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AN EXPRESS ASSESSMENT METHOD FOR MEAT QUALITY AND SAFETY

Tamara Kalyuzhnaya ^{a*}, Larisa Karpenko ^a, Diana Orlova ^a,
Alexander Drozd ^b, Valentine Urban ^a

^a Department of Veterinary Sanitary Expertise, St. Petersburg State Academy of Veterinary Medicine, RUSSIA.

^a Department of Epizootology, St. Petersburg State Academy of Veterinary Medicine, RUSSIA.

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ABSTRACT

The aim of the research was to study the reliability and acceptability of this rapid method, to evaluate its effectiveness in determining the degree of freshness of meat in an experiment. Native preparations were made from the studied samples of fresh meat and stained with hematoxylin-eosin, microscopy of which revealed whole muscle fibers with transverse striations, tightly adjacent to each other, pink-red cytoplasm due to eosin staining, whole muscle cell nuclei, violet color intensively stained Ehrlich hematoxylin. Microscopy of native preparations of stale meat and meat of dubious freshness revealed structural changes in the elements of muscle tissue, namely, the striation of the fibers is poorly distinguishable or absent, the color of the cytoplasm is uneven, there are areas with muscle fibers located randomly, not tightly adjacent to each other. The nuclei of muscle fibers are weakly colored, uneven or absent as a result of ongoing microbiological and enzymatic processes.

The method of microscopy of native meat preparations stained with hematoxylin-eosin is available, informative and allows you to assess the degree of freshness of meat and can be applied directly in places of storage and sale of products, in the conditions of production laboratories and laboratories for veterinary and sanitary examination of food markets independently or in a set of assessment methods quality and safety of meat.

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1. INTRODUCTION

One of the popular and favorite food products among consumers is animal meat. Due to its nutritional value and rich taste, meat is a part of all kinds of meat delicacies, semi-finished products, and culinary products. Enjoy the true bouquet and variety of meat products is possible only if they are

prepared from fresh, benign raw materials. It is widely known that meat is a perishable product. So, for example, in accordance with GOST 31476-2012 "Pigs for slaughter. Pork in carcasses and half carcasses. Specifications" and GOST 34120-2017 "Cattle for slaughter. Beef and veal in carcasses, half carcasses and quarters. Technical conditions" permissible terms and conditions of product storage are established, under which quality and safety indicators are maintained at a high level.

As a rule, meat spoilage develops if these conditions are not observed as a result of the intensive development of lactic acid, putrefactive microorganisms, micrococci, yeast, molds, etc., including the totality of intestinal microbiota (Chen et al., 2019). The greatest danger to people's health when consuming poor-quality meat products is represented by meat of doubtful freshness, since such products do not have pronounced organoleptic signs of spoilage, can easily be masked in a retail network, for example, marinating for barbecue, making culinary products, etc. At the same time, meat of dubious freshness accumulates in itself products of the primary breakdown of proteins, which adversely affect the digestion of people and cause its upset (Yushina, 2018).

In connection with the aforementioned quality specialists in large network companies, veterinarians representing the state and departmental veterinary services in food markets, processing enterprises should pay special attention to the quality control of raw meat, including in terms of its freshness. The meat freshness control methodology is regulated by GOST 7269-2015 "Meat. Sampling methods and organoleptic methods for determining freshness" and GOST 23392-2016 "Meat. Methods of chemical and microscopic analysis of freshness" and defines organoleptic, physico-chemical and microscopic research methods, including indicators such as appearance, color, condition of meat on the surface and section, consistency, smell, transparency and aroma of broth vapor using a cooking sample, products primary protein breakdown in the reaction with copper sulfate, volatile fatty acids, the number of microorganisms and the degree of decomposition of muscle tissue during microscopy of smear-prints (Donkova, 2018).

In addition, in cases of non-compliance with the veterinary and sanitary rules for the production and circulation of meat raw materials, as well as in the framework of state monitoring, an analysis of microbiological safety is required according to the indicators provided for in the Technical Regulation of the Customs Union 034/2013 "On Meat and Products", which include KMAFAnM, BGKP, *S.aureus*, bacteria of the genus *Proteus*, yeast and mold, sulfite-reducing clostridia (Kalyuzhnaya, 2019).

