LIPID DAMAGE DURING FROZEN STORAGE OF GADIFORM SPECIES CAPTURED IN DIFFERENT SEASONS

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SUMMARY

Quality loss of two gadiform fish species (blue whiting, *Micromesistius poutassou*; hake, *Merluccius merluccius*) during frozen storage (–30°C and –10°C; up to 12 months) was studied. For it, hydrolytic (formation of free fatty acids, FFA) and oxidative (conjugated dienes, peroxide and interaction compound formation) lipid damage was analysed. For both species, individual fishes captured in two different trials (May and November) were considered. An increasing (p<0.05) lipid hydrolysis and oxidation (peroxide and interaction compound formation) was observed for all kinds of samples throughout the frozen storage. Interaction compound detection by fluorescence analysis showed the best correlation values with storage time. Some higher (p<0.05) hydrolysis development could be observed in hake captured in May than in its counterpart from the November trial, while frozen blue whiting did not provide definite differences for FFA formation between both trials. Concerning peroxide formation, higher (p<0.05) values were obtained for individual blue whiting and hake captured in November when compared to their corresponding May fish for both frozen storage conditions. Interaction compound formation was also found higher (p<0.05) for November hake fish than for its counterpart captured in May, while blue whiting did not provide definite differences between trials.

**Running Title:** Lipid damage in frozen gadiform species

**Keywords:** Hake, blue whiting, freezing, oxidation, hydrolysis, season, temperature
1. INTRODUCTION

Gadiforms is a large group of fish species (cod, hake, pollack, haddock, whiting, saithe, etc.) that represent an important percentage of the overall fish catching [1] and consumption [2] in most European countries. Thus, in addition to being commercialised as round fresh fish or fillets, most frozen fishery products like fillets, fish fingers and surimis are made from whole or minced muscle of these fish [3].

Frozen storage has been widely employed to maintain fish properties before consumption or further use in other technological processes [4, 5]. In the case of frozen gadiform species, quality loss has been mainly associated with formaldehyde formation and its implication in quality loss [6, 7]. However, lipid hydrolysis and oxidation have been shown to occur and become an important factor of gadiform fish acceptance during the frozen storage as influencing the sensory quality [8], protein denaturation, texture changes, functionality loss [9-11] and formation of complexes between oxidised lipids and proteins [12, 13].

Marine species have shown wide lipid content and composition variations as a result of endogenous and exogenous effects [14]. Related to exogenous effects, the catching season has shown to play a key role regarding temperature, feeding availability and other external factors in different types of marine fatty species [15, 16]. According to the great incidence of lipid damage on fish quality, an important effect of the seasonal variations on damage development has been reported in processed marine fatty species [17-19]. Concerning lean fish species, studies of lipid content and composition variation as a result of the catching season [20, 21] and its further effect on food quality [22, 23] have been scarce, so that definite conclusions are not yet available.
The present work aims to the lipid damage evolution during frozen storage of two commercial gadiform fish species (blue whiting and hake). The effect of the time and temperature of storage on lipid hydrolysis and oxidation is analysed. Comparison between individual fishes captured at two different seasons is encountered.

2. MATERIALS AND METHODS

2.1. Raw material, processing and sampling

Blue whiting (*Micromesistius poutassou*; body length range: 25-28 cm) and hake (*Merluccius merluccius*; body length range: 39-44 cm) were captured at local fishing banks close to north-western Spain and kept on ice (10 h) until they arrived at the laboratory. Both species were studied at two different catching times: spring (May trial) and autumn (November trial). Two seasons were chosen because of their different external factors encountered (namely, temperature and feeding availability) and their possible different effect on lipid damage evolution during further processing. For each fish species studied, individuals of the same size and from the same capture zone were purchased in both trials. Individual fish gonads were at the 5th/6th stage (blue whiting) and at the 4th/5th stage (hake) of Maier’s scale of gonad maturity.

In both trials, individual fishes were eviscerated, beheaded, filleted and packaged in polyethylene bags. For hake experiments, two individual fishes were employed for each sampling point, while three fishes were employed in the case of blue whiting. All fish fillets were placed in a freezing room at −40ºC; after 48 hours, the fillets were distributed into two storage temperatures: −30ºC and −10ºC. For each fish species, storage temperature and trial, fillets were divided into three batches (n=3).
which were studied separately during the whole experiment to assess the statistical
study.

