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A DSC STUDY ON THE EFFECT OF MARINATION ON THE STABILITY OF SKIN COLLAGEN FROM CHICKEN WINGS

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Background. Marination is a good method to enhance attractiveness of chicken wings, which are considered by consumers as least attractive. Sensory value of marinated wings is dependent largely on the flavour of skin, because they contain proportionally more skin than other carcass elements. Moreover, skin constitutes a natural barrier, which may facilitate or hinder the penetration of marinade components, depending on the conformation state of proteins it is composed of primarily collagen. The aim of the study was to investigate the effect of specific marinades on thermal stability of collagen – the main component of skin proteins using differential scanning calorimetry (DSC).
Material and methods. Chicken wings were marinated using model marinades, marinades prepared according to original recipes and ready-to-use marinades used in industrial practice. Marinated skin samples were weighted (approx. 10 mg) and analyzed by DSC. Heating rate was 5°C/min, within the range from 20 to 100°C.
Results. In analyses using model marinades denaturation temperature (T_{max}) of collagen was reduced by approx. 3°C and enthalpy (ΔH) was lowered by approx. 40%. An even bigger reduction of collagen denaturation temperature (by approx. 7°C) and enthalpy ΔH (by approx. 48%) was found after the application of marinades prepared following original recipes (W1, W2, W3). In turn, the application of model marinades containing NaCl and organic acids (acetic or citric) resulted in stabilization of collagen, which was manifested by an increase of enthalpy (ΔH) by approx. 50% (for marinade containing 2% citric acid).
Conclusions. Temperature and enthalpy of collagen denaturation was dependent on type of marinade. The extent of collagen denaturation affects nutritional and sensory value. Considering that time and temperature of intensive heat treatment are important factors in the formation process of carcinogenic compounds i.e. heterocyclic aromatic amines, it bis important to choose these marinades, which reduce the denaturation temperature and enthalpy of proteins.

Key words: collagen, chicken skin, marination, denaturation, DSC

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INTRODUCTION

Wings are these chicken meat elements, which are considered by consumers as less desirable than such poultry carcass elements as breast fillets, thighs or drumsticks. Marination is performed in order to improve organoleptic attributes of meat, primarily its taste, aroma and texture, as well as enhance microbiological safety thanks to a reduction of pH and limited formation of heterocyclic aromatic amines in the course of thermal processing. Wings are elements containing the highest proportion of skin among all culinary chicken meat elements. Skin in wings accounts for approx. 22%, i.e. twice the amount in thighs or drumsticks, thus sensory attributes of this element are to a considerable extent dependent on skin. Skin constitutes a natural barrier, which may facilitate or hinder the penetration of marinade to meat tissue, depending on the conformation state of proteins it is made up of. The primary component of skin is a protein, collagen, which is found at approx. 20% in porcine skin and at 14-25% in the skin of fish [Sikorski 2007]. Chicken skin contains about 9-13% protein consisting mostly of collagen (60-80%), dependly on age [Judge and Aberle 1982], dietary differences [Kafri et al. 1985, Bonifer and Froning 1996, Smolinska et al. 1988]. This protein is composed of a triple helix, with the isoelectric point pI 7-7.5 [Wallace 1990, Rosenblatt et al. 1993]. As a result of water absorption it swells – the lower its cross-linking and the more distant pH of the medium is from pI, the more it swells. In the course of heating collagen fibres first swell, which weakens protein-protein bonds. Next, under the influence of heating collagen fibres are denatured, which is manifested in fibre shrinkage, which may be as high as 75%. Further heating in the presence of water results in the transition of cross-linked collagen into gelatin – a mixture of thermal degradation products of collagen, composed of fragments with different molecular weights. The essence of collagen denaturation consists mainly in the removal of intracellular hydrogen bonds, stabilizing the natural structure of proteins and hydrophobic bonds. As a result of denaturation the helix is transformed into a coil. Studies on the behaviour of proteins in thermal processes are made possible thanks to the use of differential scanning calorimetry (DSC).

