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Influence of frozen storage on textural properties of sardine (*Sardina pilchardus*) mince gels

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This paper examines the influence of freezing temperature (−40°C, −18°C) and frozen storage temperature (−18°C, −12°C) on gels made from washed, minced muscle of sardine (*Sardina pilchardus* R). In gels frozen at −18°C, there was loss of water-holding capacity (WHC), elasticity, hardness and cohesiveness and a slight increase in thiobarbituric acid index. The gel strength did not differ from that of the chilled gel. The WHC decreased in all lots during storage. The greatest increase in rancidity occurred in gels stored at −12°C, particularly those frozen at 18°C. Gel strength, hardness, cohesiveness and elasticity all proved more stable in gels frozen at −40°C irrespective of storage temperature. © 1997 Elsevier Science Ltd

INTRODUCTION

Although pelagic fish species such as sardine abound in Spanish coastal waters, the ratio of consumption to volume of catch is low. However, their nutritional value is high, and technologies have therefore been developed to produce gelled shellfish analogues of high nutritional value, especially protein, in order to maximize the yield of such catches. Preparation of this type of product takes advantage of the gel-forming capacity of myofibrillar proteins (Lanier, 1986).

Most products made from fish muscle are marketed in the frozen state. During freezing, the muscle is unstable, especially when minced, and undergoes a number of alterations. These induce protein aggregation, thus causing hardening of the muscle (Shenouda, 1980). We therefore need to identify suitable freezing parameters in order to minimize rheological and biochemical changes in gels, which in the present case constituted the end-product. The rate of freezing and the conditions of storage affect the structure of the frozen tissue. Among the possible factors causing freeze-denaturation are ions and ice crystals, chemical interactions of proteins, and binding of fatty acids and lipid oxidation products (Sikorsky & Pan, 1994).

Given that gelation is accompanied by protein aggregation, it is possible that gels are not as strongly affected by freezing as muscle. However, very little work has been published on freezing of fish gels (Kato *et al.*, 1993; Alvarez *et al.*, 1990).

This paper examines the influence of freezing temperature and frozen storage temperature on biochemical textural characteristics of gels made from sardine muscle. Two freezing rates were assayed: quick-freezing (−40°C) and slow-freezing (−18°C). Storage temperatures were −12°C and −18°C.

MATERIALS AND METHODS

The fish used in these experiments were sardine of the species *Sardina pilchardus* (Walbaum), caught off the Mediterranean coast in April. The fish arrived at the Instituto del Frío 1 day after capture. Fish mince was prepared using the following procedure. Sardines were headed, gutted and washed. Skin bones were removed with a Baader Model 694 (Lübeck, Germany) deboning machine. Muscle was minced and held for 10 min at 0–3°C in an aqueous solution of 0.5% bicarbonate (3:1, solution:minced muscle) with constant stirring. The solution was left for 10 min. Excess water was then removed using a screw press. As cryoprotectants, 4% sorbitol and 0.2% tripolyphosphate were added. The mince was kept chilled (12 h) pending commencement of gel preparation.

Homogenization of muscle

Washed sardine mince was semi-thawed and placed in a refrigerated vacuum homogenizer (Model. UM5; Ste-

phan u. Söhne, Germany). The muscle was ground for 1 min (rotor angular velocity 3000 rpm). Sodium chloride (2.5%) was then added with sufficient crushed ice to give the required final gel moisture and the mixture was homogenized for 5 min at 1500 rpm under vacuum. The final moisture content was adjusted to 79%.

Heat treatment

The resulting homogenates were stuffed into flexible casings (Krehalon; Soplaril Hispania, Barcelona), 40 μm thick, 3.5 cm diameter and 20 cm long. The sausages were first placed in a waterbath at 37°C for 30 min, then moved to another bath at 90°C for 50 min. The finished gels were kept overnight at 4°C.

Freezing procedure

The gels were frozen in a tunnel freezer (Frigoscandia) in two different lots, determined by the freezing temperature: quick (−40°C) slow (−18°C).

Proximate analysis and protein functionality

To characterize the washed mince, moisture, ashes, crude fat and crude protein were determined by AOAC (1975) methods. The analysis did not show 4% added sorbitol as cryoprotectant. The results were averages of three determinations expressed as percentage of muscle mince. As functionality index, apparent viscosity (Borderías *et al.*, 1985), protein solubility (Ironside & Love, 1958) and emulsion capacity were determined (Borderías *et al.*, 1985).

