POLISH JOURNAL OF FOOD AND NUTRITION SCIENCES
www.pan.olsztyn.pl/journal/
e-mail: joan@pan.olsztyn.pl
Pol. J. Food Nutr. Sci.
2007, Vol. 57, No. 4, pp. 457–463

EFFECT OF CHILL STORAGE TIME ON PROTEOLYSIS AND LIPID OXIDATION IN VACUUM-PACKED MUSCLES FROM DUCK

Ewa Przysiężna

Department of Animal Food Technology, University of Economics, Wrocław

Key words: drakes, breast muscles, leg muscles, free amino acids, proteolytic activity, TBARS, vacuum-packaging, chill storage

Proteolysis and lipid oxidation in the vacuum-packed leg and breast muscles from Mullard drakes stored at 1°C were studied. As proteolysis indicators there were determined proteolytic activity, contents of amino nitrogen and free amino acids (FAA), and TBARS values as indicators of oxidative changes. Changes were also determined in the proteolytic activity, TBARS values and contents of: amino nitrogen and FAA. As a result, 18 FAA were found in breast muscles and 19 in leg and their total contents after 1 day of storage were 184.39 mg/100 g tissue, and 233.33 mg/100 g tissue (respectively). In the case of breast muscles a significant increase in the content of detected FAA (except Pro) was noted after 13 days of storage and in the leg muscles (except Asp) after 5 days of storage. It was established that the proteolytic activity decreased (*ca*. 38%) in breast after 18 days and in leg (*ca.* 30%) after 5 days of storage. The content of amino nitrogen significantly increased in breast muscles after 18 days and in leg muscles after 5 days of storage. During storage, TBARS values were observed to increase continuously in breast muscles, whereas in legs they first increased after 5 days and then were observed to decrease.

INTRODUCTION

Raw meat normally cannot be chill stored for extended periods because of: food spoilage induced by psychrotrophic bacteria, texture degradation caused by the presence of endogenous proteases, and lipid oxidation. The reactions involved in the proteolysis of meat proteins are catalyzed by endogenous enzymes together with proteases produced by microorganisms. During the spoilage of meat and other raw products of animal origin the FAA initially present in the substrate are first degraded to produce volatile metabolites with off odour. 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. FAA are thus apparently well suited for monitoring the microbial spoilage of protein-rich foodstuffs [Schmitt & Schmidt-Lorenz, 1992].

Lipids play a key role in many quality traits of meat products, including nutritional value and sensory properties (flavour in particular), because they are both solvents and precursors of aroma compounds. Lipid oxidation (often measured with TBARS test) is one of the main causes of quality deterioration in meat during storage and processing resulting in rancidity. The changes in quality are manifested by adverse changes in flavour, colour, texture, a decrease in nutritive value, and the possible production of toxic compounds [Buckley *et al*., 1995; Fernandez *et al*., 1997; Gray *et al*., 1996; Morrissey *et al*., 1998] and limit the shelf-life of meat and meat products [Kanner, 1994]. Lipid oxidation has negative effects on the quality of muscles-based food and often determines the shelf life of meat and meat products. Progression of lipid oxidation is influenced by a number of factors, such as the content and state of muscle pro-oxidants (iron, myoglobin), muscle antioxidant levels (tocopherol), fat content and fatty acids profiles, degree of processing (mincing, heating), and storage conditions (time, temperature, packaging).

Lipid oxidation is one of the principal chemical reactions occurring in chill storage of muscle. It is initiated in the membrane – bound phospholipids [Morrissey *et al*., 1994], which are susceptible to peroxidation due to their high concentration of long chain polyunsaturated fatty acids. Poultry meat is more susceptible to oxidative rancidity than red meat because of its higher content of polyunsaturated fatty acids. Oxidation occurs as a result of the reaction between atmospheric oxygen and unsaturated fatty acids, particularly polyenoic fatty acids [Kanner *et al*., 1988]. Vacuum-packaging reduces the amount of oxygen in the package and reduces lipid oxidation [Fernandez-Espla & O'Neill, 1993]. The development of packaging can extend shelf life and prevent chemical deterioration of food products. Vacuum-packaging continues to be in many cases the most cost effective packaging strategy. In the USA, the use of vacuum packages increased by 3% over two years (10% in 2002 to 13% in 2004) [Eilert, 2005].

