Effect of Marination on the Thermodynamic Properties of Chicken Muscle Proteins Studied by DSC

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Abstract

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The marination of meat is a method applied to improve the sensory values like tenderness and juiciness of meat, and to enhance microbiological safety of the product. The effects of specific marinades on the thermal stability of the muscle proteins using differential scanning calorimetry (DSC) was examined. Various marinades were tested, composed mainly of NaCl as well as triphosphates and organic acids, self made marinades, and ready-to-use marinades used in industrial practice. As a result of the experiment conducted, it was found that all marinades used changed significantly the thermal stability of muscle proteins. The use of sodium chloride and sodium triphosphate for marination caused a reduction of enthalpy and denaturation temperature of myosin and actin. However, a greater influence on the stability of muscle proteins was observed with marinades containing organic acids (acetic and citric). The most significant reduction of the denaturation temperatures and enthalpy (to the lowest level of 0.56 J/g) was found for self made marinade composed of 20.7% cider vinegar and 16% lemon juice.

Keywords: myosin; actin; chicken meat; marinades; denaturation; DSC

The highest consumer desirability is recorded for such parts of poultry carcasses as breast fillet, thighs or drumsticks, while the lowest for wings. Marination may enhance and diversify the potential cooking uses for these parts. It is a practice applied in the poultry industry and the catering sector. Marinades consist of functional additives, herbs, and spices. Marinated products are sold as convenience foods most frequently to be grilled. The objective of marination is to improve the organoleptic properties of meat, mainly its taste, aroma, and texture including tenderness and juiciness, as well as to enhance microbiological safety thanks to the reduction of pH (Xiong & Kupski 1999a,b; Kerr *et al*. 2000; Smith & Acton 2001). Tenderness and juiciness are the most important attributes of meat quality, which are developed during *post mortem* aging (Tomaszewska-Gras *et al*. 2011). Myosin and actin proteins are the most responsible for the tenderness and juiciness of meat. The thermal stability of these proteins can be studied using differential scanning calorimetry (DSC). At present, DSC is one of the most frequently applied techniques in the analysis of the thermal stability of biological systems such as e.g. meat (Tomaszewska-Gras & Konieczny 2010; Kovačević *et al*. 2011). A particular advantage of this technique is related to the potential to analyse proteins in the natural state, without the need to dissolve or extract proteins. In earlier studies on meat proteins using DSC, Wright and Wilding (1984) determined the denaturation temperature and enthalpy of the whole muscle and individual proteins in rabbit meat i.e. myosin, actin, and sarcoplasmic proteins. In the case of the muscle analysis, they obtained three peaks. Similar studies were conducted with other animal species. Xiong *et al*. (1987) used DSC to compare the

thermal denaturation of muscle proteins from beef, pork, lamb, and from chicken breast and thigh. They observed that the mammalian and chicken thigh muscles exhibited three transitions, whereas chicken breast muscle demonstrated a thermal curve with five peaks, which was confirmed also by Kijowski and Mast (1988a). As a result of all these studies, endothermal peaks were obtained corresponding to the denaturation of myosin (54–58°C), sarcoplasmic proteins, and collagen (60–68°C), as well as actin (71–83°C). The increased ionic strength and changes in pH may alter the native state of proteins and their thermal stability. In most conducted studies, where DSC was applied to analyse the effect of pH and ionic strength (Quinn *et al*. 1980; Wright & Wilding 1984; Kijowski & Mast 1988b; Barbut & Findlay 1991), model marinade solutions were used, containing a maximum of three chemical compounds. Sodium chloride and phosphates are used in marinades as functional additives in order to enhance the water holding capacity of meat and reduce the thermal drip during thermal processing. Sodium chloride causes swelling of muscle fibres, resulting in an increased water holding capacity. Polyphosphates enhance the effect of salt thanks to an even greater reduction of the thermal drip. The combined action of NaCl and phosphates has not been fully clarified (Kijowski & Mast 1988b). Other components of marinades include organic acids, whose task is to reduce pH and extend the shelf life. However, the pH value of meat plays a key role for its capacity to hold water and stabilise proteins. Marinades due to their function have a varied composition, they frequently contain sodium chloride, phosphates, and organic acids, different spices, herbs, and other natural components. These compounds influence the spatial structure of proteins and their stability. Thus the aim of this study was to verify, with the use of DSC, the action on proteins performed by specific marinades, i.e. model ones composed mainly of NaCl as well as polyphosphates and organic acids, self-made marinades, and ready-to-use marinades used in industrial practice.

