Cryoprotective Effect of Trehalose and Maltose on Washed and Frozen Stored Beef Meat

Dragan KOVAČEVIĆ and Krešimir MASTANJEVIĆ

Faculty of Food Technology, University of J. J. Strossmayer in Osijek, Osijek, Croatia

Abstract

Kovačević D., Mastanjević K. (2011): **Cryoprotective effect of trehalose and maltose on washed and frozen stored beef meat**. Czech J. Food Sci., **29**: 15–23.

The cryoprotective effects of trehalose and maltose (w = 2–10%) on washed beef meat were investigated. Washed beef meat produced from fresh beef meat was frozen and stored for 360 days at –30°C. Myofibrillar protein functional stability was monitored by salt extractable protein (SEP) and differential scanning calorimetry (DSC). Salt extractable protein (SEP) showed that the addition of trehalose and maltose causeda smaller loss of protein solubility during the frozen storage. Peak thermal transition (T_p) and denaturation enthalpy (ΔH) of myofibrillar proteins were evaluated. Differential scanning calorimetry (DSC) revealed a shift in the peak thermal transition temperature (T_p) of myosin and actin to higher temperature as the mass fractions of trehalose and maltose increased. The transitions enthalpies of myosin and actin of the washed beef meat samples showed a higher increase with the increase of mass fraction of trehalose then of that of maltose. Since the value of denaturation enthalpy is directly related to the amount of native proteins, higher values of ΔH point to higher cryoprotective effects of trehalose.

Keywords: thermal transitions temperature; DSC; SEP; washed beef meat; trehalose; maltose

Washed beef meat (WBM) is surimi-like product made from red beef meat. The process for making surimi-like product from beef, using modified technology from fish surimi (Park *et al*. 1996), results in a semi-purified protein fraction containing a high concentration of myofibrillar proteins. Freezing has become one of the most frequently used preservation methods for meat and meat products. To protect myofibrillar proteins from freeze-denaturation and during frozen storage and to maintain its possible high processability, cryoprotectants, such as disaccharides, polysaccharides, polyalcohols, acids, or polyphosphates are generally added (Park *et al*. 1988; Mac Donald & Lanier 1991). The most commonly used instrumental methods for the determination of the cryoprotective effects of the added substances are the measurement of myofibrillar protein solubility SEP (Salt extractable protein) (Sych *et al*. 1990), Ca^{2+}ATP-ase activity, unfrozen water by Nuclear Magnet Resonance (NMR), and transition temperatures and enthalpy of myofibrillar proteins by Differential scanning calorimetry (DSC) (Sych *et al.* 1990; Park *et al.* 1993; Park 1994; Yang & Froning 1994; Kijowski & Richardson 1996; Stangierski & Kijowski 2003). Differential scanning calorimetry (DSC) is a useful technique for studying the thermal behaviour of muscle proteins (Barbut & Findlay 1991). The changes in the protein structure during DSC analysis are referred to as transition changes, and peak temperatures at these transitions are used to represent the transition temperatures (Findlay & Barbut 1990). Trehalose (D-glucopyranosyl-α(1→1)-D-glucopyranoside) is a non-reducing disaccharide with a low calorific value and low sweetness, i.e. only 45% of that of sucrose (Colaco *et al*. 1994). Because of its ability to form strong hydrogen bonds

with the polar group of biomolecules and a very high glass transition temperature, trehalose has superior preservation properties as compared to other sugars (Patist & Zoerb 2005). Trehalose has been found to have protective effects against thermal inactivation of enzymes (Miller *et al.*1998) and freeze drying of microorganisms (Leslie *et al.* 1995). (Osako *et al*. 2005) investigated the gel forming ability, unfrozen water content, and Ca^{2+} ATPase activity of horse mackerel surimi during freezing and frozen storage upon the additions of trehalose, sucrose, glucose, and sorbitol, and they concluded that trehalose had a similar cryoprotective effect as sucrose and sorbitol. However, Zhou *et al.* (2006) reported that the addition of 8% of trehalose to fish myofibrillar proteins (tilapia) had a better cryoprotective effect, especially on salt extractable protein (SEP), total sulfhydryl and disulfhydryl bonds contents, and gel forming ability over 24 weeks of frozen storage then 8% of sucrose/sorbitol (1:1) mixture. So far, no information regarding the cryoprotective effect of trehalose on washed beef meat (WBM) has been reported. The aim of this work is to investigate with Differential scanning calorimetry (DSC) and the measurement of myofibrillar protein solubility SEP (Salt extractable protein) the cryoprotective effects of trehalose and maltose on washed beef meat (WBM).

MATERIAL AND METHODS

The samples of WBM were prepared in the laboratory from beef mainly (*m. psoas major*) by the modified procedure of Park *et al*. (1996). Instead of tap water, distilled water was used for washing and leaching. The samples were mixed with a) trehalose (w = 2–10%) and b) maltose (w = 2–10%). The mass fractions were determined as percents of total mass. The pH level was measured in a homogenate of the sample with distilled water (1:10, p/v) using pH/Ion 510 Bench pH/Ion/mV Meter (Eutech Instruments Pte Ltd/ Oakton Instruments, Vernon Hills, USA). Water activity (a_w) was determined using a Rotronic Hygrolab 3 (Rotronic AG, Bassersdorf, Switzerland) at room temperature. The FoodScan Meat Analyser was used to determine moisture, total protein, and total fat according to the AOAC (2007). The samples were packed in polyethylene bags, frozen, and stored at –30°C. Freeze denaturation was evaluated after 30, 90, 180, and 360 days by salt extractable protein and differential scanning calorimetry analysis. The initial measurements were conducted in WBM samples before freezing.

