# TENDERNESS AND PROTEIN CHANGES OF PORK IN RELATION TO PIG GENOTYPE AND POSTMORTEM GLYCOLYSIS PHENOTYPE

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## Introduction

Pork shows quite large variations in tenderness, and these variations are believed to be caused by differences in postmortem metabolism. The most common defects of pork meat quality are watery meat (PSE), RSE and "acid" (or RN) meat (Warner et al. 1997, Monin & Sellier 1985). PSE meat usually lacks tenderness (Kemp et al. 1976, Boles et al. 1992, Pospiech et al. 1999), and this has been associated with slower postmortem changes in the cytoskeletal proteins (Boles et al. 1992, Pospiech et al. 1997). The "acid" meat is usually found to be more tender in comparison to normal quality meat (RFN) (Lundström et al. 1994) and the postmortem tenderness improvement occurs a little faster than in normal (RFN) meat. The improved tenderness of "acid" meat as compared to RFN has been explained to result from very low ultimate pH, which may favor higher activity of muscle proteases. PSE meat also has low ultimate pH values and it reaches this pH even faster after death. The aim of the current paper was to relate changes in the myofibrillar proteins to postmortem tenderness. We examined the postmortem changes of two main proteins (Tn-T and titin) believed to be responsible for tenderness (Greaser et al. 2000) and how these changes related to meat quality (PSE, "acid" meat, RFN).

## Material and methods

The investigations were carried out on 60 pigs. Classification into the different groups was based on rate of postmortem pH decline and drip loss of muscle at 24 hours postmortem (fast glycolyzing - FG, low pH meat - LpH, slow glycolyzing - SG) and genetic testing (homozygote mutant [$RN^- RN^-$], heterozygote [$RN^- rn^+$], homozygote normal [$rn^+ rn^+$]). The fast glycolyzing muscle will often become PSE, slow glycolyzing muscle usually is normal, and the low pH meat is often due to the RN mutation. Measurements of pH value were carried out at 45-60 minutes after slaughter ($pH_1$) and after chilling the carcasses on the following day ($pH_2$). Water holding capacity was evaluated by measuring the drip loss from the muscle samples removed 24 hours after chilling. The $pH_1$ value of SG meat was above 5.8 and the $pH_2$ above 5.5. In case of FG a very rapid drop of pH value in tissue directly after slaughter was observed ($pH_1 < 5.8$). Meat was considered as LpH if its $pH_1$ value was between 5.9 and 6.3, the $pH_2$ was below 5.5, and the drip loss was greater than 2.5%. Meat tenderness was analyzed using a Warner-Bratzler shear device after 1 and 9 days of cold storage. Protein analyses were conducted at six postmortem times: 5 minutes, 45 minutes, 3 hours, 6 hours, 24 hours and 9 days. Collected samples were frozen in liquid nitrogen and stored at $-70^0$C until analysis. The electrophoretic evaluation of proteins was conducted on washed myofibrils. These were obtained using modified rigor buffer (Fritz & Greaser 1991) [0.05M KCl, 0.05M Tris (pH 7.5), 5mM EGTA and 2mM $NaN_3$] with addition of protease inhibitors (0.1mM PMSF, 0.1mM leupeptin and 1mM benzamidine). Protein separations were carried out using agarose (1.5% of SeaKem Gold agarose, pH 8.5) according to the method adapted from Wu and Kusukawa (1998) and polyacrylamide gels with addition of urea (15% acrylamide, pH 8.8 resolving gel and 8M urea). The first type of gel allowed excellent separation of proteins with molecular weights above 200kDa, while the second one was more suitable for separation of smaller molecules, especially Tn-T and its degradation products. Densitometric evaluation of separated proteins was conducted on scanned images using IPLab software. Results were statistically analyzed with help of the STATISTICA program (Stanisz 1998).

## Results and discussion

SG meat was characterized by the highest mean $pH_1$ value, whereas LpH meat had the lowest $pH_2$ value (see table). The largest drip loss was observed in watery meat, but the difference in values between LpH and FG meat was very small and statistically insignificant. The above relationships confirm previous reports (Koćwin-Podsiadła et al. 1998, van Laack & Kauffman 1999, Lundström et al. 1994) and reflect typical effects of postmortem glycolysis on properties of pork quality. The comparisons of the degradation process of the titin show that this protein was more slowly degraded in FG muscles, which is consistent with results from previous studies with pork (Boles et al. 1992) and turkey meat (Pospiech et al. 1997). Differences between the rate of titin degradation in SG meat and LpH meat were insignificant (Fig. 1). A slightly different pattern of change occurred in the breakdown of Tn-T. The highest rate of degradation was observed in FG and LpH muscles, especially during the first 3 to 6 hours after slaughter (Fig. 2). In the final stage of investigation (9 days after slaughter) the value of the ratio for all types of meat was similar. Thus it appears that earlier acidification might have caused faster Tn-T degradation shortly after the slaughter, but the process of protein degradation continued further so that, at the end of the storage, the degradation of Tn-T is similar in all types of meat. Described protein changes probably affected meat tenderness. FG meat was least tender while LpH and SG were most tender and very similar (see table). LpH meat occurrence is mostly associated with a mutation in main gene described as $RN^-$ (Monin & Sellier 1985). It may occur in one or two alleles. Influence of this gene was tested in 16 pigs of the same Hampshire background genetics. Meat quality comparison showed that in case of mutation the acidification was the largest at 24 hours after slaughter. The size of drip loss was also largest in these groups (table). The variation in titin degradation rate between these groups was very small (Fig. 3). In case of Tn-T, more degradation occurred in meat from pigs with the $RN^-$ mutation at 24 hours postmortem while less was found in comparison to the normal meat at 9 days after death (Fig. 4). Meat tenderness of pigs with the $RN^-$ gene was usually better than in meat from pigs

