Cryoprotective effect of trehalose on washed chicken meat

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Abstract The cryoprotective effects of trehalose ($w=0$ –10%) on washed chicken meat (WCM) were investigated. WCM was produced from broiler, frozen and stored for 360 days on -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 caused smaller decrease in protein solubility during frozen storage. Peak thermal transition (T_p) and denaturation enthalpy (ΔH) of myofibrillar proteins were evaluated. Differential scanning calorimetry (DSC) revealed a shift in peak thermal transition temperature (T_p) of myosin and actin to higher temperature as the mass fraction of trehalose increases. The transitions enthalpies of myosin and actin of WCM samples showed higher increase with the increase of mass fraction of trehalose. Since the value of denaturation enthalpy is directly related to amount of native proteins, higher values of ΔH indicates to the higher cryoprotective effects of trehalose.

Keywords Thermal transitions temperature · Cryoprotection · DSC · SEP · Washed chicken meat · Trehalose

Introduction

Washed chicken meat (WCM), produced with modified technology from fish surimi, Dawson et al. (1988), and are

characterized by very good technological properties, such as high water holding capacity and high ability to form strong gels after being heated. The most frequently used food preservation technique for this kind of food product is freezing. To protect myofibrillar proteins from freeze-denaturation and during frozen storage and maintain its possibly high processability, cryoprotectants, such as disaccharides, polysaccharides, polyalcohol's, acids, polyphosphates are generally added (Park et al. 1988; MacDonald and Lanier 1991). Most commonly used methods for determination cryoprotective effects of added substances are measurement of myofibrillar protein solubility SEP (Salt extractable protein), 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 1994; Kijowski and Richardson 1996; Stangierski and Kijowski 2003; Day and Dora 2010; Nopianti et al. 2011). Differential scanning calorimetry (DSC) is a useful technique for studying thermal behaviour of muscle proteins (Barbut and Findlay 1991). Changes in the protein structure during DSC analysis are referred as transition changes, and peak temperatures at these transitions are used to represent transition temperatures (Findlay and Barbut 1990).

Trehalose (D-glucopyranosyl- α (1 $\rightarrow$ 1)-D-glucopyranoside) is a non-reducing disaccharide with low caloric value and low sweetness, only 45% of that of sucrose (Colaco et al. 1994). Because of its ability to form strong hydrogen bonds with the polar group of the biomolecules and higher glass transition temperature, trehalose has superior preservation properties as compared to other sugars (Patist and Zoerb 2005). Trehalose has been found to have protective effect against thermal inactivation of enzymes (Miller et al. 1998) and freeze-drying of microorganisms (Leslie et al.

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1995). Osako et al. (2005) investigated gel forming ability, unfrozen water content, Ca^{2+} ATPase activity of horse mackerel surimi, during freezing and frozen storage, upon addition of trehalose, sucrose, glucose and sorbitol and concluded that trehalose had similar cryoprotective effect as sucrose and sorbitol. However, Zhou et al. (2006) reported that addition of 8% of trehalose to fish myofibrillar proteins (tilapia) had better cryoprotective effect, especially on salt extractable protein (SEP), total sulfhydryl and disulfhydryl bond content and gel forming ability over 24 weeks of frozen then 8% of sucrose/sorbitol (1:1) mixture. So far, no information regarding the cryoprotective effect of trehalose on chicken myofibrillar proteins has been reported. The purpose of this work is to investigate with Differential scanning calorimetry (DSC) and measurement of myofibrillar protein solubility SEP (Salt extractable protein), cryoprotective effects of trehalose on washed chicken meat (WCM).

Material and methods

Samples of washed chicken meat (WCM) were prepared in the laboratory from broiler (mainly lat. *Pectoralis major M.* and *Pectoralis minor M.*) by the procedure of Yang and Froning (1992) with modifications. Instead tap water, distilled water was used for washing and leaching. Samples were mixed with trehalose ($w=0\text{--}10\%$). Mass fractions were determined as percent of total mass. The pH level was measured in a homogenate of the sample with distilled water (1:10) with pH/Ion 510 – Bench pH/Ion/mV Meter (Eutech Instruments Pte Ltd/Oakton Instruments, 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. 04. (2007). Samples were packed in polyethylene bags and stored at $-30\text{ }^{\circ}\text{C}$. Freeze denaturation was evaluated after 30, 90, 180 and 360 days by salt extractable protein (SEP) and differential scanning calorimetry (DSC) analysis. Initial measurements were conducted in WCM samples before freezing.

