JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Possible Role for Cryostabilizers in Preventing Protein and Lipid Alterations in Frozen-Stored Minced Muscle of Atlantic Mackerel

JUAN J. RODRÍGUEZ-HERRERA,* MARTA BERNÁRDEZ, GABRIEL SAMPEDRO, MARTA L. CABO, AND LAURA PASTORIZA

Instituto de Investigaciones Marinas (CSIC), Eduardo Cabello 6, 36208 Vigo, Spain

Adding DE 18 maltodextrin (80 g kg^{-1}) to high-fat minced mackerel was highly effective against lipid oxidation and protein and color changes during frozen storage. It increased the temperature of ice-melting onset (T_m') and decreased freeze concentration of solutes in the unfrozen water (UFW) phase, which would have allowed it to effectively slow such perturbations. This maltodextrin showed a higher effectiveness against lipid oxidation, but was slightly less effective in preventing the loss of protein solubility than common cryoprotectants, that is, an equiproportional mixture of sucrose and sorbitol. Such differences in effectiveness were much higher in low-fat minces, in which lipid oxidation proceeded to a much lower extent. Consequently, prior to replacing traditional cryoprotectants with maltodextrins, it should be known which processes limit the shelf life of the food. Decreasing (from 80 to 50 g kg^{-1}) the proportion of maltodextrin added to high-fat minced mackerel showed that although it affected only slightly the effectiveness against lipid oxidation, it did notably affect the effectiveness in preventing the loss of protein solubility and color changes. Therefore, such a decrease could be accepted only if lipid oxidation is the most limiting process of shelf life, but does not seem appropriate when protein changes are important.

KEYWORDS: Frozen-stored minced fatty fish; lipid oxidation; cryostabilization; maltodextrin

INTRODUCTION

Nowadays, there is an increasing interest in developing high-value-added fish products—with a long shelf life—by using minces made from fatty fish (*1*−*3*). Such interest has been stimulated by the nutritional relevance associated with their high *n*-3 poly-unsaturated fatty acid content, the consumption of which is considered to have a number of beneficial effects on human health (*4*−*6*), as well as by the depletion and overexploitation of many important fish stocks throughout the world (*7*). Notwithstanding, fish lipids, and particularly poly-unsaturated fatty acids, are very prone to oxidation in the frozen state (*8*−*11*), which becomes a major limitation to the development of such products.

Freezing and frozen storage are important techniques for long-term preservation of fish, but several alterations still take place. In minced fish such alterations are enhanced by the disruption of muscle integrity, which allows an intimate contact among cellular compounds and the access of oxygen (*12*, *13*). Consequently, a great volume of the catches of fatty fish are used to produce fish oil and meal (*1*, *7*). Previous results had shown a great potential for cryostabilization as a technology to prevent protein alterations in frozen-stored minced gadoids, mainly due to the inhibitory effect of several maltodextrins on the production of formaldehyde, which thus reduced its subsequent interactions with muscle compounds (*14*−*16*). Additionally, a 25 DE maltodextrin had been shown to effectively prevent protein denaturation in horse mackerel surimi stored at −18 °C (*17*). However, our studies found that a DE 18 maltodextrin was the most effective among a series of maltodextrins in preventing protein alterations in minced blue whiting muscle. Consequently, it was considered to be logical to question if cryostabilization by using this maltodextrin could be also applied to prevent alterations taking place in frozen-stored minced fatty fish, because it could be very useful in the development of more stable and nutritive food products from fatty fish minces.

MATERIALS AND METHODS

Chemicals. Maltodextrins were supplied free of charge by Cerestar (Barcelona, Spain). Sucrose, sorbitol, sodium dodecyl sulfate (SDS), 2-thiobarbituric acid (TBA), ammonium thiocyanate, butylated hydroxytoluene (BHT), and 1,1,3,3-tetraethoxypropane were purchased from Sigma-Aldrich Co. All other reagents were of analytical grade.

Experimental Design. Atlantic mackerel (*Scomber scombrus*), caught in offshore Galician waters, were gutted and beheaded within the first 24 h postcapture, and fillets were inmediately taken off, skinned, and minced in a meat mincer (Cutter DITO SAMA K-35). Subsequently, minces were manually mixed with DE 18 maltodextrin or an equiproportional mixture of sucrose and sorbitol—used traditionally as cryoprotectant by the minced fish industry—at a concentration of 80 g kg^{-1}. DE 28 maltodextrin was also used in one study (see below). A batch with no additive was used as a control.

* Author to whom correspondence should be addressed (telephone 34.986.214465; fax 34.986.292762; e-mail juanherrera@iim.csic.es).

10.1021/jf0581271 CCC: $33.50
Published on Web 04/06/2006

Samples (~50 g) about 0.5−0.8 cm thick were placed in individual plastic bags, which were sealed and then frozen in a freezer cabinet set at −50 °C. One day later, they were transferred to freezer cabinets set at −10 or −20 °C. Samples were periodically taken out of freezers and subjected to a number of analyses. Measurements were carried out in triplicate.

Protein Solubility. Protein solubility in salt medium was determined as described in Herrera et al. (*16*). Approximately 30 g of minced muscle was homogenized in 300 mL of 0.7 M NaCl and 25 mM phosphate buffer, pH 7.0. Homogenates were centrifuged at 15000*g* (15 min, 3 °C), and the supernatant was collected. An aliquot of the supernatant was mixed with 0.9 M trichloroacetic acid [ratio 1:2 (w/w)] and centrifuged at 10000*g* (15 min, 5 °C). The precipitate was collected, dried to constant weight at 100 °C, and accurately weighed (±0.1 mg). The nitrogen content of the dried precipitate was measured according to the Kjeldahl method by using an automatic Kjeltec 2300 analyzer unit (Foss Tecator AB). The salt-soluble protein content (grams per kilogram) was calculated by accepting a conversion factor of 6.25 betweeen nitrogen and protein contents. Protein solubility was determined as the percentage ratio between salt-soluble protein content and total protein content, the latter being determined in noncentrifuged homogenates.

Color. Tristimulus reflectance color (*L*, *a*, and *b* values) was measured by using a Minolta Chroma meter CR-200 as described in Hunter (*18*). At least six measurements were made on each replicate.

Peroxide Value. The peroxide value was determined according to a modification of the method developed by Ueda et al. (*19*). Briefly, after extraction according to a modified Folch method (*20*), fish lipids (~4 mg or less) were desolventized under a gentle stream of nitrogen. Lipids were inmediately redissolved in 0.2 mL of hexane, and 5.0 mL of ethanol and 0.1 mL of ammonium thyocyanate were added. Subsequently, 0.1 mL of ferrous chloride was added, and then nitrogen was flushed to fill the headspace of the test tubes. The reaction mixtures were left to stand for 7 min at room temperature, and absorbance was read at 500 nm. The concentration of ferric ion (milligrams per milliliter) was determined by reference to a calibration curve from a standard solution of ferric chloride (0−0.6 mg/mL).

All reagents and solvents were deoxygenized prior to use.

Peroxide value was calculated according to the equation

$$PV = [Fe^{3+}]\ (mg/mL)/(55.84 \times mg\ of\ lipids)$$

55.84 being the atomic weight of iron. Results are expressed as milliequivalents of Fe per kilogram of lipids.

