# Quality Loss Related to Rancidity Development During Frozen Storage of Horse Mackerel (*Trachurus trachurus*)

**Santiago P. Aubourg*, Carmen Piñeiro, and Mª Jesús González**

Instituto de Investigaciones Marinas Consejo Superior de Investigaciónes Cientificas, 36208-Vigo, Spain

**ABSTRACT:** The development of rancidity and its effect on quality loss were studied in frozen horse mackerel (*Trachurus trachurus*). Two different kinds of fish products (whole fish and fillets) were stored at a commercial freezer temperature (–20°C) for up to 12 mon and were compared to samples stored at a much lower temperature (–80°C). Analyses included: lipid hydrolysis (FFA formation) and oxidation (PV, thiobarbituric acid index, fluorescent compound formation), loss of endogenous antioxidant (α-tocopherol), protein changes (electrophoretic analysis of sarcoplasmic and SDS-soluble fractions), and sensory analysis (skin, eyes, gills, flesh odor, consistency, flesh appearance). According to biochemical indices, fillets stored at –20°C showed susceptibility to rancidity development, leading to a shelf life of 1 mon, whereas whole fish at the same temperature were still edible at month 5. The use of a low temperature (–80°C) inhibited rancidity development, leading to good-quality (whole fish) and fair-quality (fillets) fish products at the end of the experiment. The application of protective treatments especially designed to prevent lipid oxidation is encouraged when commercializing this species in the frozen state.

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**KEY WORDS:** Fillets, frozen storage, horse mackerel, quality, rancidity, sensory analysis, shelf life, whole fish.

In the last few decades, marine foods have captured great attention from the consumer because of their positive role in human health and nutrition (1,2). Marine species are known to give rise to a wide range of products of great economic importance in many countries. However, the fishing industry is suffering from dwindling stocks of traditional species as a result of drastic changes in their availability. As a result, fish technologists and the fish trade have turned their attention to some unconventional sources of raw material (3,4).

One such species is horse mackerel (*Trachurus trachurus*), a medium-fat species that is abundant in the northeast Atlantic Ocean (5). Efforts have been made to use it in the manufacture of several products such as smoked (6), canned (7), chilled (8), and frozen (9) fish.

Freezing and frozen storage have been used largely to retain fish sensory and nutritional properties (10,11). However, marine species have shown a highly unsaturated lipid composition and the important presence of prooxidant molecules that facilitate the development of rancidity (12,13). Under frozen conditions, lipid oxidation compounds are produced that interact with proteins, leading to protein denaturation (14), nutritional losses (15), and modification of electrophoretic profiles of proteins (16), while endogenous antioxidant systems have been shown to be partially lost (17).

The present work concerns horse mackerel (*T. trachurus*) and its commercialization as a frozen product. For that, two different kinds of fish products (whole fish and fillets) were stored at a commercial freezer temperature (–20°C) and compared with the corresponding controls held at –80°C. Analyses included lipid hydrolysis and oxidation damage, endogenous α-tocopherol assessment, protein electrophoretic profile changes, and sensory acceptance. The study was carried out over 12 mon of frozen storage so that much attention could be given to quality changes and, accordingly, to the shelf life.

## MATERIALS AND METHODS

*Raw fish, sampling, and processing*. Fresh horse mackerel (*T. trachurus*) were obtained in a local market 10 h after being caught. During this time and until arrival at the laboratory, the fish were kept on ice. The length of the fish was in the range 18–24 cm; the weight was in the range of 250–280 g. The fish were divided into two groups. One was directly packaged in polyethylene bags and frozen at –80°C, and will be considered as the *whole fish* product (including head, organs, and skin) in the present work. The other group was carefully dressed, filleted by hand, washed with water, packaged in polyethylene bags, and frozen at –80°C; this group will be considered as the *fillet* product (deboned but not skinned) in the present work. After 24 h at –80°C, half of the whole fish and half of the fillets were placed at –20°C; the remaining material continued to be kept at –80°C. Sampling was undertaken on the raw fish material and at months 1, 3, 5, 7, 9, and 12 of frozen storage at –80 and –20°C on both kinds of products (whole fish and fillets) after being thawed overnight at 2°C. At each temperature and for each kind of fish product, three different batches were considered. These were studied separately to achieve statistical reliability.

*Sensory analyses*. Sensory analyses were conducted by a taste panel consisting of five experienced judges, according to the guidelines presented in Table 1 (18). Four categories were ranked: highest quality (E), good quality (A), fair quality (B), and rejectable quality (C). Sensory assessment of the whole

*To whom correspondence should be addressed at Instituto de Investigaciones Marinas Consejo Superior de Investigaciónes Cientificas, Spain. E-mail: saubourg@iim.csic.es

**TABLE 1**
**Scale Employed for Evaluating Quality of Frozen Horse 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 discolored 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; gray pupil |
| Gills | Bright red; without odor; lamina perfectly separated | Rose-colored; without odor; lamina adhered in groups | Slightly pale; fishy odor; lamina adhered in groups | Greyish-yellow color; intense ammonia odor; lamina totally adhered |
| Flesh odor | Sharply seaweedy and shellfish | Weakly seaweedy and shellfish | Slightly sour and incipient rancidity | Sharply sour and rancid |
| Consistency | 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 |

fish samples included the following parameters: skin, eyes, gills, flesh odor, consistency, and flesh appearance. In the case of fillets, the following attributes were considered: skin, flesh odor, consistency, and flesh appearance.