Salt soluble proteins (SEP)

Soluble proteins were extracted by the procedure of Li and Wick, Li and Wick (2001) with modifications. 1 g of sample with 6 ml Standard brine STB solution Li and Wick (2001), was mixed with vortex mixer (Vibromix 10, Tehnica, Slovenia) at $4\text{ }^{\circ}\text{C}$ for 30 min. The salt soluble proteins were recovered in the supernatant following centrifugation at $10\,000\times g$, $4\text{ }^{\circ}\text{C}$, 15 min. in a Heraeus Multifuge 3 L-R. The Bio-Rad Protein Assay (Bio-Rad

Laboratories) was used to estimate protein concentration in the resulting supernatants using bovine albumin as a protein standard. Percent salt extractable protein (SEP) was expressed as the concentration of salt extractable protein (mgml^{-1}), estimated by Bio-Rad analysis.

DSC measurements

Differential scanning calorimetry (DSC) was performed on Mettler Toledo DSC 822e differential scanning calorimeter equipped with STAR^c software. Samples of ca. 15 mg ($\pm 1\text{ mg}$) weighed and sealed into standard aluminium pans ($40\text{ }\mu\text{l}$) and scanned over the range from 25 to $95\text{ }^{\circ}\text{C}$ at a heating rate of $10\text{ }^{\circ}\text{Cmin}^{-1}$, using empty standard aluminium pan as a reference. The peak temperatures (T_p) were determined from DSC curves. The changes in enthalpy ($\Delta H\text{ Jg}^{-1}$), associated with the denaturation of proteins, were determined by measuring the area under the DSC curves using STAR^c software. Transition enthalpies were expressed on the total mass fraction of protein.

Statistical analysis

Three determinations for basic chemical composition, pH, a_w , peak temperatures (T_p), transition enthalpies and SEP were measured from each sample. Experimental data were analyzed 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 ver. 7.0 StatSoft Inc. Tulsa, OK. USA.

Results and discussion

The mean basic chemical composition, pH and a_w values of individual samples of WCM did not vary significantly and amounted to $86.17\%\pm 0.58$ water, $13.06\%\pm 0.58$ protein, $0.73\%\pm 0.07$ fat, 6.95 ± 0.04 pH and $0.98\pm 0.01\text{ }a_w$.

Salt soluble proteins (SEP)

The changes in salt extractable protein (SEP) of WCM mixed with trehalose ($w=0\text{--}10\%$) during frozen storage at $-30\text{ }^{\circ}\text{C}$ are shown in Fig. 1. The highest decreases of SEP in all samples were in the first 30 days, especially for sample without addition trehalose. SEP of the control sample after 360 day of frozen storage was reduced to 53.85% of initial value for fresh WCM, which is higher compared with results reported by Zhou et al. (2006) which reported 44.8% loss of fish (tilapia) protein solubility after 24 weeks of frozen storage, which can be explained by the nature of sample. For WCM samples with addition of 2, 4, 6, 8 and 10% of trehalose SEP of 4.10, 4.24, 4.55, 4.85 and 5.05 mgml^{-1}

Fig. 1 Changes in salt extractable protein (SEP) of washed chicken meat (WCM) mixed with trehalose (%) as a function of storage time at -30°C (mean $\pm$ standard deviation of three samples)

were observed, and were decreased to 57.34, 59, 46, 67.91, 68.40 and 70.72 of its initial SEP values, respectively. From the SEP results it can be noted that addition of trehalose in all mass fraction resulted with lowered SEP, compared with the control at day 0, this can be explained by the dilution effect of trehalose on the protein content in WCM.

Generally, control sample showed largest SEP loss throughout the storage up to 360 days, samples with 2, 4, 6, 8 and 10% of trehalose showed smaller loss of salt protein solubility (significant at $P < 0.05$) as the mass of trehalose increases during frozen storage for all frozen time intervals (Fig. 1). Myofibrillar protein denaturation frozen storage expressed by the loss of protein solubility during is a result of formation of hydrogen or hydrophobic bonds, as well as disulfide bonds and ionic interaction (Sych et al. 1990; MacDonald and Lanier 1991; Sych et al. 1991; Auh et al. 1999).

