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# Effect of Neutral and Oxidized Lipids on Protein Functionality in Megrim (*Lepidorhombus whiffiagonis* W.) and Sardine (*Sardina pilchardus* R.) during Frozen Storage

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*ABSTRACT*

*The effect of added cod liver oil and oxidized cod liver oil on protein solubility, apparent viscosity and emulsifying capacity was measured during frozen storage (−18°C) of vacuum packed minced muscle of two non-dimethylamine (DMA)-forming species, megrim (*Lepidorhombus whiffiagonis *W) and sardine (*Sardinia pilchardus *R.). The aim was to establish the role of both unoxidized and oxidized lipids in protein functionality in the absence of interference due to the effect of formaldehyde on the proteins. The results showed that, with the addition of cod-liver oil (CLO), the three protein functional properties were equal to, or slightly lower than, the untreated controls in both species. Samples with oxidized cod-liver oil (OCLO) added showed lower protein solubility and viscosity, whereas the emulsifying capacity values were higher than in the controls. Hence, in non-DMA-producing fish species, no protective effect on fish protein can be attributed to neutral lipids.*

## INTRODUCTION

During frozen storage of fish, myofibrillar proteins can undergo a number of changes in texture and functionality. Among the different factors to which such deterioration is attributed are formaldehyde (FA) in certain low fat-content species (Castell *et al.*, 1973) and the intermediate and final products of lipid oxidation (Lilliard, 1987).



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It has been postulated that certain factors, such as neutral lipids, may stabilize proteins against deterioration (Olley *et al.*, 1962), although there are some contradictory data in model systems (Shenouda, 1980; Andou *et al.*, 1980, 1981).

Several studies carried out in our laboratory showed an apparent protective effect of both neutral and oxidized lipids in minced hake stored at −18°C (Careche & Tejada, 1990). These results lead us to postulate that the protective role attributed to lipids is not due only to a direct effect of lipids on proteins, but more importantly to the inhibition of formaldehyde/dimethylamine (DMA) formation in the hake mince, an effect which is most apparent when oxidized lipids are added. The authors therefore felt it necessary to establish the role of neutral and oxidized lipids in non-DMA-forming species in order to confirm the proposed hypothesis.

The aim of this work was to study the effect of both neutral and oxidized lipids in non-DMA-forming species: megrim (*Lepidorhombus whiffiagonis* W.) with naturally-occurring medium fat content and sardine (*Sardina pilchardus* R.) with naturally-occurring high fat content.

## MATERIALS AND METHODS

### Sample preparation

Megrim (*Lepidorhombus whiffiagonis* W.) and sardine (*Sardina pilchardus* R.) were caught, respectively, on the Galician Shelf in October 1985 and in the Mediterranean area in June 1987, and kept on ice for 24 h prior to use. Megrim samples consisted of seventy individuals with a mean length of 34 cm and a mean weight of 500 g after gutting, while the sardines totalled 43 kg, with mean length and weight of 13 cm and 31 g. The fish were headed, gutted, washed and minced in a Baader model 694 apparatus using a drum with 3-mm diameter holes.

The percent compositions of the megrim and sardine minces were, respectively, as follows: crude protein, 16·8 and 17·3; crude fat, 3·9 and 12·6; moisture, 79·0 and 67·8; ash, 0·8 and 1·2, pH was 6·95 and 5·98, respectively.

Three aliquots of mince were prepared for each species, to make up the following lots: 3% w/w cod liver oil (CLO) added; 3% w/w oxidized cod liver oil (OCLO) added, and untreated controls. The conditions of CLO oxidation are described in a previous paper (Careche & Tejada, 1990).

The temperature of the muscle was kept below 7·5°C during handling. The lots obtained were packaged on trays containing approximately 600 g, frozen in a horizontal plate freezer until the thermal centre temperature reached −18°C, vacuum-packed and stored at −18°C for about 370 days.

## Analyses

Crude fat was initially determined by a modification of the method of Bligh and Dyer (1959), using dichloromethane instead of chloroform, as suggested by Knudsen *et al.* (1985); crude protein (AOAC, no. 24024 (1975)), dimethylamine (DMA) values (Dyer & Mounsey, 1945), 2-thiobarbituric acid value (TBA) (Lemon, 1975), percentage of soluble protein (PS/PT) (Ironside & Love, 1958) apparent viscosity ($\eta_{app}$) (Borderías *et al.*, 1985) and emulsifying capacity (EC) (Tejada *et al.*, 1987) were analysed periodically throughout the storage period. Peroxide value (POV) (UNE, 1973) and TBA number (Sinhuber & Yu, 1977) were measured in the added CLO and OCLO. A one-way analysis of variance (Guenther, 1964) of the periodically-studied variables was performed using a BMDP IV program on a CDC CYBER 180/855 computer. The degree of significance was established for $P < 0{\cdot}05$.

