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Using Histological Analysis to Detect Mincemeat Falsification

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ABSTRACT

The article considers the problem of the quality and falsification of semi-finished meat products in the Russian Federation. Samples of artificially falsified minced meat controlled by intact ground beef used as material for analysis. Shredded liver, kidneys, lungs, udder, diaphragm are introduced into the test samples. The tests were carried out by the histological method, according to GOST 19496-2013. By using histological analysis of minced striated muscles obtained images showed striation characteristic of skeletal musculature. In the cytoplasm of individual myocytes, we found that amount of Sarcocystis was relatively high. Comparative analysis mincemeat with minced skeletal and diaphragmatic muscles revealed that it was hard to detect as falsification. However, it's possible given some morphological differences between skeletal and diaphragmatic muscle fibers. In the process of microscopy of minced meat samples, without falsified by-products, inclusions of the corresponding tissues are easily visualized. As a result, we can conclude that histological analysis is a reasonably reliable way to determine the composition of chopped meat products. Unfortunately, the current standard is the mandatory histological identification of semi-finished products only if there is a disagreement on the structure of the raw material. That is, the problem of falsification lies not only in the dishonesty of producers but also in the presence of regulatory requirements governing the requirements for meat and meat products.

Key words: Convenience foods, Edible co-products, Meat quality, Myocytes, Sarcocystis, Microscopy

INTRODUCTION

The falsification and low quality of meat products is a global problem in various regions of the Russian Federation and the world (Kozlova 2012; Gokulakrishnan *et al*., 2015; Lisitsyn *et al*., 2016). Reduction of the number of cattle and a reduction of beef production (Khvyla *et al*., 2010; Yurchak *et al*., 2016; Boukili *et al*., 2019; Nkosi *et al*., 2020). Financial difficulties experienced by livestock farms. Reorganization of large meat processing enterprises and the establishment of private enterprises of lower capacity. Restriction of import products - these and other factors lead to a decrease in the quality of raw materials, semi-finished products, meat, and meat products.

With all the variety of meat products, in our opinion, the most relevant is the study of the quality of minced meat. These meat products are in high demand among consumers and serve as raw materials for the manufacture of semi-finished meat products and finished culinary meat products, which in turn are the favorite foods of the population (Bhat *et al*., 2016; Bansal *et al*., 2017). It knew that high-quality falsification is carried out by replacing the main components and introducing various ingredients into the product that are not provided for by the recipe (Lisitsyn *et al*., 2014; Bhat *et al*., 2016; Yacoub and Sadek, 2017). As the analysis of publications on the topic of falsification showed, the question of introducing plant materials, food additives, and cheaper meat of slaughtered (and not only) animals into meat products has been most thoroughly studied (Kumar *et al*., 2015; Razzak *et al*., 2015; Tibola *et al*., 2018; Tulyakova and Shibanova, 2019). For example, starch, gums, vegetable gelling agents, flour can find in the composition of the tested products. Previous research showed the most of the popularity of unconventional raw materials using in cooked sausages was carrageenan, and in dumplings was soy protein products (Ali *et al*., 2015). Svechnikova *et al*. (2020) focused on the qualitative falsification of minced meat with soy isolates and suggest using the ELISA expert test to detect this type of forgery (Rahman *et al*., 2014; Arnautov, 2016). Undoubtedly, ELISA diagnostics is expressive and less labor-intensive compared to the histological method for detecting soy protein in meat products. Recommended using a BIC analysis to identify and prevent cases of falsification at enterprises. Using it can control the composition of sophisticated food additives. The PCR method is most famous worldwide for detecting fraud (Gorlov *et al*., 2009; Gushchin 2017; Hossain *et al*., 2019).

