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CRYOPROTECTIVE EFFECT OF MALTOSE ON WASHED AND FROZEN STORED CHICKEN MEAT

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Abstract

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The cryoprotective effects of maltose (w = 0-10%) on washed chicken meat (WCM) were investigated. WCM was produced from broiler meat, 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 maltose caused smaller decrease of protein solubility during frozen storage. The peak transitions temperatures (T_p) and denaturation enthalpies (ΔH) of myosin and actin of WCM samples showed increase during frozen storage (-30°C) 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.

Key words: thermal transitions temperature, cryoprotection, DSC, SEP, washed chicken meat, maltose
Abbreviations: DSC - Differential scanning calorimetry; SEP - Salt extractable protein; WCM - washed chicken meat

Introduction

Washed chicken meat (WCM) 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 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; Yang and Froning, 1994; Kijowski and Richardson, 1996; Stangierski and Kijowski, 2003). 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). 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

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(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 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 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) 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°C). The FoodScan Meat Analyser (Foss, Denmark) was used to determine moisture, total protein and total fat according to the AOAC 2007. 04. 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 (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, (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, Tehtnica, Slovenia) at 4ºC for 30 min. The salt soluble proteins were recovered in the supernatant following centrifugation at 10 000 × g at 4ºC, 15 min. in a Heraeus Multifuge 3L-R (Heraeus AG, Germany). The Bio-Rad Protein Assay (Bio-Rad Laboratories Gmbh, Muenchen, Germany) 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 ($mgml^{-1}$), estimated by Bio-Rad analysis.

DSC measurements

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

Statistical analysis

Three determinations for basic chemical composition, pH, a_w, peak temperatures (T_p), denaturation enthalpies (ΔH Jg^{-1}) and SEP concentrations 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% ± 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)

The changes in salt extractable protein (SEP) of WCM mixed with maltose (w = 0 - 10%) during frozen storage at -30ºC are shown in Figure 1. The highest decreases of SEP in all samples were in the first 30 days, especially for sample with no additives. SEP of the control sample after 360 day of frozen storage was reduced to 44.01%. For WCM samples with addition of 2, 4, 6, 8 and 10% of maltose SEP of 6.94, 7.03, 6.05,

6.99 and 7.07 mgml^{-1} were observed, and having been decreased to, after 360 day of frozen storage, 53.45, 56.04, 58,99, 61.65 and 63.36% of its initial SEP values, respectively. From the SEP results it can be noted that addition of maltose in all mass fraction resulted with lowered SEP, compared with the control at day 0, this can be explained by the dilution effect of maltose 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 maltose showed smaller loss of salt protein solubility for all frozen storage time intervals (Figure 1). Myofibrillar protein denaturation during 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). In this study, the addition of maltose caused smaller loss of chicken myofibrillar protein solubility, for all frozen storage time intervals.

Differential scanning calorimetry

Differential scanning calorimetry thermogram's of WCM mixed with maltose (w = 0-10%) after 360 days of frozen storage are illustrated in Figure 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 (Figure 1) are related to the thermal denaturation of myosin and actin. Peak thermal temperatures (T_p) of WCM myosin and actin mixed with maltose (w = 0-10 %) at 0, 30, 90, 180 and 360 day of frozen storage are presented in Table 1. Values of peak thermal temperatures (T_p) of myosin and actin of control sample were different then values of raw chicken breast meat reported by Kijowski and Mast (1988), Murphy et al. (1998), 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 (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 maltose, but not as of frozen storage time. Highest value of myosins and actins T_p showed the samples of WCM mixed with 10% of maltose for all storage time intervals. T_p of actin transitions vary significantly ($P<0.05$) with addition of maltose but not with frozen storage time (Table 1). T_p of myosin shows higher shift by increase of mass fraction of maltose then a T_p of actin for all samples and all frozen storage time intervals (Table 1).

