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TITIN AND TROPONIN T CHANGES IN RELATION TO TENDERNESS OF MEAT FROM PIGS OF VARIOUS MEATINESS

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Native titin and troponin T undergo slower *postmortem* degradation in the *musculus longissimus dorsi* from pig carcasses of high meatiness (HM) (>55%) than in that from pig carcasses of low meatiness (LM). This was confirmed by western blotting of both proteins. More extensive protein degradation observed in muscles from LM pigs might be associated with their faster tenderisation, in comparison with changes recorded in the meat from HM pigs. Two-way statistical analysis of variance revealed that these processes were significantly affected by the meatiness of pigs and the time of meat storage after slaughter, although the former of these factors had more pronounced influence.

INTRODUCTION

Pork, in comparison with meat from other farm animals, is usually considered to be tender [Dransfield *et al.*, 1981]. This may result from its fairly fast *postmortem* aging and relatively high marbling. However, at present pork – especially from meaty pigs – is often evaluated as less tender [Wood *et al.*, 1995; Grześkowiak *et al.*, 1998]. There are several reasons for its lower tenderness. The main factor may be associated with changes in the degradation of muscle proteins, although it is worth mentioning that higher meatiness need not necessarily be connected with slower protein breakdown [Goll, 1991; Koohmaraie *et al.*, 2002]. Titin and troponin T (Tn-T) belong to the most important proteins responsible for meat tenderisation [Taylor *et al.*, 1995; Greaser *et al.*, 2000]. *Postmortem* changes of Tn-T are associated with the appearance of Tn-T degradation products characterised by a molecular weight of 28–30 kDa [Penny & Dransfield, 1979; Ho *et al.*, 1994]. In the case of native titin (T1), its degradation leads to gradual disappearance with the increasing staining intensity of its degradation products, especially the T2 unit whose molecular weight is around 2400 kDa. In the case of pork, the rate of this process depends on the type/quality of meat [Boles *et al.*, 1992]. In comparison with normal quality muscles, a slower degradation was found in PSE muscles.

The aim of this research was to evaluate the relationships between the tenderness of meat derived from carcasses of high (>55%) and low meatiness (<50%) and *postmortem* changes of titin and troponin T, the main proteins responsible for the process of tenderisation during *postmortem* aging.

MATERIAL AND METHODS

The investigations were conducted on 32 pigs, which were divided into two groups according to the meat content in the carcass: above 55% – HM (17) and below 50% – LM (15). The ULTRA-FOM 200 apparatus was used to evaluate the meatiness of carcasses. The lumbar part of the *m. longissimus dorsi* served as experimental material. Only muscles with normal quality properties were taken into consideration [Kauffman *et al.*, 1993; Borzuta & Pospiech, 1999]. Quality assessment was performed by measurements of pH_1 (45 min) and pH_2 (24 h after slaughter), using a portable pH-meter, type Handylab 2 with a combined glass-calomel electrode, type Schott L6880, and by measurements of electrical conductivity performed 90 min and 24 h after slaughter using a PQM apparatus. Loins were deboned, weighed, vacuum packed and sampled for further analysis.

Samples designated for the instrumental evaluation of tenderness were heated in a water bath until a temperature of 70°C was reached inside slices. Tenderness evaluation was conducted on meat cuts perpendicular to the muscle fiber layout using an Instron 1140 apparatus equipped with a Warner-Bratzler device. The size of cross section was 1 cm^2.

Proteins of the washed myofibrillar fraction isolated from muscle samples taken after 48 and 168 h of storage at 4°C underwent electrophoretic analysis on polyacrylamide gel (15% or 12%) with the addition of urea (8 mol/L) [Fritz & Greaser, 1991; Pospiech *et al.*, 2000a]. This allowed to observe almost all myofibrillar proteins on the same gel. In addition, it was easier to evaluate degradation changes in troponin T (Tn-T) because tropomyosin was moved before it, and the degradation products of Tn-T (DPTn-T) were