Differential scanning calorimetry

Differential scanning calorimetry thermogram's of WCM mixed with trehalose ($w=0$ –10%) after 360 days of frozen storage are illustrated in Fig. 2. WCM thermogram normally contained two endothermic transitions. Referring to previous DSC studies of similar samples (Wright and Wilding 1984; Sych et al. 1990; Barbut and Findlay 1991; Kijowski and Richardson 1996; Fernandez-Martin 2007) it can be assumed that two peaks in this study are related to the thermal denaturation of myosin and actin. Denaturation temperatures (T_p) of myosin and actin for WCM with trehalose ($w=0$ –10%) at 0, 30, 90, 180 and 360 day of frozen storage are presented in Table 1. Values of denaturation temperatures (T_p) of myosin and actin were different then values of raw chicken breast meat reported by

Fig. 2 Differential scanning calorimetry thermograms (DSC) of washed chicken meat stored for 360 days at -30°C as a function of mass fraction of trehalose (%) (mean of three samples)

Kijowski and Mast (1988), Murphy et al. (1998) and Bircan and Barringer (2002). Similar results was reported by Kijowski and Richardson (1996) for washed mechanically deboned poultry meat, this could be explained by concentration of myofibrillar protein by washing and different pH and ionic environment when compared to the raw state of muscle (Wright and Wilding 1984; Xiong et al. 1987; Lesiow and Xiong 2001). Analysis of variance of myosin T_p showed that myosin's T_p varied significantly ($P < 0.05$) as a function of mass fraction of trehalose, but not as of frozen storage time (Sych et al. 1990; Herrera et al. 2001) (Table 1). These shifts in T_p of myosin to the higher values as the mass fraction of trehalose increases can be interpreted as a stabilization of myofibrillar proteins since a higher temperature was required to denature these proteins (Sych et al. 1990; Sych et al. 1991). Highest shift in T_p of myosin shows the samples of WCM mixed with 10% of trehalose for all storage time intervals. T_p of actin transitions vary significantly ($P < 0.05$) with addition of trehalose but not with frozen storage time (Table 1). T_p of myosin shows higher shift by increase of mass fraction of trehalose then a T_p of actin for all samples and all time intervals (Table 1) (Sych et al. 1990; Sych et al. 1991). The method of expressing peak enthalpies ΔH was adopted to provide an estimate of the quantity of native proteins. Denaturation enthalpies of myosin and actin for WCM samples with addition of trehalose ($w=0$ –10%), during 360 days of frozen storage, are shown in Table 2. The values of ΔH of myosin and actin showed decrease with the increase of storage time (Sych et al. 1990; Herrera et al. 2001; Stangierski and Kijowski 2003), highest decreases of

Table 1 Denaturation temperatures (T_p) (°C) of myosin and actin of washed chicken meat (WCM) samples

Trehalose (%)	Storage time (days)				
	0	30	90	180	360
Myosin					
0	60.9 ^a ±0.10	59.9 ^a ±0.14	55.4 ^a ±0.03	55.0 ^a ±0.30	55.0 ^a ±0.30
2	62.8 ^a ±0.11	60.8 ^b ±0.04	60.5 ^b ±0.09	61.5 ^b ±0.32	61.5 ^b ±0.32
4	63.3 ^b ±0.03	61.8 ^c ±0.05	60.9 ^c ±0.12	62.3 ^c ±0.09	62.3 ^c ±0.09
6	63.5 ^c ±0.09	62.2 ^d ±0.03	61.7 ^d ±0.33	62.6 ^d ±0.09	62.6 ^d ±0.09
8	63.8 ^d ±0.07	62.4 ^e ±0.07	62.2 ^e ±0.12	63.1 ^e ±0.12	63.0 ^e ±0.12
10	66.2 ^e ±0.14	63.1 ^f ±0.07	62.8 ^f ±0.09	63.7 ^f ±0.10	63.7 ^f ±0.10
Actin					
0	69.0 ^a ±0.07	71.3 ^a ±0.34	70.7 ^a ±0.11	70.6 ^a ±0.19	70.6 ^a ±0.19
2	72.2 ^a ±0.08	71.7 ^a ±0.56	71.9 ^b ±0.05	72.3 ^b ±0.03	72.3 ^b ±0.03
4	72.3 ^a ±0.08	72.4 ^b ±0.19	72.8 ^c ±0.12	72.7 ^c ±0.03	72.7 ^c ±0.03
6	72.8 ^b ±0.10	73.1 ^c ±0.23	73.4 ^d ±0.13	73.1 ^d ±0.12	73.1 ^d ±0.12
8	73.6 ^c ±0.10	73.7 ^d ±0.15	73.6 ^e ±0.05	73.6 ^e ±0.36	73.6 ^e ±0.36
10	74.0 ^d ±0.12	74.1 ^e ±0.29	73.7 ^{ef} ±0.14	74.3 ^{ef} ±0.05	74.3 ^{ef} ±0.05