Meat of ducks is attractive for consumers due to its high sensory and nutritive values. The available literature pro-

Author's address for correspondence: Ewa Przysiężna, Department of Animal Food Technology, University of Economics, ul. Komandorska 118/120, 53-345 Wrocław, Poland; tel. (48 71) 36 80 264; e-mail: ewa.przysiezna@ae.wroc.pl

vides mainly a description of duck meat quality or methods of improving its quality. There are no adequate data in literature concerning the effect of vacuum-packaging and chill storage on protease activity and lipid oxidation of duck muscles. This was the reason for undertaking the investigation of this subject.

This contribution is a part of the work on changes in the quality of Mullard drakes meat caused by ageing under vacuum condition at 1°C. The earlier contributions refer to microbiological contamination and odour [Kosek *et al*., 1998] and some functional and sensory attributes [Skrabka-Błotnicka *et al*., 2003].

The aim of this study was to investigate the effect of storage time at 1°C on proteolysis and lipid oxidation in vacuum--packed muscles from Mullard drakes.

MATERIALS AND METHODS

Materials preparation. Breast and leg muscles weighing *ca*. 280 g and 140 g, respectively, from 12-week old industrially killed Mullard drakes were used. The breast and leg portions (with skin and with skin and bones, respectively) were vacuum packed into PA/PE bags (with an oxygen transmission rate of 27.7 cm^3 m^{-2} 24 h^{-1} atm.$^{-1}$) 24 h after slaughter using a packaging machine MULTIVAC model A300/52. The samples were stored in a refrigerator at 1°C±0.5. The breast samples were taken for examination after 1, 6, 13 and 18 days and leg samples after 1, 5 and 11 days of storage. Six breast or six leg muscles were tested each time. Two series were investigated. The breast and leg muscles were not examined at the same time. Breast muscles were investigated first. Taking into consideration the fact that the leg muscles stored for 1 day were more contaminated than the breast muscles, the time of storage between sequential examinations was cut in relation to breast muscles. The samples were determined for: proteolytic activity, contents of amino nitrogen, free amino acids, and TBARS values (thiobarbituric acid-reactive substances).

Sample preparation for analyses. Protease activity was measured in a homogenate with hemoglobin as a substrate (pH 3.8, a sample was incubated in a shaking water bath at 37°C for 30 min) [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 an 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 & Toldra [1991] with certain modification. Ground tissue (8 g) and 40 mL of 0.1 mol/L HCl were homogenised 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 of 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.

The amino nitrogen was determined as follows: 10 g of ground tissue was placed in 90 mL of distilled water, cooled in an ice bath and homogenised for 2 min. The mixture was stored in a refrigerator overnight, and was then filtered with Whatman 1 paper filters. The amino nitrogen was measured in the filtrate, and expressed in mg N/g tissue [Mejbaum-Katzenellenbogen & Mochnacka, 1969].

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).

Statistical analysis. Data were analysed statistically using the Student's t-test to determine significant differences between the mean values of control and the examined samples at a significance level of $p<0.05$. The control samples were breast and leg muscles vacuum-packed after 1 day of storage.

RESULTS AND DISCUSSION

Proteolytic activity

The proteolytic activity of both muscles after 1 day of storage was 208 UH/g tissue (Table 1). During storage at 1°C the proteolytic activity in breast muscles decreased significantly after 18 days by about 38% as compared to breast muscles after 1 day of storage (Table 1). In the case of leg muscles the proteolytic activity decreased significantly by about 30% and 50% after 5 and 11 days, respectively, as compared to 1 day of storage (Table 1). The content of amino nitrogen significantly increased in breast muscles after 18 days and in leg muscles after 5 days of storage (Table 1).