GOST 19496-2013 "Meat and meat products. The method of histological examination" regulates the control of the degree of freshness of meat by histological examination of samples, which allows us to draw a conclusion about the freshness of products on the structure of muscle tissue, namely, on the structure and relative position of muscle fibers, the integrity of the nuclei, the intensity of color of tissue elements (Merkucheva, et al., 2016). This method is accurate, but very difficult to implement, takes a long time, which prevents it from being used in practical work directly under production conditions (Orlova et al., 2019; Tokarev et al., 2019).

In recent years, a number of new and non-invasive imaging methods have appeared, such as optical imaging, ultrasound imaging, tomographic imaging, thermal imaging and odor imaging, which have shown great potential in assessing quality and safety (Shchebentovska, et al., 2015). In world practice, the development and implementation of methods based on the interaction of highly sensitive and selective chemosensors and a nanocomposite (Pavase, et al., 2018), spectral analysis,

including near-infrared spectroscopy (NIRS), hyperspectral imaging (HSI) and Raman spectroscopy (RS) (Alshejari, et al., 2017), as well as a mobile electronic analyzer, the so-called electronic nose (Yushina, 2018).

As an alternative to the classical standardized method for determining the degree of freshness of meat by the histological method, we proposed an express method for the production of native meat preparations, which allows one to evaluate the structure of muscle tissue, which is available in execution and does not require special equipment and specialist qualifications (Khvylya, et al., 2012). The aim of the research was to study the reliability and acceptability of this rapid method, to evaluate its effectiveness in determining the degree of freshness of meat in an experiment.

2. MATERIALS AND METHODS

As research materials, we used 20 samples of meat from agricultural and commercial animals, as well as poultry: pork - 4 samples; beef - 3; elk meat - 2; nutria - 3; broiler chickens - 5; turkeys - 3.

At the first stage of the research, the degree of freshness of all meat samples was assessed according to organoleptic, physico-chemical and microscopic indicators by methods regulated by current regulatory and technical documents, as well as a micro picture of native meat preparations stained in accordance with GOST 19496-2013.

During organoleptic studies of meat, the appearance, color, condition of the muscles on the surface and section, consistency, smell, transparency and aroma of the broth vapor were evaluated using a boiling test according to GOST 7269-2015.

Appearance and color were determined by visual inspection, immediately after cutting the muscles in the deeper layers. At the same time, attention was drawn to the stickiness of the meat from the surface and its moisture content in the cut by applying filter paper to the cut surface and evaluating the wet traces remaining on it. The consistency of the meat was determined by pressing on its surface with a spatula and observing the rate of leveling of the resulting fossa. The smell was evaluated from the surface, as well as in the deeper layers during the cut; special attention was paid to the smell of meat adjacent to the bone. In addition, the color, smell and texture of fat were established by squeezing and rubbing it between the fingers.

To make a cooking sample, the meat was ground with scissors to the state of mincemeat, while the connective and fatty tissue were separated and a 20 g sample was taken, placed in a 100 cm³ conical flask, 60 cm³ of distilled water was added, the flask was covered with a watch glass, put in a water bath and heated to temperature 80-85°C. When the first vapors of the broth appeared, its smell was evaluated, specificity, presence of extraneous odors, sour or putrid odor were noted. The transparency of the broth, the presence of flakes in it, was evaluated in transmitted light, pouring the broth into a measuring cylinder.

Chemical and microscopic analysis of meat freshness was carried out in accordance with the requirements of GOST 23392-2016, GOST 20235.1-74, determining the products of the primary protein breakdown in the reaction with copper sulfate, the presence of ammonia and ammonium salts with Nessler's reagent, the number of microorganisms and the degree of decomposition of muscle tissue under microscopy fingerprints.

When setting up the reaction with copper sulfate, hot broth prepared in the sample was used to

identify the products of primary protein breakdown, which was filtered through a funnel with a dense layer of cotton wool 0.5 cm thick in a test tube placed in a container with cold water. Then, 3 drops of copper sulfate of mass concentration of $50 \text{ g} / \text{dm}^3$ were added to the filtered extract, stirred by shaking, and the transparency of the filtrate was evaluated after 5 minutes.

