CHANGES IN STRUCTURE AND MYOFIBRILLAR PROTEINS MUSCLES *M. PSOAS MAJOR* AND *M. SEMITENDINOSUS* DURING COLD STORAGE AS INFLUENCED BY CATTLE AGE

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Background

During post rigor cold storage significant changes take place in structure of muscle fibres and in intramuscular connective tissue. In consequence, meat tenderness increases and the flavour profile of meat is formed (Zamora et al. 1996; Palka 2000). Myofibrils of muscle fibre divide into smaller fragments, main structural changes take places in the disc regions of sarcomers, cytoskeletal proteins and costamers (Pospiech & Grześ 1997; Boyer-Berri & Greaser 1998). The main reason changes in the myofibrils structure is activating influence of calcium ions on proteolitic calpaines (Ouali 1992) and degradation of proteoglycans in *endomysium* and *perimysium* (Nishimura et al. 1996). White muscle fibres have more extensive and active of sarcoplasmic reticulum that red ones (Newbold 1980). The scope of the structural changes and rate of the cold storage ageing of different muscles originating from animals of different maturity can vary.

Objective

The objective of the study was determination of changes in muscle structure and myofibril protein composition of *m. psoas major* (PM) and *m. semitendinosus* (ST) obtained from calves, heifers and cows carcasses during 12-days cold storage ageing.

Methods

Samples for histology analysis were obtained directly from carcasses of calves, heifers and cows 3 hr after slaughter and from muscles aged 6 and 12 days at 4°C taken from carcasses at 24 hr after slaughter. Samples were preserved in 2,5% glutaric aldehyde (pH = 7,2) and in 1% solution of osmium acid, dehydrated in ethanol and melt in epoxy resin Epon 812. Muscle fragments of 1 μm breadth, designated for observation with light microscope Ampliwal by Carl Zeiss Jena, were coloured with Azur II and methylene blue, photographic documentation was enlarged 460 fold. Muscle fragments 1 nm breadth designated for observation with electron microscope Tesla 500, were placed onto copper nets and contrasted with uranyl acetate and lead citrate, photographic documentation was enlarged 13 500x.

Samples for determination of myofibril proteins composition were obtained from PM and ST muscles on 1st, 6th and 12th day of cold storage. Samples were homogenised in rigor buffer (75 mM KCl, 10 mM KH_2PO_4, 2 mM $MgCl_2$) of pH 7,0 containing 0,1 mM PMSF (Fritz et al. 1989). Myofibril sediment, after twice washing it with rigor buffer, was analysed with electrophoresis in the vertical position with two-layer 12% polyacryloamide gel using assembly SE 250. Concentration of protein fractions was measured with densitometer GS 365W by Hoefer Scientific Instruments. Electrophoresis and identification of myofibril protein fractions was carried out similarly to procedure described by Fritz et al. (1989).

Results and discussion

Three hours after slaughter the muscle fibres and myofibrils were close to each other and sarcomas had intact structure of bands A and I, M line and discs Z.

On the 6th day of cold storage an increase of distance between fibres themselves and between sarcolemma and myofibrils was observed. I bands of sarcomeres were elongated, the contour of M line was less clear and partial disintegration of Z discs was observed.

On the 12th day of cold storage spaces between fibres and spaces between sarcolemma and myofibrils increased further. Sarcomeres' structure was disintegrated more, there were numerous cracks in I band regions, Z discs showed increased breakdown. Changes in structure of investigated bovine muscles PM and ST during cold storage were similar to those described by other authors (Minelli et al. 1995), however, they were more intensive in calves' and heifers' muscles than in cows' muscles.

Electrophoresis of myofibrils showed 18 protein fractions, 13 of them were identified as typical myofibril proteins, and for others molecular weights were estimated.

Contractile proteins (myosin and actin) content in PM and ST muscles was similar and did not depend on maturity of animals or time of cold storage.