Salt soluble proteins (SEP). Soluble proteins were extracted by the procedure of Li and Wick (2001), with modifications. 1 g of sample with 6 ml Standard brine STB solution (Li & Wick 2001), was mixed in a vortex mixer (Vibromix 10, Tehtnica, Slovenia) at 4°C for 30 minutes. The salt soluble proteins were recovered in the supernatant by means of centrifugation at 10 000× g, 4°C, 15 min, in a Heraeus Multifuge 3L-R. The Bio-Rad Protein Assay (Bio-Rad Laboratories GmbH, Munchen, Germany) was used to estimate the protein concentration in the resulting supernatants using bovine albumin as a protein standard. Salt extractable protein was expressed as the concentration of salt extractable protein (mg/ml), estimated by Bio-Rad analysis.

DSC measurements. Differential scanning calorimetry was performed on Mettler Toledo DSC 822^e differential scanning calorimeter equipped with $STAR^e$ software. The samples of ca 15 mg (± 1 mg) were weighed and sealed into standard aluminium pans (40 µl) and scanned over the rage from 25°C to 95°C at a heating rate of 10°C/min, using empty standard aluminium pan as a reference. The peak temperatures (T_p) were determined from DSC curves. The changes in enthalpy (ΔH, J/g), associated with the denaturation of proteins, were determined by measuring the areas under the DSC curves using $STAR^e$ software. Transition enthalpies were expressed on the total mass fraction of protein.

Statistical analysis. Three determinations of basic chemical composition, pH, a_w, peak temperatures (T_p), transition enthalpies (ΔH), and SEP were performed with each sample. The experimental data were analysed by the analysis of variance (ANOVA) and Fisher's least significant difference (LSD), with significance defined at $P < 0.05$. Statistical analysis was carried out with Statistica Version 7.0 (StatSoft Inc., Tulsa, USA).

RESULTS AND DISCUSSION

The mean basic chemical compositions, pH, and a_w values of the individual samples of WBM did not vary significantly and amounted to 81.96 ± 0.36% water, 15.13 ± 0.23% protein, 2.00 ± 0.06% fat, pH 6.80 ± 0.05, and a_w 0.96 ± 0.02.

Salt soluble proteins (SEP)

The changes in salt extractable protein of WBM mixed with trehalose and maltose (2–10%) during frozen storage at –30°C are shown in Table 1. The highest decrease of SEP occurred in all samples in the first 30 days, especially in the sample without the addition of either trehalose or maltose. SEP in the control sample after 360 days of frozen storage was reduced to 34.21% of the initial value, which is lower compared with the results obtained by Zhou *et al.* (2006), who reported 44.8% loss of fish (tilapia) protein solubility after 24 weeks of frozen storage. This can be explained by the nature of the sample and a higher protein content in the initial sample. For WBM samples with the additions of 2%, 4%, 6%, 8%, and 10% of trehalose, after 360 days of frozen storage SEP of 3.32, 3.41, 3.56, 3.67, and 3.74 mg/ml, respectively, was observed, having been decreased to 71.70%, 70.16%, 73.58%, 75.98%, and 76.01% of the initial SEP values, respectively. In the samples mixed with 2%, 4%, 6%, 8%, and 10% of maltose the initial SEP values were reduced to 66.51%, 69.26%, 72.13%, 74.00%, and 76.84% respectively. From the SEP results it can be derived that the additions of trehalose and maltose in all mass fractions resulted in lowered SEP, as compared with the control on day 0; this can be explained by the dilution effect of trehalose on the protein content in WBM.

Generally, the control sample showed the greatest SEP loss throughout the storage up to 360 days, the samples with 2%, 4%, 6%, 8%, and 10% of trehalose or maltose showed smaller decreases of salt protein solubility (significant at $P < 0.05$) as the mass of trehalose or maltose increased during frozen storage for all frozen time intervals (Table 1). Myofibrillar protein denaturation during frozen storage expressed by the loss of protein solubility is a result of hydrogen or hydrophobic bonds formation, as well as of that of disulfide bonds, and of ionic interactions (Sych *et al.* 1990, 1991; MacDonald & Lanier 1991; Auh *et al.* 1999). SEP of WBM decreased quasi linearly as a function of storage time. In general, the samples with the addition of trehalose (w = 2–10%) showed smaller decreases of protein solubility then did the samples with the addition of maltose (w = 2–10%) for all frozen time intervals (Table 1).