Thiobarbituric Acid Reactive Substances (TBA-RS) Index. The content of TBA-RS was determined following the method of Pikul et al. (*21*) with some modifications. After being desolventized under a gentle stream of nitrogen, lipids (~3−5 mg) were redissolved in 0.1 mL of chloroform. Then 0.2 mL of 81 g kg^{-1} SDS was added, and the mixture was vigorously vortexed. Subsequently, 3.0 mL of 4 g kg^{-1} TBA in 10% acetic acid, 0.7 mL of distilled water, and 0.1 mL of 20 g kg^{-1} BHT in ethanol were pipetted, and the whole mixture was vortexed further. Test tubes were heated at 100 °C for 1 h, cooled rapidly to stop the reaction, and centrifuged at 2000*g* for 15 min. Absorbance was read at 535 nm.

The index of TBA-RS (grams of malonaldehyde per kilogram of lipid**)** was determined by reference to a calibration curve constructed from a standard solution of 1,1,3,3-tetraethoxypropane (0−6 μg in the reaction mixture).

Free Fatty Acid Content. The content of free fatty acids was determined according to the Lowry and Tinsley method (*22*) as modified by Bernárdez et al. (*23*), which uses cyclohexane in place of benzene as solvent in which lipids are dissolved.

Thermal Analysis. Samples were subjected to differential scanning calorimetry (DSC) by using a DSC Pyris 1 (Perkin-Elmer) equipped with an Intracooler IIP mechanical accessory. Heat flow and melting point were calibrated from the melting endotherm of indium and the melting temperature of water. An empty pan was always used as a reference.

Samples (10−15 mg) were accurately weighed into 50 μL aluminum pans using a high-precision balance (±0.0001 g) and immediatly sealed with a Universal press (Perkin-Elmer). Once inside the calorimetric cells, pans were allowed to stand for 1 min at 10 °C and then cooled to −55 °C at a rate of 20 °C/min, being maintained at this temperature for 3 min. Afterward, samples were heated to 10 °C at 5 °C/min, and this latter thermogram was subjected to analysis. Scans were carried out in triplicate.

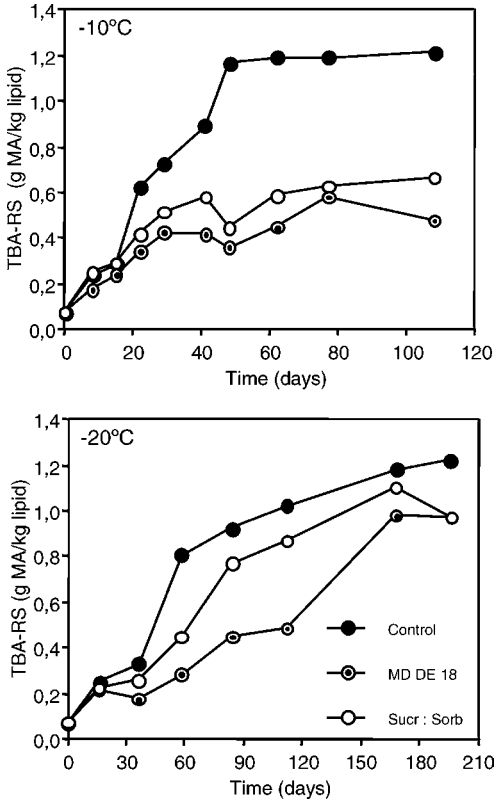

Figure 1. Thiobarbituric acid reactive substances in high-fat minced mackerel muscle during frozen storage at −10 or −20 °C.

The ice-melting onset was defined as the onset temperature of the transition just preceding the ice-melting endotherm, and its value (T_m') was determined from the intersection of the tangents to the baseline and to the leading side of the transition. Freeze concentration of solutes in the unfrozen water (UFW) phase was determined from the differential cumulative percentage variation of the ice-melting endotherm area, which was obtained by means of the Pyris software by setting as integration limits a temperature slightly lower than T_m' and another one slightly above the ice-melting endotherm (*15*).

Statistical Analysis. The results obtained for the different batches were subjected to an analysis of variance at each sampling period by means of a Student's *t* test with a significance level of 95%. Analysis of variance were carried out with the help of the software Microsoft Excel version 5.0a for Power Macintosh. Whenever statistical differences between any pair of batches were found, they are shown in tables by means of different letters. The periods of storage during which the same differences among all batches remained were grouped. The periods of storage at which there were no statistical differences between any pair of batches were left out of the tables.

RESULTS AND DISCUSSION

Effects on Lipid and Protein Alterations of High-Fat Minced Mackerel. The addition of DE 18 maltodextrin to high-fat (145 g of lipids kg^{-1}) minced mackerel muscle was highly effective in preventing lipid oxidation during frozen storage at both −10 and −20 °C (**Figures 1** and **2**). Thus, the peroxide value (PV) and the index of thiobarbituric acid reactive substances (TBA-RS) were significantly lower for the cryostabilized samples than for the control throughout the period of storage (**Tables 1** and **2**). The mixture of sucrose and sorbitol had a significant effect against lipid oxidation, too. A recent study (*24*) has shown some antioxidant activity of sugars and polyhydric alcohols in fish oil emulsions, too. However, in the

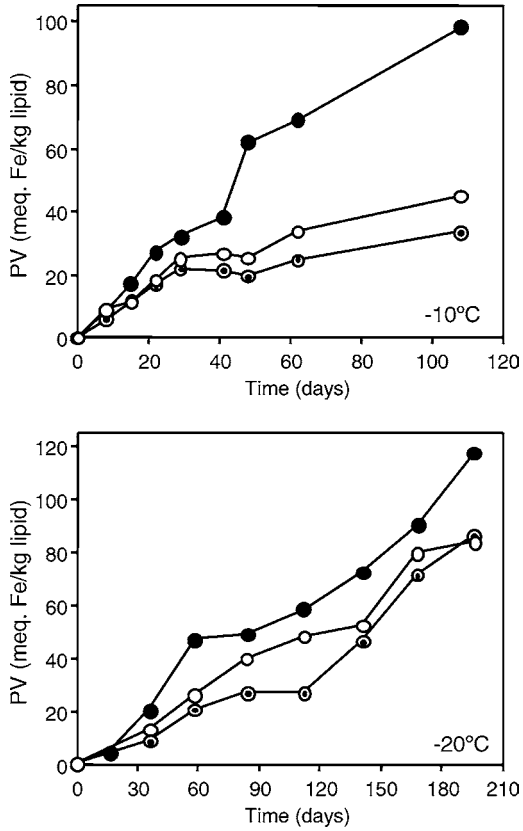

Figure 2. Peroxide value in high-fat minced mackerel muscle during frozen storage at −10 or −20 °C. Symbols as in **Figure 1**.

Table 1. Statistical Differences in Peroxide Value among the Batches of High-Fat Minced Mackerel Stored at −10 (**a**) and −20 °C (**b**)[a]

time (days)	control	MD DE 18	sucrose sorbitol
		(**a**) −10 °C	
8	a	b	a
15–29	a	b	b
41–108	a	b	c
		(**b**) −20 °C	
36–112	a	b	c
141–196	a	b	b

[a] Different letters within the same row show significant differences ($\alpha = 0.05$).

Table 2. Statistical Differences in Thiobarbituric Acid Reactive Substances among the Batches of High-Fat Minced Mackerel Stored at −10 (**a**) and −20 °C (**b**)[a]

time (days)	control	MD DE 18	sucrose sorbitol
		(**a**) −10 °C	
8–15	a	b	a
22–108	a	b	c
		(**b**) −20 °C	
16	a	b	b
36–112	a	b	c
168	a	b	ab
196	a	b	b

[a] Different letters within the same row show significant differences ($\alpha = 0.05$).

present study the maltodextrin was significantly more effective than the mixture of sucrose and sorbitol during most of the storage period at both temperatures. Fujii et al. (*25*) had also reported that dextrin increased the stability to autoxidation when compared with mono- and disaccharides.

