# STUDY AND IDENTIFICATION OF MAIN PROTEINS AND PEPTIDES TO DETERMINE THE CONTENT OF MUSCLE PROTEIN IN STRUCTURELESS COOKED PRODUCTS BY THE METHOD OF TWO-DIMENSIONAL ELECTROPHORESIS FOLLOWED BY THE TIME-OF-FLIGHT MASS SPECTROMETRY IDENTIFICATION

**N. L. Vostrikova[a],*, I. M. Chernukha[a], A. V. Kulikovskiy[a], and S. S. Shishkin[b]**

[a] The Gorbatov's All-Russian Meat Research Institute,
Talalikhina Str. 26, Moscow, 109316 Russian Federation

[b] The Federal Research Centre "Fundamentals of Biotechnology" of the Russian Academy of Sciences,
Leninsky Ave. 33, build. 2, Moscow, 119071 Russian Federation

* e-mail: nvostrikova@list.ru

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**Abstract:** Proteomic technologies in the modern laboratory practice proved to be very efficient to reveal biochemical changes in meat products, such as changes in heat-resistant and species-specific proteins that have the ability to become the relevant bio-markers. Several tissue-specific proteins were identified in the work under review using proteomic technologies in tested samples of meat and in specially manufactured sausage products that may be used as individual biomarkers to verify conformity of meat products to the alleged composition. Also, individual non-muscle proteins (soya and chicken protein) were determined in test samples of meat products apart from species-specific muscle proteins that may act as functional ingredients used in cooking process. Overall, total of more than 200 protein fractions were identified in the completed studies by the mass spectrometry method which are described in this review in part. The results obtained will be used to draft the procedure for quantitative evaluation of the meat component content in structureless cooked products (cooked sausages) as well as to draw proteomic protein charts of the native meat stock used to manufacture goods as per GOST (State Standard). Studies conducted in the range of this discipline will help to formulate and considerably develop approaches to identify and evaluate protein markers of quality, functionality and safety of meat for processing and processed meat products.

**Keywords**: proteomics, two-dimensional electrophoresis, bio-markers, mass spectrometry

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## INTRODUCTION

All biosignatures and biosignature formation mechanisms are controlled including both the gene activity and proteins, the gene expression products.

Currently, the characteristics and gene functional properties are the area of great challenge for the postgenomic age. Postgenomic tools and technologies apply integral, variable experimental approaches that may be characterized as complex biosystems.

Proteomics is the essential keystone to describe the genom functionality as all other functional genom tools, including transcriptomics and metabolomics. The purpose of proteome study is the genom information conversion to the efficient conception of biological mechanisms for scholars to create and realize hypotheses to find solutions to various problems regarding safe and quality food production [2, 3, 6, 7].

As for agricultural sciences, as well as for all other biosciences, introduction of proteomics and other postgenomic tools is vital to understand processes that occur in the multicomponent matrix named "meat".

It is specified currently that all quality properties of meat are of quite complicated origin. Despite the fact that many properties are being studied intensely, molecular mechanisms to form these properties are still unclear up to date. Consequently, there is a demand in novel approaches to clarify the way the meat quality properties form.

The use of proteomic strategy in studies of molecular mechanisms to form quality properties of the meat stock is the vital stage to produce high-quality animal products and to stabilize the production process more efficiently [8, 9, 11].

Most proteomic works are performed using two-dimensional electrophoresis (2-DE). This is the method to keep significance for proteomic researches. However, the volume of work to be completed requires methods and the equipment with the known high

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capacity, information content and sensitivity. Today, most scientists of world-wide reputation involved in proteomics sphere are assured that the combination of high performance liquid chromatography (HPLC) and tandem mass-spectrometry (MS/MS) may result in the quicker breakthrough in proteomics [1].

This work was aimed to create the integrated methodological approach to determine the content of the muscle protein in structureless cooked products by using two-dimensional electrophoresis followed by the time-of-flight mass spectrometry identification of confirmatory marker proteins.

## OBJECTS AND METHODS OF STUDY

Experimental studies were conducted in the laboratory of "Scientific and Methodological Works, Biological and Analytical Researches" of "The Gorbatov's All-Russian Meat Research Institute" in collaboration with "Protein Research" Laboratory of Federal Research Center of Biotechnology, Russian Academy of Sciences.

The following methods were used in the work:

– Protein fractionation with 2-DE by O'Farrell using ampholytes with isoelectric points in a pH gradient.

Proper modification of two-dimensional electrophoresis by O'Farrell as the main proteomic technologies which, in particular, used isoelectric focusing of ampholyte pH gradient (IEF-PAGE) and nonequilibrium pH gel electrophoresis (NEPHGE).

– Protein fractionation with two-dimensional electrophoresis by O'Farrell using isoelectric focusing in the immobilized pH gradient (IPG- PAGE)

When using IPG-PAGE of two-dimentional electrophoresis modification, the fractionation in the first direction (isoelectric focusiong) was performed in so-called strips – manufactured strips of polyacrylamide, conjugated with immobilines that ensured pH gradient within the range from 3 to 10 (13 cm Immobiline$^{TM}$ DryStrip pH 3–10, "GE Healthcare") on the Ettan IPGphor 3 [4, 8].

– Mass spectrometric methods to identify proteins

Protein fractions selected for identification were cut from gel plates obtained by the two-dimensional electrophoresis. Gel sections were grinded, the containing protein was hydrolyzed with the trypsin and tryptic peptides were extracted for identification using time-of-flight mass spectroscopy on the matrix (MALDI-TOF).