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The histological analysis allows to determine the presence of unconventional raw materials in products from mincemeat, and prove the fact of falsification. It is essential by certification of fresh meat, semi-finished products, and finished culinary products (Berlingieri *et al*., 2007; Bragina *et al*., 2013; Lisitsyn *et al*., 2014). Besides, when analyzing histological sections, the researcher can determine the presence of by-products in the culinary meat product, assess the quality of the feedstock by the structure of muscle fibers, the distribution, and color of the nuclei, differentiate the muscles, establish storage conditions for the products. In this regard, we consider it relevant to perform a histological examination of artificially falsified by-products of minced meat.

MATERIALS AND METHODS

This study was conducted at the Perm Agrarian and Technological University during 2019. As a research material used samples of artificially falsified mincemeat, intact ground beef served as a control and test samples with minced liver, kidneys, lungs, udder, and diaphragm.

In work, we used the histological method of research, according to GOST 19496-2013. Pieces took for examination (1.5×1.5 cm in size) placed in plastic cassettes for cutting with dimensions of 2.8×4.0×0.5 cm. The tissue sections in the cartridge poured with 10% neutral buffered formalin (pH 7.0-7.2). Then, the material carried out on alcohols of increasing strength for dehydration and tissue densification using a histoprocessor - a LEICA TP 1020 automatic machine (Leica Biosystems Nussloch GmbH, Germany) with a given wiring cycle of 18 hours.

Pieces were poured into highly pure paraffin (histomix medium) with a melting point of 56 ° C. For this, use the Thermo scientific Histostar apparatus (Thermo Fisher Scientific, USA). Sections of 2-3 microns thick made from the obtained paraffin blocks on a Microm HM 325 microtome semi-automatic machine (MICROM International GmbH, Germany). Microscopy of histological preparations carried out on a MT 5300 optical microscope (Meiji Techno, Japan). All samples had examined in five replicates. Statistica analyzed all data.

RESULTS AND DISCUSSION

As a result of the microscopy of the control samples of mincemeat, it determined that skeletal muscle prevailed in the product. Muscle fibers are in the form of fragments, in some places preserved in structure without clearly distinguishable striation. Figure 1a shows that some cells often deformed in waves. The nuclei were located in the peripheral parts of the cells, flattened, with a hyperchromic hue.

In other fields of view, the cells deprived of nuclei, the thickness of muscle fibers increased, the cytoplasm is colored unevenly, with alternating pale zones and hyper-eosinophilic sites (Fig. 1b).

Sarcocystis located in the cytoplasm of individual myocytes (Fig. 2). The occurrence of these protozoa in the minced meat samples under study turned out to be relatively high.

The intermuscular stroma was edematous, fibrous structures, fragments of vascular walls, and fat cells traced in it (Fig. 3).

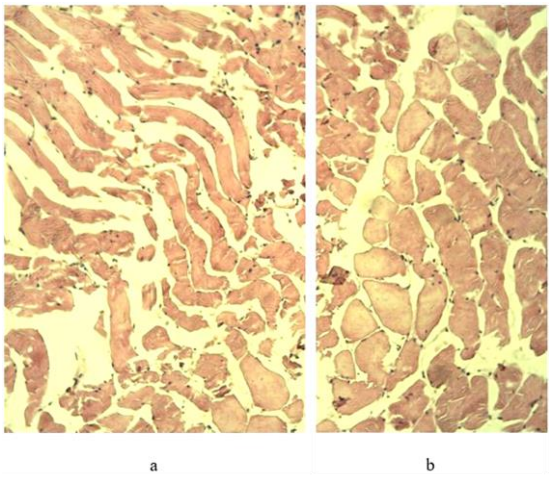

Fig. 1: Skeletal muscle microscopy. x100. Hematoxylin and Eosin (H & E) stain

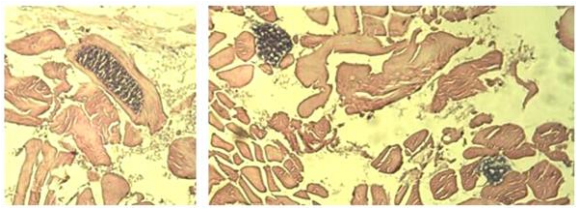

Fig. 2: A parasite of the genus Sarcocystis in cattle myocyte. Increase: about. x10, approx. x10. H & E stain