Table 1. Changes in salt extractable protein SEP (mg/ml) of WBM mixed with trehalose and maltose as a function of frozen storage time

	Days of frozen storage				
	0	30	90	180	360
Trehalose (%)					
0	$4.997^{dA} \pm 0.05$	$3.46^{aB} \pm 0.04$	$3.31^{aC} \pm 0.01$	$3.27^{aC} \pm 0.01$	$3.27^{aC} \pm 0.02$
2	$4.63^{aA} \pm 0.22$	$3.72^{bB} \pm 0.02$	$3.41^{bB} \pm 0.61$	$3.36^{bB} \pm 0.01$	$3.32^{aB} \pm 0.01$
4	$4.86^{bcA} \pm 0.08$	$3.72^{bB} \pm 0.01$	$3.55^{cC} \pm 0.03$	$3.50^{cC} \pm 0.02$	$3.41^{bD} \pm 0.06$
6	$4.82^{bA} \pm 0.02$	$3.88^{cB} \pm 0.03$	$3.77^{dC} \pm 0.02$	$3.63^{dD} \pm 0.02$	$3.56^{cE} \pm 0.00$
8	$4.83^{bA} \pm 0.03$	$3.89^{cB} \pm 0.01$	$3.85^{eC} \pm 0.02$	$3.75^{eC} \pm 0.03$	$3.67^{dD} \pm 0.03$
10	$4.92^{cdA} \pm 0.06$	$3.98^{dB} \pm 0.04$	$3.90^{fC} \pm 0.01$	$3.81^{fD} \pm 0.01$	$3.74^{eE} \pm 0.01$
Maltose (%)					
0	$4.97^{dA} \pm 0.05$	$3.22^{aB} \pm 0.03$	$3.15^{aC} \pm 0.02$	$3.01^{aCD} \pm 0.02$	$2.93^{aD} \pm 0.02$
2	$4.54^{bA} \pm 0.05$	$3.39^{bB} \pm 0.02$	$3.22^{bC} \pm 0.01$	$3.10^{bC} \pm 0.01$	$3.02^{bD} \pm 0.01$
4	$4.49^{abA} \pm 0.02$	$3.48^{cB} \pm 0.01$	$3.40^{cC} \pm 0.59$	$3.30^{cD} \pm 0.01$	$3.11^{cE} \pm 0.01$
6	$4.45^{aA} \pm 0.02$	$3.63^{dB} \pm 0.02$	$3.48^{dC} \pm 0.02$	$3.35^{dD} \pm 0.01$	$3.21^{dE} \pm 0.01$
8	$4.50^{abA} \pm 0.06$	$3.73^{eB} \pm 0.02$	$3.58^{eC} \pm 0.01$	$3.45^{eD} \pm 0.04$	$3.33^{eE} \pm 0.03$
10	$4.49^{abA} \pm 0.03$	$3.81^{fB} \pm 0.01$	$3.70^{fC} \pm 0.02$	$3.63^{fD} \pm 0.02$	$3.45^{fE} \pm 0.03$

Values are means ± SD of triplicate; values in the same column or row with different superscripts a–f and A–F are significantly different ($P < 0.05$)

Differential scanning calorimetry

Differential scanning calorimetry thermograms of WBM for each treatment after 360 days of frozen storage are illustrated in Figure 1. WBM thermogram normally contained two endothermic transitions. Referring to the previous DSC studies of similar samples (Wright & Wilding 1984; Sych *et al*. 1990; Barbut & Findlay 1991; Stuart *et al*. 1991; Park *et al*. 1993; Bircan & Barringer 2002; Aktas *et al*. 2005; Brunton *et al*. 2006; Fernandez-Martin 2007), it can be assumed that two peaks in this study are related to the thermal denaturation of myosin and actin. Peak thermal temperatures (T_p) of myosin and actin for WBM with trehalose and maltose (w = 2–10%) at 0, 30, 90, 180, and 360 day of frozen storage are presented in Tables 2 and 3. The values of peak thermal temperatures (T_p) of myosin and actin were different then the values of beef meat reported by Barbut and Findlay (1991), Bircan and Barringer (2000), and Brunton *et al*. (2006). Similar results were reported by Yang and Froning (1994), and Kijowski and Richardson (1996) for washed mechanically deboned poultry meat. This could be explained by the concentration of myofibrillar protein through washing and different pH and ionic environment when compared to the raw state of muscle (Wright & Wilding 1984; Xiong *et al*. 1987; Lesiow & Xiong 2001). The analysis of variance of myosin T_p showed that myosin T_p varied significantly ($P < 0.05$) as a function of the mass fraction of trehalose, but not as that of frozen storage time (Sych *et al*. 1990) (Table 2). T_p of myosin of WBM samples mixed with maltose varied significantly ($P < 0.05$) as a function of the mass fraction of trehalose and as a function of time (Table 3) (Herrera *et al*. 2001). These shifts in T_p of myosin to higher values as the mass fraction of trehalose and maltose increases can be interpreted as stabilisation of myofibrillar proteins since a higher temperature is required to denature these proteins (Sych *et al*. 1990, 1991). The highest shift in myosin T_p was shown by the samples of WBM mixed with 10% of trehalose for all storage time intervals. Generally, T_p of myosin for WBM mixed with trehalose showed greater shifts then the samples mixed with maltose for all frozen storage time intervals (Tables 2 and 3). T_p of actin transitions vary significantly ($P < 0.05$) with the addition of trehalose but not with frozen storage time (Table 2). As to the samples mixed with maltose, T_p of actin transitions vary significantly ($P < 0.05$) with the increase of the mass fraction

