Praca oryginalna *Original paper*

Relationship between post mortem desmin degradation and meat quality of poultry breast muscle

DOROTA WOJTYSIAK, KATARZYNA POŁTOWICZ*, JANUSZ KARASIŃSKI**

Department of Reproduction and Animal Anatomy, Faculty of Animal Breeding and Biology, Agricultural University, Al. Mickiewicza 24/28, 30-059 Kraków, Poland
*Department of Animal Genetics and Breeding, National Research Institute of Animal Production, Krakowska 1, 32-083 Balice, Poland
**Department of Cytology and Histology, Institute of Zoology, Jagiellonian University, Ingardena 6, 30-060 Kraków, Poland

Wojtysiak D., Połtowicz K., Karasiński J.
Relationship between post mortem desmin degradation and meat quality of poultry breast muscle

Summary

The objective of the study was to determine the level of *post mortem* intact desmin and compare the intact desmin with physico-chemical properties of poultry breast muscle during 168 hours cool storage at +4°C. The research was conducted on 6 40-day-old Ross 308 broiler chickens slaughtered at a similar body weight. Muscle samples were taken immediately after slaughter of animals (15 min) and on 24, 96 and 168 hours after slaughter. The *post mortem* intact desmin and degradation products were determined using SDS-PAGE and Western blot analysis. The following technological parameters of meat were also determined: pH, colour L*a*b*, drip loss, thermal loss, free water, and shear force.

The current findings indicate that the fastest rate of desmin degradation and the greatest changes in the physico-chemical properties of meat occurred within the first 24 h of storage. Longer storage of meat had no effect on meat quality traits except drip loss. Moreover, our results indicate that levels of intact desmin positively related with drip loss values and negatively with pH and shear force.

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

The cytoskeleton of animal cells is composed of three major fibre systems: microfilaments (6 nm), microtubules (24 nm) and intermediate filaments (10 nm). According to Helliwell (5), desmin is the main cytoskeletal protein of skeletal muscle and is located in a collar around the myofibrils at the level of the Z-disc, forming transverse and longitudinal links between adjacent myofibrils. Moreover, desmin is connected at the periphery of the fibre to a complex network of proteins that stabilizes the sarcolemma and transmits force from the contractile apparatus to the basement membrane and endomysial tissues. After an animal's death, muscle intracellular metabolism undergoes radical changes. It has been documented that cytoskeletal and other structural proteins are degraded during storage of meat, and it has been speculated that their degradation is important to meat quality, especially meat tenderness (9-11, 18). Degradation of intermediate filaments may tenderize meat by facilitating the separation of myofibrils, thereby weakening the lateral strength of meat. Recently it was demonstrated that cytoskeletal proteolysis could play an important role in drip loss of pork during *post mortem* storage (1, 6, 9, 10, 24). Skeletal muscles are mainly composed of water and proteins, thus proteome analysis can provide much information on structures and functions of proteins involved in several mechanisms that determine meat quality. Unfortunately, proteomic studies related to meat quality traits are limited, especially in poultry (14). 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 characteristics include water-holding capacity, tenderness, colour, and meat physico-chemical properties. Some of them, e.g. meat colour, are important sensory characteristics on which consumers often base product selection and judge quality. The most critical quality factor associated with consumer 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 characteristics that occur during processing and storage have already been reported by many authors, most are related to changes between distinct points in time (e.g. 2 and 24 h *post mortem*) or for only the first 24 or 48 h *post mortem* (3, 4,

19). Additionally, proteomic tools may help to better understand factors affecting meat traits and find new information on its quality.

The objective of the study was to determine the level of *post mortem* intact desmin and to compare the intact desmin with meat quality traits of poultry breast muscles during 168 h of cool storage at +4°C.

Material and methods

Breast muscle (*pectoralis superficialis*) of Ross 308 broiler chickens was 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 36-400) diets for broiler. On day 40 of rearing, 6 males of similar body weight (2590 g on average) were randomly chosen from the flock.

To determine desmin degradation pattern and meat quality traits, a sample of breast muscle was taken immediately after the slaughter of the birds (15 min) and after storage of meat for 24 h, 96 h and 168 h at +4°C. Immediately after the slaughter of the animals right and left breast muscles were cut from the carcasses. The right fillet of each carcass was cut into four parts and used for determination of thermal loss and Warner--Bratzler shear force. One part was used immediately after slaughter (15 min). The three other parts of the muscles were vacuum packed and stored for 1, 4 and 7 days at +4°C, respectively. The left fillet of each carcass was divided into two parts and vacuum packed. One half was used for desmin analysis and determination of pH, free water and colour L*a*b*. The other half of each left fillet was used for the determination of drip loss. All measurements were determined after 15 min and 24, 96 and 168 hours of storage at +4°C. After storage, samples for desmin degradation pattern were immediately frozen in liquid nitrogen and stored at –80°C until analysis.

