Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2021 18(3): 2278-2283. **OPEN ACCESS**

Express method to manufacture native preparations to determine the Turkey meat freshness degree

Tamara Kalyuzhnaya, Alexander Drozd, Diana Orlova*, Manya Mkrtchyan

Saint Petersburg State University of Veterinary Medicine, 5, Chernigovskaya st., St. Petersburg 196084, **Russia**

***Correspondence:** orlovad@spsuvm.ru **Received 15-03-2021, Revised: 08-07-2021, Accepted: 12-08-2021 e-Published: 23-08-2021**

The article presents the results of applying the express method to manufacture native preparations to determine the degree of freshness of turkey meat during the veterinary and sanitary examination. Along with meat obtained from farm animals, poultry meat is in great demand, particularly turkey meat. However, turkey meat in the trade network can easily undergo spoilage, facilitated by violations of temperature and humidity storage conditions. The methods regulated by the current GOST requires a comprehensive assessment of organoleptic, physicochemical, and histological indicators, preparation for the study, the use of expensive equipment, a large amount of time and labor, which makes it difficult to use them in the conditions of real circulation of food products. Therefore, the development and implementation of an express method remain relevant, which can be an alternative to the histological method. The research aimed to assess the effectiveness of the proposed express method to manufacture native preparations in determining the degree of freshness of meat. The research materials used 73 samples of turkey meat. At the first stage of the research, the freshness degree of all meat samples was determined by organoleptic, physicochemical, and microscopic indicators by methods regulated in the current regulatory and technical documents, by micro-picture native meat preparations, colored by GOST 31931-2012.At the next stage, samples of turkey meat, placed in individual polymer sealed containers, were stored in a refrigerator at a temperature of + 2 ... + 40C, with a relative humidity of 80-85%. Evaluation of the degree of freshness of turkey meat was carried out using the above methods and compared the results obtained with the results obtained in the study of fresh meat. As a result of the research, identification criteria were determined. Such as the number of areas with transverse striation of muscle fibers, with muscle fibers tightly adjacent to each other, with uniformly colored cytoplasm, with colored nuclei. These criteria make it possible to determine the category of meat freshness using the proposed express method during the veterinary-sanitary examination.

Keywords: turkey meat, veterinary and sanitary examination, quality and safety, freshness, express method, native preparations.

INTRODUCTION

Currently, much attention is paid to the quality and safety of food products, including meat, an integral part of the consumer basket. Along with meat obtained from farm animals, poultry meat is in great demand, particularly turkey meat. Consumer interest in turkey meat is related to its dietary properties. Compared to broiler meat, turkey meat is characterized by a higher yield - 70%, high content of amino acids such as valine, isoleucine, and tryptophan, and low content of adipose tissue. At the same time, it has a high content of polyunsaturated fatty acids, such as oleic, linoleic, palmitoleic. However, turkey meat in trade in the trade network can easily undergo spoilage, facilitated by violations of temperature and humidity storage conditions (Kalyuzhnaya, 2019).

In the current GOST 31473-2012 "Turkey meat (carcasses and parts thereof). General technical conditions (Reissue)" set the conditions, shelf life, and quality parameters of turkey meat. However, for profit, unscrupulous producers can use expired turkey meat to make culinary products intended for direct consumption, which in turn can cause poisoning for consumers. For preventing the meat sale of stale and dubious freshness in the trading network, it is necessary to assess its quality and safety in actual product circulation conditions. Methods to determine the turkey meat freshness are set out in GOST 31470-2012 "Poultry meat, by-products, and semi-finished products from poultry meat. Methods of organoleptic and physicochemical studies" and establish the definition of such indicators as appearance and color, consistency, the smell of meat on the surface and cut, transparency and aroma of broth vapor using a cooking test, products of primary protein breakdown in reaction with Nessler's reagent, volatile fatty acids, acid and peroxide values of fat, peroxidase activity. Also, according to GOST 19496-2013 "Meat and meat products. Method of histological research", methods of histological analysis of meat freshness are determined by qualitative assessment of the state of structural elements of muscle tissue based on microstructural characteristics of freshness degree or meat spoilage. However, the methods proposed in the current GOST require a comprehensive assessment for all indicators, preparation for the study, for example, the preparation of reagents, the use of expensive equipment, and a significant amount of time and labor. These disadvantages make it challenging to use some methods for determining the freshness of turkey meat, for example, histological, in laboratories for veterinary and sanitary examination at food markets, in laboratories at processing plants.

