Effect of Enzymatic Modification on Frozen Chicken Surimi

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Abstract

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The effects were assessed of the addition of microbial origin transglutaminase (MTG) and modification time of proteins in frozen chicken surimi on its texture, rheological attributes, molecular dynamics of water, water activity, and thermal properties of proteins. Surimi was produced from mechanically separated poultry meat. The amount of 3 g/kg MTG were added to samples of fresh, frozen (stored at –22°C for 30 days), and defrosted surimi which were incubated for a max. 9 h at 15°C. The highest values of the analysed texture attributes and rheological modules were recorded in the case of the sample enzymatically modified before freezing. The values of spin-lattice relaxation time were significantly higher in the control system. In comparison to non-modified surimi, the samples treated with MTG were characterised by a considerable increase in the equilibrium water activity. After 7 h modification, the value of enthalpy for surimi protein was by approx. 30% lower in comparison to the control sample.

Keywords: transglutaminase; texture; rheological; NMR; water activity; DSC

A fresh myofibril preparation (MP) from mechanically recovered poultry meat (MRPM) contained a considerable amount of water (approx. 83–87%), resulting in its susceptibility to adverse microbiological and chemical changes. Freezing or lyophilisation may significantly reduce these changes. However, meat freezing and frozen storage result, among other things, in the denaturation of myofibrilar proteins which, as a consequence, leads to a significant deterioration of its functional properties (Park & Morrissey 2000; Herrera & Sampedro 2002; Stangierski & Kijowski 2003; Somjit *et al.* 2005). Physical processes occurring in raw materials in the course of frozen storage result from the changes in the contained water. The most significant of these changes is the phase transition of water into ice, constituting the essence of freezing and being the primary cause of all other physical changes and their consequences modifying the final quality of raw materials.

In order to minimise the adverse consequences of freezing, substances stabilising muscle proteins are used, e.g. polysaccharides, polyols, acids, polyphosphates, and carragheenans (Kijowski & Richardson 1996; Krala & Dziomdziora 2000; Stangierski & Kijowski 2003). Carbohydrates stabilise actomyosin in fish surimi, hinder the release of water from proteins, increase water surface tension, and prevent the deterioration of protein solubility (Herrera & Sampedro 2002). However, a high addition of sucrose (typically 6–8%) results in an excessive sensation of sweetness, sometimes considered unacceptable (particularly in Europe), as well as in darkening frozen surimi. Thus, research is being conducted to reduce its concentration in cryoprotectant mixtures or to replace sucrose and sorbitol with other substances. Hydrocolloids, including carragheens, polyphosphates, or protein hydrolysates, frequently added to processed meat in order to improve the binding of the final product,

texture, or water holding capacity, do not exhibit definitely confirmed cryoprotectant properties (Sych *et al.* 1990a,b; Uijttenboogart *et al.* 1993; Stangierski & Kijowski 2003; Stangierski 2009). There are no unambiguous opinions on the cryoprotectant properties of transglutaminase in relation to animal proteins. The studies conducted on fish surimi with the addition of an enzymatic preparation did not show an advantageous role of TG during freezing or confirm the dominant role of transglutaminase in the frozen structuring of ground fish meat (Joseph *et al.* 1994; Kołakowski 2005). In turn, in earlier studies Sakamoto *et al.* (1995) indicated an improved quality of gels obtained from frozen surimi following the addition of microbial transglutaminase.

The investigations presented in this study are a continuation of the analyses concerning the effect of enzymatic modification on rheological attributes and dynamics of water binding by unfrozen proteins of myofibril preparation from mechanically recovered poultry meat (Stangierski *et al*. 2012). The aim of this paper was to analyse physico-chemical properties of myofibril proteins subjected to transglutaminase treatment and a 30-day frozen storage. Physico-chemical properties and the molecular dynamics of water binding by the system of poultry proteins were assessed determining rheological attributes, conducting NMR relaxation analyses, testing the texture of myofibril preparations, measuring the equilibrium activity of water, and applying differential scanning calorimetry (DSC).

MATERIAL AND METHODS

Detailed characteristics of the experimental materials and testing methods applied in this part of the study were specifically described by Stangierski *et al*. (2012). The direct experimental material was myofibrilar preparation (MP) (synonym – chicken surimi) manufactured from mechanically recovered chicken meat.

