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Differential lipid damage in various muscle zones of frozen hake (*Merluccius merluccius*)

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Abstract A comparison of the lipid damage produced in different hake zones was carried out during frozen storage at -11 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, at the end of storage reaching values of about 40% (for the light muscle zones) and 12% (for the dark muscle) of the total lipids at -11 °C. Significant ($P < 0.05$) correlation value ($r = 0.67$ – 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 the regression lines slopes in the different zones showed that a lower ($P < 0.05$) lipolytic 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 them 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

Introduction

During frozen storage of fish, the 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 shortens the shelf life of fish products [1, 2].

Lipid damage studies during frozen storage have been mostly focused on fatty fish species [3–5]. In the case of lean fish such as gadoid species, quality loss has been mainly associated with formaldehyde (FA) formation; however, lipid hydrolysis and oxidation have been 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 frozen storage of lean fish species [11, 12].

Fish constituents have been shown to be inhomogeneously 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 the 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 of 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 damage measured in four different zones of hake muscle during 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 and -11 °C. At each storage temperature, hake was divided into three batches that were analysed separately during the whole experiment. Individual hake of each batch were taken for subsequent analysis at the following storage times: 0, 1, 3, 6 and 12 months.

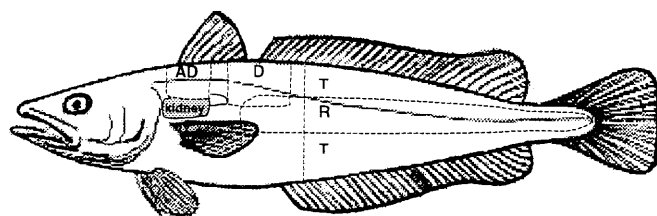


Fig. 1 Situation in the hake body of the different muscle zones considered in the present study: dorsal (D), tail (T), anterodorsal (AD) and dark (R)

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

Water and lipid contents. The water content was determined by weight difference of the homogenized muscle (1–2 g) before and after 24 h at 105 °C; the results were calculated as g of water/100 g of muscle. Lipids were extracted by the Bligh and Dyer [19] method; results were calculated as g of lipids/100 g of muscle.

Determination of lipid damage. Conjugated diene (CD) formation was measured at 233 nm [20]. The 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].

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

Fluorescence analysis. A Perkin-Elmer LS 3B fluorescence spectrophotometer was employed. Fluorescence formation was studied at 393/463 and 327/415 nm according to previous experience [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 sulfate solution (1 µg/ml in 0.05 M H₂SO₄). The fluores-

cence shift (δF) was calculated as the ratio between both RF values: $\delta F = RF_{393/463 \text{ nm}}/RF_{327/415 \text{ nm}}$ 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 the slope comparison of FFA in the four muscle zones were carried out according to Glantz and Slinker [27].

Results

Water content ranged between 79 and 82% in the three light muscle zones. The dark muscle showed a lower proportion and was 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 obtained in any of the four zones as a result of the frozen storage. Lipid content 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 content [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 during the different time/temperature conditions (Table 1). It could be assumed that conjugated diene (CD) formation was the same in all of them. However, the dark muscle showed, in all cases, lower levels than the other three zones.

Table 1 Conjugated diene values^a obtained in the four muscle zones under the different time/temperature conditions

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

^a 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 parentheses

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 until the end of the storage. This lack of CD increase, in spite of increasing the storage time, could be explained by the fact that CDs have been reported to be relatively unstable and capable of interacting with other constituents present in muscle, leading to relatively lower levels of 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 could be observed 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 from each other. In most cases, higher values were obtained for the dark muscle, which showed a higher oxidation develop-

ment, similar to that 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 difference was observed for each of the light muscle zones as a result of the storage temperature.

Considering the time/temperature evolution of the TBA-i values obtained, a progressive increase until 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 fish muscle, leading to a decrease in the TBA-i detection in spite of the increasing 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 1-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

Table 2 Thiobarbituric acid values^a obtained in the four muscle zones under 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 (1.014)	0.17 b (0.069)	0	0.08 b (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)

^a 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 parentheses

Table 3 Fluorescence ratio values^a obtained in the four muscle zones under 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)

^a 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 parentheses

than for the light ones, showing again as in the case of the TBA-i a higher oxidation level; this higher δF value was maintained until the end of 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, few significant differences were obtained in the case of the three light zones until month 6, which was followed by a general increase at the end of storage at both temperatures. In the case of the dark muscle, few 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 up to the end of 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, at the end of storage reaching values around 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, especially 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 content than the other zones. The initial FFA value in the dark zone was very low because of the large proportion of neutral lipid classes responsible for energy accumulation in lipid form [18, 32]. The AD 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.

Table 4 Free fatty acids content^a obtained in the four muscle zones under the different time/temperature conditions

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

^a 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 parentheses

Discussion

Lipid damage has been studied through hydrolytic and oxidative changes. Hydrolytic activity provided the best assessment of lipid damage in the present experiment. Previous experiences of frozen storage of 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.

Concerning the origin of FFA during frozen storage, some authors have attributed a decrease in the phospholipid 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

Table 5 Correlation values between the free fatty acids content and the frozen storage time in the four muscle zones at both storage temperatures^a

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

^a Significant ($P < 0.05$) values were obtained in all cases

phospholipids. According to the comparison study of the regression lines slopes, the 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 planned by assessing the molecules involved such as the lipid classes (FFA, total phospholipids, triglycerides) and the hydrolytic enzymes (lipases, phospholipases, triglyceride hydrolases).

The formation of FFA itself does not lead to nutritional losses [13]. However, it has been proved that the accumulation of FFA in frozen fish is related to some extent with the 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 accordingly affect fish quality during all kinds of fish processes [38, 39].

On the other hand, 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 a zone, oxidation can be favoured by factors such as the amount of lipid and the presence of prooxidant components such as myoglobin [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 the recommendation of some authors that the dark muscle should be removed before processing and storage without making a significant weight loss in the final product [2, 13].

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