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EFFECT OF CHILLING STORAGE TIME ON THE PROTEOLYSIS AND LIPID OXIDATION IN VACUUM-PACKED TURKEY BREAST MUSCLES

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Key words: turkeys' breast muscles, free amino acids, proteolytic activity, TBARS, vacuum-packing

Changes of free amino acid contents, proteolytic activity, and TBARS values in the vacuum-packed breast muscles of turkey stored at 1°C for 19 days were studied. Eighteen free amino acids were identified. Their total content after 1 day of storage was 171.36 mg/100 g tissue, proteolytic activity was 48.29 UH/g tissue, and TBARS values reached 0.66 mg MA/kg tissue. After 11 days, the contents of free amino acids and proteolytic activity significantly increased (by *ca.* 52% and 25%, respectively) whereas TBARS values decreased (by *ca.* 33%). After 19 days of storage the contents of free amino acids and TBARS values increased (by *ca.* 116% and 15%, respectively), whereas the proteolytic activity did not significantly differ from a control sample. While storing turkey breast muscles, a continuous increase in the concentrations of all detected free amino acids was observed except for Arg and Trp which were stable over the entire experimental period and for Tyr whose concentration increased significantly already after 4 days of storage and remained at this level throughout the storage period. The contents of free Asp, Asn, Gly, Thr, Ala, Val, Met, Phe, Ile, Leu, Lys, Pro and the percentage contents of free Asp, Ala, Val, Met, Phe, Ile, Leu, Lys could be regarded as indicators of storage time or spoilage.

INTRODUCTION

Raw meat normally cannot be stored for extended periods under refrigeration (1–2°C) because of the proliferation of psychrotropic food spoilage of microorganism and texture degradation caused *i.a.* by the presence of endogenous proteolytic enzymes [Lakritz, 1988]. During the spoilage of muscles and other raw products of animal origin, the free amino acids initially present in the substrate are first degraded to produce volatile metabolites with off odours. It is only in the advanced stage of spoilage that additional amino acids are made available for microbial metabolism from the enzymatic degradation of proteins and peptides. In the final processes of protein degradation by bacterial enzyme, decarboxylation of free amino acids results in the formation of biogenic amines. The consumption of food containing high amounts of some biogenic amines can have a toxicological effect. On the other hand, polyamines play important roles in multiple physiological functions of humans and animals [Brink *et al.*, 1990; Bardócz *et al.*, 1993].

During the cooking process heterocyclic amines [Pais *et al.*, 1999] and aromatic amines mutagens are formed from the present free amino acids. As reported by Hatch *et al.* [1992], they are likely to effect carcinogenesis in human beings.

Lipid oxidation is one of the major causes of deterioration in meat and meat products. The major factors affecting the deterioration of meat quality through lipid oxidation include the composition of phospholipids, the amount of polyunsaturated fatty acids in meat, the presence of free metal ions, oxygen, heme pigments, mechanical processes, cooking and the addition of salt during processing procedures [Kanner *et al.*, 1988 a, b]. Poultry meat is more susceptible to oxidative rancidity than red meat due to its higher content of polyunsaturated fatty acids.

The development of packaging can improve shelf life and prevent chemical deterioration of food products caused by lipid oxidation and pigment autoxidation. Molecular oxygen is an integral part of the lipid peroxidation process. Therefore, vacuum-packaging reduces oxidation as it decreases the contact between a product and atmospheric oxygen [Fernandez-Espla & O'Neill, 1993].

The paper is a fragment of some a more complex study into "The quality changes in the turkey breast muscles vacuum packed without added, with lactic acid, with natrium lactate and storage at 1°C", concerning microbiological changes of sensory and functional properties of turkey breast muscles. The earlier manuscript informs about microbiological contamination. Consequently the maximum storage time at 1°C for vacuum-packed turkey breast muscles was found to be 9 days [Pipek *et al.*, 1999]. The remaining results are being prepared.

The aim of this work was to investigate the effect of storage time at 1°C on the proteolysis and lipid oxidation in the vacuum-packed breast muscles of turkeys.

MATERIAL AND METHODS

Material. Fifteen breast muscles (*ca.* 1 kg each) were isolated from industrially slaughtered turkeys and vacuum-

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-packed into bags 24 h after slaughter. All the samples were stored in a refrigerator at 1°C±0.5. Three breast samples were taken for examination after storage for 1, 4, 11, 14 and 19 days. Two series were investigated. The samples were determined: proteolytic activity, contents of free amino acids, and TBARS values (thiobarbituric acid-reactive substances).