On MP samples obtained in the presented study, analyses were conducted to determine the texture, rheological attributes by the dynamic method (DMA), while spin-lattice and spin-spin relaxation times were measured by NMR and equilibrium water activity was also determined (see methods in publication Stangierski *et al*. 2012). Moreover, thermal analyses of proteins were conducted applying differential scanning calorimetry (DSC) with the use of a Perkin-Elmer device Model DSC 7 (Norwalk, USA). The preparation samples of 16 ± 1 mg were heated at a rate of 5°C/minute. Within the 2–110°C temperature range, an empty capsule was used as a reference sample. Two standards, i.e. gallium and indium, were used in the calibration of temperature and enthalpy (Perkin-Elmer Co., Norwalk, USA).

Sample preparation for analyses. A portion of the obtained myofibril preparation before freezing was modified enzymatically using microbial transglutaminase (MTG) added in the amount of 3 g/kg, at 15°C for 6 or 9 hours. The difference in the protein modification time resulted from the analytical specificity of the research methods applied. The MP enzymatic modification was performed using a preparation of commercial name ACTIVA WM of the Ajinomoto Co. Ltd. (Barentz, Poland). The applied preparation was in powder form and contained 1% transglutaminase of microbiological origin (*Streptoverticillium* sp.) of 100 IU/g activity and 99% maltodextrine used as the enzyme carrier (Ajinomoto's specifications). One unit was the amount of the enzyme which catalysed the formation of 1 μmol of hydroxamic acid/min at 37°C.

The control sample and the modified sample were frozen and stored for 30 days at –22 ± 1°C. After sample defrosting to a temperature of approx. 15°C, a portion of the control material was subjected to MTG treatment under conditions specified above. Thus five sample variants were obtained:

(1) the control sample, not modified enzymatically (MP),
(2) the control sample modified enzymatically (MP-M),
(3) the frozen control sample, not modified enzymatically (MP-F),
(4) sample subjected to enzymatic modification before freezing (MP-MbF),
(5) myofibril preparation sample enzymatically treated after defrosting (MP-MaD).

Statistical analysis. In order to compare the significance of differences between mean values, statistical verification was performed using for this purpose Duncan's test and NIR or Kruskal-Walis test for non-parametrical data. Depending on the needs, single- (ANOVA) and multi-factorial (MANOVA) analysis of correlation were employed. To verify the research hypothesis assumed, $P \leq 0.05$

significance level of inference was adopted. Statistical analysis was performed using STATISTICA PL Version 9.0 software by StatSoft.

RESULTS

The process of freezing and subsequent 30-day cold storage of the myofibril preparation had a significant effect on the deterioration of its texture parameters. The greatest, adverse changes were observed in the control sample. On the basis of the results recorded, a particularly advantageous effect of the MTG addition and incubation time on the mechanical properties of the myofibril preparation was be found both before and after frozen storage (Table 1). The highest values of the analysed texture attributes were found in case of the PM sample modified enzymatically before freezing. This is connected with the formation of strong cross-linking bonds between protein molecules, which considerably improve the texture of the system (Ahhmed *et al.* 2009). It may be assumed that the bonds formed between proteins in the course of protein incubation with the enzyme are so stable that the process of freezing and subsequent storage at low temperatures does not damage them, making it possible to preserve

Table 1. Texture parameters observed for the control system of myofibrilar preparation and transglutaminase modified proteins after defrosting (n = 6; ± standard deviation)

