PE7.08 Water-holding capacity of chicken gizzard stored at cold temperatures 101.00

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***Abstract*: The water-holding capacity (WHC), and toughness (shear force) of chicken gizzard were evaluated during post mortem storage for 4.5, 7, 12, 24, 48, 72 and 96 h at 4 °C. Degradation of the cytoskeletal proteins desmin, talin and vinculin were monitored by SDS-PAGE and Western blotting during the same designated storage period. The WHC of the gizzards decreased significantly from 12 h to 72 h of storage, but by 96 h the WHC was restored to the level measured after storage for 12 h. The shear force value of the gizzards increased rapidly until 12 h and then decreased until 24 h, with a further slight decrease by 48 h. Degradation products of desmin, talin and vinculin appeared at 96 h, 12 h and 48 h post mortem, respectively. The intensity of immunolabeling for desmin, talin and vinculin after storage for 96 h decreased to 51 %, 25 % and 52 % of the initial value. The appearance of desmin degradation products was accompanied by an increase in WHC. It suggests that the post mortem degradation of desmin is involved in the increase of WHC in chicken gizzard during storage at 4 °C, and talin and vinculin may be involved.**

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I. INTRODUCTION

The water-holding capacity (WHC) is one of the important factors that determine the quality of meat. High purge losses decrease profits because of weight loss. In addition to the loss of salable weight, purge loss entails the loss of a significant amount of protein [1]. There is experimental evidence that supports the suggestion that the proteolysis of key cytoskeletal proteins, such as the intermediate filament protein desmin, may be related to drip production [2]. Degradation of these proteins at an early time post mortem would certainly allow water that is expelled from the intramyofibrillar spaces to remain in the muscle cell for a longer period of time. It is possible that the reduced degradation of the proteins that attach the myofibril to the cell membrane results in increased shrinking of the muscle cell, which is ultimately translated into drip loss.

Thus, water-holding is regarded as an important quality trait of meat and meat products. Offal products such as stomach and intestine from beef, pork and poultry are used as fresh meat and meat products for human consumption. The chicken gizzard is a meat by-product that contains the proteins desmin, talin and vinculin. This study was carried out to determine the quality of chicken gizzards, with a focus on the WHC and the changes of cytoskeletal proteins in smooth muscle that occur during post mortem storage at 4 °C.

II. MATERIALS AND METHODS

Chicken gizzards

Chicken gizzards were purchased from a local slaughter-house immediately after removal from 52–55 days old broiler chickens. The gizzards were cleaned and then stored at 4 °C.

Water-holding capacity.

Five gizzard samples at each time-point were used to measure WHC. Gizzards were cut into 3 mm thick slices, and then the middle part of each slice was cut to a thickness of 3 mm and a height of

10 mm. The samples were weighed and transferred to 1 ml open-ended tubes with a 90 μm pore size filter in the bottom of the tube to separate solids from exudate during centrifugation. The tubes were put into 1.5 ml centrifugation tubes and centrifuged at 4 °C for 30 min at 1,500 × g. Any loss of sample was calculated as the difference in weight before and after centrifugation. Total water content was determined by weighing centrifuged samples after heating at 100 °C for 24 h. Total water was calculated: $WHC = [1 - (W_b - W_a)/W_t] \times 100$ %

where W_b and W_a are the weight of the sample before and after centrifugation, respectively, and W_t is the total water content of the sample.

Toughness

The toughness of the chicken gizzards was evaluated as the shear force value. Samples were subjected to shear force measurements after storage at 4°C for 4.5, 7, 12, 24, 48, 72 and 96 h. Five strips of gizzard (1 cm × 1 cm × length) at each storage time-point were measured by a Rheometer using a cutter blade positioned parallel with the orientation of the muscle fibers at a speed of 1 mm/s.