To detect ammonia and ammonium salts in poultry and nutria meat as the final products of protein breakdown, a reaction was performed with the Nessler reagent. Prepared an aqueous meat extract in a ratio of 1:4. A sample of mincemeat, weighing 5 g, was placed in a conical flask with 20 cm^3 of twice boiled distilled water and insisted for 15 minutes with three shaking, after which the extract was filtered through a paper filter into a 1 cm^3 tube. 10 drops of Nessler's reagent were added to the obtained filtrate, the contents of the tube were shaken and the change in its color and transparency was observed.

For microscopic studies, the surface of the meat was fired with a swab dipped in alcohol, cut pieces with a size of $1.5 \text{ cm} \times 1.0 \text{ cm} \times 1.5 \text{ cm}$ with sterile scissors, and the surfaces of the slices were applied to a glass slide (three prints on two glass slides). The preparations were dried in air, fixed above the burner flame and stained by Gram. Microscopic examination of at least 25 fields of view under immersion at an increase in the x90 objective lens counted the number of cocci and rods, estimated the degree of decomposition of muscle tissue.

The structure of the studied meat samples was evaluated by microscopy of native muscle tissue preparations stained with hematoxylin-eosin.

For the preparation of native muscle tissue preparations, meat samples of 15-20 grams were used. The meat sample was held with tweezers and, in the direction of the muscle fibers, curved scissors with a convex side outward made a cut 2-3 mm thick and 8-10 mm long.

The obtained sections in the amount of 5-7 pieces were laid out on the bottom glass of the compressorium so that the distance between them was at least 1 cm, after which they were covered with top glass, crushing muscle tissue and fixing the preparations with screws.

Then, crushed muscle sections using dissecting needles were removed from the compressorium, placed in a porcelain cup, and stained with hematoxylin-eosin according to GOST 19496-2013. After staining, the sections were again placed in the compressorium, if necessary, 1-2 drops of a 50% aqueous solution of glycerol were applied to them and microscopic with an eyepiece enlargement of 10, objective lens of 4, 10 and 20, evaluating the structure of muscle tissue. At the same time, attention was paid to the location and structure of muscle fibers, the presence and integrity of nuclei, and the intensity of their color (Khvyła, et al, 2016).

At the next stage, the test samples were placed in individual polymer sealed containers and stored under identical conditions in a refrigerator at a temperature of $+ 2 \dots + 4 \text{ C}$, relative humidity 80-85%. During storage, the degree of meat freshness was assessed using the methods described above and the results were compared with a micro picture of native meat preparations of fresh, dubious freshness and stale.

3. RESULT AND DISCUSSION

At the beginning of the experiment, as a result of organoleptic studies, it was found that all the samples of the studied meat in appearance corresponded to this type of meat, had a natural color from white-pink in poultry to dark red elk meat. On the surface, a pronounced crust of drying was noted,

signs of mucus, rot and other microbiological and enzymatic signs were not found, in the section - moderate moisture. When pressing on the surface of the meat, the resulting fossa leveled out quickly, which corresponds to an elastic consistency. The smell of meat was rated as specific, characteristic of this type of meat. The broth staging the sample by cooking is transparent, with a specific meat smell, with large drops of fat on the surface, the samples of defrosted meat gave a washed broth with a small number of flakes during cooking. The products of the final and primary decomposition of proteins were absent in the samples; in the reaction with copper sulfate, the extract remained transparent; in the reaction with the Nessler reagent, it was yellow-green in color, transparent. Single cocci and rods were detected in smears of fingerprints; no traces of muscle tissue decay were detected.

According to organoleptic, physico-chemical and microscopic studies, all meat samples were recognized fresh and then each sample was made native preparations and stained with hematoxylin-eosin, microscopy of which revealed whole muscle fibers with transverse striation, tightly adjacent to each other see Figures 1, 2. The cytoplasm is pink-red due to eosin staining. The nuclei of muscle cells are holistic, violet in color, intensely stained with Erlich hematoxylin.

