

# Cryoprotective Effects of Lactitol, Palatinit and Polydextrose® on Cod Surimi Proteins during Frozen Storage

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## ABSTRACT

The cryoprotective effects of lactitol dihydrate, Polydextrose® and Palatinit (Isomalt) at 8% w/w in cod-surimi were compared to an industrial control containing a sucrose/sorbitol 1:1 mixture and a control without additive. Surimi was stored at  $-20^{\circ}\text{C}$  for 12 wk and examined for freeze-induced protein changes every 2 wk by salt extractable protein and differential scanning calorimetry analyses: Palatinit®, lactitol and Polydextrose® stabilized surimi proteins equally well as did the sucrose/sorbitol mixture. Salt extractable protein and myosin peak enthalpy for surimi were maintained at the same level as the industrial control. Confirming earlier results, initial  $T_{\text{max}}$ -myosin yielded information regarding surimi protein stability over extended periods of frozen storage.

## INTRODUCTION

DURING FREEZING STORAGE of fish muscle, alterations in fish myofibrillar proteins have been largely accepted as the principal cause of loss of protein functional properties (Shenouda, 1980). The retention of these functional properties, namely gel-forming ability and water holding capacity of actomyosin, are essential for manufacturing surimi-based analogs (Lee, 1984).

Extended frozen storage of surimi is made possible by the incorporation of sucrose and sorbitol which have been shown to be effective in inhibiting fish protein denaturation (Noguchi, 1974; Akahane, 1982) and by polyphosphates which also have been shown to induce stabilization of myosin (Park and Lanier, 1987; Noguchi and Matsumoto, 1971). However, the excessive sweet taste in final surimi products, resulting from the addition of sucrose and sorbitol at 4%, 4%, respectively (Lee, 1984) has received some criticism (Noguchi et al., 1975c; Park et al., 1987). Recently, interest has focused on identifying other cryoprotectants with reduced or without sweetness for use in surimi (Park et al., 1988; Park and Lanier, 1987; Noguchi and Matsumoto, 1970, 1971, 1975a,b).

The cryoprotective effects of lactitol and Palatinit® on surimi during frozen storage have not previously been examined. Lactitol and Palatinit® are bulk sweeteners or polyols derived from carbohydrates whose carbonyl group has been reduced to a hydroxyl group. Lactitol (D-galactosyl- $\beta(1 \rightarrow 4)$ -D-glucitol) is produced by catalytic hydrogenation of lactose (Ziesenitz and Siebert, 1987). Palatinit® is produced from sucrose and is an equimolar mixture of D-glucosyl- $\alpha(1 \rightarrow 6)$ -D-glucitol and D-glycosyl- $\alpha(1 \rightarrow 1)$ -D-mannitol (Sträter, 1986). As additives, lactitol and Palatinit® present several attractive properties including reduced caloric value of approximately 50%, relative sweetness of 0.40 and 0.50, respectively, compared to sucrose, essentially non-cariogenic and well tolerated by diabetics (Ziesenitz and Siebert, 1987; Den Uyl, 1987).

Palatinit® has passed a complete toxicological assessment

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(Sträter, 1986) and has been submitted for GRAS status approval in the United States. Its use has been authorized in England and Switzerland since 1983 (Bollinger, 1985). The required toxicity studies for a food additive petition for lactitol have been carried out and results appear positive. Lactitol has been identified by the Scientific Committee on Food in the EEC as a safe product with the remark that laxative activity may be observed at high intakes (Den Uyl, 1987). The joint expert committee on Food Additives of FAO/WHO (JECFA) allocated an acceptable daily intake of "not specified" (Den Uyl, 1987). Food additive petitions for lactitol have been filed in the United States, England and most European countries (Booy, 1987).

Polydextrose® is a highly branched polysaccharide prepared by the thermal polymerization of glucose (Park et al., 1987). The use of this nonsweet bulking additive as a cryoprotectant in muscle foods has been patented (Lanier and Akahane, 1986). Recently, Park et al. (1988) reported that it could substitute sucrose/sorbitol (at the same level, 8% w/w) in surimi without alteration of cryoprotective effects. Polydextrose® has been approved by FDA for use in reduced or low calorie foods.

