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DETERMINATION THE QUALITY OF MEAT, MANUFACTURED MEAT, AND MEAT PRODUCTS VIA THE HISTOLOGICAL METHOD

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ABSTRACT

Microstructural analysis methods allow not only assessing the quality but also determining the quantitative ratio of the ingredients of meat products. With the histological examination, it is possible to estimate the state of the starting material. Changes in the native morphology manifest defrosted raw materials or those subjected to autolytic processes. Improving the methods of histological studies to assess the quality and identify undeclared components (falsifications) in meat products is still relevant today. The material for the study was meat products: ripened and chilled poultry and cattle (hip) meat, semi-finished products (chicken and ground beef), finished meat products (sausage products). Studying the microstructure of beef samples found the good structuredness of muscle fibers. The endomysium has well developed, which is especially clearly seen in the cross-section. On the periphery of the muscle fibers under the sarcolemma found oval-elongated nuclei. During the histological examination of minced meat and sausages, especially when staining according to the Perls Van Gieson method, fragments of fatty and connective tissues are revealed. The developed method opens up vast opportunities for use in quality control and detection of falsifications of raw meat and meat products.

Disciplinary: Veterinary Science, Biology, Histology, Bioscience.

1 INTRODUCTION

The variety of meat products, both domestic and foreign production, on Russian food markets and the whole world continuously increases. It includes animal origin products of low, medium, and high price categories but monitoring the quality of these products mainly aimed at safety for

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consumers and rarely about product composition (Tokarev et al., 2019).

Depending on features of meat autolysis after slaughter, raw meat classifies by next type: PSE (with low final pH), DFD (with high final pH), and RSE (deceiving meat, change in the genotype of animals). Such deviations may arise from hormonal preparations during intensive fattening and violations of pre-slaughter holding technology and cattle slaughter (Orlova et al. 2020).

Histological examination is one of the methods that allows identifying constituent components of meat products, including the degree of autolytic processes in muscle fiber. Microstructural analysis techniques can be essential tools for evaluating meat quality and detecting counterfeit meat products. Using histological methods can give qualitative assessment of the animal and plant components that include into a product, as well as their percentage (Pchelkina 2016; İnce et al. 2018; Malakauskienėet al., 2016; Migaldi et al. 2016).

Application these methods are vast: from assessment meat of mechanically deboned (including poultry) to the whole assortment of semi-finished and finished meat products. The disadvantages are analysis duration up to 36-48 hours. If source material has already been subjected to mechanical and sometimes thermal effects (minced meat, semi-finished meat products, sausage, and ham-piece products, etc.), the identification process has complicated (Mokhtar et al. 2018; İnce et al, 2018; Harem et al. 2018).

The essential condition to obtain reliable results is competent sampling, the correct choice of preparation method, and unique staining methods for the test sample.

Currently, to detect undeclared components have been using a PCR reaction. Using multiplex PCR to identification the meat of several species of animals simultaneously reduces the labor intensity and cost of laboratory research (Nsubuga et al. 2004; Kalyuzhnaya et al. 2019).

However, even though in recent years, this method becomes widespread and available, it has some drawbacks. In particular, in a qualitative analysis without using quantitative standards, are detecting traces of undeclared components, which gives false-positive results.

Microstructural analysis methods make it possible to qualitatively and quantitatively determined the ingredients of meat products.

Also, thanks to histological examination, it is possible to determine the state of starting material, including defrosted raw materials or those subjected to autolytic processes, which is manifested by a change in the native morphology.

Improvement of the methods of histological research for assessing the quality of raw materials and products, as well as the identification of undeclared components (falsifications) in meat products, is still relevant today.

2 MATERIALS AND METHODS

The study was at the laboratory of the Department of Biology, Ecology, Histology of the St. Petersburg State University of Veterinary Medicine. The study material was meat products: chilled poultry and cattle (hip part), semi-finished products (chicken and ground beef), finished meat products (sausage products). Sampling was carried out by GOST (2019) based on histological

examination. The manufacture and study of histological preparations were mainly carried out by the current interstate standards (GOST, 2013; 2014) using new reagents.

