POSSIBLE WAY OF STUDYING THE STATE OF CYTOSKELETON PROTEINS OF MUSCLES IN NATIVE STATE WITH THE VIEW OF ELUCIDATING THEIR INFLUENCE ON MEAT TOUGHNESS

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A possibility of using X-ray diffraction patterns of high resolution for studying proteins of cytoskeleton of muscles in native state is discussed. A densitogram of X-ray diffraction patterns of pork m. long. dorsi with intensities and dispositions of main meridian reflexes is presented. It has been speculated that the reflexes 215 A° and 108 A°, as if "banned" during X-ray diffraction analysis of myosin, belong to proteins of cytoskeleton.

The problem of meat tenderization with the view of production of high-quality meat products is one of the most urgent in meat science. Among numerous methods as used to increase meat tenderness: mechanical treatment, action of vibrations, treatment with microbial and plant enzymes, activization of the own meat proteinases, the latter one seems the most advanced.

When one speaks about intensification of the own proteolysis of meat, natural processes of violation of integrity of proteinaceus components of muscles during postmortem ageing of meat and thus uniform and controllable processes of its softening are implied. However, our knowledge about this process is incomplete and confused. First of all, the roles of two groups of proteinases, that is calpains and cathepsins during post-mortem proteolysis of meat, as well as of the groups of proteins that are affected by these enzymes are subject to contest.

Some authors beleive that it is mainly the calpains that play a major role in meat proteolysis (Dransfield E., 1992, 1993, 1994), while others give preference to the influence of lysosomal cathepsins D, B and L. (Koohmaraie M., 1994, Quali A. 1990, Beltran J. A. et al, 1992, Barmeri V. M. H et al, 1993).

And rather contradicting opinions are expressed about the proteins being the main "target" of these enzymes, that is: the proteins of actomyosin complex, the collagen of intramuscular connective tissue or the third, little known group of proteins.

Our investigations in 1980-1990 of the natur of meat of different quality groups (N, PSE, DED) led to rather interesting results about negative correlation between the structure state of the proteins of actomyosin complex and the activity of calpains and cathepsins D, B and L. The investigations included highly diversified conditions: ageing from 2 to 96 hours, different temperatures of meat chilling post-mortem, freezing and defrosting, heat treatment to 90°C. In our studies the meat PSE served as a natural label which due to peculiarities of its biophysical and biochemical nature permitted to see what would have been concealed if only "normal", traditional meat had been studied.

Thus, it was revealed that the proteins of actomyosin complex of PSE meat contrary to common opinions in meat processing possess higher water-binding ability after freezing and subsequent thawing, than after chilling. And the activity of calpains and cathepsins D, B and L in PSE meat after freezing and thawing under the same conditions decreased considerably (Borisova M. A., et al, 1994).

Then, we have shown that in ageing up to 4 days the conformation of proteins of actomyosin complex is optimum in "normal" meat during the first two hours, and at 48 hours, while in PSE meat this is true only for the first two hours postmortem, and during further ageing, up to 24 hours the state of structure of these proteins significantly decreased and did not change up to 96 hours post-mortem.

Also using these samples we have shown that the activity of proteinases, mainly cathepsins, is much higher for PSE meat, than for normal meat and goes through the maximum at 48 h post-mortem (Borisova M. A., et al, 1994). When studying the influence of different cooling temperatures post-mortem on the activity of its own proteinases we found that chilling temperature (+4°C) is the optimum one. However, the conformation state of proteins of actomyosin complex did not depend upon cooling temperature both for PSE and N meat (Borisova M. A., et al, 1995). As we have found, the cathepsins (particularly B and L) have shown activity in relation to the collagen intramuscular connective tissue, however, only about 20% of their total activity are due to collagen.

All above mentioned allowed us to suppose that the main "attack" of the own proteinases of meat is directed to the proteins of cytoskeleton, first of all to connectin (titin), being the strongest and difficult for degradation, and being in the muscles in considerable quantities (10% of total proteins and which at the present time is considered to be primarily responsible for toughness of meat).

In the scientific literature there is a number of works where a suggestion about denaturing influence of the own meat proteinnases on connectin is supposed and about connection of this process with structural and mechanical properties of meat (King N. L., et al, 1981, 1984; Locker R. H., et al, 1982; Fritz J. D., et al, 1992). But in most investigations connectin is studied by SDS-electrophoresis, i. e. in denatured state, that certainly is accompained by a number of artefacts.