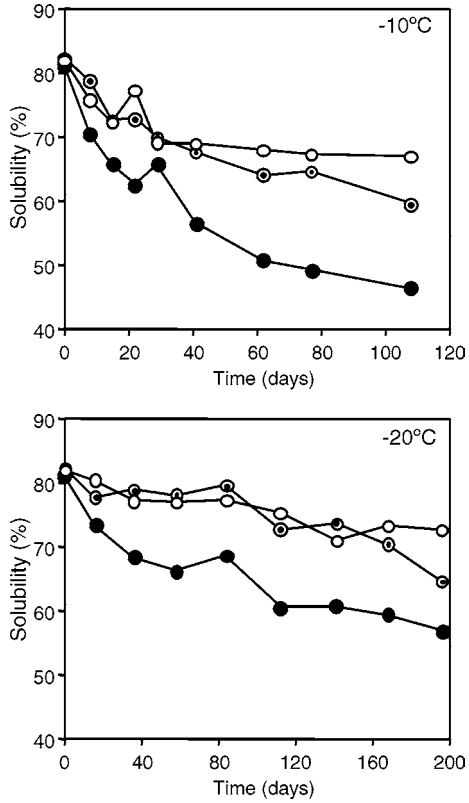

Figure 3. Solubility for high-fat minced mackerel muscle during frozen storage at −10 or −20 °C. Symbols as in **Figure 1**.

Table 3. Statistical Differences in Solubility among the Batches of High-Fat Minced Mackerel Stored at −10 (**a**) and −20 °C (**b**)[a]

time (days)	control	MD DE 18	sucrose sorbitol
		(**a**) −10 °C	
8–77	a	b	b
108	a	b	c
		(**b**) −20 °C	
16	a	ab	b
36–168	a	b	b
196	a	b	c

[a] Different letters within the same row show significant differences ($\alpha = 0.05$).

The development of high-value-added foods from fatty fish has found as an important limitation the high susceptibility of fish lipids to oxidation, especially in minced fish, due to their high content of poly-unsaturated fatty acids. It is therefore clear that the effectiveness of DE 18 maltodextrin in inhibiting lipid oxidation could be of great help in the production of such foods.

Both the mixture of sucrose and sorbitol and DE 18 maltodextrin slowed significantly the loss of protein solubility during the whole period of storage at −10 and −20 °C, but the former was slightly more effective. Thus, differences became significant after 100 and 190 days of storage, respectively (**Figure 3**; **Table 3**). These results differ from those obtained for minced blue whiting muscle. Additionally, the loss of solubility was slower and the differences between the control and the treatments and between both treatments were much lower in mackerel than in blue whiting minces. The high denaturing effect of the large amounts of formaldehyde that are produced in blue whiting minces is presumably mainly responsible for such differences (*16*).

The hydrolysis of fatty acids was found to be not very important in frozen-stored high-fat mackerel. However, DE 18

Table 4. Production of Free Fatty Acids in High-Fat Minced Mackerel during Frozen Storage at −10 (**a**) and −20 °C (**b**)[a]

time (days)	control	MD DE 18	sucrose sorbitol
		(**a**) −10 °C	
29	11.56a	12.38a	11.99a
62	20.59a	18.34b	17.04b
108	30.43a	26.35b	27.36b
		(**b**) −20 °C	
84	5.00a	2.75b	2.15b
141	5.29a	3.30b	5.10a
196	7.37a	6.35b	8.01a

[a] Different letters within the same row show significant differences ($\alpha = 0.05$).

Table 5. Statistical Differences in *a* Values among the Batches of High-Fat Minced Mackerel Stored at −10 (**a**) and −20 °C (**b**)[a]

time (days)	control	MD DE 18	sucrose sorbitol
		(**a**) −10 °C	
8–22	a	b	b
29–108	a	b	c
		(**b**) −20 °C	
58–84	a	b	c
112–196	a	b	b

[a] Different letters within the same row refer to significant differences ($\alpha = 0.05$).

maltodextrin showed some inhibiting effect at both temperatures of study (**Table 4**).

The changes in color were also slowed by DE 18 maltodextrin or the combination of sucrose and sorbitol. This was shown by measurements of the Hunter parameters (**Figure 4**). Thus, the values for *b* were significatly lower and the values for *a* were significatly higher in the treated samples than in the control during most of the period of storage at −10 and −20 °C (**Tables 5** and **6**). These results reflect that their addition delayed muscle yellowing caused by oxidative alterations and maintained the original redness for much longer. Regarding *a*, samples with DE 18 maltodextrin showed values significantly higher than those with sucrose and sorbitol from 29 days of storage at −10 °C, which reveals the higher effectiveness of the maltodextrin to preserve redness. However, significant differences between treatments were noticed only between 58 and 84 days of storage at −20 °C. Instrumental measurements of redness loss were considered to be adequate as a tool to follow haemoglobin-mediated lipid oxidation in fish flesh (*24*), so DE 18 maltodextrin may have prevented haemoglobin-mediated lipid oxidation. Also, DE 18 maltodextrin prevented yellowing slightly better than sucrose and sorbitol from 141 days of storage at −20 °C—the values for *b* of maltodextrin-containing samples were lower than those of samples with sucrose and sorbitol—