Time of modification (h)	Firmness (N)	Consistency (N·s)	Cohesiveness (N)	Index of viscosity (N·s)
MP-F				
0	$9.3^{h} \pm 0.2$	$69.7^{n} \pm 1.4$	$-6.8^{g} \pm 1.0$	$13.2^{kl} \pm 0.5$
1	$9.3^{h} \pm 0.3$	$77.2^{m} \pm 1.5$	$-7.4^{g} \pm 0.7$	$14.0^{jk} \pm 0.4$
2	$9.6^{gh} \pm 0.3$	$79.1^{lm} \pm 1.8$	$-8.9^{f} \pm 0.4$	$15.3^{hi} \pm 0.5$
3	$9.8^{gh} \pm 0.2$	$80.3^{kl} \pm 1.6$	$-8.5^{f} \pm 0.6$	$16.1^{h} \pm 0.5$
4	$9.6^{gh} \pm 0.3$	$83.1^{jk} \pm 1.4$	$-10.0^{de} \pm 0.5$	$15.9^{h} \pm 0.4$
5	$9.5^{gh} \pm 0.3$	$84.5^{ij} \pm 1.5$	$-9.9^{de} \pm 0.5$	$14.1^{j} \pm 0.2$
6	$8.7^{i} \pm 0.2$	$82.3^{hjk} \pm 1.4$	$-8.5^{f} \pm 0.7$	$14.8^{i} \pm 0.3$
MP-MbF				
0	$11.4^{d} \pm 0.2$	$84.8^{ij} \pm 1.2$	$-9.0^{ef} \pm 0.8$	$19.6^{f} \pm 0.5$
1	$11.8^{cd} \pm 0.3$	$96.2^{ef} \pm 1.6$	$-10.0^{de} \pm 0.5$	$22.2^{e} \pm 0.4$
2	$11.5^{cd} \pm 0.4$	$94.0^{fg} \pm 1.4$	$-10.5^{cd} \pm 0.9$	$23.6^{d} \pm 0.3$
3	$12.3^{c} \pm 0.3$	$99.5^{de} \pm 1.6$	$-11.4^{bc} \pm 0.8$	$32.6^{c} \pm 0.4$
4	$15.5^{a} \pm 0.3$	$119.9^{a} \pm 1.6$	$-13.3^{a} \pm 0.7$	$45.8^{a} \pm 0.4$
5	$14.9^{a} \pm 0.2$	$111.6^{b} \pm 1.1$	$-12.5^{ab} \pm 0.8$	$46.3^{a} \pm 0.5$
6	$14.3^{a} \pm 0.2$	$109.3^{b} \pm 1.6$	$-12.8^{ab} \pm 0.4$	$40.9^{b} \pm 0.4$
MP-MaD				
0	$9.8^{gh} \pm 0.2$	$84.5^{ij} \pm 1.8$	$-8.5^{f} \pm 0.5$	$12.9^{l} \pm 0.4$
1	$10.2^{fg} \pm 0.3$	$86.2^{i} \pm 1.5$	$-9.0^{ef} \pm 0.3$	$17.2^{g} \pm 0.4$
2	$10.6^{ef} \pm 0.2$	$90.0^{h} \pm 1.5$	$-10.5^{d} \pm 0.3$	$19.7^{f} \pm 0.4$
3	$11.0^{de} \pm 0.2$	$91.5^{gh} \pm 1.7$	$-11.4^{bc} \pm 0.3$	$24.0^{d} \pm 0.4$
4	$12.3^{c} \pm 0.3$	$102.9^{c} \pm 1.2$	$-11.0^{cd} \pm 0.5$	$24.2^{d} \pm 0.5$
5	$12.4^{bc} \pm 0.4$	$99.7^{d} \pm 1.7$	$-11.2^{c} \pm 0.3$	$23.6^{de} \pm 0.6$
6	$12.9^{b} \pm 0.1$	$100.3^{cd} \pm 1.9$	$-10.8^{cd} \pm 0.3$	$22.9^{e} \pm 0.3$

MP – control sample not modified enzymatically; MP-M – control samplemodified enzymatically; MP-F – frozen control sample not modified enzymatically; MP-MbF – sample subjected to enzymatic modification before freezing; MP-MaD – myofibril preparation sample enzymatically treated after defrosting; different letters in columns denote a significant difference for means at $P \leq 0.05$

superior quality attributes in comparison to the control samples.

In the case of the sample frozen with an addition of MTG preparation a relatively high increase may be observed in the values of the texture parameters after approx. 4 h of enzymatic modification of the preparation. For comparison, the highest values of the texture parameters in unfrozen MP were recorded after 2–3 h of transglutaminase modification (Stangierski *et al*. 2012). It can not be excluded that the cross-linking process of the system proteins, despite the dynamics of the process being considerably slower, occurred also during frozen storage. This seems to be confirmed also by the results of water activity determination.

An advantageous effect was also obtained with enzymatic modification of defrosted MP. All determined texture attributes of the above mentioned sample were statistically significantly greater than the values of the parameters recorded for the control sample. This may mean that the phenomenon of the primary myosin heavy chain (MHC) cross-linking occurred during the sample incubation, despite the denaturation changes of some proteins in the preparation caused by the freezing process.