A significant decrease in proteolytic activity in turkey breast muscles vacuum-packed with added lactic acid after 28 days of storage at 1°C was observed by Przysiężna [2002]. In turn, Przysiężna [2000] observed an increase and then a decrease of the proteolytic activity in vacuum-packed turkey breast muscles stored at 1°C for 14 days. An increase in the proteolytic activity in muscles during storage was also observed by Krala [1996, 1999]. The author examined chicken breast and leg muscles packed in PE bags and stored in air and in modified atmosphere (75% CO_2, 20% N_2, 5% O_2 and 80% CO_2, 20% N_2). He found that the proteolytic activity in muscles stored in modified atmosphere increased more slowly than in the samples packed in PE bags and stored in air.

The present work confirms that the changes in proteolytic

TABLE 1. Proteolytic activity, amino nitrogen, content of total FAA and TBARS values of vacuum-packed breast and leg muscles of Mullard drakes stored at 1°C.

Time of storage (days)	Proteolytic activity (UH/g tissue)	Amino nitrogen (mg N/g tissue)	Total FAA (mg/100 g tissue)	TBARS values (mg MA/kg tissue)
Breast muscles				
	$\bar{x}\pm$SD	$\bar{x}\pm$SD	$\bar{x}\pm$SD	$\bar{x}\pm$SD
1	207.87±15.04	0.80±0.06	184.39±9.86	0.48±0.03
6	199.95±12.66	0.88±0.04	214.54±11.66*	0.75±0.06*
13	211.51±4.48	0.85±0.04	265.13±20.51*	1.02±0.06*
18	129.55±12.56*	1.12±0.05*	393.89±26.18*	1.17±0.03*
Leg muscles				
	$\bar{x}\pm$SD	$\bar{x}\pm$SD	$\bar{x}\pm$SD	$\bar{x}\pm$SD
1	208.82±9.90	0.81±0.06	233.33±8.50	0.28±0.05
5	144.99±6.75*	1.04±0.08*	381.48±26.06*	0.85±0.05*
11	106.58±5.40*	1.19±0.05*	389.31±34.34*	0.79±0.05*

Data are average values of 12 samples; $\bar{x}\pm$SD – average values ± standard deviation; *significantly different from a control sample ($p< 0.05$)

activity depend on the type of muscles and the kind of acting factors, as found by others [Przysiężna & Skrabka-Błotnicka, 1996]. The activity increases due to either the release of enzymes from lysosomes or the uncovering of the new reactive centres of enzymes. The decrease of the proteolytic activity can be caused by the conformation changes of enzymes.

An increase in the content of amino nitrogen in chicken breast and leg muscles stored at 4°C was observed by Krala [1999]. An increase of amino nitrogen content was observed in the present work but it was accompanied by proteolytic activity decrease. The increase may be the result of other enzymes' activity.

Free amino acids

The following FAA were detected in vacuum-packed breast and leg muscles after 1 day of storage: aspartic acid, glutamic acid, asparagine, serine, glutamine, histidine, glycine, threonine, alanine, tyrosine, valine, methionine, tryptophane, phenylalanine, isoleucine, leucine, lysine, proline and additionally arginine in the case of leg muscles (Figures 1, 2). The total content of FAA in breast muscles was 184.39 mg/100 g tissue and in leg muscles 233.33 mg/100 g tissue (Table 1). A significant increase in the content of total FAA in breast muscles was observed after storage for 6, 13 and 18 days (by *ca.* 16%, 44% and 114%, respectively) and in leg muscles after storage