At present DSC is one of the most commonly applied techniques in the investigations on thermal stability of such biological systems as e.g. meat. A particular advantage of this technique is the possibility to examine proteins in the natural state and medium, as proteins need not be extracted or isolated. It is already known that denaturation temperature of collagen depends on many factors. These include the type and origin of collagen, as well as the age of the organism from which it was collected [Ledward et al. 1975, Judge and Aberle 1982], ionic strength [Lim 1976, Kijowski 1993], pH of the environment [Horgan et al. 1991], iminoacid content [Privalov and Tiktopulo 1970].

AIM OF THE WORK

The objective of the research was to investigate the effect of specific marinades on thermal stability of collagen – the main component of skin proteins, using differential scanning calorimetry (DSC).

MATERIAL AND METHODS

Material

Chicken wings comprised experimental material. Analyses were conducted on skin samples collected from marinated wings. Chicken wings prior to marination were subjected to the process of tenderization, which consisted in puncturing with a multi-needle device in order to increase the solution absorption area. Marination was performed by static immersion in plastic containers. The process of marination lasted for approx. 20 h under cold storage conditions (4°C). Tested model marinades with the composition presented in Table 1 are denoted as M1, M2, M3, M4, M5, M6 and M7. Moreover, analyses were conducted on self made marinades prepared following original recipes and denoted as W1, W2 and W3, as well as commercially available marinades used in industrial practice, denoted as H1, H2 and H3. The compositions of marinades are presented in Table 1.

Table 1. Composition of marinades

Meat without marinades	Symbol of marinade BM	Composition	pH of marinade
Model marinades	M1	6% NaCl	7.15
	M2	6% NaCl, 2% sodium triphosphate STPP	7.63
	M3	6% NaCl, 1% sodium triphosphate STPP, 1% citric acid	3.31
	M4	6% NaCl, 1% sodium triphosphate STPP, 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
Self made marinades	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 table oil, salt, spices, vegetables, flavour preparation, E 621 – sodium glutamate, sugar, herbs (oregano), E 331 – sodium citrate, 93.7% oil	5.45
	H2	6.3% spice mixture TEJO Composition: salt, spices, E 621 – sodium glutamate, dextrose, spice preparations 93.7% oil	5.11
	H3	FEINSCHMECKER MARINADE – commercially available flavouring sauce. Produced for BATIK	6.0

DSC – differential scanning calorimetry

Thermal analysis was conducted using a DSC 7 differential scanning calorimeter by Perkin-Elmer. Skin samples were collected from wings and they were weighed to aluminium pans (approx. 5-8 mg). In the course of investigations an empty pan was used as a reference. Heating rate was 5°C/min, within the range from 20 to 100°C. Three replications were performed for each sample. In the description of the denaturation process two parameters were used: temperature T_{max} (°C), which is the meaximum peak temperature and enthalpy ΔH (J/g), defining the amount of heat required for denaturation of proteins, determined as the area of the denaturation peak.

Statistical calculations

Statistical calculations were performed using STATISTICA 6.0 and Microsoft Excel 2003 software. A one-way analysis of variance ANOVA was conducted.

RESULTS

The first stage of investigations consisted of analyses concerning the effect of seven model marinades on the volume of enthalpy and denaturation transition temperature of collagen. Enthalpy of thermal transition of proteins in meat is the energy, which was collected in the course of thermal denaturation of proteins. It is an endothermic process. Figure 1 presents thermal curves for model marinades, while Table 2 contains values of enthalpy (ΔH) and temperatures (T_{max}) of denaturation processes of collagen in samples with model marinades (M1 ... M7). Figures 2 and 3 present thermal curves of collagen denaturation in samples with self-made marinades: W1, W2 and W3 as well as commercial marinades H1, H2 and H3. Table 3 gives results of determinations of enthalpy and denaturation temperatures for marinades W1, W2, W3, H1, H2 and H3.

The process of collagen denaturation consists in the disruption of secondary and tertiary structure of collagen and it occurs in two stages, since the helix structure collapses first and next the structure is degraded to components of smaller molecular weights [Reich 1970]. Thermodynamic properties of collagen were first studied by Finch and Ledward et al. [1975] and Hellauer and Winkler [1975], who found denaturation temperature of collagen within the range of approx. 61-65°C. In the non-marinated sample (BM) denaturation of collagen in broiler chicken skin was observed at 57°C, at enthalpy of this process of 3.14 J/g skin (Table 2). Similar temperatures for broiler chickens within the range from 57.8 to 62.6°C were recorded by Wattanachant et al. [2005]. Kijowski [1993] found denaturation of collagen in the skin of 1.5-year old hens at 69.4°C. Denaturation temperature of collagen increases with age, which is caused by a higher degree of collagen cross-linking. Differences in denaturation temperatures of collagen in literature may result from different conditions of analyses, i.e. different heating rates and different methods of sample preparation.