3 THE HISTOLOGICAL EXAMINATION

The histological examination by the classical method: samples of 1.5x1.5x0.4cm fixed in 10% neutral formalin within 24-48 hours. Fixed samples have been subjected to dehydration in several stages by placing them in a solution of isopropyl alcohol with nonionic surfactants. For compaction and blocks production, the material has been embedding by paraffin Histomix. Each sample has been obtaining histological sections with a thickness of 3-3.5μm on a Rotmik-2 rotary microtome. Subsequently, they have been fixing on glass slides and stained.

3.1 MICROSTRUCTURAL STUDIES

Microstructural studies have been using hematoxylin and eosin, which has allowed identifying the primary tissue and cellular structural features. Staining steps: dewaxing of sections in xylene (2 times for 4 minutes), rehydration in 96% ethyl alcohol (2 times for 4 minutes), washing in distilled water (5-7 minutes), stained with Mayer's hematoxylin (5 minutes), rinsing the sections in running water, staining with an aqueous solution of eosin (5 minutes), rinse in running water, dehydration in 96% ethyl alcohol (2 times for 4 minutes each), clarification of the sections in xylene (2 times for 4 minutes each), the conclusion of the parts under the coverslip using a mounting medium.

3.2 COLLAGEN FIBERS DIFFERENTIATION

Differentiation of collagen fibers and other components of the connective tissue, the staining method was used according to the Perls Van Gieson method (solution of potassium ferrocyanide and picrofuchsin, according to Van Gieson).

Method description: dewaxing of sections in xylene (2 times for 4 minutes), rehydration in 96% ethyl alcohol (2 times for 4 minutes), rinsing in distilled water (5-7 minutes), then placing the sections in a solution of potassium ferrocyanide (20 minutes), then thoroughly rinsing in distilled water, applying picrofuchsin on samples according to Van Gieson (10 minutes), again rinsing in distilled water, rehydrating in ethyl alcohol of increasing concentration, clarifying the sections in xylene and putting under cover glass using mounting environment.

The study of histological preparations and their photographing carried out on a light microscope LOMO MIKMED-5 using a camera LOMO MS-3.

4 RESULTS AND DISCUSSION

As a result of research has been found that muscle tissue forms the basis of most meat products and is contained in sufficient quantities. Striated muscle tissue, in combination with connective tissue, fatty tissue, bone tissue, nerve fibers, blood, and lymphatic vessels, forms the basis of meat.

Studying the microstructure of beef samples found the good structuredness of muscle fibers (see Figure 1). The endomysium has sufficiently developed, which has especially clearly seen in the cross-section (see Figure 1C). Along the periphery of muscle fibers under the sarcolemma found the oval-elongated nuclei.

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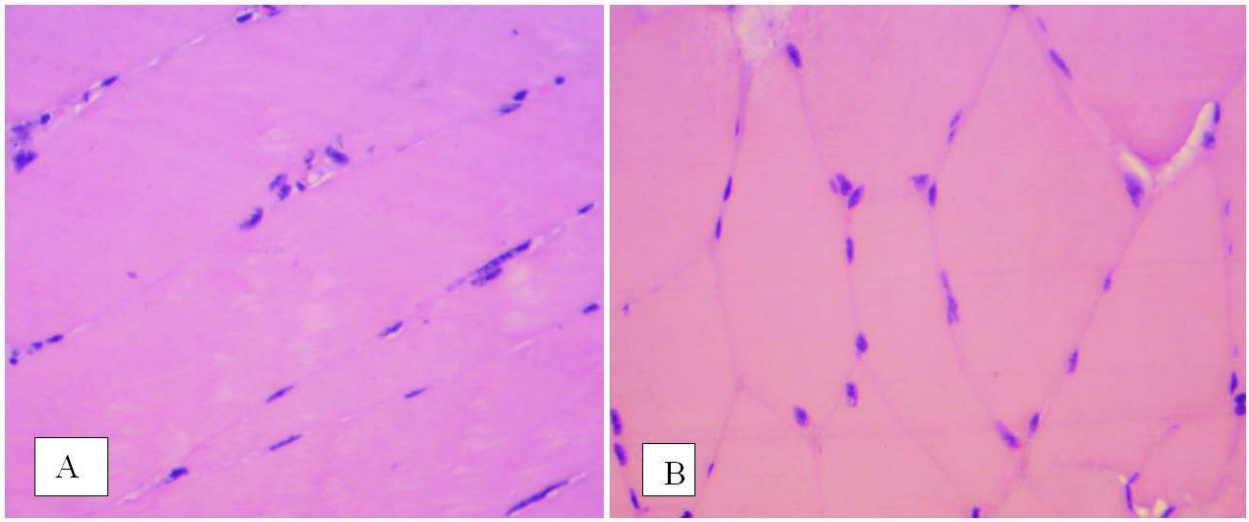