MATERIAL AND METHODS

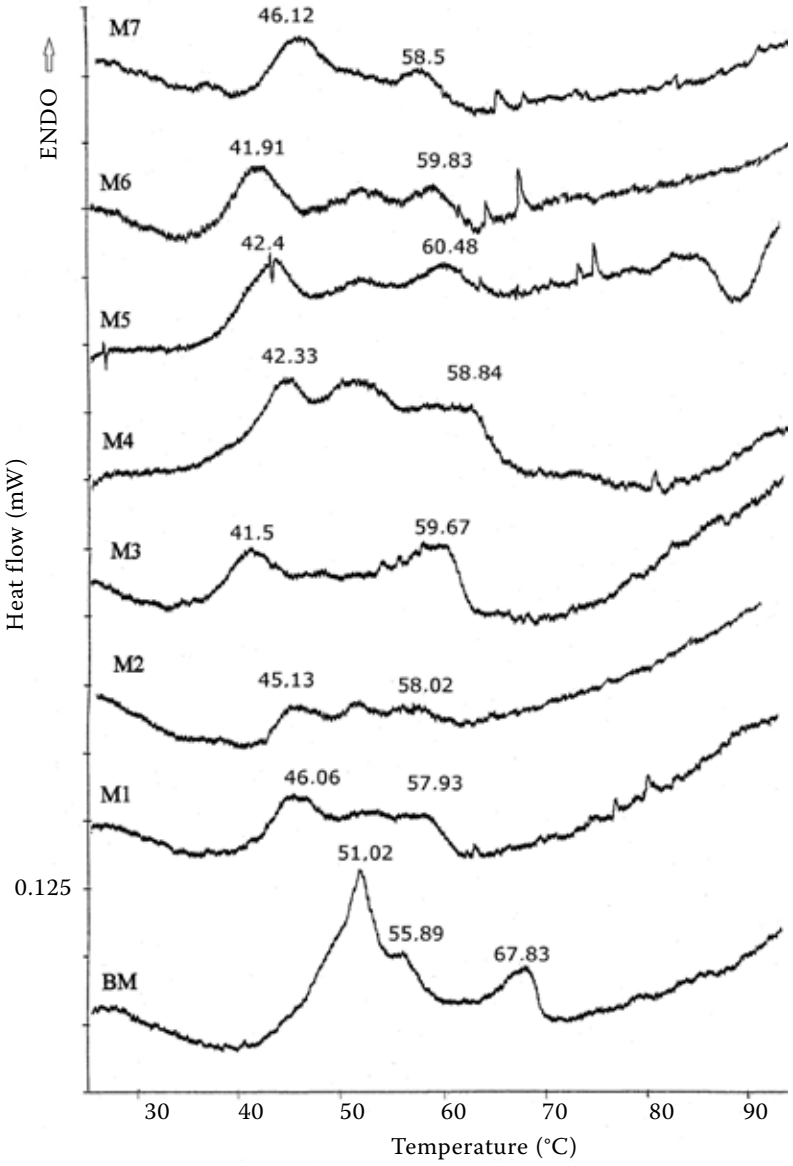
Experimental material comprised chicken wings. The analyses were conducted on the samples of muscles collected from marinated wings.