*Composition analyses*. Once fish products (whole fish and fillets) had been subjected to sensory analysis, the white muscle was separated, minced, and homogenized to carry out the biochemical analyses. Water content was determined by weight difference between the homogenized muscle (1–2 g) before and after oven-drying for 24 h at 105°C. Results were calculated as g water/100 g muscle. Lipids were extracted from the muscle by the method of Bligh and Dyer (19). Results are expressed as g lipids/100 g wet muscle.

The sarcoplasmic fraction from muscle proteins was obtained by extraction in a low-ionic-strength buffer composed of 10 mM Tris-HCl pH 7.2 + 50 mM pentamethyl sulfonic acid. Samples of 500 mg of muscle were homogenized for 60 s in 4 mL of solution buffer, according to Piñeiro *et al.* (20). Afterward, extracts were centrifuged at 12,500 × *g* for 15 min in a JA20.1 rotor (J221-M centrifuge; Beckman-Coulter) at 4°C, and the supernatants were recovered. The protein concentration in extracts was determined by means of the protein microassay method (Bio-Rad Laboratories Inc., Hercules, CA). A standard curve constructed on BSA was used as the reference. The extracts were maintained at –80°C until electrophoretic analysis.

Solubilization of muscle protein was also made in a 2% SDS buffer (2% wt/vol SDS/0.1 DTT/60 mM Tris-HCl, pH 7.5) using the same conditions mentioned for the sarcoplasmic protein extraction (20) except that before the centrifugation at 4°C, the SDS extracts were boiled at 100°C for 2 min, homogenized for 30 s, and then maintained at room temperature. The protein content was determined by indirect measurement at 280 nm (20). SDS extracts were maintained at –20°C until analysis.

*Lipid damage indices*. FFA content was determined by the method of Lowry and Tinsley (21) based on complex formation with cupric acetate-pyridine. Results are expressed as g FFA/100 g lipids. PV, expressed as meq oxygen/kg lipids, was determined by the ferric thiocyanate method (22). The thiobarbituric acid index (TBA-i) (mg malondialdehyde/kg fish tissue) was determined according to the method of Vyncke (23). Fluorescence formation (Perkin-Elmer LS 3B) at 327/415 nm and 393/463 nm was studied as described elsewhere (24,25). 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 sulfate solution (1 μg/mL in 0.05 M $H_2SO_4$) at the corresponding wavelength. The fluorescence ratio (FR) was obtained from the lipid extract analysis according to the following calculation: $FR = RF_{393–463nm}/RF_{327–415nm}$.

*Tocopherol assessment.* Tocopherol isomers were analyzed

according to the method of Cabrini *et al.* (26). The presence of the different tocopherol isomers was checked. Only the α-tocopherol isomer was detected in the subject horse mackerel samples, and its content was expressed as μg/100 g fish muscle.

*Protein electrophoretic studies*. Electrophoretic analysis was carried out by means of horizontal commercial SDS-PAGE gels. Samples were mixed with sample buffer according to the Laemmli (27) procedure. Because of their higher resolution and reproducibility, precast polyacrylamide 245 × 110 × 1 mm commercial gels (Excel-Gel SDS Homogeneous 15%, Amersham Biosciences, Uppsala, Sweden) for horizontal electrophoresis were selected. Anode and cathode buffer strips (Amersham Biosciences) were also employed. Electrophoretic studies were performed in a Multiphor II electrophoresis system (Amersham Biosciences) provided with a MultiTemp III refrigerated bath circulator (Amersham Biosciences). Running conditions were 1000 V/40 mA/40 W, at 15°C for 165 min. Once the bromophenol blue had reached the anode, gels were fixed and stained by a standard silver staining protocol (Amersham Biosciences). A low M.W. protein standard (14–94 kDa) from Amersham Biosciences was employed as the reference.

*Statistical analyses*. Data from the different biochemical measurements were subjected to one-way ANOVA ($P < 0.05$); comparison of means was performed using a least squares difference method (28). Correlation analyses and the Spearman test for nonparametric correlations were also performed (28).

## RESULTS AND DISCUSSION

Water content ranged between 74 and 78% in all samples. Lipid content ranged between 2.2 and 3.7% on a wet basis. Differences in both constituents may be explained as a result of fish-to-fish variation, and not as arising from frozen storage conditions (time and temperature) and the kind of fish product (whole fish and fillets). Comparison of the present results with previous research showed a higher water content for horse mackerel than for fattier fish species (sardine) (24) and a lower water content than for leaner fish species (blue whiting, cod, and haddock) (25,29), in accordance with an inverse ratio between water and lipid matter.

The protein content extracted with low ionic strength buffer ranged from 2.5 to 3.5 g/100 g wet muscle. Small variations in its content could be explained as fish-to-fish differences and not arising from frozen storage conditions.

*Lipid hydrolysis*. In the present study, the FFA content of the raw material (Table 2) was similar to that of fatty fish species (tuna, sardine) (24,30) and lower than that of lean fish species (blue whiting, haddock, cod) (25,29).

