A Possible Role For Cryostabilisers In Preventing Protein And Lipid Alterations In Frozen-Stored Minced Muscle Of Atlantic Mackerel

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RUNNING title header: Cryostabilization of frozen-stored minced mackerel

ABSTRACT

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 (Tm') and decreased freeze-concentration of solutes in the unfrozen-water (UFW) phase, which would have allowed it to effectively slow down 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 i.e. a 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 cryoprotectans with maltodextrins it should be known which process(es) limit the shelflife of the food.

Decreasing (from 80 g kg^{-1} to 50 g kg^{-1}) the proportion of maltodextrin added to high-fat minced mackerel showed that though it only affected slightly the effectiveness against lipid oxidation, it did notably the effectiveness in preventing a the loss of protein solubility and color changes. Therefore, such decrease could only be accepted if lipid oxidation is the most limiting process of shelflife, 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 shelflife- by using minces made from fatty fish (*1,2,3*). Such interest has been stimulated by the nutritional relevance associated to 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 (*4,5,6*), as well as by the depletion and over-explotation 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 (*8,9,10,11*), which becomes a major limitation to develop such products. Freezing and frozen storage are one of the most 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, majorly due to inhibitory effect of several maltodextrins on the production of formaldehyde, which thus reduced its subsequent interactions with muscle compounds (*14,15,16*). 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 logical to raise if cryostabilization by using this maltodextrin could be also applied to prevent alterations taking place in frozen-stored minced fatty fish, since it could be very useful to develop more stable and nutritive.food products from fatty fish minces.

MATERIALS AND METHODS

Experimental design

Atlantic mackerel (*Scomber scombrus*), caught in offshore galician waters, were gutted and beheaded within the first 24 h post-capture 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 (Cerestar Ibérica, S.A.) 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.

Samples (ca. 50g) 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°C 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*).

Color

Tristimulus reflectance color (*L*, *a*, *b*) 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 extracting according to a modified Folch method *(20),* fish lipids (ca. 4 mg or less) were desolventised under a gentle stream of nitrogen. Lipids were inmediately re-dissolved 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 up the headspace of the test tubes. The reaction mixtures were left to stand for 7 min at room temperature and absorbance was read out at 500 nm. The concentration of ferric ion (mg/mL) 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 deoxigenized prior to use.

Peroxide value was calculated according to the following equation:

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

being 55.84 the atomic weight of iron.

Results are expressed as meq. of Fe per kg of lipids.

Thiobarbituric-reactive substances index

The content of thiobarbituric-reactive substances was determined following the method of Pikul et al *(21)* with some modifications. After being desolventised under a gentle stream of nitrogen, lipids (ca. 3-5 mg) were re-disolved in 0.1 ml of chloroform. Then 0.2 ml of 81 g kg^{-1} sodium dodecyl sulfate (SDS) were 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} butyl-hydroxytoluene (BHT) in ethanol were pipetted and the whole mixture was vortexed further. Test tubes were heated at 100°C for one hour, cooled down rapidly to stop the reaction and centrifuged at 2000xg for 15 min. Absorbance was read out at 535 nm.

The index of thiobarbituric-reactive substances (g malonaldehyde per kg 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 reaction mixture).

Free fatty acid content

The content of free fatty acids was determined as described in Bernárdez et al *(22*).

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%. These analyses were carried out with the help of the software Microsoft Excel Version 5.0a for Power Macintosh.

RESULTS AND DISCUSSION

1. Effects on lipid and protein alterations of high-fat minced mackerel

The addition of DE 18 maltodextrin to high-fat (145g 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 and the index of thiobarbituric-reactive substances were significantly lower for the cryostabilised 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 *(23)* has shown some antioxidant activity of sugars and polyhydric alcohols in fish oil emulsions too. However, in the 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. *(24)* had also reported that dextrin increased the stability to autoxidation when compared with mono- and di-saccharides. No differences were noticed in the content of any fatty acid -as determined by gas chromatography- among samples with very different degree of lipid oxidation (results not shown).

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 a great help to produce such foods.

Both the mixture of sucrose and sorbitol and DE 18 maltodextrin slowed down significantly the loss of protein solubility during the whole period of storage at -10 and -20°C (**Figure 3**), but the former was slightly more effective. Differences became significant after 100 and 190 days of storage, respectively (**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 major responsible for such differences *(16)*. The hydrolysis of free fatty acids was found to be not very important in frozen-stored high-fat mackerel (**Table 4**). And though it was found that DE 18 maltodextrin inhibited this process at -20°C, there should be considered that the low level of production might have been source of mistake.

The changes in color were also slowed down 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 5-7**). These results reflect that their addition delayed muscle yellowing caused by oxidative alterations and maintained the original redness for much longer. However, whereas DE 18 maltodextrin prevented yellowing slightly

better than sucrose and sorbitol at -20°C from 139 days of storage -the values for *b* of maltodextrin-containing samples were lower than those of samples with sucrose and sorbitol-, the mixture of sucrose and sorbitol was slightly more effective from 76 days of storage at -10°C. Regarding *a*, samples with DE 18 maltodextrin showed values significantly higher than those with sucrose and sorbitol from 28 days of storage at -10°C -significant differences between treatments were only noticed between 58 and 84 days of storage at -20°C-, which reveals a better ability of DE 18 maltodextrin to preserve redness. Instrumental measurements of redness loss were considered to be adequate as a tool to follow haemoglobin-mediated lipid oxidation in fish flesh *(25)* so that DE 18 maltodextrin would have best prevented haemoglobin-mediated lipid oxidation. Slight differences between treated samples and control were also found in lightness (*L*), which occasionally became significant at -10°C (never at -20°C). 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 (Tm') from -27,5°C to -24°C, which implies a lower molecular mobility in maltodextrin-containing minces than in the control. A similar increased had been previously reported for minced blue whiting muscle *(26)*. It also decreased freeze-concentration of solutes in the unfrozen-water (UFW) phase by increasing the proportion of unfrozen water respect to the total freezable water content at the temperatures of study (as shown and by DSC scans in **Figure 5**) and by "diluting" the freeze-concentration of muscle components in the UFW (*16*). On the opposite, the addition of sucrose and sorbitol has some plasticising effect, diminishing Tm' to -31.5°C, but decreased freeze-concentration of solutes in the unfrozen-water (UFW) phase. Accordingly, DE 18 maltodextrin and the mixture of sucrose are able to slow down diffusion-limited processes effectively, the former's ability being higher. This are

clearly the cases of lipid oxidation, which is a typical diffusion-limited reaction, and hydrolysis of free fatty acids.