Table 1. Composition of marinades

	Sample	Composition	pH
Meat without marinade	BM		
Model marinades	M1	6% NaCl	7.15
	M2	6% NaCl, 2% sodium triphosphate (TPP)	7.63
	M3	6% NaCl, 1% sodium triphosphate (TPP), 1% citric acid	3.31
	M4	6% NaCl, 1% sodium triphosphate (TPP), 1% acetic acid	6.42
	M5	6% NaCl, 2% citric acid	1.6
	M6	6% NaCl, 2% acetic acid	3.45
	M7	6% curing salt	7.28
Marinades prepared according to the authros' recipes	W1	11.7% olive oil, 27.4% brown sugar; 20.7% cider vinegar; 16.1% lemon juice; 3.9% dried garlic; 2.4% NaCl; 17.9% mustard	3.09
	W2	14.6% brown sugar; 58.3% coca-cola; 19% onion; 1.5% dried garlic; 4% soy sauce; 2.2% NaCl; 0.4% pepper	4.39
	W3	21.6% honey; 1.7% olive oil; 7.2% mustard; 2.4% lemon juice; 48% pineapple juice; 11% ketchup; 1.5% NaCl	3.36
Ready-to-use marinades	H1	6.3% flavour preparation POLSMAKI containing cooking oil, salt, spices, vegetables, flavour preparation, 621-sodium glutamate, sugar, herbs (oregano), E331-sodium citrate; 93.7% oil	5.45
	H2	6.3% spice blend TEJO containing salt, spices, E621-sodium glutamate, dextrose, spice agents; 93.7% oil	5.11
	H3	FEINSCHMECKER MARINADE – commercially available flavouring sauce	6.0

The chicken wings, prior to marination, were subjected to tenderisation, which consisted in needling with a multineedle machine in order to enhance the rate of marinade components migration. Marination was performed by static soaking in plastic containers and lasted approx. 20 h under refrigeration conditions (4°C). Model marinades of formulations presented in Table 1, marked as M1, M2, M3, M4, M5, M6, and M7, were tested in this study. Marinades prepared according to the recipes of Bailey (1986) were also used, marked as W1, W2, and W3, while ready-to-use commercial marinades were marked as H1, H2, and H3.

DSC – differential scanning calorimetry. Thermal analysis was conducted using a differential scanning calorimeter DSC 7 by Perkin-Elmer (Perkin Elmer, Norwalk, USA). The meat samples were collected from the same position in the wings and weighed (approx. 10 mg) into aluminum sample pans (Perkin Elmer, Norwalk, USA; 20 µ, No. 210-0062). An empty capsule was used as the reference sample. The heating rate was 5°C/min, within the range from 20°C to 100°C. Three replications were performed with each sample. As a result of DSC analyses, thermal curves were obtained. The enthalpy of denaturation ΔH (J/g) and temperature of maximum transition T_{max} (°C) were calculated with the data analysis software supplied by Perkin Elmer. Enthalpy (ΔH) was determined by measuring the area under the DSC curve and expressed in J/g of protein. Protein analyses were performed by Kjeldahl procedure using a Kjeltec system. A factor of 6.25 was used to convert nitrogen to protein.



BM – without marinade, M1–M7 – type of model marinades; composition of marinades shown in Table 1; heating rate 5°C/min

Figure 1. DSC thermograms of marinated chicken meat from wings

Statistical calculations. Statistical calculations were performed using computer software STATISTICA 6.0 and Microsoft Excel 2003. The experimental data were analysed by the one-way analysis of variance (ANOVA) and Fisher's least significant difference with significance defined at $P < 0.05$.

