PROTEOMICS AS A TOOL FOR STUDYING MEAT AUTOLYSIS

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Abstract – The protein profiles of beef and pork *M. longissimus dorsi* in the autolysis process were obtained by the methods of one-dimensional gel electrophoresis in 12.5% polyacrylamide gel in the presence of sodium dodecyl sulfate and two-dimensional electrophoresis with isoelectrofocusing in ampholine and immobiline pH gradients. Protein detection on two-dimensional electrophoregrams was carried out by staining with Coomassie R-250 and silver nitrate followed by MALDI-TOF MS and MS/MS. We established an increase in the fragments of such proteins as pyruvate kinase, α-enolase, muscle creatine phosphokinase and glyceraldehyde-3-phosphate dehydrogenase, troponin I, adenilate kinase, α-B-crystallin, myosin light chains and cofilin 2.

Key Words – proteomics, two-dimensional electrophoresis, meat, autolysis.

I. INTRODUCTION

Proteins, in particular, animal proteins, are structural and functional elements of all living organisms. They perform several functions, among which a special attention should be paid to the regulatory function highlighting a role of biologically active peptides. These peptides are formed as a result of the proteolytic or autolytic processes occurring in meat. It is obvious that in the autolysis process a reliably high number of polypeptides are formed. At present, many polypeptide substances containing about 2-30 amino acids, which existed initially in meat raw material or were formed during technological processing, were extracted from meat raw material, especially from beef, chicken and pork muscle proteins. A range of short peptides was revealed, which demonstrated hypotensive, opioid, antioxidant, antithrombotic and other biological effects that influence several pathogenetic mechanisms underlying the development of pathological processes [1].

It is difficult to predict a result of an enzyme impact during autolysis. This is associated with the fact that occurrence of autolytical processes has an irregular character; while a result of the fermentolysis process has a particular structure [2]. Therefore, it is of great interest to study the mechanisms of formation of biologically active substances having the peptide nature during the autolytical changes in the muscle tissue.

II. MATERIALS AND METHODS

Beef and pork *m. longissimus dorsi* were the subject of investigation. The samples of beef *m. longissimus dorsi* were stored at a temperature of 4±2°C for 12 days in a vacuum container. The samples of pork *m. longissimus dorsi* were taken from unpacked half-carcasses stored at a temperature of 2±2°C for 5 days in conditions of the industrial refrigerator. The samples for examination were taken on the 0, 3rd, and 5th days. The pH values, water binding capacity [3], moisture content by the gravimetric method, protein content by the titrimetric method according to Kjeldahl and drip losses as the difference in weight were determined.

One-dimensional electrophoresis was carried out in 12.5% polyacrylamide gel in the presence of sodium dodecyl sulfate (SDS) in a chamber VE-10 (Helicon, USA) by the O`Farrell method [4]. As a standard for electrophoresis, we used a marker from Thermo, USA, which was a mixture of 11 recombinant proteins in a molecular weight range of 250-5 kDa. Staining was performed using Coomassie R-250 with our own modification [5] and silver nitrate. A protein fraction selected for identification was cut from gel plates obtained by

two-dimensional electrophoresis. Gel areas were minced, protein contained in them was hydrolyzed by trypsin and tryptic peptides were extracted for identification by matrix-assisted laser desorption/ionization (MALDI-TOF).

In MS/MS analysis, mass-spectra of fragments were recorded on the mass spectrometer Bruker Utraflex in the tandem mode (TOF-TOF) with detection of positive ions.

Protein identification was carried out using the software Mascot, option Peptide Fingerprint (Matrix Science, USA) with an accuracy of MH^+ mass detection of 0.01% (taking into consideration the possibility of cystein modification by acrylamide and methionine oxidation)

The protein content was analyzed using the UniProt Protein Database [6].

III. RESULTS AND DISCUSSION

The results of the physico-chemical analysis, functional characteristics of pork *m. longissimus dorsi* at the beginning of the experiment (24 hours after slaughter) and in the autolysis process are presented in Table 1.

Table 1 Physico-chemical composition of pork *m. longissimus dorsi* during autolysis

Indicator	Period of autolysis		
	24 hours	72 hours	120 hours
moisture, %	75.35±0.2	74.5±0.3	73.62±0.2
protein, %	22.4±0.52	23.3±0.67	23.3±0.55
WBC,%	92.05±0.33	89.72±0.25	87.66±0.21
drip losses, %	16.16±0.32	16.79±0.18	17.24±0.44

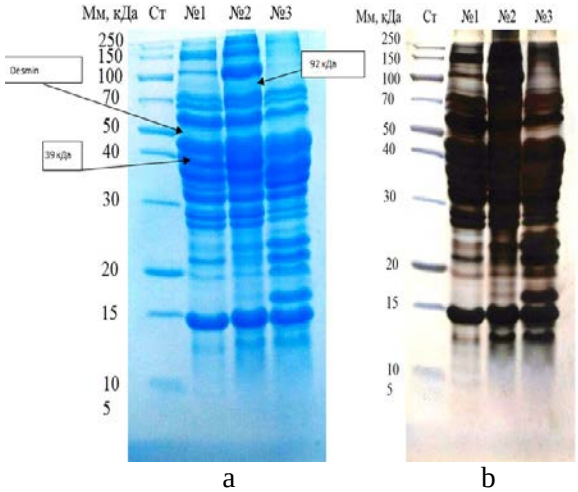
As a result of the performed investigations of meat raw material at different degrees of autolysis, it was found that changes in the total protein amount were within insignificant deviations, and moisture decreased according to the corresponding structural changes in muscle fibers and release of free moisture (meat juice).