The deterioration of certain functional properties of proteins determined after freezing and frozen storage was caused, among other things, by the lower myosin solubility and to a lesser extent by that of actin. This was confirmed in DSC thermodynamic analyses conducted on poultry surimi (Kijowski & Richardson 1996; Stangierski 2009). Previously, Smith *et al.* (1990) on the basis of the results of their studies on myofibrils isolated from chicken muscles suggested that the reduced protein solubility may have been caused by the presence of slight amounts of lipids and their oxidation products.

Additionally, the correlation coefficient r between the individual texture parameters in the frozen myofibril preparation was analysed at $P \leq 0.05$. We observed a strong positive correlation between firmness and index of viscosity (0.95), consistency and index of viscosity (0.93), as well as firmness and consistency (0.92). In turn, a weak negative correlation of firmness with the other texture parameters was recorded, amounting on average to –0.65.

Rheological properties. Freezing and frozen storage of protein preparation systems, either with the enzyme addition or without it, resulted in a marked diversification of their mechanical and rheological attributes. A particularly advantageous effect on

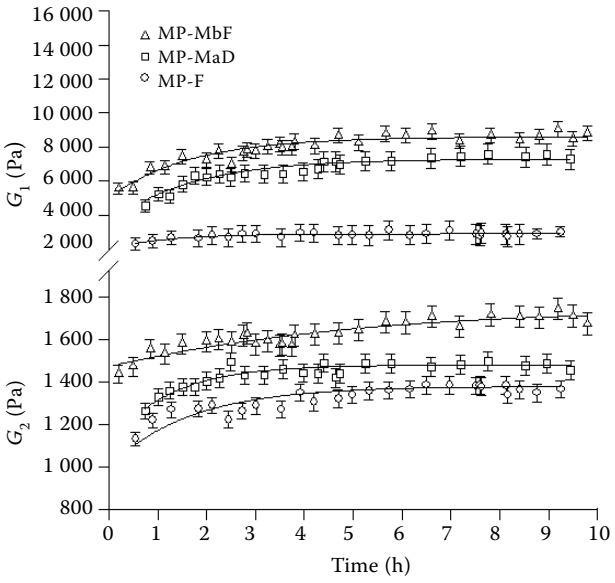

Figure. 1. Kinetics of values changes of the dynamic elasticity modulus G_1 and the loss modulus G_2 during the incubation process of myofibrilar preparation (n = 5)

MP-MbF – sample subjected to enzymatic modification before freezing; MP-MaD – myofibril preparation sample enzymatically treated after defrosting; MP-F – frozen control sample not modified enzymatically

rheological attributes of MP was observed with the enzyme addition as well as incubation time both before and after frozen storage. Moduli of

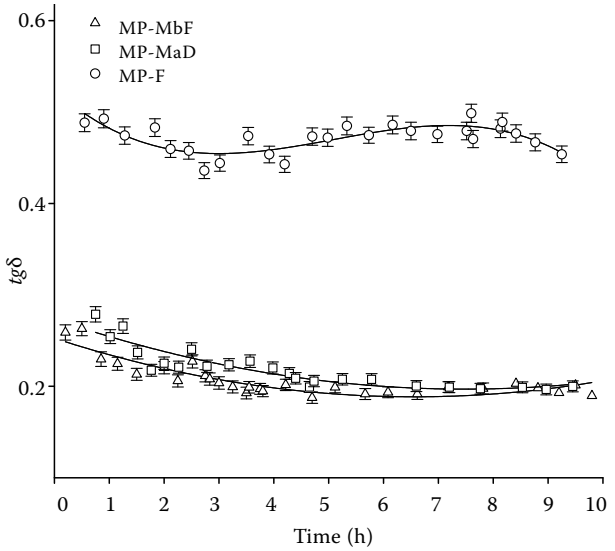

Figure 2. Kinetics of value changes of the lost tangent *tg*δ during the incubation process of myofibrilar preparation (n = 5)

MP-MbF – sample subjected to enzymatic modification before freezing; MP-MaD – myofibril preparation sample enzymatically treated after defrosting; MP-F – frozen control sample not modified enzymatically

Table 2. Mean values and standard deviation of the crosslinking kinetics constant k obtained as a result of fitting of theoretical curves in accordance with Eq. (1) to experimental values (n = 5; ± standard deviation)