Sample preparation for analyses. Protease activity was measured in homogenate with hemoglobin (pH 3.8) as a substrate [Anson, 1938]. The activity was expressed in UH/g tissue.

Extraction of enzymes: 10 g of ground tissue was placed into 50 mL of 0.25 mol/L sucrose solution containing 0.02 mol/L KCl, cooled in ice bath and homogenised at 4000 rpm for 2 min at 0°C [Moeller *et al.*, 1976].

Extraction of free amino acids was carried out according to the method described by Aristoy and Toldra [1991] with certain modification. Ground tissue (8 g) and 40 mL of 0.1 mol/L HCl were homogenized at 5°C for 8 min and centrifuged at 10 000 × g for 20 min. The supernatant was filtrated through glass wool and collected for further procedure. The 2 mL samples were mixed with 6 mL acetonitrile. The mixture was allowed to stand for 30 min before centrifugation at 10 000 × g for 15 min. Supernatant (720 μL) was mixed with 80 μL of the internal standard solution (sarcosine and norvaline).

The free amino acids composition was measured using a Hewlett Packard HPLC chromatograph AminoQuant II/M Standard sensitivity with the HP 1090 M Liquid Chromatograph Diode Array detector and HPLC Chemstation (Pascal Series). The procedure was described in the Operator's Handbook [1990]. The content of free amino acids was expressed in mg/100 g tissue.

TBARS values were measured according to the procedure of Salih *et al.* [1987] with some modifications. Ground tissue (10 g) was homogenised for 2 min (4 000 rpm) with 34.25 mL of 4% cold perchloric acid (*ca.* 4°C). The butylated hydroxytoluen (BHT), dissolved in 98% ethanol, was added prior to homogenisation (0.75 mL). The homogenate was filtered with the Whatman 1 paper filters. Filtrate (5 mL) was mixed with 5 mL of 20 mmol/L TBA (thiobarbituric acid) and incubated in boiling water for 1 h. Then it was cooled for 10 min. in running water. The absorbance was determined at 532 nm against a blank containing 5 mL of 4% perchloric acid and 5 mL of TBA solution. The TBARS volumes were calculated as described by Pikul [1993] and expressed as mg malondialdehyde/kg meat (mg MA/kg meat).

The obtained data were analysed statistically. The Student's t-test was used to compare mean values to determine significant differences between the average values of control and the examined samples. The control samples were breast muscles vacuum-packed after 1 day of storage.

RESULTS

The study demonstrated that during storage at 1°C the proteolytic activity in vacuum-packed turkey breast muscles increased significantly after 11 and 14 days (by *ca.* 25% and 40%, respectively) as compared to breast muscles after 1 day of storage, then decreased and after 19 days was observed not differ significantly in comparison to turkey breast muscles stored for 1 day (Table 1).

The following free amino acids (FAA) were detected in vacuum-packed turkey breast muscles after 1 day of storage: aspartic acid, glutamic acid, asparagine, serine, histidine, glycine, threonine, alanine, arginine, tyrosine, valine, methionine, tryptophan, phenylalanine, isoleucine, leucine, lysine, proline (Figure 1). The total content of FAA was 171.36 mg/100 g tissue (Table 1).

TABLE 1. Proteolytic activity, contents of free amino acids, and TBARS values in the vacuum-packed turkey breast muscles stored at 1°C.

Time of storage (days)	Proteolytic activity (UH/g tissue)		Total FAA (mg/100 g tissue)		TBARS values (mg MA/kg tissue)	
	x	SD	x	SD	x	SD
1	48.29	5.26	171.36	4.20	0.66	0.04
4	54.55	5.26	170.85	3.41	0.55*	0.06
11	60.63*	6.07	260.15*	20.58	0.44*	0.04
14	68.46*	4.78	285.40*	1.78	0.66	0.06
19	54.54	4.20	370.22*	6.83	0.76*	0.06

x – mean of six samples; SD – standard deviation; * – significantly different from the sample stored for 1 day ($p<0.05$)

A significant increase in the content of total FAA was observed after storage of turkey breast muscles for 11, 14, 19 days (by *ca.* 52%, 60% and 116%, respectively) as compared to those stored for 1 day. The free Glu (29.35 mg/100 g tissue) and Trp (36.54 mg/100 g tissue) were the major FAA in the breast muscles after 1 day of storage (Figure 1). After 4 days of storage it was evident that the concentrations of individual FAA were similar to those recorded after 1 day of storage except for Tyr. While storing turkey breast muscles, a continuous increase in the concentrations of all detected free amino acids was observed except for Arg and Trp which were stable over the entire experimental period and for Tyr whose concentration increased significantly already after 4 days of storage and remained at this level throughout the storage period. The content of free amino acids was observed to change to a little extent during the storage period, *i.e.* an increased concentration was observed only for Ser and His after 19 days of storage.

