no changes till the end of the storage. This increase lack, in spite of increasing the storage time, could be explained by the fact that conjugated dienes have been reported to be relatively unstable and capable of interacting with other constituents present in the muscle leading to relatively lower

levels in the CD detection (23, 29, 30). In the case of the dark muscle, higher values are obtained in the -11°C samples than in the corresponding -18°C ones; in the light muscles however, no differences as a result of the storage temperature can be pointed out after 3 months of storage.

Concerning the secondary lipid oxidation, the three white muscles showed relatively low TBA-i values (Table 2) compared to frozen fatty fish results (3, 25) and could not be significantly differentiated between themselves. In most cases higher values were obtained for the dark muscle, that showed a higher oxidation development, similar to the one obtained for a light muscle from a fatty fish species. The dark muscle showed higher values for samples stored at -11°C than for the corresponding -18°C ones. Little differences where obtained for each of the light muscle zones as a result of the storage temperature.

Considering the time/temperature evolution of TBA-i values obtained, a progressive increase till month 6 was observed for the four zones. Then, after 12 months, a slight decrease was observed that could be explained by the fact that TBA reactive substances are prone to interact with biological constituents present in the fish muscle leading to a decrease in the TBA-i detection in spite of increasing the fish damage (20, 31).

Interaction compounds formed by reaction between lipid oxidation products and biological amino constituents were measured by the fluorescence ratio values (23-25) (Table 3). The one month sampling did not provide significant differences between the four zones. However, after 3 months of storage at -11°C higher δF values were obtained in the case of the dark muscle than in the light ones, showing again as in the case of the TBA-i a higher oxidation level; this higher δF value was maintained till the end of the storage. At -18°C very little differences were obtained between the four zones.

If we consider the time/temperature evolution of the δF value in each zone, little significant differences were obtained in the case of the three light zones till month 6, that was followed by a general increase at the end of the storage at both temperatures. In the case of the dark muscle little significant differences were obtained at -18°C as a result of the storage time, although an increasing tendency could be inferred; at -11°C, an increase was observed after 3 months followed by no changes till the end of the storage.

Lipid hydrolysis

It can be observed (Table 4) that hydrolytic activity has been very strong in the four zones as a result of the time/temperature conditions of the present experiment. As a general tendency in all zones considered, hydrolysis increased progressively with time and temperature, reaching at the end of the storage values round 40% (for the light muscle zones) and 12% (for the dark muscle) of total lipids at -11°C. Higher FFA contents were observed at -11°C than at -18°C in the four muscle zones, specially after 6 and 12 months.

Comparison of the different muscle zones showed very little differences between the three light zones. However, the dark muscle showed in all cases lower FFA contents than the other zones. Initial FFA value in the dark zone was very low because of the big proportion of neutral lipid classes responsible for the energy accumulation in the lipid form (18, 32). The anterodorsal zone showed the highest FFA initial level; however, after 3 months of storage at both temperatures, no differences were obtained compared to the two other light zones.

DISCUSSION

Lipid damage has been studied through the hydrolytic and oxidative changes. Hydrolytic activity provided the best assessment of lipid damage along the present experiment. Previous experiences on frozen storage of a lean fish agree with this result (33, 34), where this kind of damage determination is presented as a valuable tool in order to assess quality. Table 5 shows the correlation values obtained between FFA content and the storage time at both temperatures for the four muscle zones. In all cases, significant ($p < 0.05$) values were obtained.

In order to compare the rates of hydrolytic activity of the different muscle zones, a statistical comparison of the regression lines slopes was carried out (27). As a result, no differences ($p<0.01$) in the hydrolytic activity were obtained between the three light muscles at both temperatures. However, the dark muscle showed a lower ($p<0.05$) slope than the three light muscles at both temperatures. No differences in the rates of FFA formation were obtained as a result of the storage temperature in each of the muscle zones.

About the origin of FFA during the frozen storage, some authors have attributed a decrease of the phospholipids content in lean fish as the only source of FFA (35, 36). However, de Koning et al. (37) attributed this FFA formation to hydrolysis of both neutral lipids and phospholipids. According to the comparison study of regression lines slopes, present results indicate that the zone with the lowest phospholipid proportion (dark muscle) (18) shows the lowest hydrolytic activity. Further research focused on the hydrolytic mechanism of light and dark muscles is intended by assessing molecules involved such as lipid classes (FFA, total phospholipids, triglycerides) and hydrolytic enzymes (lipases, phospholipases, triglyceride hydrolases).

