

Cryoprotection of myofibrillar preparation from poultry meat

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The aim of the study was to assess the effectiveness of cryoprotective action of selected substances on essential functional properties of frozen stored myofibrillar preparation. The experimental material was a myofibrillar preparation obtained from mechanically recovered poultry meat (MRPM) by washing and separation of fat and connective tissue. Changes occurring during frozen storage were investigated in the preparation without and with the addition of the following substances: 0.2% carrageen, 0.25% sodium tripolyphosphate mixture and 8% polydextrose, and additionally 0.3% enzymatic preparation (ACTIVA WM) containing microbial transglutaminase (MTG). Samples with the addition of ACTIVA were incubated at the temperature of $7 \pm 1^\circ\text{C}$ for 1, 3, 5 and 24 h. All samples were stored at the temperature of approx. -23°C for 2, 30, 60 and 90 days. The smallest change in protein solubility was observed in samples with added TPP and polydextrose, followed by those with carrageen and MTG after 1 h incubation. Along with the extension of incubation time of samples containing the enzyme a gradual decrease was found in the amount of soluble protein. Also the results of the investigations obtained using the DSC technique showed the most advantageous protective effect, i.e. causing the smallest decrease in enthalpy values of samples during freezer storage, in case of the addition of TPP and polydextrose mixture. Analysis of cooking loss volume from gels showed that carrageen was the most advantageous addition to the myofibrillar preparation. Moreover, a gradual increase was observed in the volume of thermal drip along with the extension of incubation time of the protein isolate with preparation ACTIVA. Results of gel texture testing indicated that the most advantageous effect on gel quality was found for ACTIVA applied in 3 and 5 h incubation with the myofibrils. Assuming that among the analyzed properties the most important were thermal drip and gel texture, 0.3% MTG at incubation time of 3 hours was considered the most advantageous additive.

Keywords: myofibrillar preparation; cryoprotectants, transglutaminase, texture

Introduction

Mechanically recovered poultry meat is a processing material with rather limited technological processability. One of the methods to utilize mechanically recovered poultry meat (MRPM) is the production of protein preparations using the technology of fish surimi. A myofibrillar preparation produced by washing MRPM several times with water exhibits very good technological properties, primarily a high capacity to form strong gels after heating. Freezing and frozen storage have become the most popular storage techniques in case of comminuted fish meat. However, freezing and frozen storage of fish surimi as well as washed MRPM result in protein denaturation and as a consequence a deterioration of functional properties, such as protein solubility, water holding capacity after heating and the capacity to form gels of desirable quality (Park and Morrissery, 2000; Herrera and Sampedro, 2002; Stangierski and Kijowski, 2003; Somjit *et al.*, 2005). In the washing process water soluble components, which stabilize myofibrillar protein during freezing and frozen storage, were removed from MRPM (Jiang *et al.*, 1987). Among all muscle protein myofibrillar protein, especially myosin,

exhibits the highest susceptibility to damage cause by freezing. In order to reduce disadvantageous effects of freezing substances stabilizing muscle protein are used, e.g. polysaccharides, polyalcohols, acids, polyphosphates and carrageenans (MacDonald and Lanier, 1991; Matsumoto and Noruchi, 1992; Park, 1994; Kijowski and Richardson, 1996b; Krala and Dziomdziora, 2000; Stangierski and Kijowski, 2003). At present most frequently 10% sucrose and 0.2 – 0.5% polyphosphates or 10% 1:1 (w/w) sucrose and sorbitol mixture with a small admixture of polyphosphates are added to fish surimi before freezing. However, such a high addition of sucrose (usually 6-8%) results in excessive, sometimes unacceptable (especially in Europe) sensation of sweetness and the darkening of frozen surimi. For this reason studies are being continued to lower its concentration in cryoprotectant mixtures and to replace sucrose and sorbitol with other substances.

For example, carrageenans belonging to the category of hydrocolloids and polyphosphates, commonly added to processed meat to improve its texture, product binding or water holding capacity, do not have unambiguously confirmed cryoprotective properties (Park *et al.*, 1993; Uijttenboogart *et al.*, 1993; Sych *et al.*, 1990ab; Stangierski and Kijowski, 2003). The protective action of polyphosphates is the effect of numerous factors, among which the biggest importance is ascribed to their effect on myosin dissociation, solubility of myofibrillar protein, swelling of protein found in the insoluble fraction and binding of ions in bivalent metals. Recently food industry has focused on the application of transglutaminase (EC 2.3.2.13). In the meat processing sector a practical application has been found for protein cross-linking using transglutaminase, both endogenous, found especially in fish meat and that added in the course of processing. In case of preparation ACTIVA WM possible protective properties towards protein may be connected with the presence of large amounts of maltodextrin in the preparation (99%), which is used to protect fish protein (Carvajal *et al.*, 1999).