The identification of low or nonsweet cryoprotectants for surimi would be beneficial to the surimi industry. The present study compared the cryoprotective effects of lactitol, Palatinit® and Polydextrose® to an industrial control containing sucrose/sorbitol and a control without additive, on cod-surimi proteins during frozen storage.

## MATERIALS & METHODS

### Production of experimental surimi

Cod fillets (*Gadus morhua*), originating from the Gaspé région (Quebec, Canada), were purchased fresh from "Pêcheries St. Laurent" (Quebec, Canada). Surimi preparation was based on the technology described by Lee (1984). As detailed in Sych et al. (1990), the following steps were carried out at a temperature of less than  $10^{\circ}\text{C}$ : (1) mincing fillets (4 mm); (2) washing mince 3 times with cold water (pH 7.0) at 1:3 mince: water, with the final wash containing 0.2% NaCl; (3) dewatering by filtering through a plastic cheese cloth; (4) final dewatering in a basket centrifuge. Final surimi (83.4% moisture) was divided into experimental units and the following treatments assigned at random: T1—control—no additive; T2—8% w/w sucrose/sorbitol 1:1 mixture (sucrose-Lantic, sorbitol-Atkemics, Montreal, Canada); T3—8% w/w Polydextrose (Polydextrose® K, neutralized to pH 7.0 with potassium bicarbonate, Pfizer Canada, Inc., Quebec, Canada); T4—8% w/w Palatinit® (Palatinit Gmbh, Obrigheim/Pfalz, West Germany); T5—8% w/w lactitol (lactitol dihydrate, CCA biochem b.v., Gorinchem, Holland).

Final surimi for treatments T2 to T5 contained 8% w/w of the respective additives. Samples were vacuum-packaged, rapidly frozen at  $-34^{\circ}\text{C}$  and stored at  $-20 \pm 2^{\circ}\text{C}$  for 12 weeks. The entire experiment was repeated. Freeze denaturation of proteins was evaluated every 2 weeks by salt extractable protein and differential scanning calorimetry analyses. For the initial storage period, these parameters were measured prior to freezing of surimi samples.

### Moisture content, total protein and pH of surimi samples

Moisture content determination of surimi was similar to the AOAC (1980) method for meat products. Eight grams of surimi was dried in

Table 1—Average moisture and total protein of experimental surimi

Treatments	Moisture (%) <sup>a</sup>		Total protein nitrogen (%) <sup>c</sup>	
	R1 <sup>b</sup>	R2	R1	R2
T1-control	83.7	83.2	16.3	16.8
T2-8% sucrose/sorbitol	76.5	76.7	14.5	14.4
T3-8% Polydextrose®	77.0	76.1	14.7	15.8
T4-8% Palatinit®	76.4	75.9	14.4	14.9
T5-8% lactitol	77.1	77.3	15.1	14.7

<sup>a</sup> Reported values are averages of triplicate analyses,  $S_x = 0.31\%$

<sup>b</sup> R1, R2, repetitions 1 and 2

<sup>c</sup> Reported values are averages of duplicate analyses,  $S_x = 0.36\%$

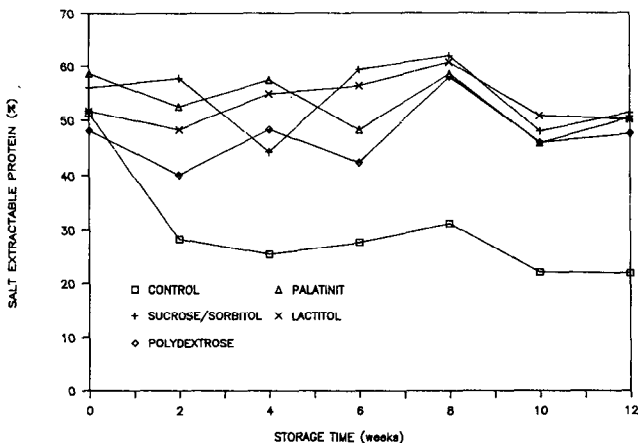


Fig. 1.—Changes in salt extractable protein of cod surimi as a function of storage time at  $-20^{\circ}\text{C}$  for the various experimental treatments: T1 - control, T2 - 8% sucrose/sorbitol, T3 - 8% Polydextrose®, T4 - 8% Palatinit®, T5 - 8% lactitol, ( $S_x = 6.7\%$ ).