A continuous decrease in the percentage of free Trp, Pro; a continuous increase in the percentage of free Asp, Ala, Val, Met, Phe, Ile, Leu, Lys as well as a decrease in Tyr of turkey breast muscles were observed after 11 days of storage.

The contents of free Asp, Asn, Gly, Thr, Ala, Val, Met, Phe, Ile, Leu, Lys, Pro and the percentage contents of free Asp, Ala, Val, Met, Phe, Ile, Leu, Lys could be regarded as indicators of storage time or spoilage.

After 1 day of storage, the TBARS value in the vacuum-packed turkey breast muscles was 0.66 mg MA/kg tissue (Table 1). A significant decrease in TBARS values (by *ca.* 17% and 33%) was observed after 4 and 11 days of storage respectively. Upon longer storage, TBARS values increased and after 19 days of storage were higher by *ca.* 15% in comparison with turkey breast muscles stored for 1 day.

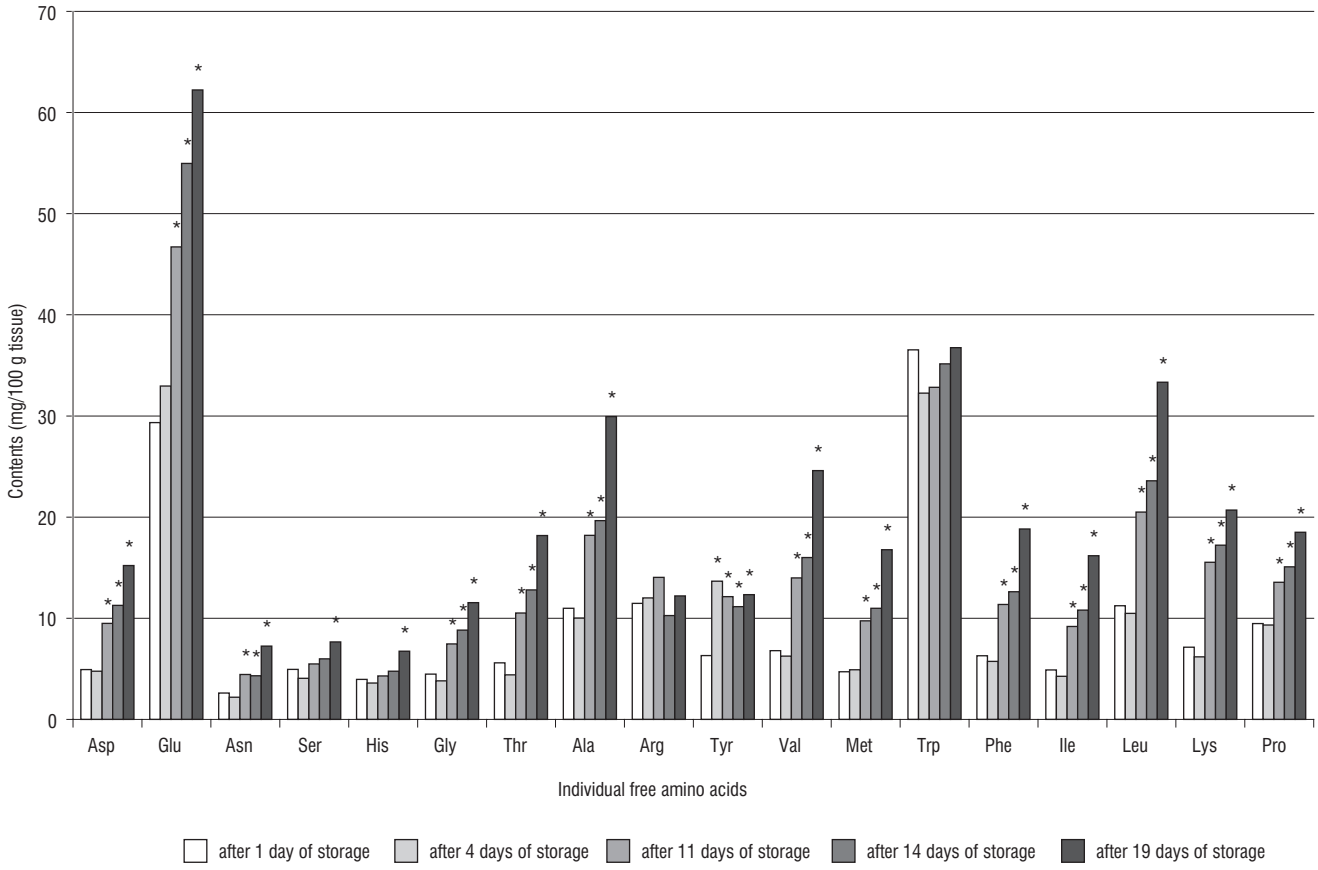

FIGURE 1. Free amino acid contents in breast muscles of turkey vacuum-packed and stored at 1°C (means of 12 samples; * significantly different from the sample stored for 1 day, $p<0.05$).

DISCUSSION

The proteolytic activity in the vacuum-packed breast muscles of turkey stored at 1°C for 1 day was lower than that in breast and leg muscles of Mullard duck vacuum-packed and stored at 1°C after 1 day (*ca.* 208 UH/g tissue) [Przysiężna, 1999]. Upon storage, the proteolytic activity decreased after 5 days in the case of leg muscles and after 18 days in the case of breast muscles of Mullard duck [Przysiężna, 1999]. A significant decrease in the proteolytic activity was observed in turkey breast muscles vacuum-packed with added lactic acid after 28 days of storage at 1°C [Przysiężna, 2002]. An increase of the proteolytic activity in muscles during storage was observed by Krala [1996]. The author examined chicken breast and leg muscles packed in PE bags and stored in the air and under modified atmosphere. He found that the proteolytic activity in muscles stored under modified atmosphere increased more slowly than in the samples packed in PE bags and stored in the air.