Protein changes, however, follow a much more complex pattern, as they are driven by a collective mechanism associated to a hierarchy of freedom degrees of the different structural elements conforming the protein (*27*). Furthermore, low DE maltodextrins 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.

2. Effects on lipid and protein alterations of low-fat minced mackerel

The lipid content of fatty fish is subjected to seasonal variations, related mostly to spawning and feeding. Considering that the results of the first experience 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, it was raised if the addition of maltodextrins was adequate to preserve low-fat (52.5 g 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 (*28*), 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**), but no significant differences were found between them (**Table 8**). On the opposite, 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 down diffusion-limited reactions in frozen systems, and it was also suggested for small solutes, e.g. sucrose and sorbitol *(29).* 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 differences in the effectiveness of sucrose and sorbitol against lipid oxidation between low- and high-fat fish has to be explained by intrinsic factors of the system. In high-fat fish, lipid oxidation comes fundamentally from triglycerides, which are dispersed as small droplets within the muscle structure,whereas membrane lipids have an major role in the oxidation of lipids of low-fat fish and, consequently, this process takes place to a much lower extent. 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. To this respect, Oldenhof et al. *(30)* have pointed out that sucrose seems to function by direct interaction with biomolecules, whereas maltodextrins would acte as an osmotically inactive bulking agent strengthening of the glassy matrix. Nevertheless, further studies will be needed to find out the reason for this lack of effectiveness.

The mixture of sucrose and sorbitol, however, slowed down the loss of solubility to a significantly higher extent than both maltodextrins, particularly than DE 18 maltodextrin (**Figure 7 and 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 the hydrolysis of free fatty acids, but no differences were found among treatments (**Figure 8, Table 10**). In accordance with the prevalence 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, though no clear trend has yet been reported concerning if it favours 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. On the opposite, the samples with maltodextrins showed higher values for *a* than the samples with sucrose and sorbitol from 210 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 no differences were appreciated among treatments.

These studies have shown that maltodextrins protect minced muscle of fatty fish against freeze-induced lipid oxidation better 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 fish, whereas 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 firstly recommended to prevent freeze-induced perturbations in high-fat 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 cryoprotectans with maltodextrins it should be known which process(es) limit the shelflife of the food.

3. 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 *(31)* led 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 lipids kg^{-1}) minced mackerel muscle during frozen storage. This study was also carried out at -10°C, and a non-treated 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.

On the opposite, the addition of 50g kg^{-1} DE 18 maltodextrin to minced mackerel muscle was not too effective in preventing the loss of protein solubility (**Figure 10**). In general, solubility was significantly lower in 50 g kg^{-1} DE 18 maltodextrin- 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 (*32*). However, solubility only was occasionally higher in 50 g kg^{-1} DE 18 maltodextrin-containing than in control samples, whereas increasing the

proportion of maltodextrin added from 50 to 80 g kg^{-1} led to significant differences during all the 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 than those of 80 g kg^{-1} DE 18 maltodextrin-containing samples. On the opposite, the values for *b* were significantly higher than those of 80 g kg^{-1} DE 18 maltodextrin-containing samples (also than samples with sucrose and sorbitol) from 14 days of storage, but not than those of 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 seem to be responsible for yellowing too. Lightness of 50 g kg^{-1} DE 18 maltodextrin-containing minces were intermediate, and no significantly differences were found respect to the control and 80 g kg^{-1} -cryoprotectant containing minces.

The proportion of maltodextrin added affected significantly both Tm' and freeze-concentration. Thus, decreasing the proportion of maltodextrin (from 80 g kg^{-1} to 50 g kg^{-1}) increased Tm' to a lower extent, i.e. -25°C, which agrees with the linearity between maltodextrin concentration and Tm' shown by Herrera et al *(23).* The effect on freeze-concentration is also clear. Reducing the proportion of maltodextrin added does not only give rise to a much higher freeze-concentration of solutes at a fixed proportion of unfrozen water -respect to the freezable water content-, but a comparison of the percentage integral variation of the ice melting endotherm areas of minces with 50 g kg^{-1} and 80 g kg^{-1} maltodextrin (**Figure 5**) reveals that it makes a lower proportion of water of the freezable water content be unfrozen at the temperature of study and therefore freeze-concentration of water-soluble muscle components be higher.

Consequently, a lower Tm' and a higher freeze-concentration of solutes would give explanation to the lower effectiveness of reducing the proportion of maltodextrin added. Decreasing the concentration of maltodextrin from 80 g kg^{-1} to 50 g kg^{-1} reduced slightly the effectiveness of this cryostabiliser in preventing lipid oxidation, but affected to a greater extent its effectiveness against freeze-induced perturbations in protein solubility and color. Therefore, such decrease could be accepted when lipid oxidation is the most limiting process of shelflife, but it does not seem appropriate when protein changes are important.

SAFETY

Organic solvents should be handled under fume hood conditions.