RESULTS AND DISCUSSION

In the first stage of the study, analyses were conducted for the effects of seven model marinades on the enthalpy and denaturation temperatures of muscle proteins. Enthalpy of the thermal transition of proteins in meat is the energy, which is absorbed in the course of thermal denaturation of proteins. It is an endothermic process. Figure 1 presents the thermal curves obtained with the model marinades, while Table 2 contains the recorded values of enthalpy (ΔH) and temperatures (T_{max}) of denaturation for myofibrillar proteins, i.e. myosin and actin. DSC thermogram of the non-marinated sample of meat from chicken wings is shown in Figure 1. On the thermal curve, three endothermic transitions are visible, which can be ascribed to the denaturation temperatures (T_{max}) of myosin (51°C), sarcoplasmic and connective tissue proteins (56°C), and actin (68°C). Typical transition temperatures of chicken breast muscle (pectoralis major) can range from 49°C to 57°C for myosin and its subunits, 61–67°C for sarcoplasmic proteins, and 76–79°C for actin (Xiong *et al.* 1987; Kijowski & Mast 1988; Wang & Smith 1994; Murphy *et al.* 1998; Wattanachant *et al.* 2005; Bertram *et al.* 2006). On the basis of the collected results (Table 2), it may be stated that the values of enthalpy and denaturation temperature in all marinated samples differed significantly ($P < 0.05$) from the non-marinated sample, for which the highest values of enthalpy and denaturation temperatures of myosin and actin were recorded. In sample M1 with the addition of sodium chloride (6% NaCl), the effect of enthalpy reduction of observed from 9.56 J/g to 4.57 J/g as well as a shift in the denaturation temperature by approx. 5°C for myosin and 10°C for actin. The reduction of enthalpy and denaturation temperature is consistent with the results published by Kijowski and Mast (1988b) who observed a decrease in the denaturation temperature by 5–6°C for myosin and actin in chicken meat samples containing 4% NaCl and a reduction of enthalpy by approx. 60%. The destabilising effect of NaCl was observed by those authors already at a 1% concentration of sodium chloride. A reduction of the denaturation temperature and enthalpy of beef, rabbit, and pork proteins caused by of NaCl was observed also by Quinn *et al.* (1980) and Barbut and Findlay (1991). On the basis of the results of earlier studies (Quinn *et al.* 1980; Kijowski & Mast 1988b; Barbut & Findlay 1991) as well as those presented here (Table 2), it was stated that the higher is NaCl concentration, the greater is the reduction of thermal stability of muscle proteins, which start to be denatured at much lower temperatures. The analyses using DSC conducted on sample M2 with the addition of 6% NaCl and 2%

Table 2. Denaturation enthalpies and temperatures of myofibrillar proteins from chicken wings marinated with model marinades, heating rate 5°C/minute

Sample	pH	Enthalpy ΔH (J/g protein)	Temperature T_{max} (°C)	
			myosin	actin
BM		$9.56^{C} \pm 0.6$	$51.02^{C} \pm 1.1$	$67.83^{D} \pm 0.47$
M1 (NaCl)	7.15	$4.57^{A,B} \pm 0.2$	$46.06^{B} \pm 0.21$	$57.93^{B} \pm 0.4$
M2 (NaCl, TPP)	7.63	$3.54^{A} \pm 0.64$	$45.13^{B} \pm 0.2$	$58.02^{B} \pm 0.72$
M3 (NaCl, TPP, citric acid)	3.31	$4.08^{A,B} \pm 1.48$	$41.5^{A} \pm 1.1$	$59.67^{B,C} \pm 0.92$
M4 (NaCl, TPP, acetic acid)	6.42	$5.62^{B} \pm 1.28$	$42.33^{A} \pm 1.76$	$58.84^{B,C} \pm 1.27$
M5 (NaCl, citric acid)	1.6	$4.41^{A,B} \pm 1.32$	$42.4^{A} \pm 1.22$	$60.48^{C} \pm 2.01$
M6 (NaCl, acetic acid)	3.45	$5.49^{B} \pm 1.08$	$41.91^{A} \pm 0.65$	$59.83^{B,C} \pm 1.15$
M7 (curing salt)	7.28	$4.45^{A,B} \pm 1.16$	$46.12^{B} \pm 0.86$	$58.5^{B,C} \pm 0.3$

$^{A–D}$identical letters next to mean values of enthalpy indicate a lack of significant differences at $P < 0.05$; BM – non-marinated sample; ± standard deviation for $n = 3$