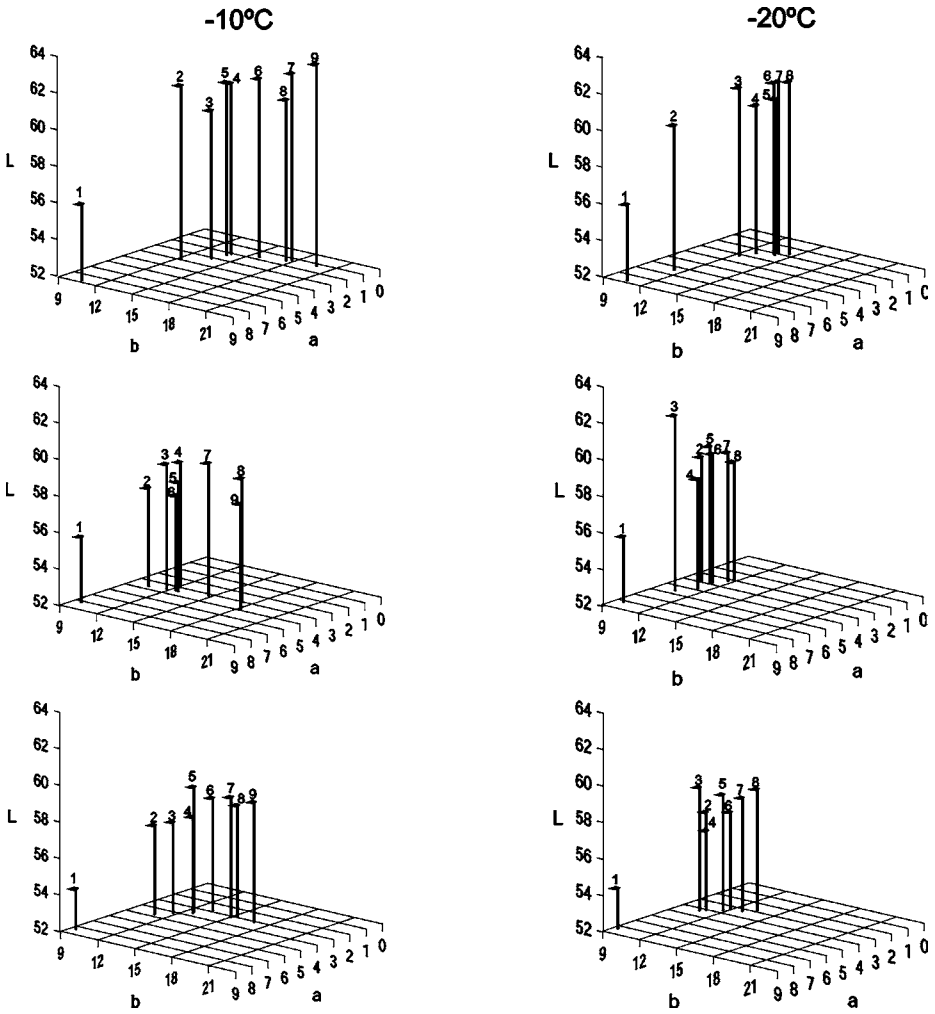

Figure 4. Hunter color values for high-fat minced mackerel muscle during frozen storage at −10 °C (left) or −20 °C (right): control sample (top); sample with MD DE 18 (middle); sample with sucrose and sorbitol (bottom). Numbers above bars correspond to the following sampling periods: at −10 °C, 1 (0 days, nonfrozen), 2 (8 days), 3 (15 days), 4 (22 days), 5 (29 days), 6 (41 days), 7 (62 days), 8 (77 days), and 9 (108 days); at −20 °C, 1 (0 days, nonfrozen), 2 (36 days), 3 (58 days), 4 (84 days), 5 (112 days), 6 (141 days), 7 (168 days), and 8 (196 days).

Table 6. Statistical Differences in *b* Values among the Batches of High-Fat Minced Mackerel Stored at −10 (**a**) and −20 °C (**b**)[a]

time (days)	control	MD DE 18	sucrose sorbitol
		(**a**) −10 °C	
0–29	a	b	b
41	a	a	b
62–108	a	b	c
		(**b**) −20 °C	
58–112	a	b	b
141–196	a	b	c

[a] Different letters within the same row refer to significant differences ($\alpha = 0.05$).

Table 7. Statistical Differences in Ligthness (*L*) of High-Fat Minced Mackerel Stored at −10 °C

time (days)	control	MD DE 18	sucrose sorbitol
108	a	b	b

whereas the mixture of sucrose and sorbitol was more effective from 41 days of storage at −10 °C.

Slight differences between treated samples and control were also found in lightness (*L*), which occasionally became significant at −10 °C (never at −20 °C) (**Table 7**). Subsequent studies showed such significant differences much more clearly (see below).

The addition of 80 g kg^{-1} DE 18 maltodextrin increased the temperature of ice-melting onset (T_m') from −27.5 to −24 °C, which implies a lower molecular mobility in maltodextrin-containing minces than in the control. A similar increase had been previously reported for minced blue whiting muscle (*27*). It also decreased freeze concentration of solutes in the UFW phase by increasing the proportion of unfrozen water with respect to the total freezable water content at the temperatures of study (as shown by DSC scans in **Figure 5**) and by "diluting" the freeze concentration of muscle components in the UFW (*16*). In contrast, the addition of sucrose and sorbitol has some plasticizing effect, diminishing T_m' to −31.5 °C, but decreased the freeze concentration of solutes in the UFW phase. Accordingly, DE 18 maltodextrin and the mixture of sucrose are able to slow diffusion-limited processes effectively, the former's ability being higher. This is clearly the case of lipid oxidation, which is a typical diffusion-limited reaction, and hydrolysis of fatty acids.

Protein changes, however, follow a much more complex pattern, as they are driven by a collective mechanism associated with a hierarchy of freedom degrees of the different structural elements forming the protein (*26*). Furthermore, low DE maltodextrins can cause some perturbations in fish proteins (*14*) so that it should not be discarded that DE 18 maltodextrin could cause some slight perturbations of the native protein architecture, which would give rise to long-term changes.

Effects on Lipid and Protein Alterations of Low-Fat Minced Mackerel. The lipid content of fatty fish is subject to seasonal variations, related mostly to spawning and feeding. Considering that the results of the first experiment showed that DE 18 maltodextrin reduced lipid oxidation in high-fat minced mackerel muscle during frozen storage, but was slightly less effective than the mixture of sucrose and sorbitol against the loss of solubility after some time in the frozen state, the question was raised if the addition of maltodextrins was adequate to preserve low-fat (52.5 g of lipids kg^{-1}) minced mackerel muscle in the frozen state. This study was carried out only at −10 °C for practical purposes.

Bearing in mind that low DE maltodextrins interfere with protein gelification (*29*) and that it could affect protein structure and therefore protein solubility, a DE 28 maltodextrin was also included in this second experiment. It might be expected that such an effect was lower, as DE was higher.

Both maltodextrins prevented lipid oxidation effectively (**Figure 6**; **Table 8**). In contrast, minces with sucrose and sorbitol showed occasionally PVs or TBA-RS indices higher than the control, which differs from results obtained in high-fat minced mackerel. An important effect on molecular kinetics has been considered to be the reason for high molecular weight polymers to slow diffusion-limited reactions in frozen systems, and it was also suggested for small solutes, for example, sucrose and sorbitol (*30*). Later, it was proposed that small solutes had a diluting effect on the freeze concentration of reactants in the unfrozen water phase (*15*). However, the difference in the effectiveness of sucrose and sorbitol against lipid oxidation between low- and high-fat fish seems to be related to intrinsic factors of the system. In high-fat fish, lipid oxidation came fundamentally from triglycerides, which amounted to ∼95% of the total lipid content (as determined by thin-layer chromatography) and are dispersed as small droplets within the muscle structure. Membrane lipids have an important role in the oxidation of lipids of low-fat fish, as phospholipids accounted for 20% of the total lipid content. The results of these two studies show clearly that sucrose and sorbitol do not prevent the oxidation of membrane lipids at relatively high temperatures of frozen storage. It seems thus that sucrose and sorbitol do not prevent lipid oxidation in low-fat fish by the same mechanism(s) as maltodextrins. In this respect, Oldenhof et al. (*31*) have pointed out that sucrose seems to function by direct interaction with biomolecules, whereas maltodextrins would act as an osmotically inactive bulking agent causing strengthening of the glassy matrix. Nevertheless, further studies will be needed to find the reason for this lack of effectiveness.

The mixture of sucrose and sorbitol, however, slowed the loss of solubility to a significantly higher extent than both maltodextrins, particularly DE 18 maltodextrin (**Figure 7**; **Table 9**). Nevertheless, no significant differences were found between DE 18 and DE 28 maltodextrins.

Maltodextrins as well as the mixture of sucrose and sorbitol diminished significantly the hydrolysis of fatty acids, but no differences were found among treatments (**Figure 8**; **Table 10**). In accordance with the higher content of membrane lipids, the production of free fatty acids had a much higher relevance in low-fat than in high-fat minced mackerel. It has been pointed out that the production of free fatty acids has some effects on protein alterations and lipid oxidation, although no clear trend has yet been reported as to whether it favors or hinders such processes.

