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Morphological structure properties of the musculus longissimus dorsi in pigs in the process of autolysis

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A multitude of physical and bio-chemical processes take place during autolysis, including ATP break-up (dephosphorylation) that leads to glycogen phosphorylation, intensive accumulation of myofibrillar proteins, concentration of lactic (and phosphorous) acids that affect the state of muscle proteins. This, in turn, affects the technological properties of meat. Histological examination of the longest back muscle of pigs showed a distinct cross-striation of muscle fibers. Histological examination of the longest back muscle of pigs showed a distinct cross-striation of muscle fibers. During autolysis, micro-cracks appeared with minor breaks in sarcolemma, and only some muscle fibers areas had contraction nodes. During autolysis, micro-cracks appeared with minor breaks in sarcolemma, and only some muscle fibers areas had contraction nodes. Raw meat, which was classified as DFD (dark, sticky) according to the quality criteria, had a different structure. Cross-section of its fibers were rounder, fibers were tightly placed to each other, cell structures only slightly damaged. Analysis of the histological and ultrastructural changes in pork of different quality groups shows that the processes of deterioration of muscles in PSE and DFD groups cause significant changes in optical and geometrical properties of the surface and near-surface layers and affect the development of autolytic processes and intensity of destructive changes.

Key words: *musculus longissimus dorsi; autolysis; micro-structural changes; myofibrils; sarcolemma; sarcomere; nucleus; destruction.*

Особливості морфологічної будови найдовшого м'язу спини свиней у процесі автолізу

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У статті представлені результати морфологічних, ультраструктурних змін та особливостей поверхні м'язових волокон найдовшого м'язу спини свиней різних якісних груп. У процесі автолізу в м'язовій тканині запускається цілий ряд фізико-хімічних та біохімічних процесів – це розпад АТФ, внаслідок дефосфорилювання якої проходить процес фосфоролізу глікогену, інтенсивне скорочення міофібрилярних білків, накопичення молочної (і фосфорної) кислоти, які впливають на стан м'язових білків, що, в свою чергу, формує технологічні властивості м'яса. Усі ці процеси найкраще прослідковуються в перші години після забою тварин. При гістологічному дослідженні найдовшого м'язу спини свиней виявляли чітку поперечну посмугованість м'язових волокон. У процесі автолізу з'являлись мікротріщини з незначними розривами сарколеми, і лише в окремих м'язових волокнах відзначали ділянки з вузлами скорочення. М'язова тканина, яку за якісними характеристиками віднесли до групи PSE характеризувалась тоненькими м'язовими волокнами в яких чіткість деструктивних автолітичних процесів проявлялась інтенсивніше. М'язи PSE гістологічно виглядали достатньо рихлими, набряклими, поперечна посмугованість не чітко проглядалась, кількість мікротріщин візуально збільшувалась, у порівнянні з м'ясом якісних показників

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NOR. Самі м'язові волокна вільно розміщувались одні біля одних, ядра клітин без видимих змін, а сполучнотканинні прошарки були децю розширені. М'ясна сировина, яку класифікують за якісними показниками як DFD (темна, клейка), за своєю структурою відрізнялась тим, що волокна на поперечному зрізі були круглішими, щільно прилягали одні до одних, клітинні структури незначно зруйновані. На позовжних зрізах м'язові волокна розміщувались лінійно, і лише в окремих ділянках можна було спостерігати незначну хвилястість. Електронно-мікроскопічними дослідженнями м'язів в період розвитку помертвого задубіння, доводилось виявляти сильне скорочення міофібрил м'язового волокна, що проявлялось зменшенням розмірів І-дисків та потовщенням Z-пластинок. Проте, відзначали посилене набухання мітохондрій, вкорочення крист, в окремих клітинах навіть спостерігали гомогенізацію і лізис матриксу. У сполучній тканині виявляли розволокнення і розпушення волокнистих структур.

Ключові слова: найдовший м'яз спини, автоліз, мікроструктурні зміни, міофібрили, сарколема, саркомер, ядро, деструкція.

Особенности морфологического строения длиннейшей мышцы спины свиней в процессе автолиза