Figure 1: (A) Longitudinal and (B) transverse sections of skeletal muscle tissue (beef) (stain with hematoxylin and eosin; magnification x600).

The skeletal muscle tissue of birds has a similar structure (Figure 2). However, as expected, the connective tissue layer between the first-order muscle bundles is very poorly developed, and the endomysium is hardly visible. Also, in the muscle fibers of birds, the nuclei are located not only along the periphery but also have a central location, which seen when studying a histological specimen in a longitudinal section (see Figure 2A).

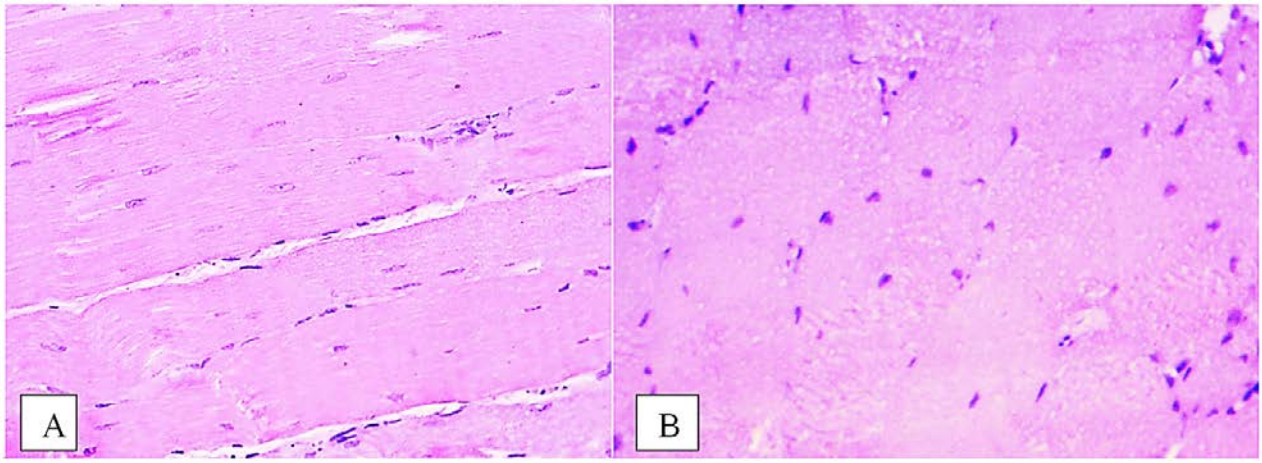

Figure 2: Chicken muscle tissue. (A) Longitudinal and (B) cross-sections (stain with hematoxylin and eosin; magnification x400).

Also, we have researched meat purchased in retail stores. When chilled beef is histologically examined, it is determined that it had previously frozen. Ice crystals appear on freezing and storage. They have located both between muscle fibers and within them. Their presence leads to varying degrees of sarcolemma destruction, which makes it possible to determine the quality and technological properties of raw meat. In some cases, defrosted meat retains muscle tissue defects resulting from the formation of ice crystals, which makes it possible to differentiate the raw meat that has been frozen and defrosted (see Figure 3).