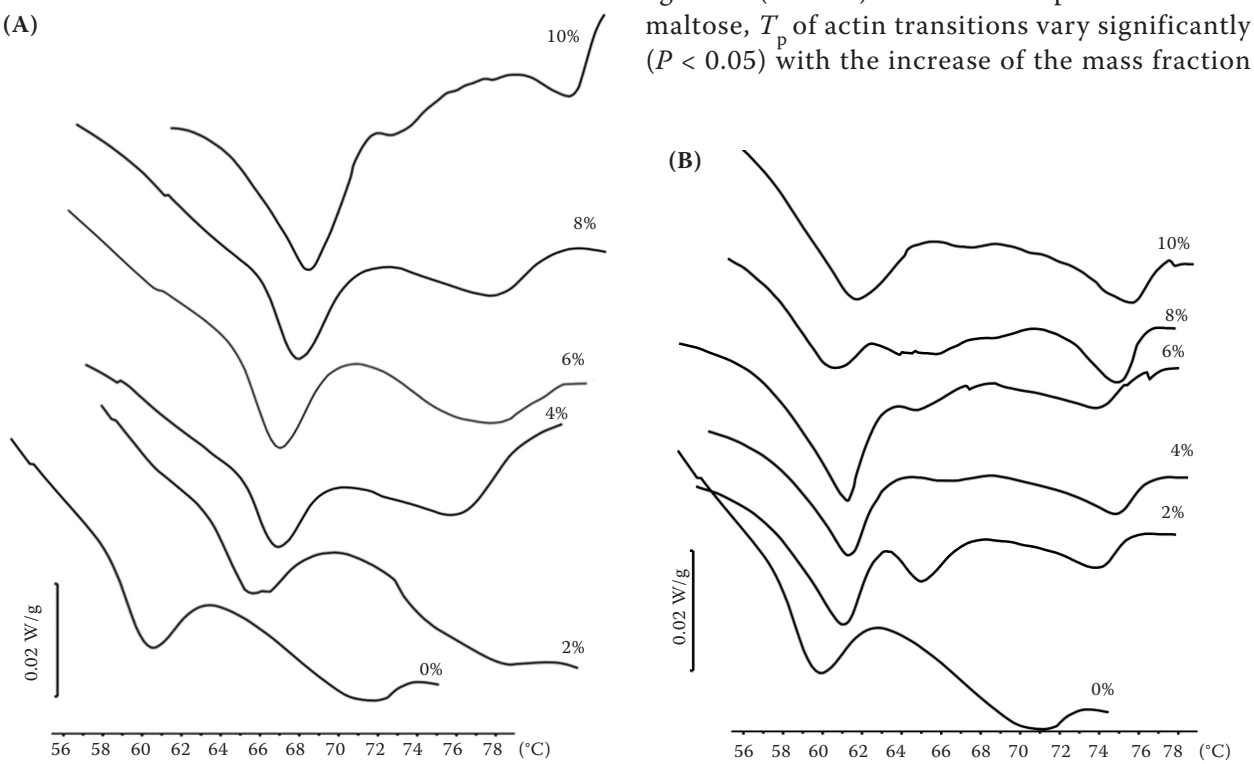

Figure 1. DSC thermograms of WBM stored for 360 days at –30°C as a function of mass fraction of trehalose(**A**) and maltose (**B**)

Table 2. Temperatures of thermal transition (T_p) of myosin and actin during frozen storage

Trehalose (%)	Days of frozen storage				
	0	30	90	180	360
Myosin					
0	$62.24^{aA} \pm 0.19$	$61.17^{aC} \pm 0.21$	$62.15^{a\,B} \pm 0.30$	$60.24^{aD} \pm 0.10$	$60.23^{aD} \pm 0.33$
2	$65.69^{b} \pm 0.10$	$64.79^{b} \pm 0.08$	$65.04^{b} \pm 0.29$	$64.97^{b} \pm 0.10$	$64.27^{b} \pm 0.05$
4	$66.27^{cA} \pm 0.11$	$65.27^{cD} \pm 0.07$	$65.56^{c\,B} \pm 0.04$	$65.28^{cC} \pm 0.05$	$65.20^{cE} \pm 0.11$
6	$66.71^{dA} \pm 0.04$	$65.81^{dC} \pm 0.29$	$65.73^{cdB} \pm 0.24$	$65.66^{dC} \pm 0.11$	$65.77^{dD} \pm 0.01$
8	$66.96^{eA} \pm 0.08$	$66.31^{eA} \pm 0.10$	$65.83^{cdA} \pm 0.05$	$66.16^{eA} \pm 0.02$	$66.97^{eA} \pm 0.05$
10	$67.23^{fA} \pm 0.12$	$66.60^{eB} \pm 0.28$	$67.12^{d\,D} \pm 0.07$	$67.46^{fC} \pm 0.09$	$67.29^{fA} \pm 0.11$
Actin					
0	$73.22^{aA} \pm 0.12$	$71.89^{aB} \pm 0.37$	$73.38^{aB} \pm 0.09$	$72.81^{aC} \pm 0.45$	$71.74^{aB} \pm 0.32$
2	$74.71^{bA} \pm 0.03$	$73.83^{bB} \pm 0.06$	$73.87^{b\,B} \pm 0.13$	$72.92^{aD} \pm 0.14$	$73.24^{bC} \pm 0.04$
4	$75.26^{cA} \pm 0.12$	$73.95^{bD} \pm 0.14$	$74.12^{cC} \pm 0.07$	$73.31^{bE} \pm 0.42$	$74.46^{cB} \pm 0.08$
6	$76.38^{dA} \pm 0.08$	$74.58^{cC} \pm 0.24$	$74.50^{dD} \pm 0.08$	$74.21^{cE} \pm 0.16$	$74.86^{dB} \pm 0.09$
8	$76.55^{eA} \pm 0.11$	$75.36^{dB} \pm 0.13$	$74.82^{eD} \pm 0.04$	$74.66^{dE} \pm 0.29$	$75.32^{eC} \pm 0.11$
10	$76.88^{fA} \pm 0.04$	$75.43^{dC} \pm 0.30$	$75.09^{fE} \pm 0.12$	$75.19^{eD} \pm 0.13$	$76.03^{fB} \pm 0.17$