## RESULTS AND DISCUSSION

Table 1 shows the initial conditions of the lipids added to the megrim and sardine minces, and the lipid content of the minces after treatment.

Tables 2 and 3 show the analyses of variance of the variables studied periodically in megrim (Table 2) and sardine (Table 3) for the different storage times and conditions.

The maximum DMA values for megrim and sardine after 370 days storage at −18°C were below 0·20 and 0·86 mg *N*-DMA/100 g, respectively,

**TABLE 1**
Initial Analyses of Lipids Added to the Minces and Final Lipid Content of the Minces after Treatment

| *Peroxide value (POV) (meq/kg)* | *TBA value (μmol/100 g)* | *Fat content of mince after treatment (%)* |
|---|---|---|
| CLO (M) 18 | 298 | 6·9 |
| OCLO (M) 760 | 2954 | 6·3 |
| CLO (S) 10 | 118 | 15·4 |
| OCLO (S) 951 | 1078 | 15·5 |

CLO, Cod liver oil added to minced megrim (M) or minced sardine (S).
OCLO, Oxidized cod liver oil added to minced megrim (M) or minced sardine (S).

**TABLE 2**
Analysis of Variance of the Variables Studied in Minced Megrim for the Different Storage Times and Conditions

| *Variable* | *Lots* | *Storage time (days)* | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | *0* | *26* | *54* | *96* | *165* | *255* | *365* |
| TBA value | MC | *a/x* | *b/x* | *c/x* | *d/x* | *e/x* | *f/x* | *g/x* |
| | MN | *ab/y* | *a/x* | *b/x* | *b/x* | *c/y* | *d/y* | *e/y* |
| | MO | *a/z* | *b/y* | *b/y* | *c/y* | *d/z* | *d/z* | *e/z* |
| % Protein solubility | MC | *a/x* | *b/x* | *c/x* | *d/x* | *e/x* | *e/x* | *f/x* |
| | MN | *a/x* | *b/y* | *c/y* | *c/x* | *d/y* | *e/x* | *f/x* |
| | MO | *a/y* | *b/z* | *c/y* | *d/y* | *e/x* | *f/y* | *g/x* |
| Apparent viscosity | MC | *a/x* | *b/x* | *b/x* | *c/x* | *d/x* | *e/x* | *f/x* |
| | MN | *a/y* | *b/y* | *c/y* | *d/y* | *e/y* | *f/y* | *g/y* |
| | MO | *a/z* | *b/z* | *c/z* | *d/z* | *e/z* | *f/z* | *g/z* |
| Emulsifying capacity | MC | *a/x* | *b/x* | *c/xy* | *c/x* | *d/xz* | *d/x* | *d/x* |
| | MN | *a/yw* | *a/x* | *b/y* | *b/x* | *c/x* | *c/x* | *c/x* |
| | MO | *a/xz* | *b/xy* | *c/x* | *d/x* | *d/y* | *e/y* | *d/y* |

Different letters in each file (*a, b, c*) and in each column (*x, y, z*) indicate significant differences ($P < 0.05$).
MC, control sample.
MN, 3% cod liver oil added.
MO, 3% oxidized cod liver oil added.

in all lots. These values confirm published data (Hebard *et al.*, 1982) to the effect that these species are classified as non-DMA-producing.

## 2-Thiobarbituric acid index

Figures 1(a) and 1(b) show the observed TBA values for minced megrim and sardine, respectively, during frozen storage. Whereas TBA values for minced megrim control, and, with neutral lipids added, increased evenly during frozen storage, these values decreased for the oxidized lipid added samples. The same pattern, which is normal in advanced states of rancidity (Melton, 1983), was observed for the sardine samples. In minced sardine the values oscillated noticeably in the oxidized lipid-added samples, probably as a result of the presence of other thiobarbituric reactive substances (TBARS) in the sample (Careche & Tejada, 1988).

Although statistically significant differences were observed between all three treatments, the oxidized lipid treatment differed from the control far more than did the neutral lipid treatment.