Sample preparation and SDS-PAGE

Five gizzard samples (0.5 g) at each storage time-point were added to 5 ml of rigor buffer (RB; 75 mM KCl, 10 mM KH_2PO_4, 2 mM $MgCl_2$, 2 mM EGTA, pH 7.0) and homogenized with a Polytron. The homogenate was centrifuged at 10,000 × *g* for 10min at 4°C, and the supernatant was discarded. Then 5 ml of fresh RB was added to the pellet and the homogenization was repeated. This process was done 3 times to obtain the final precipitate, which was added to 10 ml of RB and the protein concentration, as measured by a biuret method, was adjusted to 6 mg/ml. Samples were mixed 1:1 (final concentration of protein 3mg/ml) with standard sample buffer (8 M urea, 2 M thiourea, 3% (w/v) SDS, 75 mM Dl-dithothreitol, 25 mM Tris–HCl, pH 6.8, and heated at 100°C for 3 min in a dry bath heater, cooled, and applied to the electrophoresis gel. The determination of desmin and vinculin was done in 10 % polyacrylamide separating gels, and a 7.5% polyacrylamide separating gel was used for the determination of talin.

Transfer conditions

Gels for desmin, talin and vinculin were transferred to polyvinylidene difluoride (PVDF) membranes using a semi-dry transfer unit model BE-300 (BIO CRAFT, Tokyo, Japan) at a constant current of 144 mA for 30, 30 and 60 min, respectively. The EzBlot (ATTO, Tokyo, Japan) reagents were used for the transfer buffer.

Western blotting

After transfer, the membranes were blocked overnight at room temperature in EzBlock in TTBS (20 mM Tris, 500 mM NaCl, 0.05 % (v/v) Tween20) and incubated for 1 h at room temperature with the primary antibody. The membranes were then rinsed thoroughly with TTBS and incubated as a 1:1000 dilution of phosphatase-conjugated secondary antibody in TTBS for 1 h at room temperature. After thorough rinsing in TBS, the protein bands were visualized using the AP Conjugate Substrate Kit. The primary antibodies used in the Western blotting procedure included monoclonal anti-desmin, monoclonal anti-talin (8d4, Sigma-Aldrich, Saint Louis, MO, USA) and monoclonal anti-vinculin diluted 1:800, 1:400 and 1:400 in TTBS containing EzBlock, respectively. The staining intensity of each protein was analyzed using ImageJ (NIH, Bethesda, MD, USA).

III. RESULTS AND DISCUSSION

Water-holding capacity during post mortem storage

The WHC of chicken gizzard during post mortem storage is shown in Figure 1. There was no significant change of WHC between 4.5 h and 24 h post mortem but it was decreased drastically by 72 h. Finally, at 96 h it was restored to the level found at 4.5 h. These results are in agreement with those in the literature: [3] reported that the WHC of pork decreases the first 2 days (24 h to 48 h) post mortem and then it eventually increased during post mortem storage for 10 days (240 h). The results of the present study suggested that the WHC of chicken gizzard during the first 48 h post mortem is similar to that of pork, but the subsequent increase in the WHC of chicken gizzard is more rapid than that of pork.

It is most likely that the more protein degradation is increased, the more WHC increases. The WHC of chicken gizzards stored at 4 °C was increased while desmin and talin, as well as vinculin, were degraded. Interestingly, this indicates that degradation of protein in smooth muscles of poultry is of great importance, as this mechanism may improve the physicochemical properties of chicken even when stored at 4 °C for 4 days.

Toughness during post mortem storage

Figure 2 shows the post mortem toughness of chicken gizzard stored at 4 °C. The shear force value of the gizzards increased rapidly during the

first 12 h and then decreased until 24 h, and it was further decreased slightly until 48 h; however, the value from 48 h to 96 h post mortem was stable.

As muscle goes into rigor, cross-bridges form between the thick and thin filaments. The water accumulates in the extracellular space between muscle fiber bundles and, at a later stage, between single muscle fibers. Water drains slowly from these extracellular compartments to the surface, where it forms drip [4].

Meat tenderness decreases markedly during the first 24 h post mortem and then increases rapidly between 24 h and 72 h post mortem. It is likely that rigor mortis of chicken skeletal muscle is achieved faster than that in other animals, such as cow, sheep and pig (beef, lamb and pork are collectively referred to as red meat). We suggest that rigor mortis occurs slightly more slowly in the smooth muscle than in the skeletal muscle of chicken.

Degradation of desmin, talin and vinculin during post mortem storage

Western blotting for desmin of chicken gizzard stored at 4 °C for 96 h is shown in Figure 3-1. The molecular mass of desmin degradation products that appeared at 96 h post mortem were about 45 kDa and 38 kDa. The band intensity of intact desmin from chicken gizzard stored at 4 °C for 96 h is shown in Figure 4. The intensity of immuno-labeling for desmin decreased to 51 % of the value at 12 h after 96 h post mortem storage.