Hydrolysis developed gradually at –20°C in both kinds of products, so that a good correlation value with time was obtained ($r^2 = 0.94$ and 0.89 for whole fish and fillets, respectively). The use of a much lower temperature such as –80°C showed a preservative effect by inhibiting lipid hydrolysis development, so that lower ($P < 0.05$) FFA values were obtained in all cases by comparing –80°C with –20°C samples. The kind of product (whole fish and fillet) did not exert an important effect on lipid hydrolysis.

Examining the extent of lipid hydrolysis was deemed important to the study because of the high lipid hydrolysis development previously observed in horse mackerel during frozen storage (9) and also because of the great influence of lipid hydrolysis on lipid oxidation (31) and on protein denaturation (14).

*Lipid oxidation*. Peroxide formation (Table 3) was higher ($P < 0.05$) for fillets at –20°C than for the three other samples throughout the experiment, which could be explained as a combination of a higher temperature and a greater surface area being exposed to air. At the end of the storage time, whole fish

**TABLE 2**
**FFA[a] Determination in Frozen Horse Mackerel**

| Frozen storage time (mon) | WP-80 | WP-20 | F-80 | F-20 |
|---|---|---|---|---|
| Raw fish | 0.60 a (0.290) | 0.60 a (0.290) | 0.60 a,b (0.290) | 0.60 a (0.290) |
| 1 | $^{W}$ 0.35 a (0.153) | $^{X}$ 1.20 a (0.404) | $^{W}$ 0.61 a,b (0.184) | $^{Y}$ 1.92 a,b (0.104) |
| 3 | $^{W}$ 0.39 a (0.116) | $^{X}$ 2.47 a,b (0.528) | $^{W}$ 0.57 a (0.260) | $^{X}$ 3.22 b (0.696) |
| 5 | $^{W}$ 1.10 b (0.290) | $^{X}$ 3.75 b,c (0.575) | $^{W}$ 1.27 b (0.226) | $^{X}$ 3.78 b (0.469) |
| 7 | $^{W}$ 0.73 a (0.221) | $^{Y}$ 5.85 c,d (1.584) | $^{W}$ 0.66 a,b (0.252) | $^{X}$ 3.99 b (1.069) |
| 9 | $^{W}$ 0.58 a (0.198) | $^{X}$ 8.35 d,e (2.428) | $^{W}$ 1.24 b (0.424) | $^{X}$ 6.67 c (2.191) |
| 12 | $^{W}$ 0.37 a (0.200) | $^{X}$ 10.09 e (2.001) | $^{W}$ 0.57 a (0.411) | $^{X}$ 8.30 c (2.056) |

[a]Expressed as g FFA/100 g lipids. Mean values of three independent determinations; SD are indicated in parentheses. For each column, means followed by different letters (a–e) are significantly different ($P < 0.05$). For each row, means preceded by different superscripts (W–Y) are significantly different ($P < 0.05$). Sample abbreviations: WP-80 (whole fish stored at –80°C), WP-20 (whole fish stored at –20°C), F-80 (fillets stored at –80°C), and F-20 (fillets stored at –20°C).

**TABLE 3**
**PV[a] Assessment in Frozen Horse Mackerel**

| Frozen storage time (mon) | WP-80 | WP-20 | F-80 | F-20 |
|---|---|---|---|---|
| Raw fish | 3.59 a,b (0.283) | 3.59 a,b (0.283) | 3.59 a (0.283) | 3.59 a (0.283) |
| 1 | $^{W}$ 3.09 a (1.179) | $^{W}$ 2.68 a (0.635) | $^{W}$ 3.97 a (1.796) | $^{X}$ 16.02 b (1.758) |
| 3 | $^{X}$ 8.13 c,d (0.294) | $^{X}$ 10.14 a,b,c (2.825) | $^{W}$ 4.32 a (1.382) | $^{Y}$ 58.26 e (2.616) |
| 5 | $^{W}$ 9.35 c,d (2.747) | $^{W}$ 9.87 a,b,c (2.744) | $^{W}$ 4.56 a (1.150) | $^{X}$ 33.63 c,d (7.637) |
| 7 | $^{W,X}$ 8.62 c,d (0.939) | $^{X}$ 14.48 c (4.326) | $^{W}$ 7.44 b (1.293) | $^{X}$ 26.86 c (0.623) |
| 9 | $^{W}$ 11.49 d (3.130) | $^{W}$ 12.63 b,c (4.050) | $^{W}$ 10.92 c (1.343) | $^{X}$ 39.33 d (6.010) |
| 12 | $^{W}$ 6.63 b,c (2.036) | $^{X}$ 31.77 d (1.676) | $^{W}$ 3.84 a (0.987) | $^{Y}$ 87.44 f (10.709) |

[a]Expressed as meq oxygen/kg lipids. Mean values of three independent determinations; SD are indicated in parentheses. For each column, means followed by different letters (a–f) are significantly different ($P < 0.05$). For each row, means preceded by different superscripts (W–Y) are significantly different ($P < 0.05$). For abbreviations see Table 2.

kept at –20°C showed an increase in PV that was significantly higher ($P < 0.05$) than in the two –80°C samples that were assessed. In both fish products kept at –80°C, a peroxide breakdown could be assessed at the end of the experiment. Fair correlation values with time were obtained for samples kept at –20°C ($r^2 = 0.82$ and 0.74 for whole fish and fillets, respectively). The use of a low temperature, i.e., –80°C, inhibited the formation of peroxides so that values higher than 12 meq oxygen/kg lipids were not obtained.

