**RANCIDITY DEVELOPMENT DURING MACKEREL
(*Scomber scombrus*) FROZEN STORAGE: EFFECT OF
CATCHING SEASON AND COMMERCIAL
PRESENTATION**

Santiago P. Aubourg^{a,*}, Alicia Rodríguez^b and José M. Gallardo^a

^a*Department of Seafood Chemistry, Institute for Marine Research (IIM-CSIC), Vigo (Spain).*

^b*Department of Food Science and Chemical Technology. University of Chilli, Santiago (Chilli).*

* Correspondent: FAX: +34986292762; e-mail: saubourg@iim.csic.es

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SUMMARY

Hydrolytic and oxidative rancidity development and its effect on quality loss were studied in frozen mackerel (*Scomber scombrus*) by biochemical and sensory indices. The effect of the lipid content on fish damage during a commercial freezer temperature (-20°C) was studied for up to 12 months; thus, mackerel caught at two different times of the year (May and November) was checked, being May included in the period of minimum lipid content, while November is known to correspond to the maximum lipid content time. The study was also focused to two different kinds of fish products (whole fish and fillets). An increasing lipid hydrolysis was observed for all kinds of samples during the frozen storage; no differences ($p>0.05$) between whole fish and fillets were found for free fatty acid formation; however, mackerel from May showed a higher ($p<0.05$) hydrolysis development than its counterpart from November. An increasing lipid oxidation (peroxide value and thiobarbituric acid index) was observed for all kinds of samples during the frozen storage. Fillet lipid oxidation was found higher ($p<0.05$) than in whole fish. Fattier mackerel (November fish) showed a higher ($p<0.05$) oxidation development than its counterpart from May in the case of fillet products. Fluorescence assessment related to interaction compound formation showed to be higher ($p<0.05$) in fillets than in whole fish, although was bigger ($p<0.05$) in May samples. Sensory analysis corroborated the biochemical analyses, so that fillets showed a shorter shelf-life (1 and 3 months for November and May fish, respectively) than their counterpart whole fish (5 months for November and May mackerel) pieces.

1. INTRODUCTION

Freezing and frozen storage have been largely used to retain fish sensory and nutritional properties [1, 2]. However, the presence in fish species of a highly unsaturated lipid composition and a great content of prooxidant molecules have led during the frozen storage to an important enzymatic and nonenzymatic rancidity development that strongly influences the shelf-life of fish products [3, 4].

Fatty fish is attracting a great attention because of the positive role of marine lipids ($\omega 3$ polyunsaturated fatty acids, particularly) on human nutrition and health [5, 6]. However, fatty species undergo large variations of their lipid contents along the different seasons, being the highest content for the Autumn period, while the lowest one is found for Spring when one considers northern species [7, 8]. Since a great effect of lipid damage on fatty fish quality has been proved [9, 10], a special attention should be accorded to the possible effect that the catching season (lipid content, namely) may have on the processed fatty fish quality.

Small pelagic fatty fish species can constitute food products of great economic importance in many European countries [11]. One abundant such species in both North Atlantic coasts is Atlantic mackerel (*Scomber scombrus*) belonging to the *Scombridae* family [12]. Although it is recognised as a healthy food, remains underutilised [13] basically because of its poor frozen shelf-life. Thus, previous research has shown an important endogenous prooxidant activity [14, 15] and quality loss during its frozen storage as fillet [16-18] and whole fish [19] products. However, a comparative study of quality loss in both kinds of distribution products has not been carried out up to now.

The present work concerns mackerel (*Scomber scombrus*) trading as a frozen product. The study is focused to investigate the effect of the lipid content on the quality loss during the frozen storage. For it, mackerel fish from two different seasons was studied. The research also implies the two most common distribution fish products (whole fish and fillets). Analyses comprise hydrolytic and oxidative rancidity development, which are monitorised up to 12 months of frozen (-20°C) storage by biochemical and sensory indices.

2. MATERIALS AND METHODS

2.1. Raw fish, sampling and processing

Fresh mackerel (*Scomber scombrus*) was obtained in May 2002 and in November 2002 in a local market 10 h after being caught. During this time and until arrival at the laboratory, the fish was kept on ice. Gonads of fish collected were at 5th/6th stage of the Maier's scale of gonad maturity. The length of the fish was in the range 19-24 cm; the weight was in the range 260-290 g. These two times of the year were chosen because of their opposite lipid content, being May included in the period of minimum content, while November corresponds to the maximum content time [20, 21].