Values are means ± SD of triplicate; values in the same column with different superscripts a–f and A-F are significantly different ($P < 0.05$)

(Table 3). T_p of myosin shows a greater shift due to the increase of the mass fractions of trehalose and maltose then does T_p of actin with all samples and all time intervals (Tables 2 and 3) (Sych *et al*. 1990; Herrera *et al*. 2001). The method of expressing peak enthalpies ΔH was adopted to provide an estimate of the quantity of native proteins. Enthalpies of myosin and actin transitions for WBM samples with the addition of trehalose and maltose during 360 days of frozen storage are shown in Figures 4–6. The values of ΔH of myosin and actin showed a decrease with the increase of

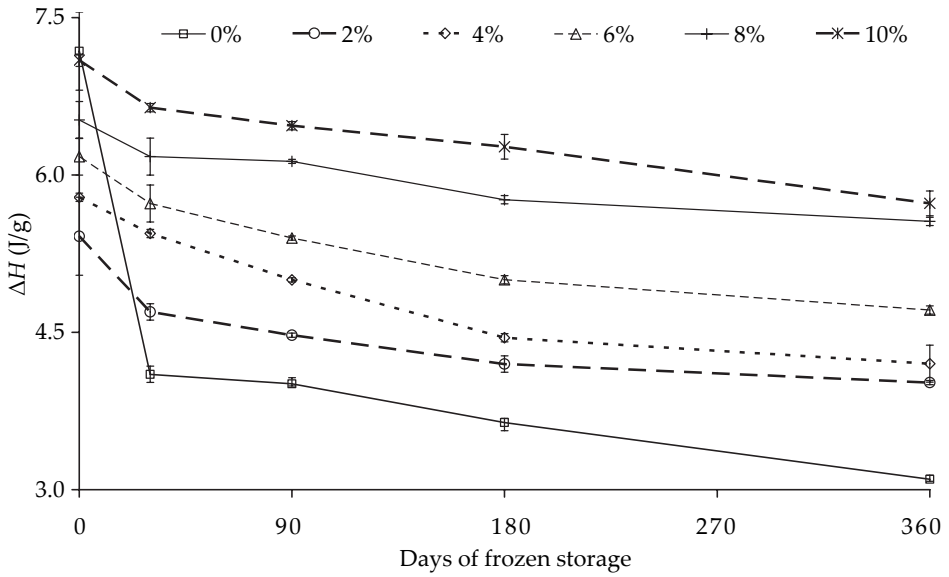

Figure 2. Changes in enthalpy of WBM myosin as a function of mass fraction of trehalose and frozen storage time

Table 3. Temperatures of thermal transition (T_p) of myosin and actin during frozen storage

Maltose (%)	Days of frozen storage				
	0	30	90	180	360
Myosin					
0	$62.24^{aA} \pm 0.19$	$61.17^{aC} \pm 0.21$	$62.15^{aB} \pm 0.30$	$60.24^{aE} \pm 0.10$	$60.23^{aD} \pm 0.33$
2	$62.48^{bA} \pm 0.28$	$61.25^{aC} \pm 0.08$	$62.21^{aB} \pm 0.03$	$60.65^{aD} \pm 0.08$	$60.84^{bE} \pm 0.08$
4	$62.73^{cA} \pm 0.30$	$61.41^{bC} \pm 0.04$	$62.42^{b\,B} \pm 0.20$	$61.04^{bD} \pm 0.08$	$61.26^{cD} \pm 0.04$
6	$63.04^{d} \pm 0.16$	$61.84^{c} \pm 0.11$	$62.86^{c} \pm 0.53$	$61.77^{c} \pm 0.05$	$61.74^{d} \pm 0.11$
8	$63.53^{eA} \pm 0.12$	$62.08^{dE} \pm 0.06$	$63.17^{dB} \pm 0.35$	$62.16^{dD} \pm 0.57$	$62.56^{eC} \pm 0.06$
10	$63.83^{fC} \pm 0.07$	$63.97^{eA} \pm 0.05$	$63.87^{eB} \pm 0.07$	$63.06^{eD} \pm 0.06$	$63.27^{fE} \pm 0.05$
Actin					
0	$73.22^{aA} \pm 0.12$	$71.89^{aB} \pm 0.37$	$72.08^{aB} \pm 0.09$	$71.01^{a\,C} \pm 0.45$	$71.74^{aB} \pm 0.32$
2	$73.24^{aA} \pm 0.05$	$72.16^{aC} \pm 0.07$	$72.24^{aB} \pm 0.09$	$71.23^{aE} \pm 0.04$	$72.06^{bD} \pm 0.09$
4	$72.34^{bA} \pm 0.17$	$73.09^{bA} \pm 0.03$	$72.71^{bA} \pm 0.06$	$72.32^{aA} \pm 0.05$	$72.44^{cA} \pm 0.07$
6	$72.81^{cE} \pm 0.10$	$73.73^{cA} \pm 0.18$	$73.02^{cC} \pm 0.10$	$72.87^{bD} \pm 0.15$	$73.16^{dB} \pm 0.12$
8	$73.65^{dC} \pm 0.11$	$74.42^{dA} \pm 0.10$	$73.51^{dD} \pm 0.02$	$73.51^{cD} \pm 0.26$	$73.93^{eB} \pm 0.10$
10	$74.20^{eC} \pm 0.04$	$74.88^{eA} \pm 0.05$	$73.74^{eE} \pm 0.09$	$73.91^{dD} \pm 0.15$	$74.65^{fB} \pm 0.05$