At MS/MS analysis, mass-spectrum of fragments was recorded using MALDI-TOF mass spectrometer Bruker Ultraflex in the tandem (TOF-TOF) mode to detect positive ions.

Proteins were identified by the Mascot Software, Peptide Fingerprint ("Matrix Science", USA) option, with the weighing accuracy rate $MH^+$ equal to 0.01% (assuming cysteine modification with acrylamide and methionine acidizing), as well as per database of the National Center of Biotechnological Information of USA (NCBI, address: http://www.ncbi.nlm.nih.gov) [8].

Samples of muscular tissue of pork and beef as well as test batches of cooked sausage goods were used for the analysis manufactured in compliance with the GOST and samples of sausage goods of similar products purchased in the retail network.

## RESULTS AND DISCUSSION

It is known that the minimal and sometimes soft changes in the composition or structure of protein components may decisively affect the meat properties. Biological processes that result in delicacy changes that is the most vital property of the pork include proteolytic transformations that occur in the meat protein system during rigidity, aging and further storage as cooled or frozen [10].

The meat quality is closely related to biological characteristics of the animal. Thereat, it is borne in upon that the meat quality characteristics such as delicacy, water-binding capacity, fractional structure, autolytic changes and others are complicated and multi-component systems and thus, they would be characterized in detail based on experimental approaches and technologies aimed to simultaneous study of multiple genes and proteins in parallel [12].

The proteomics aims to identify molecular markers usually named bio-markers that allow earlier and more accurate diagnostics of diseases in medicine, for instance. Currently, bio-marker search and identification is vital since bio-markers may be used to improve the larger range of characteristics, including methods to be used for meat production and processing.

Nevertheless, identification of the muscle protein content in structureless cooked products upon thermal treatment is a great challenge.

General concepts have been formed throughout several decades that proteomic technologies allowing transition to qualitative improvement of results when protein cell extracts and tissue samples are tested. This general conclusion is proved by results of this work.

During this cycle of works, the comparative study was performed to evaluate the efficiency of 2-DE methods where isoelectric focusing in ampholyn (IEF-PAGE) or immobiline (IPG-PAGE) pH 3–10 gradients are used. As an example, the Fig. 1 shows results obtained from fractionation using two 2-DE modifications above of the pig muscle tissue samples.

When comparing Fig. 1a and Fig. 1b, it can benoted that both modifications revealed almost similar fraction arrangement belonging to actins, tropomyosins and myosin light chain At the same time, the qualitative abundance of actin on two-dimensional electropho-regrams obtained by IPG-PAGE, was considerably higher than that in fraction obtained by IEF-PAGE. Apparently, the reason of this difference is the origin aggregation of proteins with the trace seen on the left edge of IEF-PAGE two-dimensional electrophoregram (Fig. 1a).

It is meanwhile seen that in the central and the right sections of 2-DE IEF-PAGE, more fractions are registered than on IPG-PAGE 2-DE. Measurements made specially showed that isoelectric focusing performed in normal conditions on strips with immobiline with pH gradient 3–10 does not result in segregation (and further identification) of proteins with $pI \geq 6.2–6.5$. In this view, most proteomic tests

performed later used the proper 2-DE modification described above that included isoelectric focusing in ampholyn pH gradient.

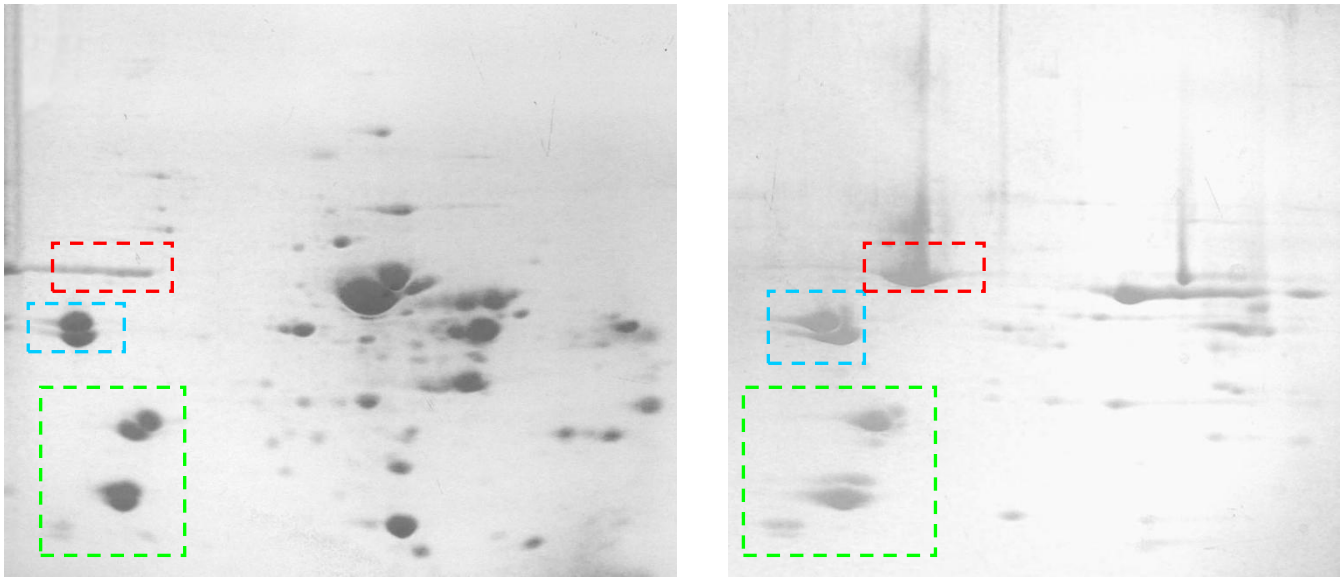
IPG-PAGE modification was use din special analysis to characterize the delicate electrophoretic features of proteins of pI < 6.0. The Fig. 2 below shows the test results of electrophoretic features of tropomyosin and myosin light chains in samples of various pig muscles.