Changes of the proteolytic activity are determined by the kind of muscles and the kind of acting factors [Przysiężna & Skrabka-Błotnicka, 1996]. It is a common knowledge that the activity increases due to either release of enzymes from lysosomes or uncovering the new reactive centres of enzymes. The decrease of the proteolytic activity can be caused by the conformation changes of enzymes.

It results from literature that the content of FAA in muscles depends on the kind of muscles and time of storage. The FAA content has been reported to account for *ca.* 2.30 mg/g of meat in the raw breast muscles of turkey [Pais *et al.*, 1999], and for 184 mg/100 g and 233 mg/100 g in vacuum-packed breast and leg muscles respectively, of force-fed Mullard duck after 1 day of storage at 1°C [Przysiężna, 1999]. Karasawa *et al.* [1990] found 19 free amino acids in breast and leg muscles of chicken. During storage at 4°C, little changes were found in free amino acids: Tau, Cys, His and Trp, a continuous increase in: Gly, Ala, Val, Met, Ile, Leu, Phe and Lys and a decrease after an increase in: Asp, Thr, Ser, Gly, Tyr, Arg and Pro.

A more rapid increase in free amino acid content during storage at 1°C was observed in leg than in breast muscles of vacuum-packed force-fed Mullard ducks [Przysiężna, 1999]. During storage, the increase in free amino acid content was observed by Krala [1992] in breast muscles of chickens packed in PE bags and stored at 4°C in the air and under modified atmosphere. He found out that the content of free amino acids increased more slowly in the muscles stored under modified atmosphere than in those stored in the air.

The significant increase in the total contents of free amino acids in turkey breast muscles vacuum-packed with added lactic acid and stored at 1°C was observed by Przysiężna [2002].

The free Glu (29.35 mg/100 g tissue) is one of the major FAA in vacuum-packed turkey breast muscles stored for 1 day at 1°C. The concentration of free Glu is lower in other meat, thus during storage it was reported to account for: 6.03 mg/100 g in beef (at 4°C for 4 days), 3.53 mg/100 g in pork (1 day of storage), and 12.95 mg/100 g in chicken (0 day of storage) [Nishimura *et al.*, 1988].

