Effect of storage on desmin degradation and physico-chemical properties of poultry breast meat

D. WOJTYSIAK[1] and K. POŁTOWICZ[2]

[1]Department of Reproduction and Animal Anatomy, Agricultural University, 30-059 Kraków, Poland; [2]Department of Animal Genetics and Breeding, National Research Institute of Animal Production, 32-083 Balice, Poland.
Corresponding author: poltowicz@izoo.krakow.pl

The aim of the study was to determine the postmortem desmin degradation and changes in the physico-chemical properties of poultry meat during 7-day cool storage at +4°C. Breast muscles of 40-day-old Ross 308 chickens reared under commercial environmental conditions and fed standard starter, grower and finisher diets for broilers were investigated. On day 40 of rearing, 5 males of similar body weight (2590 g on average) were randomly chosen from the flock. On the day of slaughter and on the 1st, 4th and 7th day after slaughter, the desmin degradation pattern in breast muscles was studied using Western blot analysis after SDS-PAGE. The following technological parameters of meat were determined: pH, L*a*b* colour (Minolta CR310), drip loss, thermal loss, water holding capacity, and Warner-Bratzler shear force (Instron 5542). The results were analysed statistically using variance analysis and Duncan's test.
The fastest degradation of desmin and the greatest changes in the physico-chemical properties of meat occurred within the first 24 h of storage. 15 min postmortem, breast muscles of the chickens were characterized by a high pH (6.64) and low water holding capacity (11.11%). The highest shear force was found on the day of slaughter (102.44 N) but during the following days it decreased significantly to 44.65 N and 34.26 N on days 1 and 7, respectively. A significant decrease in pH during the first 24 h postmortem was accompanied by a significant decrease in water holding capacity of muscles. As a result of storage, meat lightness (L*) increased significantly from 52.73 on the day of slaughter to 55.5 at the end of the experiment. Yellowness was increased by 2.28 units. After an initial growth, redness (a*) decreased to the value observed at the start of the experiment. The study showed that poultry meat quality traits are fixed during the first 24 h of storage, while longer storage at +4°C does not significantly affect texture, pH or water holding capacity.

Keywords: broiler chickens; breast muscles; cool storage; desmin; meat quality

Introduction

After an animal's death, muscle intracellular metabolism undergoes radical changes. Postmortem processes in the muscular tissue determine the subsequent meat quality traits (Schreus, 2000). Skeletal muscles are mainly composed of water and proteins, thus proteome analysis can give much information on structures and functions of proteins involved in several mechanisms which determine meat quality. Unfortunately, proteomic studies related to meat quality traits are limited, especially in poultry. In mammals, meat quality has been given already a new highlight with proteomic study. Lametsch and Bendixien (2001) reported changes in proteome patterns between samples of porcine muscles taken from slaughter to 48 h postmortem and identified more than 20 proteins which can be used as meat quality markers (Lametsch et al., 2002 and 2003). Results obtained by Remignon et al. (2006) indicated a relationship between the presence or absence of myosin heavy chain, actin fragments and glyceraldehyde-3 phosphate dehydrogenase (GAPDH) enzyme in normal- or fast glycolysing turkey breast muscles, meat aging and meat tenderness.

Muscle cells contain cytoskeletal elements, which form a continuous intracellular network spanning the entire muscle fibre. Desmin filaments are muscle-specific intermediate filaments located at the periphery of the Z-discs, and they have been postulated to play a critical role in the lateral registration of myofibrils (Wang and Ramirez-Mitchell, 1983). Previous studies have shown that desmin and other cytoskeletal proteins are degraded during storage of meat, and it has been speculated that their degradation is important to meat quality, especially meat tenderness and water holding capacity (Koohmaraie et al., 1995; Morrison et al., 1998; Therkildsen et al., 2002; Melody et al., 2004). The rate of postmortem proteolysis of several important structural muscle proteins is considered a determinant factor in postmortem tenderization (Taylor et al., 1995).

Meat quality depends on several factors such as genetics, rearing conditions, handling of animals during transportation and slaughter and also handling of meat during storage and processing. The most important meat quality characteristics include water holding capacity, tenderness, colour, and meat processing ability. Some of them, e.g. meat colour, are important sensory characteristics by which consumers often base product selection and judge quality. The most critical quality factor associated with consumer's satisfaction is probably texture. It is affected by the maturity of the connective tissues and by the contractile state of the myofibrillar proteins. Although changes of meat characteristic that occur during processing and storage have been already reported by many authors, most are related to changes between distinct points in time (e.g. 2 and 24 h postmortem) or for only the first 24 or 48 h postmortem (Cavitt et al., 2005; Thielke et al., 2005). Additionally, proteomic tools may help to better understand factors affecting poultry meat traits and find new information on its quality.