At both times (May and November), the fish were divided into two groups. One of them was directly packaged in polyethylene bags (without high-pressure or modified atmosphere) and frozen at -80°C, and would be considered as the whole fish product (including head and organs). The other group was carefully dressed, filleted by hand (layer thickness about 0.75-1.25 cm), washed with water, packaged in polyethylene bags (without high-pressure or modified atmosphere), and frozen at -80°C; this group would be

considered as the fillet product (deboned but not skinned). Each whole fish and fillet piece was packaged individually. After 24 h at -80°C both whole fish and fillet products were placed in a freezer at -20°C . Sampling was undertaken on the starting fish material and at months 1, 3, 5, 7, 9 and 12 of frozen storage at -20°C . In both experiments (May and November) and for each kind of fish product, three different and independent batches (same vessel and catch day) were considered ($n=3$) and studied separately to achieve the statistical reliability.

2.2. Water and lipid contents

Water content was determined by weight difference between the homogenised fish muscle (1-2 g) and after 24 hr at 105°C . Results were calculated as g water/100 g muscle.

Lipids were extracted by the Bligh and Dyer method [22]. Quantification results are expressed as g total lipids/100 g wet muscle.

2.3. Lipid damage indices

Free fatty acid (FFA) content was determined by the Lowry and Tinsley [23] method based on complex formation with cupric acetate-pyridine. Results are expressed as g FFA/100 g lipids.

The peroxide value (PV) was determined according to the ferric thiocyanate method [24]. Results are expressed as meq active oxygen / kg lipids.

The thiobarbituric acid index (TBA-i) was determined according to the method of Vyncke [25]. Results are expressed as mg malondialdehyde / kg fish tissue.

2.4. Interaction compound formation

Interaction compounds (tertiary oxidation compounds) produced between oxidised lipids and nucleophilic compounds present in the fish muscle (free amino acids, peptides, proteins, aminated phospholipids) [26, 27] were determined by fluorescence formation.

Fluorescence formation (Perkin-Elmer LS 3B) at 327/415 nm and 393/463 nm was studied as described elsewhere [28]. The relative fluorescence (RF) was calculated as follows: $RF = F/F_{st}$, where F is the fluorescence measured at each excitation/emission pair, and F_{st} is the fluorescence intensity of a quinine sulphate solution (1 µg/ml in 0.05 M H₂SO₄) at the corresponding wavelength. The fluorescence ratio (FR) was obtained from the lipid extract analysis, according to the following calculation: $FR = RF_{393/463nm} / RF_{327/415nm}$.

2.5. Sensory analysis

Sensory analysis was conducted by a taste panel consisting of five experienced judges, according to the guidelines presented in Table 1 [29]. Four categories were ranked: highest quality (E), good quality (A), fair quality (B) and rejectable quality (C). Sensory assessment of the whole fish samples included the following parameters: skin, eyes, gills, odour, texture and flesh appearance. In the case of fillets, skin, odour and flesh appearance were only considered.

2.6. Statistical analysis

The Statgraphics Plus (version 4.0, software package, Manugistics Inc., Rockville, MA, USA) was used for investigating the statistical significance difference at the 95%

confidence level of the biochemical results obtained. Analyses included one-way ANOVA by the least significance difference (LSD) method and multivariate comparisons by the Tuckey test. The Spearman test ($p < 0.05$) for non-parametric correlations was performed in the case of sensory assessments [30].

3. RESULTS AND DISCUSSION

3.1. Water and lipid contents

A lower lipid content in white muscle was obtained for mackerel captured in May (range 1.0%-3.5%) than for mackerel corresponding to the November experiment (range 6.5%-10.0%). This result agrees with previous research concerning the lipid content distribution for mackerel [20, 21] and other northern species [7, 8].

Comparison of the water content of mackerel fish between both periods led to the opposite conclusion than for the lipid matter, according to an already known inverse ratio between both constituents [31]. Water content in May fish (74-78%) was found similar to medium fat species (horse mackerel) [32], while the water content from the November catching (65-73%) was found relatively low, similar to fatty fish species [28].

The time of frozen storage and the kind of product (whole fish and fillets) did not exert a significant ($p > 0.05$) effect on both constituents. For each kind of product, differences observed may be explained as a result of fish-to-fish variation.

3.2. Lipid hydrolysis

In the present study, the FFA content of the starting fish from the May experiment (0.45 ± 0.13) was found higher ($p < 0.05$) than its counterpart from November (0.12 ± 0.03). Both values were included in the same range than other fatty fish species (tuna, sardine) [28, 33] and were found lower than those obtained for lean fish species (haddock, cod, Atlantic pomfret) [34, 35].

A significant ($p < 0.05$) hydrolysis development was observed for both kinds of mackerel products from May and November during the frozen storage (Figure 1). FFA formation was found bigger ($p < 0.05$) for fish products from May than in their counterparts from November. May samples (whole fish and fillets) provided a sharp increase till month 3 and then remained relatively constant till the end of the experiment, while November fish led to a slow and gradual increase during the storage. Comparison between both kinds of products (whole fish and fillets) did not provide significant ($p > 0.05$) differences in FFA formation neither in leaner or fattier mackerel.

