E EFFECT OF CRYOPROTECTANTS DURING FROZEN STORAGE ON GELLING PROPERTIES OF MYOFIBRILLAR PROTEIN ISOLATES .

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INTRODUCTION

y scientific work on isolation and use of mechanically deboned poultry meat (MDPM) (Dawson *et al.*, 1989, Hernandez *et al.*, 1986, reurs *et al.*, 1991, Trziszka *et al.*, 1991a/b, 1992, Uijttenboogaart *et al.*, 1992) have shown great potential for the industrial production rotein preparations like 'Chicken-surimi' or 'Chicken Myofibrillar Protein Isolate'. A large problem with these types of protein isolates orage because myofibrillar proteins are rather susceptible for microbiological deterioration even under cooled circumstances.

and meat products are commonly preserved by freezing, since under these conditions there is relatively little deterioration in product ity even after prolonged storage (Barbut and Mittal, 1990; Krivchenia and Fennema, 1988; Sikorski *et al.*, 1976). Muscle tissue structure fected to a greater extent by freezing and frozen storage than are its chemical properties. This is mainly due to crystallization of water e muscle tissue, especially the size and location of ice crystals produced (Sebranek, 1982). Frozen storage of finely comminuted meat ucts requires particular attention due to the possible occurrence of fat oxidation and freezing-induced protein denaturation which markedly ces the functional properties of frozen products.

wiarowicz *et al.*. (1990, 1991) found that enhanced oxidative changes occurring in mechanically deboned poultry meat (MDPM) during en storage caused a marked deterioration in the functional properties of proteins. They suggested that frozen storage of MDPM should be extended beyond 2 to 3 months.

ation of myofibrillar proteins (MPI) from MDPM, as an alternative to the conventional utilization of by-products in further processed try products, gives manufacturers the opportunity to produce MPI on an industrial scale (Dawson *et al.*; 1989, Schreurs *et al.*, 1991; szka *et al.*, 1992). However MPI can only be commercially applied in products provided it will not lose its functional properties during en storage.

possibility to overcome these problems is frozen storage in the presence of cryoprotectants. In the fish industry, surimi is usually stored en in the presence of sucrose, sorbitol and polyphosphates (Lee, 1984).

ur earlier study (Trziszka *et al.*, 1991) it was found that the addition of sucrose and sorbitol as well as monosodium glutamate (0.5%) tetrasodium pyrophosphate (0.3%) to MPI significantly increased gelling properties during heating. The use of mono- and disaccharides increase sweetness, simultaneously decreasing the usability of the endproduct (Sych *et al.*,1990, Sych *et al.*, 1991). For this reason studies other protectants capable of inhibiting freeze induced protein denaturation in MPI are required. Of particular interest are the studies on use of polydextrose during frozen storage of fish surimi (Lanier and Akahane, 1986, Park *et al.*, 1988), and the use of other high molecular ght carbohydrates (HMWC) (MacDonald & Lanier, 1991) like starch.

t recent studies on protein protection during frozen storage have focussed on 4 groups of compounds, i.e. carbohydrates, polyalcohols, ein hydrolyzates and hydrocolloids (Sych *et al.*, 1990).

of major importance that the protein isolates can be stored for extended periods of time without significant changes and that functional perties will be retained as much as possible.

first scope of our investigations was to determine the possibility of frozen storage of MPI obtained by procedures used in earlier eriments (Trziszka *et al.*, 1991). The studies included the effects of various cryoprotectants and drastic changes of the conditions i.e. posure to several cycles of freezing and thawing over a storage period of several weeks.

second scope of this study was to obtain insight in the changes taking place in myofibrillar protein isolates with and without cryoprotectants ing extended storage time at -21 °C.

MATERIALS AND METHODS

experimental material consisted of MPI obtained by one-stage extraction of mechanically deboned chicken meat (MDCM) in 0.075 M HCO_3 using a separator (Westfalia Separator AG, type SB-7-06-076, Oelde, Germany) according to a procedure described by Trziszka ., (1991).

