Strategy for the study of the proteome in animal muscle tissue

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Abstract. The existing approaches to the methodology of using proteomic and mass-selective methods for the analysis of the component composition of meat product proteins are systematized, formalized and modified. The scientific and practical foundations of a systemic proteomic strategy for identifying the protein composition of meat raw materials and the authenticity of meat products have been developed. A scheme for constructing proteomic maps and spectra of identified proteins and peptides of meat and meat products using bioinformatic data processing has been developed, and a scheme for choosing a research methodology as a tool for identifying and confirming the composition of meat products has been formed.

1. Introduction
One of the main problems of modern production of meat products is the quality of raw meat, which depends on various factors, including genetic components, conditions of transportation, production and processing. The most significant components of meat are proteins, the total content, structure and functional state of which is constantly changing as part of this complex biological system, with a large number of interacting components. Recently, there has been an intensification of research on the patterns and mechanisms of transformations of biomolecules, in particular proteins, under the influence of endogenous and exogenous factors. The science of meat is developing especially intensively in this direction. In particular, study is intensified on the influence of the protein phenotype, that is, the individual combination of external and internal factors on the change in protein expression and, as a result, the change in the composition and properties of animal tissues. Mammalian tissue samples usually contain from 10,000 to 30,000 different types of proteins, so it is necessary to use a wide range of methods in order to simultaneously prepare, separate and quantify the results of the synthesis of these thousands of proteins. The study of changes in proteins during autolysis, or under the influence of heat treatment, and the possibility of quantitative and qualitative determination of the composition of raw meat or finished meat product and its components determine the ways the acquired knowledge is practically used. These aspects, together with the confirmation of food safety, form the scientific basis for monitoring raw materials and food products, which is becoming an increasingly urgent task worldwide. Proteomics provides identification and quantitative determination of all proteins in the sample, and most importantly, monitoring of their changes.

Proteomics should become an effective tool for solving this problem. Proteomics is the main direction of functional genomics, within which it is possible to conduct a highly reliable analysis of meat products. With the help of this methodology, impressive results have already been obtained in almost all areas of biomedical research. Currently widely used methods of studying the proteome – two-dimensional electrophoresis with subsequent identification of the detected proteins by mass spectrometry and interpretation of data using bioinformatics, require refinement and adaptation for the study of animal tissues and meat products. All these methods have their
own individual advantages and limitations, and none of them is equally well suited for analyzing all the types of proteins present in a complex tissue sample. The complexity of adapting the methods to complex meat products is explained by (1) high-temperature heat treatment leading to protein denaturation; (2) the multicomponent composition of the meat product, in which muscle, milk, egg and vegetable proteins can be present simultaneously, while being from several species of animals, plants or poultry; (3) the need to identify proteins with one molecular weight or one pI.

Therefore, the use of bioanalytical methods for proteomic studies of meat product samples is an actual and modern approach. Such studies are conducted in order to establish the functional role of each of the identified proteins. Depending on the type of organism and the metabolic state of the cells, the proteome can contain from several thousand to hundreds of thousands of proteins. Many of them undergo gene expression, the nature of which is often impossible to predict [1].

One of the most promising areas of research on changes in proteins under the influence of technological processing is the identification, study and determination of the possibility of practical application of biomarkers of these changes.

It is obvious that over the past five years, the number of proteomic studies aimed at studying meat proteins has increased significantly, which opens up the possibility of their routine use in control and analytical laboratories engaged in the analysis of the quality and safety of meat products in the future. In the database of the V. M. Gorbatov Federal Research Center for Food Systems of the Russian Academy of Sciences, work was carried out to develop a scientific and practical model for identifying tissue- and species-specific substances of protein nature in meat products, on the basis of which tools were developed to confirm the authenticity of meat products and a product passport developed according to generally accepted regulatory documentation.

Of undoubted scientific interest is the potential: of using the proteomic strategy as a tool for studying the protein profile of objects of plant and animal origin; of identifying biomarkers of technological processes, for qualitative and quantitative identification of the composition of raw materials and finished meat and meat-growing products and; for studying the mechanisms of proteome changes for the directed formation of the specified characteristics of animal raw materials.

2. Materials and Methods

Research on the identification and identification of muscle proteins of meat raw materials and processed products included the study of the protein composition of muscle tissue of various types of animals and poultry and meat products produced from them. Experimental studies were carried out in accordance with the tasks set at the V. M. Gorbatov Food Systems Research Center of the Russian Academy of Sciences in the Research and Testing Center. Some stages of the work were performed on the basis of the FITC of Biotechnology of the Russian Academy of Sciences.

In accordance with the purpose of the work, the objects of research were:
- m. longissimus dorsi: pork, beef, horse meat, camel meat;
- pectoral muscles of poultry (chicken, turkey);
- model minced meat from m. longissimus dorsi animals and poultry pectoral muscles in a 1:1 combination;

Experimental batches of meat products were manufactured in industrial conditions of the meat processing plant, in accordance with GOST R 52196-2011 and GOST 33673-2015, and also were taken from the retail network (various manufacturers), including those developed according to technical specifications.