Adding maltodextrins or sucrose and sorbitol significantly delayed increases in *b* and decreases in *a* in low-fat minced mackerel frozen-stored at −10 °C (**Figure 9**; **Table 11**). No significant differences were found among treatments during the first 111 days of storage. Subsequently, only the mixture of sucrose and sorbitol had still some effect on yellowing. In contrast, the samples with maltodextrins showed higher values for *a* than the samples with sucrose and sorbitol from 212 days of storage. Unlike in high-fat minced mackerel, the values for *L* were significantly higher in the control than in treated samples throughout the period of storage, but differences among treatments were hardly appreciated.

These studies have shown that maltodextrins protect minced muscle of fatty fish against freeze-induced lipid oxidation better

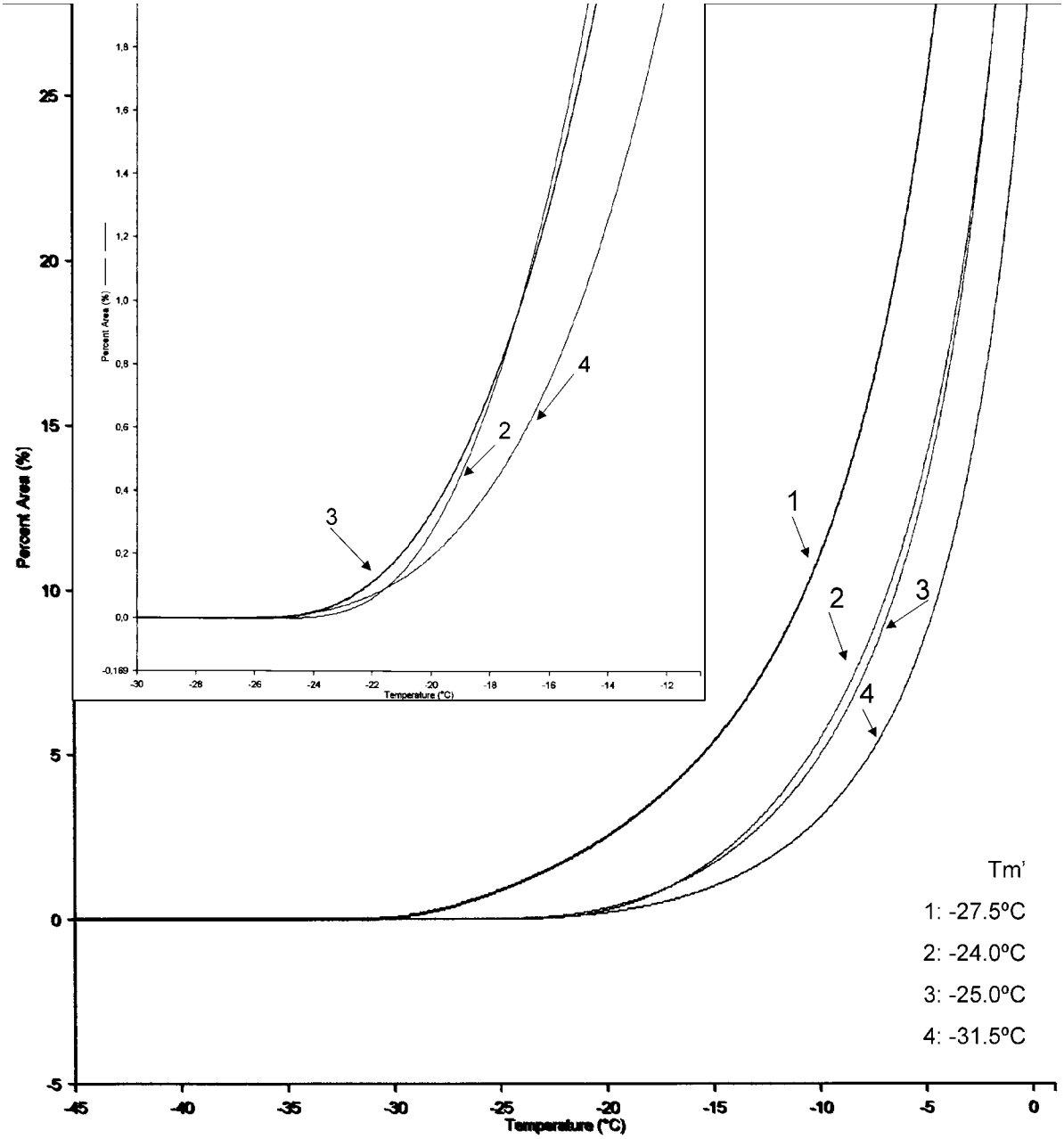

Figure 5. Differential cumulative percentage variation of the ice-melting endotherm area and maximally freeze-concentrated temperature of ice-melting onset for minced mackerel muscle. Notations: control sample (1); sample with 8% MD DE 18 (2); sample with 5% MD DE 18 (3); sample with sucrose and sorbitol (4).

than traditional cryoprotectants, but unlike in frozen minced muscle of gadoids, they do not seem to do so against freeze-induced protein perturbations. The oxidation of lipids has a great importance in high-fat minced fish, in which only slight differences were appreciated between the effectiveness of DE 18 maltodextrin and the mixture of sucrose and sorbitol in preventing protein changes. Consequently, the use of a proper maltodextrin would be first recommended to prevent freeze-induced perturbations in high-fat minced fish. On the contrary, the loss of solubility seemed to be faster in low-fat fish, in which lipid oxidation proceeded to a much lower extent. Consequently, prior to replacing traditional cryoprotectants with maltodextrins, it should be known which processes limit the shelf life of the food.

Effects of Reducing Maltodextrin Concentration on Protein Alterations in High-Fat Minced Mackerel. Studies combining maltodextrins and antioxidants to prevent oxidative processes taking place in high-fat minced fish more effectively (*32*) led us to consider the chance of reducing the proportion of maltodextrin added. Such reduction would be economically and technically interesting, but there is a need to find out how it would affect the protein fraction. We therefore examined subsequently the effectiveness of two different concentrations of DE 18 maltodextrin (50 and 80 g kg^{-1}) in preventing freeze-induced perturbations taking place in high-fat (132.5 g of lipids kg^{-1}) minced mackerel muscle during frozen storage. This third study was also carried out at −10 °C, and a nontreated control as well as a reference treatment consisting of a mixture of sucrose and sorbitol were again included.

A slightly lower effectiveness against lipid oxidation was shown when the concentration of DE 18 maltodextrin was reduced (**Table 12**). Nevertheless, lipid oxidation was much slower in samples with 50 g kg^{-1} DE 18 maltodextrin than in those with 80 g kg^{-1} sucrose and sorbitol or in the control, which reflects its high effectiveness against oxidative processes.

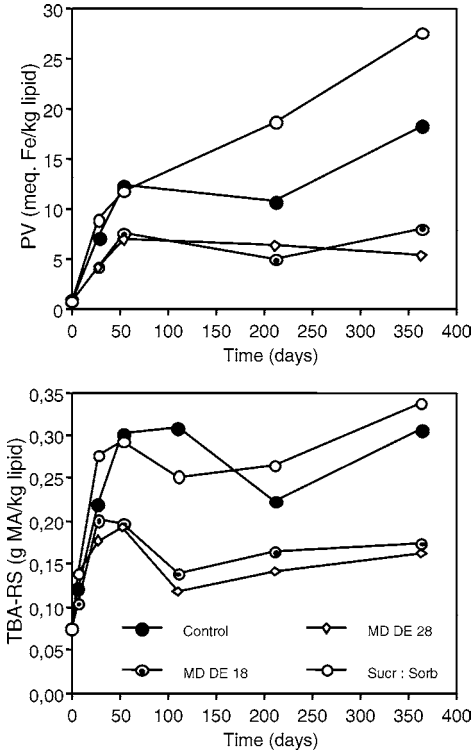

Figure 6. Peroxide value (top) and thiobarbituric acid reactive-substances (bottom) in low-fat minced mackerel muscle during frozen storage at −10 °C.