As it is seen in Fig. 2, IPG-PAGE modification visually demonstrates tissue-specific features of this protein groups.

Essential for experimental validation of proteomic technologies is the potential of 2-DE along with mass spectrometry methods to reveal and identify chicken and vegetable protein in meat products (cooked meat products) manufactured as per reference documentation. The relevant results are shown below in Fig. 3 and Fig. 4.

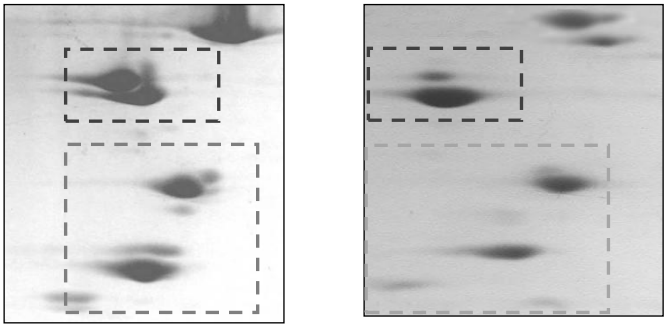
In particular, the Fig. 3a shows the fragment of 2-DE protein extract of "Lyubitelskaya" sausage where the fraction №1 was identified as the chicken pyruvate kinase by MALDI method. The reference fractions no. 2 and no. 3 for this case were earlier identified as pyruvate kinase and the isoform of M creatine kinase *Sus scrofa*. The mass spectrum of tryptic peptides for fraction no. 1 is given in Fig. 3b.

The fraction “Gcn” shown in Fig. 4 was similarly identified as the soya protein glycinin (4249566 Protein NCBI).



(a) (b)

**Fig. 1.** Two-dimensional electrophoregrams obtained by fractionation of the pig muscle tissue samples by 2-DE modifications with the help of isoelectric focusing in ampholyn (a) and immobiline (b) pH 3–10 gradients. Dashed rectangles show zones of muscle protein anatomic location: actin – red; tropomyosins – blue, myosin light chain – green.



(a) (b)

**Fig. 2.** Two-dimensional electrophoregram fragments obtained by fractionation of samples of various pig muscles (a – skeletal muscles, b – cardiac muscle) by 2-DE modification using the isoelectric focusing in the immobiline pH 3–10 gradient. Dashed rectangles show arrangement zones; tropomyosins – in blue and myosin light chains – in green.

