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<b>Authors(s)</b>	Anese, M., Gormley, T. R. (Thomas Ronan)
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# Effects of Dairy Ingredients on some Chemical, Physico-chemical and Functional Properties of Minced Fish during Freezing and Frozen Storage

M. Anese\* and R. Gormley

Teagasc, The National Food Centre, Dunsinea, Castleknock, Dublin 15 (Ireland)

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*A number of dairy ingredients have been selected based on their potential to act as cryoprotectants and have been added to four different fish mince types in order to slow undesired changes in the fish due to freeze/thaw cycles. Changes in fish chemical composition, colour, pH, water-holding capacity (WHC) and texture of fish gels, due to the added dairy ingredients, were investigated. The addition of the dairy ingredients affected the pH and the colour of the fish minces. For almost all of the fish types studied, the lower the fish WHC values, the greater the texture values for the gels.*

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## Introduction

Freezing and frozen storage are used extensively for the preservation of fish and fishery products. Although microbial growth and almost all chemical reactions can be temporarily slowed by low temperature, freezing and frozen storage may be responsible for many chemical and physical changes in fish, which can affect the functional and sensory properties of the products (1). These changes are mainly caused by alterations in fish myofibrillar proteins during frozen storage as a result of the formation of intermolecular cross-linkages, and consequently the aggregation and denaturation of actomyosin (2-5). During freezing, the decrease in the amount of liquid water available to the proteins, as well as the increase in electrolyte concentration and mechanical damage of muscle structures, caused by ice crystal growth are considered to be the main causes of protein denaturation in frozen fish (6-8). The retention of functional properties, in particular gel-forming ability and water-holding capacity (WHC), is important for manufacturing fish-based products.

Extension of shelf-life of fish during frozen storage can be achieved by the incorporation of ingredients (e.g. cryoprotectants) that are able to prevent ice crystal growth and the migration of water molecules from the protein, thus stabilizing the protein in its native form during frozen storage (6). Some cryoprotectants, such as mono- and disaccharides, glycerol, sorbitol, some salts, ascorbic acid, citric acid, carboxymethyl cellulose, gums or their combinations, are satisfactorily used to freeze-preserve fish, and also fruit and poultry (6). The

cryoprotectant effect of some nonfish proteins, for example milk protein derivatives and egg white, in surimi and fish-based products has been reported by several authors (9-15). Although the study on the functional and physico-chemical properties of nonfish proteins proved useful in some cases for predicting gel-forming ability and other functional properties of fish-based products, a good cryoprotectant effect can be obtained only when a synergism between nonfish and fish proteins has been established (10-12).

The objective of the current study was to investigate a number of dairy ingredients, selected on the basis of their potential to act as cryoprotectants, as improvers of the quality of frozen fish mince. Cod and haddock were chosen as examples of low-fat white fish, and salmon and spent salmon as examples of fish with different fat and moisture contents.

## Materials and Methods

### Preparation of minced fish

Frozen fillets of cod (*Gadus morhua*), haddock (*Gadus aeglefinus*), salmon and spent salmon (*Salmo salar*), obtained from fish processors, were used as starting material. Fish fillets were skinned and minced (the drum having 5mm diameter perforations [Kenwood]). To the minced fish, 80g/kg of dairy ingredients were added on a weight basis. The ingredients were powders of lactose (LAC), skim milk (SKI), 90% demineralized whey (DEM), milk protein isolate (MPI), whey (WHE), 355g/kg whey protein concentrate (W35), 815g/kg whey protein concentrate (W80), spray-dried calcium caseinate (CCA) and spray-dried sodium caseinate (NCA), all obtained from Kerry Ingredients

\*To whom correspondence should be addressed. Present address: Dipartimento di Scienze degli Alimenti, University of Udine, Via Marangoni 97, 33100 Udine (Italy).

