LIPID DAMAGE DETECTION DURING THE FROZEN STORAGE OF AN UNDERUTILIZED FISH SPECIES

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ABSTRACT

Lipid damage was studied on an underutilized fish species (blue whiting, *Micromesistius poutassou*). Blue whiting fillets were frozen at –40ºC and stored at –30ºC and –10ºC up to one year. Primary and secondary lipid oxidation products, interaction compounds and lipid hydrolysis were studied on the fish white muscle. At –30ºC most of the lipid damage indices tested (free fatty acids, peroxide value, conjugated dienes, thiobarbituric acid index and fluorescence detection) showed a significant ($p<0.05$) correlation with the storage time. However, as the fish damage increased (–10ºC condition) only the free fatty acids and fluorescence detections provided satisfactory correlations with the storage time (r^2 = 0.94 and r^2 = 0.92, respectively), while reliability of the remaining indices decreased in order to assess lipid damage and fish deteriorative changes.

Key Words: Blue whiting, fish food, frozen storage, lipid oxidation and hydrolysis, underutilized species

Running Title: Lipid damage during fish frozen storage

INTRODUCTION

Fish and other marine species give rise to products of great economic importance in many countries. During processing and storage fish quality may decline as a result of several factors. One of the most important concerns the oxidation of the highly unsaturated lipids (Ackman, 1989) directly related to the production of off flavors and odors (Hsieh and Kinsella, 1989; Harris and Tall, 1994).

Frozen storage has been widely employed to retain fish properties before it is consumed or employed in other technological processes (Pigott and Tucker, 1987; Erickson, 1997). During the frozen storage of fish lipid hydrolysis and oxidation have been shown to occur and become an important factor of fish acceptance as influencing rancidity development, protein denaturation and texture changes (Mackie, 1993; Verma et al., 1995).

The fish industry is actually suffering from dwindling stocks of traditional species as a result of drastic changes in their availability. As a result, fish technologists and fish trade have turned their attention to some unconventional sources of raw material (Flick et al., 1992; Shahidi and Venugopal, 1997). One of such species is the blue whiting (*Micromesistius poutassou*), a gadoid abundant in the northeast Atlantic (Dagbjartson, 1975; FAO Inform, 1998).

Related to blue whiting, efforts have been made to employ it in the manufacture of a large number of restructured products from fish mince (Kolakowski and Wianecki, 1990; Borderías et al., 1997). However, since it is also directly consumed, research has been carried out on the fish as such; the selenium and phosphorus contents have been quantified (Oehlenschläger, 1990a, 1990b), protein changes have been studied during

frozen storage (Huidobro and Tejada, 1995) and quality changes during chilling storage have been determined (Smith et al., 1980; Aubourg et al., 1998a).

The present study is focused on lipid damage caused by freezing at –40ºC and frozen storage up to one year at two temperatures (-30ºC and -10ºC) of blue whiting fillets. Detection of primary and secondary lipid oxidation products, interaction compounds and lipid hydrolysis was carried out.

MATERIALS AND METHODS

Raw material, processing and sampling

Fresh blue whiting (*Micromesistius poutassou*) were obtained 10 hr after catching. Upon arrival in our laboratory, individual fish were eviscerated, beheaded, filleted and frozen at -40°C. The fish fillets (80-100g each) were then distributed into two storage temperatures: -10°C and -30°C; the first temperature is often employed during sale and distribution and produces accelerated changes in lipids, while the second one would serve as control. For each storage temperature, fillets were divided into three batches that were studied separately during the whole experiment. Analyses on fish were carried out on the white muscle of the raw material employed and at 1, 3, 5, 7, 9 and 12 months of frozen storage at both temperatures.

Water and lipid contents

Water content was determined by weight difference of the homogenized muscle (1-2 g) before and after 24 hours at 105°C. Results were calculated as g water/ 100g

1 muscle. Lipids were extracted from the homogenized white muscle by the Bligh and
2 Dyer (1959) method. Results were calculated as g lipids/ 100g wet muscle.
3
4 **Lipid damage measurements**

5 Free fatty acid (FFA) content was determined by the Lowry and Tinsley (1976)
6 method based on complex formation with cupric acetate-pyridine. Results are expressed
7 as g FFA/ 100g lipids.