It should be noted that, in general, the TBA indices for the sardine minces

**TABLE 3**
Analysis of Variance of the Variables Studied in Minced Sardine for the Different Storage Times and Conditions

| *Variable* | *Lots* | *Storage time (days)* | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | *0* | *28* | *70* | *116* | *162* | *274* | *365* |
| TBA value | SC | *a/x* | *b/x* | *c/x* | *b/x* | *d/x* | *e/x* | *f/x* |
| | SN | *a/y* | *b/y* | *c/y* | *be/x* | *d/y* | *e/y* | *f/y* |
| | SO | *a/z* | *b/z* | *c/z* | *d/y* | *a/z* | *e/z* | *f/z* |
| % Protein solubility | SC | *a/x* | *b/x* | *c/x* | *d/x* | *ed/x* | *be/x* | *be/x* |
| | SN | *a/x* | *b/y* | *c/y* | *d/y* | *e/y* | *d/y* | *e/y* |
| | SO | *a/y* | *b/z* | *b/x* | *b/z* | *bc/z* | *d/z* | *d/z* |
| Apparent viscosity | SC | *a/x* | *b/x* | *c/x* | *d/x* | *e/x* | *f/x* | *f/x* |
| | SN | *a/x* | *b/y* | *c/y* | *d/y* | *d/y* | *e/y* | *e/y* |
| | SO | *a/y* | *b/z* | *c/z* | *d/y* | *e/z* | *f/z* | *f/z* |
| Emulsifying capacity | SC | *a/x* | *b/x* | *c/x* | *c/x* | *c/x* | *d/x* | *e/x* |
| | SN | *a/xy* | *b/x* | *c/y* | *d/y* | *c/y* | *e/y* | *f/y* |
| | SO | *a/y* | *b/x* | *c/z* | *b/z* | *c/z* | *d/z* | *e/z* |

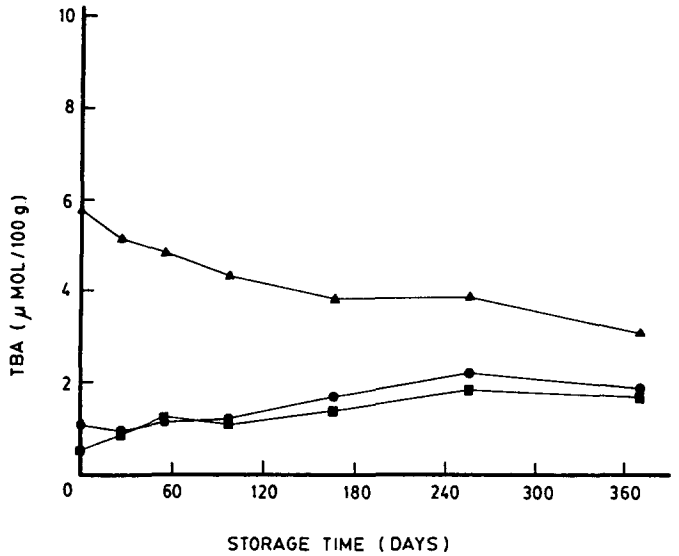
Different letters in each file (*a*, *b*, *c*) and in each column (*x*, *y*, *z*) indicate significant differences ($P < 0.05$).
SC, control sample.
SN, 3% cod liver oil added.
SO, 3% oxidized cod liver oil added.

with oxidized lipids added were much higher initially than for the equivalent megrim minces, despite the fact that the TBA value of the added cod liver oil was lower than that of the megrim (Table 1). We believe that these higher values in the oxidized lipid-added sardine mince have their origin in the action of the peroxides existing in the added rancid oil, which swiftly trigger a chain reaction accelerating the oxidation of the natural lipids in this species, oxidation being more intense because of the higher lipid content.