**Table 1** Moisture and total protein content of ingredients added to minced fish

Ingredient <sup>a</sup>	Moisture (g/kg)	Total protein (g/kg)
LAC	0.6	4
SKI	33.8	377
DEM	15.4	137
MPI	45.5	565
WHE	10.2	142
W35	31.0	355
W80	30.4	815
CCA	40.9	931
NCA	44.0	916

<sup>a</sup>LAC = lactose; SKI = skim milk; DEM = 90% demineralized whey; MPI = milk protein isolate; WHE = whey; W35 = whey protein concentrate, (355g/kg); W80 = whey protein concentrate (815g/kg); CCA = calcium caseinate; NCA = sodium caseinate.

(Listowel, Co. Kerry, Ireland). **Table 1** shows the moisture and protein content of the ingredients used. Each mixture was blended in a Kenwood blender for 3 min at constant rate. The prepared samples were packaged in plastic bags (each package containing about 300 g) and gently pressed in order to obtain low-thickness samples and exclude air bubbles. Samples without dairy ingredients were used as controls (CON). Samples were subjected to three freeze/thaw cycles (accelerated tests). Each freeze/thaw cycle was carried out by freezing samples in an air blast freezer at -35°C for about 2 h and thawing at 3 °C overnight. Freezing and thawing rates were recorded using a Grant Squirrel Datalogger (Grant Instruments Ltd, Barrington, Cambridge, U.K.). Data were then transferred to an expansion unit (Epson HX-20, Epson Corporation, Japan) and plotted.

#### Preparation of gels

Gels were prepared according to the modified method of Hastings (16). Thawed minced samples were mixed with 30 g/kg NaCl in a food blender (Kenwood) for 3 min. Mixtures were then packed into 40 mm diameter polyvinylidene chloride (PVDC) casings, using a hand sausage stuffer. Sausages (about 20 cm long) were cooked in a water-bath at 90 °C for 40 min and cooled in cold running water for 10 min. Samples were stored at 2 °C overnight and were then allowed to equilibrate at room temperature before analysis.

#### Moisture content

Samples were dried at 70 °C and 74.7 mPa (for about 15 h) in a vacuum oven (Edwards High Vacuum B 240, Manor Royal Crawley, Sussex, U.K.) to constant weight. The moisture content was determined by weight difference and expressed as g/kg of initial sample weight. Measurements were made in duplicate, and the difference in results between two determinations carried out on the same sample did not exceed 50 g/kg moisture.

#### Fat content

AOAC Official Method of Analysis (17) was used to determine the fat content of the fish. Analyses were performed in duplicate for each sample, and the difference in results between two determinations carried out on the same sample did not exceed 50 g/kg fat.

#### Total protein

Total protein was determined in duplicate using the well-known Leco FP-428 Determinator System (Leco Corporation, St. Joseph, MI, U.S.A.). This is a micro-processor-based, software-controlled instrument for the determination of nitrogen in a variety of materials. Carefully weighed samples (averaging 0.4 to 0.5 g) were combusted in a hot furnace (950 °C) in the presence of pure oxygen. The gases of combustion were passed through a thermoelectric cooler to remove most of the water. A 10 mL aliquot of the sample mixture was passed through hot copper to remove oxygen, then through Lecosorb (NaOH on non-fibrous silicate carrier) and Anhydrone (MgClO<sub>4</sub>) columns (Leco Corporation, St. Joseph, MI, U.S.A.) to remove carbon dioxide and water, respectively. The remaining nitrogen was measured by a thermal conductivity cell. Before analysis, the instrument was calibrated using EDTA as standard (95.9 g/kg nitrogen). The final result was expressed as g protein per kg initial sample, using a conversion factor of 6.25 for fish and 6.38 for milk derivatives. Measurements were made in duplicate, and the difference in results between two determinations carried out on the same sample did not exceed 10 g/kg protein.

#### pH

The pH of the minced samples was estimated according to the method of Sych *et al.* (18). Samples (5 g) were mixed with 10 mL deionized water (single analysis). The pH was measured with a pH-meter (PHM 82 Standard pH-meter, Radiometer, Copenhagen, Denmark) at room temperature.