In the meat samples, the pH value in the autolysis process practically did not change, i.e., the glycolytic changes after slaughter were pronounced weakly due to the low content of glycogen in muscles. In the course of autolysis, the WBC value was reduced by 4.8%, which, in turn, was also confirmed by drip losses. Muscle protein solubility decreased (an isoelectric point was at 4.7-5.4) and the level of their hydration decreased, which was confirmed by a reduction in water binding capacity from 92.05±0.33% to 87.66±0.21 %.

It is obvious that it is during the autolysis process when protein destruction (in particular, destruction of specific myofibrillar proteins and cytoskeletal proteins, titin and nebulin) and polypeptide formation occur. It was shown that protein destruction begins 6 hours after slaughter [7].

At the same time, it is not completely clear what exactly causes this destruction: whether intrinsic meat enzymes, fragmentation caused by amino acid modification by chemical factors [8] or direct hydrolysis under the action of weakly acidic and acidic environment in the muscle tissue. Troponin T, nebulin and cypher/ZASP proteins are essential participants of postmortem proteolysis in the muscle tissue of vertebrate mammals. For example, cypher proteins are localized at the Z-disc and link α-actinin [2]

The results of the electrophoretic analysis are presented in Fig.1.



a b

Figure 1. Electrophoregram of beef *m. longissimus dorsi* proteins

Staining: a - Coomassie R-250, b - silver nitrate

Ст – markers of molecular weights; 1 –beef (0 days); 2 – beef (9 days); 3 – beef (9 days, thermally treated)

As can be seen on the electrophoregrams of the beef samples after 9 days of autolysis, the intensity of the band corresponding to desmin (about 53 kDA) declined and a fragment of troponin T (about 29.5 kDa) appeared. An increase in the intensity of this band was especially noticeable on

the electrophoregram of thermally treated beef after 9 days of autolysis.

The 39-kDa component was present in the samples on the 1st and 9th day of autolysis but was absent after thermal treatment (sample 3 in Fig.1). The pH value had a negative correlation with changes in troponin T and positive correlation with the 30-kDa component.

In thermally treated beef, an appearance of a band with a molecular weight of 90 kDa was revealed after 9 days.

Therefore, the autolytic processes can facilitate formation of low molecular weight fractions of proteins with the tissue specific properties.

In the muscle tissue, high proteinase activity was observed 24 hours after slaughter. The calpain activity was maximal during the first hours after animal slaughter.

Then, the samples of pork *m. longissimus dorsi* on the 1st, 3rd and 5th days of autolysis were studied by the method of mass-spectrometry (MALDI-TOF MS and MS/MS). Several markers of autolytic changes were found (Fig. 2, Table 1).

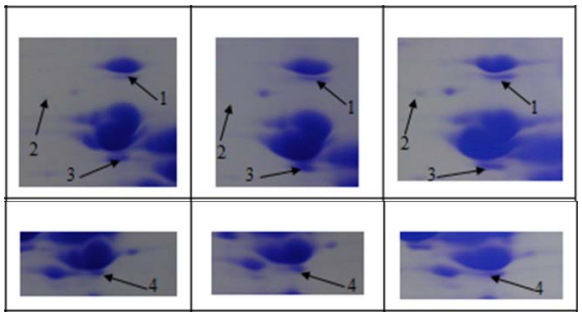


Figure 2. Mass spectrometric identification of protein fractions in pork *m. longissimus dorsi* on 1, 3 and 5 days of autolysis

The results of identification showed that the clearest sign of autolysis was the appearance of fragments of fast skeletal muscle troponin T. In the presence of the common troponin T fraction (identified earlier), the appearance and increase in three additional fragments with different molecular weight and pI were detected.

The peptides of the C- and N-terminal end of a molecule successively ceased to be detected by the mass spectra of tryptic peptides. We also observed an increase in the fragments of such proteins as pyruvate kinase, α-enolase, muscle creatine phosphokinase and glyceraldehyde-3-phosphate dehydrogenase (Fig.2. fractions 1-4 Table 2), troponin I, adenilate kinase, α-B-crystallin, myosin light chains and cofilin 2.

Table 2 The results of the mass-spectrometric identification of protein fractions in pork *m. longissimus dorsi* on the 1st, 3rd and 5th days of autolysis

Protein; certain synonyms, (*gene symbol*)	Numbers in Protein NCBI*	Мм/pI (exper.)
1.pyruvate kinase PKM isoform X6 (PKM2)	545841009	58,0/6,80
2.alpha-enolase (ATP5A1)	927145216	52,0/5,80
3.creatine kinase M-type (CKM)	194018722	41,0/6,60
4.glyceraldehyde-3-phosphate dehydrogenase (GPDH)	329744642	34,0/7,30

Understanding of changes in proteins, which are induced during technological processing and the cause of high variability in processing results will significantly help to improve processing technologies. Multiple studies showed that variations in the rate of post-slaughter glycolysis in different carcasses led to ageing of meat with different tenderness [8].

However, there is no clear understanding of mechanisms of post-mortem changes and further studies in this direction are necessary.

IV. CONCLUSION

Meat quality is determined by complex relationships between biological and environmental factors. However, it is often classified according to appearance and color of raw material as well as taste of finished products. This is reasonable both for production and consumers, but can be insufficiently accurate for classification of comparable proteomic groups and can lead to too complex variations within compared data arrays.

Biomarkers for the traits of muscle tissue growth and development, processes of proteolytic changes after slaughter can be used in animal breeding for targeted correction of metabolic processes as an instrument for decision making and meat quality management.

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