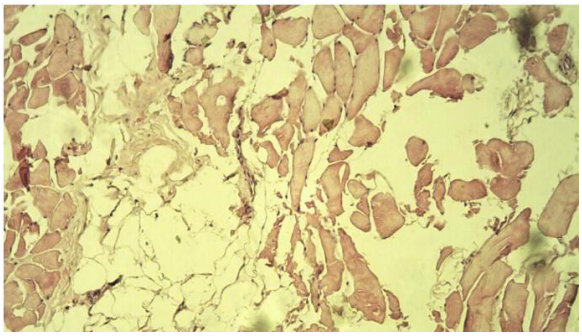

Fig. 3: Minced meat microscopy (beef). Skeletal muscle and fat cells. x100. H & E stain

It knew that the diaphragm belongs to the food by-products of the first category, and, consequently, the cost of this raw material is significantly lower than the meat of 1, 2, and 3 grades. Unscrupulous manufacturers can use a significant difference in the cost of meat of the diaphragm and skeletal muscles for high-quality falsification of minced meat and minced meat semi-finished products. Our research we have no apparent histological differences between the diaphragmatic and skeletal muscles, as follows from Fig. 4.

The muscle fibers of the diaphragm in the preparation located in the form of fragments of longitudinal and transverse sections of different thicknesses. The characteristic structure of muscle fiber preserved in places without clearly distinct striation. On the longitudinal sections, it is noticeable that part of the myocytes, as well as in the minced meat prepared from skeletal muscles, are wave-like deformed. The nuclei are located in the peripheral parts of the cells, flattened, with a hyperchromic hue.

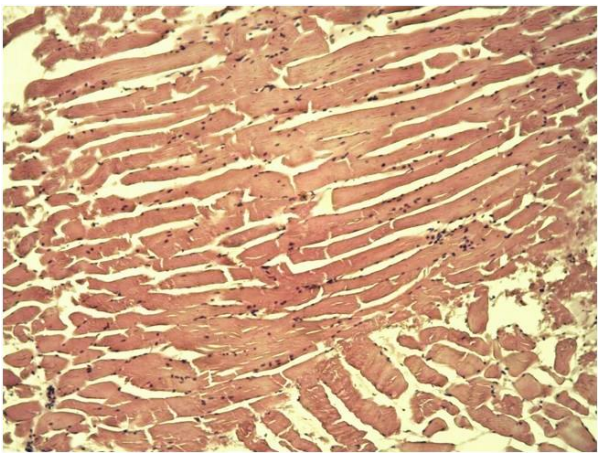

Fig. 4: Microscopy of muscle fibers of the diaphragm. x100. H & E stain.

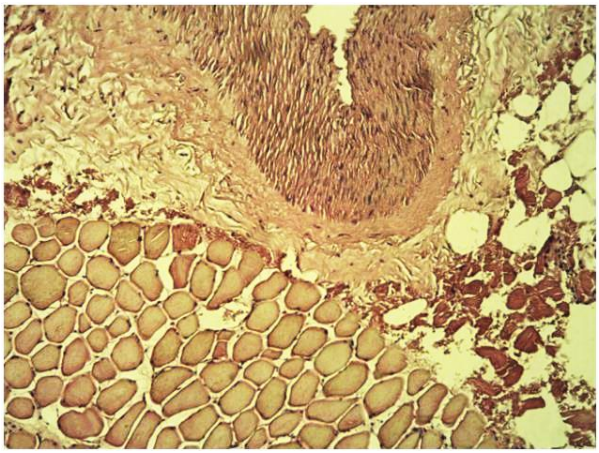

Fig. 5: Microscopy of muscle fibers and the tendon center of the diaphragm. x100. H & E stain.