Water-holding capacity (WHC)

The WHC was determined according to the procedure described by Montero and Gómez-Guillén (1996) and expressed as the percentage of water retained per 100 g of water present in the gel prior to centrifugation. All determinations were carried out in quadruplicate.

TBA (2-thiobarbituric acid) index

The method of Vyncke (1970) was followed. The results are expressed as μmol malonaldehyde per 100 g of sample. Determinations were performed in quadruplicate.

Table 1. Proximate analysis and pH of washed muscle and unwashed muscle

	Fat (%)	Total protein (%)	Ash (%)	Moisture (%)	pH
Washed	4.2±0.4	17.7±0.8	1.4±0.0	74.5±0.6	7.3±0.0
Unwashed	1.1±0.1	13.0±0.2	0.9±0.1	81.7±0.2	7.5±0.1

Analysis does not show sorbitol (4%) added as a cryoprotectant in washed muscle.

Puncture test

The puncture test was determined according to the procedure described by Gómez-Guillén *et al.* (1996). The gel strength was determined by multiplying breaking force (N) by breaking deformation (mm). All determinations were performed at least in quadruplicate.

Compression tests

Both texture profile and compression–relaxation tests are described by Gómez-Guillén *et al.* (1996). All the determinations were performed at least in quadruplicate.

Statistical analysis of data

Two-way analysis of variance was carried out for the different samples. The computer program used was Statgraphics (STSC Inc., Rockville, USA). The difference of means between pairs was resolved by means of confidence intervals using a least significant difference (LSD) range test. Level of significance was set for $P \leq 0.05$.

RESULTS

Proximate analysis and protein functionality of the washed mince

Table 1 shows the proximate composition of minced sardine muscle before and after washing.

The moisture protein contents of unwashed muscle were within the normal ranges for this species. Washing removed approximately 3% of fat. It also caused a reduction in total protein content through elimination of some water-soluble sarcoplasmic proteins a relative increase in muscle moisture. These findings are consistent with the literature (Suzuki, 1986; Gómez-Guillén *et al.*, 1996; Huidobro *et al.*, 1992).

Values of pH were relatively high (7–7.5). In unwashed muscle this could have been affected by season of capture, as sardine spawn in April and May. During the spawning season, glycogen reserves in the muscle are at a minimum, so that there is virtually no conversion to lactic acid and hence no reduction in pH. These findings are consistent with Toyoda *et al.* (1992).

The bicarbonate solution, used during washing to encourage solubilization and partial elimination of sarcoplasmic proteins (Lanier, 1986) and fat, raised the pH slightly.

The relatively low fat content found in the unwashed muscle is typical of this species at the time of year when the specimens were caught, which was during the sardine spawning season (Nunes *et al.*, 1990).

As regards functional properties (Table 2), soluble protein values were similar to those reported by Gómez-

Table 2. Functional properties of washed muscle

Soluble protein (%)	Viscosity (cP)	Emulsifying capacity
62.5 ± 4.29	15 600 ± 259.89	153.72 ± 0.52

Guillén *et al.* (1996) for muscle with high gel-forming capacity. Values of viscosity and emulsifying capacity were very high, which is consistent with a protein having a high degree of functionality. A number of authors (Huidobro *et al.*, 1990; Borderías *et al.*, 1985) working with either minced or whole fish have reported that viscosity and emulsifying capacity are indicative of the degree of alteration of the protein.

There are numerous factors influencing the quality of any washed minced muscle. Important among these are: season caught, physiology, state of preservation of the sardine before mincing and ambient conditions during the process.

Effect of freezing temperature on the gel

Table 3 shows water-holding capacity (WHC), 2-thiobarbituric acid (TBA) index and rheological analysis (puncture test, texture profile analyses (TPA) and relaxation test) of the gels, both chilled and immediately after freezing at the two experimental temperatures (−40°C and −18°C). The WHC was very high in all cases, with no significant differences between the chilled gel (control) and the gel frozen at −40°C (quick-frozen). The WHC of the gel frozen at −18°C (slow-frozen) was significantly lower than that of the control. These results are in agreement with Kato *et al.* (1993), who reported a small increase in free drip in crab scallop analogues made from walleye pollack surimi after slow freezing (−10°C or −20°C), whereas there was virtually no difference after quick freezing (−35°C). Crystallization of ice may disrupt the water structures contributing to the hydrophobic adherents which participate in buttressing the native protein conformation (Sikorsky & Kolakowska, 1994).