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placed mainly below Tn-T. The extraction of myofibrillar proteins was carried out using the rigor buffer (RB) (75 mmol/L KCl, 10 mmol/L KH_2PO_4, 2 mmol/L $MgCl_2$, 2 mmol/L EGTA, pH 7.0) with the addition of 0.1 mmol/L of PMSF [Fritz & Greaser, 1991]. The percentage contents of native titin (T1), its main degradation product with a weight of 2400 kDa (T2), troponin T (Tn-T) and its degradation products (DPTn-T) were determined employing a densitometric evaluation of gels. The proteins were further analysed using the Western blotting analysis. After electrophoresis, the proteins were transferred onto Immobilon [Fritz & Greaser, 1991]. The 9D10 monoclonal anti-titin from the collection of the Muscle Biology Laboratory of the Wisconsin University was applied as an antibody against titin, whereas an anti-troponin T clone No. JLT-12 purchased from Sigma was used against Tn-T. The secondary antibody was goat anti-mouse IgG (H&L) conjugated to alkaline phosphatase from Organon Teknika.

The statistical evaluation involved the two-way analysis of variance with the aid of Statistica software [Stanisz, 1998]. Differences were defined significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

The instrumental evaluation of tenderness (Table 1) showed that meat from low meatiness (LM) pig carcasses (<50%) was characterised by better tenderness already 48 h after slaughter, and further cold storage improved it slightly only. It was necessary to use a significantly higher shear force to cut m. *longissimus dorsi* from carcasses of high meatiness (HM) pigs. Meat tenderness was found to increase with storage time. These results confirm earlier investigations, in which it was found that, irrespective of the evaluation method applied, pork from HM pigs usually received lower quality grades in comparison with the meat from LM ones [Wood *et al.*, 1995; Grześkowiak *et al.*, 1998; Szalata *et al.*, 1999; Pospiech *et al.*, 2000b]. In addition, the statistical analysis revealed (Table 2) that meat tenderness was significantly affected by pig meatiness and storage time. The interaction between these two factors was low.

The electrophoretic analysis of native titin showed significantly higher amounts of T1 in the muscle samples derived from carcasses of LM porkers both after 48 and 168 h of cold storage (Table 3). In the first period of the study, a higher proportion of the T2 band was found in the muscle samples from LM pigs than in those from HM ones. However, this relationship was found reversed after 168 h of cold storage. Taking the T2/T1 ratio as an indicator of the rate of titin degradation, it could be concluded that muscle proteolysis occurred more slowly in the *m. longissimus dorsi* from LM pigs. Since accurate discrimination between titin, its degradation products and other proteins, which can be characterised by similar molecular weights, is difficult without additional investigations, immunoblotting against titin was performed using the 9D10 anti-titin antibody. This analysis (Figure 1b, Table 5) showed that titin underwent

TABLE 1. Instrumental evaluation of meat from pigs of various meatiness.

Shear force (N/cm^2)	Meatiness (%)	
	<50	>55
48 h	$45.13^b \pm 10.61$	$56.67^a \pm 16.37$
168 h	$35.26^b \pm 8.21$	$39.31^b \pm 13.06$

$^{a, b}$ – means followed by different letters are significantly different at $p<0.05$

TABLE 2. Two-way analysis of variance of instrumental evaluation of pork tenderness.

Parameter	Source of variation	Number of degrees of freedom	Mean square	$F_{calc.}$
Shear force (N/cm^2)	meatiness (1)	1	864.94	5.55*
	time (2)	1	2637.60	16.92*
	interaction (1 x 2)	1	199.92	1.28
	error	53	155.87	

* – significance at $p<0.05$

TABLE 4. Two-way analysis of variance of selected protein contents in the washed myofibrillar fraction based on the SDS-PAGE analysis (%).

Parameter	Source of variation	Number of degrees of freedom	Mean square	$F_{calc.}$
T1	meatiness (1)	1	36.66	11.89*
	time (2)	1	0.07	0.02
	interaction (1 x 2)	1	0.77	0.25
	error	34	3.08	
T2	meatiness (1)	1	3.54	0.75
	time (2)	1	0.04	0.01
	interaction (1 x 2)	1	35.28	7.50*
	error	45	4.70	
Tn-T	meatiness (1)	1	1.08	0.48
	time (2)	1	4.09	1.83
	interaction (1 x 2)	1	8.11	3.63
	error	45	2.24	
DPTn-T	meatiness (1)	1	22.67	25.93*
	time (2)	1	7.43	8.49*
	interaction (1 x 2)	1	0.05	0.06
	error	45	0.87	

* – significance at $p<0.05$

TABLE 3. Changes in titin, troponin T and the content of their degradation products in the washed myofibrillar fraction on the basis of the SDS-PAGE analysis (%).