I was divided into four groups:

up 1.	Control, MPI without any cryoprotectants:	C
up 2.	MPI supplemented with 4% (v/w) of Karion F and 4% (w/w) of sucrose:	KAS
up 3.	MPI supplemented with 8% (w/w) polydextrose:	PDX
up 4.	MPI supplemented with 4% (v/w) Karion F and 4% (w/w) of starch:	KST

ion F® is the trade name of a food grade sorbitol syrup (70 % w/w) from Merck, Darmstadt, Germany; sucrose (Analar grade) was obtained m BDH Chemicals, Poole, UK; polydextrose® was from Pfizer; the potato starch used was obtained from a local grocery store. Mixing he cryoprotectants in the MPI occurred by using a Hobart N50 mixer during 5 minutes at speed 2. After mixing the material was portioned vacuum packed into individual plastic bags, each containing ca 300 g.

first experiment (short term storage and repeated freezing and thawing) was carried out according to scheme 1. A sample was taken n fresh MPI as well as from the 1, 2 or 3 times frozen and thawed MPI, stored respectively for 2, 3 and 4 weeks. Another sample was d at -21 °C for 4 weeks prior to thawing.

the seccond experiment (long term frozen storage) portions of each group of cryoprotectant were submitted to frozen storage at roximately -21 °C for 0, 1, 3, 6 and 9 months respectively.

Gel production and characteristics.
Twenty g of MDCM, or the appropriate MPI, containing 2.0% of NaCl were heated at 90°C for 15 minutes in a polypropylene tube (ϕ 21 length 73 mm), cooled with tap water and refrigerated at 4°C until the next day (Trziszka *et al.*, 1992).
Cooking losses of the gels were calculated from the difference in weight before and after heat treatment.
Gel strength (hardness), expressed in newtons, and springiness, expressed in mm, were determined using an Overload Dynamics food tex measuring device as described by Lyon *et al.*, (1980).
Gel elasticity (folding test) was determined by twice folding 3 mm thick gel slices (15 mm diameter) and evaluating cracking of the g using a 5-point scale established by Nippon Suisan Kaisha Ltd. (Lee, 1984).
All data were statistically analyzed by analysis of variance using the GENSTAT V program (Copyright, 1984, Lawes Agricultural Tr Rothamsted Agricultural Station, UK).

RESULTS AND DISCUSSION.

Experiment 1
The data obtained in this study are shown in the Table 1. It shows the major parameters determining the functional properties of MPI the basis of gels after heat treatment. Weight losses significantly differed depending on the cryoprotectant used. Each MPI with cryoprotectan added showed a increased loss due to prolonged storage and multiple freeze thaw treatments. The values obtained with KAS (except 0 2 weeks) and PDX proved to be significant.
The losses were the highest in the groups in which the MPI was frozen and thawed repeatedly.
Regarding the individual cryoprotectants, the weight losses using KAS and PDX were high compared with those noted with KST. KAS a PDX, cryoprotectants widely used in surimi production, did not prove to be suitable in cases where MPI was exposed to repeated freezi and thawing. KAS can be used as a cryoprotectant in MPI provided that freezing and thawing is not repeated. PDX proved to be unsuitabl as a cryoprotectant for MPI due to high cooking losses.
The low weight loss observed with KST (sorbitol and starch) present is very likely due to water binding of starch. Since a wide variety meat products is supplemented with starch as a binding agent, its addition to MPI can be beneficial for two reasons:
On one hand it functions as a gel-binder (Oakenfull, 1987) and on the other hand as a cryoprotective agent (MacDonald & Lanier, 1991
Based on these findings it seems favorable that the addition of starch is advantageous.
Gel strength, expressed in newtons is a major factor determining the texture of gels. The data in Table 1 show a few significant differenc in gel resistance (hardness) for the groups with added cryoprotectant as compared to the control. Addition of KAS and PDX showed significant increase on gel hardness after storage for 2 and 3 weeks. After that period the hardness values decreased to non-significant level as compared to the controls.
Each freezing and thawing cycle of the MPI from the control groups decreased the gel strength significantly, whereas in the cryoprotectant supplemented MPI the original gel strength values were maintained at a level twice as high as the respective control samples regardless o the drastic freezing and thawing operations.
Springiness, defined as the distance a sample recovers between first and second bite (Lyon *et al.*, 1980), is also a parameter which cannot be neglected in the evaluation of the texture of products. As can be seen from Table 1, freezing and thawing of MPI without any cryoprotectant significantly decreased the value of this parameter, thus indicating that elasticity of the gel had been reduced.
The cryoprotectants used in this experiment significantly increased springiness of gels produced from frozen stored MPI. In this respect the best cryoprotectant proved to be KST while the least effective was PDX.
The folding test, another indicator of gel springiness and commonly used in the textural analysis of surimi (Hastings, 1989, Lee, 1984) confirmed the data obtained above which means a significant loss of this property by the freezing and thawing treatment in the control group The use of cryoprotectants only resulted in a significant lower springiness in case of addition of KAS and stored for4 weeks during repeated freezing and thawing. The greatest springiness was noted for gels from MPI with added KST.
Summing up, it can be noted that MPI which is to be stored frozen should be supplemented with cryoprotectants to protect the functional properties of the proteins probably by inhibition of freeze-induced protein denaturation.
The best cryoprotectant of the three used in our experiments proved to be 4 % Karion F (sorbitol) in combination with 4 % starch although a combination of 4 % Karion F and 4 % sucrose, widely used in surimi technology, can also be used for MPI. Sorbitol and sucrose, however will impact a sweet taste to the MPI which can be a problem (Sych *et al.*, 1990).