The initial TBA value in chilled gel (control) was relatively high, as sardine is a fatty species. Montero *et al.* (1996), working on sardine mince, reported similar values to those of the present paper. According to Huidobro *et al.* (1995), TBA levels within this range in sardine muscle do not correlate with rancidity as appreciated by the senses. The increased rancidity denoted by the TBA index is due chiefly to formation of malonaldehyde by hydroperoxides from fatty acids containing three or more double bonds (Sikorsky *et al.*, 1984). The TBA index can present problems in interpretation owing to the large number of factors that interfere in measurement. But even so, it is a widely used method and the authors believe it is valid for monitoring the difference in oxidation among the various lots (Sikorsky & Kolakowska, 1994). Immediately following the freezing process, there was a significant increase in the TBA index of the slow-frozen lot but no significant difference between the quick-frozen and control lots. Huidobro *et al.* (1990), working on muscle of horse mackerel, also found that, at the outset of frozen storage, TBA levels increased with respect to the levels in chilled muscle. Rehbein and Orlick (1990) found that even severe lipid oxidation had only a slight effect on the texture of frozen minced fillets of Antarctic fish.

The gel strength of the chilled gel (control) was similar to that reported by Gómez-Guillén *et al.* (1996) for minced sardine muscle with high gel-forming capacity (Table 3). Likewise, the data for breaking force and breaking deformation were very similar. The slow-frozen lot did not differ significantly from the control in gel strength, breaking force or breaking deformation. The quick-frozen gel again did not differ significantly in breaking deformation, although breaking force was lower than in the control ($P \leq 0.05$). Kato *et al.* (1993) likewise reported no significant variation in the gel strength of commercial scallop analogues frozen at 10°C, but they did find a moderate increase in gel strength of commercial crab analogues frozen at −20°C.

After freezing there was a significant decrease in the hardness and cohesiveness of the slow-frozen gel; these parameters were not significantly different in the fast-frozen gel and the control. Kato *et al.* (1993), on the other hand, reported a slight increase in gel strength as a result of freezing. Penetration and compression testing are different rheological techniques which do not always correlate well (Burgarella *et al.*, 1985; Lee & Chung, 1989. Slow-freezing appears to cause some structural damage to gel proteins, which lose part of their water-binding ability. Rate of freezing and storage temperature are both factors that influence the formation of ice

Table 3. Influence of freeze temperature on rheological properties, water-holding capacity (WHC) and TBA (μmol MA per 100 g) index of gels

	WHC (%)	TBA index	Gel strength (N×mm)	Breaking deformation (mm)	Breaking force (N)	Hardness (N)	Cohesiveness	Elasticity (%)
Refrigeration (control)	93.20 ± 1.24a	1.03 ± 0.02a	67.43 ± 11.90a	13.61 ± 0.15a	4.92 ± 0.78a	31.22 ± 0.19a	0.725 ± 0.01a	13.40 ± 0.15a
−40°C	91.56 ± 1.49ab	1.22 ± 0.31ab	50.85 ± 5.01b	13.85 ± 1.07a	3.67 ± 0.09b	30.22 ± 0.94a	0.738 ± 0.023a	12.61 ± 0.19b
−18°C	90.53 ± 0.64b	1.37 ± 0.13b	61.10 ± 1.84a	14.76 ± 0.53a	4.583 ± 4.14b	23.78 ± 4.14b	0.695 ± 0.01b	11.80 ± 0.35c

Significant ($P \leq 0.05$) differences for the same column are indicated by different letters.
MA, malonaldehyde.

crystals (Shenouda, 1980; Sikorsky *et al.*, 1976). Slow-freezing favours the formation of ice crystals and the enlargement of their diameter (Fennema *et al.*, 1973). The portion of the water that was shed formed small ice crystals. When the gel was thawed, these caused a slight loss of the network's structural integrity, which was appreciable as a decline in textural parameters as measured by the compression test. The penetration test, on the other hand, registered no significant changes, probably because there was insufficient freezing-induced protein aggregation to cause rheological changes.