a vacuum oven ( $\leq 13.3$  KPa) to constant weight at  $95\text{--}100^{\circ}\text{C}$  in triplicate. Total protein was determined on 500 mg samples of surimi using the Kjeldahl method (AOAC, 1980; Kjeld-Foss automatic, model 16210), with a conversion factor of 6.25. The pH of surimi was estimated by mixing 5 g sample with 10 mL of deionized water (duplicate analyses). The pH was measured with a glass electrode (Fisher glass-body combination electrode) at room temperature (Poulter and Lawrie, 1977).

#### Salt extractable protein (SEP)

Salt extractable protein was carried out following the method of Dryer et al. (1950). Duplicate ten g samples of surimi were extracted overnight at  $0^{\circ}\text{C}$  in 290 mL of 5% KCl in 0.08M Tris maleate (pH 7.2). The Bio-Rad Protein Assay (Bio-Rad Laboratories) was used to estimate protein concentration in the resulting supernatants (duplicate analyses), using bovine albumin as a protein standard. Percent salt extractable protein (SEP) was expressed as the ratio of the quantity of salt extractable protein, estimated by Bio-Rad analysis, to that of total protein content. More details on the above analyses are reported in Sych et al. (1990).

#### Heat-induced denaturation

Differential scanning calorimetry was performed on a DuPont 9900 Differential Scanning Calorimeter equipped with a 910 DSC cell. Duplicate samples of ca 15 mg ( $\pm 0.1$  mg) were sealed in volatile sample pans and scanned over the range  $10\text{--}100^{\circ}\text{C}$  at a heating rate of  $10^{\circ}\text{C}/\text{min}$ , using water as a reference (ca 15 mg). Enthalpies ( $\Delta H$ ) (Joules/g protein), associated with the denaturation of proteins, were determined by measuring the area under the DSC curve, using the General Analysis Program (Anonymous, 1986) and by dividing these areas by the total protein content of the scanned samples.

A sigmoidal baseline was used to calculate the area of the first transition to compensate for the change in baseline. A linear baseline was normally used for the second transition. Peak maximum temperatures ( $T_{\text{max}}$ ) were used to describe the denaturation temperatures of proteins.

#### Statistical analysis

Statistical analysis was carried out using the Statistical analysis System (SAS, 1985). For each variable studied, an overall analysis of variance (ANOVA) was carried out on all data, as well as on treatment means at each storage period. When significant differences were found ( $P \leq 0.05$ ), a Waller-Duncan k-ratio t-test using k-ratio 100:1 which approximates  $\alpha = 0.05$  (Steel and Torrie, 1960), or Duncan's New Multiple Range test ( $P \leq 0.05$ ) was used to compare treatment means at each storage interval. Regression analysis was carried out on storage time means to test for significant variations as a function of storage time. Correlation analysis was carried out among all variables.

## RESULTS & DISCUSSION

#### Evaluation of experimental surimi

Average moisture and total protein (%) of the experimental treatments for each repetition are shown by Table 1. Surimi had an average moisture content of 83.4% before addition of additives. After addition of additives, average moisture ranged from 76.1% to 77.3%. Moisture of industrial surimi without cryoprotectants should be below 85% with high-grade surimi processed on factory ships, containing 82% moisture (Lee, 1985). Total protein was 16.6% for control surimi.

Initial pH of surimi was  $7.0 \pm 0.01$  for all treatments and these values did not significantly vary when measured after 12 wk of frozen storage at  $-20^{\circ}\text{C}$ .