In world practice, along with classical chemical and histological methods (Malak et al., 2020), techniques have been developed for determining spoilage processes by identifying amines formed during the breakdown of proteins (Triki et al. 2018; Ruiz-Capillas and Herrero 2019; Khidhir et al. 2021), among which should be noted spectrometry (Sinanoglou et al. 2018) and the use of a particular device, the so-called electronic nose (Wojnowski et al. 2019).

Therefore, the development and implementation of an express method remain relevant, which can be an alternative to the histological method.

The research aimed to assess the effectiveness of the proposed express method to manufacture native preparations in determining the degree of freshness of meat.

MATERIALS AND METHODS

The research materials used 73 samples of turkey meat. The selection of meat samples for research and sample preparation was carried out by GOST 31467-2012 "Poultry meat, by-products, and semi-finished products from poultry meat. Sampling Methods and Preparation for Testing (Amendment Edition)".

At the first stage of the research, the degree of freshness of all meat samples was determined by organoleptic, physicochemical, and microscopic indicators, by methods regulated in the current regulatory and technical documents, and also the micro-picture of native meat preparations, colored by GOST 31931-2012, was assessed.

Appearance and color, consistency, meat smell on the surface and cut, transparency and aroma of broth vapors using a cooking test, products of primary protein degradation in reaction with Nessler's reagent, volatile fatty acids, acid and peroxide values of fat, peroxidase activity was determined according to GOST 31470-2012 Poultry meat, by-products, and semi-finished products from poultry meat. Methods of organoleptic and physicochemical research".

The appearance and color were assessed visually on the surface and after cutting the muscles in the deep layers. Simultaneously, attention was paid to the stickiness of the meat surface and its moisture on the cut by applying filter paper to the cut surface and assessing the wet traces remaining on it. For determining the meat's consistency, it was pressed on its surface with a spatula, and the rate of leveling of the formed fossa was evaluated. The smell was determined on the surface and at the cut, with particular attention paid to the smell of meat adjacent to the bone. Also, the color, consistency, and odor of the adipose tissue were evaluated.

For setting a sample by cooking meat in a sample of 20 grams crushed with scissors to the state of minced meat and placed in a conical flask of 100 cm^3, 60 cm^3 of distilled water was added, the flask was covered with a watch glass, placed in a water bath, and heated to a temperature of 80-85 °C. When the first vapors of the broth appeared, its aroma was assessed, specificity, the presence of foreign odors, sour or putrid odor were noted. Please pay attention to the

transparency of the broth and the presence of flakes in it.

To detect ammonia and ammonium salts in meat, a reaction was carried out with Nessler's reagent. For this, an aqueous meat extract was prepared in a ratio of 1:4. A sample of minced meat weighing 5 g was placed in a conical flask with 20 cm^3 of bidistilled water and infused for 15 min with three times stirring, after which it was filtered through a paper filter into a 1 cm^3 tube of the extract. To the resulting filtrate was added 10 drops of Nessler's reagent, the tube contents were shaken, and the change in color and transparency was assessed.

For microscopic studies, the surface of the meat was burned with a swab soaked in alcohol, pieces of 1.5x1.0x1.5 cm in size were cut out with sterile scissors, and the sections' surfaces were applied to the slide (three prints on two slide slides). The preparations were dried in the air, fixed over a burner flame, and stained according to Gram. At least 25 visual fields were microscoped under immersion at a magnification of the objective x 90. Simultaneously, the number of cocci and rods was counted, and the degree of muscle tissue decay was assessed.