Muscle acidity was determined with a portable pH meter CyberScan10 by direct insertion of the combined glass electrode in the muscle. Water-holding capacity was determined as an amount of free water according to the filter paper press method (11). Colour values L* (lightness), a* (redness) and b* (yellowness) were measured using a Minolta CR310 colorimeter. Four readings per fillet were taken and an average reading was recorded. Drip loss was measured in duplicate samples. After thorough weighing (e = 0.001 g), samples were placed in sealed containers. After 24 h of storage, samples were removed from their individual sealed containers, towel dried, and weighed again. The chops were then placed in new sealed containers and stored for an additional hour. Following 96 h of storage samples were again towel dried and weighed. Similarly, the samples of meat were processed after 168 h of storage. For measurements of tenderness, samples of right fillets were packed separately in a plastic bag and cooked in a water bath at 95°C until the 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 muscle 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.

Intact desmin and degradation products were analysed using Western immunoblotting. Whole muscle extracts were obtained from the breast muscle by homogenizing muscle samples with 10 volumes of 50 mM Tris and 10 mM EDTA, pH 8.3 (21). 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 16 000 × 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% with 4.5% stacking gel. Discontinuous gels were run at 100 V 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. The 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. The bound primary antibody was visualized with goat anti-mouse alkaline phosphatase conjugate (Pierce Chemical Co., USA) diluted 1 : 2500, followed by detection of alkaline phosphate activity with bromo-chloro-indolyl phosphate and nitroblue tetrazolium. The intact desmin and degradation products were quantified using ImageJ 1.33 U software (National Institutes of Health, USA).

All data were analyzed using analysis of variance and Duncan's multiple range test. Additionally, correlation coefficients were calculated to determine the relationships between different parameters. Because no relationships were observed between intact desmin and meat colour (L*a*b*), free water, thermal loss, only significant relations are reported in the table.

Tab. 1. *Post mortem* changes in physico-chemical properties of breast muscle of broiler chickens. Values are expressed as means ± SD

Item	Time of storage			
	15 min	24 h	96 h	168 h
pH	6.65 ± 0.05^{A}	6.20 ± 0.11^{B}	6.13 ± 0.09^{B}	6.08 ± 0.10^{B}
Colour L*	52.27 ± 1.39^{A}	55.07 ± 1.12^{B}	56.45 ± 1.10^{B}	55.46 ± 0.76^{B}
a*	11.86 ± 1.15	13.16 ± 0.87	13.02 ± 0.90	12.37 ± 1.25
b*	9.99 ± 0.90^{Aa}	11.23 ± 0.91^{b}	12.50 ± 0.36^{B}	12.31 ± 0.84^{B}
Free water (%)	11.28 ± 0.80^{A}	31.44 ± 1.17^{B}	33.87 ± 3.02^{B}	33.71 ± 1.12^{B}
Drip loss (%)	–	0.52 ± 0.12^{A}	1.69 ± 0.16^{B}	2.72 ± 0.18^{C}
Thermal loss (%)	23.4 ± 0.11	22.83 ± 2.76	24.25 ± 0.38	26.62 ± 0.62
Shear force (N)	99.2 ± 5.79^{A}	38.86 ± 10.38^{B}	35.71 ± 16.32^{B}	31.70 ± 5.89^{B}

Explanation: Values in rows marked with different letters a, b differ at P ≤ 0.05; A, B at P ≤ 0.01

Results and discussion

Results summarized in tab. 1 demonstrate that the greatest changes in the physico-chemical properties of meat took place within the first 24 h of storage at +4°C. Shortly after slaughter (15 min), breast muscles of the broilers were characterized by a high pH, good water-holding capacity and great hardness. After 24 h of storage the pH of breast muscles decreased significantly, while longer storage had no effect on the pH of meat. Thielke et al. (19) reported that there is a rapid and gradual decrease in pH during the first 4.5 to 5 h aging of broiler breast muscles.