#### Colour

Colour measurements were carried out on minced samples using a Hunterlab model D25 Colour Difference Meter (Hunter Associates Laboratory, Inc, Fairfax, VA, U.S.A.). The instrument was standardized against a white tile before each measurement. Colour was expressed in L, a and b Hunter scale parameters, and the a and b parameters were used to compute the hue angle ( $\tan^{-1} b/a$ ) (19,20). Measurements on each sample were made in duplicate, and the difference in results between two determinations carried out on the same sample did not exceed 10% for all the Hunter parameters.

### Water-holding capacity (WHC)

WHC was determined following the modified method of Gormley *et al.* (21). About 3 to 5 g of thawed minced samples was carefully weighed into prepared centrifuge tubes containing approximately 2 cm of glass beads; the beads were supported by a cup-shaped filter-paper thimble. Samples were then centrifuged at 1500 rpm for 10 min at 10 °C (MSE Mistral 3000). The presence of beads allowed the drainage of fluid from each sample during centrifugation. After centrifugation, the samples were removed and reweighed. WHC was expressed as follows:

$$\text{WHC} = 100 - \frac{wl}{wi} \times 100 (\%) \quad \text{Eqn [1]}$$

where *wl* is the weight loss (g) after centrifugation and *wi* the initial weight of the sample.

Measurements were made in triplicate. The coefficients of variation, expressed as the percentage ratio between the standard deviation and the mean value, were less than 5%.

### Compression/recompression and puncture tests

Compression and puncture tests were made on gels following the methods of Hastings (16) and Gormley *et al.* (22). The fish sausage samples were cut into 20 mm lengths, and the cylinders were compressed by 40% of their height using a shear press (ramspeed 4.38 mm/s) fitted with a proving ring and connected, via an amplifier, to a recorder (Philips, mod. PM-8100). The samples were subjected to recompression immediately after compression. Compression and recompression values were expressed in Newtons (N). The cylinders were subsequently puncture-tested using a flat-end probe 12 mm in diameter. The breaking force, expressed in Ns, was measured at the point of rupture of the samples. Five measurements were carried out on each sample. The coefficients of variation were less than 10% for both the compression and puncture tests. Elasticity index was calculated as the percentage ratio of recompression and compression values.

### Calorimetry

Differential scanning calorimetry (DSC) was performed on a Mettler DSC 30 (Mettler, Greifensee, Switzerland). Samples were accurately weighed (approximately 12 mg) and sealed in sample pans. An empty sample pan was used as a reference. Duplicate samples were annealed in order to allow ice crystallization (23): samples were cooled to -50 °C, heated to -25 °C at 5 °C/min, cooled to -50 °C at 10 °C/min, and then scanned from -50 °C to +30 °C at a heating rate of 5 °C/min. Enthalpies ( $\Delta H$ ), expressed as J/g, associated with the melting of water were determined by integrating the area under the DSC curve, using software for thermal analysis (Mettler, Switzerland), and dividing this area by the weight of the sample. A

sigmoidal baseline was used to calculate peak areas to compensate for the change in the specific heat ( $C_p$ ) that occurs when water passes from the solid state to liquid.

### Statistical analysis

Analysis of variance was carried out for each variable for the four fish types. The least significant difference (LSD) was calculated as follows:  $\text{LSD} = \text{s.e.d} \times 2$ ; where s.e.d. is the standard error of the difference between two treatment means.

## Results and Discussion

Average moisture, total protein, fat content and pH values for the four types of fish studied are shown in Table 2. Compared with cod and haddock species, the two types of salmon contained a higher amount of fat, although the content in the spent salmon was only one third that of the high-quality salmon (Table 2); the