To determine the acid number of the fat, about 5 g was weighed into a flask. Test fat and placed it in a water bath, then poured in 50 ml of a neutralized mixture of alcohol and ether in a ratio of 1:2. The resulting solution was added 5 drops of a 1% alcoholic solution of phenolphthalein. It was rapidly titrated with 0.1 N. caustic sodium until a pink color appears that does not disappear within a minute.

To determine the fat peroxide number, a 1 g sample of the test fat was placed in a conical flask and dissolved in 20 ml of a mixture of glacial acetic acid and chloroform (1:1). The solution was added 0.5 ml of a freshly prepared saturated solution of potassium iodide and kept in the dark place for 3 min. Then, 100 ml of distilled water was added to the solution, to which 1 ml of 1% starch solution was added in advance. The released iodine was titrated with 0.01 N. sodium sulfate solution until the blue color disappears.

To determine volatile fatty acids, a sample of minced meat weighing 25 g, weighed on a laboratory balance, was placed in a round-bottomed flask. There was poured 150 cm^3 of a sulfuric acid solution with a concentration of 20 g/dm^3. The contents of the flask were mixed. The flask was closed with a stopper. A conical flask with a capacity of 250 cm^3 was placed under the refrigerator, on which a volume of 200 cm^3 was marked. Distilled water in a flat-bottomed flask was brought to a boil, and volatile fatty acids were distilled off with steam until 200 cm^3 of distillate was collected in the flask. During distillation, the weighed flask was heated. Titration of the distillate's entire volume was carried out with a 0.1 mol/dm^3 sodium hydroxide solution in a flask with phenolphthalein indicator until the appearance of a crimson color that did not disappear within 30 seconds.

The peroxidase activity was assessed using the benzidine test. For this, a sample of muscle tissue weighing 5 grams crushed to the state of minced meat, placed in a conical flask with a capacity of 100 ml, containing 20 ml of distilled water, infused for 20 minutes with occasional agitation, and filtered through a paper filter.

Then, using a pipette, 2 ml of an aqueous extract was added to the test tube, and 5 drops of an alcohol solution of benzidine with a mass fraction of 0.2% were added, the contents of the test tube were shaken, two drops of a solution of hydrogen peroxide with a mass fraction of 1% were added, and a change in the color of the contents of the tube was observed.

The micro-picture of the meat samples understudy was studied using the proposed express method to manufacture native preparations of muscle tissue, crushed between glasses into thin sections, and stained with hematoxylin-eosin (Orlova et al. 2019; Kalyuzhnaya et al. 2020; Orlova and Drozd 2020; Orlova et al.2020).

To prepare native preparations of the muscle section, we used meat samples 15-20 gr. The meat sample was held with tweezers, and a cut 2-3 mm thick and 8-10 mm long was made in the direction of the muscle fibers with curved scissors with the convex side outward.

The obtained sections in the amount of 5-7 pieces were laid out in the compressorium so that the distance between them was at least 1 cm, crushed between the glasses, and fixed with screws.

The crushed muscle sections were then removed from the compressor using dissecting needles, placed in a porcelain cup, and stained with hematoxylin-eosin according to GOST 19496-2013 "Meat and meat products. Method of histological examination".

After staining, the sections were again placed in a compressor, if necessary, 1-2 drops of a 50% aqueous solution of glycerol were applied to them and micro scoped under a microscope at an eyepiece magnification - 10, objective - 4, 10, and

20, assessing the structure of muscle tissue. Simultaneously, attention was paid to the location and integrity of muscle fibers, the presence, and the nuclei's integrity.

At the next stage, samples of turkey meat, placed in individual polymer sealed containers, were stored in a refrigerator at a temperature of + 2 ... + 40C, with a relative humidity of 80-85%. Evaluation of the degree of freshness of turkey meat was carried out using the above methods and compared the results obtained with the results obtained in the study of fresh meat.

The obtained research results were processed using Microsoft Office Excel applications, as well as by the method of variation statistics with the calculation of the arithmetic mean values of the correlation coefficient: M is the arithmetic mean, m is the error of the arithmetic mean, Student's t-test determined the reliability of differences between the samples at Microsoft Office Excel ($p < 0.05$).