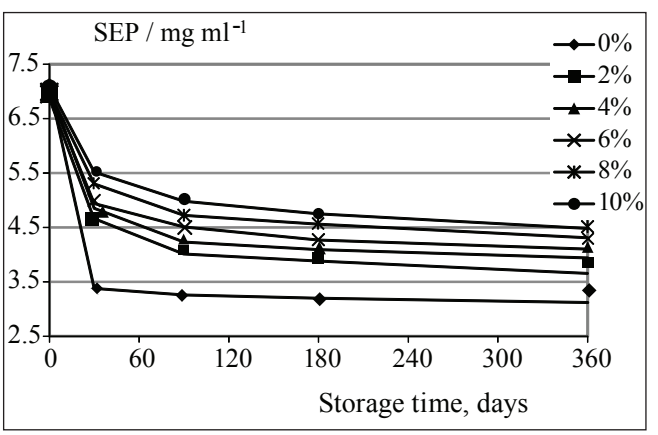

Fig. 1. Changes in salt extractable protein of WCM (washed chicken meat) mixed with maltose (w = 0-10%) as a function of storage time at -30ºC

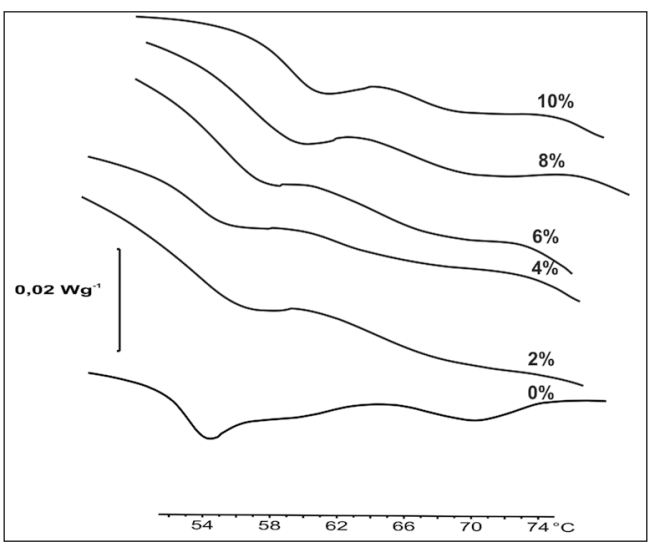

Fig. 2. DSC thermograms of WCM (washed chicken meat) stored for 360 days at -30°C as a function of mass fraction of maltose

The method of expressing peak enthalpies Δ*H* was adopted to provide an estimate of the quantity of native proteins. Enthalpies of myosin and actin denaturation for WCM samples with addition of maltose (*w* = 0-10%), during 360 days of frozen storage, are shown in Table 2. Sample of WCM without addition of maltose after 360 days of frozen storage showed 56.94% and 52.35% reduction of myosin's and actins' Δ*H*. Samples with 10% of maltose after 360 days of frozen storage showed only 32.77% reduction of myosin's and

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

Maltose, %	Storage time, days				
	0	30	90	180	360
			Myosin		
0	60.88a ± 0.10	59.91a ± 0.14	55.38a ± 0.03	59.98^{aB} ± 0.29	53.74a ± 0.23
2	61.21ab ± 0.08	61.76a ± 0.09	55.77b ± 0.15	61.50^{bA} ± 0.32	53.84a ± 0.13
4	61.44b ± 0.39	62.05b ± 0.11	56.25c ± 0.06	62.26^{cA} ± 0.09	54.74b ± 0.13
6	61.89c ± 0.16	62.20b ± 0.13	56.77d ± 0.11	62.65^{dA} ± 0.09	55.25c ± 0.10
8	62.64d ± 0.06	62.69c ± 0.11	57.66e ± 0.19	63.08^{eA} ± 0.12	55.77d ± 0.09
10	62.70d ± 0.03	62.93d ± 0.04	58.41f ± 0.17	63.69^{fA} ± 0.10	56.14e ± 0.10
			Actin		
0	68.95a ± 0.07	71.35a ± 0.34	70.73a ± 0.11	70.57a ± 0.19	70.25a ± 0.51
2	69.21b ± 0.08	72.23b ± 0.08	71.05b ± 0.10	70.66a ± 0.06	70.37a ± 0.16
4	69.49c ± 0.04	72.36bc ± 0.08	71.47c ± 0.14	70.95b ± 0.10	70.57a ± 0.09
6	69.73d ± 0.10	72.66bc ± 0.10	71.87d ± 0.10	71.53c ± 0.11	70.92b ± 0.09
8	70.01e ± 0.06	73.13c ± 0.10	72.72e ± 0.14	72.11d ± 0.14	71.41c ± 0.14
10	70.27f ± 0.13	73.43d ± 0.12	73.55f ± 0.16	72.99e ± 0.11	71.91d ± 0.19