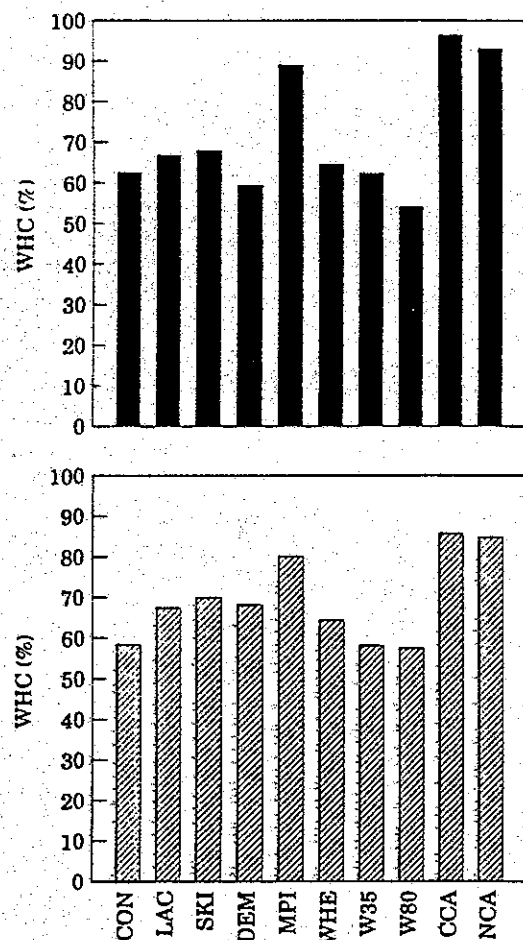


Fig. 1 Water-holding capacity (WHC) values for cod (■) and haddock (▨), with and without added dairy ingredients. CON = control; LAC = lactose; SKI = skim milk; DEM = 90% demineralized whey; WHE = whey, MPI = milk protein isolate; W35 = whey protein concentrate (355 g/kg), W80 = whey protein concentrate (815 g/kg); CCA = calcium caseinate; NCA = sodium caseinate. Differences are statistically significant if the difference between the means are greater than the least significant difference (LSD = 2.4)

higher fat content was balanced by a lower moisture content.

After addition of the dairy derivatives, average moisture ranged from 752.5 to 765.1 g/kg for cod, from 758.4 to 768.7 g/kg for haddock, from 729.5 to 745.7 g/kg for spent salmon and from 673.2 to 696.2 g/kg for salmon.

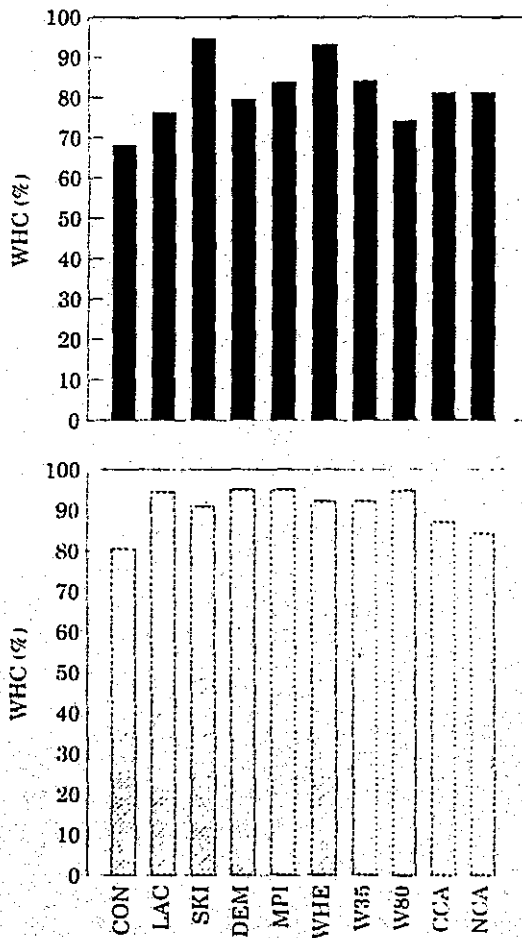


Fig. 2 Water holding capacity (WHC) values for spent salmon (■) and salmon (□) with and without added dairy ingredients. (See Fig. 1 for abbreviations)

Table 3 Hunter L and hue angle values for cod, haddock (Had), spent salmon (SS) and salmon (S), with and without added dairy ingredients