Values are means ± SD of triplicate; values in the same column or row with different superscripts a–f and A–F are significantly different ($P < 0.05$)

storage time (Herrera *et al.* 2001; Stangierski & Kijowski 2008). The highest decreases of ΔH of myosin and actin in all samples occurred in the first 30 days of frozen storage, especially in the sample without any additive. The values of ΔH for myosin and actin showed an increase with the increase of the mass fractions of trehalose and of maltose with the exception of day 0, which is in agreement with the results for mechanically deboned poultry meat mixed with different additives as reported by Stangierski and Kijowski (2008). The sample of WBM without the addition of trehalose after 360 days of frozen storage showed 56.95% and 55.61% reduction of myosin and actin

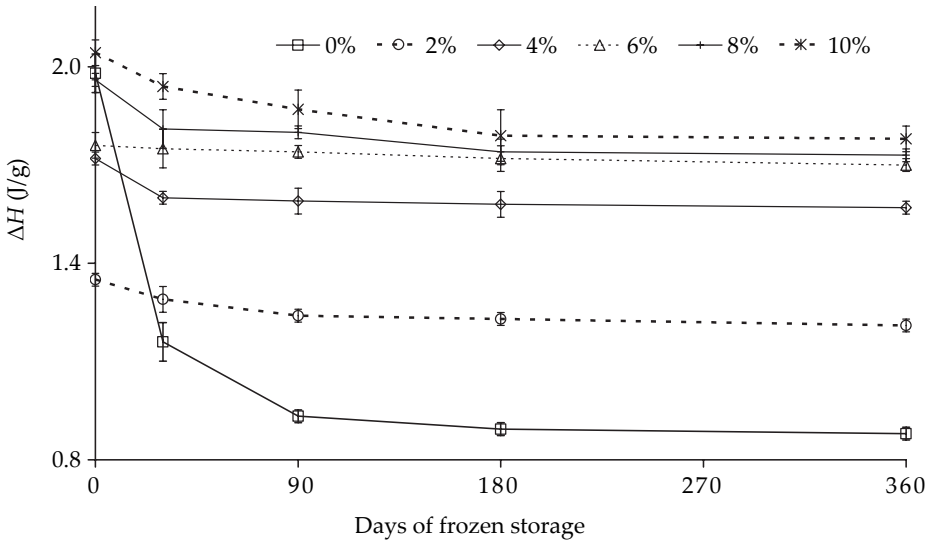

Figure 3. Changes in enthalpy of WBM actin as a function of mass fraction of trehalose and frozen storage time

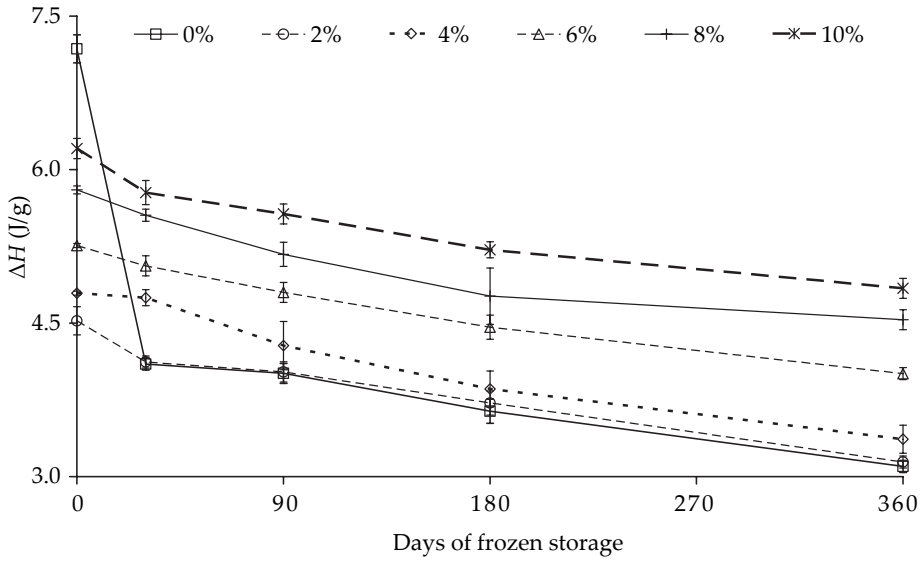

Figure 4. Changes in enthalpy of WBM myosin as a function of mass fraction of maltose and frozen storage time