A 190 kDa degradation product of talin was already present in the sample measured at 12 h post mortem and the intensity of the 190 kDa band increased gradually with storage time, especially in the sample measured at 96 h (Figure 3-2). Figures 4 showed that the intensity of immuno-labeling for the 225kDa talin decreased to 25 % of the value at 12 h after 96 h post mortem. In accord with these, in our study, the intensity of immuno-labeling for the intact talin decreased more rapidly than that for desmin or vinculin.

Western blotting for chicken gizzard vinculin stored at 4 °C for 96 h is shown in Figure 3-3. The molecular mass of the vinculin degradation product that appeared at 48 h post mortem is 90 kDa. There is a great similarity in the vinculin degradation product between red meat and the smooth muscle of chicken. A degradation product of vinculin with a molecular mass of about 90 kDa was detected in normal lamb biceps femoris muscle on day 3 post mortem [5]. In the current study, the band intensity of intact vinculin of chicken gizzard stored at 4 °C for 96 h is shown in Figure 4. The intensity of immuno-labeling for vinculin decreased to 52 % of the value at 12 h after 96 h post mortem.

Several studies have shown that there is a significant correlation between the degradation of desmin and water-holding capacity of pork [6, 7, 8, & 9]. Talin and vinculin constitute part of the costameres which are found at the sarcolemma of muscle fibers overlying the Z-disks. The costameric proteins talin and vinculin have been shown to be more extensively degraded in pork that has higher water-holding capacity [2]. Thus, it seems that degradation of inter-myofibrillar and costameric connections have the potential to improve water-holding capacity [6, 7, 8 & 2].

In our study, it was suggested that desmin, talin and vinculin in chicken gizzard may have participated in the WHC performance of smooth muscle and that μ-calpain may have a role in post mortem degradation of proteins in smooth muscle as well as in skeletal muscle.

IV. Conclusion

Desmin, talin and vinculin in chicken gizzard were degraded during post mortem storage at 4 °C. The WHC of chicken gizzard decreased until 72 h post mortem and then returned to the initial level at 96 h post mortem. This was confirmed by the results of SDS-PAGE, where desmin, talin and vinculin showed drastic degradation. This provides evidence that protein degradation in chicken gizzards is related to WHC. The data suggest that water movement in smooth muscles in chicken stored at 4 °C does not differ from that in red meat.

Regardless of other quality traits, such as microbial content and chemical parameters, we suggest that the WHC of the smooth muscle of poultry can be improved by storage at 4 °C for post mortem storage. Finally, this may increase consumer satisfaction with smooth muscles, which may lead to better profitability.

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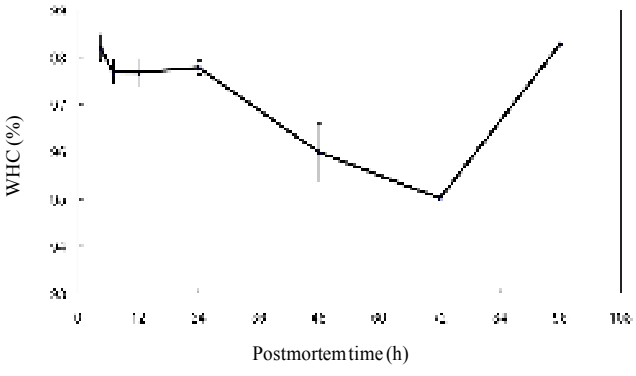

Figure 1. Water-holding capacity of the chicken gizzards stored for 4.5, 7, 12, 24, 48, 72 and 96 h postmortem at 4°C. Each value is represented as the average of 5 determinations ± SD.