Treatment <sup>a</sup>	L value <sup>b</sup>				Hue angle <sup>c</sup>			
	Cod	Had	SS	S	Cod	Had	SS	S
CON	59.6	57.8	47.7	49.0	93.0	101.1	58.8	49.0
LAC	55.7	56.5	46.4	44.5	93.1	100.6	57.3	45.3
SKI	60.9	61.4	51.4	45.7	94.8	100.4	57.1	46.2
DEM	57.6	57.7	48.0	45.5	96.2	99.9	56.9	44.7
MPI	63.0	61.6	50.4	50.0	95.6	95.3	58.9	50.9
WHE	57.1	57.2	49.2	45.6	99.8	102.0	57.1	44.6
W35	58.9	60.5	48.5	44.8	95.4	101.0	56.9	47.8
W80	58.2	58.9	47.8	47.9	92.7	91.6	58.5	50.8
CCA	58.9	57.6	49.9	50.5	93.1	97.3	53.7	47.0
NCA	60.5	53.8	48.9	49.2	95.0	93.8	52.9	49.8

<sup>a</sup>CON = control; LAC = lactose; SKI = skim milk; DEM = 90% demineralized whey; MPI = milk protein isolate; WHE = whey; W35 = whey protein concentrate. (355 g/kg); W80 = whey protein concentrate (815 g/kg); CCA = calcium caseinate; NCA = sodium caseinate.

<sup>b</sup>LSD: T=0.90, F/T=1.81.

<sup>c</sup>LSD: T=1.88, F/T=3.8.

The differences are statistically significant if the difference between the means are greater than the least significant difference (LSD) for each factor, i.e. T=treatment; F.T=interaction between fish and treatment.

Table 2 Proximate composition and pH for minces of cod, haddock (Had), spent salmon (SS) and salmon (S)

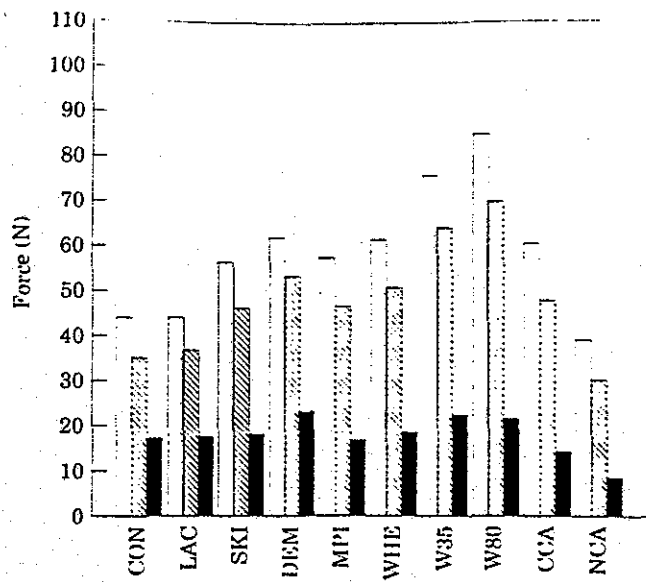
Fish type	Moisture (g/kg)	Total protein (g/kg)	Fat (g/kg)	pH
Cod	777.5	216	5	6.8
Had	781.4	213	5	6.5
SS	781.6	197	21	6.7
S	723.1	215	61	6.6

Depending on their protein content, the dairy ingredients influenced the protein content of the fish samples to a different degree. The minimum and maximum total protein values for dairy ingredient-incorporated cod, haddock, spent salmon and salmon were 177 and 219, 177 and 220, 165 and 224, and 174 and 222 g/kg, respectively.

Addition of dairy ingredients significantly affected the pH of the four fish types ( $P < 0.001$ ). However, differences in fish pH for the different treatments were small, so may not be of practical importance. The minimum and maximum pH values for cod, haddock and spent salmon were observed for the W80 and NCA treatments, respectively, and in the case of salmon for the W80 and SKI.