ACKNOWLEDGMENT

This research was supported by Interministerial Commission of Science and Technology (CICYT) during the Research Project AGL 2001-1355. We thank Alberto Gallego, Bibiana Torres and Carlos Suárez for technical assistance.

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TABLES

Table 1a: Statistical differences in PV among the batches of high-fat minced mackerel stored at -10°C

Time (days)	Control	MD DE 18	Sucrose sorbitol
8	a	b	a
15-29	a	b	b
41-108	a	b	c

Table 1b: Statistical differences in PV among the batches of high-fat minced mackerel stored at -20°C

Time (days)	Control	MD DE 18	Sucrose sorbitol
36-112	a	b	c
141-196	a	b	b

a-c: different letters within a same file show significant differences (‡=0,05).

Table 2a: Statistical differences in TBA-RS among the batches of high-fat minced mackerel stored at -10°C

Time (days)	Control	MD DE 18	Sucrose sorbitol
8-15	a	b	a
22-108	a	b	c

Table 2b: Statistical differences in TBA-RS among the batches of high-fat minced mackerel stored at -20°C

Time (days)	Control	MD DE 18	Sucrose sorbitol
16	a	b	b
36-112	a	b	c
168	a	b	ab
196	a	b	b

a-c: different letters within a same file show significant differences (‡=0,05).

Table 3a: Statistical differences in solubility among the batches of high-fat minced mackerel stored at -10°C

Time (days)	Control	MD DE 18	Sucrose sorbitol
8-77	a	b	b
108	a	b	c

Table 3b: Statistical differences in solubility among the batches of high-fat minced mackerel stored at -20°C

Time (days)	Control	MD DE 18	Sucrose sorbitol
16	a	ab	b
36-168	a	b	b
196	a	b	c

a-c: different letters within a same file show significant differences (‡=0,05).

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Table 4a: Production of free fatty acids in high-fat minced mackerel during frozen storage at -10°C

Time (days)	Control	MD DE 18	Sucrose sorbitol
29	11.56a	12.38a	11.99a
62	20.59a	18.34ab	17.04b
108	30.43a	26.35a	27.36a

Table 4b: Production of free fatty acids in high-fat minced mackerel during frozen storage at -20°C

Time (days)	Control	MD DE 18	Sucrose sorbitol
84	5.00a	2.75b	2.15b
141	5,29a	3,30b	5,10a
196	7,37a	6.35b	8,01a

a-b: different letters within a same column show significant differences (‡=0,05).

6

7

8

9

Table 5a: Statistical differences in ligthness (*L*) of high-fat minced mackerel stored at -10°C

Time (days)	Control	MD DE 18	Sucrose sorbitol
0-77	a	a	a
108	a	b	b

Table 5b: Statistical differences in ligthness (*L*) of high-fat minced mackerel stored at -20°C

Time (days)	Control	MD DE 18	Sucrose sorbitol
0-196	a	a	a

a-b: different letters within a same column show significant differences (‡=0,05).

1

Table 6a: Statistical differences in *a* among the batches of high-fat minced mackerel stored at -10°C

Time (days)	Control	MD DE 18	Sucrose sorbitol
0-8	a	a	a
15-22	a	b	b
29-108	a	b	c

Table 6b: Statistical differences in *a* among the batches of high-fat minced mackerel stored at -20°C

Time (days)	Control	MD DE 18	Sucrose sorbitol
0	a	a	a
58-84	a	b	c
112-196	a	b	b

a-c: different letters within a same column refer to significant differences (‡=0,05).

2

3

Table 7a: Statistical differences in *b* among the batches of high-fat minced mackerel stored at -10°C

Time (days)	Control	MD DE 18	Sucrose sorbitol
0-62	a	b	b
77	a	a	b
108	a	b	c

Table 7b: Statistical differences in *b* among the batches of high-fat minced mackerel stored at -20°C

Time (days)	Control	MD DE 18	Sucrose sorbitol
0	a	a	a
58-112	a	b	b
141-196	a	b	c

a-c: different letters within a same column refer to significant differences (‡=0,05).

1

Table 8a: Statistical differences in PV among the batches of frozen-stored low-fat minced mackerel

Time (days)	Control	MD DE 18	MD DE 28	Sucrose sorbitol
28-54	a	b	b	a
212-364	a	b	b	d

Table 8b: Statistical differences in TBA-RS among the batches of frozen-stored low-fat minced mackerel

Time (days)	Control	MD DE 18	MD DE 28	Sucrose sorbitol
7	a	a	a	a
28	a	ab	b	c
54	a	b	b	a
111	a	b	c	d
212	a	b	b	c
364	a	b	b	a

a-d: different letters within a same column refer to significant differences (‡=0,05).

2

3

Table 9: Statistical differences in solubility among the batches of frozen-stored low-fat minced mackerel

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-c: different letters within a same column refer to significant differences (‡=0,05).

1

Table 10: Statistical differences in the hydrolysis of free fatty acids of frozen-stored low-fat minced mackerel

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-b: different letters within a same column show significant differences (‡=0,05).

2

3

4

Table 11a: Statistical differences in *L* among the batches of frozen-stored low-fat minced mackerel

Time (days)	Control	MD DE 18	MD DE 28	Sucrose sorbitol
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

Table 11b: Statistical differences in *a* among the batches of frozen-stored low-fat minced mackerel

Time (days)	Control	MD DE 18	MD DE 28	Sucrose sorbitol
0-28	a	a	a	a
54	a	ab	b	b
78-111	a	b	b	b
212-364	a	b	b	c

Table 11c: Statistical differences in *b* among the batches of frozen-stored low-fat minced mackerel

Time (days)	Control	MD DE 18	MD DE 28	Sucrose sorbitol
0-111	a	b	b	b
212-364	a	a	a	b

a-c: different letters within a same column refer to significant differences (‡=0,05).