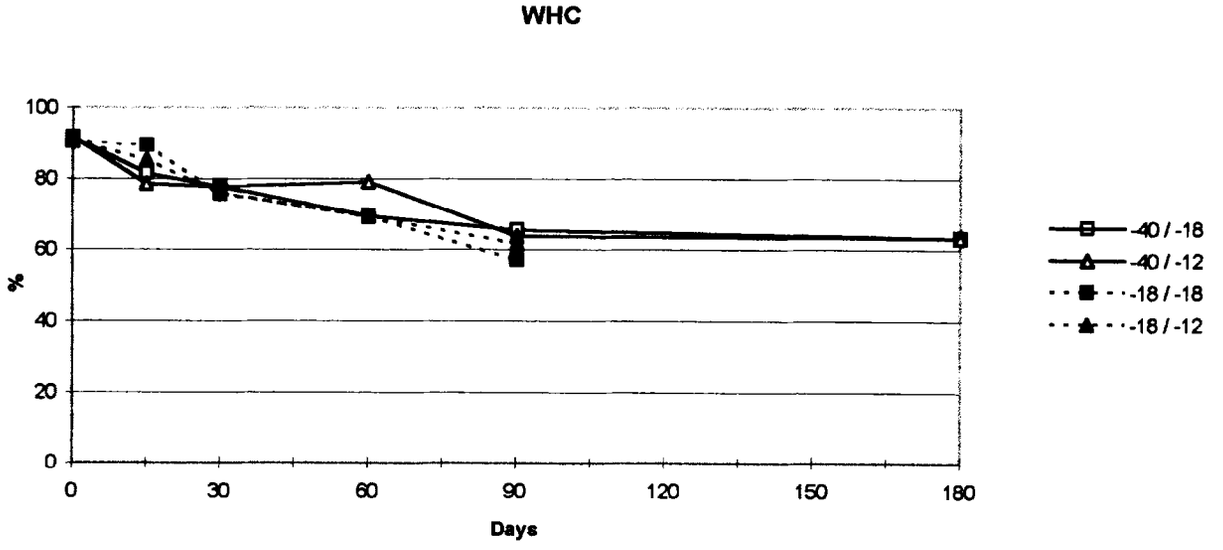
As regards elasticity (relaxation test), the control values were similar to those reported by Gómez-Guillén *et al.* (1996). Upon freezing, there was a significant decrease in both slow- and quick-frozen gels.

Effect of storage temperature

Figure 1 shows the WHC of the various gels over the frozen storage period.

The evolution of WHC was very similar in all lots, irrespective of freezing and storage temperatures. There was a marked downward tendency in WHC during the first 30 days of storage. From that point until 180 days of storage, there were no significant changes in WHC of quick-frozen gels regardless of storage temperature. After 90 days, slow-frozen gels were found to contain large numbers of ice crystals which undermined their structural integrity, and it was therefore decided to discontinue analysis of these gels. Kato *et al.* (1993) reported a pronounced increase in free drip in commercial crab and scallop analogues over the first 6 months of storage at −10°C. These authors found either no changes or only very slight changes in samples frozen at 35°C. In the present case, the WHC did not differ significantly in gels stored at different temperatures. In slow-frozen gels, however, there was considerable formation of ice crystals during the early days of storage, which did not occur in quick-frozen gels. In order to determine the WHC, the sample must be completely thawed; this means the elimination of considerable quantities of water, which are not taken into account since the purpose is to measure the WHC of the gelled proteins. This could be the reason why the various lots exhibited no significant differences in WHC.

Figure 2 shows the evolution of rancidity as determined by the TBA index. This was generally less stable in gels stored at −12°C than in those stored at −18°C. TBA was constant throughout storage ($P \leq 0.05$) in the quick-frozen gel stored at −18°C. In all other lots there were slight fluctuations in the early stages of storage, and by 90 days TBA had increased considerably. This increase was most pronounced in slow-frozen gels stored at −12°C, followed by quick-frozen gels stored at 12°C. This suggests that the increase in TBA over the storage period was influenced by both freezing rate and



Lots	0	15	30	60	90	180
-40 / -18	a/1	ab/1	b/1	b/1	b/1	b/1
-40 / -12	a/1	b/1	b/1	b/1	c/1	c/1
-18 / -18	a/1	a/1	b/1	cb/1	c/1	-
-18 / -12	a/1	a/1	ab/1	bc/1	c/1	-

Fig. 1. Water-holding capacity (WHC) of gels during frozen storage at −18°C (−40°C/−18°C; −40°C/−12°C) and at −12°C (−18°C/−18°C; −18°C/−12°C). Different letters in the same row indicate significant differences ($P \leq 0.05$) between the controls and each lot. Different numbers in the same column indicate significant differences ($P \leq 0.05$) between the lots and each control.