For the sample treated with the solution of 6% sodium chloride (Table 2) a considerable reduction was observed for enthalpy from 3.14 J/g for the non-marinated sample to 1.91 J/G (marinade M1 – 6% NaCl), and for denaturation temperature of collagen from 57°C to 54°C (Table 2). The lowest values of enthalpy (1.75 J/g) and temperature

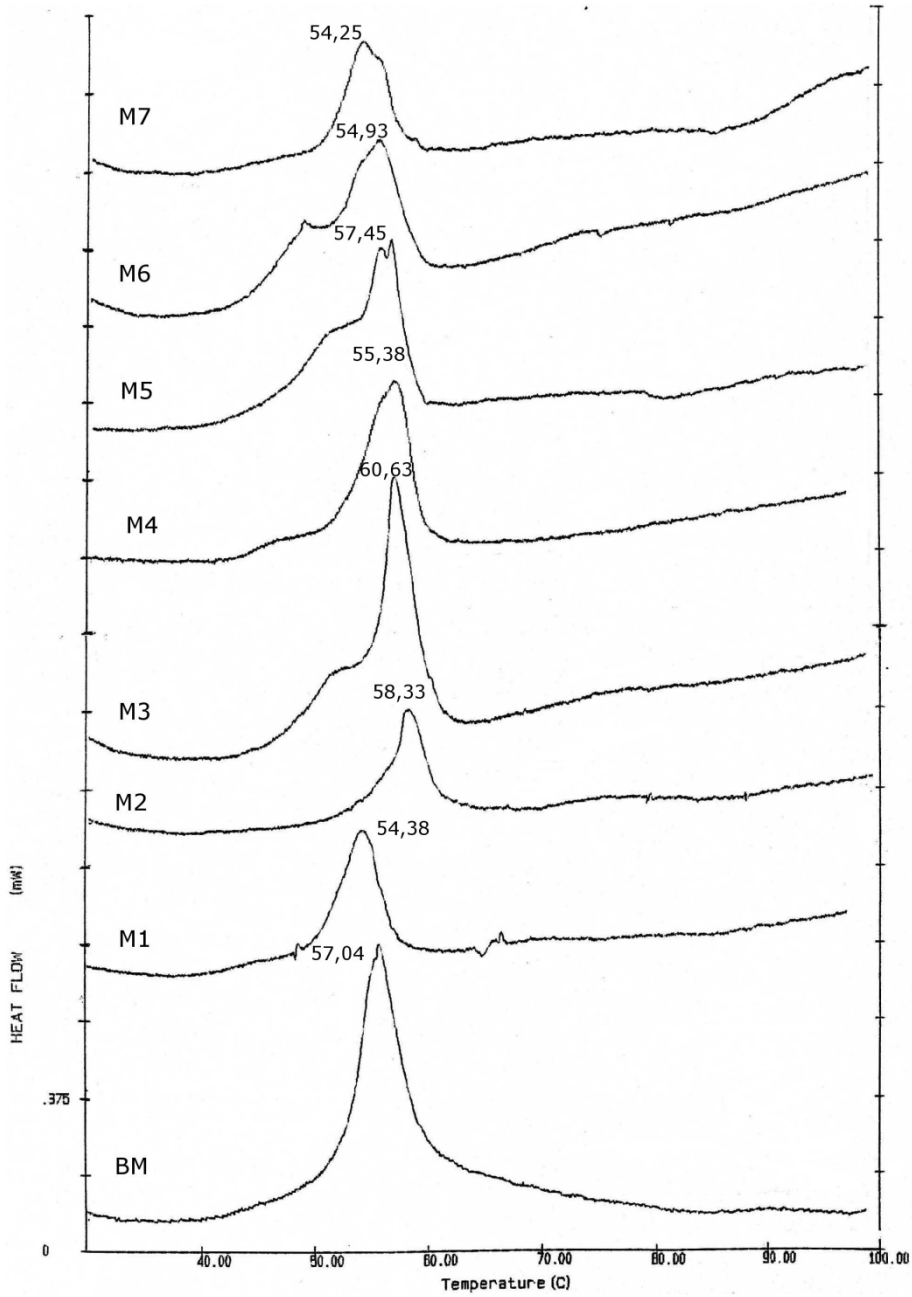

Fig. 1. DSC thermograms of collagen from marinated chicken wings: BM – without marinade, M1 ... M7 – type of model marinades, composition of marinade – Table 1; heating rate 5°C/min