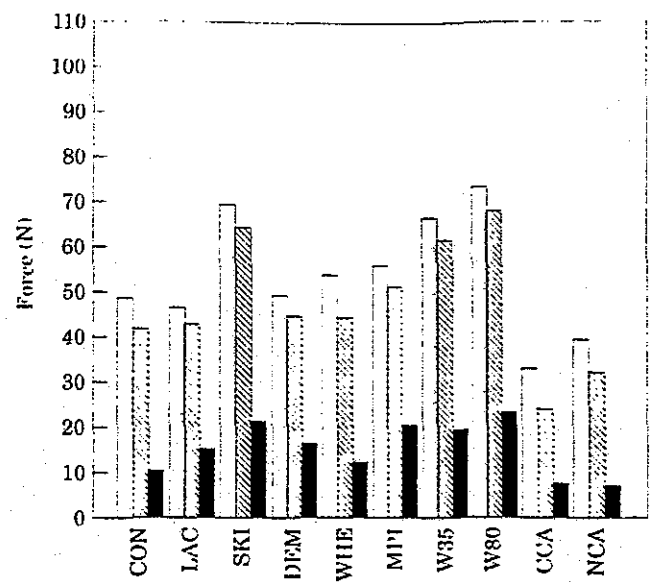
Table 3 shows Hunter L and hue angle values of the four fish types studied. The results for L and hue angle showed that the treatment effects on each of the four fish types were significant ( $P < 0.001$ ). Considering the effect of the addition of the ingredients on cod and haddock separately from that on salmon (because of the different colour of their flesh), a significant interaction between fish and treatment was found ( $P < 0.001$ ).

Figures 1 and 2 show the WHC values for cod and haddock, and salmon and spent salmon, respectively. The addition of the dairy ingredients significantly affected the WHC values for the samples ( $P < 0.001$ ). Cod showed the highest and lowest WHC values with CCA and W80 respectively. In the case of haddock, the



**Fig. 3** Compression (□), recompression (▨) and puncture (■) values for cod gels with and without added dairy ingredients. Gels prepared from fish after three freeze/thaw cycles. CON = control, LAC = lactose, SKI = skim milk, DEM = 90% demineralized whey, WHE = whey, MPI = milk protein isolate, W35 = whey protein concentrate (355 g/kg), W80 = whey protein concentrate (815 g/kg), CCA = calcium caseinate, NCA = sodium caseinate. Differences are statistically significant if the difference between the means is greater than the least significant difference (LSD). LSD = 3.5 for compression and recompression; LSD = 0.75 for puncture.

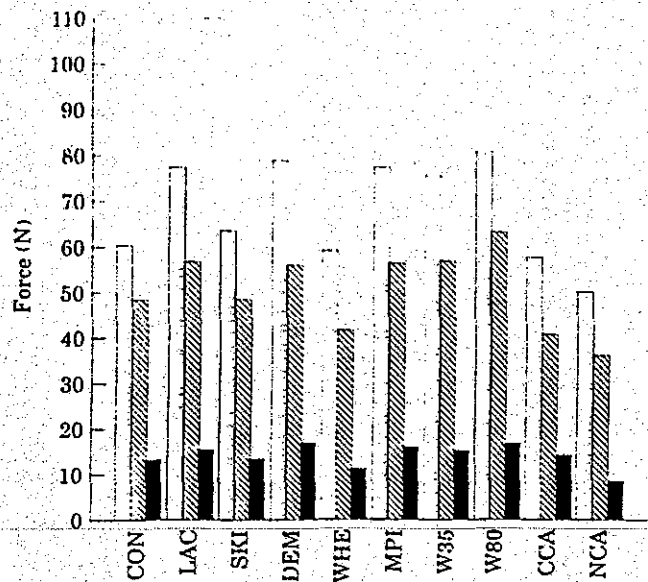
highest WHC values were found for CCA or NCA treatments (LSD < 2.4) and the lowest for W80 or W35 treatments (LSD < 2.4). Both types of salmon presented the lowest WHC value when no ingredients were added, while the highest values were obtained with the incorporation of SKI or WHE (LSD < 2.4) in