Table 8. Statistical Differences in Peroxide Values (**a**) and (**b**) Thiobarbituric Acid Reactive Substances among the Batches of Frozen-Stored Low-Fat Minced Mackerel[a]

time (days)	control	MD DE 18	MD DE 28	sucrose sorbitol
		(**a**) Peroxide Values		
28–54	a	b	b	a
212–364	a	b	c	d
		(**b**) TBA-RS		
28	a	ab	b	c
54	a	b	b	a
111	a	b	b	c
212–364	a	b	b	a

[a] Different letters within the same row refer to significant differences ($\alpha = 0.05$).

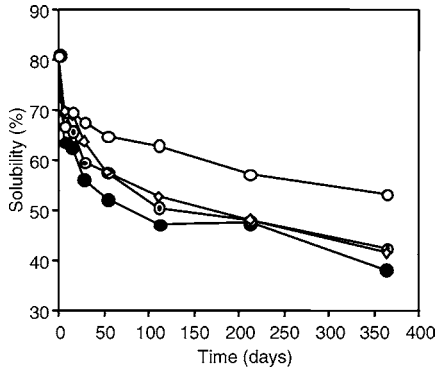

Figure 7. Solubility for low-fat minced mackerel muscle during frozen storage at −10 °C. Symbols as in **Figure 6**.

In contrast, the addition of 50 g kg^{-1} DE 18 maltodextrin to minced mackerel muscle was not very effective in preventing the loss of protein solubility (**Figure 10**). In general, solubility was significantly lower in 50 g kg^{-1} DE 18 maltodextrin-containting samples than in 80 g kg^{-1} DE 18 maltodextrin- or sucrose and sorbitol-containing samples (**Table 13**). Increasing the concentration of cryoprotectant generally results in increased cryoprotective effects (*33*). However, solubility was only occasionally higher in 50 g kg^{-1} DE 18 maltodextrin-containing samples than in the control, whereas increasing the proportion of maltodextrin added from 50 to 80 g kg^{-1} led to significant differences during the entire period of frozen storage.

Reducing the concentration of maltodextrin to 50 g kg^{-1} also had some effects on color changes (**Figure 11**). As shown in **Table 14**, the values for *a* were significantly higher than those of the control from 29 days of storage, but not those of 80 g kg^{-1} DE 18 maltodextrin-containing samples. In contrast, the values for *b* were significantly higher than those of 80 g kg^{-1} DE 18 maltodextrin-containing samples from 14 days of storage, but not those of the control. Although lipid oxidation causes yellowing, the values for *b* of samples with sucrose and sorbitol were significantly lower than those of 50 g kg^{-1} DE 18 maltodextrin-containing samples, so that protein changes could be responsible for yellowing, too. Lightness values of 50 g kg^{-1} DE 18 maltodextrin-containing minces were intermediate, and no significant differences were found with respect to 80 g kg^{-1} cryoprotectant-containing minces or generally with respect to the control.

The proportion of maltodextrin added affected significantly both T_m' and freeze concentration. Thus, decreasing the proportion of maltodextrin (from 80 to 50 g kg^{-1}) increased T_m' to a lower extent, that is, −25 °C, which agrees with the linearity

Table 9. Statistical Differences in Solubility among the Batches of Frozen-Stored Low-Fat Minced Mackerel[a]

time (days)	control	MD DE 18	MD DE 28	sucrose sorbitol
7	a	b	b	ab
15	a	b	b	b
28	a	ab	bc	c
54	a	b	b	c
111	a	ab	b	c
212	a	a	a	b
364	a	b	b	c

[a] Different letters within the same row refer to significant differences ($\alpha = 0.05$).

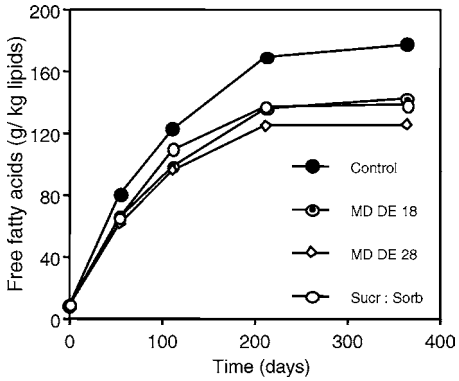

Figure 8. Hydrolysis of fatty acids in low-fat minced mackerel muscle during frozen storage at −10 °C.

Table 10. Statistical Differences in the Hydrolysis of Free Fatty Acids of Frozen-Stored Low-Fat Minced Mackerel[a]

time (days)	control	MD DE 18	MD DE 28	sucrose sorbitol
54	a	b	b	b
111	a	b	b	ab
212–364	a	b	b	b

[a] Different letters within the same row show significant differences ($\alpha = 0.05$).

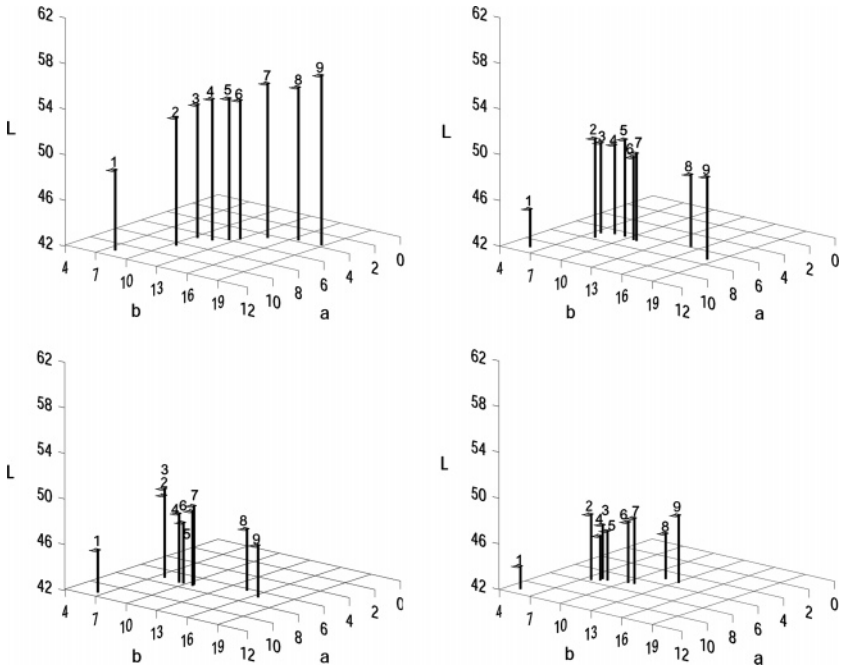

Figure 9. Hunter color values for low-fat minced mackerel muscle during frozen storage at −10 °C: control sample (upper left); sample with MD DE 18 (lower right); sample with MD DE 28 (lower left); sample with sucrose and sorbitol (lower right). Numbers above bars correspond to the following sampling periods: 1 (0 days, nonfrozen), 2 (7 days), 3 (15 days), 4 (28 days), 5 (54 days), 6 (78 days), 7 (111 days), 8 (212 days), and 9 (364 days).