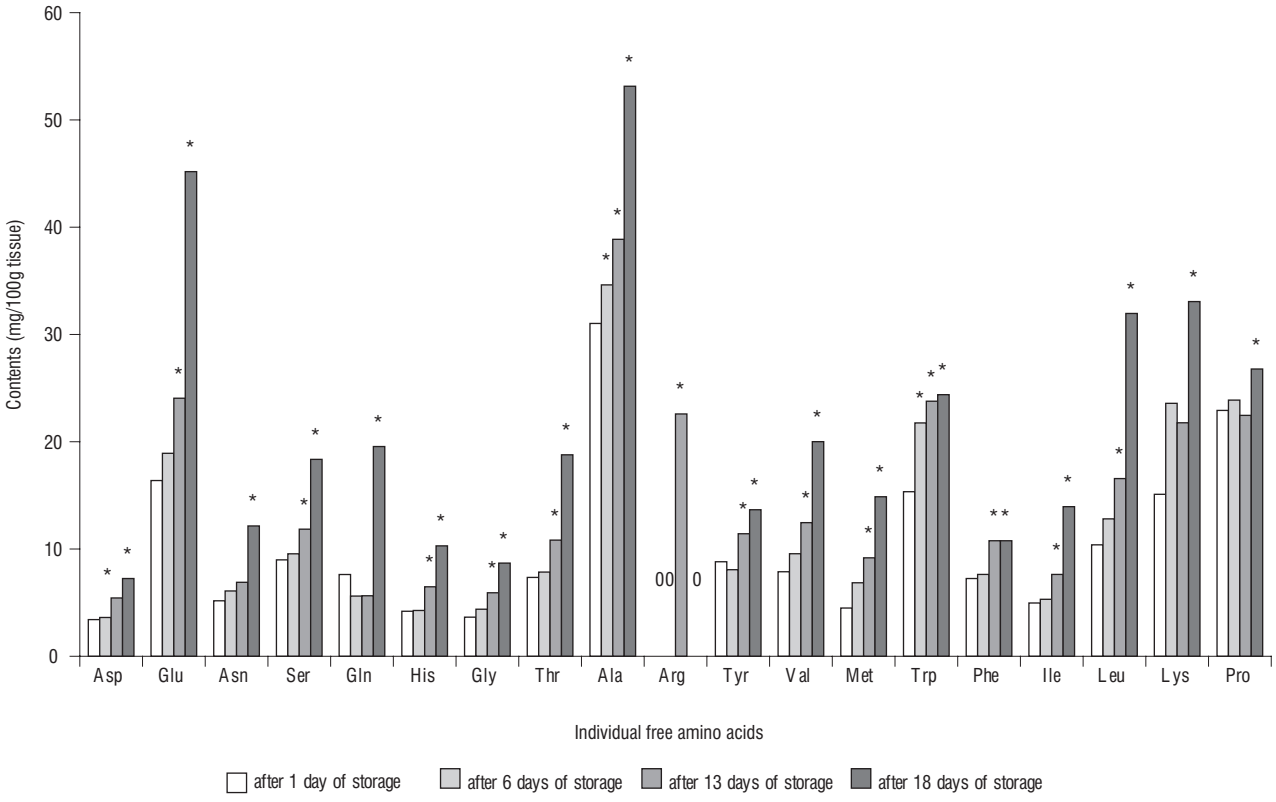

FIGURE 1. Free amino acids contents in breast muscles of Mullard drakes vacuum-packed and stored at 1°C (means of 12 samples, * – significantly different from the sample stored for 1 day ($p<0.05$)).