Meatiness of carcasses*	Time after slaughter (h)	T1	T2	Tn-T	DPTn-T
LM	48	$6.49^a \pm 2.29$	$5.87^a \pm 2.70$	2.94 ± 1.21	$4.13^a \pm 1.18$
	168	$6.08^a \pm 1.39$	$4.22^b \pm 2.07$	3.18 ± 1.41	$4.85^a \pm 0.87$
HM	48	$4.00^b \pm 1.70$	$4.70^b \pm 1.83$	4.06 ± 0.95	$2.70^b \pm 0.90$
	168	$4.22^b \pm 0.41$	$6.46^a \pm 2.07$	2.66 ± 2.06	$3.55^b \pm 0.79$

$^{a, b}$ – means in the same column followed by different letters are significantly different at $p< 0.05$, *: LM – meat from pigs of low meatiness (<50% of meat in the carcass), HM – meat from pigs of high meatiness (>55% of meat in the carcass)

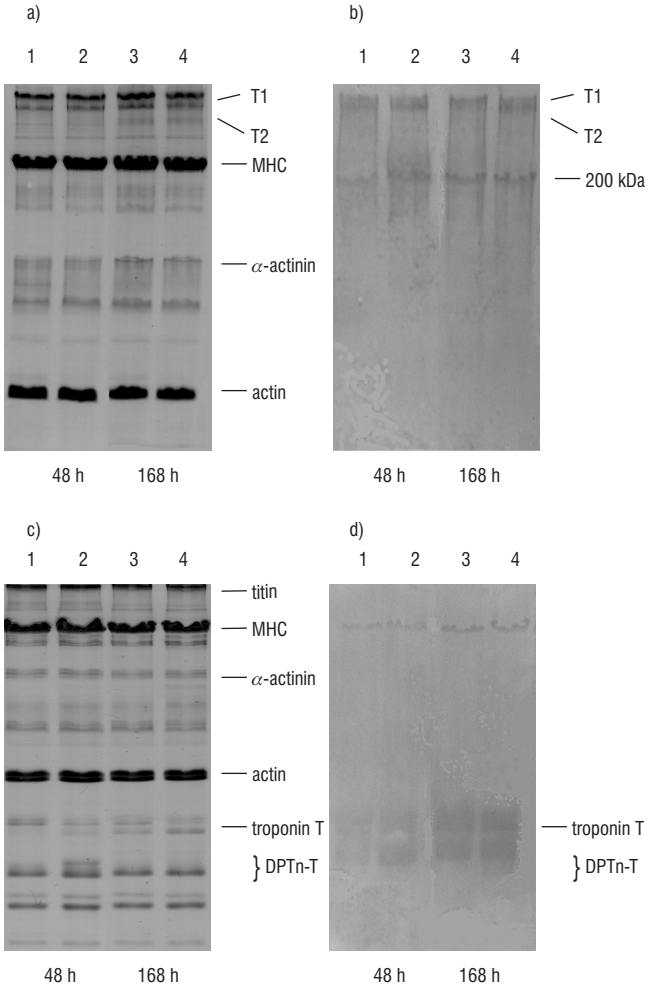

FIGURE 1. Electrophoretic separation of proteins from washed myofibrils of *m. longissimus dorsi* (a) 12% SDS-PAGE with 8 M of urea; (b) western blotting with the 9D10 titin antibody; (c) 15% SDS-PAGE with 8 M urea; (d) western blotting using troponin T antibody; Band 1 – separation of proteins from meat of pigs with meatiness >55%, 48 h; band 2 – separation of proteins from meat of pigs with meatiness <50%, 48 h; band 3 – separation of proteins from meat of pigs with meatiness >55%, 168 h; band 4 – meatiness <50%, 168 h.

a slower degradation in muscles from HM pigs. Two days after slaughter, slightly more T1, T2 and the product whose molecular weight was approximately 200 kDa were observed in the muscle samples from carcasses with over 55% meatiness than in those from LM carcasses (Table 5). After 7 days (168 h) of cold storage, a decline in the amounts of T1 and T2 in the muscles of both investigated groups of pigs was noted. Nevertheless, a higher content of native titin was found in the muscles from HM carcasses, although the changes recorded between these two groups of pork were statistically non-significant (Tables 5 and 6).