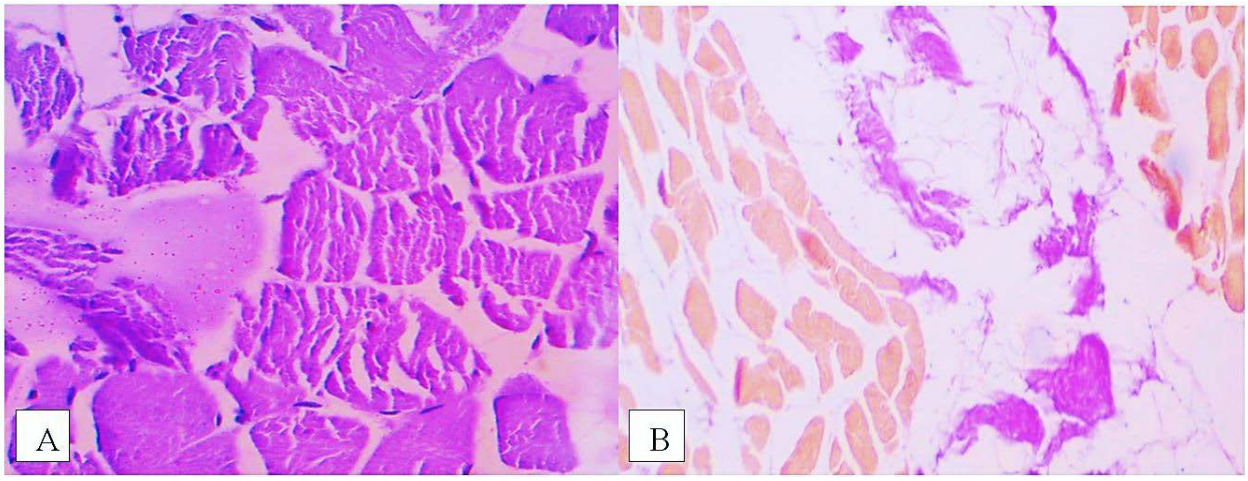

Figure 3: Frozen meat (beef). Oblique cut. (A) stain with hematoxylin and eosin and (B) stain according to Perls Van Gieson (magnification x400).

The histological examination of semi-smoked sausage mainly reveals fragments of fatty and connective tissues. The connective tissue, especially brightly detected by stained according to Perls Van Gieson. Elements of muscle tissue had been found in smaller quantities, mostly small in size with moderate destruction since, by the process of technological processing of sausages, there is a mandatory violation of the integrity of muscle structures. At the same time, there is a swelling of muscle fibers, fragmentation, and partial homogenization during the disintegration of nuclear structures and contractile fibrillar protein complexes (see Figure 4).