Secondary oxidation (Table 4) as measured by TBA-i showed results similar to those for PV. Levels developed in fillets kept at –20°C were higher and showed a continuous increase with storage time, which led to a satisfactory correlation value ($r^2 = 0.94$). The other three samples showed a maximal mean value at month 5, which was followed by a decrease. A higher ($P < 0.05$) TBA-i was obtained for whole fish kept at –20°C than for both products at –80°C at the end of the storage time. Values obtained at –20°C during the length of the experiment were relatively low compared with those related to fattier fish species (yellowfin tuna, swordfish, and sardine) (24,32).

FR showed a gradual increase for fillets and whole fish stored at –80°C and for whole fish at –20°C (Table 5). The best correlation values were obtained for the whole fish products ($r^2 = 0.89$ and 0.92, for –80 and –20°C samples, respectively). This general increase can be explained as resulting from the interaction between peroxides and TBAR substances with

**TABLE 4**
**Thiobarbituric Acid Index[a] Determination in Frozen Horse Mackerel**

| Frozen storage time (mon) | WP-80 | WP-20 | F-80 | F-20 |
|---|---|---|---|---|
| Raw fish | 0.17 a (0.035) | 0.17 a (0.035) | 0.17 a (0.035) | 0.17 a (0.035) |
| 1 | $^{W}$ 0.19 a (0.083) | $^{W}$ 0.26 a,b (0.129) | $^{W}$ 0.24 a (0.051) | $^{X}$ 0.76 b (0.190) |
| 3 | $^{W}$ 0.19 a (0.085) | $^{X}$ 0.41 b (0.113) | $^{X}$ 0.45 b (0.136) | $^{Y}$ 1.04 b,c (0.110) |
| 5 | $^{W}$ 0.72 b (0.162) | $^{W}$ 0.85 c (0.050) | $^{W}$ 0.83 c (0.170) | $^{W}$ 0.74 b (0.089) |
| 7 | $^{W}$ 0.18 a (0.051) | $^{X}$ 0.45 b (0.151) | $^{W}$ 0.20 a (0.081) | $^{Y}$ 1.29 c,d (0.056) |
| 9 | $^{W}$ 0.22 a (0.040) | $^{W}$ 0.41 b (0.145) | $^{W}$ 0.20 a (0.095) | $^{X}$ 1.60 d (0.406) |
| 12 | $^{W}$ 0.22 a (0.069) | $^{X}$ 0.75 c (0.190) | $^{W}$ 0.14 a (0.012) | $^{Y}$ 2.37 e (0.167) |

[a]Expressed as mg malondialdehyde/kg fish muscle. Mean values of three independent determinations; SD are indicated in parentheses. For each column, means followed by different letters (a–e) are significantly different ($P < 0.05$). For each row, means preceded by different superscripts (W–Y) are significantly different ($P < 0.05$). For abbreviations see Table 2.

**TABLE 5**
**Fluorescence Ratio[a] Analysis in Frozen Horse Mackerel**

| Frozen storage time (mon) | WP-80 | WP-20 | F-80 | F-20 |
|---|---|---|---|---|
| Raw fish | 0.42 a (0.015) | 0.42 a (0.015) | 0.42 a (0.015) | 0.42 a (0.015) |
| 1 | W 0.52 a,b (0.711) | W 0.70 b (0.112) | W 0.53 a (0.028) | X 1.45 b,c (0.331) |
| 3 | W 0.65 a,b (0.028) | W 0.89 b (0.223) | W 0.53 a (0.072) | X 1.79 c,d (0.291) |
| 5 | W 0.79 b (0.146) | W 0.73 b (0.157) | W,X 1.10 b,c (0.346) | X 1.35 b (0.304) |
| 7 | W 0.83 b,c (0.014) | X 1.14 c (0.133) | W,X 1.01 b,c (0.031) | Y 1.38 b,c (0.145) |
| 9 | W 1.16 c,d (0.349) | W 1.19 c,d (0.085) | W 0.85 b (0.184) | X 1.90 d (0.246) |
| 12 | W 1.28 d (0.134) | W 1.43 d (0.100) | W 1.14 c (0.201) | X 1.80 c,d (0.248) |

[a]Calculated as expressed in the Materials and Methods section. Mean values of three independent determinations; SD are indicated in parentheses. For each column, means followed by different letters (a–d) are significantly different ($P < 0.05$). For each row, means preceded by different superscripts (W–Y) are significantly different ($P < 0.05$). For abbreviations see Table 2.

nucleophilic molecules (free amino acids, peptides, proteins, aminated phospholipids) present in the fish muscle (33,34), which lead to the formation of fluorescent molecules. In the case of fillets stored at –20°C, a continuous increase was not observed; there was a decrease at month 5, and the highest mean value was determined at month 9. According to the PV and TBA-i data, a higher development of fluorescence occurred for fillets at –20°C than for the three other samples throughout the storage time, whereas no significant differences ($P > 0.05$) could be ascertained among the three others.