free of this mutation. These results are in agreement with those of Lundström et al. (1994). Faster degradation of titin is probably the most important difference between FG and LpH meat. Observations of meat from pigs with RN$^-$ gene point out that higher acidification, which is usual in this type of material, probably favored protein degradation processes. Comparison of meat tenderness and protein changes in SG and FG meat shows that the size of these changes dependents not only on quantity of formed acid but also on the time course of its increase, which is a consequence of glycolytic changes in muscle. Intense increase of meat acidity after slaughter may influence faster tenderization of meat, as long as acidity does not cause wateriness. Rapid acidification found in FG

Fig. 1 Changes of titin in pork of various quality

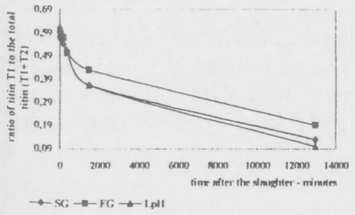



Fig. 2 Changes of troponin T in pork of various quality

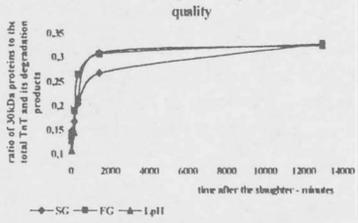
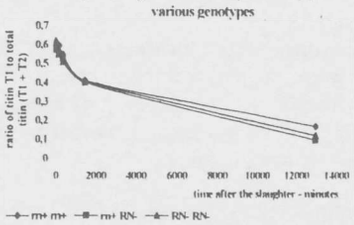



Fig. 3 Changes of the titin in pork from pigs of various genotypes



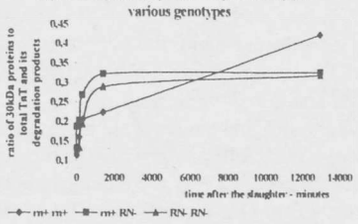

muscle decreased speed of titin degradation but stimulated Tn-T breakdown during the first day postmortem.

Changes of selected properties of meat in relation to its quality and pigs genotype

| Characteristics | Type of muscle | | | Genotype | | |
|---|---|---|---|---|---|---|
| | SG | FG | LpH | $rn^+ rn^+$ | $rn^+ RN^-$ | $RN^- RN^-$ |
| Number of pigs | 38 | 15 | 7 | 4 | 7 | 5 |
| $pH_1$ | $6.15^a$ | $5.56^b$ | $6.11^a$ | 6.06 | 5.94 | 6.06 |
| $pH_2$ | $5.57^a$ | $5.52^a$ | $5.42^b$ | $5.58^b$ | $5.43^a$ | $5.43^a$ |
| WB – 1 day (kG/cm$^2$) | 7.60 | 7.62 | 6.54 | 7.22 | 6.77 | 6.07 |
| WB – 9 days (kG/cm$^2$) | 5.27 | 5.97 | 5.26 | 5.44 | 5.20 | 4.70 |
| Drip loss (%) | $1.59^b$ | $4.81^a$ | $4.00^a$ | $1.52^b$ | $2.92^a$ | $4.99^a$ |

$^{a,b}$ - mean values marked with different indexes indicate the significant differentiation between them ($P<0.05$)

Fig. 4 Changes of troponin T in pork from pigs of various genotypes




The slightly slower but greater acidification of LpH meat probably limited titin degradation in the period beyond one day postmortem. Therefore in muscles from pigs with the RN$^-$ gene, greater titin degradation was observed than in muscles of pigs free of this mutation. Larger proteolysis of proteins in meat containing more lactic acid corresponds to the results of Fernandez & Tornberg (1992) and Watanabe & Devine (1996), who obtained more tender meat when it revealed lower pH value. It is possible, that acid pH plus higher temperature directly after the slaughter might unfold the Tn-T and make it more susceptible to proteolytic degradation. If this occurred, then perhaps the cathepsins, which operate more efficiently at lower pH, might become more involved in Tn-T degradation in the acid meat animals. Lower tenderness of FG meat, in which lactic acid forms very fast, may be due to acid denaturation of the muscle proteases. In summary, these studies indicate that the rate and extent of postmortem metabolic changes in meat, especially shortly after death, may significantly influence meat tenderness.

**Conclusions**

- Titin changes postmortem are slower in FG compared with LpH or SG muscles
- Large acidification of FG and LpH meat favored faster Tn-T degradation during the first day postmortem, but resulted in retarded breakdown during subsequent aging compared with SG meat
- Better tenderness of LpH meat on the second day after slaughter appears to be associated with greater proteolysis of Tn-T
- Titin and Tn-T postmortem changes do not always proceed in parallel

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