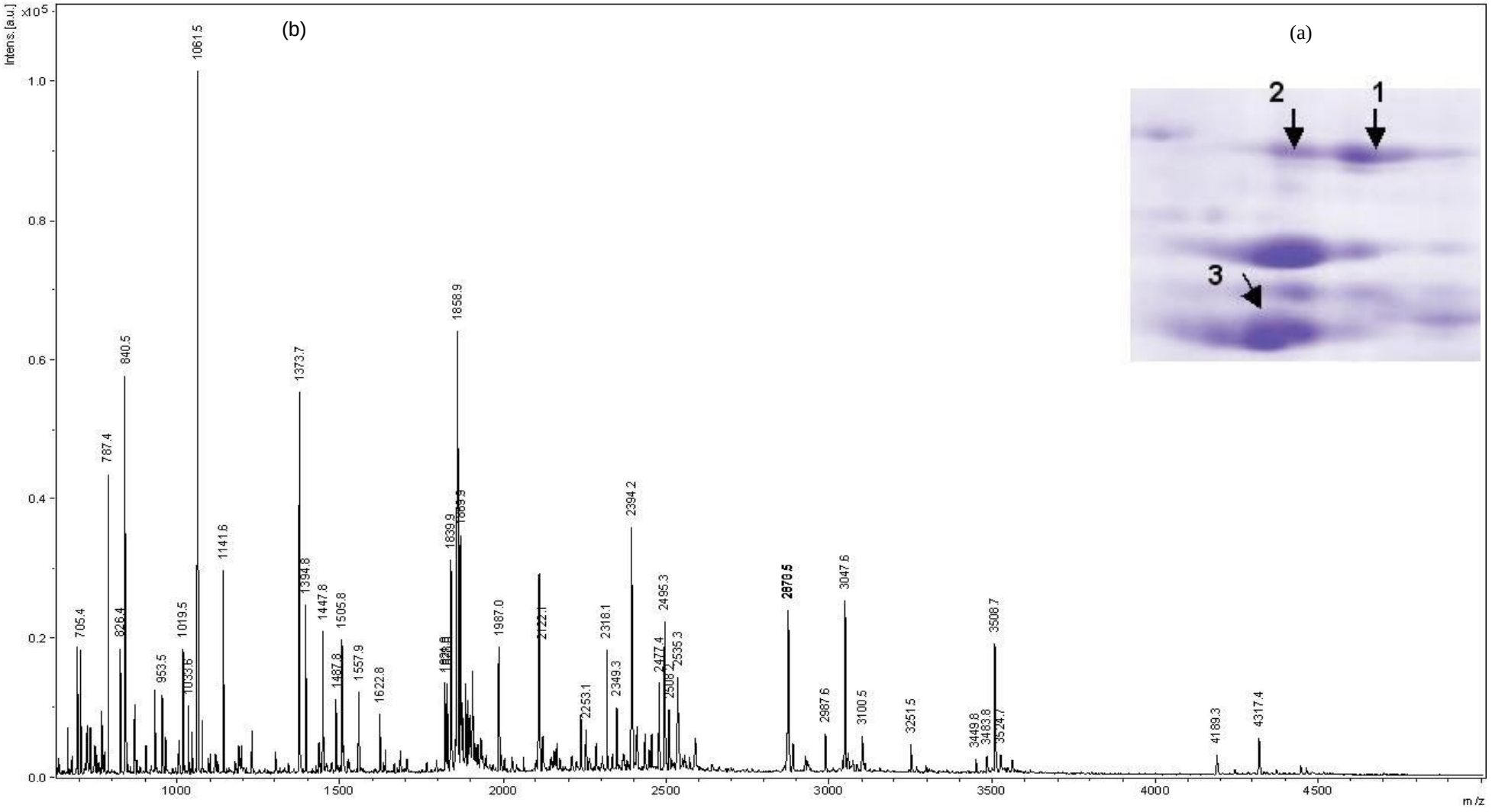



**Fig. 3.** The results of proteomic identification of chicken pyruvate kinase in the sample of "Lyubitelskaya" sausage: (a) fragment of two-dimensional electrophoregram with the identified fraction №1 and two reference fractions no. 2 (*Sus scrofa* pyruvate kinase) and no. 3 (*Sus scrofa* creatine kinase), (b) Mass spectrum of tryptic peptides obtained at MALDI-TOF MS identification of fraction № 1 as *Gallus gallus pyruvate kinase.*

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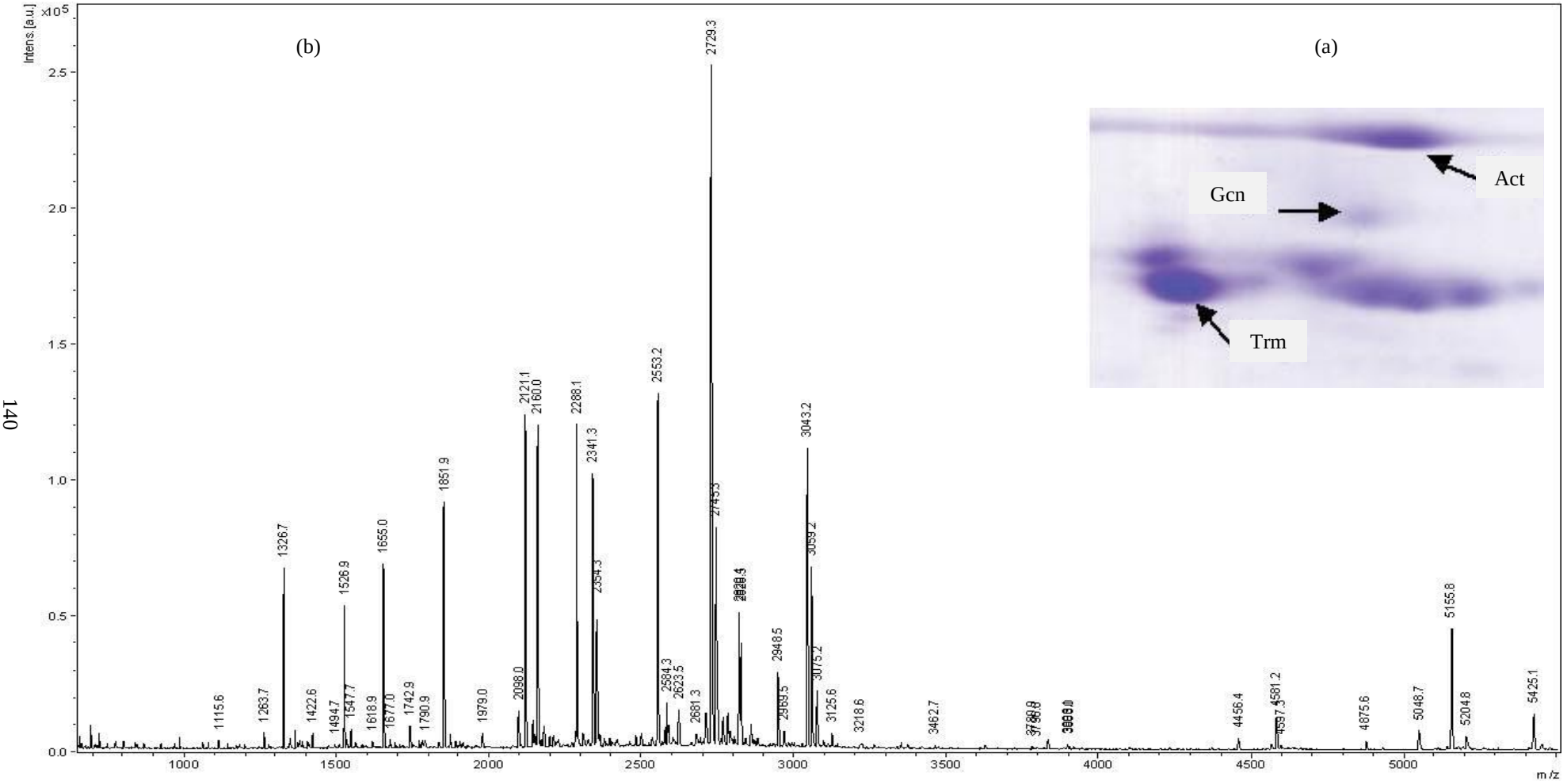


**Fig. 4.** The results of proteomic identification of soya protein glycinin (4249566 Protein NCBI) in the sample of "Lyubitelskaya" sausage: (a) fragment of two-dimensional electrophoregram with the identified fraction Gcn and two reference fractions Act (*Sus scrofa* actin) and Trm (*Sus scrofa* tropomyosin), (b) Mass spectrum of tryptic peptides obtained at MALDI-TOF MS identification of Gcn fraction as *Glycine max glycinin.*

Sum it up so far, it can be concluded that studies performed resulted in experimental validation of selected control methods for some proteolitic properties in the cooked sausage products using total biochemistry and proteomic techniques.