8 Peroxide value (PV) expressed as meq oxygen/ Kg lipid was determined by the
9 ferric thiocyanate method (Chapman and McKay, 1949).

10 Conjugated diene (CD) formation was measured at 233 nm (Kim and Labella,
11 1987). The result is expressed according to the formula: CD = B x V / w, where B is the
12 absorbance reading at 233 nm, V denotes the volume (mL) of the sample and w is the
13 mass (mg) of the lipid extract.

14 The thiobarbituric acid index (TBAI) (mg malondialdehyde/ Kg sample) was
15 determined according to Vyncke (1970).

16 Fluorescence formation (Perkin-Elmer LS 3B) at 393/463 nm and 327/415 nm
17 was studied as described earlier (Aubourg and Medina, 1997; Aubourg et al., 1998b).
18 The relative fluorescence (RF) was calculated as follows: $RF = F/F_{st}$, where F is the
19 sample fluorescence at each excitation/emission maximum, and F_{st} is the fluorescence
20 intensity of a quinine sulfate solution (1 μg/mL in 0.05 M H_2SO_4) at the corresponding
21 wavelength. The fluorescence ratio (FR) was calculated as the ratio between both RF
22 values: $FR = RF_{393/463nm} / RF_{327/415nm}$. The FR value was analyzed on the aqueous (AFR)
23 and organic (OFR) phases resulting from the lipid extraction (Bligh and Dyer, 1959).

Statistical analyses

Data from the different lipid damage measurements were subjected to the ANOVA one-way method ($p<0.05$), correlation analysis and factor analysis (principal components) (Statsoft, 1994); a varimax normalized rotation was employed for factor rotation.

RESULTS

Water contents ranged between 81 and 83 % in all samples; no differences were obtained as a result of temperature and time of storage. Lipid contents ranged between 0.43 and 0.60 % on wet basis; no significant ($p<0.05$) differences were obtained as a result of the frozen storage conditions (time and temperature).

Lipid hydrolysis

The preservative effect of the storage temperature was evident since FFA values of –30ºC samples were lower than the corresponding –10ºC ones (Tables 1 and 2).

At –30ºC, a significant increase in the FFA content was only obtained at month 5; then, no more increases were detected, showing a mean value at the end of the storage of 14.08. However, at –10ºC, a progressive increase with the storage time was observed leading at the end of the experiment to a 55.75% value for the FFA proportion in the lipid fraction; sharp increases occurred at months 1 and 5. According to previous experiments (Quaranta and Pérez, 1983; de Koning and Mol, 1991), FFA formation as a result of lipid (triglyceride and phospholipid classes) hydrolysis has provided a suitable means for assessment of fish damage during frozen storage.

1 **Lipid oxidation**

2 The PV at –30ºC was only slightly different during the first nine months of
3 storage (Table 1); a sharp increase was observed at month 12, although the PV obtained
4 (9.32) was relatively low (Pérez-Villarreal and Howgate, 1991; Vidya Sagar Reddy et
5 al., 1992) as a result of the preservative effect on fillets of the storage temperature.

6 A faster development of primary oxidation in the five first months of the
7 experiment was detected at –10ºC (Table 2), reaching the highest PV mean value at
8 month 5 (13.97). After that time, a progressive decrease was observed till month 9, that
9 could be explained as a result of decomposition of hydroperoxides into smaller
10 molecules (Melton, 1983; Cho et al., 1989).

11 Primary oxidation was also detected by the CD index. At –30ºC an increase at
12 month 5 (Table 1) was observed. The highest mean value was obtained at the end of the
13 storage, as in the case of the PV, although only small differences ($p<0.05$) were
14 observed by comparison with samples at 5-9 months. At –10ºC (Table 2) the highest
15 mean value was obtained at month 7, and that was followed by some decrease. As in the
16 case of the PV, CD value decreased with increasing time and temperature conditions as
17 a result of decomposition into smaller molecules (Cho et al., 1989; Aubourg et al.,
18 1998b).

19 Secondary lipid oxidation was studied by the TBAI. Values obtained in both
20 temperatures along the whole experiment were relatively low (Tables 1 and 2),
21 especially compared to those observed in fatty fish species (Kurade and Baranowski,
22 1987; Aubourg et al., 1998b). A general tendency could not be inferred from values
23 obtained at –30ºC; the highest value was obtained at month 5. At –10ºC, the highest
24 values were obtained at months 3 and 5; then a decrease was detected, that could be

explained as a result of combining with proteins to form polymers (Orlick et al., 1991; Vidya Sagar Reddy et al., 1992).