*α-Tocopherol assessment.* Although a decreasing trend in α-tocopherol content was obtained for both samples kept at –80°C, no significant ($P > 0.05$) differences could be assessed during frozen storage (Table 6). However, samples kept at –20°C showed a gradual decrease in α-tocopherol content in whole fish and fillet samples ($r^2 = -0.73$ and –0.82, respectively).

Comparison among the four kinds of samples indicated a lower ($P < 0.05$) α-tocopherol content in fillets at –20°C than in the other three at the end of storage, in agreement with the previously noted results on lipid oxidation (PV, TBA-i, and FR).

The present results show large individual variations in α-tocopherol content in accordance with previous research (35),

**TABLE 6**
**α-Tocopherol[a] Assessment in Frozen Horse Mackerel**

| Frozen storage time (mon) | WP-80 | WP-20 | F-80 | F-20 |
|---|---|---|---|---|
| Raw fish | 308.33 a (97.787) | 308.33 b (97.787) | 308.33 a,b (97.787) | 308.33 c (97.787) |
| 1 | W 326.67 a (23.072) | W 356.33 b (87.077) | W 304.33 a,b (143.615) | W 319.67 c (81.684) |
| 3 | W 289.03 a (12.490) | X 350.67 b (60.343) | W,X 328.17 b (73.141) | W 225.67 b,c (36.747) |
| 5 | W 273.05 a (29.103) | W 260.47 a,b (42.211) | W 306.67 a,b (81.938) | W 201.00 b,c (43.486) |
| 7 | W 225.43 a (41.373) | W 161.67 a (10.786) | W 221.47 a (30.293) | W 188.47 b,c (31.490) |
| 9 | W 225.50 a (68.891) | W 188.60 a (16.122) | W 133.93 a (5.090) | W 93.75 a,b (47.023) |
| 12 | X 237.33 a (45.927) | X 161.93 a (53.421) | X 214.00 a (75.530) | W 28.13 a (9.381) |

[a]Expressed as μg/100 g fish muscle. Mean values of three independent determinations; SD are indicated in parentheses. For each column, means followed by different letters (a–c) are significantly different ($P < 0.05$). For each row, means preceded by different superscripts (W–X) are significantly different ($P < 0.05$). For abbreviations see Table 2.

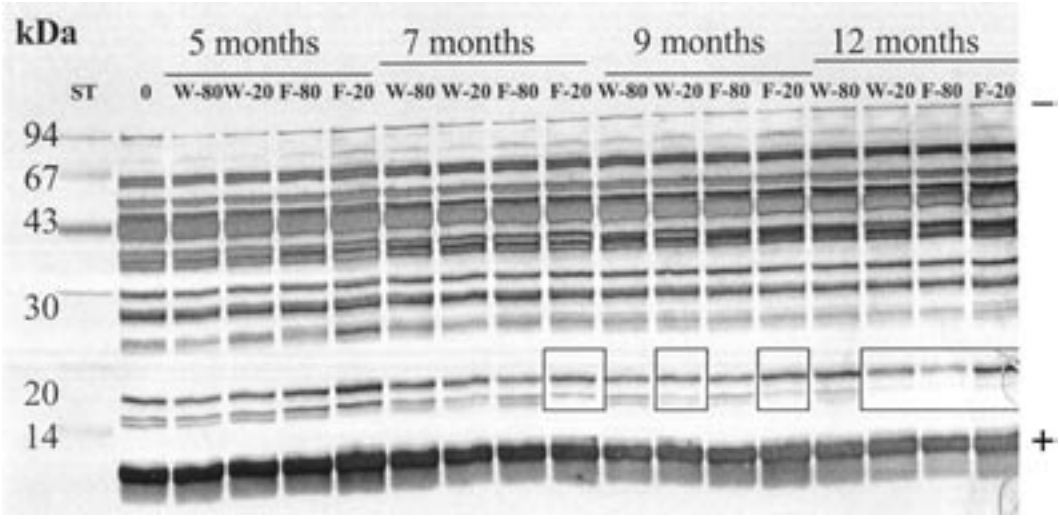


**FIG. 1.** Electrophoretic profiles obtained from analyses of sarcoplasmic protein fraction by SDS-PAGE in commercial homogeneous gels. Lane abbreviations: ST (low M.W. standard), 0 (raw fish), W-80 (whole fish stored at –80°C), W-20 (whole fish stored at –20°C), F-80 (fillets stored at –80°C), F-20 (fillets stored at –20°C). The numbers above the lanes indicate the months of frozen storage. The cathodic (–) and anodic (+) sides are indicated.

which make it difficult to establish significant differences. However, the α-tocopherol mean value of whole fish kept at –20°C was less than the corresponding values of both –80°C samples at the end of the storage, although differences were not significant ($P > 0.05$). Previous research in which more rigorous processing conditions were applied, frozen storage was at –6°C (36), and frozen storage was preceded by a chilling time (17) showed greater α-tocopherol losses than in the present case.