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

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

Maltose, %	Storage time, days				
	0	30	90	180	360
			Myosin		
0	7.20f ± 0.01	4.10a ± 0.04	4.01a ± 0.02	3.71a ± 0.05	3.10a ± 0.01
2	4.87a ± 0.02	4.18b ± 0.03	4.16a ± 0.01	3.81a ± 0.04	3.41b ± 0.04
4	5.37b ± 0.02	4.75c ± 0.06	4.22b ± 0.03	3.96b ± 0.04	3.67c ± 0.08
6	6.11c ± 0.04	4.83d ± 0.03	4.76c ± 0.05	4.46c ± 0.14	4.01d ± 0.04
8	6.64d ± 0.08	4.97e ± 0.12	4.91d ± 0.06	4.76d ± 0.14	4.53e ± 0.06
10	6.86e ± 0.05	5.79f ± 0.03	5.75e ± 0.02	5.22e ± 0.09	4.84f ± 0.04
			Actin		
0	2.12d ± 0.03	1.30a ± 0.01	1.21a ± 0.01	1.11a ± 0.03	1.01a ± 0.01
2	1.52a ± 0.03	1.35b ± 0.03	1.25a ± 0.05	1.20a ± 0.02	1.18b ± 0.02
4	1.68b ± 0.03	1.60c ± 0.06	1.55b ± 0.04	1.53b ± 0.03	1.20b ± 0.03
6	1.89c ± 0.04	1.75d ± 0.05	1.70c ± 0.03	1.69c ± 0.03	1.61c ± 0.03
8	1.91c ± 0.04	1.80e ± 0.04	1.74d ± 0.02	1.70c ± 0.04	1.68d ± 0.03
10	2.03d ± 0.02	1.90f ± 0.01	1.82e ± 0.04	1.77d ± 0.05	1.70d± 0.03

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).

19.81% actins reduction of Δ*H*. The values of Δ*H* of myosin and actin for all samples showed decrease with the increase of storage time (Sych et al., 1990; Herrera et al. 2001; Stangierski and Kijowski, 2003). Highest decreases of Δ*H* of myosin and actin in all samples were in the first 30 days, especially for sample without additive. In general, sample of WCM without addition of maltose showed highest decrease in myosin's and actins Δ*H* throughout 360 days of frozen storage. The highest values of transition enthalpies showed samples mixed with 10% of maltose, for all frozen storage time intervals, with the exception of day 0, which is in agreement with the results reported by Stangierski and Kijowski (2008). Δ*H* for myosin and actin varied significantly ($P<0.05$) as a function of mass fraction of maltose and as a function of frozen storage time (Table 2). Results of denaturation enthalpy measurements indicated that myosin is more influenced by addition of maltose and exhibits higher freezing and frozen storage damage then actin (Sych et al., 1990).

Conclusions

Differential scanning calorimetry and SEP of WCM shows that addition of maltose cause's stabilization of chicken 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 maltose increases, approve that maltose was acting according to the cryoprotecting mechanism and interacted with chicken myofibrillar proteins. Results of this study 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 maltose.

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