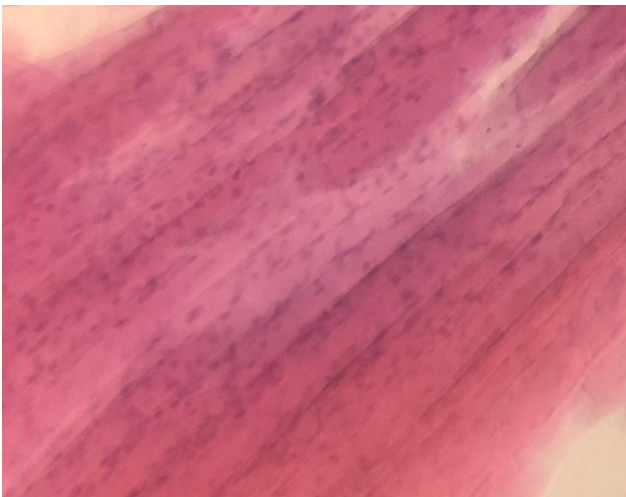


Figure 1: Micro picture of the native preparation of fresh beef. SW 80.

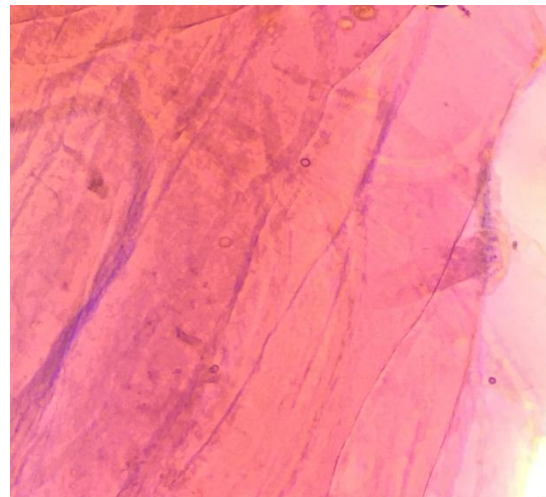


Figure 2: Micrograph of a native preparation of fresh turkey meat. SW 80.

During storage, the test meat showed signs of spoilage. From the surface and in the thickness of the meat became gray, sticky with an acidic or putrid odor of varying severity. The cut meat is wet, on the filter paper leaves a pronounced wet trace. When pressing on the surface of the meat with a spatula, the resulting fossa was leveled for 1-2 minutes or not leveled, indicating a weakening of the tissue turgor. The broth during the preparation of the sample by cooking is cloudy, with flakes, a small number of drops of fat on the surface or without it, the smell is unpleasant, sour, musty or putrid.

When formulating reactions with copper sulfate and Nessler's reagent, the presence of protein breakdown products was established, the extracts became cloudy, and in the reaction to ammonia and ammonium salts its color changed to intense yellow.

Microscopy of fingerprint smears revealed a moderate or large number of microorganisms with up to 20 or more microbial cells in the field of view, as well as traces of the breakdown of muscle fibers.

According to these indicators, the studied meat was rated as dubious freshness and stale. Microscopy of native preparations of such meat revealed structural changes in the elements of muscle tissue, namely, the striation of the fibers is poorly distinguishable or absent, the color of the cytoplasm is uneven, there are areas with muscle fibers located randomly, not tightly adjacent to each other. The nuclei of muscle fibers are weakly colored, uneven or absent as a result of ongoing microbiological and enzymatic processes see Figures 3, 4, 5, 6, 7.

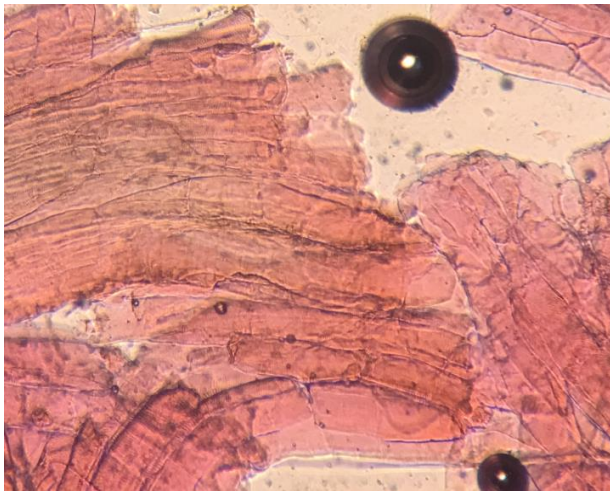


Figure 3: Micrograph of a native preparation of moose meat of doubtful freshness. SW 80.