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В статье представлены результаты морфологических, ультраструктурных изменений и особенностей поверхности мышечных волокон длиннейшей мышцы спины свиней различных качественных групп. В процессе автолиза в мышечной ткани запускается целый ряд физико-химических и биохимических процессов – это распад АТФ, в результате дефосфорилирования которой проходит процесс фосфорилиза гликогена, интенсивное сокращение миофибриллярных белков, накопление молочной (и фосфорной) кислоты, которые влияют на состояние мышечных белков, что, в свою очередь, формирует технологические свойства мяса. Все эти процессы лучше прослеживаются в первые часы после убоя животных. При гистологическом исследовании длиннейшей мышцы спины свиней выявляли четкую поперечную исчерченность мышечных волокон. В процессе автолиза появлялись микротрещины с незначительными разрывами сарколеммы, и только в отдельных мышечных волокнах отмечали участки с узлами сокращения. Мышечная ткань, которую по качественным характеристикам отнесли к группе PSE характеризовалась тонкими мышечными волокнами в которых четкость деструктивных автолитических процессов проявлялась интенсивнее. Мышцы PSE гистологически выглядели достаточно рыхлыми, набухшими, поперечная исчерченность нечетко просматривалась, количество микротрещин визуально увеличивалось по сравнению с мясом качественных показателей NOR. Сами мышечные волокна свободно размещались, ядра клеток без видимых изменений, а соединительнотканые слои были несколько расширены. Мясное сырье, которое классифицируют по качественным показателям как DFD (темное, клейкое), по своей структуре отличалось тем, что волокна на поперечном срезе были круглее, плотно прилегали друг к другу, клеточные структуры незначительно разрушены. На продольных срезах мышечные волокна располагались линейно, и только в отдельных участках можно было наблюдать незначительную волнистость. Электронно-микроскопическими исследованиями мышц в период развития помертвого окоченения, наблюдали сильное сокращение миофибрилл мышечного волокна, четко проявлялось уменьшение размеров I-дисков и утолщение Z-пластинок. Кроме того, отмечали усиленное набухание митохондрий, уменьшение количества крист, в отдельных клетках – гомогенизацию и лизис матрикса. В соединительной ткани просматривалось разрыхление волокнистых структур.

Ключевые слова: длинная мышца спины, автолиз, микроструктурные изменения, миофибриллы, сарколема, саркомер, ядро, деструкция.

Introduction

Scientists have studied the issues related to the morphological characteristics of raw meat, changes in the muscle tissue during autolysis and under various mechanical and physical impacts for several decades. It is now evident that unjustified introduction of new meat pig genotypes in the breeding and commercial production, intensive type of feeding, changes to technological parameters of rearing, hypodynamia, absence of or minimal pre-slaughter care and handling and failings of primary carcass processing, often lead to decreased natural resistance of animals, abnormal biochemical processes in muscle tissue and manifestations of genetically determined PSE and DFD defects of meat (Adutskevych and Belousov, 1971; Kuznyetsov et al., 2002; Kryshafovych et al., 2007; Kudryashov, 2007).

Raw meat qualitative properties that differ from the normal (NOR) meat are Dark Firm Dry (DFD) and Pale

Soft Exudative (PSE) – pale, soft and watery. Another quality organoleptic defect in meat quality is the pH change. The pH change is important both in one hour and 24 hours after slaughter (Liu et al., 1995).

A multitude of physical and bio-chemical processes take place during autolysis, including ATP break-up (dephosphorylation) that leads to glycogen phosphorylation, intensive accumulation of myofibrillar proteins, concentration of lactic (and phosphorous) acids that affect the state of muscle proteins. This, in turn, affects the technological properties of meat (Kudryashov, 2007).

All these processes are best traced immediately after the slaughter (3, 6, 7 hours). Therefore, there is a need for a detailed study of structural and functional changes in the muscle tissue of animals during autolysis.

The aim of our study was to examine morphological and ultrastructural changes, and properties in surface features of muscle fibers of the MLD (longest back muscle) in pigs of different quality groups.

Materials and methods

To study the morphological characteristics of raw meat during autolysis, pieces of pork (from the longest back muscle) were collected, their pH was measured at 1, 3, 24 and 48 hours after slaughter. To establish the structural changes in the muscle fibers during autolysis, we employed histologic, electron microscope research methods and studied changes of muscle fiber surface with a scanning microscope.

1. Histological study. Test samples of meat were fixed in 10% neutral formalin solution for 2 days, followed by rinsing in tap water. After rinsing the material was carried through an upward series of alcohols (70, 80, 90, 96). The sample was kept in each of the alcohol solutions for 24 h. At that a process of dehydration occurred. The next stage consisted of compacting test samples in two portions of chloroform (up to 3 h each). Then the sample was transferred to a chloroform-paraffin and kept in a thermostat at 37 °C for 1–2 h. The sample was twice transferred into paraffin in the thermostat, using a new portion each time, and each portion was kept at 56 °C for 2 h. After that the studied samples were embedded into special forms with paraffin. Using the sledge microtome, sections 5–15 mm thick were made and later stained with hematoxylin and eosin.

For electron microscope examination, muscle pieces were fixed for 2 hours in a 1.5% solution of glutaraldehyde in 0.2 M mole cacodylate buffer (pH 7.2). The samples were then rinsed in two portions of the buffer and further fixed in 1.5% solution of osmium oxide (OsO₄). After that, the samples were washed, dehydrated in increasing concentrations of ethanol, contrasted in uranyl acetate and embedded in epoxy resin – Epon-812. Ultrathin sections were contrasted by uranyl acetate and lead citrate. Samples were viewed and photographed in the electron-transmission microscope PEM-100.

2. Scanning electron microscopy study. Qualitative indicators of meat were evaluated based on the studies of its surface grains using scanning electron microscope (SEM) JEOL-T220A. Magnification in 50–350 times was used for photographing. Preparation of samples for SEM research included two procedures – fixation and metallization of the sample. Fixation is used when the surface of living tissues in samples is studied. Metallization is a procedure of spatter of thin metallic film (thickness 5–10 nm) of gold, carbon, copper etc. on the surface of the samples. Metallization of muscle tissue samples was performed in vacuum sputtering apparatus VUP-5 by thermal sputter of copper. As a result of the SEM photographing, images of the studied muscle fibers obtained, the state of the surface fibers, damage, injuries etc. were assessed.