Modulus	Type of sample	k ± SD	R^2
Elasticity G_1	MP-F	0.0064 ± 0.0008	0.89
	MP-MbF	0.0074 ± 0.0004	0.90
	MP-MaD	0.0071 ± 0.0003	0.93
Loss G_2	MP-F	0.0046 ± 0.0003	0.89
	MP-MbF	0.0054 ± 0.0004	0.88
	MP-MaD	0.0052 ± 0.0003	0.91

MP-MbF – sample subjected to enzymatic modification before freezing; MP-MaD – myofibril preparation sample enzymatically treated after defrosting; MP-F – frozen control sample not modified enzymatically

elasticity (G_1) and moduli of losses (G_2) revealed much higher values in the protein systems with the MTG addition in comparison to the control system over the entire time range of the conducted experiment (Figure 1). The highest values of the rheological attributes analysed were found in the case of the MP sample modified enzymatically before freezing. The most adverse changes were observed in the control sample. These changes were manifested by the loss of elastic properties, reflected in much higher values of $tg\delta$ (Figure 2) in relation to the enzymatically modified systems.

Frozen storage did not cause any inhibition of the cross-linking process in the system proteins, but only limited its dynamics. Similarly as it was the case with fresh MP samples (Stangierski *et al.* 2012), this phenomenon occurred with greatest intensity in the period up to approx. 3 h incubation. This was indicated by the values of constants $k(G_1)$ and $k(G_2)$ (Table 2), defining the rate of changes for components G_1 and G_2, respectively, of the combined modulus of rigidity in the analysed protein systems obtained from the goodness of fit of Eq. (1):

$$G(t) = G_0 + (G_\infty - G_0)(1 - e^{-k}) \quad (1)$$

where:

G_0, G_∞ – refer to the initial and final equilibrium values of moduli of elasticity and losses

k – constant of cross-linking kinetics and defines the rate of the structuring process

NMR analysis. The analysis of the relaxation times in the protein systems subjected to freezing

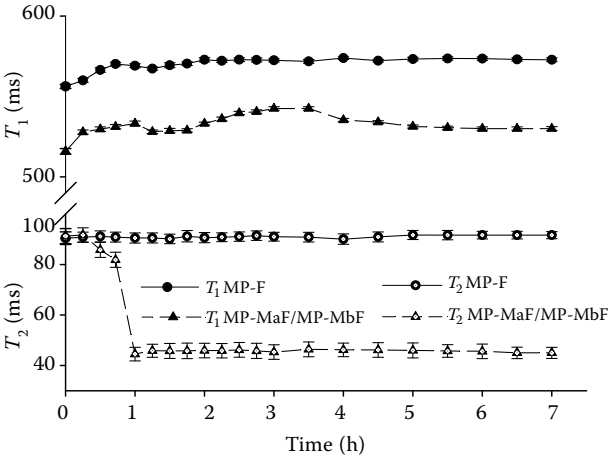

Figure. 3. Time changes in values of spin-lattice T_1 and spin-spin T_2 relaxation times in the protein incubation process of myofibrilar preparation (n = 4)

MP-F – frozen control sample not modified enzymatically; MP-MbF – sample subjected to enzymatic modification before freezing; MP-MaD – myofibril preparation sample enzymatically treated after defrosting

showed that the frozen system with the enzyme addition and the system to which MTG was added after defrosting had similar T_1 and T_2 values. This means that, from the point of view of molecular dynamics, it is not important when the transglutaminase preparation is introduced. In the protein preparation subjected to the long-term action of low temperatures, we could observe distinct differences between the values of both relaxation times in comparison to the control sample (Figure 3).

Spin-lattice relaxation times T_1 were markedly higher for the control system over the entire analysed time range of the experiment conducted. Thus it may be assumed that the enzymatic modification of the preparation proteins resulted in this system in much greater water binding. In the control sample, we observed slight minimum value of T_1 appearing after approx. 1.5 h from the onset of the analyses. After approx. 2 h up to the end of the analysed MP storage, relaxation times T_1 had similar values. The analysis of spin-spin relaxation times (T_2) in the control sample showed constant values over the entire investigated time interval. Thus it may be concluded that the freezing of protein systems results in significant structural changes in proteins, which inhibit the process of changes in molecular dynamics of water in MP preparations after defrosting.