In all cases, analyses were carried out on the homogenised white muscle of the
initial fish material employed and at 1, 3, 5, 7, 9 and 12 months of frozen storage of the
different kinds of fish samples.

2.2. Water and lipid contents

Water content was determined by weight difference of the homogenised fish
muscle (1-2 g) before and after 24 h at 105°C. Results were calculated as g water/ 100g
flesh muscle. Lipids were extracted from the fish muscle by the Bligh and Dyer [24]
method. Results were calculated as g lipid/ 100g wet flesh muscle.

2.3. Free fatty acids assessment

Free fatty acid (FFA) content was determined on the lipid extract by the Lowry
Results are expressed as g FFA/100 lipid.

2.4. Methods used for lipid oxidation measurement

Conjugated dienes (CDs) formation was measured on the lipid extract according
to the Kim and Labella [26] method. The CDs content results are expressed as
absorption coefficients (AC), according to the formula: AC = B x V / w, where B is the
absorbance reading at 233 nm of an aliquot of the lipid extract, V denotes the aliquot
volume (ml) and w is the mass (mg) of the lipid material included in the aliquot.

Peroxide value (PV) expressed as meq oxygen/ kg lipid was determined on the
lipid extract according to the ferric thiocyanate method [27].
Formation of fluorescent compounds was determined with a Perkin Elmer LS 3B fluorimeter by measurements at wavelength of excitation and emission, as previously described [28]. The relative fluorescence (RF) was calculated as follows: \( RF = \frac{F}{F_{st}} \), where \( F \) is the fluorescence measured at each excitation/emission maximum, and \( F_{st} \) is the fluorescence intensity of a quinine sulphate solution (1 µg/ml in 0.05 M \( \text{H}_2\text{SO}_4 \)) at the corresponding wavelength. The fluorescence ratio (FR) was calculated as the ratio between the two RF values: \( FR = \frac{RF_{393/463\text{nm}}}{RF_{327/415\text{nm}}} \). The FR value was determined in the aqueous phase (methanol-water layer) resulting from the lipid extraction [24].

2.5. Statistical analysis

Data from the different lipid damage measurements were subjected to the ANOVA one-way method (\( p<0.05 \)) [29]; comparison of means was performed using a least-square difference (LSD) method. Correlation analysis between storage time and lipid damage indices was also studied.

3. RESULTS AND DISCUSSION

3.1. Water and lipid contents

A lower (\( p<0.05 \)) lipid content was obtained for blue whiting captured in May than for its counterpart corresponding to the November trial (Table 1); this difference was observed in the initial fish and maintained throughout the frozen storage. When the blue whiting water content is considered, the opposite result to the one obtained for the lipid matter is concluded, according to the initial and frozen fish values (Table 1). Results agree with previous research concerning the lipid content distribution in fatty
fish species where a higher lipid content is obtained in November than in May time [16, 19]. However, lipid content differences in the present case were not so marked as for fatty species.

Despite results concerning blue whiting, hake analysis did not show differences (p>0.05) in the lipid and water contents when comparing both trials (Table 1). No effect of the catching time could be observed in both constituents.

For both fish species, the time and temperature of frozen storage did not exert a significant (p>0.05) effect on both constituent contents. For each trial, differences observed in lipid and water contents may be explained as a result of fish-to-fish variation and heterogeneity between stocks; however, values were included in the ranges expressed in Table 1.

3.2. Lipid hydrolysis

The lipid hydrolysis evolution was studied by means of the FFA assessment. For both fish species (Figures 1 and 2), an important FFA formation (p<0.05) with time was observed for samples stored at –10ºC, while a partial inhibition on lipid hydrolysis could be outlined by lowering the storage temperature to –30ºC. According to previous research carried out on frozen lean fish species [13, 30], hydrolytic activity has shown to be sensitive to the storage time and temperature. In all cases, satisfactory correlation values were obtained with storage time (Tables 2 and 3), so that this quality index could be considered an accurate tool for assessing quality loss, according to previous research [9, 31].