The aim of this study was to determine the postmortem desmin degradation and changes in the physico-chemical properties in poultry breast meat during 7-day cool storage at +4°C.

Materials and methods

Breast muscles of Ross 308 chickens were studied. Birds were reared to 40 days of age under standard environmental conditions at a stocking density of 15 birds/m^2 and fed complete starter (days 1-21), grower (days 22-35) and finisher (days 36-40) diets for broilers. On day 40 of rearing, 5 males of similar body weight (2590 g on average) were randomly chosen from the flock. Immediately after slaughter samples of *pectoralis superficialis* muscle was taken to determine the desmin degradation pattern. Desmin degradation was analysed using Western immunoblotting. Whole muscle extracts were obtained from *pectoralis superficialis* muscle by homogenizing muscle samples with 10 volumes of 50 mM Tris and 10 mM EDTA, pH 8.3 (Wheeler and Koohmaraie, 1999). The muscle homogenate was diluted 1:1 with protein denaturing buffer (4% SDS, 20% glycerol, 125 mM Tris, pH 6.8) and heated at 50°C for 20 min. Samples were centrifuged at 16000×g and protein concentration was determined using BCA assay (Sigma Chemical Co, St. Louis USA). For electrophoresis, 5 µg protein per lane was loaded and desmin was separated on 12.5% gel with 4.5% stacking gel. Discontinuous gels were run at 100V for 1.5 h. Gel was transferred to Immobilon-P transfer membrane (Millipore) overnight at 4°C and 30 mA in buffer containing 25 mM Tris, 193 mM glycine, and 15% methanol. Membrane was blocked with 10% non-fat dried milk in Tris-buffered saline pH 7.4 containing 0.05% Tween 20 for 1 h, and then incubated at room temperature with primary monoclonal antibody (NCL-DES-DERII, Novocastra, UK) at 1:250 for 1 h. Membrane was washed three times (5 min) with TTBS after each incubation. Bound primary antibody was visualized with goat anti-mouse alkaline phosphatase conjugate (Pierce Chemical Co., USA) diluted 1:2500, followed by detection of alkaline phosphatase activity with bromo-chloroindolyl phosphate and nitroblue tetrazolium. The intact desmin and degradation products were quantified using ImageJ 1.33U software (National Institutes of Health, USA). Initial muscle acidity was determined 15 min postmortem with a portable pH meter CyberScan 10 by direct insertion of the combined glass electrode in the muscle. After the completion of pH measurements, right and left *pectoralis superficialis* muscles were cut from the carcasses to determine the following technological parameters of meat: L*a*b* colour, drip loss, thermal loss, water holding capacity measured by the filter paper press method (Morrison et al., 1998), and Warner-Bratzler shear force. Colour values L* (lightness), a* (redness) and b* (yellowness) were measured on all left fillets (medial side) at the time of deboning using a Minolta CR310 colorimeter. Four readings per fillet were

taken and an average reading was recorded. After thorough weighing (e=0.001 g), samples of left muscle were placed in sealed containers. Drip loss was determined after 24 h cold storage at +4°C. For measurement of tenderness, samples of right fillets were packed separately in a plastic bag and cooked in a water bath in 95°C until core temperature reached 80°C. The samples were cooled, weighed for thermal loss determination, and prepared for the shear force measurements. One 1.27 cm diameter core was removed from each sample parallel to the fibre orientation through the thickest portion of the cooked muscle. Shear force was determined as maximum force (N) perpendicular to the fibres using Instron 5542 equipped with a Warner-Bratzler blade. Muscles were divided in such a way as to enable the measurements to be repeated in each bird on the 1st, 4th and 7th day of storage at +4°C. The results were analysed statistically using analysis of variance and Duncan's multiple range test. Additionally, phenotypic correlations (*r*) between percentage of degraded desmin and physico-chemical properties were estimated.

Results and discussion

The greatest changes in the physico-chemical properties of meat took place within the first 24 h of storage. Shortly after slaughter, breast muscles of the broilers were characterized by a high pH, good water holding capacity and great hardness. pH of breast muscles was 6.64 at 15 min postmortem and decreased significantly (P≤0.01) to 6.19 after 24 h of storage at +4°C *(Table 1)*.