#### Salt extractable protein % (SEP)

The change in surimi SEP for each treatment as a function of frozen storage at  $-20^{\circ}\text{C}$  over 12 wk is shown in Fig. 1. These values are the means of 2 repetitions. Generally, SEP for control surimi decreased rapidly early during frozen storage, while SEP for surimi treated with 8% sucrose/sorbitol (T2), 8% Polydextrose® (T3), 8% Palatinit® (T4) and 8% lactitol (T5) remained relatively stable throughout frozen storage.

An overall ANOVA on SEP data showed that the treatment and storage time effects were significant ( $P \leq 0.001$ ), accounting for 60.2% and 12.6%, respectively, of the total variation in SEP. Regression analysis showed that SEP for control samples (T1) decreased significantly as a function of storage time ( $P \leq 0.01$ ). The largest decrease occurred after 2 weeks, since SEP decreased from 51.3% to 28.3%. At 4 weeks frozen storage, a Waller-Duncan test (k-ratio = 100) showed that the SEP mean for T1 was significantly different from means for T2, T3, T4 and T5.

This rapid decrease in protein extractability during frozen storage has been noted for other fish muscle system (Jiang et al., 1987; Krivchenia and Fennema, 1988; Gill et al., 1979). Similarly, Sych et al. (1990) reported a 47.5% decrease in SEP of cod surimi without cryoprotectants, occurring mainly at 0–6 wk of frozen storage at  $-20^{\circ}\text{C}$ . Generally, surimi SEP was initially higher for all samples in the present study, in the range 51.3%–54.1%, whereas in Sych et al. (1989), surimi SEP was in the range of 38.7%–45.6% for samples treated with carbohydrate and/or polyols at equivalent levels of addition.

Palatinit-treated samples showed higher SEP than Polydextrose®- and lactitol-treated samples, but only until 4 weeks frozen storage (Fig. 1). Throughout the entire storage period, SEP for lactitol-treated samples remained slightly higher than SEP for Polydextrose®-treated samples. However, Duncan tests at each storage period did not indicate significant differences among T2, T3, T4 and T5 treatment means ( $P \leq 0.05$ ). After 12 weeks of frozen storage at  $-20^{\circ}\text{C}$ , SEP for treatments T2, T3, T4 and T5 was only slightly lower than initial values of SEP (Fig. 1). Regression analysis showed that SEP for these treatments did not significantly vary with storage time.

Salt extractable data suggested that Polydextrose®, Palatinit® and lactitol protected cod surimi proteins equally well

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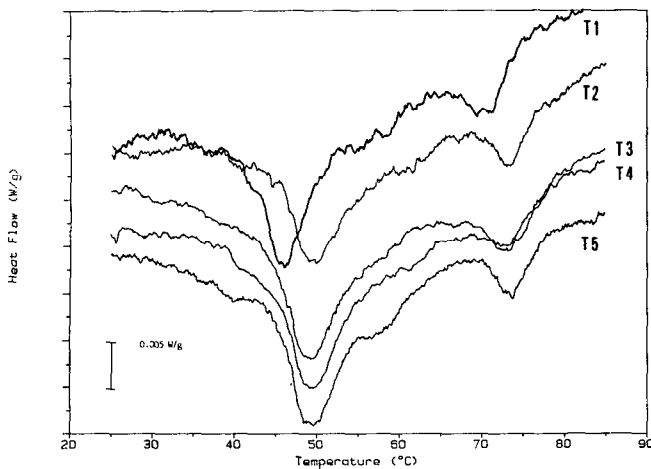


Fig. 2.—DSC thermograms of cod surimi stored for 8 wk at  $-20^{\circ}\text{C}$  for treatments: T1 - control, T2 - 8% sucrose/sorbitol, T3 - 8% Polydextrose<sup>®</sup>, T4 - 8% Palatinit<sup>®</sup> and T5 - 8% lactitol.