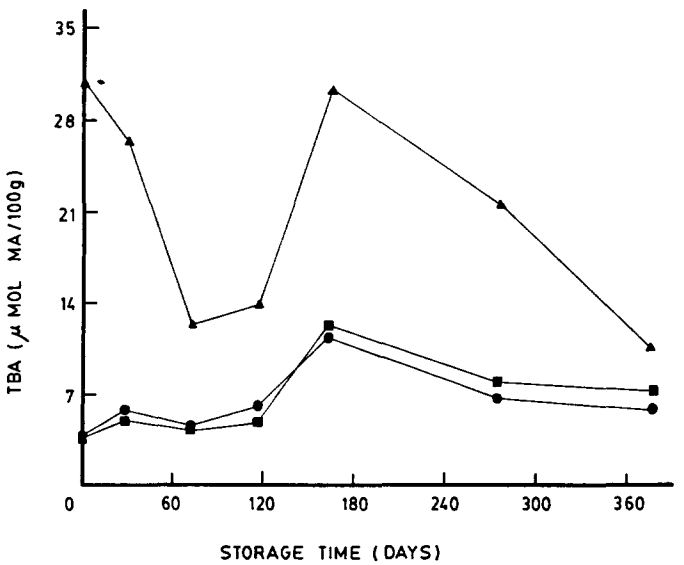
## Protein solubility

The percentages of soluble protein for megrim and sardine treatment during storage are shown in Figs 2(a) and 2(b). Minced megrim with no lipids added showed high initial solubility values, which decreased gradually and significantly during the first 165 days and thereafter remained constant. There were no significant differences in protein solubility between the CLO-added megrim and the control samples. The values for OCLO-added samples were statistically significantly lower than for the control samples.

Protein solubility values were initially lower in the minced sardine control

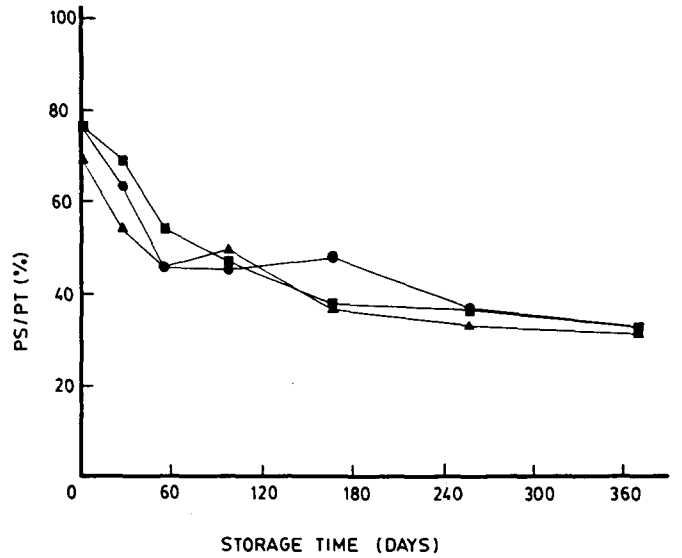



(a)

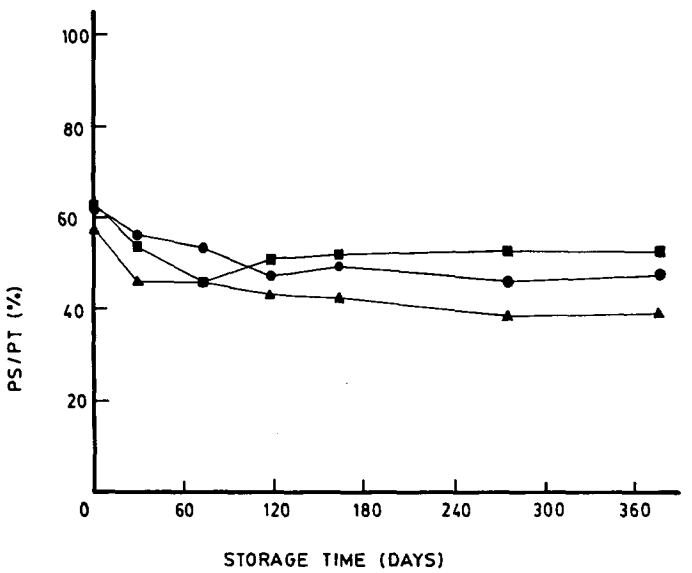



(b)

**Fig. 1.** 2-Thiobarbituric acid value (TBA) during frozen storage (−18°C) (a) minced megrim and (b) sardine. ■, Control lot; ●, 3% CLO added; ▲, 3% OCLO added.

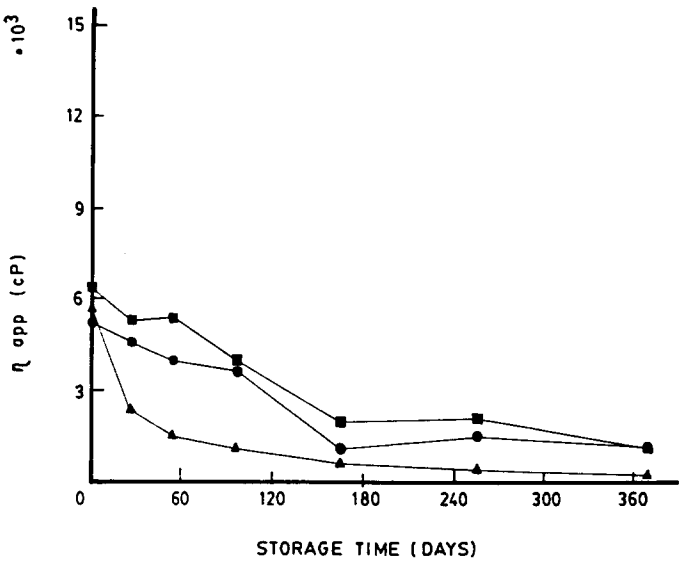



(a)