Table 11. Statistical Differences in *a* (**a**), *b* (**b**), and *L* (**c**) Values among the Batches of Frozen-Stored Low-Fat Minced Mackerel[a]

time (days)	control	MD DE 18	MD DE 28	sucrose sorbitol
		(**a**) *a* Values		
54	a	ab	b	b
78–111	a	b	b	b
212–364	a	b	b	c
		(**b**) *b* Values		
0–111	a	b	b	b
212–364	a	a	a	b
		(**c**) *L* Values		
0	a	b	b	b
7	a	ab	ab	b
15	a	b	b	b
28–54	a	b	bc	c
78–364	a	b	b	b

[a] Different letters within the same row refer to significant differences ($\alpha = 0.05$).

Table 12. Effects of Reducing Maltodextrin Concentration on Thiobarbituric Acid Reactive Substance Index in Minced Mackerel Stored at −10 °C[a]

time (days)	control	MD DE 18 (5%)	MD DE 18 (8%)	sucrose sorbitol
29	0.334a	0.249b	0.166c	0.240b
61	0.463a	0.209b	0.191b	0.362c
119	1.143a	0.434b	0.271c	0.892d

[a] Different letters within the same row refer to significant differences ($\alpha = 0.05$).

between maltodextrin concentration and T_m' shown by Herrera et al. (*27*). The effect on freeze concentration is also clear. Reducing the proportion of maltodextrin added not only gives rise to a much higher freeze concentration of solutes at a fixed proportion of unfrozen water with respect to the freezable water content, but a comparison of the differential cumulative percentage variation of the ice melting endotherm areas of minces with 50 and 80 g kg^{-1} maltodextrin (**Figure 5**) reveals that it makes

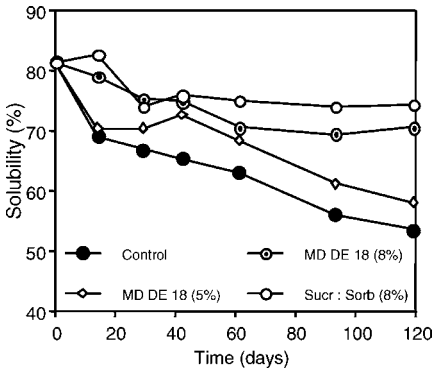

Figure 10. Solubility of high-fat minced mackerel muscle containing two diferent proportions of MD DE 18 (5 and 8%) during frozen storage at −10 °C.

Table 13. Statistical Differences in Solubility among the Batches of Minced Mackerel Stored at −10 °C in Experiment 3[a]

time (days)	control	MD DE 18 (5%)	MD DE 18 (8%)	sucrose sorbitol
14–29	a	a	b	b
42	a	b	b	b
61	a	b	bc	c
93–119	a	a	b	b

[a] Different letters within the same row refer to significant differences ($\alpha = 0.05$).

a lower proportion of water of the freezable water content be unfrozen at the temperature of study and therefore raises the freeze concentration of water-soluble muscle components. Consequently, a lower T_m' and a higher freeze concentration of solutes would explain the lower effectiveness of reducing the proportion of maltodextrin added.

Decreasing the concentration of maltodextrin from 80 to 50 g kg^{-1} reduced slightly the effectiveness of this cryostabilizer in preventing lipid oxidation, but affected to a greater extent its effectiveness against freeze-induced perturbations in protein

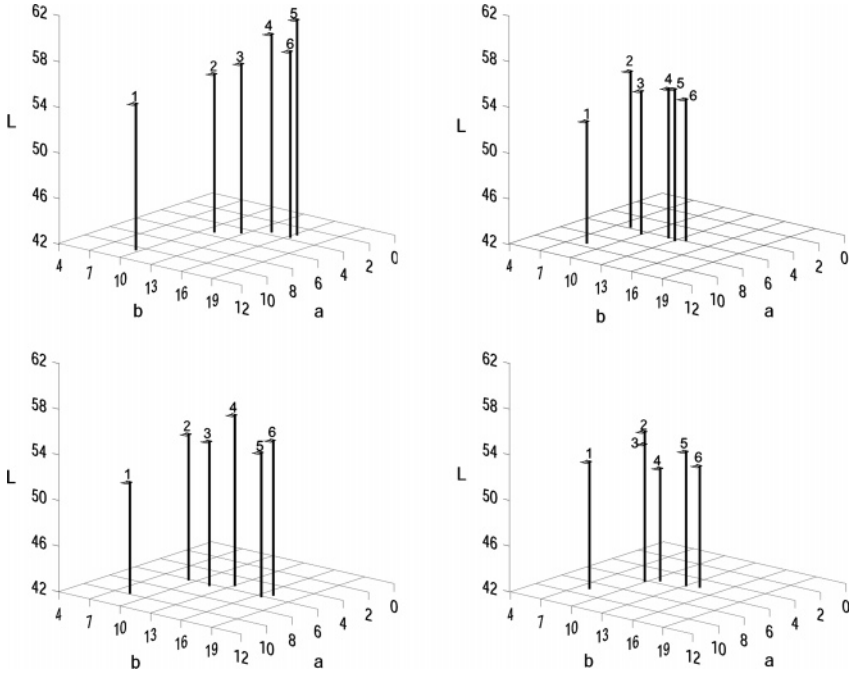

Figure 11. Hunter color values for high-fat minced mackerel muscle containing two diferent proportions of MD DE 18 (5 and 8%) during frozen storage at −10 °C: control sample (upper left); sample with 5% MD DE 18 (lower left); sample with 8% MD DE 18 (upper right); sample with sucrose and sorbitol (lower right). Numbers above bars correspond to the following sampling periods: 1 (0 days, nonfrozen), 2 (14 days), 3 (29 days), 4 (61 days), 5 (93 days), and 6 (119 days).

Table 14. Statistical Differences in *a* (**a**), *b* (**b**), and *L* (**c**) Values among the Batches of Minced Mackerel Stored at −10 °C of Experiment 3[a]

time (days)	control	MD DE 18 (5%)	MD DE 18 (8%)	sucrose sorbitol
		(**a**) *a* Values		
29–61	a	b	b	b
93	a	b	b	c
119	a	b	b	b
		(**b**) *b* Values		
0	a	ab	ab	b
14	a	b	c	ab
29	a	a	b	b
61	a	ab	bc	c
93–119	a	a	b	b
		(**c**) *L* Values		
61	a	ab	bc	c
93	a	b	b	b
119	a	ab	b	b

[a] Different letters within the same row refer to significant differences ($\alpha = 0.05$).

solubility and color. Therefore, such a decrease could be accepted when lipid oxidation is the most limiting process of shelf life, but it does not seem to be appropriate when protein changes are important.

SAFETY

Organic solvents should be handled under fume hood conditions.

ACKNOWLEDGMENT

We thank Alberto Gallego, Bibiana Torres, and Carlos Suárez for technical assistance.

LITERATURE CITED

(1) Venugopal, V.; Shahidi, F. Value-added products from underutilized fish species. *Crit. Rev. Food Sci. Nutr*. **1995**, *35* (5), 431−453.

(2) Undeland, I.; Ekstrand, B.; Lingnert, H. Lipid oxidation in minced herring (*Clupea harengus*) during frozen storage. Effect of washing and precooking. *J. Agric. Food Chem*. **1998**, *46*, 2319−2328.

(3) Hultin, H. O.; Kelleher, S. D. Surimi processing from dark muscle fish. In *Surimi and Surimi Seafood*; Park, J. W., Ed.; Dekker: New York, 2000; pp 59−77.

(4) Hunter, B. J.; Roberts, D. C. K. Potential impact of the fat composition of farmed fish on human health. *Nutr. Res*. **2000**, *20* (7), 1047−1058.