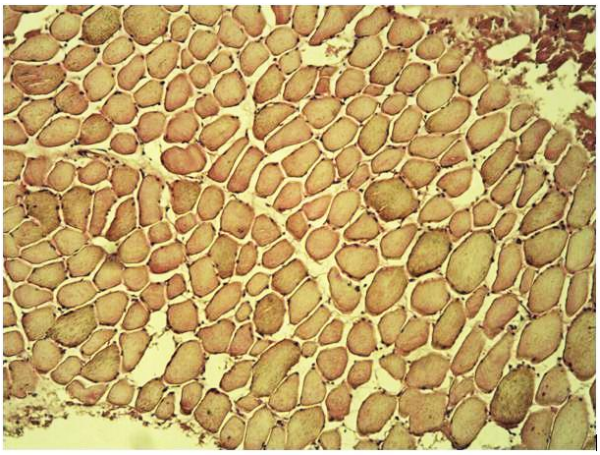

Fig. 6: Microscopy of muscle fibers of the diaphragm. Cross-section. x100. H & E stain.

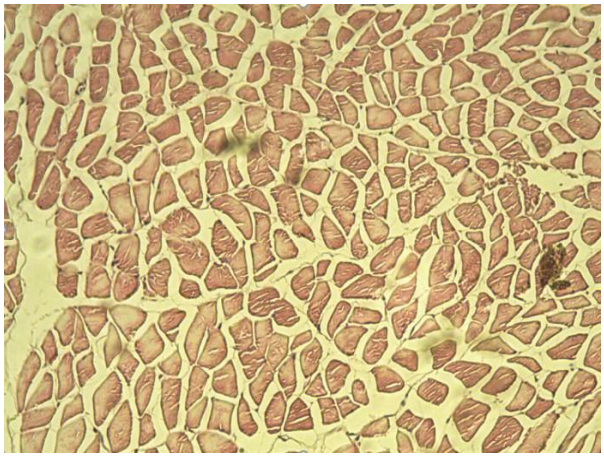

Fig. 7: Microscopy of skeletal muscle. Cross-section. x100. H & E stain.

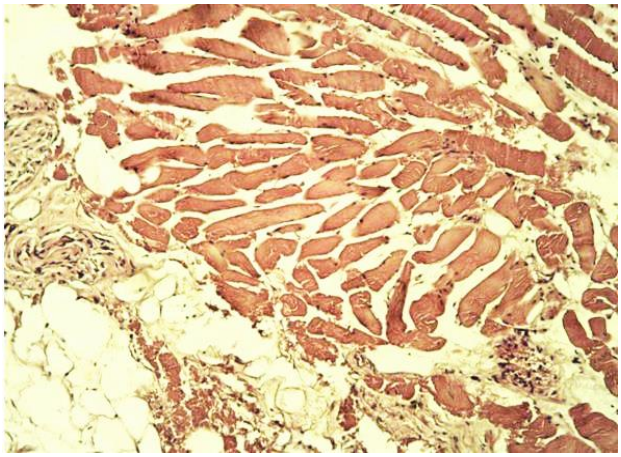

Fig. 8: Microscopy of the diaphragm. Muscle fibers and fat cells. x100. H & E stain.

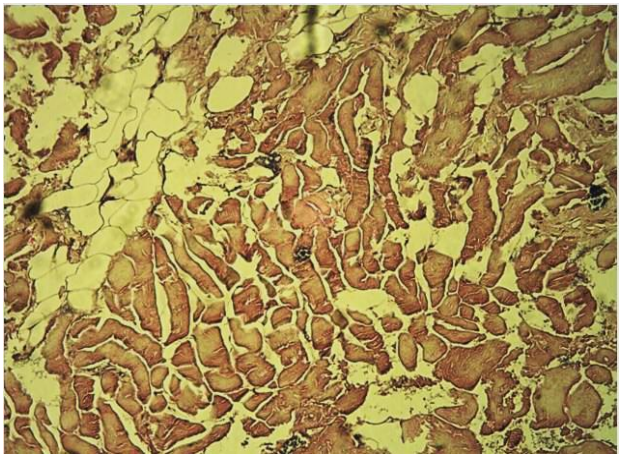

Fig. 9: Microscopy of skeletal muscle. Muscle fibers and fat cells. x100. H & E stain.

