POST MORTEM DEVELOPMENT OF MEAT TENDERNESS AS RELATED TO CHANGES IN CYTOSKELETAL PROTEINS OF CHICKEN MUSCLES

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Background

The development of meat quality is dependent on the meat ageing process, which is characterised by a loss of its structural integrity and caused by the post mortem proteolytic degradation of the so-called cytoskeletal proteins. The most important structural components involved in meat ageing are considered to be the cytoskeletal proteins, among which there are titin, nebulin, and desmin as well as vinculin (Taylor et al., 1995). Titin and nebulin form a set of longitudinal filaments and are largely responsible for the longitudinal integrity of the muscular fiber. Desmin is present in intermediate filaments connecting adjacent myofibrils at their Z-line levels and one of the many proteins (such as vinculin) composing the cell membrane's skeleton named costameres involved in linking myofibrils to the sarcolemma of the muscle (Taylor et al., 1995). The kinetics of the post mortem proteolytic degradation of these key cytoskeletal proteins is of crucial importance for meat quality development.

Objective

Presently, little is known about the changes in cytoskeletal proteins of poultry meat. The objective of this study was therefore to investigate degradation process in cytoskeletal proteins and identify marker proteins or degradation products for the purpose of monitoring the meat ageing development.

Materials and methods

Animals Commercial broilers, brand Ross were slaughtered at 6 weeks of age at the in-house processing plant of the Institute ID-DLO in the plant conditions. The 0 hour samples for electrophoresis were taken immediately after the excision of the muscles, frozen in liquid nitrogen and stored at –80°C. The skinned breast parts were kept in a cold room at 4°C for sampling at 1, 3, and 6 hours and further after 1, 2, 3, 5, and 7 days of post mortem storage. The measurements were carried out on 12 carcasses per sampling time (108 animals in total).

SDS-PAGE & Western blotting The sample for SDS-PAGE and Western blotting was prepared according to Fritz & Greaser (1991). The procedure for SDS-PAGE and Western blotting was described by Tomaszewska-Gras et al. (1997). The gels and the blots were scanned on a Hewlett Packard The image scans were subsequently processed as densitometer traces on a tabletop computer equipped with the Quantiscan program from Biosoft.

Shear force measurements The shear force measurement was carried out according to Tomaszewska- Gras et al. (1999).

Statistical analysis For the shear force measurements, the simple one way ANOVA was used. According to the Spearman rank correlation module, results of optical density of different bands on western blots were converted after the classification to scores in the following manner: – = 0, ± = 1, + = 2, ++ = 3, +++ = 4.

Results and discussion

Figure 1 shows the results of western blots from 7.5% gels of meat proteins developed with polyclonal anti-titin and monoclonal anti-nebulin antibodies, respectively. All blots showed an increasing degradation of all proteins analysed during the post mortem ageing of chicken breast muscle. On the titin blot of the breast muscle, four clearly visible degradation products of titin were identified, referred to as Tb_a, Tb_b, Tb_c and Tb_d. Components Tb_a, Tb_b and Tb_c with a molecular weight of approximately 1400, 700 and 450 kDa respectively, already appeared at 3 hours p.m. The fourth component Tb_d, (1200 kDa), appeared 5 days p.m. just below titin T_2. Tanabe et al. (1992) identified a 1200 kDa component and suggested that this component originates from T_1, and is composed of the Z-line side part of the T_1 molecule. Western blot results of nebulin, as shown in Figure 1, demonstrated that in the breast muscle the degradation process started after 3 hours. After 6 hours p.m. the cleavage of nebulin occurred into two bands (800 kDa and 600 kDa). From the blots it can be concluded that the breakdown of nebulin was most remarkable between 3 and 48 hours, when intact nebulin was degraded and disappeared. This fact is consistent with previous reports, where the degradation of nebulin was analysed by SDS-PAGE (Tomaszewska-Gras et al., 1997).

Figure 1 shows also results of western blots from 12.5% gels with meat proteins developed with polyclonal anti-desmin and monoclonal anti-vinculin antibodies. The results showed that in the breast muscle, the degradation process of desmin started within 3 hours of storage. Below intact desmin, a faint band of approximately 47 kDa appeared, referred to as component D_a. Later a new band was visible on the blot, designated D_b, (40 kDa). The most remarkable degradation of desmin in the breast muscle occurred between 6 and 72 h p.m. In other studies of desmin degradation by Western blotting analysis, bovine meat was used (Taylor et al., 1995). The degradation of vinculin (Figure 1) was completed in the breast muscle within 3 days p. m. The comparison of chicken muscle with the fast ageing bovine *Semimembranosus muscle* (Taylor et al., 1995), showed that vinculin disappeared at the same time.