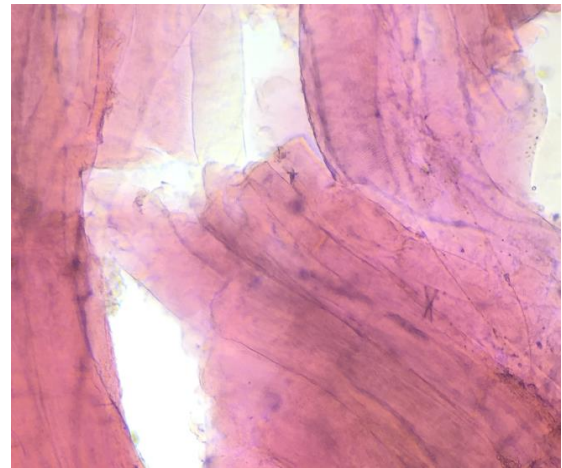


Figure 4: Micrograph of a native preparation of broiler meat of doubtful freshness. SW 80.

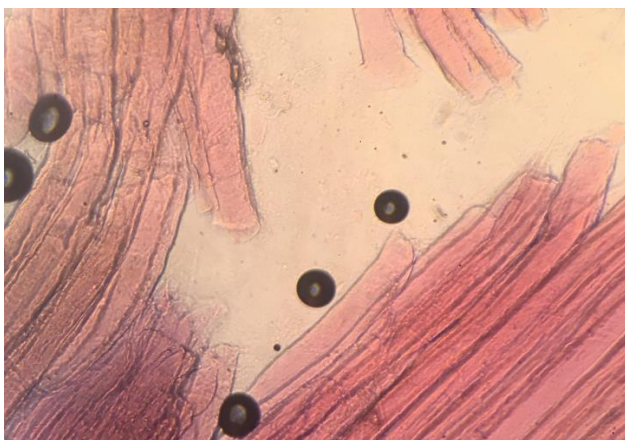


Figure 5: Micrograph of the native preparation of stale pork. SW 80.



Figure 6: Micrograph of the native preparation of stale nutria meat. SW 80.