When performing the work, a set of generally accepted, standard and modified proteomic methods was used, including descriptive, analytical, inductive and deductive methods in general domestic and foreign literature, which were used to ascertain the existing methodologies for identifying tissue- and species-specific substances of protein-peptide nature in meat products.
The research was based on an extraction-fractional approach to conducting proteomic studies, with further mass spectrometric identification using the bottom-up methodology and bioinformatic interpretation of the results obtained, characteristic of meat products [2-6].

The identification of protein fractions on two-dimensional electrophoregrams (2DE) was carried out after trypsinolysis by MALDI-TOF MS and MS/MS mass spectrometry on a MALDI-time-of-flight Ultraflex mass spectrometer (Bruker, Germany) in the mass range of 500-8000 Da. The analysis of the obtained mass spectra of tryptic peptides was performed using the Mascot program (Matrix Science, USA), with an accuracy of determining the mass of MN+ equal to 0.01%, searching the databases of the National Center for Biotechnological Information of the USA (NCBI) [7]. Individual proteins and peptides were determined by manual processing by comparing amino acid sequences during protein sequencing. In the comparative analysis of the proteomic profiles of the presented samples, the information modules “Cow skeletal muscle proteins (Bos taurus)”, “Pig skeletal muscle proteins (Sus scrofa)”, “Horse skeletal muscle proteins (Equus caballus)” and “Camel skeletal muscle proteins (Camelus bactrianus)” of the multilevel database “Proteomics of muscle organs” were used (http://mp.inbi.ras.ru). The poultry were additionally identified using NCBI databases.

3. Results and Discussion

As a result of the research conducted to modify classical methodologies for fractionation and identification of the composition of muscle proteins from farm animals and poultry, special approaches were developed [8-10]. They reflect the use of proteomic methods for the analysis of muscle proteins in the study of meat raw materials, and the identification and identification of tissue- and species-specific substances of protein nature in muscle tissue, which laid the scientific and practical foundations to create a systematic proteomic strategy for identifying the protein composition of meat products. The hierarchical scheme of the practical implementation of the strategy for studying the muscle proteome of farm animals and poultry is shown in Figure 1.
Figure 1. The hierarchical scheme showing practical implementation of the research strategy for the muscle proteome

The construction of proteomic maps of proteins in raw materials and meat products is based on the complex application of individual monomethodologies such as:
- electrophoresis (1D, 2D) – obtaining a protein pattern from a sample
- time-of-flight mass spectrometry – obtaining the spectrum of detected proteins and peptides from a sample;
- bioinformatic data processing – comparison of the amino acid sequence of proteins with the database of decoded genomes of similar species and identification by the corresponding transcripts (in the absence of information in the database), to identify each protein from the resulting pattern;
- analysis and archiving of the received information.

Bioinformatic data processing was used not only as an evidence base for the identification of proteins and peptides from samples, but also for the quantitative identification of the identified fractions.

The bioinformatic interpretation of the results obtained made it possible to formulate and significantly expand approaches to the identification and quantitative determination of protein markers that signify the quality, functionality and safety of meat raw materials (detection of falsification, determination of the presence of allergens) in finished meat products.

According to the obtained data, the information is systematized using bioinformatics methods, which is successfully integrated into the proteomic strategy used in the system of multi-level control of the origin of raw materials in meat products. It seemed interesting to form an assessment system for the control of the composition of meat products, aimed at identifying cases of violation
of established recipes by a set of methods for a two-level system of screening and arbitration. Figure 2 shows a multi-level system for the selection of methods previously evaluated for the identification of the composition of meat products.

Figure 2. A two-level control system for the composition of meat products

Thus, a review of existing methodological approaches and their experimental confirmation revealed the absence of one specific method that would solve such an urgent problem as the quantitative determination of undeclared components in meat products. In accordance with the prospects for inclusion in the system of multi-level control of the composition of meat products, ICA and/or ELISA (inexpensive methods, with a high level of reliability) is recommended as a screening method, and LC-MS as an arbitration (confirming) identification method (p <0.05). When analyzing the complementarity of the considered methods, it is worth using two or three methods together:
- IFA/IHA: within the framework of production control, in the context of rapid decision-making;
- IFA/MS: within the framework of confirmatory (arbitration) control, as the most highly reliable method;
- Identification of the ICA/2D/MS biomarker: within the framework of confirmatory (contradictory) control, as the most indicative confirmation.

4. Conclusion
Modern analytical technologies based on a systematic approach to analysis are required to study interspecific and intraspecific features of meat proteins and their transformation during maturation and technological processing. Proteomics opens up wide opportunities in this direction as a methodology for studying proteins in a certain system and at a certain time, which allows identification of complex patterns between the state of proteins, and the functional and technological properties of raw materials and processing methods. Proteomics also enables
development of accurate analytical methods for searching for biomarkers and identifying unfair practices. Therefore, the introduction of proteomics should be considered as an important step towards achieving higher quality of raw materials and sustainable production development.

As a result of the conducted research, a comprehensive approach has been developed to identify species- and tissue-specific substances of a protein nature when controlling a meat product. The proposed proteomic strategy in the study of the introduction of qualitative indicators in meat raw materials will combine the identification of the protein composition of meat products with a scheme for the identification and determination of the composition of protein and non-protein components in products, as well as additives of vegetable or animal origin.

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