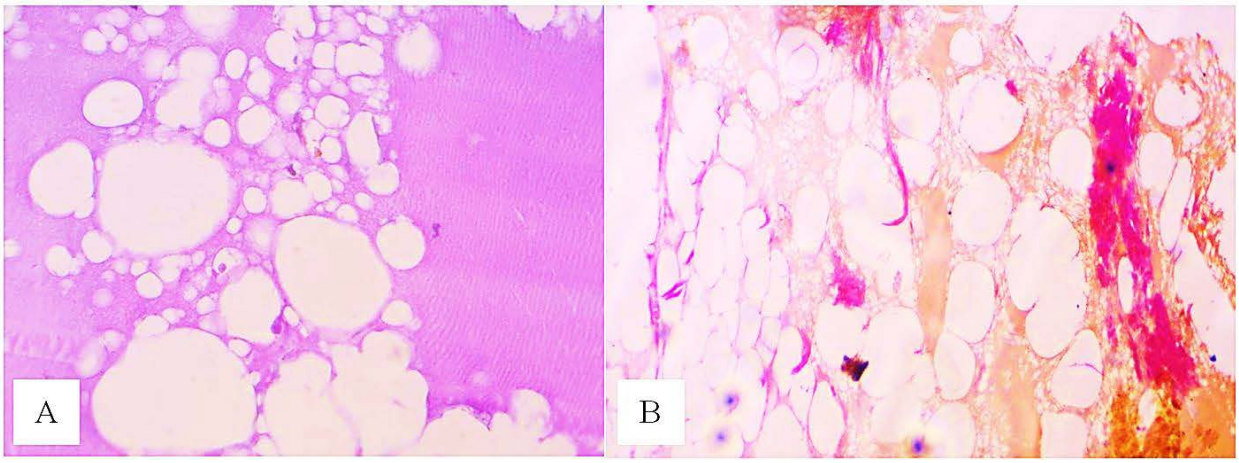

Figure 4: Semi-smoked sausage, (A) Longitudinal section, (B) stain with hematoxylin and eosin and stain according to Perls Van Gieson (magnification x400).

By the histological examination of minced meat, had observed the different orientation of muscle fibers (transverse, oblique, and longitudinal), which is due to the process of grinding the raw material (see Figure 5).

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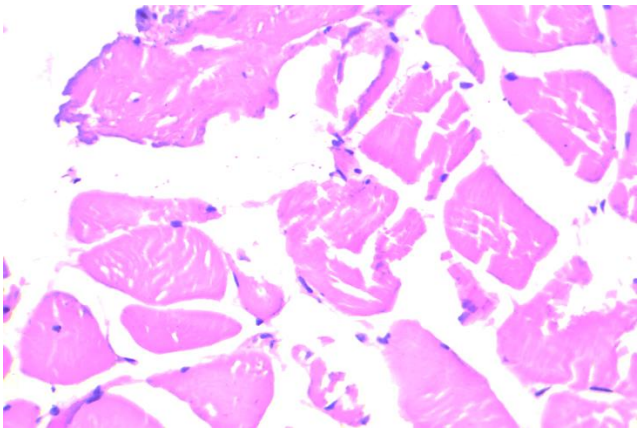

Figure 5: Microstructure of minced beef.
(Stain with hematoxylin and eosin; magnification x400).

At the same time, the process of mechanical meat processing leads to a decrease in its porosity in comparison with an initial state of meat raw materials. On transverse sections, the shape of the muscle fibers is polygonal with little roundness. On longitudinal sections in the muscle fibers revealed a distinct transverse striation, although, in some areas, it has partially replaced by a longitudinal one. Against the background of the typical linear shape of muscle fibers, one can also find the predominant wavy textures deformed during the technological impact in the manufacture of minced meat. Part of the muscle fibers fragmented into segments of different lengths.

The border is distinguishable between individual muscle fibers, represented by endomysium, and two sarcolemmas of adjacent muscle fibers. The nuclei of the cells are elongated-oval with fairly distinguished chromatin.

A completely different histological picture observed when analyzing the results of light microscopy of minced meat from chicken meat. Studies have shown that it consists mainly of fragments of muscle tissue, including muscle fibers, muscle bundles of the first and second orders, as well as the connective tissue stroma, which unites them into single whole elements.

The semi-finished products also contain a small number of crushed fragments of fatty tissue, revealed in the form of individual lipocytes and their small groups. The general architectonics of fatty tissue preserved, its microstructure characterized by moderate signs of destruction as a result of technological processing.

5 CONCLUSION

Thus, histological methods can widely be using in quality control of various meat products. This method offers a sovereign or additional research to identify both counterfeit meat products and to evaluate the quality of raw materials. However, when carrying out microstructural analysis, it is necessary to take into account the type of meat products under study, as well as their constituent components. The methods of histological examination make it possible to control the quality of meat products effectively. Using this method allows us to determine the type and condition of meat raw materials and the presence of various technological additives of plant origin due to the peculiarities of the structure of the arrangement of nuclei in muscle fibers.

6 AVAILABILITY OF DATA AND MATERIAL

Data can be made available by contacting the corresponding authors

7 AVAILABILITY OF DATA AND MATERIAL

The corresponding author will be liable to provide information regarding this paper.

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