1

Table 12: Effects of reducing maltodextrin concentration on TBA-RS index in minced mackerel stored at -10ºC

Time (days)	Control	MD DE 18 (5%)	MD DE 18 (8%)	Sucroses orbitol
29	0,334a	0,249b	0,166c	0,240b
61	0,403a	0,209b	0,191b	0,286c
119	1,143a	0,434b	0,271c	0,892d

a-d: different letters within a same column refer to significant differences (‡=0,05).

2

3

Table 13: Statistical differences in solubility among the batches of minced mackerel stored at -10ºC in expt 3

Time (days)	Control	MD DE 18 (5%)	MD DE 18 (8%)	Sucrose sorbitol
0-29	a	a	b	b
42	a	b	b	b
61	a	b	bc	c
93-119	a	a	b	b

a-c: different letters within a same column refer to significant differences (‡=0,05).

1

Table 14a: Statistical differences in *L* among the batches of minced mackerel stored at -10ºC of exp. 3

Time (days)	Control	MD DE 18 (5%)	MD DE 18 (8%)	Sucroses orbitol
0-29	a	a	a	a
61	a	ab	bc	c
93	a	b	b	b
119	a	ab	b	b

Table 14b: Statistical differences in *a* among the batches of minced mackerel stored at -10ºC of exp. 3

Time (days)	Control	MD DE 18 (5%)	MD DE 18 (8%)	Sucrose sorbitol
0-14	a	a	a	a
29-61	a	b	b	b
93	a	b	b	b
119	a	b	b	b

Table 14c: Statistical differences in *b* among the batches of minced mackerel stored at -10ºC of exp. 3

Time (days)	Control	MD DE 18 (5%)	MD DE 18 (8%)	Sucrose sorbitol
0	a	a	a	a
14	a	b	c	b
29	a	a	b	b
61	a	ab	bc	c
93	a	a	b	b
119	a	a	b	b

a-c: different letters within a same column refer to significant differences (‡=0,05).

2

3

4

5

Figure Captions

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

Figure 2: Peroxide value in high-fat minced mackerel muscle during frozen storage at -10°C or -20°C.

Figure 3: Solubility for high-fat minced mackerel muscle during frozen storage at -10°C or -20°C.

Figure 4: Hunter color values for high-fat minced mackerel muscle during frozen storage at -10°C (left-hand graphs) or -20°C (right-hand graphs). Control sample (upper graphs); sample with MD DE 18 (middle graphs); and sample with sucrose and sorbitol (lower graphs). Numbers above bars correspond to the following sampling periods:
- At -10°C: 1 (0 days, non-frozen), 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, non-frozen), 2 (36 days), 3 (58 days), 4 (84 days), 5 (112 days), 6 (141 days), 7 (168 days), 8 (196 days).

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

Figure 6: Peroxide value (upper graph) and thiobarbituric acid reactive-substances (lower graph) in low-fat minced mackerel muscle during frozen storage at -10°C.

Figure 7: Solubility for low-fat minced mackerel muscle during frozen storage at -10°C.

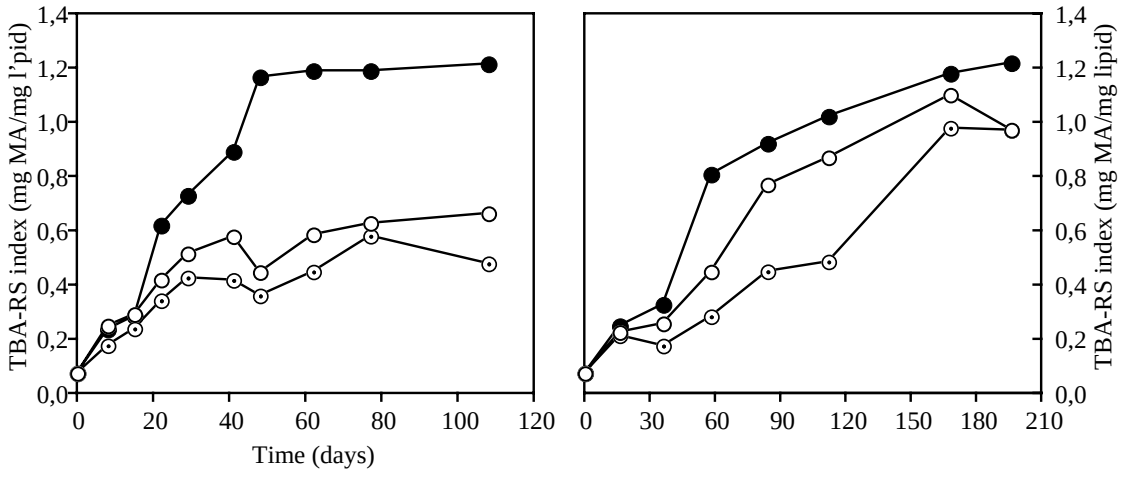
Figure 8: Hydrolysis of free fatty acids in low-fat minced mackerel muscle during frozen storage at -10°C.

Figure 9: Hunter color values for low-fat minced mackerel muscle during frozen storage at -10°C. Control sample (upper left graph); sample with MD DE 18 (lower right graph); sample with MD DE 28 (lower left graph); and sample with sucrose and sorbitol (lower right graph). Numbers above bars correspond to the following sampling periods: 1 (0 days, non-frozen), 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).

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.