Renerre *et al.* [1999] also reported low initial TBA values in turkey meat. The TBARS values in the drakes' vacuum-packed breast and leg muscles during storage significantly increased. Quicker changes were observed in leg than in the breast muscles [Przysiężna, 1999]. An increase in TBA values was observed in poultry meat during storage [Kilic &Richards, 2003; Alasnier *et al.*, 2000; Tang *et al.*, 2001a, b; Karpińska *et al.*, 2001], in chicken meat after heating and after 2, 4 and 6 days of refrigerated storage at 4–6°C [Pikul, 1999], and in beef steak [Noriham *et al.*, 2004], but Salih *et al.* [1989] reported that the TBA values of the refrigerated raw thigh turkey muscles did not increase significantly after 7 days of storage. On the other hand many authors observed a decrease in TBARS values during refrigerated storage of vacuum-packaged: turkey breast rolls [Smith & Alvarez, 1988], breast and leg muscles of turkey (4°C) between time 0 to 1 week [Higgins *et al.*, 1998], rabbit meat after 10 days of storage at 1°C under atmosphere without oxygen [Berruga *et al.*, 2004]. A decrease in TBA number generally occurs after a certain storage interval due to the formation of secondary products of lipid oxidation which do not react with the TBA reagent or to the reaction of malondialdehyde with protein [Melton, 1983]. Malondialdehyde and other TBARS were metabolized by bacteria as they reached spoilage levels in pork [Lebepe *et al.*, 1990]. This may be attributed to decomposition of TBA reactive substance to other products of lipid oxidation by chemical or microbial mechanisms, as could be expected in samples undergoing rapid spoilage. It is likely that microbial spoilage has an influence on lipid oxidation and TBARS values [Branen, 1978], as it was observed in breast muscles of turkey vacuum-packed and stored at 1°C [Pipek *et al.*, 1999] and in this work.

CONCLUSION

The results obtained suggest that in turkey breast muscles stored for 11 days the changes were slower than in those between 11 and 19 days of storage. Vacuum packaging reduced lipid oxidation in turkey breast muscles during storage at 1°C and thus may extend their shelf-life.

The content of free Asp, Asn, Gly, Thr, Ala, Val, Met, Phe, Ile, Leu, Lys, Pro and the percentage content of free Asp, Ala, Val, Met, Phe, Ile, Leu, Lys could be regarded as indicators of storage time or spoilage.

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WPŁYW CZASU CHŁODNICZEGO PRZECHOWYWANIA NA PROTEOLIZĘ I UTLENIANIE LIPIDÓW W PRÓŻNIOWO ZAPAKOWANYCH MIĘŚNIACH PIERSIOWYCH INDYKÓW

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Badano zmiany zawartości wolnych aminokwasów, aktywności proteolitycznej i wskaźnika TBARS w piersiowych mięśniach indyka pakowanych próżniowo i przechowywanych w 1°C przez 19 dni. Oznaczono zawartość 18 wolnych aminokwasów. Całkowita ich zawartość po 1 dniu przechowywania wynosiła 171.36 mg/100 g tkanki. Aktywność proteolityczna próby kontrolnej wynosiła 48.29 UH/g tkanki, a wskaźnik TBARS 0.66 mg MA/kg tkanki. Po 11 dniach zaobserwowano istotny wzrost aktywności proteolitycznej i zawartość wolnych aminokwasów (o 25%, o 52% odpowiednio), a wskaźnik TBARS zmniejszył się (33%) (tab. 1). Po 19 dniach przechowywania stwierdzono wzrost zawartości wolnych aminokwasów i wskaźnika TBARS (o 116%, o 15% odpowiednio), a aktywność proteolityczna nie różniła się istotnie od próby kontrolnej. Podczas przechowywania mięśni piersiowych indyka zaobserwowano stały wzrost ilości wolnych aminokwasów z wyjątkiem Arg i Trp, których zawartości były stabilne przez cały badany okres czasu i Tyr, której ilość istotnie wzrosła jedynie po 4 dniach przechowywania i pozostała na tym poziomie w dalszym okresie czasu. Zawartość wolnych Asp, Asn, Gly, Thr, Ala, Val, Met, Phe, Ile, Leu, Lys, Pro oraz procentowy udział wolnych Asp, Ala, Val, Met, Phe, Ile, Leu, Lys może być wskaźnikiem czasu przechowywania lub wskaźnikiem zepsucia mięśni piersiowych indyka (rys. 1).