A significant decrease in pH was accompanied by a significant increase in free

water value and drip loss value. Higher free water value indicates poorer water-holding properties. Free water values of breast muscles increased significantly only after 24 h *post mortem* (from 11.28 to 34.44 at 15 min and 24 h *post mortem*, respectively). During the next days, there were no significant differences in free water values. The highest drip loss (0.52%) was found during the first 24 h of storage. During the next days, it ranged, on the average, from 0.39% per day (between 24 h and 96 h of storage) to 0.34% per day (between 96 h and 168 h), totaling 2.72% after 168 h. The highest weight loss during the first 24 h of storage was probably the result of rigor mortis. It is clear that early *post mortem* events including rate and extent of pH decline, proteolysis and even protein oxidation are crucial in influencing the ability of meat to retain moisture. Schafer et al. (15) showed a significant relationship between drip loss and pH *post mortem*. These results agree with a previous study of Bee et al. (1), who reported higher drip losses combined with more rapid pH decline *post mortem* in the *m. longissimus* of pigs.

Thermal losses were of similar value on the day of slaughter and after 24 h of storage and showed an upward tendency to 168 h of the trial.

As a result of storage, meat lightness (L*) and yellowness (b*) increased significantly only after 24 h *post mortem*. At the same time, there were no significant differences in redness (a*) values. However, after an initial increase, a tendency toward decreasing redness value to the value obtained at the start of the experiment was observed. Meat lightness was possibly also related to its increased acidity. Similar results were obtained by Boulianne and King (2), 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 *post mortem* (12, 13). They reported that L* value of breast muscles increased dramatically during the first hours of processing. In contrast with our results, Yang and Chen (23) found lightness and redness to decrease in breast and leg muscles of chickens during storage. According to Petracci and Fletcher (12) differences in a* values for broiler chicken breast muscles showed no real trend for the first 12 h *post mortem*, 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 *post mortem*, during which the values tend to decrease. From 24 to 192 h *post mortem* the trend was similar. Similarly, the results obtained in our study, in which meat colour changed especially during the first 24 h *post mortem*, are in general agreement with the previous study (12).

Additionally, the present study showed the highest shear force on the day of slaughter, next after 24 h of storage it decreased significantly, which is connected with rigor mortis. During the following days, storage of meat had no effect on shear force values. According to Schreus (16), the increase in meat tenderness observed on the first day after slaughter is due to *post mortem* degradation of muscle proteins. Concurrent to our findings, Thielke et al. (19) reported that higher shear force values were found in the fillets aged up to 3 h *post mortem*. Further

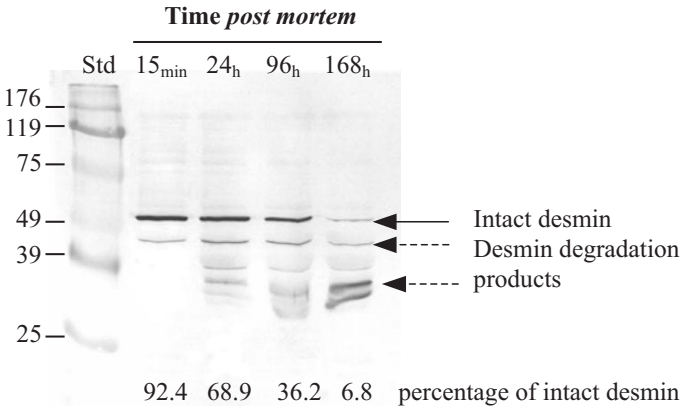

Fig. 1. Western blot analysis of desmin in breast muscle at 15 min and 24, 96 and 168 hours *post mortem*, and percentage of intact desmin. The molecular weights (in kDa) of standard proteins (Std) are indicated on the left

aging led to a significant decrease in shear force values (i.e., to a significant increase in tenderness).

Analysis by Western immunoblotting revealed that desmin was degraded gradually with time *post mortem* (fig. 1), but the fastest degradation of desmin was found in the breast muscle within the first 24 h of storage. This degradation is not universal because different cytoskeletal proteins are subject to different rates and degrees of degradation (8, 11, 20). Differences in *post mortem* desmin degradation are probably connected with the differences in muscle structure. Morrison et al. (11) showed that slow, red type I fibres contain higher levels of desmin than fast, white type IIB fibres. Therefore the levels of desmin could be caused by differences in muscle fibre composition.