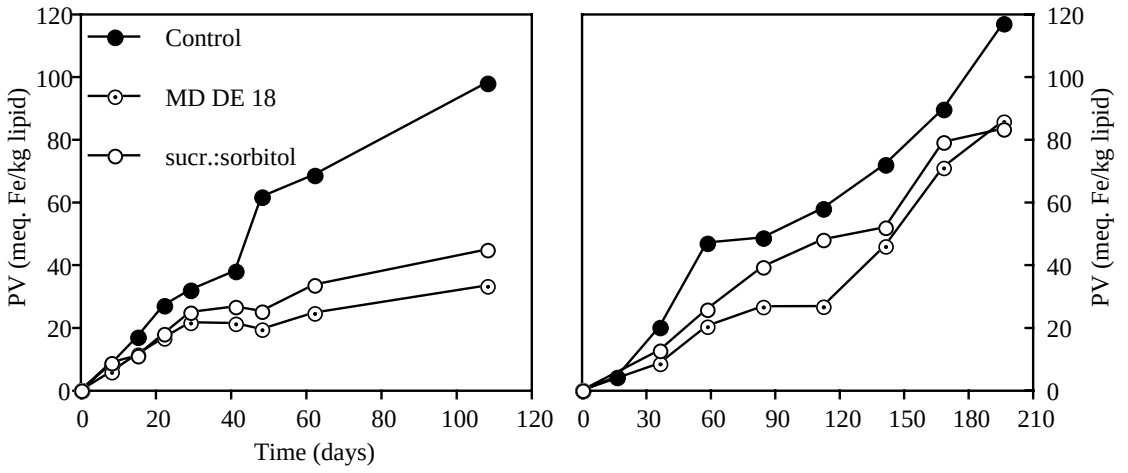
Figure 11: Hunter color values for high-fat minced mackerel muscle containing two diferent proportions of MD DE 18 (5% and 8%) during frozen storage at -10°C. Control sample (upper left graph); sample with 5% MD DE 18 (lower left graph); sample with 8% MD DE 18 (upper right graph); and sample with sucrose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

1


TBA-RS index (mg MA/mg l'pid)
TBA-RS index (mg MA/mg lipid)
Time (days)

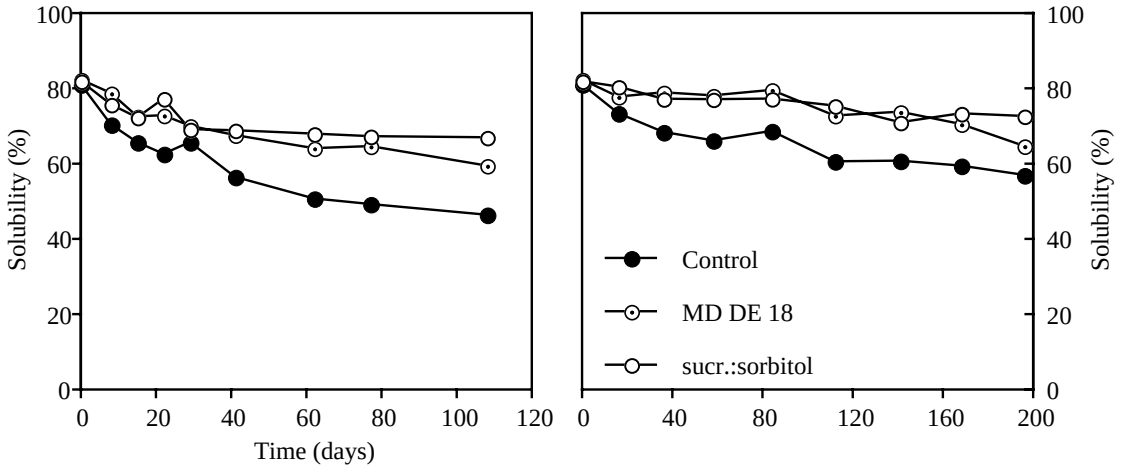
2

3


Control
MD DE 18
sucr.:sorbitol
PV (meq. Fe/kg lipid)
Time (days)

4

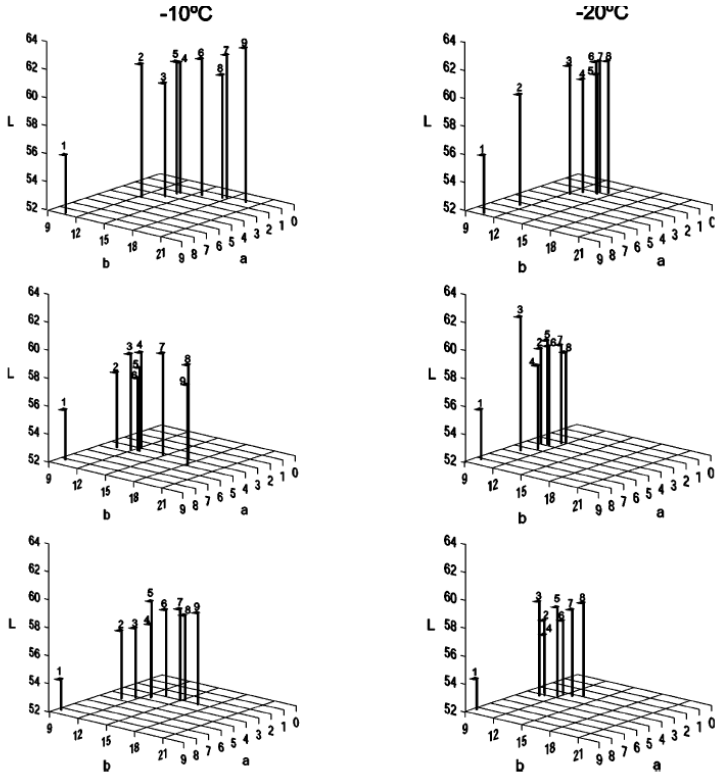
5


Solubility (%)
Control
MD DE 18
sucr.:sorbitol
Time (days)