The application on a commercial scale of synthetic cryoprotectants, despite their sometimes positive action, is rather unlikely due to their high price. For this reason it is necessary to carry out further research in order to find and confirm the effects of the action of new stabilizers which may possibly be applied in frozen storage of meat. Thus the aim of the study was also to determine the effectiveness of the addition of ACTIVA to a frozen myofibrillar preparation.

Materials and methods

Experimental material consisted of poultry meat mechanically recovered from the dorsal part of unfrozen chicken carcasses in an RM 500 deboner by Lima. MRPM was washed with a 1% aqueous sodium chloride solution and next with water (MRPM : water; 1:3) and fat and connective tissue were separated according to the specification in the patent claim (Kijowski *et al.*, 1996). The obtained myofibrillar preparation was mixed with the addition of the following substances: 0.2% carrageen (Satiagel RPI 515 – Degussa Food Ingredients), a mixture of 0.25% sodium tripolyphosphate (TPP-Sigma) and 8% polydextrose (Litesse® – Danisco), and additionally 0.3% enzymatic preparation containing microbial transglutaminase (MTG) (ACTIVA WM – Ajinomoto Co. Ltd.). Samples with the addition of ACTIVA were incubated at the temperature of $7\pm 1^\circ\text{C}$ for 1, 3, 5 and 24 h. All samples after being mixed with additives were frozen and stored for 2, 30, 60 and 90 days at $-23 \pm 1^\circ\text{C}$.

The basic chemical composition of the myofibrillar preparation was assayed using standard methods developed for meat materials.

Protein solubility was tested according to Helander (1961) extracting protein from samples with the use of 0.1M phosphate buffer with pH 7.4. The amount of protein dissolved in the buffer was expressed as a percentage of total protein contained in the sample.

Thermodynamic properties of the protein preparation were tested using a DSC 7 differential scanning calorimeter by Perkin-Elmer. Preparation samples of 16 ± 1 mg were heated at $10^\circ\text{C}/\text{min}$. within the temperature range of $20\text{--}110^\circ\text{C}$. An empty capsule was used as a reference sample. Two standards were used for the calibration of temperatures and enthalpy: gallium and indium (Merck).

The preparation of protein gels – 2.5% sodium chloride were added to the sample and mixed thoroughly. Test tubes with contents were heated in a water bath at 80°C for 25 min. Samples with formed gels were cooled and stored at $4\text{--}5^\circ\text{C}$ until the next day.

The volume of cooking loss was calculated on the basis of the difference in weight of the sample before heating and that of the heated sample. The volume of cooking loss was expressed as a percentage in relation to the weight of the fresh sample.

Texture was analyzed in a Texture Analyser TA-XT2i (Great Britain) using the stress relaxation test making it possible to determine firmness and elasticity. A sample placed between two parallel plates with the height and diameter of 20 mm was compressed to 50% its height and remained in that position under constant load for 1 min.

Statistical calculations included the values of means, standard deviations and analyses of variance, performed using the STATISTICA software.

Results and discussion

The mean basic chemical composition and pH values of individual samples of myofibrillar preparations did not differ significantly and amounted to 83.9% water, 14.5% protein, 1.0% fat, 0.9% ash, pH 6.53. Only a sample with the addition of 0.25%TTP and 8% polydextrose distinctly differed from the others in terms of contents of water (78.3%) and protein (12.5%).

On the basis of protein solubility no statistically significant effect was found for the analyzed compounds on this functional property of protein determined before sample freezing (*Table 1*).

Table 1. Protein content in myofibrillar preparations (MP) soluble in phosphate buffer [% of total protein].