Formation of FFA itself does not lead to nutritional losses (13). However, it has been proved that accumulation of FFA in frozen fish is related in some extent with lack

of acceptability of frozen fish, because FFA are known to cause texture deterioration by interacting with proteins (8-10). Also hydrolysis is reported to enhance the formation of lipid oxidation products and affect accordingly, fish quality during all kinds of fish processes (38-39).

On the other side, the relatively low lipid content found in the light muscle zones has led to a low oxidation development. Higher values for the lipid oxidation indices (TBA-i and δF value) were obtained for the dark muscle. In such zone oxidation can be favoured by factors such as the amount of lipids and the presence of prooxidant components as mioglobine (2, 3, 13).

As a result, significant ($p<0.05$) correlations were obtained for the frozen storage time with the TBA-i and δF value at -18°C ($r = 0.57$ and $r = 0.75$, respectively) and at -11°C ($r = 0.68$ and $r = 0.65$, respectively) for the dark muscle. In the three light zones, the corresponding correlation results were poorer. This differential lipid oxidation between dark and light muscle zones agrees with recommendation of some authors that the dark muscle should be removed before processing and storage without meaning an important weight loss in the final product (2, 13).

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FIGURE LEGEND

Figure 1. Situation in the hake body of the different muscle zones considered in the present study: dorsal (D), tail (T), anterodorsal (AD) and dark (R).

TABLE 1: Conjugated Diene values* obtained in the four muscle zones in the different time/temperature conditions

Frozen Temperature: -18°C				Storage Time (months)	Frozen Temperature: -11°C			
Dorsal	Tail	Anterodorsal	Dark		Dorsal	Tail	Anterodorsal	Dark
2.18 b (0.426)	2.33 b (0.502)	4.66 c (1.100)	0.94 a (0.246)	0	2.18 b (0.426)	2.33 b (0.502)	4.66 c (1.100)	0.94 a (0.246)
4.54 bc (0.592)	5.85 c (0.059)	3.79 b (1.303)	1.01 a (0.621)	1	10.69 b (3.516)	10.53 b (3.179)	12.26 b (0.986)	1.53 a (0.637)
6.97 b (0.264)	5.26 b (1.589)	5.27 b (0.093)	0.98 a (0.344)	3	4.71 b (0.842)	3.65 b (0.774)	5.32 b (1.281)	1.03 a (0.686)
4.51 b (0.955)	4.36 b (0.591)	4.87 b (0.809)	0.72 a (0.046)	6	4.06 b (0.995)	3.47 b (1.018)	4.87 b (0.508)	1.04 a (0.569)
5.45 b (0.733)	5.06 b (0.243)	6.03 b (2.543)	0.86 a (0.261)	12	4.89 b (1.240)	4.12 b (0.562)	5.54 b (0.511)	1.12 a (0.350)

* Means of three independent determinations. For each frozen temperature, values in the same row followed by different letters are significantly different ($p<0.05$). Standard deviations are indicated in brackets.

TABLE 2: Thiobarbituric acid values* obtained in the four muscle zones in the different time/temperature conditions

Frozen Temperature: -18°C				Storage Time (months)	Frozen Temperature: -11°C			
Dorsal	Tail	Anterodorsal	Dark		Dorsal	Tail	Anterodorsal	Dark
0.08 ab (0.086)	0.05 a (0.028)	0.06 ab (0.014)	0.17 b (0.069)	0	0.08 ab (0.086)	0.05 a (0.028)	0.06 ab (0.014)	0.17 b (0.069)
0.13 a (0.031)	0.15 a (0.049)	0.21 a (0.186)	0.47 b (0.170)	1	0.09 a (0.106)	0.06 a (0.030)	0.12 a (0.015)	0.56 b (0.162)
0.10 a (0.022)	0.14 a (0.072)	0.25 a (0.048)	1.12 b (0.394)	3	0.08 a (0.082)	0.05 a (0.023)	0.10 a (0.082)	4.48 b (2.303)
0.53 (0.272)	1.09 (0.177)	0.25 (0.012)	3.75 (2.842)	6	3.23 ab (1.611)	0.65 a (0.259)	2.09 a (2.324)	6.24 b (1.574)
0.68 ab (0.471)	0.28 a (0.269)	0.57 a (0.402)	2.91 b (2.360)	12	0.47 a (0.275)	0.59 a (0.085)	0.61 a (0.596)	4.93 b (1.521)

* Means of three independent determinations. For each frozen temperature, values in the same row followed by different letters are significantly different ($p<0.05$). Standard deviations are indicated in brackets.