Fluorescent compounds formation

Interaction compounds were studied by means of fluorescent properties in the aqueous and organic phases resulting from the Bligh and Dyer (1959) extraction. The organic phase (lipid extract) study provided no changes for the OFR value along the whole experiment at –30ºC (Table 1). At –10ºC (Table 2), a gradual increase was observed till month 7; then a decrease was detected at the end of the storage.

The AFR value showed no changes during the first 5 months at both temperatures. However, a sharp increase at months 7 and 9 was observed at both storage conditions, showing a bigger fluorescent compound development at –10ºC than at –30ºC, as a result of a higher fish damage at the highest temperature.

Comparison of results in both phases show that when the time and temperature of storage increased, fluorescent compounds responsible for the fluorescence ratio value became progressively more soluble in the aqueous phase, so that an increasing AFR/OFR ratio with the storage time was detected in both temperatures (Aubourg et al., 1998b).

Correlation and multivariate analyses

The storage time and the different lipid damage indices were tested for correlation. At –30ºC (Table 3), the storage time showed the best correlations with parameters such as FFA ($r^2 = 0.89$), AFR ($r^2 = 0.88$) and CD ($r^2 = 0.87$). Between lipid indices the most satisfactory results were obtained by comparing the FFA content with the CD index ($r^2 = 0.79$) and the AFR value ($r^2 = 0.79$).

1 Some different results were obtained at –10ºC (Table 4). The storage time
2 showed again satisfactory correlations with the FFA (r^2 = 0.94) and AFR (r^2 = 0.92)
3 values, but not with the CD index (r^2 = 0.68). At the same time, comparison of the
4 different lipid damage indices showed that the best correlation value was obtained
5 between the FFA content and the AFR value (r^2 = 0.92); other correlations were not as
6 satisfactory.
7 Principal component analysis showed that 77.24 and 80.63 % of the variability
8 observed at –30ºC and –10ºC storage, respectively, could be explained by two factors.
9 Corresponding factor loadings are graphically displayed in Figures 1 and 2. Figure 1 (-
10 30ºC) shows that many parameters (ST, FFA, CD, PV and AFR) are grouped together at
11 high loading in the Factor 1 axis. In the case of the –10ºC storage (Figure 2), parameters
12 such as ST, FFA, AFR and CD show again a high loading in the Factor 1 axis, and are
13 now grouped with the OFR value instead of the PV, as in the –30ºC case.
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16 **DISCUSSION**
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18 Fish quality decreases during frozen storage as a result of increasing time and
19 temperature of storage (Mackie, 1993; Sotelo et al., 1995). The preservative effect of
20 temperature on lipid damage was evident in the present experiment; both the FFA
21 content and AFR value showed a higher development at -10ºC than at -30ºC and led to
22 satisfactory correlation values with storage time at both test temperatures. Reliability of
23 the remaining indices decreased at the -10ºC temperature. As an explanation,
24 degradation products that are measured in such indices (peroxides, conjugated dienes
25 and thiobarbituric acid reactive substances) can either be destroyed or interact with

other constituents, so that the determination cannot always provide an accurate method for the quality assessment (Melton, 1983; Cho et al., 1989; Orlick et al., 1991).

According to the general theory, lipid oxidation compounds have reacted with nucleophilic biological constituents (proteins, peptides, free amino acids and phospholipids) and caused the formation of interaction compounds with fluorescent properties (Gardner, 1979; Leake and Karel, 1985). Fluorescence detection by the AFR value has already provided a good assessment of quality changes in previous research where fatty fish processing (frozen storage and canning) was tested (Aubourg and Medina, 1997; Aubourg et al., 1998b).

At the same time, hydrolytic activity was also shown to be sensitive to the time of storage at both temperatures reaching a very high FFA value at –10ºC. Formation of FFA itself does not lead to nutritional losses. However, it has been proven that accumulation of FFA in frozen fish is related to some extent with lack of acceptability of frozen fish, because FFA are known to cause texture deterioration by interacting with proteins (Mackie, 1993; Sotelo et al., 1995) and have shown to be strongly interrelated with lipid oxidation (Miyashita and Takagi, 1986; Han and Liston, 1988).