Results and discussion

Analysis of changes in muscle tissue's active acidity during maturation in the first hours after slaughter showed

that the pH of the MLD (back longest muscle) meat in studied pigs ranged within the norm between pH 6.1–6.3. A gradual decrease in pH (5.5–6.03) was observed in the process of muscle tissue maturation.

Histological examination of the longest back muscle of pigs showed a distinct cross-striation of muscle fibers. During autolysis, micro-cracks appeared with minor breaks in sarcolemma, and only some muscle fibers areas had contraction nodes. On cross sections, muscle fibers had well-defined polygonal shape. Perimysium demonstrated well-defined areas with blood vessels and clusters of nerve nodes, as well as of lipocytes, which formed perimysium layers.

Muscle tissue, which has been attributed to the PSE group because of its qualitative characteristics, was characterized by thin muscle fibers, in which the definition of destructive autolytic processes was more prominent. Histologically, PSE muscles looked quite loose, swollen, cross striation was not clearly defined, the number of micro-cracks was visually increased in comparison with quality indicators of NOR meat. Muscle fibers were loosely placed next to each other, cells' nuclei had no visible changes and connective tissue layers were slightly widened.

Raw meat, which was classified as DFD (dark, sticky) according to the quality criteria, had a different structure. Cross-section of its fibers were rounder, fibers were tightly placed to each other, cell structures only slightly damaged. Muscle fibers in longitudinal sections were placed linearly with slight undulation being observed only in some areas. Muscle fibers had polygonal shape with slight roundness. Transverse and longitudinal striation in this meat quality group was much less demarcated and noticeable only in some areas. Most of the muscles had indistinct or virtually homogenized internal structure.

In the first hours after the slaughter, muscle tissues with active acidity within pH 5.8–6.2 clearly showed microstructural signs of after-slaughter relaxation: swollen muscle fibers were placed linearly, with rare undulation; demarcation between fibres noticeable only by tracing rod-like nuclei with distinct chromatin structure under the sarcolemma. Cross striation was well pronounced with longitudinal striation slightly flattened. Muscle myofibrils were relaxed. No changes in sarcolemma and nuclei structures were observed during this period.

Thus, during autolysis, typical changes take place in cell structures and they correlate with time. Physical, chemical and biochemical processes during autolysis cause lowering pH to almost constant level. It is also accompanied by the increased numbers of muscle fibers breaks and distances between them.

Analysis of the histological and ultrastructural changes in pork of different quality groups shows that the processes of deterioration of muscles in PSE and DFD groups cause significant changes in optical and geometrical properties of the surface and near-surface layers and affect the development of autolytic processes and intensity of destructive changes.

Pic. 1. Muscle cross-section. NOR quality group. Tightly packed muscle fibers with significantly enhanced connective tissue layer. Hematoxylin and eosin. X 400

Pic. 2. Muscle cross-section. NOR quality group. Increased cracks, expansion of connective tissue layers. Hematoxylin and eosin. X 400

Pic. 3. Microstructure of muscle fibers of the DFD quality group. Tightly packed muscle fibers. Hematoxylin and eosin. x 400

Pic. 4. Myofibrils of muscle fibers are relaxed, are parallel to each other. The first hours after the slaughter. x 32 000

Pic. 5. Breakages of muscle fibres myofibrils after 24 hours after the slaughter. x 24 000

Pic. 6. Local myofibrils destruction. x 32 000

Pic. 7. Nucleus of muscle fibers. First few hours after the slaughter. Oval shapes with condensed chromatin close to nucleolemma (nuclear membrane). x 8000

Pic. 8. Nucleus of muscle fibers. 24 hours after the slaughter. Chromatinolysis. x 8000

Pic. 9. Loosening of muscle fibers. Pronounced waviness and deformation of individual fibers. PSE quality group. SEM. x 200

Pic. 10. Tight packing of muscle fibers of the DFD quality group. SEM. x 200

Pic. 11. Smooth surface of the muscle fibers of the NOR quality group. Tight packing of muscle fibers. SEM. x 500

Pic. 12. Muscle cross-section. PSE quality group. Undulating surface and loose placement of muscle fibers. SEM. x 500

Pic. 13. Wavy, undulating surface of the muscle fibers of the DFD quality group. Tight packing. SEM. x 500

Conclusions

The studies established that in 48 hours after the slaughter, the majority of pork muscle fibers NOR have undergone partial deformation and destructive changes. At the same time, muscle fibers of PSE meat were in the initial stages of autolysis and were characterized by severe transverse striation, numerous changes such as micro-cracks, breakages and fragmentation of fibers, ultra-structural fragmentation of myofibrils and destruction of organelles.

Follow up research

To test the method of spatial-temporal speckle correlation in order to establish the relationship between structural and functional changes in the muscle tissue of different quality groups and destructive processes and activity coefficients of bio-speckles.

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