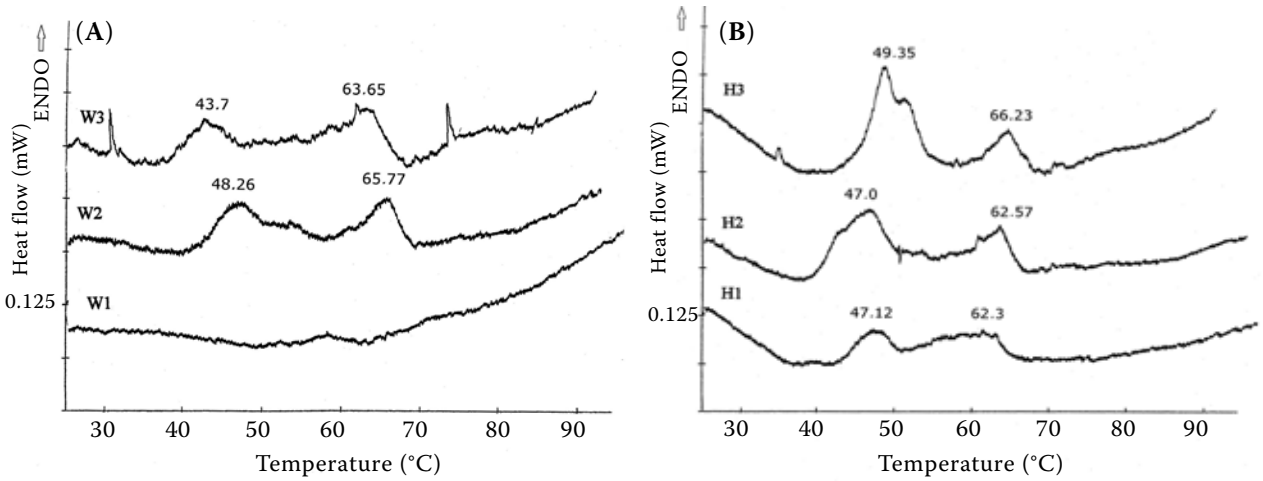


Figure 2. DSC thermograms of marinated chicken meat from wings (**A**) samples W1, W2, W3 – self made marinades and (**B**) samples H1, H2, H3 – ready-to-use marinades; composition of marinades shown in Table 1 (heating rate 5°C/min)

sodium triphosphate (TPP) showed that at such a high NaCl concentration phosphates cease to have a significant effect on the temperature and enthalpy of protein denaturation. The values of enthalpy and temperature were significantly lower for this sample than those for the non-marinated sample (BM), but they did not differ significantly from that with only 6% NaCl ($P < 0.05$) applied. Kijowski and Mast (1988b) investigated the effect of the salt sodium triphosphate alone on the stability of proteins in chicken breast muscles and found that a 1% TPP addition causes stabilisation of myosin, increases its denaturation temperature, and reduces the denaturation temperature of actin. However, they claimed that the stability of breast muscle proteins is more considerably affected by NaCl than by sodium triphosphate. Moreover, it was established (Findlay & Barbut 1990) that NaCl reduces denaturation temperature of myosin at a concentration from 0.5 to 2.5% in the presence of 0.2–0.6% TPP, while TPP enhances its stability (ΔH). The effect of the addition of organic acids (citric and acetic) was investigated in the samples containing marinades M3, M4, M5, and M6 (the composition of marinades shown in Table 1). The samples containing organic acids exhibited a significant reduction of myosin denaturation temperature from 51.02°C to approx. 42°C on average, and of actin denaturation temperature from 68°C to 60°C. In the samples containing citric acid (M3 and M5) a greater reduction of enthalpy values was observed (in M5 – 4.41 J/g and M3 – 4.08 J/g) than in those containing acetic acid, where it was 5.62 J/g for M5 and 5.49 J/g for M6. The observed values did not differ significantly ($P < 0.05$) from the sample containing