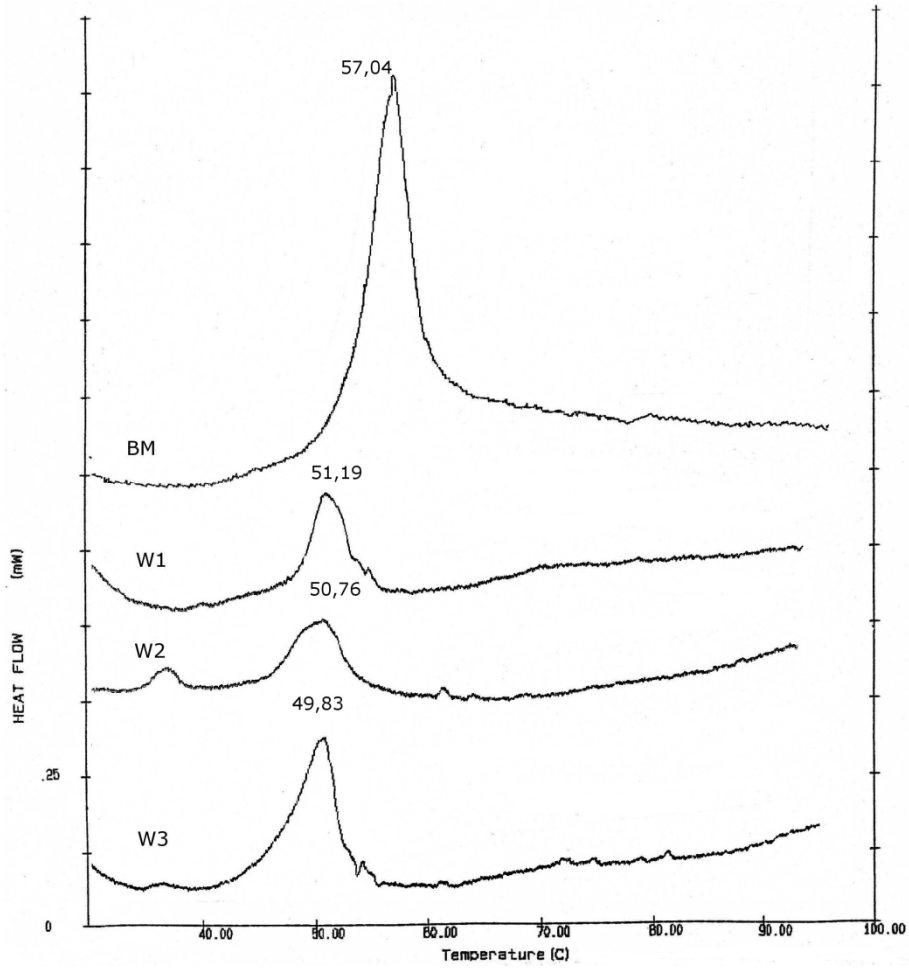

Fig. 2. DSC thermograms of collagen from marinated chicken wings: W1, W2, W3 – self made marinades, composition of marinades – Table 1; heating rate 5°C/min

(54°C) were recorded for the sample treated with 6% curing salt solution, although these values did not differ significantly from those recorded for marinade M2 (NaCl, STPP) of 1.83 J/g. This might indicate that sodium chloride in combination with nitrate (III) or triphosphate had the biggest effect on a reduction of collagen stability. Lim [1976] observed that salts of sodium, bromide and sodium chloride as well as magnesium chloride cause a reduction of denaturation temperature. Kijowski [1993] observed that soaking in the 2% NaCl solution reduced the denaturation temperature of collagen by 2.8°C from 69.4°C to 66.6°C. Reduction of denaturation temperature in diluted salt solutions is caused by swelling of collagen, which causes a weakening of protein-protein interactions. However Finch and Ledward [1973] stated that in diluted solutions of KCl and KF salts with a concentration of less than 0.3M a decrease was found for denaturation temperature of collagen, while at a higher concentration of salt (0.3-2.0M)