In our studies we tried to investigate the proteins of cytoskeleton (connectin) in the native state, that certainly may give rather unequiocally interpretable results about their real state and influence on meat toughness. For this purposes we used a method of X-ray (diffraction) analysis of high resolution.

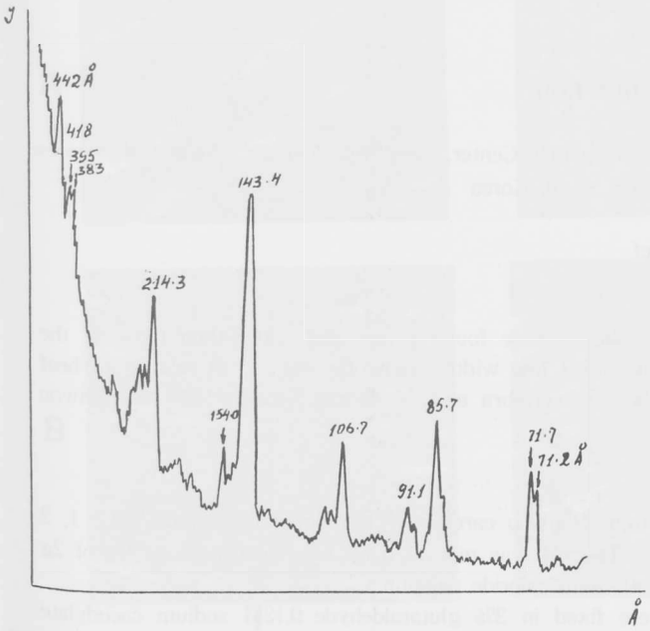

Fig. 1. Densitogram of intensity distribution of reflexes along the meridian of X-ray pattern of pork long dorsi miofibrils at 96 post mortem.

Materials and methods The method as used by us supposes high resolution of X-ray diffraction representation and makes it possible at selective physical and chemical effects on individual structural components of sarcomere to register the appearing changes with high sensitivity. An X-ray diffraction system as developed by us with two mutually perpendicular monochromators, located at the generator of X-radiation with the rotating inlet of type GX - 20 was used. The objects of investigations were preparations of myofibrils, isolated from pork m. long dorsi, according to method of Lusby et al (1983).

Results and discussion

Fig. 1 shows a densitogram of the meridian direction of the X-ray patterns as obtained by us. When interpreting such X-ray patterns it is common to consider that the main dissipating material is in the proteins of contractile apparatus of the muscles, in thick and thin filaments, therefore, a whole number of reflexes on X-ray patterns is identified with these structures.

It is common to think that the main X-ray dissipating electronic mass is located in heavy meromyosin, constituting "bridges". The main period of identity of such "bridges" is 430 A° and its orders are: 215, 143, 108, 86, 72 A°, etc. However, the electronic microscopy of myosin shows, that the symmetry of arrangement of the bridges imposes a so called "ban" on the appearance of the reflexes 215 and 108 A° on the X-ray patterns. Nevertheless on X-ray patterns obtained be us these reflexes are evident and may be related to structures similar in symmetry to the symmetry of myosin "bridges", but not coinciding with it. It is quite possible that such a symmetry may be with connectin, organized into the third type of the muscle filaments - "gap" - filaments. Much work is to be done with respect to elucidation and relation of X-ray patterns with the proteins of cytoskeleton in native state. But we think that the right way for studying these unique proteins is found by us.

References

1. Koohmaraie M (1994) Meat Sci. 36, 93-104
2. Quali A. (1990), J of Muscle Foods I, 129-165
3. Beltran J. A. et al (1992) Meat Sci. 32, 299-306
4. Barnieri V. M. H. et al (1993) 39th ICoMst, Calgary, Canada, ses. 3, 148
5. Borisova M. A. et al (1994) 40th IComst, Hague, Holland, ses. IY, 34
6. Borisova M. A. et al (1995) 41th ICoMST, San Antonic, USA, ses, E-4, 547
7. King et al (1984) Meat Sci, 11, 27-43
8. King N. L. et al (1981) Meat Sci, 5, 389-396
9. Locker R. H. et al (1982), meat Sci, 7, 93-107
10. Fritz J. D. et al (1992) J. of Muscle Foods, 3, 133-140
11. Lusby M. L. et al (1992) J of Food Sce, 48, 1787