Table 1 Results of breast meat quality evaluation

Item	Day of storage			
	0	1	4	7
pH	6.64^{A}±0.05	6.19^{B}±0.12	6.10^{B}±0.10	6.06^{B}±0.10
WHC (%)	11.11^{A}±0.80	31.27^{B}±1.19	34.13^{B}±3.04	33.86^{B}±1.02
Drip loss (%)	0	0.39^{A}±0.13	1.16^{B}±0.16	1.39^{B}±0.19
Thermal loss (%)	23.28±0.11	22.64±2.84	24.25±0.35	26.37±0.50
Colour L*	52.73^{A}3±1.43	55.35^{B}±1.14	56.24^{B}±1.10	55.50^{B}±0.79
a*	11.90±1.15	13.11±0.85	13.07±0.91	12.37±1.33
b*	10.05^{Aa}±0.90	11.40^{b}±0.91	12.44^{B}±0.39	12.33^{B}±0.85
Shear force (N)	102.44^{A}±5.88	44.65^{B}±11.58	43.92^{B}±16.42	34.26^{B}±5.12

Values in rows marked with different letters $^{a, b}$ differ at P≤0.05; $^{A, B}$ at P≤0.01

Concurrent to our findings, Thielke et al. (2005) reported that there has been a rapid and gradual decrease in pH during the first 4.5 to 5 h aging of broiler breast muscles, and it has been hypothesized that such a rise in pH may be a result of intensive postmortem proteolysis (Ristic, 1978).

A significant decrease in pH was accompanied by a significantly decreased water holding capacity of the muscles. Between the 1st and 7th day of storage, breast muscle acidity was decreasing slightly, by an average of 0.02 units per day. Water holding capacity of breast muscles was 11.11% when measured approx. 30 min. postmortem and increased to 31.27% after 24 h (P≤0.01). During the next days, values of water holding capacity continued to increase slightly. The highest drip loss (0.39%) was found during the first 24 h of storage. During the next days, it ranged on the average from 0.26% per day (between the 1st and 4th day of storage) to 0.08% per day (between the 4th and 7th day), totalling 1.39% after 7 days. It is clear that early postmortem events including rate and extent of pH decline, proteolysis and even protein oxidation are key to influencing the ability of meat to retain moisture (Huff-Lonergan and Lonergan, 2005).

Thermal losses were of similar value on the day of slaughter and after 24 h of storage and showed an upward tendency from day 4 of the trial.

As a result of storage, meat lightness (L*) increased significantly from 52.73 on the day of slaughter to 55.50 at the end of the trial. At the same time, yellowness (b*) was increased by 2.8 units (P≤0.01). After an initial increase, redness (a*) decreased to the value obtained at the start of the experiment. Meat lightness was possibly also related to its increased acidity. Similar results were obtained by Boulianne and King (1995), who showed a relationship between lightness of refrigerated breast fillets of chickens and the loss of heme pigments. The fact that poultry meat colour changes

during storage is well established. Numerous papers reported changes in turkey and broiler breast meat colour, as measured at various times postmortem (Mallina et al., 2000; Owens et al., 2000; Quiao et al., 2001; Petracci and Fletcher, 2002). They reported that L* value of breast muscles increased dramatically during the first hours of processing. In contrast with our results, Yang and Chen (1993) found lightness and redness to decrease in breast and leg muscles of chickens during storage. According to Petracci and Fletcher (2002) differences in a* values for broiler chicken breast muscles showed no real trend for the first 12 h postmortem, but from 24 to 192 h they tended to decrease. The pattern of changes in b* values was very similar during the first 12 h postmortem, during which the values tend to decrease. From 24 to 192 h postmortem the trend was similar. These results are in general agreement with ours, in which meat colour changed especially during the first 24 h postmortem.