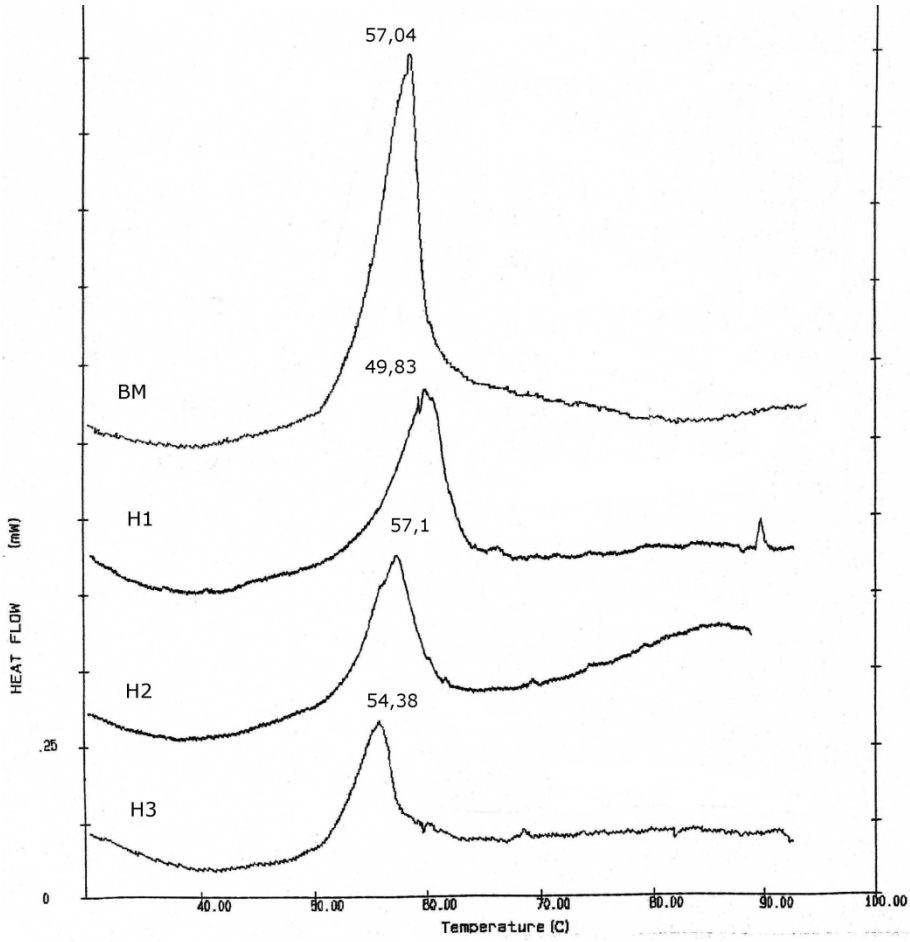

Fig. 3. DSC thermograms of collagen from marinated chicken wings: H1, H2, H3 – ready-to-use marinades, composition of marinades – Table 1; heating rate 5°C/min

this effect was the opposite, i.e. it was stabilizing, which was manifested by an increase in denaturation temperature of collagen. For 2M KCl solution the denaturation temperature of collagen was observed to increase from 67°C to 70°C.

Table 2 presents also results of analyses on collagen stability with the use of marinades containing, apart from NaCl, also organic acids (M3, M4, M5, M6), which pH was much lower than the isoelectric point of collagen (pI = 7.0-7.5) [Wallace 1990]. For marinades containing organic acids (M3, M4, M5, M6) higher enthalpy (ΔH) was observed in comparison to the non-marinated sample BM (Table 2), with denaturation temperatures of collagen maintained at a similar level as for the non-marinated sample. The highest value of enthalpy of 4.74 J/g was recorded for marinade M5 containing 2% citric acid and 6% NaCl.