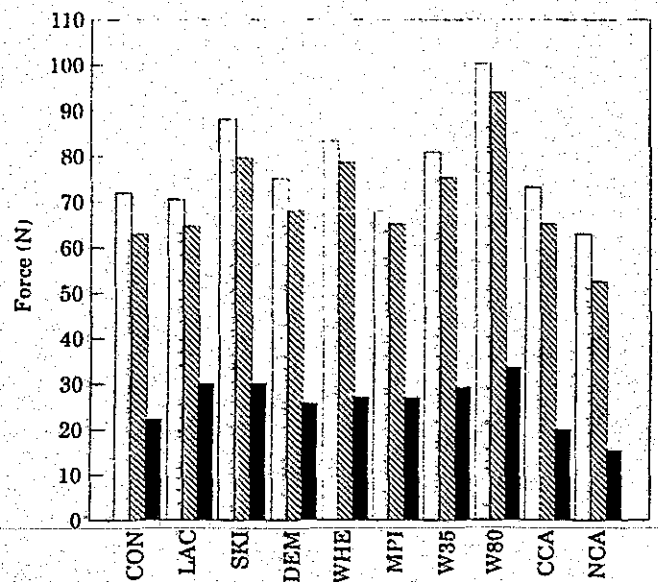
**Fig. 5** Compression (□), recompression (▨) and puncture (■) values for spent salmon gels with and without added dairy ingredients. Gels prepared from fish after three freeze/thaw cycles. (See Fig. 3 for abbreviations)

the case of the spent salmon, and LAC, DEM, MPI or W80 (LSD < 2.4) for salmon.

Figures 3, 4, 5 and 6 show compression, recompression and puncture data for the fish-based gels. Haddock had greater compression, recompression and puncture values than did cod in the case of added LAC, SKI, DEM, MPI and NCA (Figs 3 and 4). Comparing Figs 5 and 6, it can be observed that the force values for salmon were higher than those for spent salmon. This can be attributed to the different quality of the starting materials. For each of the four fish types considered, the W80-incorporated fish gels broke at significantly higher ( $P < 0.001$ ) compressive forces than did the control fish



**Fig. 4** Compression (□), recompression (▨) and puncture (■) values for haddock gels with and without added dairy ingredients. Gels prepared from fish after three freeze/thaw cycles. (See Fig. 3 for abbreviations)



**Fig. 6** Compression (□), recompression (▨) and puncture (■) values for salmon gels with and without added dairy ingredients. Gels prepared from fish after three freeze/thaw cycles. (See Fig. 3 for abbreviations)

**Table 4** Elasticity index values, expressed as the ratio of recompression and compression values  $\times 100$ , for fish-based gels. For each measurement, the standard deviation values are reported in parentheses

Treatment <sup>a</sup>	Elasticity index (%)			
	Cod <sup>b</sup>	Had <sup>b</sup>	SS <sup>b</sup>	S <sup>b</sup>
CON	81.5ad(4.5)	81.0a (4.2)	86.5a(2.1)	87.5a(2.1)
LAC	83.0ac(0.8)	73.5b (0.7)	92.0b(0.2)	91.5b(0.7)
SKI	82.0ac(1.5)	76.0c (0.2)	92.5b(0.7)	91.0b(0.2)
DEM	86.0b (0.3)	70.5d (2.1)	91.5b(0.7)	90.0b(1.4)
MPI	81.0ad(0.2)	70.5d (2.1)	81.0c(2.8)	94.0c(2.8)
WHE	82.5ac(0.5)	73.0b (1.4)	91.5b(0.7)	95.0c(2.8)
W35	84.0bc(0.6)	76.0c (0.2)	92.5b(0.7)	93.5c(0.7)
W80	81.1ad(0.9)	79.0a (1.4)	91.5b(1.0)	94.0c(0.2)
CCA	79.0de(0.2)	71.0bd(1.4)	80.5c(0.7)	89.5a(0.7)
NCA	77.0e (4.7)	72.5bd(2.1)	72.5e(4.8)	83.5d(3.5)

<sup>a</sup>CON = control; LAC = lactose; SKI = skim milk; DEM = 90% demineralized whey; MPI = milk protein isolate; WHE = whey; W35 = whey protein concentrate, (355 g/kg); W80 = whey protein concentrate (815 g/kg); CCA = calcium caseinate; NCA = sodium caseinate.

<sup>b</sup>Had = haddock; SS = spent salmon; S = salmon.