A tendency towards an increase in the contribution of the band with a molecular weight of around 200 kDa was noted in the course of cold storage, although slightly more of this protein was found in the muscles derived from carcasses of HM pigs in comparison with the muscles derived from LM ones (Table 5).

The immunoblotting analysis of titin showed that during cold storage the content of T1 and its degradation products of high molecular weight (≥200 kDa) decreased, as compared to the total content of native titin and its degradation products (Table 5). At 48 h after slaughter T1, T2 and 200 kDa protein which reacted with the 9D10 anti-titin monoclonal antibody made up 41.23% of all proteins in muscle tissue from LM pigs, whereas after 168 h – only 37.76% (Table 5, Figure 1b). In the case of muscle samples derived from HM pig carcasses, the process of titin degradation was slower during the first two days of storage as compared to the group of LM pigs, although the differences were statistically non-significant. High molecular weight proteins (≥200 kDa) which reacted with the titin monoclonal antibody made up 47.73% after 48 h and 38.96% after 168 h of storage (Table 5). During further cold storage, titin degradation products of lower molecular weight appeared in both groups of muscles, although their slightly higher quantities were recorded in muscles derived from LM pig carcasses than from the HM ones. The statistical analysis (Table 6) of

TABLE 6. Two-way analysis of variance of changes in titin and the content of its degradation products in the washed myofibrillar fraction based on the western blotting analysis (%).

Parameter	Source of variation	Number of degrees of freedom	Mean square	$F_{calc.}$
T1	meatiness (1)	1	53.30	5.34
	time (2)	1	3.29	0.33
	interaction (1 x 2)	1	0.20	0.02
	error	4	9.98	
T2	meatiness (1)	1	5.64	0.28
	time (2)	1	67.74	3.38
	interaction (1 x 2)	1	21.06	1.05
	error	4	20.05	
200 kDa	meatiness (1)	1	0.27	0.22
	time (2)	1	1.93	1.58
	interaction (1 x 2)	1	0.16	0.13
	error	4	1.22	
≥200 kDa	meatiness (1)	1	29.65	2.84
	time (2)	1	74.91	7.18
	interaction (1 x 2)	1	14.05	1.35
	error	4	10.44	

* – levels of factors and interactions did not reveal differentation even at $p<0.05$

TABLE 5. Changes in titin and the content of its degradation products in the washed myofibrillar fraction based on the western blotting analysis (%).

Meatiness of carcasses*	Time after slaughter (h)	T1	T2	200 kDa	≥200 kDa (altogether)
LM	48	10.93 ± 1.00	17.15 ± 6.51	13.15 ± 0.21	41.23 ± 5.71
	168	9.34 ± 0.29	14.58 ± 0.28	13.85 ± 1.90	37.76 ± 1.89
HM	48	15.78 ± 6.22	18.72 ± 6.07	13.23 ± 0.04	47.73 ± 0.11
	168	14.81 ± 0.31	9.65 ± 0.95	14.50 ± 1.11	38.96 ± 2.37

* – explanations as in Table 2

changes in the amount of T1 revealed that they were significantly affected by the meatiness of pigs.