*Protein electrophoretic analysis*. At month 7 of frozen storage, the fillets stored at –20°C showed some changes in the sarcoplasmic protein profiles obtained from the low ionic strength buffer, according to the SDS-PAGE analysis (Fig. 1). In this profile, one band (18 kDa M.W.) disappeared from this date until the end of the trial. The same behavior was observed in whole fish stored at –20°C (months 9 and 12) and for the fillet samples at the end of the frozen storage at –80°C.

In the case of SDS-soluble proteins, the analyses by 15% homogeneous SDS-PAGE showed several changes in the protein profiles. These changes are especially remarkable at the end of the storage (Fig. 2) between M.W. of 30–20 and 20–14 kDa in whole fish and fillets kept frozen at –20°C, the degree of damage being greater in the case of fillets than in whole fish. These two M.W. ranges could correspond to different myosin light chains. The acrylamide concentration used in the present gels does not allow separation of proteins with a M.W. above 100 kDa, such as the myosin heavy chains.

Under the effect of SDS, the myosin molecule is known (37,38) to dissociate into subunits of high and low M.W. that can be separated by electrophoretic techniques. Thus, in the frozen state (38), there have been more changes in the electrophoretic profiles from SDS-soluble proteins than in the sarcoplasmic fraction, as a result of the myofibrillar fraction of seafoods being most susceptible during frozen storage. However, some low-weight myofibrillar proteins also can be extracted with low ionic strength buffers, such as the one employed in the present study. This kind of muscle protein can be denatured by many factors, such as temperature changes and the presence of oxidized lipids and of formaldehyde. In these kinds of studies, SDS-PAGE has been widely used because it enables the determination of protein M.W. and the solubilization of denatured proteins (39).

*Sensory analysis.* Scores obtained for the different kinds of samples throughout the experiment are indicated in Table 7.

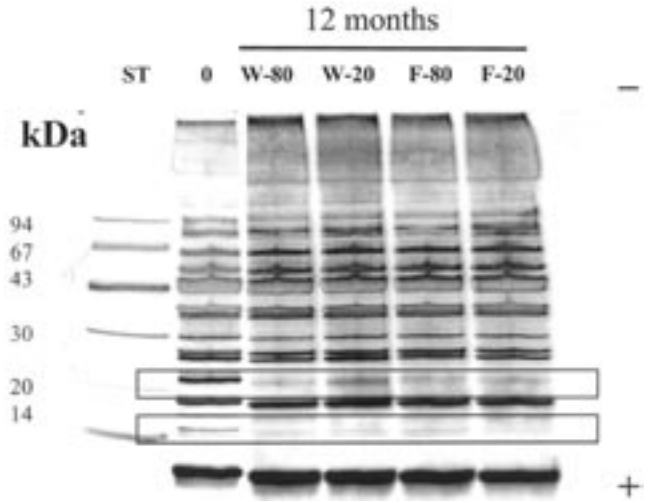


**FIG. 2.** Electrophoretic profiles obtained from analyses of SDS-soluble muscle proteins and separated in SDS-PAGE commercial homogeneous (15%) gels. For abbreviations see Figure 1.

**TABLE 7**
**Sensory Acceptance[a] of Frozen Horse Mackerel**

| Frozen storage time (mon) | WP-80 | WP-20 | F-80 | F-20 |
|---|---|---|---|---|
| Raw fish | E | E | E | E |
| 1 | E | A | E | B |
| 3 | E | B | A | C |
| 5 | A | B | A | C |
| 7 | A | C | A | C |
| 9 | A | C | B | C |
| 12 | A | C | B | C |

[a]Category marks according to Table 1. For abbreviations see Table 2.

Since different sensory parameters were analyzed in each kind of sample, Table 7 shows the lowest mark obtained at each sampling time.

Although a gradual decrease in acceptance could be assessed for the four kinds of products during the experiment, damage rates were considerably different according to the storage temperature and to the kind of product, where fillets deteriorate faster than whole fish.

The sensory analysis showed a short shelf life for the fillets stored at –20°C; they were unacceptable at month 3, whereas at –20°C whole fish were still acceptable at month 5. In the case of whole fish, the limiting attribute appeared to be the rancid odor development, it was the development of both the rancid odor and yellowish color. Both products kept at –80°C tested as still edible at the end of the storage, since whole fish were good quality (A range) and fillets were fair quality (B range).

Sensory acceptance showed good correlation with the storage time, especially in the case of samples kept at –80°C ($r^2$ = 0.87 and 0.95, whole fish and fillets, respectively). Comparison of sensory marks with biochemical indices showed the best correlation values for whole fish samples kept at –80°C ($r^2$ = 0.83 with FR) and at –20°C ($r^2$ = 0.93 and 0.90, with FFA and FR, respectively).

According to sensory assessment, frozen horse mackerel as a fillet product has a short shelf life (1 mon) when stored at a commercial freezer temperature (–20°C); however, whole fish products maintained a longer shelf life, being edible at month 5. These results agreed with biochemical indices related to lipid oxidation development (PV, TBA-i, FR), endogenous antioxidant loss (α-tocopherol), and SDS-soluble protein fraction, but not with lipid hydrolysis formation, where no effect of the product type was observed. The employment of the much lower temperature of –80°C prevented rancidity development, although its use is impractical because of commercial expenses.