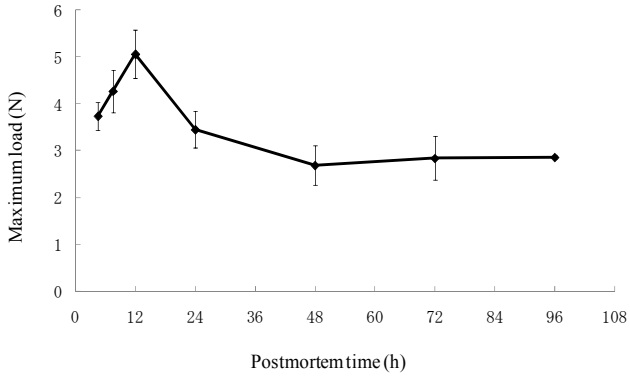

Figure 2. Change in shear force value of the chicken gizzards stored for 4.5, 7, 12, 24, 48, 72 and 96 h postmortem at 4°C. Each value is represented as the average of 5 determinations ± SD.

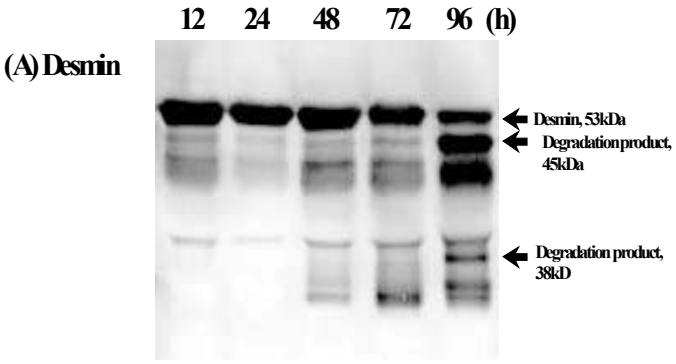

Figure 3-1. Western blotting of desmin (a), talin (b) and vinculin (c) degradation during postmortem storage of chicken gizzard stored at 4°C. Some new developed degradation products are indicated with arrows.

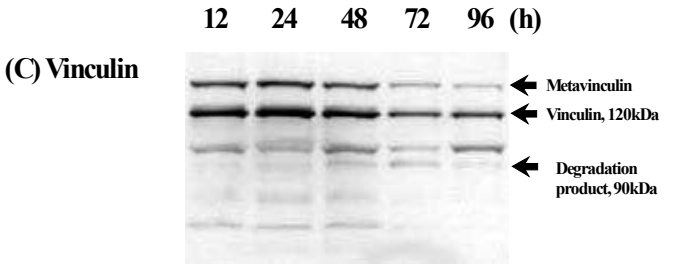

Figure 3-3. Western blotting of desmin (a), talin (b) and vinculin (c) degradation during postmortem storage of chicken gizzard stored at 4°C. Each degradation products are indicated

with arrows.

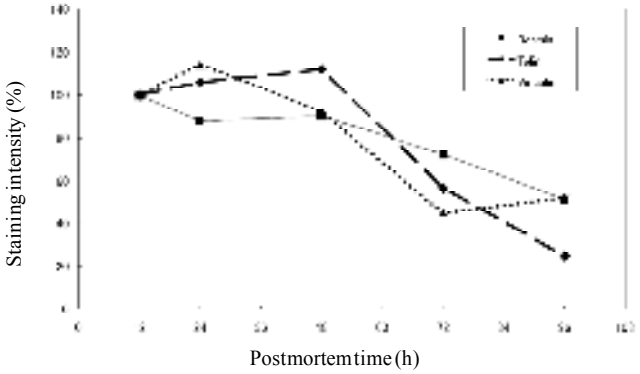

Figure 4. Staining intensity of intact protein bands on PVDF membrane after blotting for desmin (circles), talin (rectangles) and vinculin (triangles) in chicken gizzard stored at 4°C for 12, 24, 48, 72 and 96 h.

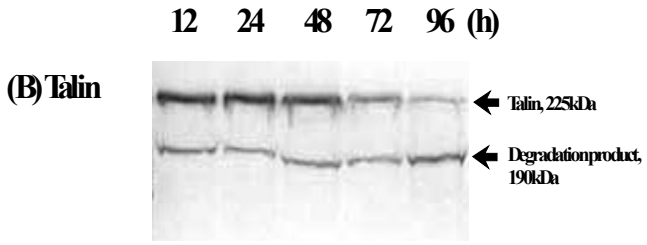

Figure 3-2. Western blotting of desmin (a), talin (b) and vinculin (c) degradation during postmortem storage of chicken gizzard stored at 4°C. Each degradation products are indicated with arrows.