Table 2—Overall means of  $T_{\text{max}}$  for the denaturation of protein components in surimi stored at  $-20^{\circ}\text{C}$  for 12 wk<sup>a</sup>

Treatments	$T_{\text{max}}$ - myosin <sup>b</sup>		$T_{\text{max}}$ - actin <sup>c</sup>	
	(°C)			
T1 Control	46.1b		71.2 b	
T2 8% sucrose/sorbitol	48.7a	(2.6) <sup>d</sup>	72.8 a	(1.6)
T3 8% Polydextrose <sup>®</sup>	49.0a	(2.9)	73.0 a	(1.8)
T4 8% Palatinit <sup>®</sup>	49.1a	(3.0)	73.2 a	(2.0)
T5 8% lactitol	49.1a	(3.0)	72.9 a	(1.7)

<sup>a</sup> Reported values are averages of repetitions and storage times ( $n=14$ ); means with the same letter are not significantly different following a Duncan test ( $P \leq 0.05$ )

<sup>b</sup>  $S_x$  (average standard deviation of repetitions) = 0.73°C

<sup>c</sup>  $S_x = 1.01^{\circ}\text{C}$

<sup>d</sup> Values in parentheses indicate the increase in  $T_{\text{max}}$  resulting from the treatments, compared to T1.

from freeze denaturation when compared to sucrose/sorbitol, when incorporated in surimi at 8% w/w level. This latter treatment is currently used in surimi manufacture.

Park et al. (1988) recently reported that Polydextrose<sup>®</sup> could be substituted for the sucrose and/or sorbitol currently added to surimi to provide the same cryoprotection. After 8 months storage at  $-28^{\circ}\text{C}$ , salt soluble protein had similarly decreased by approximately 20% and 18% for Alaska Pollock surimi (without phosphates) treated with Polydextrose<sup>®</sup> and sucrose/sorbitol, respectively (8% w/w).

### Heat-induced denaturation

Differential scanning calorimetry thermograms for each treatment after 8 weeks of frozen storage are illustrated in Fig. 2. Thermograms normally contained two endothermic transitions for cod surimi as was observed by Sych et al. (1990). By referring to previous DSC studies for fish muscle or surimi (Park and Lanier, 1989; Poulter et al., 1985; Wu et al. 1985) and to studies comparing purified protein fractions to whole muscle (Wright et al., 1977), it can be assumed that the two peaks of the present study are related to the thermal denaturation of myosin and actin. Wu et al. (1985) reported three endothermic peaks for Atlantic croaker surimi attributed to the denaturation of myosin at  $43^{\circ}\text{C}$  and  $54^{\circ}\text{C}$ , and to actin at  $71^{\circ}\text{C}$ . Poulter et al. (1985) observed a low temperature DSC transition for cod muscle at ca  $42^{\circ}\text{C}$  assumed to represent the denaturation of myosin. A second peak at  $53^{\circ}\text{C}$  was thought to be due to either a further myosin transition or to a sarcoplasmic protein transition.

An overall ANOVA on all  $T_{\text{max}}$  data followed by regression analysis on storage time means showed that  $T_{\text{max}}$  for myosin and actin varied significantly as a function of treatment but not

as a function of time ( $P \leq 0.01$ ). Data were, therefore, presented as overall means of  $T_{\text{max}}$  for the denaturation of myosin and actin (Table 2). The overall mean of  $T_{\text{max}}$ -myosin for the surimi control ( $46.1^{\circ}\text{C}$ ) falls in between the first two DSC transitions at ca  $42^{\circ}\text{C}$  and ca  $53^{\circ}\text{C}$ , reported for cod muscle by Poulter et al. (1985). However, Wright and Wilding (1984) noted that myosin may show one or more peaks on DSC thermograms depending on chemical environment (pH, ionic strength) and on thermal input.  $T_{\text{max}}$  values for DSC transitions depend on the heating rate employed which varies among studies (Wright et al., 1977).