RESULTS AND DISCUSSION

At the first stage, according to the organoleptic examination results, it was established that all samples of turkey meat had a pale pink or pink color, depending on the location of the muscle tissue. On the carcasses' surface, a pronounced drying crust was noted, the absence of spoilage signs, such as mucus or decay, an elastic consistency of muscle tissue, a specific smell of meat, both on the surface and the cut. When evaluating the cooking sample, the broth's transparency, its specific aroma, and large drops of fat on the surface were noted.

As a result of the reaction with Nessler's reagent, the extract remained transparent. It acquired a yellow-green color, which indicates the absence of products of the final degradation of proteins.

The benzidine test for peroxidase activity was positive since the extract acquired a blue-green color, which turned brown-brown within 1–2 min.

In smears, prints revealed single cocci and rods and the absence of traces of decay of muscle tissue.

The amount of VFA in the turkey meat samples was 3.37±0.11 ml KOH. The peroxide value of fat is 0.0065±0.0012% I2, and the acid value is 0.86±0.09 mg KOH.

Based on the analysis of the results obtained, all samples of turkey meat were found fresh. Further, to assess the effectiveness of the proposed method of manufacturing native preparations in determining the freshness of meat, they were made and micro scoped.

Microscopy of fresh turkey meat in native preparations revealed intact muscle fibers tightly adjacent to each other with transverse striation, without tears, stratification. The cytoplasm of muscle fibers is intensely and uniformly colored bright pink. Purple nuclei are visible along the periphery of muscle cells (Fig. 1). The structure of the muscle tissue is preserved, no signs of cell and nuclear decay were found.

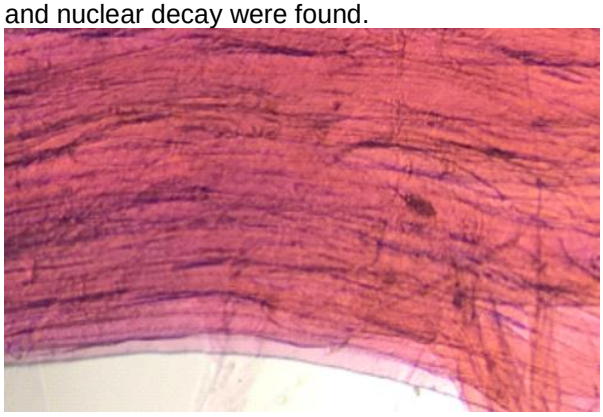

Figure 1: Micro-picture of fresh turkey meat in native preparations.

At the second stage of the research, signs of spoilage during turkey meat storage were determined.

An organoleptic examination revealed a change in the color and consistency of meat, the presence of stickiness on the surface, sour or putrid odor of varying intensity. As a result of setting the sample by cooking, the broth is cloudy, with flakes, a small amount of fat drops on the surface or without it, with an unpleasant, sour, musty, or putrid aroma.

When staging reactions with Nessler's reagent, protein degradation products were established since the extract was turbid, intense yellow in color.

Microscopy of smears-prints revealed up to 20 or more microbial cells in the field of view and traces of decay of muscle fibers.

When the benzidine test for peroxidase activity was performed, the extract acquired a blue-green color, turning into brown-brown in less than 1 min, or immediately acquired a brown-brown color. The amount of VFA in the samples of turkey meat averaged 9.28±0.13 ml of KOH for the entire experiment period, which is 2.8 times more than the amount of VFA established at the first stage of the research.