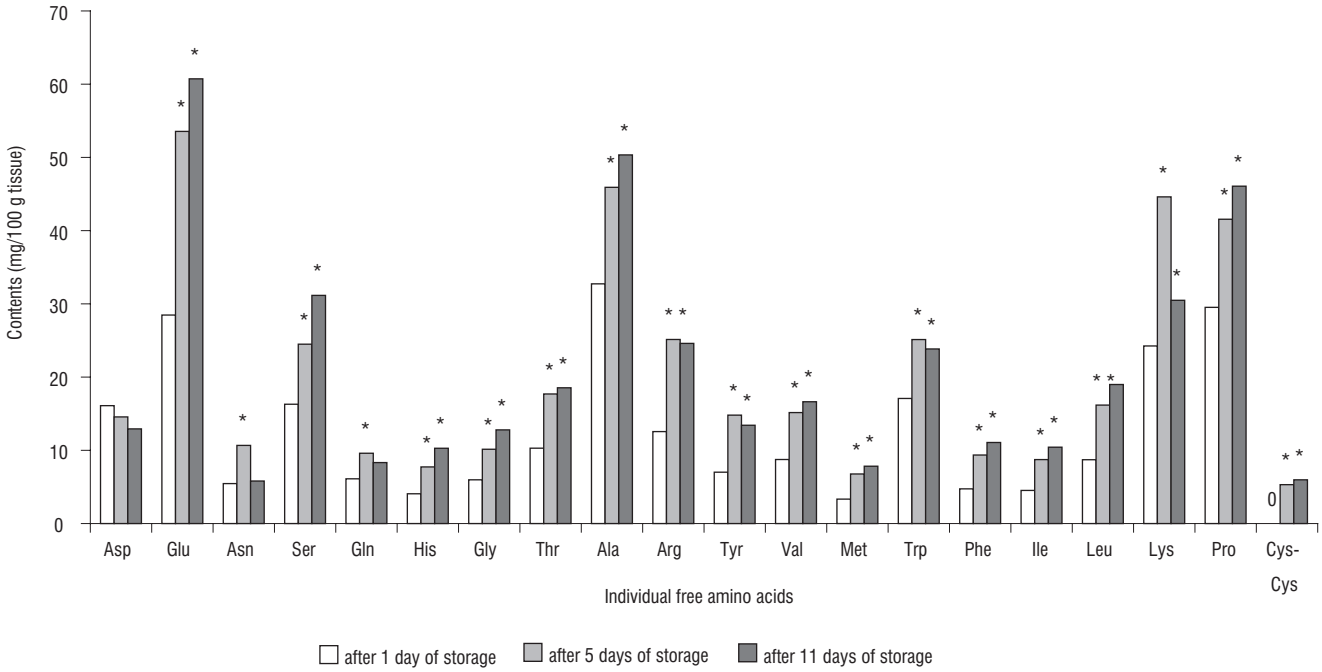

FIGURE 2. Free amino acids contents in leg muscles of Mullard drakes vacuum-packed and stored at 1°C (means of 12 samples, * – significantly different from the sample stored for 1 day ($p<0.05$)).

for 5 and 11 days (by *ca.* 63% and 67%, respectively) in comparison to samples stored for 1 day.

The free Ala (31.03.mg/100 g tissue), Pro (22.92 mg/100 g tissue) and Glu (16.38 mg/100 g tissue), Trp (15.34 mg/100 g tissue), Lys (15.10 mg/100 g tissue) were the major FAA in the breast muscles after 1 day of storage (Figure 1). During storage an increase was observed in the total and all individual FAA contents. The content of the following FAA: Asp, Glu, Ser, His, Gly, Thr, Ala, Arg, Tyr, Val, Met, Trp, Phe, Ile, Leu in breast muscles of vacuum-packed Mullard drakes stored at 1°C could be regarded as an indicator of the time of storage (Figure 1). However, they were present, except for Arg, in the vacuum-packed breast muscles stored at 1°C for 1 day, and their concentration increased during storage. The major free amino acids in the leg muscles after 1 day of storage were: Glu (28.47 mg/100 g tissue), Ala (32.73 mg/100 g tissue), Lys 24.25 mg/100 g tissue), and Pro (29.52 mg/100 g tissue) (Figure 2). While storing drakes' leg muscles changes in the concentration of all FAA were observed except for Asp and Cys-Cys which appeared after 5 days of storage. A continuous increase was observed in free Glu, Ser, His, Gly, Tre, Ala, Val, Met, Phe, Ile, Leu and Pro. A decrease in contents after an increase was observed only in free Asn, Gln, Tyr and Lys in leg muscles of drakes. The contents of the FAA such as Glu, Ser, His, Gly, Thr, Ala, Arg, Tyr, Val, Met, Trp, Phe, Ile, Leu, Lys, Pro and Cys-Cys in the case of leg muscles of drakes could be regarded as indicators of the time of storage (Figure 2).