Examining the extent of lipid hydrolysis was deemed important to the study because of the high lipid hydrolysis development previously observed in mackerel during frozen storage [19, 36] and also because of the great influence of lipid hydrolysis on lipid oxidation [37] and on protein denaturation [38].

3.3. Lipid oxidation

Primary oxidation was followed by the PV assessment. A significant ($p < 0.05$) oxidation development was observed for both kinds of mackerel products obtained in May and in November during the frozen storage (Figure 2).

Comparison of both catching time experiments showed a higher ($p<0.05$) peroxide formation for fattier mackerel in the case of fillets, where values above 10 were obtained for the 3-12 month period; however, a big increase was observed at month 7 for May fillets ($PV = 18.21\pm3.02$) that was followed by a peroxide breakdown period. Concerning whole fish samples, a sharp peroxide formation was observed at the end of the experiment for the November samples, so that a higher ($p<0.05$) value than in the May samples was obtained at this time.

Comparison of both kinds of commercial products showed a higher ($p<0.05$) primary oxidation compound formation in fillets than in their counterpart whole fish samples for the November trial. In the case of the May samples, a higher ($p<0.05$) PV was obtained for fillets at month 7. This difference may be explained as a result of a greater exposition surface of fish muscle to air during the frozen storage and agrees to previous research on horse mackerel [32].

Secondary lipid oxidation was studied according to the TBA-i assessment. A significant ($p<0.05$) oxidation development was observed for both kinds of mackerel products from May and November during the frozen storage (Figure 3). According to the results obtained for primary oxidation, comparison of both catching times showed a higher ($p<0.05$) TBA-i for mackerel caught in November in the case of fillets, which agrees with the fact that a higher lipid content is present in fish from the November trial. However, whole fish comparison did not provide significant ($p>0.05$) differences.

Comparison of both kinds of distribution products showed a higher ($p<0.05$) secondary oxidation compound formation in fillets than in their counterpart whole fish samples for the November trial. In the case of the May samples, a higher ($p<0.05$) TBA-i was obtained for fillets at months 9 and 12. As in the PV assessment, this difference may be

explained as a result of a greater exposition surface of the fish muscle to air in the case of fillets. For both experiments, whole fish sample TBA-i were always below 0.62, while this value was surpassed from months 9 (May experiment) and 5 (November experiment) for fillet samples.

3.4. Interaction compound formation

A significant ($p < 0.05$) fluorescence development was observed for leaner and fatter mackerel during the frozen storage (Figure 4).

In both catching times, fillet samples showed a higher ($p < 0.05$) fluorescence development than their counterpart whole fish samples, except after 12 months for the May mackerel. According to the results obtained for the primary and secondary lipid oxidation, interaction compound formation also led to a higher development in fillet samples. Thus, the three lipid damage assessments agree with a higher oxidation development in fillets, probably as a result of a higher surface exposition to air.

However, comparison of both catching times showed a higher ($p < 0.05$) fluorescence development for mackerel caught in May (whole fish and fillets), apparently in contrast to results obtained from primary and secondary lipid oxidation assessments for fillet samples. As it has been expressed, fluorescent compound formation depends not only on primary and secondary lipid oxidation compound formation, but also on the presence of nucleophilic compounds in the fish muscle. In this sense, an important effect of the kind of amine compound has been reported [39]. Differences in nucleophilic compound composition in the mackerel white muscle between May and November times may be responsible for the higher fluorescence development obtained for the May samples.

3.5. Sensory analysis

Different attributes were analysed. Scores obtained for the May and November mackerel experiments are expressed in Tables 2 and 3, respectively. In the case of whole fish, general aspect included skin, gills and eyes. For all kinds of samples, decreasing scores in all attributes were obtained as long as the storage time increased. Good correlation values between the storage time and the different attributes were obtained in all cases ($r^2 = 0.90-0.95$) except for fillets from the November catching, where only fair values were obtained ($r^2 = 0.80-0.90$).

Fillet samples showed a shorter shelf-life time than their counterpart whole fish samples both in May and November experiments. Shelf-life times for whole fish products were not different for samples caught in May and November (5 months in both cases), according to lipid damage (PV and TBA-i) indices that showed no significant ($p > 0.05$) differences. However, shelf-life of fillets from May samples was higher (3 months) than in their counterparts from November samples (1 month), according to the previously expressed results concerning the biochemical indices assessments.

The limiting factor for whole fish samples was odour and flesh appearance (May catching) and odour, flesh appearance and general aspect (November catching). In the case of fillets, odour was found to be the limiting factor for the May samples, while odour and flesh appearance were the limiting factors for the November samples.