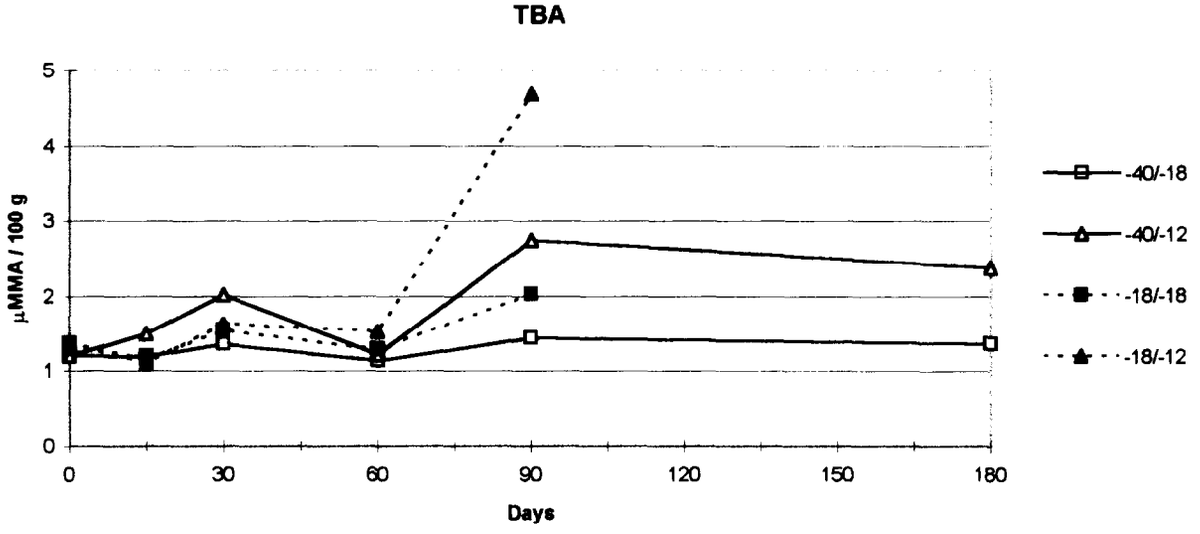
storage temperature. From 90 days until the end of the storage period, TBA did not vary in quick-frozen gels, irrespective of storage temperature. The recorded levels of rancidity were within the ranges reported for other species with comparable fat content, and in no case was rancidity appreciable to the senses (Huidobro *et al.*, 1995). The TBA value correlated well with extractability of salt-soluble protein and texture in fatty and lean herring (Kolakowska *et al.*, 1992).

Figure 3 shows gel strength, breaking force and breaking deformation of the various gels during storage at −18°C and −12°C. Evolution of these parameters was more uniform throughout the storage period in quick-frozen gels, irrespective of storage temperature. Up to 90 days, gel strength exhibited a slight downward tendency ($P \leq 0.05$), and breaking deformation a much sharper one ($P \leq 0.05$). From that point until the end of storage, gel strength remained constant. In the two quick-frozen lots, breaking deformation increased significantly and breaking force remained practically constant.

The slow-frozen gels did appear to be affected by storage temperature; gel strength values were least stable in those stored at −12°C, as evidenced by fluctuations up to 60 days, at which point breaking deformation was particularly affected. There was a general increase in gel strength, breaking force and breaking deformation over the first 30 days of storage. This could have been due to the fact that most of the ice crystal formation took place during this period, only growth taking place for the remainder. Crystal formation is caused by the removal of heat by freezing and, in the process, ice crystals are formed from water bound to proteins. Dehydration of the proteins forming the protein network, which lose their water-binding ability, favours crystal formation and also protein aggregation, which increases the density of the protein matrix and hence breaking force (Sikorsky & Kolakowska, 1994). This effect was most apparent in the slow-frozen gel stored at −12°C. Kato *et al.* (1993) also reported a considerable increase in gel strength in commercial crab analogues during 2 months of storage at −10°C, and a more moderate increase in the same products stored at 20°C. In the present case, however, there was a significant decrease in breaking deformation from day 30 to day 90, except for the slow-frozen gel stored at −12°C, for which this value was similar to that of the first month. Breaking force values generally fluctuated considerably, probably due to the difficulty of rheological analysis resulting from large cavities left by ice crystal formation.