Comparison between individual fishes from both catching times did not provide definite differences on FFA formation. In the case of blue whiting (Figure 1), opposite results are obtained depending on the time and temperature of storage considered. Thus,
a higher (p<0.05) FFA formation in November samples during a first storage period (months 1 and 3, and months 3 and 5 for –30°C and –10°C fish samples, respectively) is observed, while a higher FFA formation (p<0.05) in fish of the May trial was obtained for samples stored at –10°C during the 9-12 month period. For hake fish, individuals from the May catching led to a higher hydrolysis development than their corresponding November samples after 5 and 12 months of storage at –10°C; however, when the –30°C storage is considered, November fish stored during 1 month showed a higher (p<0.05) FFA content than in May fish in agreement with a higher (p<0.05) FFA value for the initial fish (Figure 2).

Enzymatic lipid hydrolysis has been shown to occur during fish frozen storage [10, 32]. Accumulation of FFA has been related to some extent to lack of acceptability, because FFA are known to have detrimental effects on ATPase activity, protein solubility, relative viscosity [33], cause texture deterioration by interacting with proteins [10, 11] and oxidise faster than higher molecular weight lipid classes (namely, triglycerides and phospholipids) by providing a greater accessibility (lower steric hindrance) to oxygen and other pro-oxidant molecules [34, 35].

The interaction of lipolysis and lipid oxidation is a particularly intriguing area of study as triglyceride hydrolysis has shown to lead to increased oxidation, while phospholipid hydrolysis produces the opposite effect [32, 36]. The release of FFA from a triacylglycerol matrix may accelerate their interaction with oxidative catalysts and hence accelerate the rate of lipid oxidation and generation of off flavours [37]; this pro-oxidant effect has been explained on the basis of a catalytic effect of the carboxyl group on the formation of free radicals by the decomposition of hydroperoxides [38]. In contrast, free fatty acid liberation from phospholipids would lead to a decreased
interaction between oxidised and oxidisable fatty acids within the membrane matrix, thus inhibiting free-radical propagation reactions [37, 39].

3.3. Lipid oxidation

The lipid oxidation evolution was studied by means of the CDs and peroxide content and by assessment of the fluorescent compound formation.

For both fish species, individuals captured in May showed a progressive CDs formation (Table 4) with time for both storage conditions, so that fair correlation values with time were obtained for blue whiting ($r^2 = 0.82$ and $0.95$; Table 2) and hake ($r^2 = 0.92$ and $0.94$; Table 3). For both November fish trials, the CDs content analysis did not provide an accurate assessment of rancidity development (Tables 2 and 3), since CDs values decreased in some cases with increasing time and temperature. Blue whiting from the November experiment provided a maximum CDs formation at the 1-5 month period when stored at $-30^\circ C$ and a clear tendency could not be outlined in samples kept at $-10^\circ C$. For November hake fish, a maximum CDs formation could be observed at the 3-7 month period for both storage temperatures, that was followed by a CDs content decrease. This breakdown has been reported to be more likely to be produced in cases of advanced rancidity development and can be explained by the fact that CDs compounds are produced during the first steps of oxidation development, being relatively unstable and susceptible to decompose into smaller molecules that are capable of interacting with other constituents present in muscle [28, 40, 41].

The CDs content analysis (Table 4) showed a higher formation at months 1 and 3 for the blue whiting November trial than in the case of its corresponding May experiment at both temperatures. However, this tendency was changed in the 7-12 month period, so that a higher CDs content was observed for blue whiting samples.
corresponding to the May trial for both storage temperatures. When hake is considered, comparison between May and November samples showed higher values for those corresponding to the May trial in most cases.

A progressive peroxide formation with time (Table 5) could be outlined in all cases, except for May blue whiting and November hake when being both kept frozen at –10°C ($r^2 = 0.87-0.95$; Tables 2 and 3). In such two cases, the highest values were obtained in the 5-7 month period and were followed by a PV decrease. For both fish species, a higher (p<0.05) peroxide formation was obtained in fish stored at –10°C than in its counterpart stored at –30°C when considering the 3-7 month period; at the end of the experiment, higher peroxide mean values were obtained in all cases for fish individuals stored at –30°C than in their corresponding samples kept at –10°C. According to the above mentioned CDs breakdown, instability of peroxide molecules can also explain the PV content decrease in advanced stages of rancidity, so that breakdown into smaller molecules (secondary lipid oxidation compounds) would be expected to undergo [28, 40, 41].