If longer shelf life times are commercially required (for fillets, especially), protective treatments such as vacuum packaging, modified atmosphere packaging, and natural antioxidant application (40,41) are recommended to prevent lipid oxidation development and its concomitant effects on sensory acceptance, myofibrillar profile changes, and endogenous antioxidant loss.

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## REFERENCES

1. Kinsella, J.E., Dietary Fats and Cardiovascular Disease, in *Seafoods and Fish Oils in Human Health and Disease*, edited by J.E. Kinsella, Marcel Dekker, New York, 1987, pp. 1–23.
2. Illingworth, D., and D. Ullmann, Effects of Omega-3 Fatty Acids on Risk Factors for Cardiovascular Disease, in *Omega-3 Fatty Acids in Health and Disease*, edited by R.S. Lees and M. Karel, Marcel Dekker, New York, 1990, pp. 39–69.
3. Flick, G.J., A.P. Bimbo, L.G. Enríquez, and R.L. Ory, Potential and Physical Properties of Underutilized Species, Menhaden, in *Advances in Seafood Biochemistry. Composition and Quality*, edited by G. Flick and D. Kitts, Technomic, Lancaster, PA, 1992, pp. 1–23.
4. Shahidi, F., and V. Venugopal, Water-Soluble Protein Prepared from Underutilized Fish Species, in *Seafood Safety, Processing and Biotechnology*, edited by F. Shahidi, Y. Jones, and D.D. Kitts, Technomic, Lancaster, PA, 1997, pp. 181–186.
5. FAO, *Fishery Statistics, Yearbook 2000*, Food and Agriculture Organization of the United Nations, Rome, 2002, Vol. 90/1, pp. 245–248.
6. García, I., B. Pérez-Villarreal, and R. Pozo, Valorización de Especies Pesqueras Infrautilizadas, *Alimentación, Equipos y Tecnología* (January/February):145–149 (1996).
7. Münkner, W., Zur rationellen Verwertung der Rohware Fisch. II. Die Konservenherstellung aus Fischfarsch, *Fischerei-Forschung Rostock 25*:120–122 (1987).
8. Aubourg, S.P., Damage Detection in Horse Mackerel (*Trachurus trachurus*) During Chilled Storage, *J. Am. Oil Chem. Soc. 78*:857–862 (2001).
9. Aubourg, S.P., I. Lehmann, and J.M. Gallardo, Effect of Previous Chilled Storage on Rancidity Development in Frozen Horse Mackerel (*Trachurus trachurus*), *J. Sci. Food Agric. 82*:1764–1771 (2002).
10. Pigott, G.M., and B.W. Tucker, Science Opens New Horizons for Marine Lipids in Human Nutrition, *Food Rev. Int. 3*:105–138 (1987).
11. Erickson, M.C., Lipid Oxidation: Flavor and Nutritional Quality Deterioration in Frozen Foods, in *Quality in Frozen Food*, edited by M.C. Erickson and Y.-C. Hung, Chapman & Hall, New York, 1997, pp. 141–173.
12. Mohri, S., S.Y. Cho, Y. Endo, and K. Fujimoto, Linoleate 13(*S*)-Lipoxygenase in Sardine Skin, *J. Agric. Food Chem. 40*:573–576 (1992).
13. Richards, M.P., and H.O. Hultin, Contributions of Blood and Blood Components to Lipid Oxidation in Fish Muscle, *J. Agric. Food Chem. 50*:555–564 (2002).
14. Sikorski, Z.E., and A. Kolakowska, Changes in Protein in Frozen Stored Fish, in *Seafood Proteins*, edited by Z.E. Sikorski, B.S. Pan, and F. Shahidi, Chapman & Hall, New York, 1994, pp. 99–112.
15. Castrillón, A., E. Álvarez-Pontes, M.T. García, and P. Navarro, Influence of Frozen Storage and Defrosting on the Chemical and Nutritional Quality of Sardine (*Clupea pilchardus*), *J. Sci. Food Agric. 70*:29–34 (1996).
16. Saeed, S., and N.K. Howell, Effect of Lipid Oxidation and Frozen Storage on Muscle Proteins of Atlantic Mackerel (*Scomber scombrus*), *J. Sci. Food Agric. 82*:579–586 (2002).
17. Undeland, I., and H. Lingnert, Lipid Oxidation in Fillets of Herring (*Clupea harengus*) During Frozen Storage. Influence of