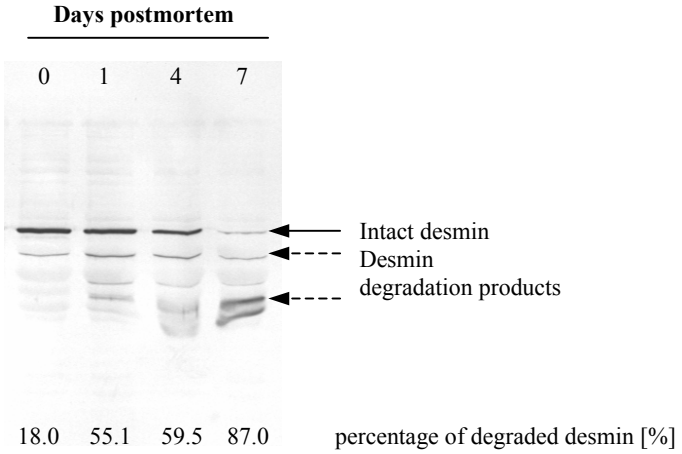

Figure 1 Western blot analysis of desmin in *m. pectoralis superficialis* at 0, 1, 4 and 7 days postmortem, and percentage of degraded desmin.

The highest shear force was found on the day of slaughter (102.44 N) but during the following days it decreased significantly ($P \leq 0.01$) to 44.65 N and 34.26 N at 1 and 7 days of storage, respectively. According to Schreus (2000), the increase in meat tenderness observed on the first day after slaughter is due to postmortem degradation of muscle proteins. Concurrent to our findings, Thielke et al. (2005) reported that higher shear force values were found in the fillets aged up to 3 h postmortem. Further aging led to a significant decrease in shear force values (i.e., to a significant increase in tenderness).

Western immunoblotting revealed that desmin was degraded gradually with time postmortem *(Figure 1)*, but the fastest degradation of desmin was found in *pectoralis superficialis* muscle within the first 24 h of storage. Similarly, earlier studies have shown that cytoskeletal protein in bovine, pig, and poultry muscles are degraded during postmortem storage. This degradation is not universal because different cytoskeletal proteins are subject to different rates and degrees of degradation (Hwan and Bandman, 1989; Taylor et al., 1995; Morrison et al., 1998; Van Laack et al., 2000). Differences in postmortem desmin degradation are probably connected with the differences in muscle structure. Morrison et al. (1998) showed that slow, red type I fibres contain higher levels of desmin than fast, white type IIB fibres, then the levels of desmin could be caused by differences in muscle fibre composition. In the present study phenotypic correlations between physico-chemical properties of meat, i.e. pH, L*a*b* colour, thermal loss, water holding capacity, and shear force and percentage of degraded desmin were never significant. On the other hand, a phenotypic correlation between percentage of degraded desmin and drip loss was positive (0.36, $P \leq 0.05$), in contrast to a negative correlation between percentage of degraded desmin and Warner-Bratzler shear force (-0.39, $P \leq 0.05$). The fact that degradation of the muscle protein (such as desmin) in postmortem muscle is associated with indices of meat tenderness confirms the earlier results of Huff-Lonergan et al. (1996), Taylor et al. (1995) and Melody et al. (2004). In literature, one may find diverse opinions. Therkildsen et al. (2002) showed that the tenderness differences or the tenderization pattern obtained by different growth rates were not related to the degradation of desmin. Taylor and Koohmaraie (1998) have shown that

the attachment of myofibrils to the costamers, of which desmin is part, disappears even in *callipyge* lambs postmortem although the meat is still tough. Thus, desmin degradation might not be of any significance for the tenderness of a specific muscle from animals with the same genetic background and the same postmortem treatment (Therkildsen et al., 2002). Meat tenderness is probably associated not only with degradation of the muscle protein but also is connected with numerous factors, such as collagen content and sarcomere length, which contribute to the tenderness of muscle (Wheeler et al., 2000). Moreover, the earlier study by Melody et al. (2004) showed, in contrast to the present results, that desmin degradation is associated with water holding capacity. On the other hand, muscle protein degradation may also be associated with drip loss. Previous studies have suggested that reduced degradation of protein (such as desmin) that ties the myofibril to cell membrane may allow shrinkage of the myofibril to result in shrinkage of the muscle cell. This shrinkage opens drip channels and results in increased drip loss (Morrison et al., 1998; Kristensen and Purslow 2001; Rowe et al., 2001). Therefore, increased degradation of desmin could prevent myofibril shrinkage from being effectively transmitted to the entire cell and would allow more moisture to reside in the tissue.

The study showed that rate of desmin degradation and poultry meat quality traits are fixed during the first 24 h of storage, while longer storage at +4°C does not affect texture, pH and water holding capacity significantly. Moreover, percentage of degraded desmin is associated with drip loss and shear force of breast muscle in poultry.

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