TABLE 3: Fluorescence ratio values* obtained in the four muscle zones in the different time/temperature conditions

Frozen Temperature: -18°C				Storage Time (months)	Frozen Temperature: -11°C			
Dorsal	Tail	Anterodorsal	Dark		Dorsal	Tail	Anterodorsal	Dark
0.47 (0.114)	0.51 (0.105)	0.35 (0.131)	0.45 (0.245)	0	0.47 (0.114)	0.51 (0.105)	0.35 (0.131)	0.45 (0.245)
0.31 (0.052)	0.30 (0.070)	0.30 (0.075)	0.36 (0.077)	1	0.29 (0.021)	0.31 (0.111)	0.27 (0.047)	0.35 (0.079)
0.35 (0.328)	0.19 (0.009)	0.20 (0.060)	0.42 (0.016)	3	0.22 a (0.023)	0.25 a (0.072)	0.22 a (0.006)	2.14 b (1.588)
0.30 a (0.153)	0.30 a (0.095)	0.28 a (0.026)	0.64 b (0.032)	6	0.38 a (0.081)	0.52 ab (0.303)	0.56 ab (0.070)	1.49 b (1.273)
1.00 (0.538)	0.65 (0.239)	0.83 (0.261)	0.90 (0.342)	12	0.75 a (0.252)	0.61 a (0.099)	0.85 a (0.152)	1.54 b (0.060)

* Means of three independent determinations. For each frozen temperature, values in the same row followed by different letters are significantly different ($p<0.05$). Standard deviations are indicated in brackets.

TABLE 4: Free fatty acids content* obtained in the four muscle zones in the different time/temperature conditions

Frozen Temperature: -18°C				Storage Time (months)	Frozen Temperature: -11°C			
Dorsal	Tail	Anterodorsal	Dark		Dorsal	Tail	Anterodorsal	Dark
5.10 b (1.519)	4.43 b (0.943)	8.80 c (1.554)	1.44 a (1.115)	0	5.10 b (1.519)	4.43 b (0.943)	8.80 c (1.554)	1.44 a (1.115)
16.20 b (0.312)	17.16 b (2.994)	16.10 b (1.811)	2.72 a (1.273)	1	16.08 ab (4.257)	25.22 bc (1.681)	33.56 c (9.914)	7.18 a (2.349)
22.54 b (7.303)	21.26 b (3.311)	20.74 b (1.435)	4.17 a (1.612)	3	26.52 b (5.687)	22.51 b (6.528)	26.63 b (3.398)	4.67 a (0.481)
22.93 b (2.888)	21.41 b (3.476)	26.64 b (6.616)	3.60 a (0.656)	6	33.49 c (2.159)	27.22 b (1.273)	32.61 c (4.284)	9.53 a (1.577)
30.52 b (1.490)	27.00 b (2.407)	24.59 b (7.329)	7.85 a (2.219)	12	39.54 b (13.824)	42.18 b (9.042)	40.75 b (4.407)	12.49 a (4.370)

* Means of three independent determinations. For each frozen temperature, values in the same row followed by different letters are significantly different ($p<0.05$). Standard deviations are indicated in brackets.

TABLE 5

Correlation values between the free fatty acids content and the frozen storage time in the four muscle zones at both storage temperatures*

Muscle Zone	Temperature: -18°C	Temperature: -11°C
Dorsal	0.82	0.82
Tail	0.77	0.83
Anterodorsal	0.67	0.74
Dark	0.85	0.79

* Significant ($p < 0.05$) values were obtained in all cases.

FIGURE 15

Situation in the hake body of the different muscle zones considered: Dorsal (D), Tail (T), anterodorsal (AD) and dark (D)