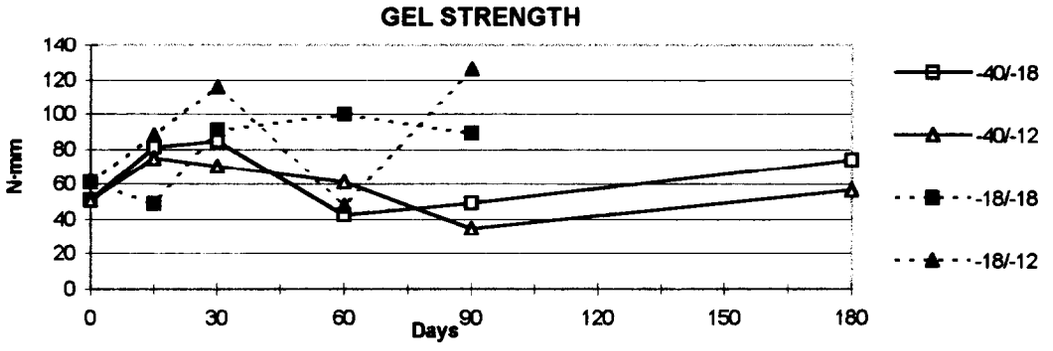
In any event, values were lower and more constant over the storage period in quick-frozen than in slow-frozen gels irrespective of storage temperature, which suggests that freezing temperature is a decisive factor in subsequent behaviour of gels in frozen storage.

Figure 4 shows hardness, cohesiveness and elasticity of gels over storage at −18°C and −12°C. Evolution of

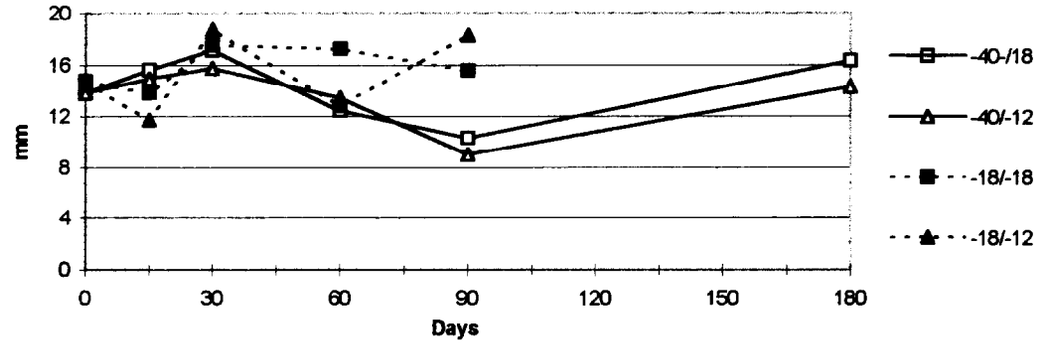


Lots	0	15	30	60	90	180
-40/-18	ab/1	a/1	b/1	a/1	b/1	b/1
-40/-12	a/1	b/2	c/2	a/1	d/1	cd/2
-18/-18	a/1	b/1	c/3	a/2	d/3	-
-18/-12	a/1	b/1	c/3	ac/3	d/4	-

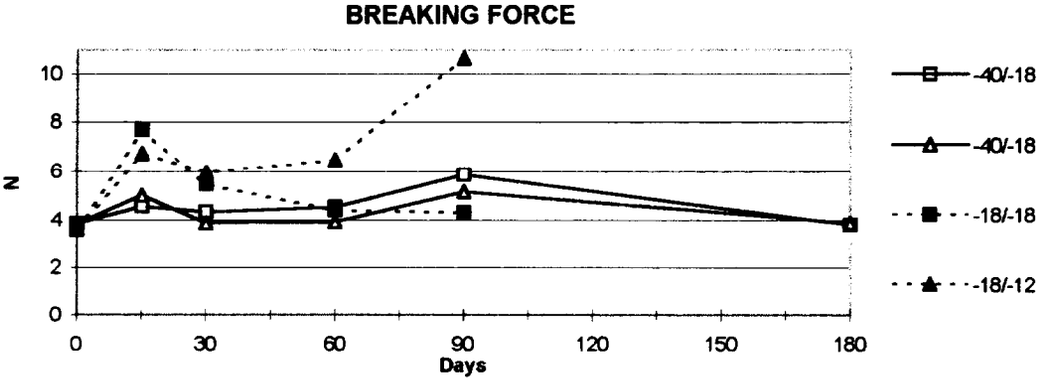
Fig. 2. TBA (as malonaldehyde) of gels during frozen storage at −18°C (−40°C/−18°C; −40°C/−12°C) and at −12°C (−18°C/−18°C; −18°C/−12°C). Different letters in the same row indicate significants differences ($P \leq 0.05$) between the controls and each lot. Different numbers in the same column indicate significant differences ($P \leq 0.05$) between the lots and each control.