Example of identification and validation of some selected protein fractions by the time-of-flight mass spectroscopy followed by interpretation based on the protein molecule database (DB) available (Fig. 5).

In total, the results of proteomic analysis of the proteins contained in the muscle tissue of the Sus scrofa pig were used as the basis to form the new information module in the early created database "Proteomics of muscle organs" ("PMO" DB, http://mp.inbi.ras.ru) with the relevant title.

As it is seen from results shown in Table 1, the data on most identified proteins available in public databases are based on the relevant transcripts of analysis material only. Accordingly, the data of proteomic analysis of such proteins (marked with **** in Table 1) is then the vital conclusive proof of their availability obtained on the protein level. At the same time, some results of mass spectrometric identification (marked as ****** in the Table 1) of similar and individual proteins due to the lack of information required supplementary studies.

However, the identification results for a variety of main contractile proteins, such as α-tropomyosin, some myosin light chains, desmin and other happened to be highly valid and quite convincing.

Differences sometimes reported in standard and experimental values of Mm and pI might be associated with the calculation performed on the basis primary protein structure data that were extrapolated from information of the respective transcript. As the consequence, the post-synthetic modifications of the proposed protein were neglected, and in particular, extraction of the signal sequence. In individual cases, when the direct data on the same protein in another mammal species were available in public databases, the appropriate adjustment was possible. So, the data by the transcript (F1SGH5) only were provided for the pig protein "Mitochondrial beta sub-unit E1 of pyruvate dehydrogenase component" (no. 9 in Table 1) in the UniProt Database, while there were direct results of the male elk that evidenced on extraction of first 30 amino-acid residue off the sequence during the protein migration in the mitochondrion (P11966). Determination of standard values of Mm and pl for the fraction no. 9 subject to potential extraction of the same section of amine acide sequence resulted in 35.90 and 5.38 and these values were notably closer to experimental values (33.50/5.50) as compared with values initially measured by the transcript (39.00/6.20). Apparently, the differences in standard and experimental values of Mm sometimes reported on abnormal electrophoretic protein lability at SDS-electrophoresis or might result from "fragmentation" of the full-length protein product predicted by the relevant transcript (for example, for the fraction no. 24).

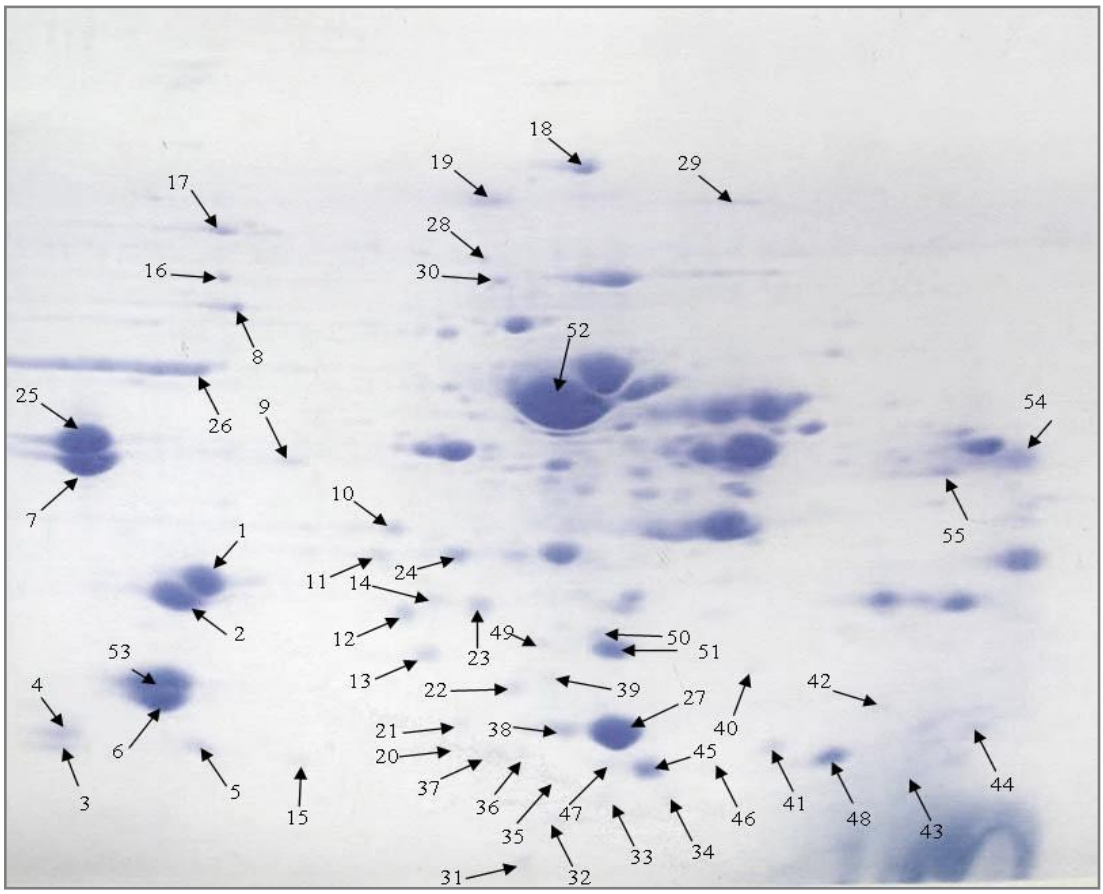


**Fig. 5.** Two-dimentional electrophoregram of proteins in the pig muscle tissue *Sus scrofa*. Arrows indicate the proteins identified. Identification results are given in the Table 1.