Table 2. Denaturation enthalpies and temperatures of skin collagen from chicken wings marinated using model marinades; heating rate 5°C/min

Number of marinade	pH of marinade	Composition	Mean enthalpy J/g skin	T_{max} of collagen °C
BM			3.14 ±0.9 B	57.04 ±0.07 B, C
M1	7.15	6% NaCl	1.91 ±0.65 A	54.38 ±1.1 A
M2	7.63	6% NaCl, 2% STPP	1.83 ±1.02 A	58.33 ±0.03 C, D
M3	3.31	6% NaCl, 1% STPP, 1% citric acid	3.74 ±1.7 B, C	60.63 ±3.67 D
M4	6.42	6% NaCl, 1% STPP, 1% acetic acid	3.93 ±1.1 B, C	55.38 ±1.36 A, B
M5	1.6	6% NaCl, 2% citric acid	4.74 ±0.6 C	57.45 ±2.92 B, C
M6	3.45	6% NaCl, 2% acetic acid	3.82 ±0.54 B, C	54.93 ±1.73 A, B
M7	7.28	6% curing salt (NaCl, 0.5% $NaNO_2$)	1.75 ±0.6 A	54.25 ±0.22 A

A-D – identical letters next to values of mean enthalpy indicate a lack of significant differences at significance level $P < 0.05$, ±standard deviation for $n = 3$.

BM – non-marinated sample.

STPP – sodium tripolyphosphate.

Arganosa and Marriott [1989] observed a reduction of collagen denaturation temperature after the application of acetic, lactic or citric acid by approx. 2-5°C. In turn, Kijowski [1993] recorded a reduction of temperature by approx. 24°C after the application of 1.5% acetic and lactic acids. Miles et al. [1995] stated that the extent of the fall of temperature was heating rate dependent, but the enthalpy of the transition was not dependent on scanning rate.

As it can be visible on the Figure 1, peaks for marinades containing acids (M3, M4, M5, M6) are not so sharp as for non-marinated sample. This suggests that organic acids produce subunits of collagen with different denaturation temperatures. Especially for marinades containing 2% citic acid (M5) and 2% acetic acid (M6) two peaks were detectable on thermograms. Stability of collagen depends to the highest degree, as it is reported by Privalov [1982], on the number of inter- and intracellular hydrogen bonds in collagen structure; at the same time, that author excluded the decisive role of hydrophobic bonds in the stabilization of collagen. Privalov [1982] showed that the higher the number of hydrogen bonds, the higher the stability of collagen and the higher the value of enthalpy for the denaturation process.

Marinades prepared following original recipes (W1, W2, W3) and those used in industrial practice (H1, H2, H3) contain much higher amounts of components affecting ionic strength and pH than model marinades. Marinades prepared using lemon juice, e.g. marinade W1 – 16% (assuming the content of approx. 5% citric acid in juice), contained approx. 0.8% citric acid. All tested marinades (W1, W2, W3, H1, H2, H3) caused a reduction of thermal stability of collagen. Marinades W1, W2 and W3, where denaturation temperature and enthalpy of collagen were significantly reduced to a lower level than that recorded for model marinades, has a particularly evident effect.

Table 3. Denaturation enthalpies and temperatures of collagen from skin of chicken wings marinated using original marinades and commercial marinades; heating rate 5°C/min

Number of marinade	pH of marinade	Composition	Mean enthalpy J/g	T_{max} of collagen °C
BM			3.14 ±0.9 A	57.04 ±0.07 C
W1	3.09	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	1.8 ±0.46 B	51.19 ±0.8 A
W2	4.39	14.6% brown sugar, 58.3% Coca-cola, 19% onion, 1.5% dried garlic, 4% soy sauce, 2.2% NaCl, 0.4% pepper	1.72 ±1.1 B	50.76 ±0.77 A
W3	3.36	21.6% honey, 1.7% olive oil, 7.2% mustard, 2.4% lemon juice, 48% pineapple juice, 11% ketchup, 1.5% NaCl	1.64 ±0.48 B	49.83 ±1.04 A
H1	5.45	6.3% flavour preparation POLSMAKI containing vegetable oil, salt, spices, vegetables, flavour preparation, E 621 – sodium glutamate, sugar, herbs (oregano), E 331 – sodium citrate 93.7% oil	1.54 ±0.38 B	57.7 ±1.86 C
H2	5.11	6.3% flavour mixture TEJO Composition: salt, spices, E 621 – sodium glutamate, dextrose, flavour preparation 93.7% oil	2.02 ±0.85 A. B	57.1 ±1.72 C
H3	6.0	FEINSCHMECKER MARINADE – commercially available flavouring sauce. Produced for BATIK	2.65 ±0.87 A. B	54.38 ±1.1 B