Table 3. Denaturation enthalpies and temperatures of myofibrillar proteins from chicken wings marinated using self-made marinades and ready-to-use, commercially available marinades; heating rate 5°C/min

Sample	pH	Enthalpy ΔH (J/g protein)	Temperature T_{max} (°C)	
			myosin	actin
BM		$9.56^{D} \pm 0.6$	$51.02^{D} \pm 1.1$	$67.83^{D} \pm 0.47$
W1	3.09	$0.56^{A} \pm 0.2$	X	$55.03^{A} \pm 2.45$
W2	4.39	$7.84^{C,D} \pm 2.8$	$48.26^{B,C} \pm 1.01$	$65.77^{C,D} \pm 1.66$
W3	3.36	$4.76^{B} \pm 1.2$	$43.7^{A} \pm 0.85$	$63.65^{B,C} \pm 1.18$
H1	5.45	$4.64^{B} \pm 1.68$	$47.12^{A,B} \pm 0.43$	$62.3^{B} \pm 2.29$
H2	5.11	$6.01^{B,C} \pm 1.96$	$47.00^{A} \pm 0.1$	$62.57^{B} \pm 1.4$
H3	6.0	$6.68^{C,D} \pm 2,68$	$49.35^{C} \pm 0.21$	$66.23^{C,D} \pm 1.12$

$^{A–D}$identical letters next to values of mean enthalpy indicates a lack of significant differences at $P < 0.05$; BM – non-marinated sample; ± standard deviation for $n = 3$

6% NaCl, but they differed significantly from the non-marinated sample BM. When comparing the marinades containing 1% and 2% citric acid, no significant differences were found between them in terms of the volume of enthalpy and denaturation temperature. For comparison, Table 3 presents the values of enthalpy and temperature and Figures 2 shows the thermal curves obtained as a result of heating the samples treated with self-made marinades (W1, W2, W3) as well as ready-to-use commercially available marinades (H1, H2, H3). Marinades W1, W2, W3 were characterised by a low pH from 3.09 to 4.39, they contained natural components such as sugar, olive, spices, vinegar, lemon juice, or pineapples. Marinade W1 was characterised by the strongest denaturing action, myosin was completely denatured, with no peak corresponding to it having been observed, both enthalpy and denaturation temperature of actin had the lowest values. A significant reduction of actin denaturation temperature by 12°C was found in relation to the non-marinated sample. Marinade W2 containing considerable amounts of brown sugar (14%) and coca-cola (58%) had the least destabilising effect on proteins. Moreover, analyses were also conducted for the effects of the commercially available marinades used in industrial practice (H1, H2, H3) on the proteins stability. These marinades were characterised by pH ranging from 5.11 to 6.00. Marinades H1 and H2 caused a significant reduction of the enthalpy values (to 4.64 and 6.01 J/g) and of the denaturation temperature of myosin (by approx. 4°C) and actin (by approx. 3°C) ($P < 0.05$). Marinade H3, whose formulation was not made available by the producer, affected the changes in the muscle proteins stability to the lowest degree.

Summing up, it was found that the marination process affects thermal properties of muscle proteins. Marinated meat samples (apart from samples W2 and H3) were characterised by a significantly lower ($P < 0.05$) enthalpy and lower denaturation temperatures of myosin and actin. The reduced denaturation temperature of both myosin and actin in marinated meat in comparison to non-marinated meat indicates that marinades affect the proteins conformation. Based on these results, it can be concluded that the factors required for the initiation of the changes in myofibrillar proteins thermal stability include ionic strength and pH. A strong effect on muscle proteins was found with marinades containing organic acids such as citric acid, apple acid (in cider vinegar), and acetic acid. A particularly drastic denaturation as a result of marination was recorded for marinade W1, with pH 3.09, containing cider vinegar and lemon juice. For this marinade, enthalpy was found at the lowest level of 0.56 J/g, which was caused by the complete denaturation of myosin and actin. Moreover, the greatest shift recorded in the denaturation temperature of actin, i.e. by 12°C, was also caused by this marinade.

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