Certainly, the chemical environment of cod surimi differs from that of cod muscle. In surimi, the washing and leaching steps result in the removal of most of the sarcoplasmic proteins, as well as fat, and other proteolytic degradation products present in fish muscle such as trimethylamine, free amino acids and other low molecular weight compounds. Different DSC thermogram patterns for cod muscle and surimi might, therefore, be expected. Park and Lanier (1989) recently observed different thermograms for Tilapia muscle and Tilapia surimi.  $T_{\text{max}}$  of the second transition decreased from  $78.6^{\circ}\text{C}$  to  $74^{\circ}\text{C}$  during the transformation of Tilapia muscle into tilapia surimi. These workers did not report a significant change in  $T_{\text{max}}$  of the first peak for Tilapia muscle at  $58.7^{\circ}\text{C}$  during surimi processing. Wright et al. (1977) observed that the positions of DSC transitions in whole rabbit muscle were not exactly the same as those of its protein fractions, due to differences in pH and ionic strength.

$T_{\text{max}}$ -myosin overall means for surimi treated with sucrose/sorbitol, Polydextrose<sup>®</sup>, Palatinit<sup>®</sup> and lactitol at 8% w/w were significantly higher than the  $T_{\text{max}}$  mean of the control (T1), ( $P \leq 0.05$ ) (Table 2). These results suggest a stabilization of myosin upon the addition of these additives to cod surimi. Park and Lanier (1987) reported that addition of Polydextrose<sup>®</sup> or sucrose/sorbitol to Jumping Mullet myofibrils at 8% w/w (with 0.5% sodium tripolyphosphate) produced a change from two steps ( $37.3^{\circ}\text{C}$  and  $55.8^{\circ}\text{C}$ ) to a one step ( $57.6^{\circ}\text{C}$ ) denaturation for myosin. This was interpreted as stabilization of the myosin conformation since a higher temperature was required to denature this protein. Sych et al. (1990) reported a 2.1–2.9°C increase compared to control  $T_{\text{max}}$ -myosin when cod-surimi was treated with a sucrose/sorbitol 1:1 mixture, sorbitol, sucrose, glycerol and glucose syrup at 8% w/w.

The overall mean of  $T_{\text{max}}$ -actin for the control surimi was detected at  $71.2^{\circ}\text{C}$ .  $T_{\text{max}}$ -actin for surimi samples treated with sucrose/sorbitol, Polydextrose<sup>®</sup>, Palatinit<sup>®</sup> and lactitol were also significantly higher ( $P \leq 0.05$ ) than the  $T_{\text{max}}$  for the control (Table 2). It appeared that these treatments (T2–T5) had a greater stabilizing effect on myosin than on actin in cod surimi. This can be illustrated by the greater increase obtained for  $T_{\text{max}}$ -myosin ( $T_{\text{max}} = 2.6\text{--}3.0^{\circ}\text{C}$ ) compared to the change in  $T_{\text{max}}$ -actin ( $T_{\text{max}} = 1.6\text{--}2.0^{\circ}\text{C}$ ) for each treatment T2, T3, T4 and T5 compared to the control  $T_{\text{max}}$  (Table 2). Sych et al. (1990) reported slightly lower increases in  $T_{\text{max}}$ -actin, in the range of 1.3–1.7°C, compared to control  $T_{\text{max}}$ -actin, following addition of sucrose/sorbitol, sorbitol, sucrose, glycerol and glucose syrup at 8% w/w in cod-surimi.

The method of expressing peak enthalpies in Joules/g protein ( $\Delta H$ ) was adopted to provide an estimate of the quantity of native protein in surimi samples and to monitor the change in this parameter as a function of frozen storage. In a recent study (Sych et al., 1990), a significant correlation was found between  $\Delta H$  for the myosin transition from DSC thermograms and salt extractable protein data (SEP) for frozen cod surimi.