Type of sample	Storage time [days]				
	0	2	30	60	90
MP	81,1 ^{abA} ± 2,67	81,0 ^{aA} ± 1,71	77,7 ^{abA} ± 0,88	70,1 ^{bB} ± 0,99	66,3 ^{bC} ± 1,26
MP + 0,2% carrageen	84,2 ^{aA} ± 2,09	82,5 ^{aAB} ± 1,67	80,0 ^{aBC} ± 2,45	77,4 ^{aCD} ± 0,78	75,5 ^{aD} ± 0,89
MP + 0,25 TPP + 8% polydextrose	81,2 ^{abA} ± 0,69	79,7 ^{aA} ± 2,46	77,5 ^{abAB} ± 1,91	74,1 ^{aB} ± 1,61	74,3 ^{aB} ± 2,71
MP + 0,3% MTG 1 h	83,5 ^{aA} ± 2,06	80,2 ^{aAB} ± 2,67	76,0 ^{bBC} ± 0,87	74,7 ^{aC} ± 1,91	74,7 ^{aC} ± 2,54
MP + 0,3% MTG 3 h	78,0 ^{bA} ± 0,99	72,9 ^{bB} ± 1,29	68,2 ^{cC} ± 0,48	67,4 ^{cC} ± 1,48	66,8 ^{bC} ± 1,47
MP + 0,3% MTG 5 h	76,6 ^{bcA} ± 1,53	73,7 ^{bA} ± 0,86	68,1 ^{cB} ± 1,61	65,0 ^{cB} ± 2,70	57,0 ^{cC} ± 1,47
MP + 0,3% MTG 24 h	71,9 ^{cA} ± 3,69	70,4 ^{bAB} ± 1,54	65,4 ^{cBC} ± 2,56	60,9 ^{dCD} ± 2,09	59,8 ^{cD} ± 1,34

a...d – the same superscripts in columns indicate no statistically significant differences among mean values ($p < 0,05$) ($n=6$).

A...D – the same superscripts in rows indicate no statistically significant differences among mean values ($p < 0,05$) ($n=6$).

However, the analysis of variance showed the effect of frozen storage time and the type of the sample on protein solubility. Moreover, the effect of preparation incubation time with the enzyme on protein solubility was observed. Longer storage i.e. 30, 60 and 90 days, resulted in a gradual decrease of protein solubility in all samples. Summing up it may be stated that no statistically significant differences were observed in the amount of soluble protein assayed after 90 days of frozen storage in samples with the addition of carrageen, TPP with polydextrose and MTG after 1 h incubation.

A deterioration of protein solubility in the myofibrillar preparation assayed after freezing and frozen storage is caused by a lower solubility of myosin and to a smaller extent of actin. This was confirmed in DSC thermodynamic tests conducted on poultry surimi (Kijowski and Richardson, 1996a). In turn, Smith *et al.* (1990) suggested on the basis of the results of their tests on myofibrils isolated from chicken muscles that the cause of a decrease in protein solubility may be the presence of small amounts of lipids and products of their oxidation. An increase in lipid oxidation may be correlated with a lowering of protein solubility.

The addition of each of the substances to the preparation resulted in a decrease of enthalpy value in comparison to the control (*Table 2*). A significant effect on the lowering of heat of transition was also found for myofibrillar preparation incubation time with the enzyme. The lowest values were found after 24 h incubation. Freezing and successive frozen storage periods resulted in a further gradual

lowering of heat of transition of analyzed samples. On the basis of obtained results it may be found that the process of freezing and frozen storage resulted in the lowest decrease of enthalpy for a sample with the addition of TTP and polydextrose amounting to approx. 18% in relation to values before freezing.

Table 2. Total enthalpy of thermal transition in fresh and frozen myofibrillar preparations [J/g of protein].

Type of sample	Storage time [days]				
	0	2	30	60	90
MP	17,2 ^{aA} ± 0,14	16,7 ^{bB} ± 0,26	10,6 ^{cC} ± 0,12	9,7 ^{cD} ± 0,09	9,2 ^{eE} ± 0,27
MP + 0,2% carrageen	15,5 ^{bA} ± 0,21	16,0 ^{cA} ± 0,07	11,5 ^{bB} ± 0,18	10,6 ^{bcC} ± 0,13	10,4 ^{bcC} ± 0,64
MP + 0,25 TPP + 8% polydextrose	15,8 ^{bB} ± 0,56	17,0 ^{aA} ± 0,14	14,3 ^{aC} ± 0,17	13,4 ^{aCD} ± 0,99	12,7 ^{aD} ± 0,23
MP + 0,3% MTG 1 h	15,3 ^{bA} ± 0,06	15,4 ^{dA} ± 0,09	11,4 ^{bB} ± 0,25	11,5 ^{bB} ± 1,07	10,9 ^{bB} ± 0,51
MP + 0,3% MTG 3 h	13,5 ^{eA} ± 0,14	12,9 ^{fA} ± 0,21	10,4 ^{eB} ± 0,17	10,0 ^{bcBC} ± 0,22	9,9 ^{cdC} ± 0,16
MP + 0,3% MTG 5 h	13,6 ^{eA} ± 0,19	14,0 ^{eA} ± 0,06	10,4 ^{eB} ± 0,09	9,6 ^{cC} ± 0,51	9,2 ^{deC} ± 0,31
MP + 0,3% MTG 24 h	12,6 ^{dA} ± 0,17	13,7 ^{eA} ± 0,12	10,7 ^{eB} ± 0,09	9,3 ^{cC} ± 1,10	9,2 ^{deC} ± 0,29

a...f - the same superscripts in columns indicate no statistically significant differences among mean values ($p < 0,05$) ($n=6$).