Part of the myofibrils of the diaphragm is devoid of nuclei, the thickness of muscle fibers increased, the cytoplasm is colored unevenly, with alternating pale areas and hyper-eosinophilic sites. There are no nuclei, and the cell wall is intermittent, can be traced by fragments. Sarcocystis located in the cytoplasm of single myocytes.

The intermuscular stroma of some parts of the diaphragm was edematous, with fragments of vascular walls and fat cells. In some fields of view, tendons visualized, representing closely spaced fibrous structures with rare cellular elements, in which elongated, flattened nuclei were visible (Fig. 5).

Histological examination of the diaphragm revealed extensive structures of fibrous tissue, represented by dense collagen fibers, sections of the walls of arterial and venous vessels, often with the destruction of the fibers.

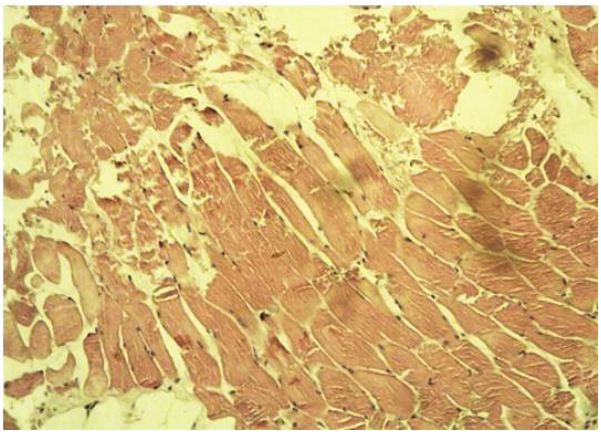

Fig. 10: Minced meat microscopy (skeletal muscles and diaphragmatic muscles). Longitudinal section. x100. H & E stain

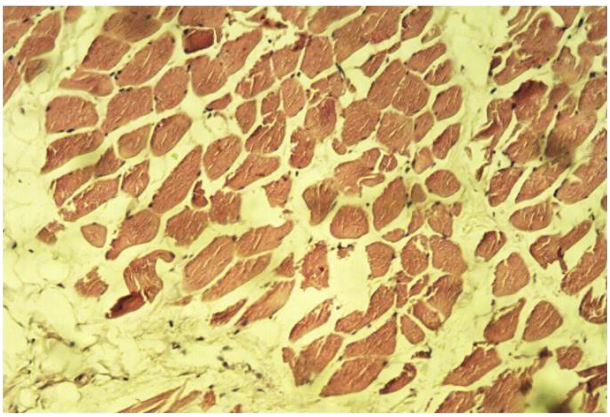

Fig. 11: Minced meat microscopy (skeletal muscles and diaphragmatic muscles). Cross-section. x100. H & E stain

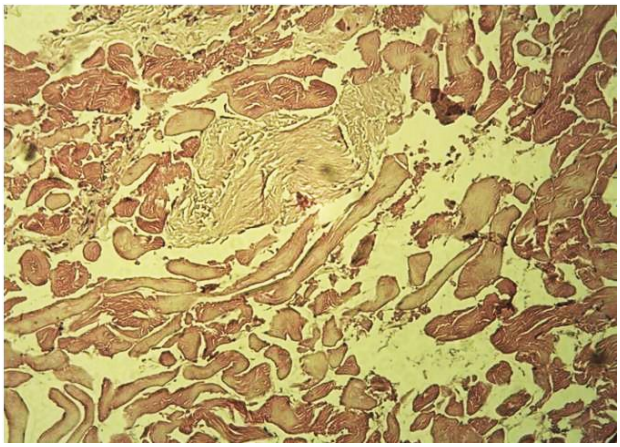

Fig. 12: Microscopy of artificially falsified minced meat (skeletal muscle and breast tissue). x100. Hematoxylin-eosin stain