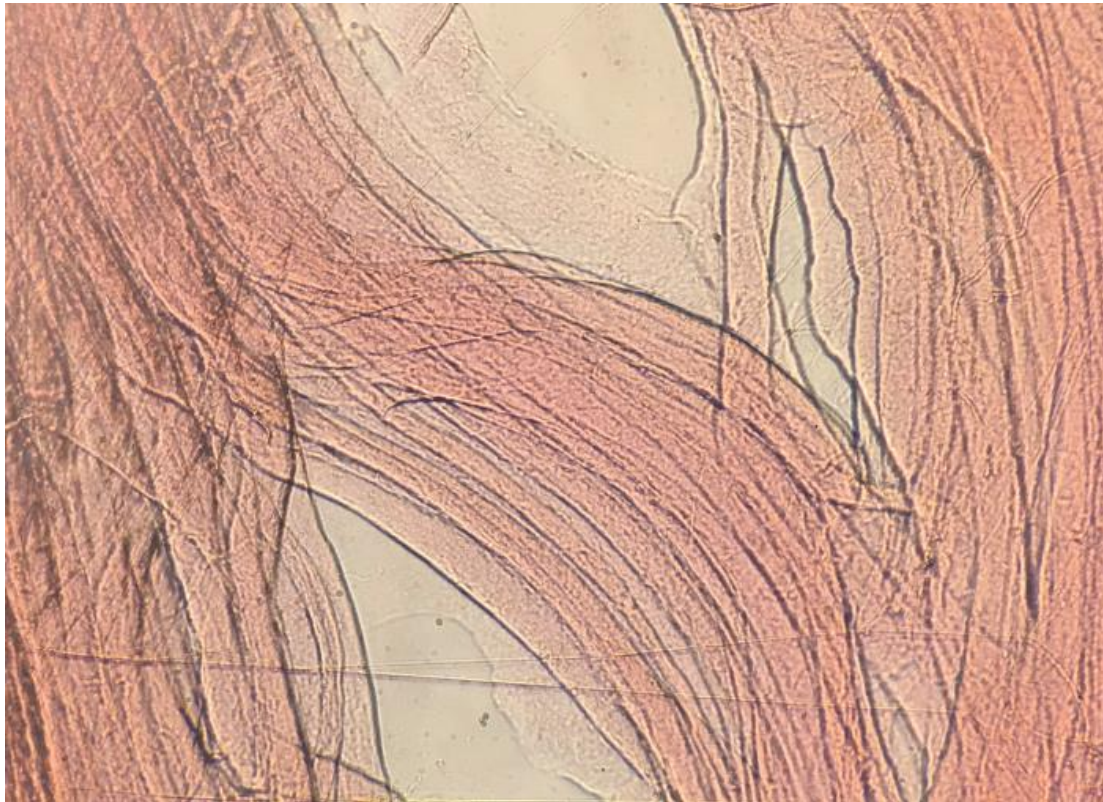


Figure 7: Micrograph of a native preparation of stale beef. SW 80.

4. CONCLUSION

Chilled meat is a perishable product and undergoes changes under the influence of microorganisms and its own enzymes in a short time. As a result of spoilage, meat not only loses its biological properties but can also pose a risk to human health, which confirms the relevance of assessing the freshness of raw meat, especially in places of sale.

Existing methods of researching meat for freshness are reliable and require an integrated approach, and therefore cannot always be fully reproduced with input control and circulation of products. In addition, in a retail network, “poor quality” raw materials can be “masked” by pickling, making semi-finished products using food additives and ingredients, which will not allow a reliable organoleptic assessment by an expert and is a violation of Russian law and consumer rights.

According to the research results, the microscopy method of native meat preparations stained with hematoxylin-eosin is available, informative and allows you to assess the degree of meat freshness by the structure of muscle tissue, the location and striation of muscle fibers, the color intensity of nuclei and cytoplasm and can be applied directly to the storage and sale of products, in the conditions of production laboratories and laboratories of veterinary sanitary examination of food markets independently or in a set of quality assessment methods and meat safety.

5. AVAILABILITY OF DATA AND MATERIAL

Data can be made available by contacting the corresponding authors

6. ACKNOWLEDGEMENT

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and associations: “Development of a methodology for the determination of chilled food products (meat, poultry, fish) obtained from fresh raw materials” on the topic Development of an express method for determining the thermal state of meat and fish by the structure of muscle fibers”.

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Kalyuzhnaya Tamara is an Assistant Professor at the Department of Veterinary Sanitary Expertise, St. Petersburg State Academy of Veterinary Medicine. Her scientific interest is Assessment the Quality and Safety of Food Products.



Larisa Karpenko is a Member of the Academic Council of St. Petersburg State University of Economics and Dissertation Council D.220.059.02. Her research interests include the study of Biochemical, Immunological and Endocrinological Indicators in Domestic and Farm Animals in Normal and Non-communicable Diseases.



Diana Orlova is an Associate Professor, St. Petersburg State Academy of Veterinary Medicine. She is a Candidate of Veterinary Sciences. Her scientific interests are issues of Assessing the Quality, Safety and Naturalness of Animal and Vegetable Products, Methods for Determining their Falsification, the use of Food Additives in the Food Industry that have a Beneficial Effect on Human Health.



Dr. Alexander Drozd is an Associate Professor and Head of the Department of Epizootology, St. Petersburg State Academy of Veterinary Medicine. He holds a Doctor of Veterinary Sciences degree. His scientific interests are issues of Assessing the Quality, Safety and Naturalness of Animal and Vegetable Products, Methods for Determining their Falsification, the Use of Food Additives in the Food Industry that have a Beneficial Effect on Human Health.



Valentine Urban is an Associate Professor at the Department of Veterinary Sanitary Expertise, St. Petersburg State Academy of Veterinary Medicine, Russia. She is a Candidate of Veterinary Sciences. Her scientific interest are the Methods of Veterinary Sanitary Examination of Food Products in Terms of Quality and Safety, Technical Regulation and Standardization of Food Raw Materials, Production and Selection, as well as ensuring Food Safety and Identifying Risks of Various Origin in accordance with the requirements of the HACCP System.