For blue whiting, comparison between both trials showed in most cases a higher peroxide content in November samples at both temperatures than in their counterpart individuals from May trial. In the case of hake, comparison between May and November trial samples showed higher mean values for fish captured in November for both storage temperatures; differences were significant at all storage times when considering the –30°C storage of both trials.

Present results on oxidation development (CDs formation and breakdown and peroxide formation) agree to previous research [19] carried out on frozen mackerel (whole fish and fillets) where a higher rancidity development was observed for individual fishes captured in November when compared to fish captured in May. A
similar result was also obtained when studying the rancidity development in frozen herring (*Clupea harengus*) captured at different catching times [17].

Freeze storage is known to be associated with fish lipid oxidation processes where different kinds of endogenous enzymes may be involved [5, 42]. Freezing and thawing may cause lysis of mitochondria and lysosomes and alter the distribution of enzymes and factors affecting the rate of enzyme reactions in tissues, so that deteriorative damage in frozen fish could be accelerated. At the same time, presence of such endogenous deteriorative enzymes may be influenced by a wide range of internal and external factors [36, 37]. Among the external factors, the catching season encountered in the present experiment has shown an important effect in the temperature and feeding habits and intensity, and accordingly, in the deteriorative enzyme content and composition. This different endogenous enzyme presence may be responsible for a different damage degree during the frozen storage.

A progressive FR increase (p<0.05) with storage time (Figures 3 and 4) was observed in all kinds of frozen fish. This increase was higher (p<0.05) in the case of samples stored at –10ºC than in their corresponding fish individuals stored at –30ºC, according to a preservative effect of temperature on lipid damage as previously reported for gadiform fish species [13, 30].

Among the different lipid damage indices tested in the present study, FR value provided the most satisfactory correlation values with time ($r^2 = 0.81-0.99$ in all cases; Tables 3 and 4). This parameter (FR value) had already proved to be an accurate tool for assessing fish quality loss during different commercial process [19, 28]. As a quality index, it is based on the interaction compound formation between lipid oxidation products (electrophilic substrates) and protein-like molecules (nucleophilic substrates) [41, 43] leading to interaction compounds with fluorescent properties. Such interaction
compounds should undergo a fluorescence shift towards higher wavelength maxima as a result of an increasing lipid damage and accordingly, an increasing fish product damage. This fluorescence shift was proposed to be measured by the FR value [28], as being the ratio between a higher (393/463 nm) and a lower (327/415 nm) excitation/emission pair (see Materials and Methods section). In addition, previous experimental evidence has demonstrated that fluorescent substances formed from oxidised membrane lipids remain attached to the amino constituents and result in compounds that are quite insoluble in organic solvents [28, 44]. Accordingly, the FR assessment in the present experiment was carried out on the resulting methanol-water layer from the lipid extraction (Materials and Methods section) [24].

Concerning the comparison between both catching times, hake samples corresponding to both frozen temperatures showed a higher (p<0.05) fluorescence formation in November individual fishes than in their counterparts corresponding to May sampling; indeed, a higher (p<0.05) FR value was detected for November fish samples stored at –30°C than in May samples stored at –10°C when considering fish samples stored 1 and 3 months.

In the case of blue whiting, some higher (p<0.05) fluorescence formation in fish corresponding to the May trial at months 7 (–10°C storage) and 9 (–30°C storage), but lower (p<0.05) at months 3 and 5 (–10°C storage) and at month 3 (–30°C storage) were obtained. Accordingly, a definite different tendency in fluorescence formation between frozen fish corresponding to both trials could not be concluded for blue whiting.

As it has been mentioned above, fluorescent compound formation depends not only on primary and secondary lipid oxidation compound formation, but also on the presence of nucleophilic molecules in the fish muscle. In this sense, amine compounds have been mentioned to play a catalytical effect on the condensation reaction between
lipid oxidation compounds [45, 46], being accorded an important effect of amine
structure on the fluorescent compound formation [47]. Indeed, an interesting
relationship has been observed between formation of interaction compounds during the
storage/processing of foods and the pigmented and fluorescent granules found in human
and animal tissues (lipofuscin) [48, 49].