Water activity. When analysing the changes in water activity in protein systems, equilibrium

Table 3. Mean values and standard deviation of the equilibrium activity a_{oc} obtained for the control sample and for the sample of transglutaminase modified myofibrilar preparation after defrosting (n = 5; ± standard deviation)

Incubation time (h)	MP-F	MP-MbF	MP-MaD
1.5	0.901 ± 0.002	0.934 ± 0.002	0.920 ± 0.002
2.3	0.896 ± 0.001	0.937 ± 0.002	0.921 ± 0.002
4.0	0.906 ± 0.002	0.938 ± 0.002	0.919 ± 0.002
4.9	0.907 ± 0.003	0.939 ± 0.003	0.918 ± 0.002
5.8	0.902 ± 0.002	0.939 ± 0.002	0.917 ± 0.001

MP-F – frozen control sample not modified enzymatically; MP-MbF – sample subjected to enzymatic modification before freezing; MP-MaD – myofibril preparation sample enzymatically treated after defrosting

values were obtained for water activity a_{oc} in the samples of proteins subjected to the action of the enzyme before and after freezing. Time changes in a_{oc} values are presented in Table 3. Similarly as in the case of the non-frozen myofibril preparation (Stangierski *et al*. 2012), during the measurement session of several hours no significant changes in water activity were observed in any of the samples. In contrast, as it results from the recorded values of equilibrium water activity, they differed significantly between the individual samples.

In comparison to the non-modified preparation, transglutaminase-treated samples were characterised by a considerable increase in the equilibrium water activity. At the same time, the preparation modified after defrosting had lower values of this parameter than those resulting from the changes caused by enzymatic modification of the identical preparation subjected to freezing. This seems to indicate changes, caused by freezing, in the protein structure of the preparation, reducing the effectiveness of transglutaminase.

DSC analysis. The heating of biological systems, including MP, causes several endothermal processes, resulting in a change in partial thermal capacity. On all thermograms two primary peaks of transitions were found, where T_1 corresponded to the range of temperatures from 56.11°C to 56.47°C. In turn, for T_2 the differences in the temperatures of transition were non-significant and fell for all incubation periods into the range of 68.45°C to 68.94°C. The temperature values presented above pertain to unfrozen MP with the addition of transglutaminase. No significant differences were found in the recorded temperatures for the control sample, while the mean temperatures T_1 and T_2 were 56.49°C and 68.95°C, respectively. The recorded temperatures of transition correspond to the denaturation temperatures of myosin and actin. In the case of the sample with MTG addition, a reduction of temperature T_1 (although not significant statistically) by approx. 0.5°C (after 3 h modification) was found in relation to the control. A decrease in the temperature of transition for myosin may indicate a lower thermostability of the system. Thus it may be assumed that, with an extension of the modification time, the process of polymerisation occurs in the myosin heavy chain (MHC), resulting in a reduction of its temperature of transition. A much greater lowering of temperatures of transition for myofibrillar proteins obtained from bovine hearts under the effect of the enzyme was reported in a study by Ramírez-Suárez *et al.* (2001).

Table 4. Values of enthalpy (J/g of sample) of myofibrilar preparation depending on modification time (n = 4; ± standard deviation)

Time of modification (h)	Type of sample				
	MP	MP-M	MP-F	MP-MbF	MP-MaD
0	$2.31 \pm 0.04^{c,A,B}$	$1.89 \pm 0.03^{b,C}$	$0.78 \pm 0.04^{a,C}$	$0.81 \pm 0.04^{a,C}$	$0.79 \pm 0.04^{a,C}$
1	$2.33 \pm 0.17^{c,B}$	$1.88 \pm 0.06^{b,C}$	$0.72 \pm 0.06^{a,B,C}$	$0.79 \pm 0.02^{a,C}$	$0.71 \pm 0.02^{a,C}$
3	$2.26 \pm 0.04^{d,A,B}$	$1.74 \pm 0.03^{c,B}$	$0.68 \pm 0.02^{b,A,B}$	$0.52 \pm 0.04^{a,B}$	$0.47 \pm 0.04^{a,B}$
5	$2.25 \pm 0.03^{e,A,B}$	$1.67 \pm 0.03^{d,A,B}$	$0.61 \pm 0.04^{c,A}$	$0.45 \pm 0.04^{b,A,B}$	$0.31 \pm 0.04^{a,A}$
7	$2.09 \pm 0.12^{d,A}$	$1.59 \pm 0.04^{c,A}$	$0.62 \pm 0.03^{b,A}$	$0.39 \pm 0.05^{b,A}$	$0.23 \pm 0.04^{a,A}$