In the present study, significant correlation coefficients between percentage of intact desmin and physico-chemical properties of meat are presented in tab. 2. The intensity of intact desmin at 15 min, 24 h, 96 h and 168 h *post mortem* was positively correlated with the drip loss. By contrast, intact desmin at 15 min, 24 h and 96 h *post mortem* was negatively correlated with pH and shear force values, whereas no correlations were found at 168 h of storage. On the other hand, no significant correlation coefficients were observed between percentage of intact desmin and meat colour (L*a*b*), free water, and thermal loss during 168 h of storage. The fact that degradation of the muscle protein (such as desmin) in *post mortem* muscle is associated with meat tenderness confirms the earlier results of Huff-Lonergan et al. (7). Likewise,

Tab. 2. Correlation coefficients between level of intact desmin and pH, drip loss and shear force of breast muscle of broiler chickens

	pH	Drip loss	Shear force
Intact desmin 15 min	–0.18*	0.26**	–0.21*
Intact desmin 24 h	–0.23*	0.32**	–0.29*
Intact desmin 96 h	–0.17*	0.25*	–0.12*
Intact desmin 168 h	ns	0.16*	ns

Explanation: * significant at $P \leq 0.05$, ** at $P \leq 0.01$; ns – not significant

Melody et al. (10) found that level of intact desmin was associated with meat tenderness. The literature contains varying opinions. Therkildsen et al. (18) showed that the differences in tenderness or the tenderization pattern obtained by different growth rates were not related to the degradation of desmin. Taylor and Koohmaraie (17) showed that the attachment of myofibrils to the costamers, of which desmin is part, disappears even in *callipyge* lambs *post mortem*, although the meat is still tough. Thus, the level of desmin might not be of any significance for the tenderness of a specific muscle from animals with the same genetic background and the same *post mortem* treatment (18). The presence (or lack) of a significant correlation between meat tenderness and level of intact desmin 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 (22). Recently, it has been recognized that *post mortem* degradation of muscle proteins not only determines meat tenderness but affects the extent of drip formation (1, 6, 9, 11, 24) and may work in concert with biophysical forces to influence water-holding capacity (10, 24). Additionally, previous studies have suggested that reduced degradation of the desmin that ties the myofibril to the 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 (9, 11). 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 (10). Positive correlations between intact desmin and drip loss observed in the current study during *post mortem* storage are in agreement with the study of Kristensen and Purslow (9) and Zhang et al., (24), who showed that the rate of desmin degradation was associated with drip loss value in pork. However, Schafer et al. (15) described that variations in the degradation of desmin only account for 13% of variation in drip loss using PLSI (Partial Least Squares) models and concluded that the degree of degradation of desmin was not correlated with drip loss. But it is noteworthy that Schafer et al. (15) only measured the early structural changes of cytoskeletal proteins from 3 to 24 h. Moreover, a significant relationship between intact desmin and pH of meat observed in the present study agrees with the findings of Melody et al. (10) who reported greater desmin degradation in the *m. longissimus* in pigs compared to the *m. psoas major*, which at the same time displayed a more rapid pH decline *post mortem* compared to *m. longissimus*. Bee et al. (1) suggested that pH decline in early *post mortem* muscles may account for a portion of the extent of proteolytic disruption of intermediate filaments and costamere, which may dictate the degree of muscle fibre shrinkage that is caused by myofibrillar shrinkage. This would have an impact on the amount of intracellular water shifted toward extracellular water during rigor development, thus affecting water loss during aging (9). Moreover, Huff-Lonergan and Lonergan (6) suggested that because desmin is a known μ-calpain substrate, it is reasonable to hypothesize that calpain autolysis and activation may explain a portion of the variation of desmin degradation and could subsequently influence drip loss.

It is concluded that the fastest rate of desmin degradation and the greatest changes in the physico-chemical properties of meat occurred within the first 24 h of storage. Furthermore, the high levels of intact desmin were associated with high levels of drip loss and low levels of pH and shear force values.