ΔH. The samples with 10% of trehalose and of maltose after 360 days of frozen storage showed the reduction of ΔH of only 19.26% and 12.74% in the case of myosin and 21.93% and 12.82% on the case of actin. These results indicated that myosin is influenced to a greater extent by the addition of trehalose and maltose and exhibits higher freezing and frozen storage damage in comparison with actin (Sych *et al*. 1990). In general, the sample of WBM without the additions of trehalose and maltose showed the highest decrease in myosin and actin ΔH throughout 360 days of frozen storage (Sych *et al*. 1990; Herrera *et al*. 2001; Stangierski & Kijowski 2003, 2008). The highest values of transition enthalpies were observed in the samples mixed with 10% of trehalose, for all frozen storage time intervals.

ΔH of myosin varied significantly ($P < 0.05$) as a function of the mass fraction of trehalose and that of maltose, and as a function of frozen storage time (Figures 3 and 5). With actin, ΔH varied significantly ($P < 0.05$) as a function of the mass fraction of trehalose and that of maltose (Figures 3 and 5) and as a function of frozen storage time.

The results of this study presented above indicated that it is possible to reduce the negative effects of freezing and of frozen storage on the functional properties of washed beef myofibrillar proteins by the addition of trehalose and maltose.

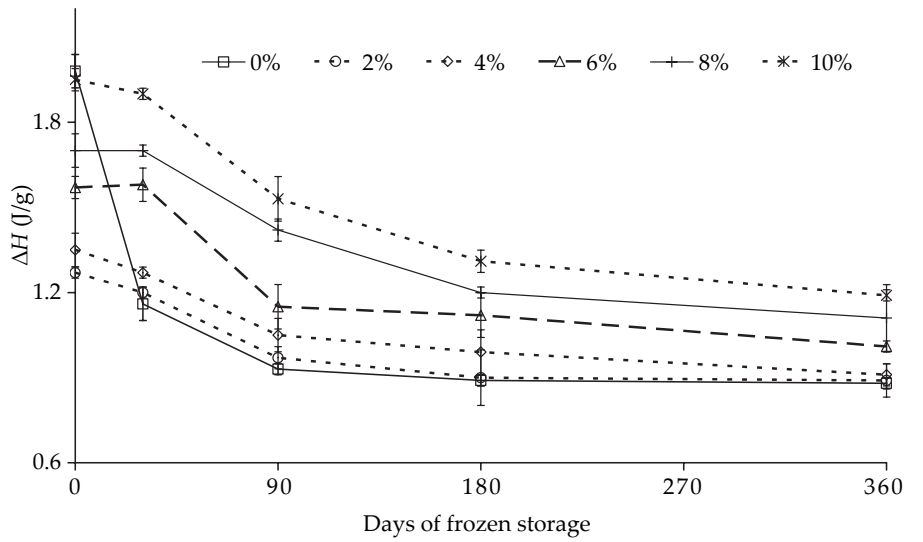

Figure 5. Changes in enthalpy of WBM actin as a function of mass fraction of maltose and frozen storage time

CONCLUSIONS

Differential scanning calorimetry and SEP of WBM shows that the addition of trehalose and maltose results in stabilisation of washed beef myofibrillar proteins during frozen storage.

The smaller loss of myofibrillar protein solubility, shift in thermal transition temperatures of myosin and actin to higher temperatures and increase of enthalpies of myosin and actin transitions as the mass fractions of trehalose and maltose increase, prove that both trehalose and maltose act according to the cryoprotecting mechanism and interact with beef myofibrillar proteins. The higher values of transition temperatures and denaturation enthalpies indicated that trehalose has a better cryoprotective effect on beef myofibrillar protein during frozen storage than maltose.

References

Aktas N., Aksu M.I., Kaya M. (2005): Changes in myofibrillar proteins during processing of pastirma (Turkish dry meat product) produced with commercial starter cultures. Food Chemistry, **90**: 649–654.

AOAC (2007). Determination of Fat, Moisture and Protein in Meat and Meat Products by Spectroscopy/Near Infrared Spectroscopy. 2007.04. In: Official Methods of Analysis. 18th Ed. Association of Official Analytical Chemists, Gaithersburg.

Auh J.H., Lee H.G., Kim J.W., Kim J.C., Yoon H.S., Park K.H. (1999): Highly concentrated branched oligosaccharides as cryoprotectant for surimi. Journal of Food Science, **64**: 418–422.

Barbut S., Findlay C.J. (1991): Influence of sodium, potassium and magnesium-chloride on thermal-properties of beef muscle. Journal of Food Science, **56**: 180–182.

Bircan C., Barringer S.A. (2002): Determination of protein denaturation of muscle foods using the dielectric properties. Journal of Food Science, **67**: 202–205.

Brunton N.P., Lyng J.G., Zhang L., Jacquier,J.C.(2006): The use of dielectric properties and other physical analyses for assessing protein denaturation in beef biceps femoris muscle during cooking from 5 to 85 degrees C. Meat Science, **72**: 236–244.

Colaco C.A.L.S., Smith C.J.S., Sen S., Roser D.H., Newman Y., Ring S., Roser B.J. (1994): Chemistry of protein stabilization by trehalose. Formulation and Delivery of Proteins and Peptides, **567**: 222–240.