Values are means±SD of triplicate. Values in the same column with different superscripts (a–f) are significantly different ($P<0.05$)

ΔH of myosin and actin in all samples were in the first 30 days, especially for sample without additive. Values of ΔH for myosin and actin showed increase with the increase of mass fraction of trehalose, with the exceptions of day 0, which is in agreement with the results reported by Stangierski and Kijowski (2008). Sample of WCM without addition of trehalose after 360 days of frozen storage showed 46.15 and 28.17% reduction of myosin's and actins' ΔH . Samples with 10% of trehalose after 360 days of frozen storage showed only 29.27% reduction of myosin's and 9.40% actins reduction of ΔH . These results indicated that myosin is more influenced by addition of trehalose and exhibits higher freezing and frozen storage

damage than actin (Sych et al. 1990). In general, sample of WCM without addition of trehalose showed highest decrease in myosin's and actins ΔH throughout 360 days of frozen storage. The highest values of transition enthalpies showed samples mixed with 10% of trehalose, for all frozen storage time intervals.

ΔH of myosin and actin for WCM samples varied significantly ($P<0.05$) as a function of mass fraction of trehalose and as a function of frozen storage time (Table 2).

Results of these study presented above indicated that is possible to reduce negative effects of freezing and frozen storage on the functional properties of chicken myofibrillar proteins by the addition of trehalose.

Table 2 Denaturation enthalpies of myosin and actin of washed chicken meat (WCM) samples (Jg^{-1} of protein)

Trehalose (%)	Storage time (days)				
	0	30	90	180	360
Myosin					
0	7.15 ^{fA} ±0.02	4.33 ^{aB} ±0.03	4.16 ^{aC} ±0.04	4.04 ^{aD} ±0.06	3.85 ^{aE} ±0.03
2	5.17 ^{aA} ±0.03	5.06 ^{bB} ±0.04	4.35 ^{bC} ±0.03	4.22 ^{bD} ±0.01	4.10 ^{bE} ±0.02
4	5.65 ^{bA} ±0.01	5.13 ^{bcB} ±0.04	4.65 ^{cC} ±0.01	4.32 ^{cD} ±0.04	4.24 ^{cE} ±0.01
6	6.34 ^{cA} ±0.03	5.22 ^{cB} ±0.02	4.98 ^{dC} ±0.01	4.84 ^{dD} ±0.01	4.55 ^{dE} ±0.09
8	7.09 ^{dA} ±0.01	5.35 ^{dB} ±0.10	5.16 ^{eC} ±0.01	4.99 ^{eD} ±0.06	4.85 ^{eE} ±0.02
10	7.14 ^{dA} ±0.01	6.17 ^{eB} ±0.02	5.36 ^{fC} ±0.02	5.23 ^{fD} ±0.02	5.05 ^{fE} ±0.06
Actin					
0	2.13 ^{eA} ±0.01	1.91 ^{aB} ±0.01	1.82 ^{aC} ±0.01	1.71 ^{aD} ±0.03	1.53 ^{aE} ±0.03
2	1.94 ^{aA} ±0.01	1.94 ^{bB} ±0.01	1.88 ^{bC} ±0.01	1.77 ^{bD} ±0.03	1.61 ^{bE} ±0.04
4	1.97 ^{bA} ±0.02	1.95 ^{cB} ±0.01	1.92 ^{cC} ±0.02	1.85 ^{cD} ±0.03	1.7 ^{cE} ±0.01
6	2.03 ^{cA} ±0.03	2.02 ^{dB} ±0.01	2.02 ^{dC} ±0.01	1.92 ^{dD} ±0.03	1.76 ^{dE} ±0.01
8	2.06 ^{dA} ±0.01	2.05 ^{eB} ±0.01	2.03 ^{deC} ±0.01	1.96 ^{dD} ±0.03	1.85 ^{eD} ±0.01
10	2.13 ^{eA} ±0.02	2.08 ^{fB} ±0.06	2.05 ^{efC} ±0.03	2.04 ^{eD} ±0.03	1.93 ^{fE} ±0.01

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

Conclusions

Differential scanning calorimetry and SEP of WCM shows that addition of trehalose cause's stabilization of myofibrillar proteins during frozen storage.

The smaller loss of myofibrillar protein solubility, the shift in thermal transition temperature of myosin and actin to higher temperature and increase of enthalpies of myosin and actin transition as the mass fraction of trehalose increases, approve that trehalose was acting according to the cryoprotecting mechanism and interacted with chicken myofibrillar proteins.

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