During frozen storage of lean fish such as gadoid species most attention has been given to the formaldehyde formation and its implication in quality loss (Gill et al., 1979; Sotelo et al., 1995). However, lipid hydrolysis and oxidation have also been shown to occur in such processes and become an important factor of fish acceptance as influencing protein denaturation, texture changes and functionality loss (Careche and Tejada, 1990; Mackie, 1993).

Present results obtained on an underutilized gadoid fish species reinforce the role of lipid damage detection during lean fish frozen storage when monitoring quality changes. Lipids from blue whiting fillets were sensitive to temperature and time of

storage, since quality changes could be assessed by free fatty acid detection and fluorescent compounds formation.

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FIGURE LEGENDS

Figure 1: Principal component analysis for different parameters (storage time, **ST**; free fatty acids, **FFA**; peroxide value, **PV**; conjugated dienes, **CD**; thiobarbituric acid index, **TBAI**; organic fluorescence ratio, **OFR**; aqueous fluorescence ratio, **AFR**) measured at –30ºC storage.

Figure 2: Principal component analysis for different parameters (**ST, FFA, PV, CD, TBAI, OFR, AFR**) measured at –10ºC storage. Abbreviations as indicated in Figure 1.

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2 **TABLE 1**: Lipid damage detection* during blue whiting frozen (–30ºC) storage**
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ST (months)	**FFA**	**PV**	**CD**	**TBAI**	**OFR**	**AFR**
0	4.97 a	3.13 a	1.64 abc	0.32 b	0.69	1.25 a
1	6.07 a	3.85 ab	1.08 a	0.13 a	0.79	1.19 a
3	5.23 a	4.05 abc	1.23 ab	0.62 cd	0.62	1.23 a
5	10.30 b	5.27 c	3.37 de	0.74 d	0.69	1.54 a
7	14.84 b	4.37 abc	3.22 cde	0.56 c	0.79	4.56 b
9	13.35 b	4.96 bc	2.88 bcd	0.32 b	0.68	7.72 c
12	14.08 b	9.32 d	4.46 e	0.60 cd	0.58	7.46 c

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18 * Abbreviations: **ST** (storage time), **FFA** (free fatty acids), **PV**, (peroxide value), **CD**
19 (conjugated dienes), **TBAI** (thiobarbituric acid index), **OFR** and **AFR**
20 (fluorescence ratio values in the organic and aqueous phases, respectively).
21 ** Means of three independent determinations. Values in the same column followed by
22 different letters are significantly different ($p<0.05$).
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2 **TABLE 2**: Lipid damage detection* during blue whiting frozen (–10ºC) storage**
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ST (months)	FFA	PV	CD	TBAI	OFR	AFR
0	4.97 a	3.13 a	1.64 a	0.32 a	0.69 a	1.25 a
1	21.66 b	5.26 ab	1.85 ab	0.20 a	0.76 ab	1.59 a
3	19.30 b	6.00 b	1.48 a	1.07 b	1.00 ab	1.33 a
5	37.82 c	13.97 d	1.70 a	1.61 b	1.25 bc	1.75 a
7	48.26 d	11.22 c	3.46 d	0.12 a	1.95 d	15.45 b
9	53.26 de	5.26 ab	2.60 bc	0.19 a	1.74 bcd	21.88 c
12	55.75 e	6.47 b	2.89 cd	0.21 a	1.07 ab	20.36 bc

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15 * Abbreviations as indicated in Table 1.
16 ** Means of three independent determinations. Values in the same column followed by
17 different letters are significantly different ($p<0.05$).
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2 **TABLE 3**: Correlation matrix for different parameters (storage time and lipid damage
3 estimations)* measured during blue whiting frozen (–30ºC) storage**
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	FFA	PV	CD	TBAI	OFR	AFR
ST	**0.89**	**0.80**	**0.87**	**0.59**	-0.29	**0.88**
FFA		**0.56**	**0.79**	**0.57**	-0.15	**0.79**
PV			**0.76**	**0.56**	-0.23	**0.61**
CD				**0.57**	-0.08	**0.76**
TBAI					-0.28	0.33
OFR						-0.20