6

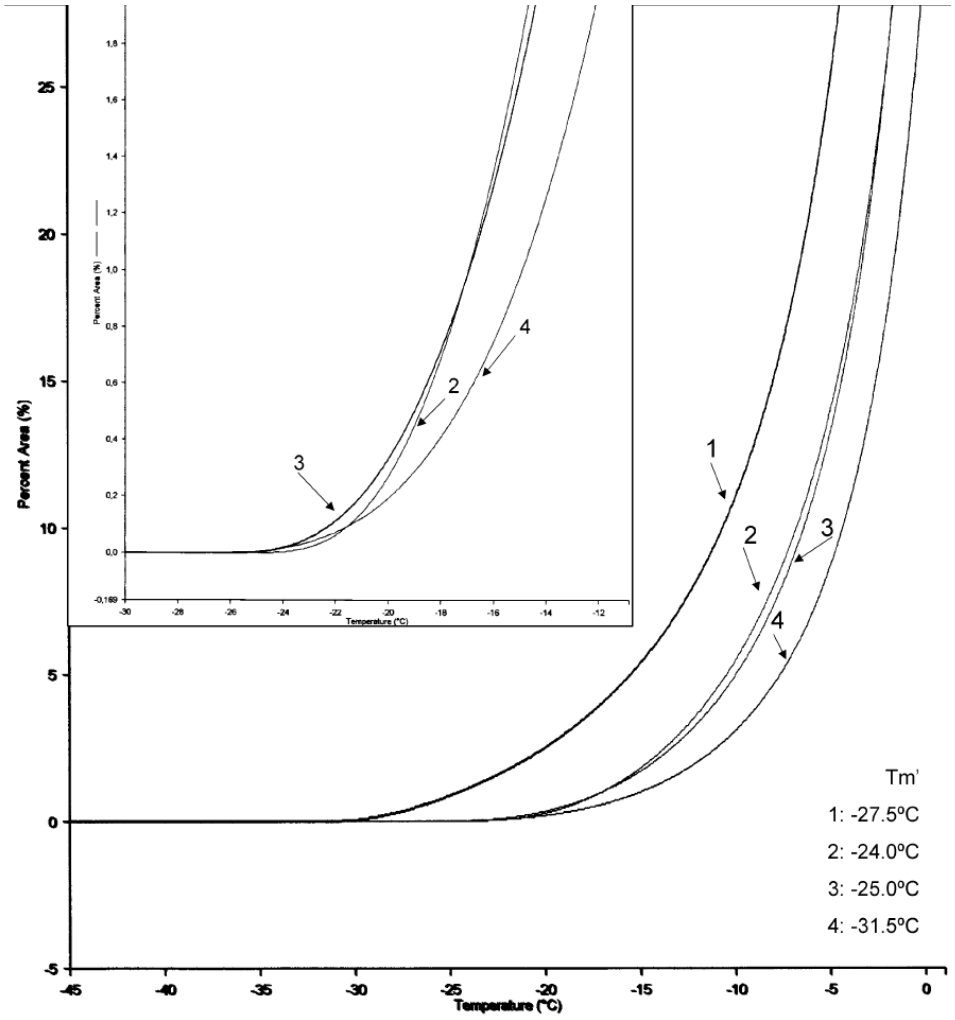
7

1



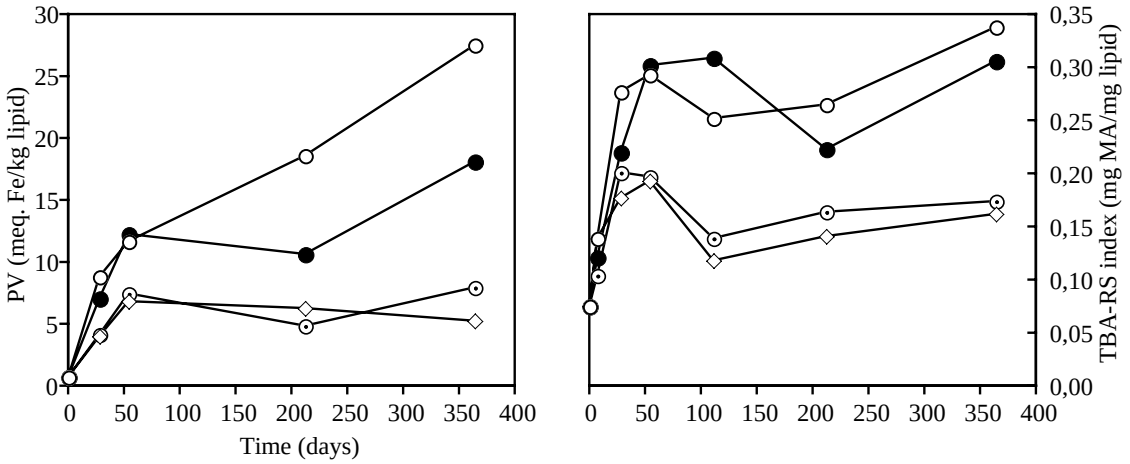
2

3


Percent Area (%)
Temperature (°C)
Tm'
1: -27.5ºC
2: -24.0ºC
3: -25.0ºC
4: -31.5ºC