Figure 1 shows also the results of the shear force measurements in breast muscles with different post mortem time in relation to western blots results of protein degradation. Within 24 hours post mortem, the breast muscle exhibits its final shear force, however,

the maximum shear force is reached after 6 hours post mortem. After 24 hours also the significant increase in intensity of degradation products of titin, desmin and nebulin was observed.

In order to identify possible candidate marker fragments, the semi-quantitative scores for relative intensity of the different proteins and cleavage products on the western blots were converted to numerical scores as described under Materials and Methods. Subsequently, the Spearman rank correlation coefficients were calculated for the relationships between these density scores and shear force measurements (tab. 1). In the breast muscle the course of degradation fragment of titin Tb_c, nebulin fragment Nb_b and desmin fragmnet Db_c showed very high correlation coefficients with shear force measurements. The decrease of vinculin showed no significant correlation with any of the traits.

In conclusion it can be stated that the degradation of specific myofibrillar cytoskeletal proteins, as studied in the present work, follows the pattern of post mortem tenderization and as such can be utilised as an indicator for meat ageing. Titin, nebulin as well as desmin degradations follow a course that is consistent with myofibrillar fragmentation (Tomaszewska-Gras et al., 1999) as well as shear force. However, it has to be taken into account that processes involved in rigor mortis development such as pH decrease can interfere with these relationships. More research is needed to identify specific proteolytic fragments of titin, nebulin and desmin, which can serve as specific markers for the estimation of the degree of proteolysis. Once these components are identified, specific probes can be developed to quickly and easily monitor the course of meat ageing. Using western blot analysis we have identified some possible candidate protein fragments that might prove useful for this purpose.

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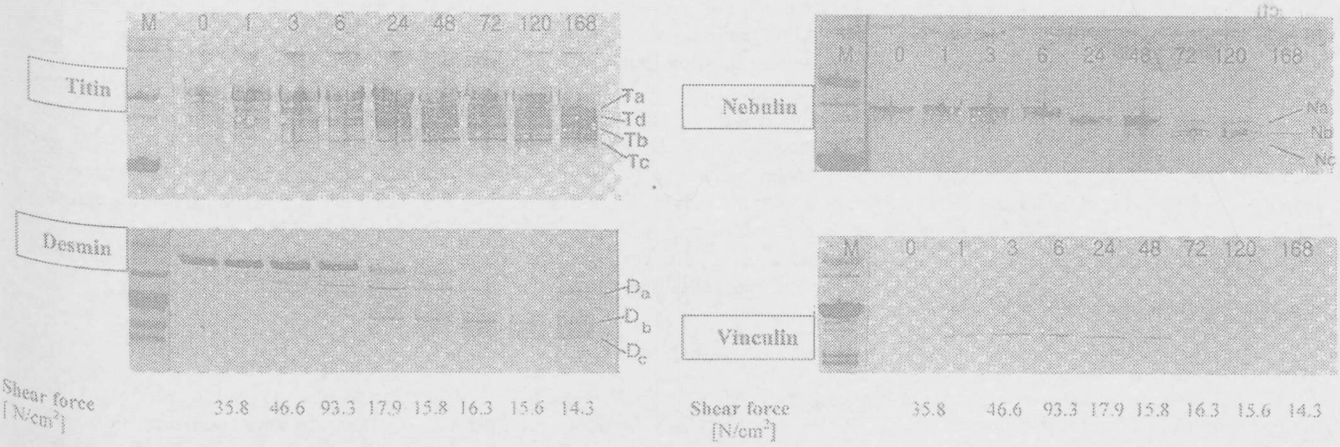

Figure 1 Western blotting of four cytoskeletal proteins at different time *post mortem* (0,1,3, ...hours) using polyclonal antibody to titin and desmin and monoclonal antibody to nebulin and vinculin in breast and leg chicken muscles. **M** – SDS- PAGE of sample at „0" time.

	Intact protein or degradation product X					
	intact	Xb_a	Xb_b	Xb_c	Xb_d	Xb_e
Titin	0.577	0.013	-0.784*	0.914**	0.069	-0.519
Nebulin	0.823*	-0.447	-0.784*			
Desmin	-0.945**	-0.210	-0.664	-0.882**		
Vinculin	0.546					

Table 1 Correlation coefficients of relationships between shear force of breast chicken muscles and relative optical density scores of proteins and proteolytic fragments on Western blots. Significance level: * - the correlation is significant at the 0.05 level; ** - the correlation is significant at the 0.01