Lots	0	15	30	60	90	180
-40/-18	ab/1	a/1	a/1,2	b/1	ab/1	a/1
-40/12	a/1	a/1	a/1	a/1	a/1	a/1
-18/-18	a/1	a/1	b/1,2	b/2	b/2	
-18/-12	a/1	b/2	c/2	a/1	c/3	

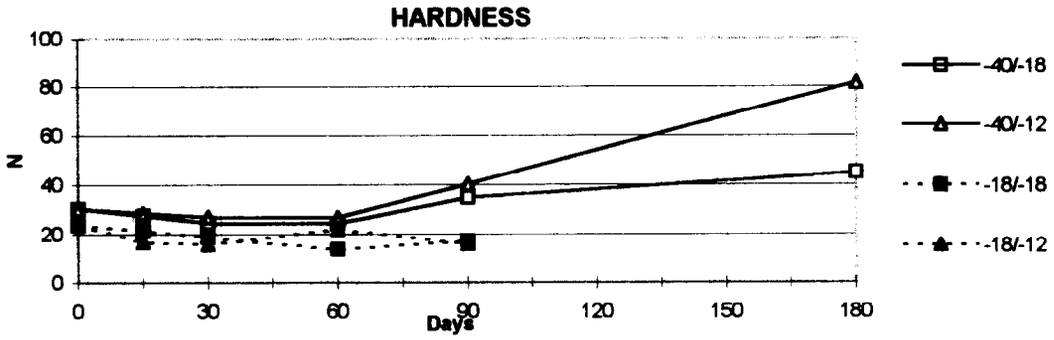


Lots	0	18	30	60	90	180
-40/-18	a/1	ab/1	b/1,2	ac/1	c/1	ab/1
-40/-12	a/1	ab/1	a/1,2	b/1	c/1	ab/2
-18/-18	a/1	a/1	b/1	b/2	ab/2	
-18/-12	a/1	b/2	bc/2	a/1	c/3	

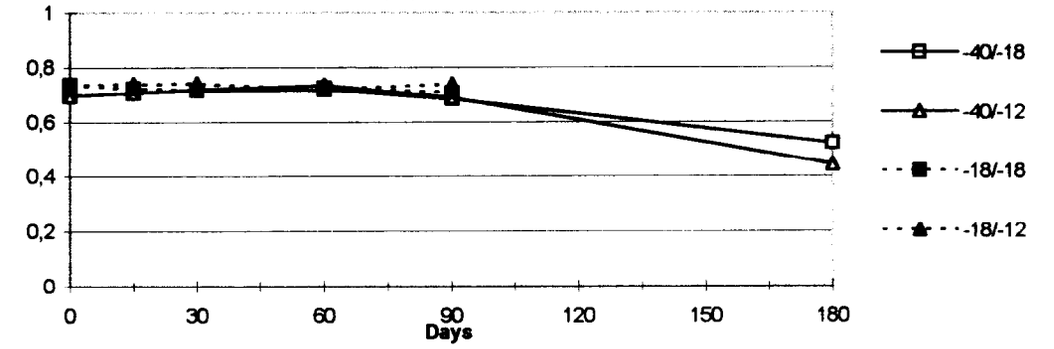


Lots	0	15	30	60	90	180
-40/-18	ab/1	a/1	ab/1,2	b/1	ab/1,2	ab/1
-40/-12	a/1	a/1,2	a/1	a/1,2	a/1	a/1
-18/-18	a/1	a/2	a/1,2	a/2	a/2	
-18/-12	a/1	b/3	c/2	a/1	c/2	

Fig. 3. Gel strength, breaking deformation and breaking force of gels during frozen storage at −18°C (−40°C/−18°C; −40°C/12°C) and at −12°C (−18°C/−18°C; −18°C/−12°C). Different letters in the same row indicate significant differences ($P \leq 0.05$) between the controls and each lot. Different numbers in the same column indicate significant differences ($P \leq 0.05$) between the lots and each control.