MP – control sample not modified enzymatically; MP-M – control samplemodified enzymatically; MP-F – frozen control sample not modified enzymatically; MP-MbF – sample subjected to enzymatic modification before freezing; MP-MaD – myofibril preparation sample enzymatically treated after defrosting; different letters in columns (A–C) and rows (a–e) denote a significant difference for means at $P \leq 0.05$

Freezing did not result in statistically significant changes in the temperature of transition T_1, which on average amounted to 56.71°C. The latter of the temperatures, T_2, increased slightly, reaching the mean value of 69.76°C. The greatest changes in the temperatures of transitions were recorded in the case of the samples modified with transglutaminase. A significant reduction of T_1 was found with an extension of the incubation time to 7 h, by 1.01 and 4.15°C, respectively, for the sample of MP frozen with MTG and for the myofibril preparation enzymatically treated after defrosting. In turn, in the case of temperature T_2, corresponding to the actin temperature of transition, a similar increase by 1.5°C on average was found in both MP samples.

Table 4 presents the course of changes in the values of enthalpy (ΔH) for the analysed samples. The control sample after the first hour of incubation had the highest value of total enthalpy, amounting on average to 2.25 J/g sample. It should be observed that the MTG addition to frozen MP proteins resulted in the heat of transition, recorded as early as after 1-h modification, being statistically significantly lower in comparison to that of the control by almost 20%. A very dynamic reduction in the value of enthalpy for MP proteins was observed up to the 3rd h of incubation. After 7-h modification, the heat of transition for MP proteins was by approx. 30% lower in comparison to the control. It needs to be stressed that the reduction of the enthalpy value was influenced by the enzyme addition, modification time, and the process of freezing, resulting in partial denaturation of myofibrillar proteins. Structural changes caused by protein denaturation probably resulted in much weaker cross-linking of proteins after defrosting under the influence of transglutaminase.

The effects of MTG addition on the thermal properties of proteins, recorded in this study, were similar to those reported by Aktaş and Kiliç (2005) for myofibril preparations obtained from beef. An addition of microbial transglutaminase caused a statistically significant reduction of the heat of transition for myosin. No reduction was observed for the ΔH value determined for actin. What is more, similarly to the presented study, those authors stated a decrease in the temperature of transition for myosin in the fresh preparation on average by approx. 1°C. The primary changes in the thermal properties of transglutaminase-treated myofibrillar proteins are ascribed to the polymerisation of myosin (Ramírez-Suárez & Xiong 2003; Stangierski 2009). It is known that the polymerisation of proteins, occurring under the influence of the enzyme, is an exothermal process and leads to a reduction of enthalpy.

CONCLUSION

Freezing and frozen storage have destructive effects on the protein systems. Frozen storage of MP causes an increase in ionic strength, and as a consequence the migration of frozen water from the myofibril medium and partial dehydration of myofibrilar proteins. At the same time, the process of freezing denaturation alters the surface of proteins, and thus also changes the number of sorption sites for water molecules. This is manifested in changes in relaxation times T_1 and T_2. As a consequence, this leads to the deterioration of functional properties of proteins, e.g. solubility, water holding capacity after heating, and the capacity to form gels of desirable quality.

The addition of transglutaminase to the tested protein systems causes as a result of freezing out of water an increase in the concentration of the proteins responsible for the formation of matrix cross-linking. This directly influences variations in the values of both the modulus of elasticity G_1 and $tg\delta$ between transglutaminase-modified protein systems and the control. Another aspect of the enzyme addition needs to be emphasised. Frozen storage of myofibrillar protein preparations with the addition of transglutaminase improves their elastic properties in comparison to analogous systems not containing the above mentioned enzyme.

It needs to be stressed that the addition of transglutaminase preparation to MP proteins after defrosting had an advantageous effect on the properties of the protein isolate, investigated in this study, in comparison to the control. It may be assumed that freezing and subsequent frozen storage of MP did not cause complete denaturation of proteins. Thus the enzyme addition could have had an advantageous effect by improving the rheological attributes of the preparation, cross-linking the myofibrillar proteins still available for this process. This may also be connected with the presence of high amounts of maltodextrin in the enzymatic preparation used in the analyses, used as a cryoprotectant in the production of surimi not only from fish, but also from other raw materials.

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