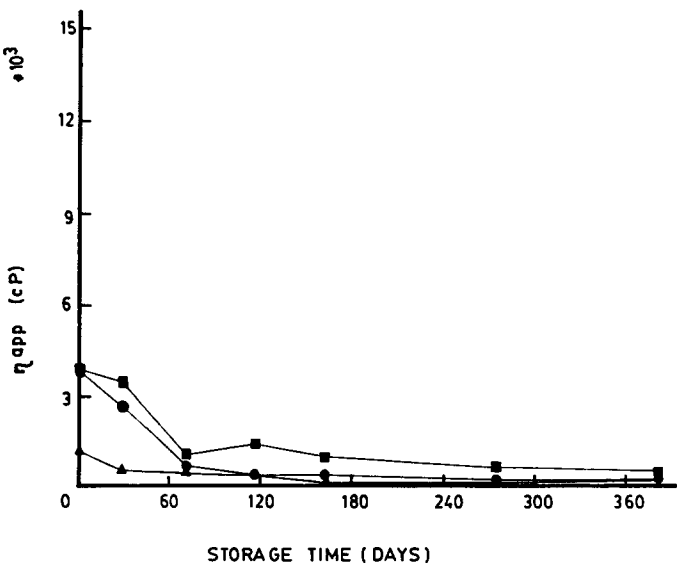



(b)

**Fig. 2.** % Protein solubility (PS/PT) during frozen storage (−18°C) of (a) minced megrim and (b) sardine. ■, Control lot; ●, 3% CLO added; ▲, 3% OCLO added.




(a)




(b)

**Fig. 3.** Apparent viscosity ($\eta_{app}$) during frozen storage (−18°C) of (a) minced megrim and (b) sardine. ■, Control lot; ●, 3% CLO added; ▲, 3% OCLO added.

than in the megrim, but were within the normal value range reported for this species (Baldrati *et al.*, 1982). During frozen storage solubility decreased only slightly. However, for the CLO-added minced sardine, although initially protein solubility was similar to, or higher than, that of the control, the value soon became significantly lower and remained so during the frozen storage period. The samples treated with oxidized lipids showed statistically lower protein solubility values than those treated with neutral lipids throughout the period studied.

**Apparent viscosity**

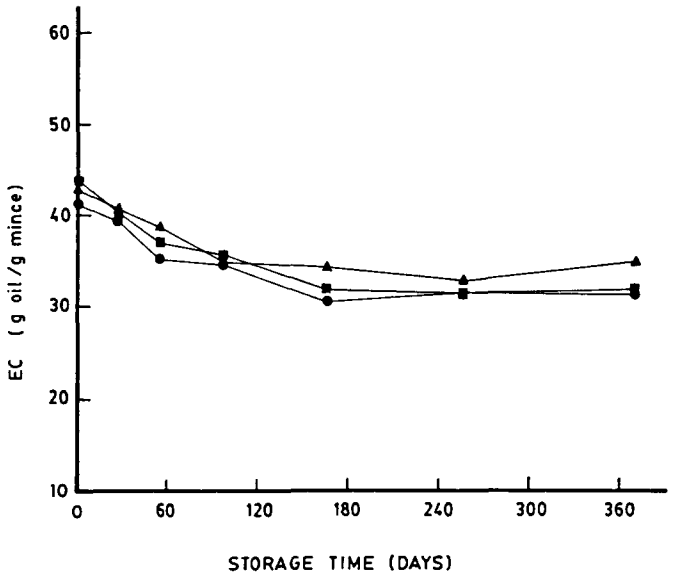
Throughout the storage period, apparent viscosity (Fig. 3(a)) was highest in the minced megrim control sample and lowest in the OCLO-added samples. In all samples, the greatest decrease took place in the first 160 days. For minced sardine samples (Fig. 3(b)), the initial values were lower than those found for megrim, in inverse relation to the amount of fat contained in either species, as in previous findings (Jiménez-Colmenero *et al.*, 1988), the greatest decrease taking place during the first 70 days of storage. In the CLO-added samples, values were similar to those of the control sample initially but significantly lower in all subsequent samplings. The OCLO-added samples, on the other hand, showed very low apparent viscosity values from the outset of storage, and then declined very slowly for the rest of the storage period.