**Table 1**. *Beginning*. Proteins identified by mass spectrometric methods (MALDI-TOF MS and MS/MS) on two-dimensional electrophoregram of protein extracts from the pig muscle tissue (*Sus scrofa*)

| No. in the 2D electroph oregram | Name of protein, some synonyms, including the English name, (*gene symbol*), **description in database "PMO"** | Numbers in Protein NCBI and/or *UniProt* | Score/No. match peptides* | % coincidence** | Mm/pI (exp.)*** | Mm/pI (stand.)*** |
|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 1 | Myosin light chain 3, Myosin light chain 1, slow-twitch muscle B/ventricular isoform (*MYL3*), **MLC1s/v** | 332656187, 311268794**** | 204/20 | 81 | 22.0/5.24 | 21.8/5.00 |
| 2 | Myosin light chain, MLC1f, MLC1F/MLC3F (*MYL1*) **MLC1f** | 157427687, 117660874**** | 211(273)/12 | 93 | 21.0/4.90 | 21.0/4.90 |
| 3 | Light chain of myosin 1/3, sceleto-muscular shortcutelectrophoretic isoform, **MYL1 variant 3** (*MYL1*) **MLC3f-ei** | 157427687 / **A1XQT8** | 136/18 | 78 | 16.5/4.63 | 16.7/4.63 |
| 4 | Light chain myosin 1/3, sceleto-muscular shortcut isoform, **MYL1 variant 3** (*MYL1*) **MLC3f** | 157427687 / **A1XQT8** | 268/32 | 92 | 16.8/4.63 | 16.7/4.63 |
| 5 | Cytochrome C oxidase Va isoform 1-like, mitochondrial (***COX5A********) **COX5A-l** | 350586831**** | 145/15 | 62 | 16.7/5.15 | 16.7/6.42 |
| 6 | Myosin regulatory light chain 2, skeletal muscle isoform MLC2B (*MYLPF*) **MLC2** | 54607195**** | 269/50 | 95 | 19.0/4.89 | 19.0/4.89 |
| 7 | Tropomyosin isoform alpha 1, α-tropomyosin (*TPM1*) **TPM1** | 148222268 / **P42639** | 155/24 | 58 | 33.5/4.71 | 32.7/4.71 |
| 8 | Desmin (*DES*) **DES** | 2959454 / **P02540** | 375/43 | 90 | 53.0/5.25 | 52.6/5.21 |
| 9 | Pyruvate dehydrogenase E1 component subunit beta, mitochondrial (*PDHB*) **PDHB** | 346986351 / **F1SGH5****** | 257/29 | 60 | 33.5/5.50 | 39.2/6.20 (35.9/5.38) ****** |
| 10 | Light chain myosin 6B, PREDICTED: myosin light chain 6B [Sus scrofa] (MYL6B) MYL6B | 194037529**** | 376/40 | 98 | 24.0/5.90 | 24.0/5.53 |
| 11 | Heat-shock protein HSP27, beta 1 isoform, (HSP27) HSPB1 | 55926209 / Q5S1U1 | 470/20 | 98 | 23.0/5.85 | 23.0/6.23 |
| 12 | Subunit d of mitochondrial F0 complex of ATP synthase (ATP5H) ATP5H | 347658971**** | 639/35 | 97 | 18.5/6.00 | 18.5/5.99 |
| 13 | Hypothetic protein containing the barred domain, gene product of locus LOC494560, Hp-cry | 311257410**** | 371/12 | 77 | 19.0/6.05 | 17.4/5.35 |



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**Table 1**. *Continued*. Proteins identified by mass spectrometric methods (MALDI-TOF MS and MS/MS) on two-dimensional electrophoregram of protein extracts from the pig muscle tissue (*Sus scrofa*)

| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|
| 14 | Protein DJ-1 (*PARK7*) **DJ-1** | 118403904 / **Q0R678****** | 286/24 | 83 | 20.5/6.15 | 19.9/6.33 |
| 15 | Protein binding fatty acids 3, cardiac/muscle isoform (***FABP3***) **FABP3** | 374637318 / **O02772** | 145/12 | 59 | 15.0/5.50 | 14.7/6.11 |
| 16 | Chaperonin, heat-shock protein 60, HSP 60 mitochondrial (*HSPD1*) **HSPD1** | 359811347 / **F1SMZ7****** | 339/47 | 69 | 61.0/5.30 | 61.0/5.70 |
| 17 | Heat-shock protein 70 isoform 8, HSP70 (*HSPA8*) **HSPA8** | 345441750 / | 540/38 | 54 | 66.0/5.27 | 71.0/5.37 |
| 18 | Aconitase mitochondrial (***ACO2***) **ACO2** | 47522738 / **P16276** | 268/52 | 61 | 86.0/6.80 | 85.7/8.24 ****** |
| 19 | Serum transferrin, PREDICTED: serotransferrin isoform 1 (*TF*) **TF** | 350591529****/ **P09571** | 329/44 | 59 | 79.0/6.70 ****** | 78.8/7.57 |
| 20 | Fatty acid-binding protein, epidermal (*FABP5*) **FABP5** | 89886167 / **Q2EN74****** | 122/12 | 48 | 16.5/6.45 | 15.2/6.60 |
| 21 | Superoxide dismutase 1 (SOD1) **SOD1** | 15082144**** / **P04178** | 86/25 | 72 | 17.0/6.40 | 15.8/6.04 |
| 22 | Ubiquitin-conjugating enzyme E2 variant 2 [Sus scrofa] (*UBE2V2*)****** **UBE2V2** | 343432604 / **I3L6T2****** | 63/8 | 40 | 19.5/6.75 | 16.4/7.79 |
| 23 | PREDICTED: adenylate kinase isoenzyme 1 [Sus scrofa] (***AK1***) **AK1** | 350579688**** / **P00571** | 516/54 | 87 | 21.0/6.50 | 21.6/8.38 |
| 24 | PREDICTED: heat shock protein beta-1-like isoform 1 [Sus scrofa] ****** | 335284210**** | 213/19 | 83 | 23.0/ 6.40 | 29.7/9.51 ****** |
| 25 | Tropomyosin beta chain (*TPM2*) **TPM2** | 194018702 / **A1X899****** | 105/14 | 29 | 34.0/4.80 | 33.4/4.62 |
| 26 | Actin alfa skeletomuscular (***ACTA1***) **ACTA1** | **/ P68137** | 209/22 | 49 | 43.0/5.23 | 42.5/5.23 |
| 27 | Myoglobine (MB) **MB** | **47523546 / P02189** | 157/13 | 77 | 17.0/7.00 | 17.1/6.76 |
| 28 | PREDICTED: stress-induced-phosphoprotein 1-like [Sus scrofa] (STIP1), **STIP1-pig******** | **335281609****** | 191/35 | 56 | 63.0/6.50 | 62.4/6.36 |
| 29 | Alfa subunit of mitochondrial trifunctional enzyme (HADHA) **HADHA** | **47522754 / Q29554** | 175/14 | 21 | 80.0/8.70 | 79.7/9.17 |



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**Table 1**. *Continued.* Proteins identified by mass spectrometric methods (MALDI-TOF MS and MS/MS) on two-dimensional electrophoregram of protein extracts from the pig muscle tissue (*Sus scrofa*)

| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|
| 30 | Dihydrolipoyldehydrogenase plastosome (***DLD***), **DLD** | 47522940 / **P09623** | 235/30 | 50 | 60.0/6.60 | 50.2/6.31 |
| 31 | Ubiquitin ****** **UBIQ** | 229532****** | 339/24 | 97 | 8.5/6.65 | 8.4/6.56 |
| 32 | Acyl-CoA-binding protein, Diazepam-binding inhibitor (***DBI***) **DBI** | 47523046 / **P12026** | 165/6 | 52 | 10.0/6.75 | 9.9/7.88 |
| 33 | ATPase inhibitor, electrophoretic isoform (*ATPIF1*) **ATPIF1-ei** | 148222591 | 330/27 | 62 | 12.0/7.00 | 12.1/9.34 |
| 34 | ATPase inhibitor, plastosome. (*ATPIF1*) **ATPIF1** | 148222591 | 313/26 | 62 | 12.0/7.40 | 12.1/9.34 |
| 35 | PREDICTED: histidine triad nucleotide-binding protein 1-like [Sus scrofa] (*HINT1*) **HINT1-pig** | 311250094**** | 172/17 | 94 | 13.7/6.90 | 13.7/6.36 |
| 36 | Histidine triad nucleotide-binding protein 1-like (*HINT1*) **HINT1-ei** | 311250094**** | 172/17 | 94 | 14.0/6.60 | 13.7/6.36 |
| 37 | Cytochrome C oxidase subunit 5B mitochondrial precursor (*COX5B*) **COX5B** | 55926217 / **Q5S3G4** | 185/18 | 75 | 11.5/6.65 | 10.6/6.07 |
| 38 | Myoglobine, electrophoretic isoform (*MB*) **MB-ei** | 47523546 / **P02189** | 357/29 | 99 | 17.0/6.75 | 17.1/6.75 |
| 39 | **PREDICTED: NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 7-like [Sus scrofa]** ?****** | 350580438**** / | 122/12 | 43 | 20.0/6.80 | 12.0/7.71 |
| 40 | NADH dehydrogenase 1 α-subcomplex subunit 8 (*NDUFA8*) **NDUFA8** | 298104126 / **F1SLR1****** | 279/10 | 40 | 20.0/8.20 | 20.0/8.09 |
| 41 | Ubiquinol cytochrome c reductase binding protein (***UQCRB***) | 297747368 / **H0VKS2****** | 375/33 | 89 | 16.0/8.70 | 13.6/9.07 |
| 42 | PREDICTED:Subunit 4-like beta subcomplex NADH dehydrogenase (ubiquinone) (*NDUFB4*) **NDUFB4-pig** | 335310208**** / **I3LPW0****** | 227/15 | 69 | 20.4/9.00 | 15.1/9.66 |
| 43 | ATP synthase subunit e, mitochondrial (*ATP5I*) **ATP5I** | 148887343**** / **Q9MYT8.4** | 425/17 | 98 | 8.8/9.10 | 8.2/9.30 |
| 44 | PREDICTED: cytochrome c oxidase subunit 6A2, mitochondrial-like isoform 2 (***COX6A2***) **COX6A2** | 311251232**** / | 498/5 | 31 | 11.0/10.0 | 10.8/10.58 |
| 45 | Hemoglobin β-chain (***HBB***) **HBB** | 261245058 / **P02067** | 334/25 | 89 | 16.2/7.10 | 16.2/7.10 |
| 46 | **FKBPIA-like, FK506-binding protein (***FKBP1A***)** FKBP1A-pig | 83921635**** | 170/8 + 1 msms | 89 | 14.5/7.90 | 11.9/7.88 |