Figure 3 represents mean enthalpies (two repetitions) for the myosin transition for each treatment as a function of frozen storage of cod surimi. Control sample enthalpy decreased significantly ( $P \leq 0.05$ ) from 2.70 J/g to 1.74 J/g after 12 weeks of frozen storage at  $-20^{\circ}\text{C}$ . However, the rate of change in enthalpy was greater early during frozen storage (0–4 weeks) and enthalpy remained almost constant thereafter (Fig. 3). This

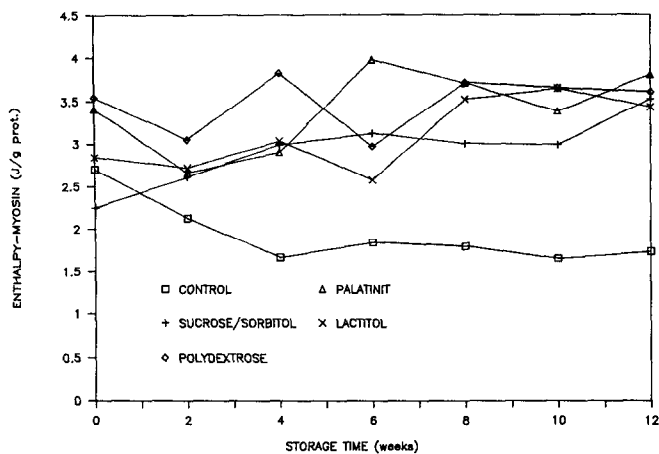


Fig. 3.—Changes in enthalpy of the myosin transition of cod surimi as a function of storage time at  $-20^{\circ}\text{C}$  for the various experimental treatments: T1 - control, T2 - 8% sucrose/sorbitol, T3 - 8% Polydextrose®, T4 - 8% Palatinit®, T5 - 8% lactitol ( $S_x = 0.65$  J/g protein).

Table 3—Overall means of enthalpies for myosin and actin transitions for surimi samples stored at  $-20^{\circ}\text{C}$  for 12 wk<sup>a</sup>

Treatments	▲ H-myosin <sup>b</sup> (J/g protein)	▲ H-actin <sup>c</sup> (J/g protein)
T1 control	1.95 d	1.09 d
T2 8% sucrose/sorbitol	2.98 e	1.12 d
T3 8% Polydextrose®	3.45 e	1.23 d
T4 8% Palatinit®	3.45 e	1.20 d
T5 8% lactitol	3.11 e	1.21 d

<sup>a</sup> Reported values are averages of repetitions and of storage times ( $n=14$ ); means with the same letter are not significantly different following a Duncan test ( $P \leq 0.05$ ).

<sup>b</sup>  $S_x$  (average standard deviation of repetitions) = 0.65 J/g protein

<sup>c</sup>  $S_x = 0.18$  J/g protein

Table 4—Correlation matrix of results of all analytical tests on cod surimi stored at  $-20^{\circ}\text{C}$  for 12 wk

	SEP (%)	$T_{\max}$ -myosin ( $^{\circ}\text{C}$ )	$T_{\max}$ -actin ( $^{\circ}\text{C}$ )	▲ H-actin (J/g protein)	▲ H-myosin (J/g protein)
SEP (%)	1.00	0.59**	0.36**	-0.07	0.53**
$T_{\max}$ -myosin ( $^{\circ}\text{C}$ )	—	1.00	0.75**	-0.03	0.65**
$T_{\max}$ -actin ( $^{\circ}\text{C}$ )	—	—	1.00	0.20	0.48**
▲ H-actin (J/g protein)	—	—	—	1.00	0.08
▲ H-myosin (J/g protein)	—	—	—	—	1.00

\*\* Significant at  $P \leq 0.01$ ,  $n = 66$  d.f.

agrees well with SEP data where the greatest loss of protein solubility occurred during 0–4 weeks of frozen storage (Fig. 1).

Regression analysis indicated that the enthalpies for treatments T2–T5 did not significantly vary with storage time. Overall means of myosin enthalpy for treatments sucrose/sorbitol, Polydextrose®, Palatinit® and lactitol, at 8% w/w in surimi were all found to be significantly different from the mean for control samples. ( $P \leq 0.05$ ) (Table 3). Overall means for lactitol, Palatinit® and Polydextrose® did not differ significantly ( $P \leq 0.05$ ). However, Polydextrose® and Palatinit® treated surimi were characterized by somewhat higher means (3.45 J/g) compared to sucrose/sorbitol treated surimi (2.98 J/g).