The results from Figs 2 and 3 support the theory that oxidized lipids are responsible for protein denaturation and aggregation during frozen storage (Shenouda, 1980) whereas the protective effect attributed to neutral lipids (Stodolnik & Knasiak, 1984) is not clear.

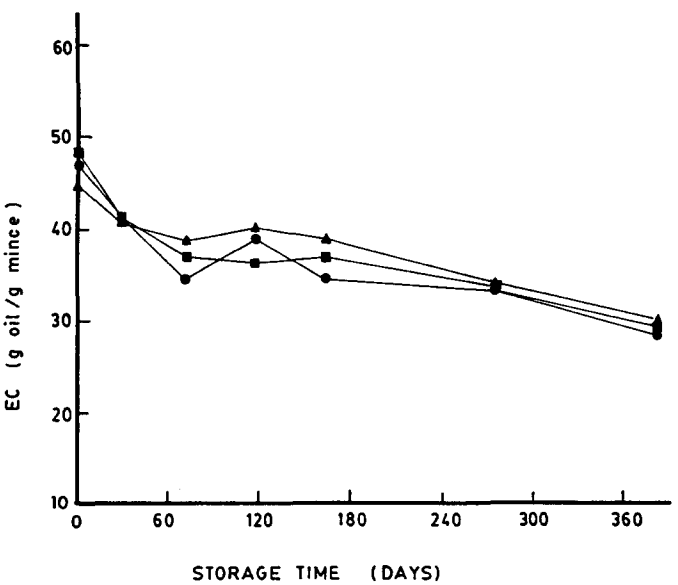
**Emulsifying capacity (EC)**

Figures 4(a) and 4(b) show the respective emulsifying capacity values for the different treatments in minced megrim and sardine. In the megrim the decrease in emulsifying capacity was slow. There was no significant difference between the control and CLO-added samples except at 0 days storage. Also, no significant difference was observed between OCLO-added and control samples for up to 90 days frozen storage; thereafter, the values for the former were significantly higher.

Emulsifying capacity in the sardine samples (Fig. 4(b)) initially showed higher values than in the megrim. The CLO-added sardine mince gave values similar to, or slightly lower than, the control sample; however, the OCLO-added samples had significantly higher values than the controls after 28 days storage. For both species this anomalous effect may be due to




(a)




(b)

**Fig. 4.** Emulsifying capacity (EC) during frozen storage (−18°C) of (a) minced megrim and (b) sardine. ■, Control lot; ●, 3% CLO added; ▲, 3% OCLO added.

the fact that the capacity of proteins to form and stabilize emulsions depends upon their solubility in water (S) (Kinsella, 1976) and their surface hydrophobicity (So) (Kato & Nakai, 1980), wherefore it is much easier to predict EC if these two properties are taken into account (Li-Chan *et al.*, 1984). With this in mind, the presence of oxidized lipids could affect the surface hydrophobicity and thus augment the proteins' capacity to emulsify fats.

The protein concentration of the homogenate used to measure viscosity and emulsifying capacity varied little over the storage period. The mean values in mg/g and in $\sigma_{n-1}$ for control, CLO- and OCLO-added lots were 31·56 (1·70), 31·28 (0·66) and 33·50 (3·50) for megrim and 33·51 (1·01), 32·90 (0·99) and 30·63 (2·11) for sardine. This implies that the variations found in viscosity and emulsifying capacity cannot be attributed to changes in protein concentration.

The results presented in this paper lead us to conclude that the addition of neutral lipids to minced megrim and sardine had no protective effect on the protein functional properties studied, while the addition of oxidized lipids had a detrimental effect on protein solubility and apparent viscosity. The results found for neutral lipids differ from many found in the literature, excepting Shenouda's finding in model systems (1980), chiefly because most of the work on the action of these lipids in intact or minced fish muscle has been conducted on species which form formaldehyde when frozen. Our results would appear to confirm the hypothesis that in DMA-forming species the apparent protective effect of neutral lipids is due to lower formaldehyde formation and hence of a less drastic loss of functionality in the presence of lipids, which is more marked where the lipids are oxidized, as has been reported by the authors for minced hake (Careche & Tejada, 1990).

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