A...E - the same superscripts in rows indicate no statistically significant differences among mean values ($p < 0,05$) ($n=6$).

The additives introduced to the myofibrillar preparation before freezing contributed to a slight increase in the volume of cooking loss from gels (*Table 3*).

Table 3. Cooking loss in gels prepared from fresh and frozen myofibrillar preparations [%].

Type of sample	Storage time [days]				
	0	2	30	60	90
MP	0,80 ^{dE} ± 0,05	1,12 ^{eD} ± 0,17	1,69 ^{eC} ± 1,06	2,26 ^{eB} ± 0,24	3,44 ^{eA} ± 0,09
MP + 0,2% carrageen	1,07 ^{bC} ± 0,38	1,08 ^{eC} ± 0,07	1,64 ^{eB} ± 0,11	1,87 ^{fA} ± 0,01	1,92 ^{gA} ± 0,05
MP + 0,25 TPP + 8% polydextrose	1,02 ^{bD} ± 0,12	0,91 ^{fD} ± 0,03	1,65 ^{eC} ± 0,17	1,79 ^{gB} ± 0,09	2,65 ^{fA} ± 0,57
MP + 0,3% MTG 1 h	0,85 ^{cdE} ± 0,08	1,26 ^{dD} ± 0,04	2,46 ^{dC} ± 0,11	3,34 ^{dB} ± 0,09	4,34 ^{dA} ± 0,26
MP + 0,3% MTG 3 h	0,97 ^{bcE} ± 0,39	3,80 ^{eD} ± 0,05	3,99 ^{eC} ± 0,08	4,60 ^{eB} ± 0,15	4,79 ^{eA} ± 0,20
MP + 0,3% MTG 5 h	1,06 ^{bE} ± 0,04	5,22 ^{bD} ± 0,16	6,11 ^{bC} ± 0,08	6,48 ^{bB} ± 0,05	6,57 ^{bA} ± 0,45
MP + 0,3% MTG 24 h	3,84 ^{aE} ± 0,03	7,15 ^{aD} ± 0,10	7,67 ^{aC} ± 0,08	8,02 ^{aB} ± 0,05	8,37 ^{aA} ± 0,19

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Moreover, a gradual increase in cooking loss may be also observed along with the extension of incubation time of the protein isolate with preparation ACTIVA, reaching the maximum value after 24 h. Analysis of the volume of cooking loss from all the analyzed gels after 2 days and in successive periods of frozen storage showed a downward trend. The addition of carrageen turned out to be the most advantageous, as weight losses from the sample after 90 days of frozen storage was slightly below 2%.

On the basis of texture results an advantageous effect of each of the compounds may be stated, especially the addition of MTG, together with the time of its incubation on gel firmness (*Table 4*). The highest value of this parameter was found in the gel sample subjected to enzyme treatment for 24 h before heating. Further increase of gel firmness was observed after the process of freezing and successive periods of frozen storage. An opposite phenomenon occurs in case of the control sample.

Table 4. Firmness of gels prepared from fresh and frozen myofibrillar preparations [N].

Type of sample	Storage time [days]				
	0	2	30	60	90
MP	26,5 ^{fA} ± 0,6	24,8 ^{eB} ± 0,8	23,6 ^{fC} ± 0,7	22,7 ^{eC} ± 0,9	18,9 ^{fD} ± 0,3
MP + 0,2% carrageen	32,0 ^{dB} ± 0,2	34,7 ^{eA} ± 0,7	31,1 ^{eB} ± 0,9	31,3 ^{dB} ± 0,3	31,3 ^{dB} ± 0,7
MP + 0,25 TPP + 8% polydextrose	28,0 ^{eC} ± 0,5	31,2 ^{dA} ± 0,6	31,3 ^{eA} ± 0,3	30,7 ^{dA} ± 0,8	29,1 ^{eB} ± 0,4
MP + 0,3% MTG 1 h	33,6 ^{eE} ± 0,6	43,4 ^{bB} ± 1,9	37,8 ^{dD} ± 0,7	40,3 ^{eC} ± 0,2	48,0 ^{eA} ± 0,7
MP + 0,3% MTG 3 h	36,9 ^{bE} ± 0,7	44,0 ^{bC} ± 0,5	42,3 ^{eD} ± 0,2	49,0 ^{bB} ± 0,5	52,7 ^{aA} ± 0,7
MP + 0,3% MTG 5 h	37,7 ^{bD} ± 0,6	46,2 ^{aC} ± 0,3	46,6 ^{bC} ± 1,2	49,5 ^{abB} ± 1,0	53,2 ^{aA} ± 0,5
MP + 0,3% MTG 24 h	41,0 ^{aD} ± 1,0	47,1 ^{aC} ± 1,4	48,7 ^{aB} ± 0,6	50,7 ^{aA} ± 0,8	51,0 ^{bA} ± 0,5