References

1. *Bee G., Anderson A. L., Lonergan S. M., Huff-Lonergan E.*: Rate and extent of pH decline affect proteolysis of cytoskeletal proteins and water-holding capacity in pork. Meat Sci. 2007, 76, 359-365.
2. *Boulianne M., King A. J.*: Biochemical and color characteristics of skinless boneless pale chicken breast. Poultry Sci. 1995, 74, 1693-1698.
3. *Cavitt L. C., Meullenet J. F., Gandhapuneni R. K., Youm G. W., Owens C. M.*: Rigor development and meat quality of large and small broilers and use of Allo-Kramer shear, needle puncture, and razor blade shear to measure texture. Poultry Sci. 2005, 84, 113-118.
4. *Gardzielewska J., Jakubowska M., Buryta B., Karamucki T., Natalczyk-Szymkowska W.*: Pomiar pH_1 a jakość mięsa kurcząt brojlerów. Medycyna Wet. 2003, 59, 426-428.
5. *Helliwell T. R.*: Muscle: Part 1 – Normal structure and function. Current Orthopaedics 1999, 13, 33-41.
6. *Huff-Lonergan E., Lonergan S. N.*: Mechanism of water-holding capacity of meat: The role of post mortem biochemical and structural changes. Meat Sci. 2005, 71, 194-204.
7. *Huff-Lonergan E., Mitsuhashi T., Beekman D. D., Parrish F. C., Olson D. G., Robson R. M.*: Proteolysis of specific muscle structural proteins by μ-calpain at low pH and temperature is similar to degradation in post mortem bovine muscle. J. Anim. Sci. 1996, 74, 993-1008.
8. *Hwan S. F., Bandman E.*: Studies of desmin and α-actinin degradation in bovine semitendinosus muscle. J. Food Sci. 1989, 54, 1426-1430.
9. *Kristensen L., Purslow P. P.*: The effect of ageing on the water-holding capacity of pork: role of cytoskeletal proteins. Meat Sci. 2001, 58, 17-23.
10. *Melody J. L., Lonergan S. M., Rowe L. J., Huiatt T. W., Mayes M. S., Huff-Lonergan E.*: Early post mortem biochemical factors influence tenderness and water-holding capacity of three porcine muscles. J. Anim. Sci. 2004, 82, 1195-1205.
11. *Morrison H. E., Mielche M. M., Purlsow P. P.*: Immunolocalisation of intermediate filament protein in porcine meat. Fibre type and muscle-specific variation during conditioning. Meat Sci. 1998, 50, 91-104.
12. *Petracci M., Fletcher D. L.*: Broiler skin and meat color changes during storage. Poultry Sci. 2002, 81, 1589-1597.
13. *Quiao M., Fletcher D. L., Smith D. P., Northcutt J. K.*: Effect of broiler breast meat color on pH, moisture, water-holding capacity, and emulsification capacity. Poultry Sci. 2001, 80, 676-680.
14. *Remignon H., Molette C., Babile R., Fernandez X.*: Current advances in proteomic analysis and its use for the resolution of poultry meat quality problems. World's Poultry Science Journal 2006, 62, 123-129.
15. *Schafer A., Rosenvold K., Purslow P. P., Andersen H. J., Henckel P.*: Physiological and structural events post mortem of importance for drip loss in pork. Meat Sci. 2002, 61, 355-366.
16. *Schreus F. J. G.*: Post-mortem changes in chicken muscle. World's Poultry Science Journal 2000, 56, 319-346.
17. *Taylor R. G., Koohmaraie M.*: Effects of post mortem storage on the ultrastructure of the endomysium and myofibrils in normal and callipyge longissimus. J. Anim. Sci. 1998, 76, 2811-2817.
18. *Therkildsen M., Larsen M. L., Bang H. G., Verstergaard M.*: Effect of growth rate on tenderness development and final tenderness of meat from Friesian calves. Animal Sci. 2002, 74, 253-264.
19. *Thielke S., Lhafi S. K., Kuhner M.*: Effect of aging prior to freezing on poultry meat tenderness. Poultry Sci. 2005, 84, 607-612.
20. *Van Lack R. L. J. M., Liu C. H., Smith M. O., Loveday H. D.*: Characteristics of pale, soft, exudative broiler breast meat. Poultry Sci. 2000, 79, 1057-1061.
21. *Wheeler T. L., Koohmaraie M.*: The extent of proteolysis is independent of sarcomere length in lamb longissimus and psoas major. J. Anim. Sci. 1999, 77, 2444-2451.
22. *Wheeler T. L., Shackelford S. D., Koohmaraie M.*: Variation in proteolysis, sarcomere length, collagen content, and tenderness among major pork muscles. Journal of Animal Sci. 2000, 78, 958-965.
23. *Yang C. C., Chen T. C.*: Effects of refrigerated storage, pH adjustment and marinade on color of raw and microwave cooked chicken meat. Poultry Sci. 1993, 72, 355-362.
24. *Zhang W. G., Lonergan S. M., Bargner M. A., Huff-Lonergan E.*: Contribution of post mortem changes of integrin, desmin and μ-calpain to variation in water holding capacity of pork. Meat Sci. 2006, 74, 578-585.

Adres autora: dr Dorota Wojtysiak, Al. Mickiewicza 24/28, 30-059 Kraków; e-mail: wojtysiakd@wp.pl