It was found out that during storage the percentage of individual FAA - calculated as a percentage of the total FAA content - Asp, Asn, Ser, His, Gly, Thr, Tyr in breast muscles and Gln, Gly, Thr, Tyr, Val, Met, Ile in leg muscles was stable. The concentrations of other detected FAA such as: Glu, Ala, Arg, Val, Met, Trp, Phe, Ile, Leu, Pro in the case of breast muscles and Asp, Glu, Ser, His, Ala, Arg, Trp, Phe, Leu and Cys-Cys in the case of leg muscles could be regarded as indicators of the time of storage. The application of FAA as indicators requires detailed research.

The content of FAA in muscles has been shown to depend on the type of muscle, time of storage and in the case of pork on quality classes [Aristoy & Toldra, 1998; Flores *et al*., 2000; Moya *et al*., 2001]. The ageing of pork meat only DFD class showed higher increases in FAA probably due to the activation of neutral aminopeptidases at the high pH of DFD meats [Moya *et al*., 2001]. The FAA in the skin of freshly slaughtered chicken was found to be 100–150 mg/100 g [Schmitt & Schmidt-Lorenz 1992], in the raw breast muscles of turkey it was about 230 mg/100 g of meat [Pais *et al*., 1999] and in vacuum-packed turkey breast muscles stored for 1 day at (1°C) it was 171.36 mg/100 g tissue [Przysiężna, 2005]. During storage an increase in FAA content was observed by Feidt *et al*. [1996] in bovine muscles with increasing ageing time, and by Krala [1992] in breast muscles of chicken packed in PE bags and stored at 4°C in air and in modified atmosphere (75% CO_2, 20% N_2, 5% O_2). The latter found that the content of FAA increased more slowly in muscles stored in modified atmosphere than in those stored in the air. A significant increase in the contents of total FAA in vacuum-packed breast muscles of turkey after 11 days storage at 1°C was observed by Przysiężna [2000] and in vacuum-packed breast muscle of turkey with added lactic acid stored for 21 days at 1°C [Przysiężna, 2002] and in chicken breast muscles Niewiarowicz *et al*. [1978]. Niewiarowicz *et al*. [1978] studied the FAA of chicken breast muscles at the start and after a week of chilled storage and observed an increase in the proportion of all amino acids except Pro. The increase of free amino acids during storage of meats was caused mainly by the actions of aminopeptidases towards peptides which were produced from meat proteins by the actions of cathepsins and calpains [Nishimura *et al*., 1996].

The content of free Glu amounted to *ca.* 16 mg/100 g tissue in the breast muscles and to *ca*. 28 mg/100 g tissue

in the case of leg muscles of Mullard drakes. 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 [Przysiężna, 2000]. Lower concentrations of free Glu have been reported in other meats, 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]. The contents of individual FAA depended on the type of muscles, time of storage and quality classes.

During storage time, the FAA increase in the examined drake muscles, at the observed decrease of proteolytic activity, may be the effect of active metabolite or active proteolytic enzymes of microbiological origin.

TBARS

After 1 day of storage, the TBARS value in the vacuum-packed breast muscles accounted for 0.48 mg MA/kg tissue and in leg muscles for 0.28 mg MA/kg tissue (Table 1).

There was a significant increase in TBARS values for both kinds of muscles, in the case of breast muscles by about 56% after 6 days, by 113% after 13 days and by 144% after 18 days, in the case of leg muscles by about 204% and 182% after 5 and 11 days, respectively (Table 1), in comparison to muscles stored for 1 day. More rapid changes were observed in leg than in the breast muscles. It may be connected with a higher content of polyunsaturated fatty acid in leg than in the breast muscles of Mullard drakes [Wołoszyn, 2002].