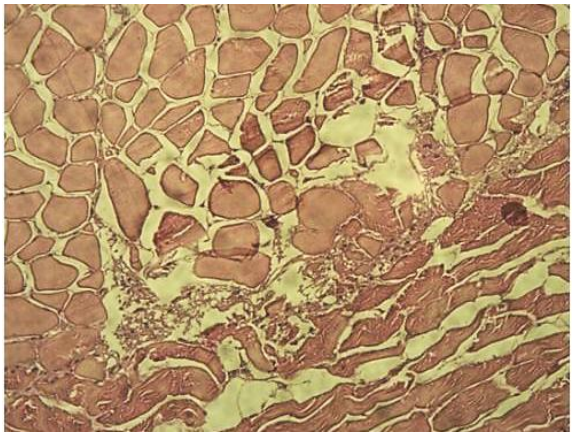

Fig. 13: Microscopy of artificially falsified minced meat (skeletal muscles and kidneys). x100. H & E stain

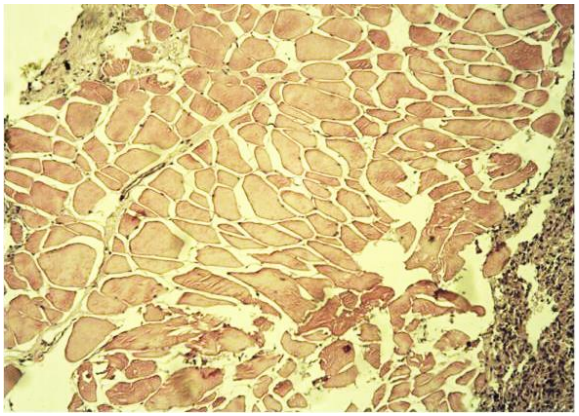

Fig. 14: Microscopy of artificially falsified minced meat (skeletal muscle and lung tissue): x100. H & E stain.

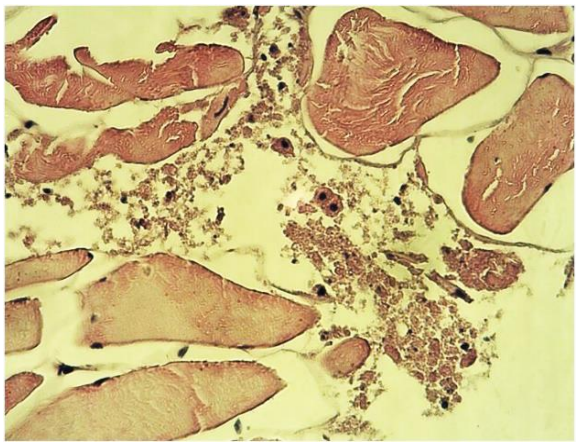

Fig. 15: Microscopy of artificially falsified minced meat (skeletal muscle and liver tissue); x100. Hematoxylin-eosin stain

Comparative histological analysis of minced skeletal and diaphragmatic muscles established that if the sternum', costal' and lumbar' parts of the diaphragm is carefully "cleansed" from the central tendon and minced, then falsification challenging to determine. However, it is possible given some morphological differences between skeletal and diaphragmatic muscle fibers (Figs. 6-11).

In addition to the diaphragm, mincemeat can be falsification by lungs, liver, kidneys, udders, etc. It should have been noting that this falsification is easy to identify by the characteristic structure of the organ (Figs. 12-15).