Prefreezing Storage, *J. Agric. Food Chem. 47*:2075–2081 (1999).
18. Council Regulations, Baremo de Clasificación de Frescura, in *Diario Oficial de las Comunidades Europeas*, European Commission, Brussels (Belgium), No. L 5/21, 1989, pp. 5–6.
19. Bligh, E., and W. Dyer, A Rapid Method of Total Lipid Extraction and Purification, *Can. J. Biochem. Physiol. 37*:911–917 (1959).
20. Piñeiro, C., J. Barros-Velázquez, R.I. Pérez-Martín, I. Martínez, T. Jacobsen, H. Rehbein, R. Kündiger, R. Mendes, M. Ettienne, M. Jerome, A. Craig, I.M. Mackie, and F. Jessen, Development of a Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis Reference Method for the Analysis and Identification of Fish Species in Raw and Heat-Processed Samples: A Collaborative Study, *Electrophoresis 20*:1425–1432 (1999).
21. Lowry, R.R., and I.J. Tinsley, Rapid Colorimetric Determination of Free Fatty Acids. *J. Am. Oil Chem. Soc. 53*:470–472 (1976).
22. Chapman, R.A., and K. McKay, The Estimation of Peroxides in Fats and Oils by the Ferric Thiocyanate Method, *Ibid. 26*:360–363 (1949).
23. Vyncke, W., Direct Determination of the Thiobarbituric Acid Value in Trichloracetic Acid Extracts of Fish as a Measure of Oxidative Rancidity, *Fette Seifen Anstrichm. 72*:1084–1087 (1970).
24. Aubourg, S.P., C.G. Sotelo, and R. Pérez-Martín, Assessment of Quality Changes in Frozen Sardine (*Sardina pilchardus*) by Fluorescence Detection, *J. Am. Oil Chem. Soc. 75*:575–580 (1998).
25. Aubourg, S.P., Lipid Damage Detection During the Frozen Storage of an Underutilized Fish Species, *Food Res. Int. 32*:497–502 (1999).
26. Cabrini, L., L. Landi, C. Stefanelli, V. Barzanti, and A.M. Sechi, Extraction of Lipid and Lipophilic Antioxidants from Fish Tissues: A Comparison Among Different Methods, *Comp. Biochem. Physiol. B. Biochem. Molec. Biol. 101*:383–386 (1992).
27. Laemmli, U., Cleavage of Structural Proteins During the Assembly of the Head of Bacteriophage T4, *Nature 227*:680–685 (1970).
28. Statsoft, *Statistica for Macintosh*, Statsoft and Its Licensors, Tulsa, OK, 1994.
29. Aubourg, S.P., and I. Medina, Influence of Storage Time and Temperature on Lipid Deterioration During Cod (*Gadus morhua*) and Haddock (*Melanogrammus aeglefinus*) Frozen Storage, *J. Sci. Food Agric. 79*:1943–1948 (1999).
30. Medina, I., R. Sacchi, and S.P. Aubourg, A $^{13}$C-NMR Study of Lipid Alterations During Fish Canning: Effect of Filling Medium, *Ibid. 69*:445–450 (1995).
31. Yoshida, H., I. Kondo, and G. Kajimoto, Participation of Free Fatty Acids in the Oxidation of Purified Soybean Oil During Microwave Heating, *J. Am. Oil Chem. Soc. 69*:1136–1140 (1992).
32. Kurade, S.A., and J.D. Baranowski, Prediction of Shelf Life of Frozen Minced Fish in Terms of Oxidative Rancidity as Measured by TBARS Number, *J. Food Sci. 52*: 300–302, 311 (1987).
33. Leake, L., and M. Karel, Nature of Fluorescent Compounds Generated by Exposure of Protein to Oxidizing Lipids, *J. Food Biochem. 9*:117–136 (1985).
34. Kikugawa, K., and M. Beppu, Involvement of Lipid Oxidation Products in the Formation of Fluorescent and Cross-Linked Proteins, *Chem. Phys. Lipids 44*:277–297 (1987).
35. Undeland, I., B. Ekstrand, and H. Lingnert, Lipid Oxidation in Herring (*Clupea harengus*) Light Muscle, Dark Muscle, and Skin, Stored Separately or as Intact Fillets, *J. Am. Oil Chem. Soc. 75*:581–590 (1998).
36. Brannan, R.C., and M.C. Erickson, Quantification of Antioxidants in Channel Catfish During Frozen Storage, *J. Agric. Food Chem. 44*:1361–1366 (1996).
37. Sikorski, Z.E., Muscle Proteins, in *Chemical-Functional Properties of Food Proteins*, edited by Z.E. Sikorski, Technomic, Basel, Switzerland, 2001, pp. 233–268.
38. Sikorski, Z.E., The Myofibrillar Proteins in Seafood, in *Seafood Proteins*, edited by Z.E. Sikorski, B.S. Pan, and F. Shahidi, Chapman & Hall, New York, 1994, pp. 40–57.
39. Sotelo, C.G., C. Piñeiro, R. Pérez-Martin, and J.M. Gallardo, Analysis of Fish and Squid Myofibrillar Proteins by Capillary Sodium Dodecyl Sulfate Gel Electrophoresis: Actin and Myosin Quantification, *Eur. Food Res. Technol. 211*: 443–448 (2000).
40. Toledo-Flores, L., and R. Zall, Methods for Extending the Storage Life of Fresh Tropical Fish, in *Advances in Seafood Biochemistry*; Composition and Quality, edited by G.J. Flick, Jr., and R.E. Martin, Technomic, Lancaster, PA, 1992, pp. 233–243.
41. Richards, M.P., S.D. Kelleher, and H.O. Hultin, Effect of Washing With or Without Antioxidants on Quality Retention of Mackerel Fillets During Refrigerated and Frozen Storage, *J. Agric. Food Chem. 46*:4363–4371 (1998).

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