Fernandez-Martin F. (2007): Bird muscles under hydrostatic high-pressure/temperature combinations – A DSC evaluation. Journal of Thermal Analysis and Calorymetry, **87**: 285–290.

Findlay C.J., Barbut S.(1990): Thermal analysis of food proteins in relation to processing data. In: Harwalkar V.R., Ma C.-Y. (eds): Thermal Analysis of Food. Elsevier Science Publishers Ltd., Barking: 92–125.

Herrera J.J., Pastoriza L., Sampedro G. (2001): A DSC study on the effects of various maltodextrins and sucrose on protein changes in frozen-stored minced blue whiting muscle. Journal of the Science of Food and Agriculture, **81**: 377–384.

Kijowski J., Richardson R.I. (1996): The effect of cryoprotectants during freezing or freeze drying upon properties of washed mechanically recovered broiler meat. International Journal of Food Science Technology, **31**: 45–54.

Lesiow T., Xiong Y.L.L. (2001): Mechanism of rheological changes in poultry myofibrillar proteins during gelation. Avian and Poultry Biology Reviews, **12**: 137–149.

Leslie S.B., Israeli E., Lighthart B., Crowe J.H., Crowe L.M. (1995): Trehalose and sucrose protect both membranes and proteins in intact bacteria during drying. Applied and Environmental Microbiology, **61**: 3592–3597.

Li C.T., Wick M. (2001): Improvement of the physicochemical properties of pale soft and exudative (PSE) pork meat products with an extract from mechanically deboned turkey meat (MDTM). Meat Science, **58**: 189–195.

MacDonald G.A., Lanier T. (1991): Carbohydrates as cryoprotectants for meats and surimi. Food Technology., **45**: 150–159.

Miller D.P., Anderson R.E., de Pablo J.J. (1998): Stabilization of lactate dehydrogenase following freeze-thawing and vacuum-drying in the presence of trehalose and borate. Pharmaceutical Research, **15**: 1215–1221.

Osako K., Hossain M.A., Kuwahara K., Nozaki Y. (2005): Effect of trehalose on the gel-forming ability, state of water and myofibril denaturation of horse mackerel *Trachurus japonicus* surimi during frozen storage. Fisheries Science, **71**: 367–373.

Park J.W. (1994): Functional protein additives in surimi gels. Journal of Food Science, **59**: 525–527.

Park J.W., Lanier T.C., Green D.P. (1988): Cryoprotective effects of sugar, polyols, and or phosphates on Alaska pollack surimi. Journal of Food Science, **53**: 1–3.

Park J.W., Lanier T.C., Pilkington D.H. (1993): Cryostabilization of functional-properties of pre-rigor and post-rigor beef by dextrose polymer and or phosphates. Journal of Food Science, **58**: 467–472.

Park S., Brewer M.S., Novakofski J., Bechtel P.J., McKeith K.F. (1996): Process and characteristic for a surimi-like material made from beef or pork. Journal of Food Science, **61**: 422–427.

Patist A., Zoerb H. (2005): Preservation mechanisms of trehalose in food and biosystems. Colloids and Surfaces B: Biointerfaces, **40**: 107–113.

Stangierski J., Kijowski J. (2003): Effect of selected commercial substances with cryoprotective activity on the quality of mechanically recovered, washed and frozen stored poultry meat. Nahrung-Food, **47**: 49–53.

Stangierski J., Kijowski J. (2008): Effect of selected substances on the properties of frozen myofibril preparation obtained from mechanically recovered poultry meat. European Food Research and Technology, **226**: 1415–1420.

Sych J., Lacroix C., Adambounou L.T., Castaigne F. (1990): Cryoprotective effects of lactitol, palatinit and polydextrose on cod surimi proteins during frozen storage. Journal of Food Science, **55**: 356–360.

Sych J., Lacroix C., Carrier M. (1991): Determination of optimal level of lactitol for surimi. Journal of Food Science, **56**: 285–290.

Wright D.J., Wilding P. (1984): Differential scanning calorimetric study of muscle and its proteins: myosin and its subfragments. Journal of the Science of Food and Agriculture, **35**: 357–372.

Xiong Y.L., Brekke C.J., Leung H.K. (1987): Thermal-denaturation of muscle proteins from different species and muscle types as studied by differential scanning calorimetry. Canadian Institute of Food Science and Technology Journal. Journal de l'Institut canadien de science et technologie alimentaires, **20**: 357–362.

Yang T.S., Froning G.W. (1994):Evaluation of protein functionality in alkali and nonalkali surimi processed mechanically deboned chicken meat. Journal of Muscle Food, **5**: 221–232.

Zhou A.M., Benjakul S., Pan K., Gong H., Liu X. (2006): Cryoprotective effects of trehalose and sodium lactate on tilapia (*Sarotherodon nilotica*) surimi during frozen storage. Food Chemistry, **96**: 96–103.

Received for publication October 28, 2009
Accepted after corrections June 1, 2010

Corresponding author:

Dr. Sc. Krešimir Mastanjević, University of J. J. Strossmayer in Osijeku, Faculty of Food Technology, Franje Kuhača 20, 31 000 Osijek, Croatia
tel.: + 385 31 224 300, e-mail: kresimir.mastanjevic@ptfos.hr