Cryoprotective effect of maltose on chicken myofibrillar proteins (CMP)

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Cryoprotective effect of maltose on chicken myofibrillar proteins (CMP)

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Summary

The cryoprotective effects of maltose on chicken myofibrillar proteins (CMP) were investigated. CMP were produced from broiler mixed with different mass fraction of maltose (w = 0 - 10 %), frozen and stored for 30 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 maltose caused smaller decrease in protein solubility after 30 days of frozen storage. Peak thermal transition temperatures (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 maltose increases. After 30 days of frozen storage transitions enthalpies (ΔH) of myosin and actin of CMP samples showed increase with the increase of mass fraction of maltose. 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 maltose on chicken myofibrillar proteins.

Keywords: thermal transitions temperatures, cryoprotection, maltose chicken myofibrillar proteins, DSC, SEP

Introduction

Washed chicken meat is surimi-like product made from chicken meat. The process for making surimi-like product from chicken, with modified technology from fish surimi (Dawson et al., 1996) results in semi-purified protein fraction containing a high concentration of myofibrillar proteins. Freezing has become one of the most frequently used preservation method for meat and meat products. To protect myofibrillar proteins from freeze-denaturation and during frozen storage and maintain its possible 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 instrumental methods for determination cryoprotective effects of added substances are measurement of myofibrillar protein solubility SEP (Salt

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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; Yang and Froning 1994; Kijowski and Richardson 1996; Stangierski and Kijowski 2008).
Maltose (D-glucopyranosyl-α (1→4)-D-glucopyranoside) is a reducing disaccharide. It has been found to have protective effect against thermal inactivation of enzymes (Kawai and Suzuki 2007) and freeze draying of microorganisms (Hamoudi et al., 2007).
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 maltose on chicken myofibrillar proteins (CMP).

Material and Methods

Samples of CMP were prepared in the laboratory from broiler (mainly *Pectoralis major M.* and *Pectoralis minor M*) by the procedure of Yang and Froning (Yang and Froning 1992) with modifications. Instead, tap water, distilled water was used for washing and leaching. Samples were mixed with maltose (w = 0 - 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, p/v) 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 (20 ± 2 ºC). 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, frozen, and stored at -30 °C. Denaturation due to freezing was evaluated after 30 days by salt extractable protein (SEP) and differential scanning calorimetry (DSC) analysis.

Salt soluble proteins (SEP)

Soluble proteins were extracted by the procedure of Li and Wick 2001 (Li and Wick 2001), with modifications. Sample of mass 1 g with 6 ml standard brine STB solution, was mixed with a vortex mixer (Vibromix 10, Tehtnica, Slovenia) at 4 ºC for 30 min. The salt soluble proteins were recovered in the supernatant following centrifugation at 10 000 × g, 4 ºC, 15 min in a Heraeus Multifuge 3L-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. Salt extractable protein (SEP) was expressed as the concentration of salt extractable protein (mg ml^{-1}), estimated by Bio-Rad analysis.

DSC measurements

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

Statistical analysis

Three determinations for basic chemical composition, pH, a_w, onset (T_o), peak (T_p) and endset (T_e) temperatures, denaturation enthalpies (ΔH) and SEP were measured for 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 average basic chemical composition, pH and a_w values of individual samples of CMP did not vary significantly and amounted to 86.17 % ± 0.58 water, 13.06 % ± 0.58 protein, 0.73 % ± 0.07 fat, 6.95 ± 0.04 pH and 0.98 ± 0.01 a_w.

Salt soluble proteins (SEP)

Myofibrillar protein denaturation during storage in the frozen state 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, Auh et al., 1999). The salt extractable protein (SEP) of CMP mixed with maltose (w = 0 - 10 %) after 30 days of frozen storage at -30 ºC are shown in Table 1. The highest values of SEP had a sample mixed with 10 % of maltose, and lowest the CMP sample without addition of maltose. SEP concentrations of CMP varied significantly ($p < 0.05$) with addition of maltose. The increase of SEP values with increase of mass fraction of maltose indicated possible cryoprotection effects of maltose on CMP.

Table 1. Salt extractable protein SEP ($mgml^{-1}$) of CMP as a function of mass fraction of maltose (w = 0 -10 %) after 30 days of frozen storage.

w (%)	SEP (mg ml^{-1})
0	$3.38^{a} \pm 0.14$
2	$4.72^{b} \pm 0.04$
4	$4.85^{bc} \pm 0.03$
6	$4.94^{b} \pm 0.02$
8	$5.30^{c} \pm 0.05$
10	$5.52^{c} \pm 0.39$

Values are means ±SD of triplicate. Values in the same row with different superscripts a-f and are significantly different ($p < 0.05$).

Differential scanning calorimetry

Differential scanning calorimetry thermogram's of CMP mixed with maltose after 30 days of storage at -30 °C are presented in Fig. 1.