Concerning the present results on hake analysis, FR value obtained has agreed to
differences found for peroxide development between both May and November samples.
However, FR assessment in blue whiting did not provide clear differences between both
fish trials, so that a varying amine content and composition may have been present in
blue whiting muscle from fish encountered in the present study and be responsible for
the lack of definite conclusions obtained in this sense.

Some correlation could be observed between FR and CDs values ($r^2 = 0.77-0.82$)
when considering the May samples for both fish species; however, for November
samples very poor correlation values were obtained as a result of the CDs breakdown in
the latest stages of the experiments. Correlation analysis between FR and PV parameters
led to some fair values when considering frozen fish stored at –30°C ($r^2 = 0.67-0.89$),
while samples stored at –10°C led to poorer results. It is concluded that fluorescent
compound formation was not accompanied by a progressive content decrease of CDs
and peroxides. Both kinds of primary lipid oxidation compounds would have continued
to be produced throughout the frozen storage while in the meantime, breakdown into
smaller molecules would also lead to interaction compound formation.
4. FINAL REMARKS

According to the FFA, CDs, PV and FR results, important lipid hydrolysis and oxidation events have developed in blue whiting and hake throughout the frozen storage at both temperatures, so that an important effect of hydrolytic and oxidant enzymes present in the fish muscle was evident.

Result comparison between both trials for each of the fish species studied has led to some marked differences in lipid oxidation development that could be explained as a result of a different deteriorative enzyme presence. Since the suitability of fish as raw material for the preparation of frozen products may partly depend on such endogenous enzyme presence, important efforts should be carried out to assess the effect of external factors such as the capture season (temperature and feeding availability, namely) on enzyme composition in fish muscle that is to be commercialised.

The effect of seasonal variability on quality of processed fish has been addressed in wild fish [17, 19] and farmed fish [18, 23] by checking the traditional quality damage indices in the resulting processed fish. Further research including the biochemical analysis of the endogenous enzyme composition at different seasons and its relationship with quality indices assessed in processed fish is expected to be carried out.

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**FIGURE LEGENDS**

Figure 1: Evolution of the free fatty acid (FFA) content in frozen (–30°C and –10°C) blue whiting captured at different times (May and November)

* Mean values of three independent determinations (n=3) are presented; bars denote standard deviations of the mean.

Figure 2: Evolution of the free fatty acid (FFA) content in frozen (–30°C and –10°C) hake captured at different times (May and November)

* Mean values of three independent determinations (n=3) are presented; bars denote standard deviations of the mean.

Figure 3: Evolution of the fluorescence ratio (FR) value in frozen (–30°C and –10°C) blue whiting captured at different times (May and November)

* Mean values of three independent determinations (n=3) are presented; bars denote standard deviations of the mean.

Figure 4: Evolution of the fluorescence ratio (FR) value in frozen (–30°C and –10°C) hake captured at different times (May and November)

* Mean values of three independent determinations (n=3) are presented; bars denote standard deviations of the mean.
Figure 1

Figure 2
Figure 3

Figure 4
### TABLE 1

Water (g/100g flesh muscle) and lipid (g/100g flesh muscle) contents in initial and frozen fish captured at different times

<table>
<thead>
<tr>
<th>Fish species</th>
<th>Catching Time</th>
<th>Lipid content * (initial fish)</th>
<th>Lipid content ** (value range in frozen fish)</th>
<th>Water content * (initial fish)</th>
<th>Water content ** (value range in frozen fish)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue whiting</td>
<td>May</td>
<td>0.43 ± 0.03 a</td>
<td>0.34–0.45</td>
<td>82.4 ± 0.4 b</td>
<td>81.5–83.3</td>
</tr>
<tr>
<td>Blue whiting</td>
<td>November</td>
<td>0.54 ± 0.04 b</td>
<td>0.47–0.57</td>
<td>78.8 ± 1.0 a</td>
<td>78.5–80.5</td>
</tr>
<tr>
<td>Hake</td>
<td>May</td>
<td>0.55 ± 0.05 a</td>
<td>0.45–0.59</td>
<td>80.6 ± 0.5 a</td>
<td>79.3–81.2</td>
</tr>
<tr>
<td>Hake</td>
<td>November</td>
<td>0.59 ± 0.07 a</td>
<td>0.49–0.61</td>
<td>81.3 ± 0.3 a</td>
<td>80.5–82.5</td>
</tr>
</tbody>
</table>

* Means of three independent determinations (n = 3) ± standard deviations. For each fish species, values followed by different letters (a, b) denote significant (p<0.05) differences between seasons.