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A...E – the same superscripts in rows indicate no statistically significant differences among mean values ($p < 0,05$) ($n = 6$).

While analyzing values of the other texture indicator, i.e. elasticity, an improvement of this property may be observed only in samples containing MTG after 3, 5 and 24 h incubation (*Table 5*).

Table 5. Elasticity of gels prepared from fresh and frozen myofibrillar preparations [%].

Type of sample	Storage time [days]				
	0	2	30	60	90
MP	54,6 ^{cA} ± 0,1	53,7 ^{cB} ± 0,5	53,6 ^{dB} ± 0,2	52,5 ^{cC} ± 0,3	51,5 ^{fD} ± 0,4
MP + 0,2% carrageen	55,3 ^{bcB} ± 0,4	54,1 ^{bcC} ± 0,3	55,3 ^{cdB} ± 0,2	56,6 ^{bA} ± 0,2	56,8 ^{eA} ± 0,5
MP + 0,25 TPP + 8% polydextrose	55,6 ^{bC} ± 0,2	56,6 ^{aB} ± 0,2	57,9 ^{aA} ± 0,2	58,3 ^{aA} ± 0,3	58,0 ^{dA} ± 0,4
MP + 0,3% MTG 1 h	55,0 ^{bcC} ± 1,1	54,5 ^{bcC} ± 1,2	57,9 ^{aB} ± 0,5	55,7 ^{bC} ± 1,1	62,2 ^{bA} ± 0,6
MP + 0,3% MTG 3 h	61,8 ^{aB} ± 0,6	54,8 ^{bcD} ± 1,5	57,1 ^{abC} ± 0,9	58,6 ^{aC} ± 1,5	64,2 ^{aA} ± 0,3
MP + 0,3% MTG 5 h	62,1 ^{aA} ± 0,3	56,6 ^{aC} ± 1,0	56,9 ^{acC} ± 1,8	59,1 ^{aB} ± 0,9	58,8 ^{cdB} ± 0,7
MP + 0,3% MTG 24 h	62,7 ^{aA} ± 0,4	55,4 ^{abC} ± 0,5	55,7 ^{bcC} ± 1,5	58,5 ^{aB} ± 0,2	59,5 ^{eB} ± 0,8

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A...D – the same superscripts in rows indicate no statistically significant differences among mean values ($p < 0,05$) ($n = 6$).

Freezing caused a statistically significant lowering of elasticity in all samples. After analyzing successive periods of frozen storage an increase was observed in elasticity for samples containing the enzyme after 1 and 3 h incubation and with the addition of carrageen and a mixture of TPP with polydextrose. In conclusion it may be stated that the addition of MTG had the most advantageous effect on gel texture in comparison to the other compounds. A significant effect of incubation time and frozen storage time on gel quality was observed.

Testing results presenting in the study show that it is possible to reduce the effect of freezing and frozen storage on functional properties of myofibrillar protein by the addition of protectants. From the theoretical point of view the role of cryoprotectants consists in the generation of the so-called vitreous state, in which no ice crystals are formed. In case of frozen products cryoprotectants are to prevent cryodiffusion of water and in this way the growth of large ice crystals at the expense of small ones. Thus, cryoprotection prevents protein dehydration, i.e. the destruction of cells of the frozen system.

Summing up the results of the study it needs to be stated that freezing and frozen storage result in a significant and along with the passing storage time also increasing deterioration of functional properties of the produced myofibrillar preparation. It is especially evident in case of the control. None of the tested additives protected all the analyzed functional properties of frozen samples to a satisfactory degree. Assuming that the most essential among the analyzed technological properties were thermal drip and gel texture, the most advantageous additive was 0.3% MTG at incubation time of 3 hours.

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