Other researchers [Taylor *et al.*, 1995; Boles *et al.*, 1992] also reported variations in the rate of titin degradation during cold storage of meat, dependent on animal species. Taylor *et al.* [1995], who analysed beef muscles, found that titin underwent partial degradation directly after slaughter. Further titin degradation takes place during the first 24 h *postmortem*, but T1 undergoes complete degradation to T2 in a period between 24 and 72 h of cold storage after slaughter. Boles *et al.* [1992] found, in washed myofibrils derived from pork muscles, a gradual disappearance of the T1 band accompanied by an increased intensity of the T2 band, when meat was stored in refrigerated conditions for 7 days after slaughter. In addition, the same researchers [Boles *et al.*, 1992] also determined variations in the rates of these processes in relation to the stress susceptibility of pigs and final meat quality. Similar results were obtained while investigating turkey muscles [Pospiech *et al.*, 1997].

The above-mentioned observations indicate that the weakening of the cytoskeletal structure may be related to titin degradation and its release from the complex of myofibrils. These processes may lead to increased tenderness and improve meat functional properties with the increase in storage time. To some degree, these changes may depend on pig meatiness.

Another protein whose *postmortem* changes are correlated with meat tenderisation is Tn-T [Penny & Dransfield, 1979; Koohmaraie *et al.*, 1984a,b; Ho *et al.*, 1994]. Electrophoretic evaluation of its percentage content on gel did not indicate significant changes during storage (Table 3, Figure 1c), although differences in its amount in the muscle samples in both swine groups sometimes made up even 25%. Significantly more DPTn-T was found in the muscle samples derived from carcasses with meatiness below 50%, both 48 and 168 h after slaughter. The process of troponin T degradation, evaluated on the basis of changes in the ratio of DPTn-T to native Tn-T, went faster in the muscles from the LM carcasses (1.4 at 48 h and 1.52 at 168 h) in comparison with the muscles derived from the HM pigs (0.66 at 48 h and 1.33 at 168 h). The degradation of Tn-T intensified with time.

Western blotting of troponin T revealed that higher quantities of native protein were found in the muscles derived from pigs with meatiness above 55%, 48 h after slaughter (Figure 1d). Many more degradation products were found in the meat samples from the group of the LM carcasses. Further storage was associated with an increase in their proportion, although slightly more degradation products of troponin T were still observed in the muscle samples derived from carcasses of meatiness below 50%. The statistical evaluation revealed that the appearance of DPTn-T was affected by meatiness and the time of meat storage (Table 4), although the former of these factors had more pronounced influence.

Reports of numerous authors [Koohmaraie *et al.*, 1984 a, b, 1995; Ho *et al.*, 1994; Huff-Lonergan *et al.*, 1996] confirm a relationship between the degree of Tn-T degradation and tenderness evaluation. The more rapid the degradation of this protein, the more tender the meat. Also a very rapid troponin T degradation in lamb and beef was reported by Hopkins and Thompson [2001], and Geesink and Koohmaraie [1999].

The results of the above-mentioned reports were corroborated in these experiments. A lower shear force was observed in the raw material derived from animals characterised by low meatiness, where the degradation of the Tn-T was more rapid.

CONCLUSIONS

The process of protein degradation, especially of titin and troponin T, was more intense in the *m. longissimus dorsi* from the LM pigs than in that from the HM ones.

More extensive protein degradation in the muscles derived from the LM pigs may indicate the existence of some links between these changes and more rapid processes of tenderisation in comparison with changes observed in the meat from the HM pigs.

The main factors which influenced the tenderisation process were the meatiness of pigs, and the time of meat storage after slaughter. Their interactions were low.

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PRZEMIANY TITINY I TROPONINY T A KRUCHOŚĆ MIĘSA ŚWIŃ O ZRÓŻNICOWANEJ MIĘSNOŚCI

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Natywna titina i troponina T w mięśniu najdłuższym grzbietu z tusz świń wysokomięsnych (HM) podlegają wolniejszej poubojowej degradacji w porównaniu do niskomięsnych (LM) świń. Powyższe zależności stwierdzono poprzez zastosowanie techniki western blottingu obu tych białek. Rozleglejsza degradacja białek w mięśniach LM może być związana z ich szybszym kruszeniem w porównaniu do zmian obserwowanych w wieprzowinie z HM świń. Dwuczynnikowa analiza wariancji wykazała, że te procesy były istotnie zależne od mięsności świń i czasu przechowywania mięsa, chociaż pierwszy z tych czynników miał bardziej znaczący wpływ.