** Each value range corresponds to fish stored during 1, 3, 5, 7, 9, and 12 months at –30ºC and –10ºC.
### TABLE 2

**Correlation coefficients*** between storage time and lipid damage indices** in frozen blue whiting captured at different times

<table>
<thead>
<tr>
<th>Storage Temperature</th>
<th>Catching Time</th>
<th>FFA</th>
<th>CDs</th>
<th>PV</th>
<th>FR</th>
</tr>
</thead>
<tbody>
<tr>
<td>–30º C</td>
<td>May</td>
<td>0.89</td>
<td>0.94</td>
<td>0.77</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.91)</td>
<td>(0.95)</td>
<td>(0.87)</td>
<td></td>
</tr>
<tr>
<td>–30º C</td>
<td>November</td>
<td>0.75</td>
<td>– 0.27</td>
<td>0.90</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.86)</td>
<td>(– 0.38)</td>
<td>(0.95)</td>
<td></td>
</tr>
<tr>
<td>–10º C</td>
<td>May</td>
<td>0.93</td>
<td>0.77</td>
<td>0.25</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.97)</td>
<td>(0.82)</td>
<td>(0.38)</td>
<td></td>
</tr>
<tr>
<td>–10º C</td>
<td>November</td>
<td>0.84</td>
<td>– 0.02</td>
<td>0.93</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.96)</td>
<td>(0.17)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Cuadratic\(^a\) and logarithmic\(^b\) correlation coefficients are expressed in brackets when superior to the linear ones.

** Abbreviations: FFA (free fatty acids), CDs (conjugated dienes), PV (peroxide value) and FR (fluorescence ratio).
**TABLE 3**

*Correlation coefficients*\(^*\) between storage time and lipid damage indices\(^**\) in frozen hake captured at different times

<table>
<thead>
<tr>
<th>Storage Temperature</th>
<th>Catching Time</th>
<th>FFA</th>
<th>CDs</th>
<th>PV</th>
<th>FR</th>
</tr>
</thead>
<tbody>
<tr>
<td>–30º C</td>
<td>May</td>
<td>0.82(^b) (0.93)</td>
<td>0.89(^b) (0.92)</td>
<td>0.95</td>
<td>0.78(^b) (0.81)</td>
</tr>
<tr>
<td>–30º C</td>
<td>November</td>
<td>0.59(^b) (0.68)</td>
<td>-0.04(^a) (0.19)</td>
<td>0.92(^a) (0.94)</td>
<td>0.88</td>
</tr>
<tr>
<td>–10º C</td>
<td>May</td>
<td>0.93(^b) (0.97)</td>
<td>0.94</td>
<td>0.83(^b) (0.90)</td>
<td>0.99</td>
</tr>
<tr>
<td>–10º C</td>
<td>November</td>
<td>0.93(^b) (0.97)</td>
<td>0.27(^b) (0.48)</td>
<td>0.70(^b) (0.78)</td>
<td>0.95</td>
</tr>
</tbody>
</table>

* Cuadratic\(^a\) and logarithmic\(^b\) *correlation coefficients* are expressed in brackets when superior to the linear ones.

** Abbreviations as specified in Table 1.
### TABLE 4

**Conjugated dienes (absorption coefficient)** formation in frozen (–30°C and –10°C) fish captured at two different times*