Significant changes and differences in levels of most of regulatory muscle proteins were not observed. During cold storage concentration of troponin T in both muscles regardless of the type of animal decreased, especially during first 6 days of storage.

Amongst cytoskeletal myofibril proteins, the most prominent changes were observed in concentration of nebulin and filamin. On the 6th and 12th day of cold storage reduction in the amount of these proteins was observed in both muscles and for all groups of cattle.

Particular attention should be paid to occurrence of protein with 30 kDa molecular weight (Table 1). This fraction appeared in larger concentrations in calves' muscles as soon as 1 day after slaughter, and its level diminished with the time of cold storage. In heifers' muscles this fraction appeared in very low concentrations 1 day after slaughter, however in cows' muscles presence of this protein was observed only on the 6th day of ageing (PM muscle) and on the 12th day (ST muscle). In the subsequent days of cold storage the level of 30 kDa protein increased and the increase was higher in case of ST muscle.

Table 1. Concentration proteins of 30 kDa molecular weight in fraction of washed myofibrils (as % of myofibril protein) during cold storage of *m. psoas major* (PM) and *m.semitendinosus* (ST) muscles obtained from calves, heifers and cows. Mean values and standard deviations.

Cattle group	Cold storage [days]					
	1		6		12	
	Muscles					
	PM	ST	PM	ST	PM	ST
Calves	1,59 ± 0,30	2,26 ± 0,49	1,13 ± 0,23	1,79 ± 0,11	1,29 ± 0,39	1,06 ± 0,41
Heifers	0,62 ± 0,33	0,33 ± 0,16	1,72 ± 0,28	2,69 ± 0,15	2,23 ± 0,46	3,49 ± 0,17
Cows	0,00	0,00	0,23	0,00	1,29	1,43

Conclusions

1. During cold storage ageing of bovine muscles significant degradation changes take place in both intercellular spaces and in the myofibril structure.
2. Scope and rate of degradation of muscle structure during cold storage are similar for muscles *m. psoas major* and *m. semitendinosus*, but the changes are more intensive in calves' muscles than in cows' muscles.
3. Appearance of protein fraction of molecular weight of 30 kDa can be an indicator of the rate of cold storage ageing of meat.

References

1. Boyer-Berri C., Greaser M.L. 1998. Effect of postmortem storage on the Z-line region of titin in bovine muscle. J. Animal Sci., 76, 1034 – 1044.
2. Fritz J. D., Swartz D. R., Greaser M. L. 1989: Factors affecting polyacrylamide electrophoresis and electroblottinng of high molecular weight myofibrillar proteins. Anal. Biochem., 180, 205-211.
3. Minelli G., Couliolo J., Vignon X., Monin G. 1995: Post mortem changes in the mechanical properties and ultrastructure of the *Longissimus* in two porcine breeds. J. Muscle Food, 6, 313-326.
4. Newbold R.P. 1980. Proc. 32nd Ann. Recip. Meat Conf., p. 70 (Amer. Meat Inst.: Chicago).
5. Nishimura T., Hattori A., Takahashi K. 1996: Relationship between degradation of proteoglycans and weakening of the intramuscular connective tissue during postmortem ageing of meat. Meat Sci., 42, 251-260.
6. Ouali A. 1992: Proteolytic and physicochemical mechanism involved in meat texture development. Biochimie, 74, 251-265.
7. Palka K. 2000: Zmiany w mikrostrukturze i teksturze mięsni bydlęcych podczas dojrzewania poubojowego i ogrzewania. Zesz. Nauk. AR w Krakowie, rozprawy, 270.
8. Pospiech E., Grześ B. 1997: Wybrane białka cytoszkieletowe i ich rola w kształtowaniu właściwości funkcjonalnych tkanki mięśniowej. Żywność, Technologia, Jakość, 4, 3(12), 5-19.
9. Zamora F., Debiton E., Lepetit J., Lebert A., Dransfield E., Uoali A.: Predicting variability of ageing and toughness in beef *m. longissimus lumborum et thoracis*. Meat Sci., 43, 321-333.