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**Table 1**. *Ending.* Proteins identified by mass spectrometric methods (MALDI-TOF MS and MS/MS) on two-dimensional electrophoregram of protein extracts from the pig muscle tissue (*Sus scrofa*)

| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|
| 47 | Hemoglobin β-chain electrophoretic isoform (***HBB***) **HBB-ei** | **/по P02067** | 157/18 | 87 | 16.5/6.95 | 16.2/7.10 |
| 48 | Hemoglobin α-chain (*HBA*) **HBA** | /by **P01965** | 324/27 | 80 | 15.5/8.76 | 15.0/8.76 |
| 49 | Crystallin αB, electrophoretic isoform (*CRYAB*) **CRYAB-ei** | 335294877 | 159/5 | 14 | 21.0/6.70 | 21.1/6.76 |
| 50 | PREDICTED: phosphatidylethanolamine-binding protein 1-like [Sus scrofa] (***PEBP1***)****** **PEBP1-pig** | 311270662**** / | 553/31 | 99 | 20,8/6,90 | 21.0/6.96 |
| 51 | αB-crystallin (*CRYAB*) **CRYAB** | 335294877**** / **Q7M2W6** | 260/19 | 86 | 20.6/6.95 | 20.1/6.76 |
| 52 | Creatine kinase, subunit M (***CKM***) **CKM** | 194018722 / **Q5XLD3** | 356/40 | 80 | 43.0/6.60 | 43.0/6.61 |
| 53 | Myosin, light chain myosin isoform 2V (*MLC2V*) **MLC2V** | 47523262 / **Q8MHY0** | 270/27 | 89 | 19.9/4.85 | 18.9/4.86 |
| 54 | Four and a half LIM domains 1 protein, isoform C (*FHL1C*), **FHL1C** | 47523806**** /****** | 175/22 | 57 | 34.0/10.50 ****** | 33.6/8.79 |
| 55 | Four and a half LIM domains 1 protein, isoform C (*FHL1C*), **FHL1C-ei** | 47523806**** /****** | 128/19 | 51 | 33.4/9.60 ****** | 33.6/8.79 |

*Notes*. * – Score / No. match peptides – characteristics widely used in the English publication for the mass spectrometry results (Score – suitability mark or "score record", No. match peptides - number of coincided peptides) Scores are given in view of MS/MS; ** – % coincidence of revealed mass tryptic peptides with the protein sequence; *** – Mm/pI – values of molecular masses (Mm) in kilodaltons and isoelectric points (pI); **** – Predicted by transcript; ***** – Defined by analogue with other relative genes of other mammals (human and/or bovine animals); ****** – Explanations are in the text above.



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Post-synthetic modifications might influence the results of *pl* determination. Thus, it was revealed in the work that the fraction no. 11 identified as "Heat-shock protein HSP27" is phosphorylated. This modification obviously produced the decreased pl value (5.83) obtained in the experiment as compared with the standard one (6.23). Still, it should be noted that in some other cases (for example, no. 18, no. 19), the decreased experimental pl values were apparently due to the fact that individual large proteins do not reach positions relevant to their real pl values when the method of isoelectric focusing is used.

Finally, it should be emphasized that, as said above, the results of mass spectrometric identification obtained for some fractions in two-dimensional electrophoregrams of the pig muscle proteins, were only based on information on nucleotide sequences of relevant transcripts (marked **** in the Table 1). In this case, electrophoretic characteristics measured in the experiment and theoretically calculated sometimes notably differentiated.

Hence, the performed proteomic analysis shown in this article of the pig muscle tissues resulted in identification of 55 muscle proteins, that included, among others, main muscle contraction members (myosins, actin, tropomyosins), enzymes of glycolysis and other metabolic processes (aldolase, dihydrolipoyldehydrogenase, NADH-dehydrogenase and other mitochondria enzymes), heat-shock proteins as well as the new protein (putative protein containing the barred domain, gene product from locus LOC494560) and several tissue-specific proteins - potential bio-markers (desmine, creatine kinase, myoglobine).

Thereat, the literary material and proprietary experimental data gave evidence that some of identified proteins are tissue-specific and may be considered as potential bio-markers. In particular, myosin light chains, desmine, creatine kinase, myoglobine and others may be classified as such proteins.

Nevertheless, in some cases considerable difficulties were faced to collect publications on the identified protein or the relevant transcript for the Sus scrofa species. For example, experimental data obtained for the fraction no. 31 had such characteristics.

Thus, the results shown in this work indicate that the compiled experimental and literature materials were useful to form the basis to develop the assay content test method for meat proteins in structureless meat products and, in particular, in cooked sausage products.

## CONCLUSION

Application of proteomic strategy in the study of molecular mechanisms to create quality parameters of the meat raw stock is essential to produce high-quality animal products and to stabilize the production process more efficiently.

The works performed resulted in formation of the consolidated methodological approach to determine the content of the muscle protein in structureless cooked products by two-dimensional electrophoresis followed by the time-of-flight mass spectrometry identification of convincing protein markers.

It can be concluded from studies performed that the use of proteomic technologies along with new data acquisition on muscle proteins allows creating new effective control methods of meat products including, in particular, determination of the content of muscle proteins and their specificity in end products.

The experimental data obtained will be also used to generate proteomic maps of proteins in native meat stock.

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