Experiment 2
The data obtained in this study are shown in tables 2 to 6. The yield values after heating, as shown in table 2 clearly show a large (statistically significant) difference between KST and other treatments. In the product with polydextrose added large cooking losses are found. These results are comparable to those found in experiment 1 and opposed to the results found with fish surimi (Park *et al.*, 1988) where polydextrose was found to be a good cryoprotectant. Karion and sucrose seem to be better cryoprotectants.
Table 3 shows the data for gel strength measurements. Obviously the utilization of the different cryoprotectants has a large effect on the gel strength compared to the starting material. Controversial effects were observed in groups with and without cryoprotectants. During storage of the materials, gel strength increased in the cryoprotected groups as opposed to the non protected groups.
Table 4 shows the springiness values obtained. These data clearly show the increasing effect of cryoprotectants on springiness, especially the increase due to the addition of Karion and starch.
Table 5 shows the shear force values. The supplementation of MPI with Karion/starch and polydextrose results in a significant increase in gel hardness.
Table 6 shows the folding test data as a measure of elastic properties of the gels. As mentioned before this test shows similar results as do the instrumental elasticity measurements. During storage a significant deterioration of elastic properties was observed in the control group but in the cryoprotected groups the folding test values remained relatively stable. The effects observed in gels with starch as a cryoprotectant obviously can partly be attributed to the gel forming properties of the starch itself.
Based on these findings, it is clear that long term frozen storage has a detrimental effect on functional properties of MPI. Utilization of cryoprotectants clearly has a positive effect on quality of these isolates. The mechanical properties of the gels, made from materials stored

er cryoprotection, were found to be significantly better than the control gels. The best mechanical properties and the lowest cooking losses e found when Karion/starch was used as a cryoprotectant.

ization of polydextrose or sorbitol/sucrose for cryoprotection in MPI is possible but again it has to be kept in mind that the latter products result in excessive sweetness (Sych *et al.*, 1990, 1991).

her research is required to find the optimal condition for use of cryoprotectants to be added to MPI during frozen storage.

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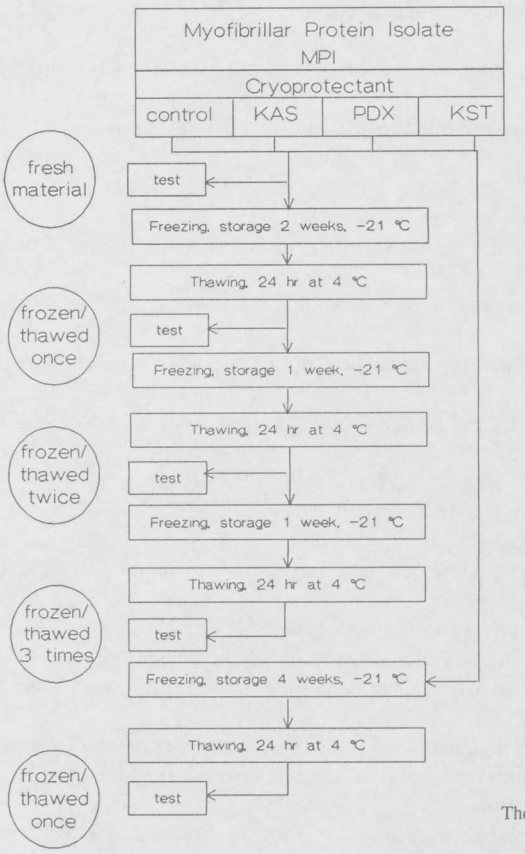
Table 4 Results of experiment 2: Springiness of gels, as the distance between first and second bite (mm).