In Fig. 12 easily visualized inclusions of breast tissue; among the fields of fibrous tissue, small segments formed by acinar structures are visible. Cubic acinus cells, the cytoplasm is weakly eosinophilic or optically transparent, the nuclei are round in way, located near the basement membrane. The structures of the ducts formed by low, flattened cells, and the cores are flattened, hyperchromic. Elements of the stroma are also visible, represented by

dense, hyper-eosinophilic structures with the presence of thick-walled vessels, sometimes whole sections, sometimes in the form of wall sections, in these gaps, seen single red blood cells.

In several fields of view, the structure of the acini and ducts not expressed, significant accumulations of cellular elements are located, randomly located, sometimes with a nuclear part, sometimes lacking nuclei.

Histological analysis samples of the mincemeat with kidney tissue revealed the presence of glomeruli and tubules. The capillary loops of the glomeruli of pronounced blood filling dilated, the capillary loops are discontinuous, in some places, the walls of the vessels destroyed, groups of freely located red blood cells are visible outside the walls. Signs of the proliferation of endothelial, mesangial cells, and cells of the outer leaf capsules not observed. Urinary spaces are significantly narrowed, contain single altered red blood cells and pinkish clumpy masses. In the tubule epithelium, widespread protein (hydropic) dystrophy, disorganization, and desquamation of epithelial cells, sometimes with a loss of tubular structure. In the gaps of the tubules are desquamated cells and homogeneous masses of pinkish color. Large branches of the renal artery of moderate blood supply. Because of careful mince kidney tissue and subsequent homogenization with mincemeat, the structures of the glomeruli and tubules broken, the cells located randomly.

Microscopy of skeletal myofibrils homogenized with pulverized pulmonary tissue revealed the presence of alveolar structures in the fields of view. The alveoli were mostly in a collapsed state. Their walls were not visible. In places they thickened, in them, we noted the cellular elements of the lymphocytic series, macrophages. In most fields of view, the alveoli homogenized, devoid of structure, and represented by cell fields. Thick-walled arteries were traced entirely or in the form of fragments with the destruction of the walls, fragmentation of fibrous structures, and widespread desquamation of the endothelium.

Liver tissue inclusions are shown in Fig. 15 as small islands. Hepatocytes randomly located, do not form structures of hepatic beams or lobules. In some places, the cell structure preserved. In some areas, the nuclei are not visible. The cytoplasm is colored unevenly, often homogenized, or slightly granular. In separate fields of vision, sections of portal tracts were visible with an excess of fibrous structures, the presence of an arterial, venous vessel, and bile duct wall. The vascular endothelium and the epithelial lining of the ducts are in a disorganized state. The cells randomly located in the lumens. In places, the walls of blood vessels and pipes are destroyed, with the rupture of fibrous formations. In separate fields of view, we noted broken fragments of the walls of the central veins.

Conclusion

Histological analysis is a reliable way to determine the composition of mincemeat and semi-finished products, but, unfortunately, GOST 32951-2014 p.6.13 implies histological identification of the semi-finished product only if there is a disagreement on the structure of the raw materials used. That is, the problem of falsification lies not only in the dishonesty of producers but also in the imperfection of regulatory documents regulating the requirements for meat and meat products. Of course, after the Russian Federation joined the WTO, many standards lost their relevance, either were revised and supplemented or introduced. In particular, TR TS 034/2013 establishes microbiological safety standards for slaughter products and meat products, requirements for physical and chemical indicators, and hygienic safety requirements for slaughter products intended for the production of meat products for baby food. We consider it necessary to clarify that the physicochemical parameters do not give a complete picture of the quality of the product, although they show the composition of the product. Identification signs do not allow to establish the fact of replacing animal fat and protein with plant ingredients. Of course, compliance with the hygienic and microbiological requirements of the technical regulation helps to prevent foodborne toxic infections and poisoning but does not protect consumers from counterfeiting. Thus, meat products sold to the population not investigated for falsification.

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