Lots	0	15	30	60	90	180
-40/-18	a/1	a/1	b/1	b/1,3	c/1	d/1
-40/-12	a/1	a/1	a/1	a/1	b/2	c/2
-18/-18	a/2	a/2	b/2	c/2	ac/3	_
-18/-12	a/2	a/2	b/2	a/3	b/3	_



Lots	0	15	30	60	90	180
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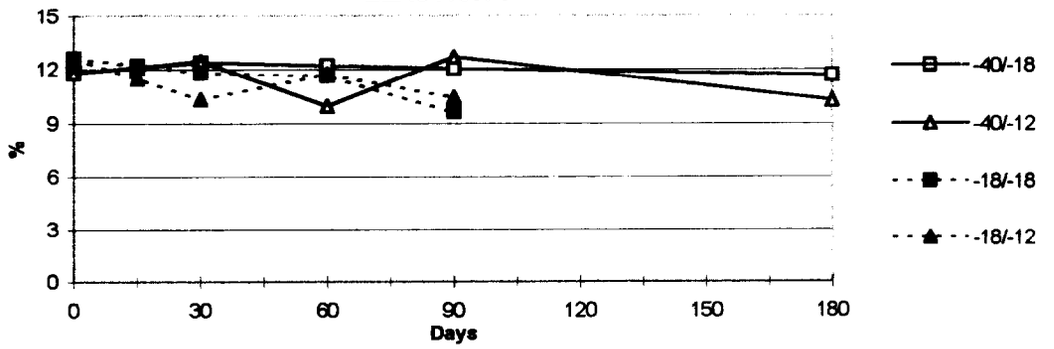
-40/-12	a/1	a/1	b/1	b/1	a/1,2	c/2
-18/-18	a/2	a/2	a/1	a/1	a/2	_
-18/-12	a/2	a/2	a/2	a/1	a/3	_



Lots	0	15	30	60	90	180
-40/-18	a/1	a/1	a/1	a/1	a/1	b/1
-40/-12	a/1	a/1	a/1	b/1	a/1	c/2
-18/-18	a/1	a/1	a/1,2	b/2	b/2	_
-18/-12	a/1	a/1	b/2	a/1	b/2	_

Fig. 4. Hardness, cohesiveness and elasticity of sardine muscle gels during frozen storage at −18°C (−40°C/−18°C; −40°C/−12°C) and at −12°C (−18°C/−18°C; −18°C/−12°C). Different letters in the same row indicate significant differences ($P \leq 0.05$) between the controls and each lot. Different numbers in the same column indicate significant differences ($P \leq 0.05$) between the lots and each control.

hardness was constant and was similar ($P \leq 0.05$) in both quick-frozen lots. As from day 90 of storage, hardness increased significantly, although to a different degree in each lot. After day 90, the increase in hardness of quick-frozen gels was twice that of slow-frozen gels, hardness values being higher in quick-frozen gels throughout the experiment. As noted earlier, slow-freezing caused structural damage to the gel network, resulting in less hardness. Variation in cohesiveness during frozen storage is shown in Fig. 4. Over the first 90 days, the cohesiveness of all gels varied only within a very narrow margin. From day 90 on, cohesiveness dropped sharply in quick-frozen gels, particularly those stored at −12°C (as already noted, and numerous ice crystals were visible to the naked eye in slow-frozen gels). According to Lanier (1986), cohesiveness is the most sensitive parameter for evaluating the state of surimi proteins. Hsieh and Regenstein (1989) reported positive correlations between hardness and cohesiveness. However, other authors (Hamann & MacDonald, 1992) have postulated mutually independent variation of these two parameters. In the present case, hardness increased and cohesiveness decreased from 90 days of storage on, although cohesiveness would appear rather to reflect deterioration of the gel network as a result of frozen storage. Variation in elasticity during frozen storage (Fig. 4) fluctuated very little over the first 2 months, irrespective of freezing rate and storage temperature. By day 90, the slow-frozen gels were significantly less elastic than the quick-frozen gels, with no significant differences in respect of storage temperature. This highlights once again the importance of the temperature at which gels are frozen. From day 90 to day 180, there was no change in the elasticity of the quick-frozen gel stored at 18°C, whereas this parameter decreased ($P \leq 0.05$) in the gel stored at −12°C.

During the first 90 days of storage, the compression parameters (hardness, elasticity and cohesiveness) of the frozen product were mainly influenced by freezing temperature. Differences arising from storage temperature were clearly appreciable only after 180 days.

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