1

2

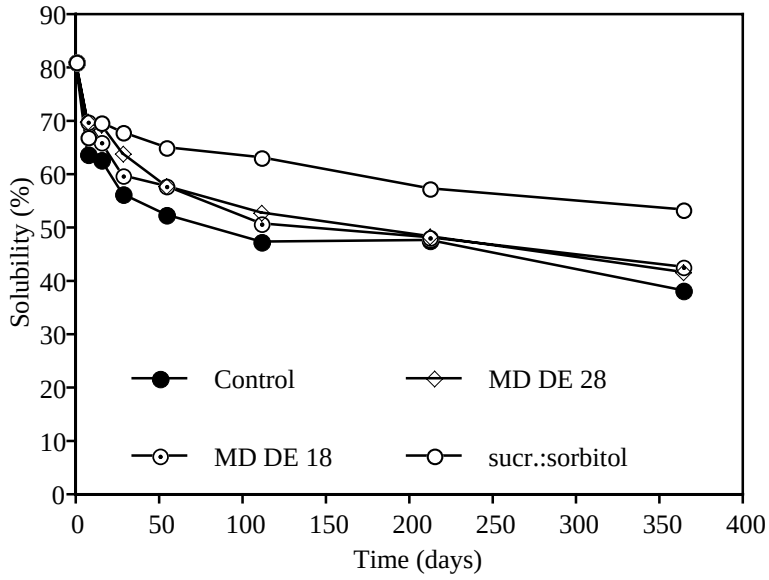

PV (meq. Fe/kg lipid)
TBA-RS index (mg MA/mg lipid)
Time (days)

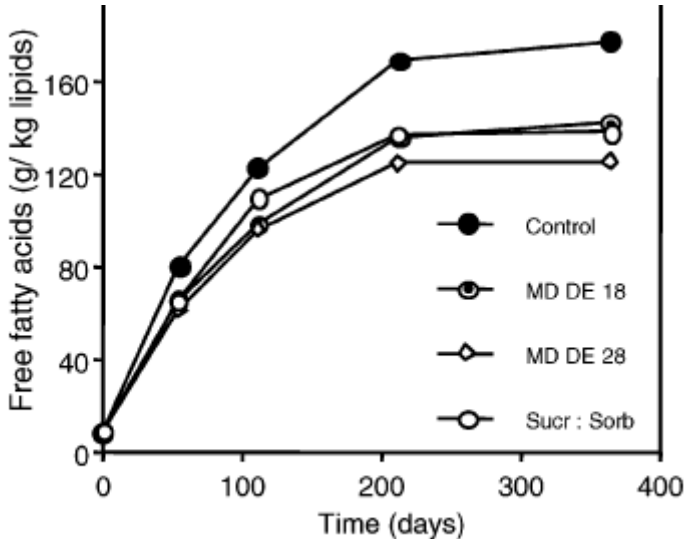
3

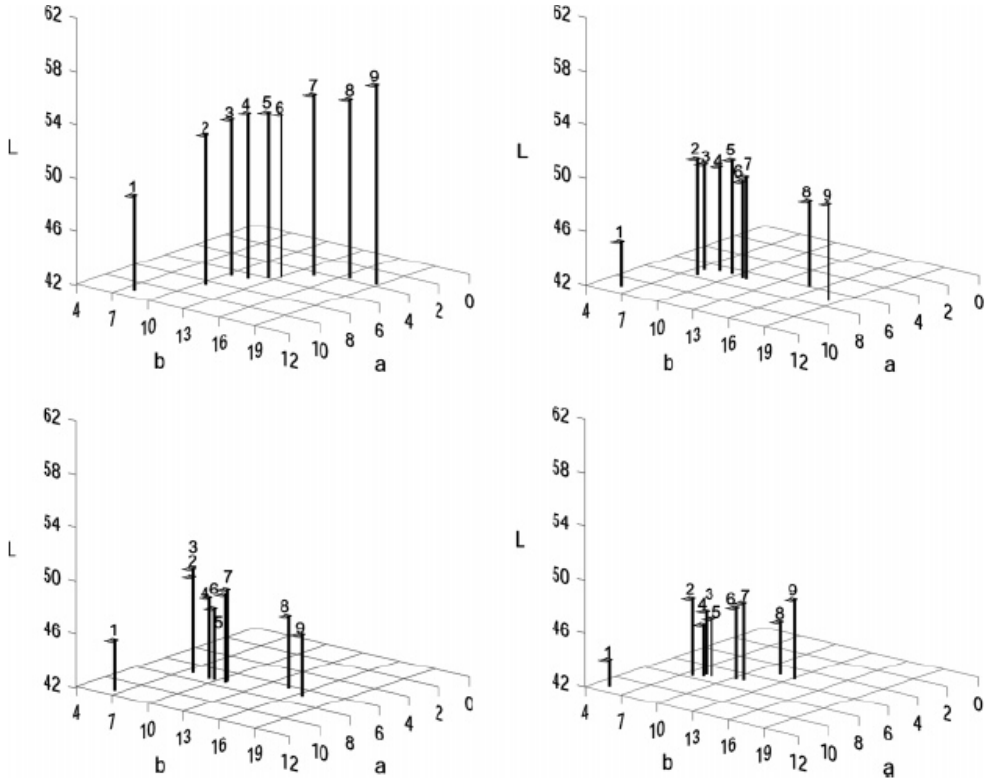
4

5

6

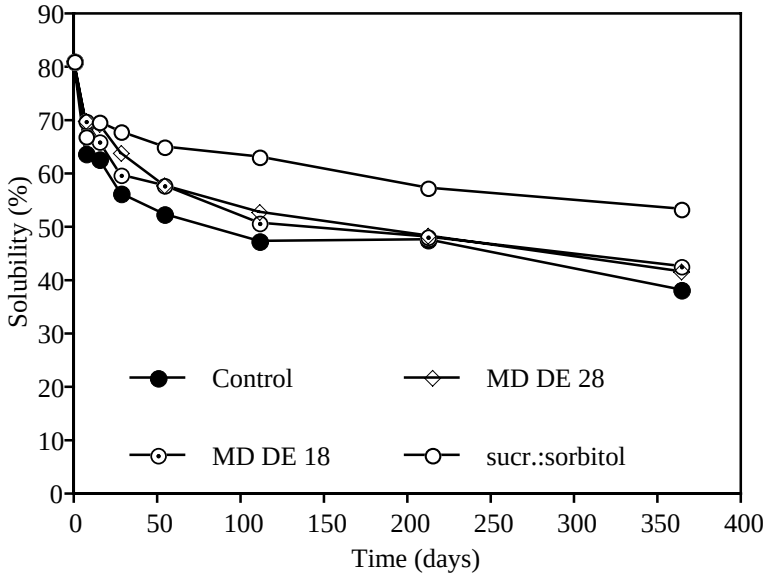

Solubility (%)
Time (days)
Control
MD DE 28
MD DE 18
sucr.:sorbitol


Free fatty acids (g/ kg lipids)
Time (days)
Control
MD DE 18
MD DE 28
Sucr : Sorb