| Storage Time (months) | Blue whiting | | Hake | | | | | | |
|-----------------------|--------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
|                       | May          | November       | May            | November       | May            | November       | May            | November       |
| Initial Fish          | 1.18 a (0.61)| 1.28 a (0.03)  | 1.18 a (0.61)  | 1.18 a (0.03)  | y 1.08 a (0.13)| z 0.39 a (0.02)| y 1.08 a (0.13)| z 0.39 a (0.02)|
| 1                     | z 1.05 a (0.08)| y 2.85 b (0.11)| z 1.91 a (0.10)| y 2.14 c (0.07)| 1.17 a (0.09)  | 1.29 b (0.53)  | 1.46 a (0.20)  | 1.79 b (0.60)  |
| 3                     | z 1.27 a (0.24)| y 2.75 b (0.10)| z 1.53 a (0.44)| y 3.73 e (0.24)| 3.52 b (0.15)  | 3.06 c (0.32)  | y 3.81 b (0.38)| z 2.50 cd (0.19)|
| 5                     | 2.34 b (0.45)| 3.03 b (0.46)  | 1.81 a (0.18)  | 1.95 bc (0.06) | y 3.81 b (0.15)| z 2.96 c (0.37)| y 3.41 b (0.22)| z 2.35 cd (0.42)|
| 7                     | y 2.71 b (0.11)| z 1.60 a (0.16)| y 3.43 c (0.39)| z 1.47 a (0.11)| 3.97 b (0.88)  | 2.95 c (0.22)  | 3.77 b (0.79)  | 2.86 d (0.34)  |
| 9                     | y 2.96 b (0.52)| z 1.62 a (0.09)| 2.64 b (0.30)  | 2.71 d (0.39)  | y 4.16 b (1.00)| z 1.60 b (0.13)| y 3.11 b (0.24)| z 1.98 bc (0.05)|
| 12                    | y 4.52 c (0.11)| z 1.88 a (0.60)| y 2.93 bc (0.32)| z 1.60 ab (0.27)| y 3.42 b (0.81)| z 1.73 b (0.17)| y 5.51 c (0.84)| z 1.76 b (0.24)|

* Means of three independent determinations (n=3); standard deviations are indicated in brackets. Mean values in the same column followed by different letters (a-e) are significantly (p<0.05) different. For each fish species, mean values preceded by different letters (y, z) indicate significant (p<0.05) differences between May and November experiment values for the same storage time and temperature.
TABLE 5

Peroxide value (meq oxygen/ kg lipid) assessment in frozen (–30°C and –10°C) fish captured at different times*

<table>
<thead>
<tr>
<th>Storage Time (months)</th>
<th>Blue whiting – 30°C</th>
<th>Blue whiting – 10°C</th>
<th>Hake – 30°C</th>
<th>Hake – 10°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>May</td>
<td>November</td>
<td>May</td>
<td>November</td>
</tr>
<tr>
<td>Initial Fish</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.20 a (0.31)</td>
<td>3.11 a (0.31)</td>
<td>3.20 a (0.31)</td>
<td>3.11 a (0.31)</td>
</tr>
<tr>
<td>1</td>
<td>3.91 ab (0.69)</td>
<td>4.18 ab (0.94)</td>
<td>z 4.28 ab (0.27)</td>
<td>y 6.69 b (1.56)</td>
</tr>
<tr>
<td>3</td>
<td>z 4.11 ab (0.32)</td>
<td>y 5.82 abc (1.04)</td>
<td>z 6.11 bc (0.70)</td>
<td>y 8.89 bc (1.62)</td>
</tr>
<tr>
<td>5</td>
<td>z 4.90 b (0.28)</td>
<td>y 7.47 c (1.77)</td>
<td>13.89 d (1.40)</td>
<td>11.24 cd (0.93)</td>
</tr>
<tr>
<td>7</td>
<td>z 4.32 ab (1.01)</td>
<td>y 7.49 bc (0.86)</td>
<td>12.38 d (1.12)</td>
<td>11.84 d (1.05)</td>
</tr>
<tr>
<td>9</td>
<td>z 4.89 b (0.62)</td>
<td>y 15.29 d (2.77)</td>
<td>z 5.31 bc (1.08)</td>
<td>y 18.48 e (0.47)</td>
</tr>
<tr>
<td>12</td>
<td>z 9.28 c (1.24)</td>
<td>y 24.72 e (3.23)</td>
<td>z 6.42 c (1.21)</td>
<td>y 20.83 f (2.08)</td>
</tr>
</tbody>
</table>

* Means of three independent determinations (n=3); standard deviations are indicated in brackets. Mean values in the same column followed by different letters (a-f) are significantly (p<0.05) different. For each fish species, mean values preceded by different letters (y, z) indicate significant (p<0.05) differences between May and November experiment values for the same storage time and temperature.