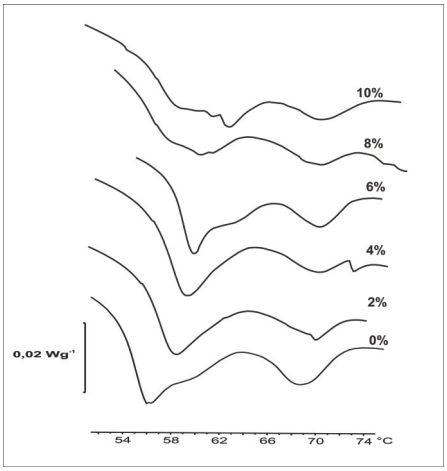

Fig. 1. DSC thermograms of CMP stored for 30 days at -30 °C as a function of mass fraction of maltose (w = 0 – 10 %)

CMP thermograms normally contained two endothermic transitions. Referring to previous DSC studies of similar samples (Kijowski and Richardson 1996; Fernandez-Martin 2007) it can be assumed that the two peaks are related to the thermal denaturation of myosin and actin. Onset (T_o), peak (T_p) and endset (T_e) of myosin and actin for CMP mixed with maltose

after 30 days of frozen storage are presented in Tables 2 and 3. Values of the peak thermal temperatures (T_p) of myosin and actin were different then values of raw chicken breast meat reported by Kijowski and Mast (1988), Murphy et al., (1998) and Bircan and Barringer (2002). 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 concentration of myofibrillar protein by washing and different pH and ionic environment when compared to the raw state of muscle (Lesiow and Xiong 2001). Analysis of variance showed that myosin's T_o, T_p and T_e varied significantly ($p < 0.05$) as a function of mass fraction of maltose (Table 2). Shifts in T_p of myosin to the higher values as the mass fraction of maltose increases can be interpreted as a stabilization of myofibrillar proteins since a higher temperature was required to denature these proteins (Sych et al., 1991; Herrera et al., 2001). Highest values of T_p of myosin shows the samples of CMP mixed with 10 % of maltose. T_p of actin transitions vary significantly ($p < 0.05$) with addition of maltose (Table 3). T_p of myosin shows higher shift by increase of mass fraction of maltose then a T_p of actin for all samples (Table 2 and 3) (Sych et al., 1990). 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 CMP samples with addition of maltose, after 30 days of frozen storage, are shown in Tables 2 and 3. Values of ΔH for myosin and actin showed increase with the increase of mass fraction of maltose, which is in agreement with the results reported by Stangierski and Kijowski (2008). The highest values of transition enthalpies showed samples mixed with 10 % of maltose. ΔH for myosin varied significantly ($p < 0.05$) as a function of mass fraction of maltose (Table 2). For actin, ΔH also varied significantly ($p < 0.05$) as a function of mass fraction of maltose (Table 3).

Results of these study presented indicate that is possible to reduce negative effects of frozen storage on the functional properties of chicken myofibrillar proteins by addition of maltose.

Table 2. Values of transitions temperatures (T_o, T_p, T_e) and denaturation enthalpies (ΔH) of CMP myosin mixed with different mass fractions of maltose (w = 0 - 10 %)

w (%)	T_o (ºC)	T_p (ºC)	T_e (ºC)	ΔH (J g^{-1})
0	$50.05^{a} \pm 0.09$	$55.38^{a} \pm 0.03$	$60.39^{a} \pm 0.05$	$4.01^{a} \pm 0.02$
2	$50.43^{a} \pm 0.60$	$55.77^{b} \pm 0.15$	$60.46^{a} \pm 0.21$	$4.16^{a} \pm 0.01$
4	$51.62^{b} \pm 0.36$	$56.25^{c} \pm 0.06$	$61.14^{b} \pm 0.10$	$4.22^{b} \pm 0.03$
6	$52.13^{bc} \pm 0.09$	$56.77^{d} \pm 0.11$	$61.50^{c} \pm 0.16$	$4.76^{c} \pm 0.05$
8	$52.63^{c} \pm 0.27$	$57.66^{e} \pm 0.19$	$61.92^{d} \pm 0.13$	$4.91^{d} \pm 0.06$
10	$53.50^{d} \pm 0.20$	$58.41^{f} \pm 0.17$	$62.54^{e} \pm 0.19$	$5.75^{e} \pm 0.02$

Values are means ±SD of triplicate. Values in the same row with different superscripts a-f and are significantly different ($p < 0.05$).

Table 3. Values of transitions temperatures (T_o, T_p, T_e) and denaturation enthalpies (ΔH) of CMP actin mixed with different mass fractions of maltose (w = 0 - 10 %)

w (%)	T_o (ºC)	T_p (ºC)	T_e (ºC)	ΔH (J g^{-1})
0	$66.23^{a} \pm 0.04$	$70.73^{a} \pm 0.11$	$71.87^{a} \pm 0.04$	$1.21^{a} \pm 0.01$
2	$66.87^{b} \pm 0.13$	$71.05^{b} \pm 0.10$	$72.15^{a} \pm 0.16$	$1.25^{a} \pm 0.05$
4	$67.43^{c} \pm 0.15$	$71.47^{c} \pm 0.14$	$72.97^{b} \pm 0.16$	$1.55^{b} \pm 0.04$
6	$67.92^{d} \pm 0.13$	$71.87^{d} \pm 0.10$	$73.47^{c} \pm 0.20$	$1.70^{c} \pm 0.03$
8	$68.48^{e} \pm 0.06$	$72.72^{e} \pm 0.14$	$73.86^{d} \pm 0.07$	$1.74^{d} \pm 0.02$
10	$68.83^{f} \pm 0.11$	$73.55^{f} \pm 0.16$	$74.95^{e} \pm 0.16$	$1.82^{e} \pm 0.04$

Values are means ±SD of triplicate. Values in the same row with different superscripts a-f and are significantly different ($p < 0.05$).

Conclusions

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 maltose increases, approve that maltose was acting according to the cryoprotecting mechanism and interacted with chicken myofibrillar proteins.

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