(5) Siddiqui, R. A.; Shaikh, S. R.; Sech, L. A.; Yount, H. R.; Stillwell, W.; Zaloga, G. P. Omega 3-fatty acids: health benefits and cellular mechanisms of action. *Mini Rev. Med. Chem*. **2004**, *4* (8), 859−871.

(6) Ruxton, C. H.; Reed, S. C.; Simpson, M. J.; Millington, K. J. The health benefits of omega-3 polyunsaturated fatty acids: a review of the evidence. *J. Hum. Nutr. Diet.* **2004**, *17* (5), 449−459.

(7) Food and Agriculture Organization Fisheries Department. *The State of World Fisheries and Aquaculture (SOFIA)*; Rome, Italy, 2004; ISBN 9251051771.

(8) Hultin, H. O. Oxidation of lipids in seafoods. In *Seafoods: Chemistry, Processing Technology and Quality*; Shahidi, F., Botta, J. R., Eds.; Blackie Academic and Profesional: London, U.K., 1993.

(9) Tall, J.; Harris, P. Rancidity in frozen fish. In *Fish Oil: Technology, Nutrition and Marketing*; 1995; pp 35−47.

(10) Kulås, E.; Olsen, E.; Ackman, R. G. Oxidation of fish lipids and its inhibition with tocopherols. In *Lipid Oxidation Pathways*; Afaf Kamal-Eldin, A., Ed.; AOCS Press: Champaign, IL, 2003; pp 37−69.

(11) Park, Y.; Kelleher, S. D.; McClements, D. J.; Decker, E. A. Incorporation and stabilization of omega-3 fatty acids in surimi made from cod, *Gadus morhua*. *J. Agric. Food Chem.* **2004**, *52*, 597−601.

(12) Hultin, H. O.; Decker, E. A.; Kelleher, S. D.; Osinchak, J. E. Control of lipid oxidation processes in minced fatty fish. In *Seafood Science and Technology*; Bligh, E. G., Ed.; 1992; pp 93−100.
(13) Pastoriza, L.; Sampedro, G.; Herrera, J. J. Effects of mincing and frozen storage on functional properties of ray muscle (*Raja clavata*). *J. Sci. Food Agric.* **1994**, *66* (1), 35−44.
(14) Herrera, J. R.; Pastoriza, L.; Sampedro, G.; Cabo, M. L. Effect of various cryostabilizers on the production and the reactivity of formaldehyde in frozen-stored minced blue whiting muscle. *J. Agric. Food Chem.* **1999**, *47*, 2386−2397.
(15) Herrera, J. R.; Pastoriza, L.; Sampedro, G. Inhibition of formaldehyde production in frozen-stored minced blue whiting (*Micromesistius poutassou*) muscle by cryostabilizers: an approach from the glassy state theory. *J. Agric. Food Chem.* **2000**, *48*, 5256−5262.
(16) Rodriguez-Herrera, J. J.; Pastoriza, L.; Sampedro, G. Effects of various cryostabilisers on protein functionality in frozen-stored minced blue whiting muscle: the importance of inhibiting formaldehyde production. *Eur. Food Res. Technol*. **2002**, *214* (5), 382−387.
(17) Dondero, M.; Gandolfo, M.; Cifuentes, A. Cryoprotective effect of maltodextrin 25 DE, milk whey and mixtures on surimi from jack mackerel (*Trachurus murphyi*). *Rev. Esp. Cien. Tec. Aliment.* **1994**, *34* (4), 389−408.
(18) Hunter, R. S. Requirements for reproducible specification and measurement of the colors of tomatoes. *Second Tomato Quality Workshop: Requirements for Reproducible Specification and Measurement of the Colors of Tomatoes*; 1976; pp 41−48.
(19) Ueda, S.; Hayashi, T.; Namiki, M. Effect of ascorbic acid on lipid autoxidation in a model food system. *Agric. Biol. Chem*. **1986**, *50*, 1−7.
(20) Reiriz, M. J.; Pastoriza, L.; Sampedro, G. Lipid changes in muscle tissue of ray (*Raja clavata*) during processing and frozen storage. *J. Agric. Food Chem.* **1992**, *40*, 484−488.
(21) Pikul, J.; Leszczynski, D. E.; Kummerow, F. A. Evaluation of three modified TBA methods for measuring lipid oxidation in chicken meat. *J. Agric. Food Chem.* **1989**, *37*, 1309−1313.
(22) Lowry, R. R.; Tinsley I. J. Rapid colorimetric determination of free fatty acids. *J. Am. Oil Chem. Soc.* **1976**, *7*, 470−472.
(23) Bernárdez, M.; Pastoriza, L.; Sampedro, G.; Herrera, J. J. R.; Cabo, M. L. Modified method for the analysis of free fatty acid in fish. *J. Agric. Food Chem.* **2005**, *53*, 1903−1906.
(24) Faraji, H.; Lindsay, R. C. Characterization of the antioxidant activity of sugars and polyhydric alcohols in fish oil emulsions. *J. Agric. Food Chem*. **2004**, *52*, 7164−7171.
(25) Fujii, N.; Hamano, M.; Yuasa, K. Inhibition of the autoxidation of methyl linoleate by protein and highly concentrated dextrin in an emulsion system. *Biosci., Biotechnol., Biochem.* **1995**, *59*, 1761−1763.
(26) Wetterskog, D.; Undeland, I. Loss of redness (*a**) as a tool to follow hemoglobin-mediated lipid oxidation in washed cod mince. *J. Agric. Food Chem*. **2004**, *52*, 7214−7221.
(27) Herrera, J. J.; Pastoriza, L.; Sampedro, G. A DSC study on the effects of various maltodextrins and sucrose on protein changes in frozen-stored minced blue whiting muscle. *J. Sci. Food Agric.* **2001**, *81*, 377−384.
(28) Sartor, G.; Johari, G. P. Structural relaxation of a vitrified high-protein food, beef, and the phase transformations of its water content. *J. Phys. Chem.* **1996**, *100*, 10450−10463.
(29) Park, J. W.; Lanier, T. C.; Green, D. P. Cryoprotective effects of sugar, polyols, and/or phosphates on Alaska Pollak surimi. *J. Food Sci*. **1988**, *53* (1), 1−3.
(30) Levine, H.; Slade, L. Beyond water activity: recent advances based on an alternative approach to the assessment of food quality and safety. *CRC Crit. Rev. Food Sci. Nutr*. **1991**, *30*, 115−360.
(31) Oldenhof, H.; Wolkers, W. F.; Fonseca, F.; Passot, S.; Marin, M. Effect of sucrose and maltodextrin on the physical properties and survival of air-dried *Lactobacillus bulgaricus*: an in situ Fourier transform infrared spectroscopy study. *Biotechnol. Prog.* **2005**, *21*, 885−892.
(32) Rodríguez-Herrera, J. R.; Bernárdez, M.; Pastoriza, L.; Sampedro, G.; Cabo, M. Combined effects of maltodextrins and antioxidants on lipid oxidation in frozen-stored Atlantic mackerel. **2005**, in preparation.
(33) Carpenter, J. F.; Crowe, J. H. The mechanism of cryoprotection of proteins by solutes. *Cryobiology* **1988**, *25* (3), 244−55.

Received for review July 25, 2005. Revised manuscript received February 2, 2006. Accepted February 15, 2006. This research was supported by Interministerial Commission of Science and Technology (CICYT) during the Research Project AGL 2001-1355.

JF0581271