Since enthalpy is directly related to the amount of native protein (g/g protein in the sample) and in this case myosin, these results imply that Palatinit®, Polydextrose® and lactitol at 8% w/w in cod surimi provided similar cryoprotection to myosin during frozen storage at  $-20^{\circ}\text{C}$  for 12 weeks. This protection to surimi appeared at least as effective as that afforded by sucrose/sorbitol.

Enthalpy for the actin transition was not found to vary significantly with treatment or storage time and overall means

were, therefore, presented in Table 3. These means ranged from 1.09 to 1.23 J/g protein for the various treatments, similar to enthalpies reported for the actin transition of cod surimi in a previous report (Sych et al., 1990).

### Correlations among analytical tests

The correlation matrix in Table 4 indicates several high correlations among analytical tests which were significant at  $P \leq 0.01$ :  $T_{\max}$ -myosin with SEP,  $T_{\max}$ -myosin with ▲H-myosin and  $T_{\max}$ -myosin with  $T_{\max}$ -actin. As observed in an earlier report, (Sych et al., 1990), ▲H-myosin was also well correlated with SEP data with a correlation coefficient of  $r = 0.53$ .

A high and significant correlation ( $r = 0.72$ ,  $p \leq 0.01$ ) was obtained between  $T_{\max}$ -myosin at zero storage and SEP at 12 wk frozen storage at  $-20^{\circ}\text{C}$ . This correlation coefficient represents the results of the present study combined with carbohydrate and polyol treatment results from Sych et al. (1990).

These results confirmed that the parameters obtained from DSC studies,  $T_{\max}$  and ▲H (particularly for myosin), provided information regarding protein stability and the quantity of native protein, respectively, of surimi during frozen storage. DSC techniques are often considered as more direct methods of analysis compared to other techniques, since DSC is capable of analyzing concentrated solutions, gels and solids, which more closely present the true situation existing in whole muscle (Wright et al., 1977). Other biochemical techniques such as protein extraction, enzymatic activity or electrophoretic analysis often require proteins to be in dilute solutions which might not be comparable to the organized protein structure of whole muscle (Wright et al., 1977).

Furthermore, the results in this report suggested that  $T_{\max}$ -myosin, measured as early as zero storage time and compared to a control could be used to predict surimi protein stability over longer periods of frozen storage. This parameter might be useful in screening tests for rapidly comparing cryoprotective effects of diverse treatments on surimi proteins. Other biochemical tests, which are more time consuming, such as protein extractability, electrophoretic analysis, enzymatic activity, viscosity and gel-forming ability could be reserved for final confirmation of significant treatment effects and for investigating mechanisms of cryoprotection.

Recently, Park et al. (1988) used protein solubility during preliminary testing of 5 starch hydrolysate products for their effectiveness on maintaining solubility of trout actomyosin during 3 wk frozen storage. Noguchi and Matsumoto (1970, 1971, 1975a,b) employed protein solubility, viscosity, sedimentation, ATPase activity and gel-forming ability analyses to test the cryoprotective effects of over 60 different substances in model systems of actomyosin.

### Mechanisms of cryoprotection

The stabilizing action of Palatinit®, lactitol and Polydextrose® may be attributed to the numerous hydroxyl groups available for hydrogen bonding with protein chains of cod surimi, leading to increased protein hydration and decreased aggregation (Noguchi, 1974). Other possible mechanisms such as hydrophobic interaction effects; reduction of the quantity of frozen water and reduction of solute concentration might also be implicated (Sych et al., 1990).

### CONCLUSIONS

BASED ON SALT extractable protein and heat-induced denaturation (DSC) results, Palatinit®, Polydextrose® and lactitol provided similar cryoprotection to the myofibrillar proteins of cod surimi and this protection appeared to be at least as effective as that afforded by sucrose/sorbitol, when considering levels of 8% w/w in surimi. Lactitol, Palatinit® and Polydextrose® present the possibility of substituting either the sugar or sor-

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bitol (in whole or in part) currently used in surimi and, thereby, reducing the sweetness of final surimi-based products. However, further studies are necessary to examine the effect of lactitol and Palatinit® on the gel-forming ability of surimi.

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