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

CONCLUSIONS

It was observed, that the denaturation process of collagen was affected by both the presence of salt (NaCl, sodium triphosphate) and the presence of organic acids (citric and acetic acids). Generally it may be stated that for samples containing only salts such as NaCl or NaCl and sodium triphosphate, or curing salt – NaCl, $NaNO_2$ (M1, M2, M7), enthalpy and denaturation temperature of collagen decreased. In turn, for samples containing organic acids higher values of enthalpy were found in comparison to the non-marinated sample. Stability of collagen in thermal processes may be changed using appropriate concentrations of salts and organic acids, with the maximum recorded reduction of temperature amounting to 7°C for marinades prepared according to original recipes (W2, W3). All presented results concerning temperature and enthalpy of collagen denaturation showed that various marinades have varying effects on the conforma-

tional state of this protein. The extent of collagen denaturation affects nutritional value, i.e. bioavailability and sensory value i.e. tenderness, of marinated elements.

Furthermore, some marinades reduced the temperature of the collagen denaturation. Time and temperature of intensive heat treatment (such as grilling or roasting) are important factors in the formation process of carcinogenic compounds i.e. heterocyclic aromatic amines.

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BADANIE ZA POMOCĄ DSC WPŁYWU MARYNOWANIA NA STABILNOŚĆ KOLAGENU ZE SKÓRY SKRZYDEŁEK KURCZĄT

Wstęp. Marynowanie jest dobrą metodą uatrakcyjnienia skrzydełek kurczęcych, które cieszą się najmniejszym zainteresowaniem spośród dostępnych elementów tuszek kurcząt. Walory sensoryczne marynowanych skrzydełek są zależne w głównej mierze od smakowitości skóry. Ponadto skóra jest naturalną barierą, która może ułatwiać lub utrudniać przenikanie składników marynaty w zależności od stanu konformacyjnego budujących ją białek, w tym głównie kolagenu. Celem pracy było zbadanie za pomocą różnicowej kalorymetrii skaningowej (DSC) wpływu określonych marynat na proces denaturacji kolagenu.
Materiał i metody. Skrzydełka kurczęce marynowano z użyciem marynat modelowych, marynat przyrządzonych według własnych receptur oraz gotowych marynat stosowanych w praktyce produkcyjnej. Do badania z użyciem kalorymetru DSC 7 firmy Perkin-Elmer pobierano ze skrzydełek próbki skóry (ok. 5-8 mg), które naważano do aluminiowych naczynek. Szybkość ogrzewania wynosiła 5°C/min, w zakresie od 20 do 100°C.
Wyniki. W badaniach z użyciem marynat modelowych (6% NaCl) uzyskano obniżenie temperatury denaturacji (T_{max}) o ok. 3°C i entalpii ΔH o ok. 40%. Jeszcze większe obniżenie temperatury denaturacji kolagenu (o ok. 7°C) i entalpii ΔH (o ok. 48%) stwierdzono po zastosowaniu marynat przygotowanych według receptur własnych (W2, W3). Natomiast zastosowanie marynat modelowych zawierających NaCl i kwasy organiczne (octowy lub cytrynowy) powodowało stabilizację kolagenu, co objawiało się wzrostem entalpii ΔH o ok. 50% (dla marynaty zawierającej 2% kwasu cytrynowego).
Wnioski. Temperatura i entalpia denaturacji kolagenu zmieniała się zależnie od rodzaju marynaty. Stopień denaturacji kolagenu wpływa na wartość żywieniową i sensoryczną marynowanych elementów. Biorąc pod uwagę, że czas i temperatura intensywnej obróbki cieplnej są ważnymi czynnikami wpływającymi na powstawanie związków kancerogennych, tj. heterocyklicznych amin aromatycznych, jest ważne, aby wybierać marynaty, które obniżają temperaturę i entalpię denaturacji białek.

Słowa kluczowe: kolagen, skóra kurcząt, marynowanie, denaturacja, analiza termiczna DSC

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