4. CONCLUSIONS

In accordance with previous research on mackerel and other fatty fish species [18, 28, 40], the present study has shown an important lipid hydrolysis and oxidation development during the frozen storage. Lipid damage and fish quality loss caused by the storage conditions have been satisfactorily assessed by biochemical damage indices (FFA, PV, TBA-i and FR) and by sensory assessment.

Results have shown a strong effect of the kind of product to be commercialised. Thus, fillets were found to be more prone to oxidation development (PV, TBA-i and FR) than their counterpart whole fish pieces, as a result of a greater accessibility of the fish muscle in fillets to oxygen from air [16, 32]. If longer shelf-life times are commercially required for fillets, protective treatments such as vacuum packaging, modified atmosphere packaging, and natural antioxidant application are recommended [17, 41, 42].

The mackerel lipid content has also shown an important effect on lipid oxidation development in the case of fillet samples; in such fish product, primary and secondary lipid oxidation were found bigger for fattier mackerel (November catching experiment) than for leaner mackerel (May experiment) and agreed to sensory assessment. However, interaction compound formation (tertiary lipid oxidation) was found bigger for May samples than in their counterpart fattier fish (November experiment). In this sense, an important effect of the nucleophilic compound composition in fish muscle is also supposed to take part, in addition to the oxidation compound presence.

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FIGURE LEGENDS

Figure 1: Free fatty acid (FFA) determination* during mackerel frozen storage**

*Mean values of three independent determinations are expressed; bars denote standard deviations of the mean.

** Sample names: WF-M and F-M (whole fish and fillets, respectively, captured in May) and WF-N and F-N (whole fish and fillets, respectively, captured in November).

Figure 2: Peroxide value (PV) assessment* during mackerel frozen storage**

*Mean values of three independent determinations are expressed; bars denote standard deviations of the mean.

** Sample names: As expressed in Figure 1.

Figure 3: Thiobarbituric acid index (TBA-i) determination* during mackerel frozen storage**

*Mean values of three independent determinations are expressed; bars denote standard deviations of the mean.

** Sample names: As expressed in Figure 1.

Figure 4: Fluorescence ratio (FR) assessment* during mackerel frozen storage**

*Mean values of three independent determinations are expressed; bars denote standard deviations of the mean.

** Sample names: As expressed in Figure 1.

TABLE 1

Scale employed for evaluating quality of frozen mackerel

Attribute	E (Highest quality)	A (Good quality)	B (Fair quality)	C (Rejectable quality)
Skin	Very intense pigmentation; transparent mucus	Insignificant pigmentation losses; slightly turbid mucus	Pigmentation discoloured and without shine; milky mucus	Important pigmentation losses; opaque mucus
Eyes	Convex; transparent cornea; bright and black pupil	Convex and slightly sunken; slightly opalescent cornea; black and cloudy pupil	Flat; opalescent cornea; opaque pupil	Concave and milky cornea; grey pupil
Gills	Bright red; without odour; lamina perfectly separated	Rose-colored; without odour; lamina adhered in groups	Slightly pale; fishy odour; lamina adhered in groups	Grey-yellowish colour; intense ammonia odour; lamina totally adhered
Odour	Sharp seaweed and shellfish	Weak seaweed and shellfish	Slightly sour and incipient rancidity	Sharply sour and rancid
Texture	Presence or partial disappearance of rigor mortis symptoms	Firm and elastic; pressure signs disappear immediately and completely	Presence of mechanical signs; elasticity notably reduced	Important shape changes due to mechanical factors
Flesh Appearance	Strongly hydrated and pink; myotomes totally adhered	Still hydrated and pink; myotomes adhered	Slightly dry and pale; myotomes adhered in groups	Yellowish and dry; myotomes totally separated

TABLE 2

Sensory acceptance* of frozen mackerel that was previously captured in May

Frozen Storage Time (months)	Whole Fish				Fillets		
	General Aspect	Odour	Texture	Flesh Appearance	Skin	Odour	Flesh Appearance
Starting fish	E	E	E	E	E	E	E
1	A	A	A	A	A	A	A
3	B	B	A	B	B	B	B
5	B	B	A	B	B	C	B
7	B	C	A	C	C	C	C
9	B	C	B	C	C	C	C
12	C	C	C	C	C	C	C

*Category marks according to Table 1.

TABLE 3

Sensory acceptance* of frozen mackerel that was previously captured in November

Frozen Storage Time (months)	Whole Fish				Fillets		
	General Aspect	Odour	Texture	Flesh Appearance	Skin	Odour	Flesh Appearance
Starting fish	E	E	E	E	E	E	E
1	A	A	A	A	B	A	B
3	B	B	A	B	B	C	C
5	B	B	A	B	C	C	C
7	C	C	B	C	C	C	C
9	C	C	C	C	C	C	C
12	C	C	C	C	C	C	C

*Category marks according to Table 1.