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16 * Abbreviations as indicated in Table 1.
17 ** Significant values (p<0.05) are expressed in bold print.
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2 **TABLE 4**: Correlation matrix for different parameters (storage time and lipid damage
3 estimations)* measured during blue whiting frozen (–10ºC) storage**
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	FFA	PV	CD	TBAI	OFR	AFR
ST	**0.94**	0.39	**0.68**	-0.32	**0.51**	**0.92**
FFA		**0.53**	**0.74**	-0.42	**0.65**	**0.92**
PV			**0.62**	-0.35	**0.52**	0.43
CD				-0.48	**0.71**	**0.63**
TBAI					-0.26	-0.43
OFR						**0.61**

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16 * Abbreviations as indicated in Table 1.
17 ** Significant values ($p<0.05$) are expressed in bold print.

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Tab. 1

Tiempo almac (meses)	Agua pre		Agua post		Líps pre	
	-30ºC	-10ºC	-30ºC	-10ºC	-30ºC	-10ºC
0	81’42	81’42	78’81 a	78’81 a	0’27 a	0’27 a
1	82’18	81’62	80’72 b	79’55 ab	0’43 b	0’45 b
3	82’55	82’75	80’07 ab	80’64 b	0’54 b	0’36 b
5	83’11	82’85	78’91 a	79’99 ab	0’45 b	0’36 b
7	81’52	81’94	80’27 b	79’71 ab	0’55 b	0’42 b
9	82’82	81’65	79’68 ab	80’10 ab	0’47 b	0’43 b
12	81’83	81’53	79’94 ab	80’86 b	0’50 b	0’43 b

Tab. 2: Pre

Tiempo almac (meses)	IP		Dienos		i-ATB	
	-30ºC	-10ºC	-30ºC	-10ºC	-30ºC	-10ºC
0	3’13 a	3’13 a	1’64 abc	1’64 a	0’32 b	0’32 a
1	3’85 ab	5’26 ab	1’08 a	1’85 ab	0’13 a	0’20 a
3	4’05 abc	6’00 b	1’23 ab	1’48 a	0’62 c	1’07 b
5	5’27 c	13’97 d	3’37 de	1’70 a	0’74 d	1’61 b
7	4’37 abc	11’22 c	3’22 cde	3’46 d	0’56 c	0’12 a
9	4’96 bc	5’26 ab	2’88 bcd	2’60 bc	0’32 b	0’19 a
12	9’32 d	6’47 b	4’46 e	2’89 cd	0’60 cd	0’21 a

Tab. 3: Post

Tiempo almac (meses)	IP		Dienos		i-ATB	
	-30ºC	-10ºC	-30ºC	-10ºC	-30ºC	-10ºC
0	3’11 a	3’11 a	1’56 a	1’56 a	0’00 a	0’00 a
1	4’18 ab	6’69 ab	2’85 b	2’14 ab	0’53 c	0’30 d
3	5’82 ab	8’89 bc	2’75 b	3’73 d	0’64 c	0’25 cd
5	7’47 ab	11’24	3’03 b	2’40 bc	0’50 bc	0’15 bc
7	9’48 b	11’84 c	1’60 a	1’47 a	0’45 bc	0’21 bcd
9	15’29 c	16’05 d	1’62 a	2’92 c	0’47 bc	0’15 bc
12	21’31 d	20’83 e	1’88 a	1’60 a	0’30 b	0’11 ab

Tab. 4: Fluos pre y post

Tiempo almac	δF_{or} pre		δF_{or} post		δF_{aq} pre	

(meses)						
	-30ºC	-10ºC	-30ºC	-10ºC	-30ºC	-10ºC
0	0’69	0’69 a	0’45 a	0’45 a	1’25 a	1’25 a
1	0’79	0’76 ab	0’56 a	0’62 ab	1’19 a	1’59 a
3	0’62	1’00 ab	0’49 a	0’79 bc	1’23 a	1’33 a
5	0’69	1’25 bc	0’82 b	0’86 c	1’54 a	1’75 a
7	0’79	1’95 d	0’93 b	0’88 c	4’56 b	15’45 b
9	0’68	1’74 bcd	0’87 b	0’97 cd	7’72 c	21’88 c
12	0’58	1’07 ab	0’69 ab	1’12 d	7’46 c	20’36 bc

1
2
3