L
62
58
54
50
46
42
b
a
4
7
10
13
16
19
12
10
8
6
4
2
0

1

2


Solubility (%)
90
80
70
60
50
40
30
20
10
0
0
50
100
150
200
250
300
350
400
Time (days)
Control
MD DE 28
MD DE 18
sucr.:sorbitol

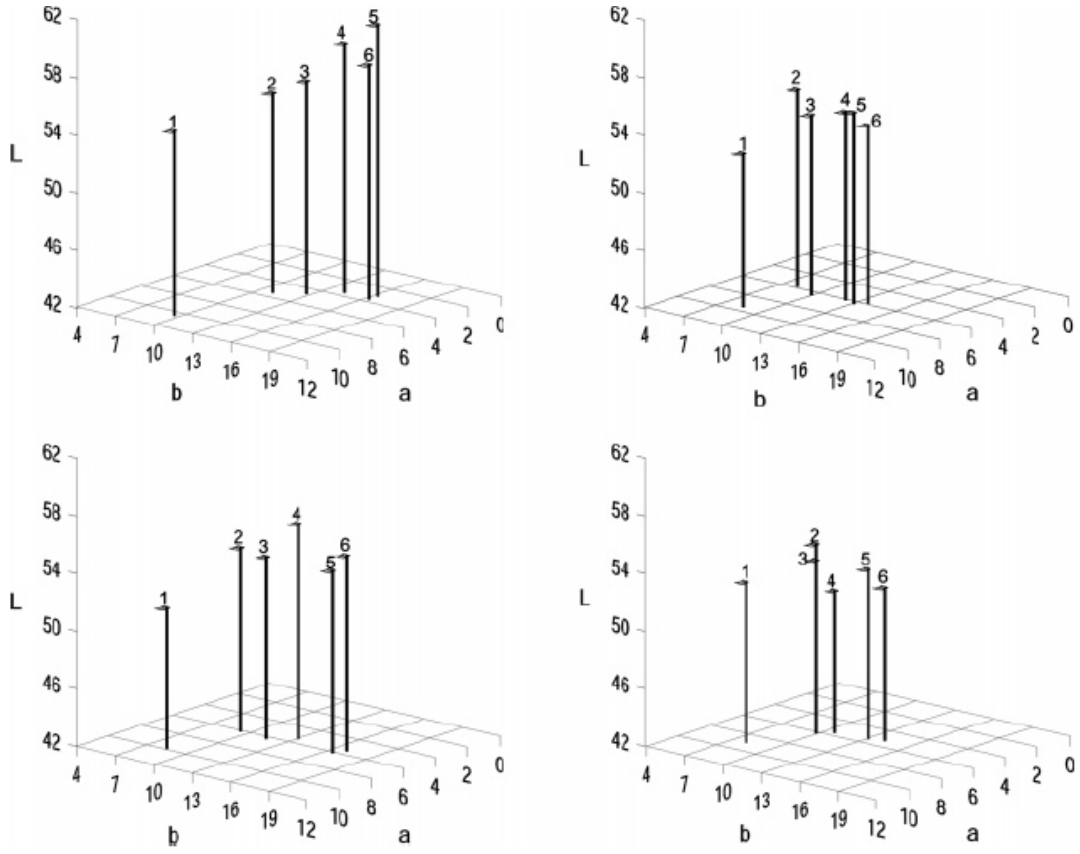
3

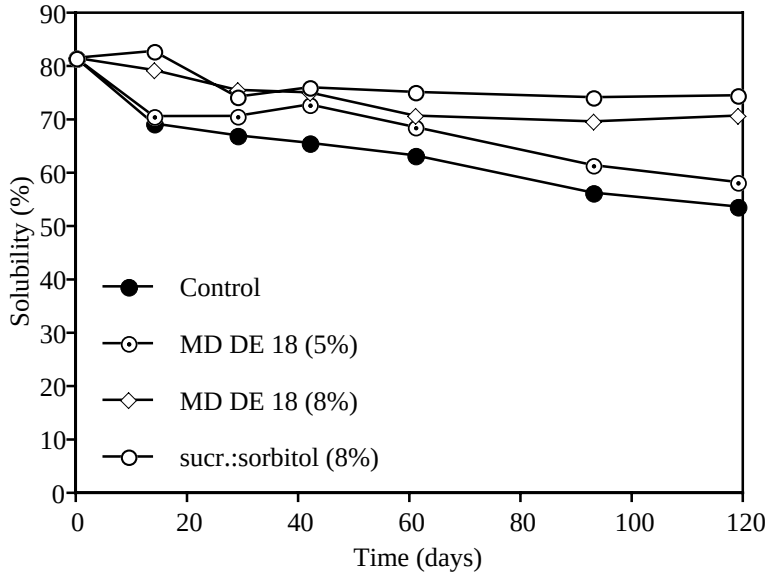
4

5

6

7


L
62
58
54
50
46
42
b
4 7 10 13 16 19
a
12 10 8 6 4 2 0
1
2
3
4
5
6


Solubility (%)
90
80
70
60
50
40
30
20
10
0
0
20
40
60
80
100
120
Time (days)
Control
MD DE 18 (5%)
MD DE 18 (8%)
sucr.:sorbitol (8%)

1