Means with the same letter within a column are not significantly different ( $P>0.05$ ).

gels. The NCA-added cod, haddock and salmon gels and the CCA-incorporated spent salmon gels required significantly lower forces to break than did the control gel ( $P<0.001$ ). The force required for recompression of samples showed a trend similar to that of compressive force. Also, the force required for penetration of samples was significantly affected by treatments ( $P<0.001$ ). The lowest breaking force was for the NCA-incorporated gels, and the highest for the DEM-added gel in the case of cod and haddock, and for the W80-incorporated gel in the case of both salmon types. Table 4 shows the elasticity index values for the various gels. They were computed as the percentage ratio of recompression and compression values and show the springiness of the gels.

From these results, it seems that, for almost all the fish types studied, the lower the fish WHC values, the greater the compressive and penetration forces for the gels. The reason may be that ingredients giving a low

**Table 5** Enthalpies (J/g), percentage of unfrozen water and percentage of water available for hydration of ingredient-incorporated cod samples. For enthalpy measurements, the standard deviation values are reported in parentheses

Treatment <sup>a</sup>	Enthalpy (J/g)	Unfrozen water (%)	Hydration water (%)
CON	229.9 (4.9)	8.9	40.1 (7th) <sup>b</sup>
SKI	198.6 (1.3)	12.8	46.1 (5th)
DEM	190.3 (5.3)	15.1	54.2 (3rd)
MPI	194.6 (1.5)	14.1	50.9 (4th)
W80	199.2 (2.5)	12.6	45.3 (6th)
CCA	169.7 (3.8)	21.5	77.5 (1st)
NCA	174.4 (5.7)	20.1	72.6 (2nd)

<sup>a</sup>CON = control; SKI = skim milk; DEM = demineralized whey (90%); MPI = milk protein isolate; W80 = whey protein concentrate (815 g/kg); CCA = calcium caseinate; NCA = sodium caseinate.

<sup>b</sup>Rank order.

fish WHC could facilitate extensive cross-linking of proteins, thereby leading to a firmer gel (14,24). Conversely, proteins, i.e. caseinates, with a very good WHC, when incorporated into fish, may distribute so finely throughout the matrix that they cause a dilution of minced fish, resulting in a weakening of the gel (10,12,14). The dilution effect of some of the dairy ingredients studied can be attributed to a strong protein-water interaction due to the hydration ability of these materials. Table 5 shows the enthalpies (J/g) of melting of water, the percentage of unfrozen water surrounding the proteins and the percentage of water available for hydration of fish proteins of ingredient-incorporated minced cod. The caseinates gave a much higher hydration value than did MPI, SKI, DEM or W80, which in turn had higher hydration values than the control sample. Moreover, it must be pointed out that, among the dairy ingredients studied, W80 had the lowest hydration value (as well as the lowest WHC value and the highest compression value).

The formation of protein-ingredient complexes, mainly through the formation of hydrogen bonds, could help to prevent fish protein aggregation and thereby inhibit denaturation during freezing and frozen storage (25). In addition, the results suggest that water for hydration, linked to the fish-dairy ingredient matrix, contributes to both the formation of an unfrozen zone surrounding the proteins (6,26) and the prevention of the migration of water molecules from the proteins. According to this hypothesis and the experimental evidence, the protection of fish proteins against freezing damage can likely be attributed to a cryoprotective effect of some of the ingredients studied, for example caseinates (27,28).

## Conclusions

The addition of dairy ingredients significantly affected the chemical composition, pH, colour, WHC and the gel texture of the four fish types. In particular, a relationship between WHC, compression and penetration forces and the amount of unfrozen water surrounding fish proteins was found. In general, the dairy ingredients (e.g. caseinates) that gave the lowest compression values had the highest WHC and hydration values, and *vice versa*. This can be attributed to the formation of a protein-ingredient complex, through hydrogen bonds, that may prevent protein-protein aggregation and the migration of water from the proteins during freezing and frozen storage. Although more research is needed, a possible application of those dairy ingredients that are effective in preventing protein denaturation could be their addition to formulated fish-based products as improvers of the quality of the frozen fish.

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