Cryoprotectant / Storage time (months)	Control	KAS	PDX	KST	Mean
0	11.7	12.0	12.1	14.2	12.5[a]
1	11.2	11.8	12.3	13.2	12.1[a]
3	11.2	12.5	12.3	13.1	12.3[a]
6	10.6	11.6	11.7	12.8	11.7[b]
9	10.8	12.2	11.7	13.2	12.0[ab]
Mean	11.2[a]	12.0[b]	12.0[b]	13.3[c]	



Scheme 1: protocol of first experiment

Table 1 Results of experiment 1

Parameter and statistics	Storage period **P** (weeks)	# of freeze /thaw treatments	Cryoprotectants **C**			
			control	KAS*)	PDX*)	KST*)
Cooking loss (g)	0		5.75	5.37	8.25	0.92
	2	+	10.42	7.50	14.17	2.00
LSD:	3	++	12.75	18.17	26.21	3.75
P = 5.09	4	+++	17.50	28.67	34.25	5.83
C = 13.26	4	+	12.62	8.13	13.83	2.33
P/C = 11.25						
Hardness [N]	0		36.39	30.55	28.66	66.25
	2	+	27.11	48.92	53.08	55.83
LSD:	3	++	24.33	52.67	45.89	49.46
P = 17.78	4	+++	19.87	41.53	34.46	53.87
C = 13.34	4	+	18.73	38.81	36.96	56.33
P/C = 24.14						
Springiness [mm]	0		14.28	12.50	13.67	14.52
	2	+	10.50	13.25	12.77	13.65
LSD:	3	++	10.98	13.38	11.80	13.50
P = 1.97	4	+++	10.21	11.15	10.00	12.38
C = 1.23	4	+	10.63	12.63	12.64	13.40
P/C = 2.57						
Folding test	0		4.04	4.42	3.58	4.67
	2	+	3.17	4.58	4.54	4.58
LSD	3	++	2.67	3.96	3.21	4.21
P = 0.51	4	+++	2.96	3.67	3.42	4.17
C = 0.80	4	+	2.95	4.04	4.04	4.12
P/C = 0.84						

The differences are statistically significant if the difference between the means are higher than LSD for each

Table 2 Results of experiment 2: Cooking loss after heating of MPI (%).

Cryoprotectant / Storage time (months)	Control	KAS	PDX	KST	Mean
0	4.90	3.83	5.60	0.75	3.77^{ab}
1	6.77	5.19	7.25	1.50	5.18^{c}
3	6.10	4.69	6.08	0.88	4.44^{bc}
6	5.27	2.67	5.06	1.25	3.55^{a}
9	6.85	3.92	7.69	1.04	4.88^{c}
Mean	5.98^{a}	4.06^{ab}	6.34^{a}	1.09^{b}	

Table 3 Results of experiment 2: Gel strength (max force in N).

Cryoprotectant / Storage time (months)	Control	KAS	PDX	KST	Mean
0	16.73	16.89	19.23	31.18	21.01^{a}
1	15.25	19.37	24.03	33.31	22.99^{abc}
3	15.81	23.27	24.84	33.41	24.33^{bc}
6	15.21	19.42	22.78	29.24	21.66^{ab}
9	14.94	22.32	25.95	35.01	24.55^{c}
Mean	15.59^{a}	20.25^{ab}	23.37^{b}	32.43^{c}	

Table 5 Results of experiment 2: Shear-Force of gels (N).

Cryoprotectant / Storage time (months)	Control	KAS	PDX	KST	Mean
0	2.11	1.89	2.18	2.91	2.27^{a}
1	2.60	2.61	3.57	4.15	3.29^{b}
3	2.26	2.57	2.95	3.46	2.81^{c}
6	2.46	2.32	2.61	3.24	2.66^{c}
9	2.33	2.33	2.76	3.14	2.64^{c}
Mean	2.35^{a}	2.35^{a}	2.81^{b}	3.38^{c}	

Table 6 Results of experiment 2: Folding test of gels.

Cryoprotectant / Storage time (months)	Control	KAS	PDX	KST	Mean
0	3.79	4.06	3.73	4.31	3.97^{a}
1	4.06	4.40	4.56	4.85	4.47^{b}
3	3.85	4.33	4.17	4.58	4.23^{ab}
6	3.35	4.27	4.33	4.67	4.16^{a}
9	3.17	4.29	4.35	4.58	4.10^{a}
Mean	3.65^{a}	4.27^{ab}	4.23^{ab}	4.06^{b}	