An increase in TBARS values was observed in poultry meat during storage [Pikul, 1999; Kilic & Richards, 2003; Alasnier *et al*., 2000; Tang *et al*., 2001a,b; Karpińska *et al*., 2001], in chicken and duck muscles with increasing final heating temperature and the length of the storage time [Hoac *et al*., 2006], in mechanically deboned turkey meat with added antioxidants and stored at –25°C [Mielnik *et. al*., 2003], in mechanically deboned turkey meat treated with high hydrostatic pressure [Tuboly *et al*., 2003]. In contrast, Salih *et al.* [1989] reported that the TBA values of chill stored 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 chill storage in vacuum-packages: turkey breast rolls [Smith & Alvarez, 1988], breast and leg muscles of turkey [Higgins *et al.,* 1998], breast muscles of turkey [Przysiężna, 2005], in rabbit meat after 10 days storage at 1°C in 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 chicken [Moerck & Ball, 1974] and 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 it could be expected in samples undergoing rapid spoilage. It is possible that microbial spoilage has an influence on lipid oxidation and TBARS values [Branen, 1978], and it was observed in vacuum-packed breast muscles of turkey stored at 1°C [Pipek *et al*., 1999; Przysiężna, 2005]. In materials of this study, the total count of anaerobic bacteria amounted to 2.3×10^7 CFU/g after 18 days of storage of breast muscles and to 8.8×10^7 CFU/g after 11 days of storage of leg muscles [Kosek *et al*., 1998

CONCLUSIONS

1. Vacuum packaging did not reduce TBARS in breast and leg muscles of Mullard drakes during storage at 1°C.

2. Significant changes of the proteolytic activity, TBARS values, as well as contents of amino nitrogen and, free amino acids in the leg muscles were observed earlier than in the breast muscles.

3. The contents of the following FAAs: Asp, Glu, Ser, His, Gly, Thr, Ala, Arg, Tyr, Val, Met, Trp, Phe, Ile, Leu in vacuum--packed breast muscles of Mullard drakes stored at 1°C as well as those of Glu, Ser, His, Gly, Thr, Ala, Arg, Tyr, Val, Met, Trp, Phe, Ile, Leu, Lys, Pro and Cys-Cys in leg muscles of drakes could be regarded as indicators of storage time or spoilage.

4. The concentrations of FAAs such as: Glu, Ala, Arg, Val, Met, Trp, Phe, Ile, Leu, Pro in the case of breast muscles and Asp, Glu, Ser, His, Ala, Arg, Trp, Phe, Leu and Cys-Cys in the case of leg muscles could be regarded as indicators of storage time or spoilage.

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Received January 2007. Revision received June and accepted July 2007.

WPŁYW CZASU CHŁODNICZEGO PRZECHOWYWANIA NA PROTEOLIZĘ I UTLENIANIE LIPIDÓW PRÓŻNIOWO ZAPAKOWANYCH MIĘŚNI KACZEK

Ewa Przysiężna

Katedra Technologii Żywności Pochodzenia Zwierzęcego, Akademia Ekonomiczna im. O. Langego, Wrocław

Badano proteolizę i utlenianie lipidów w próżniowo zapakowanych mięśniach nóg i piersi kaczorów Mullard przechowywanych w temperaturze 1°C. Oznaczono: zmiany aktywności proteolitycznej, wskaźnika TBARS i zawartości: azotu aminowego i wolnych aminokwasów (tab. 1). Zidentyfikowano 18 wolnych aminokwasów w mięśniach piersiowych (rys. 1) i 19 w mięśniach nóg (rys. 2) po 1 dniu przechowywania, a ich całkowita zawartość wynosiła 184,39 mg/100 g tkanki w mięśniach piersiowych i 233,33 mg/100 g tkanki w mięśniach nóg. Istotny wzrost zawartości indywidualnych aminokwasów w mięśniach piersiowych (za wyjątkiem Pro) zaobserwowano po 13 (rys. 1), a w mięśniach nóg (za wyjątkiem Asp) po 5 dniach przechowywania (rys. 2). Podczas przechowywania stwierdzono spadek aktywności proteolitycznej (ok. 38%) w mięśniach piersiowych (tab. 1) po 18, a w mięśniach (ok. 30%) nóg po 5 dniach (tab. 1). Istotny wzrost zawartości azotu aminowego stwierdzono po 18 dniach w mięśniach piersiowych i po 5 w mięśniach nóg (tab. 1). Wskaźnik TBARS wzrastał w mięśniach piersiowych przez cały czas przechowywania, natomiast w mięśniach nóg zaobserwowano jego wzrost po 5 dniach, a następnie spadek (tab. 1).