Table 1: Morphological characteristics of muscle tissue of turkey meat samples in native preparations (M ± m, n = 73)

Identification criteria	Fresh meat (control)	Doubtful fresh meat	Stale meat
The number of areas with transverse striation of muscle fibers, units / 20 p.z.	90,89±0,23	40,37±0,18*	0,71±0,14*
The number of areas with muscle fibers tightly adjacent to each other, units / 20 p.z.	91,09±0,12	46,24±0,13*	11,78±0,16*
The number of areas with uniformly colored cytoplasm, units / 20 p.z.	94,4±0,13	54,14±0,21*	26,91±0,15*
Number of areas with stained nuclei, units / 20 bp	92,73±0,19	62,08±0,11*	0,93±0,17*
*P < 0.05			

The peroxide value of fat was 0.095±0.019% I2 at the second stage of the research, and the acid value was 2.41±0.15 mg KOH, exceeding the analogous values established at the first stage of the studies by 14.6 times and 2.8 times, respectively.

Analyzing the results obtained, the studied samples of turkey meat were assessed as doubtful freshness and stale. When studying the microstructure of native preparations of such meat, changes in muscle tissue structural elements were found. So, the striation of muscle fibers was poorly discernible or absent, areas of chaotically located muscle fibers that were not tightly adjacent to each other were observed. The cytoplasm was colored unevenly. Muscle fiber nuclei are poorly stained or not stained due to lysis due to microbiological and enzymatic deterioration processes (Fig. 2).

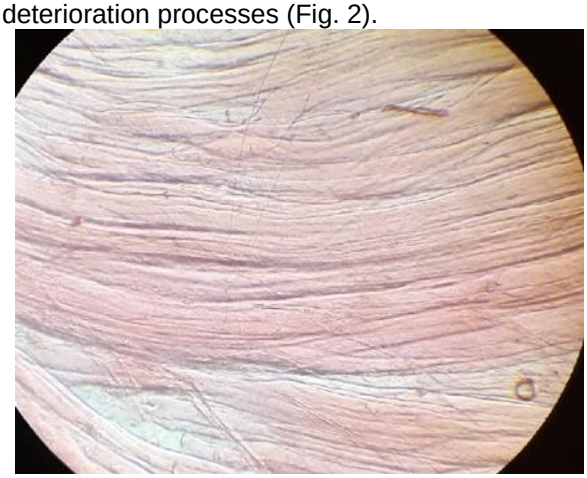

Figure 2: Micro-picture of stale turkey meat in native preparations.

The signs of spoilage revealed by microscopy of native preparations of turkey meat make it possible to consider these criteria as identification and use them in determining the category of the freshness of meat.

Analyzing the data obtained, it was found that the number of areas with transverse striations of muscle fibers in the meat of dubious freshness decreased 2.25 times compared to the same indicator in fresh meat, the number of areas with muscle fibers tightly adjacent to each other - by 1.97 times, the number of areas with uniformly stained cytoplasm - 1.74 times, and the number of areas with stained nuclei - 1.49 times.

In stale meat, a decrease in similar indicators was also established in comparison with fresh meat. Thus, the number of areas with transverse striation of muscle fibers in stale meat decreased 128 times, the number of areas with tightly adjacent muscle fibers - 7.73 times, the number of areas with uniformly colored cytoplasm - 3.51 times, and the number of areas with colored nuclei - 102.9 times.

When comparing the quantitative characteristics of the identification criteria identified in the meat of dubious freshness and stale, we found their decrease in the process of spoilage. Thus, the number of areas with transverse striation of muscle fibers in stale meat decreased 56.9 times, the number of areas with muscle fibers tightly adjacent to each other - 3.93 times, the number of areas with uniformly colored cytoplasm - 2.01 times, and the number of areas with stained nuclei - 66.8 times. The obtained values are statistically significant at $p < 0.05$.

CONCLUSION

As a result of the conducted studies, identification criteria were determined, such as the number of areas with transverse striation of muscle fibers, with muscle fibers tightly adjacent to each other, with uniformly colored cytoplasm, with colored nuclei, which make it possible to determine the category of the freshness of meat using the proposed express method during a veterinary-sanitary examination at the stages of storage and sale of products both in the

conditions of the production laboratory and in the state laboratories of the food market.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

The study was carried out with the Russian Foundation for Basic Research's financial support within the framework of scientific project No. 19-31-27001. RFBR, project number 19-31-27001, funded the reported study.

AUTHOR CONTRIBUTIONS

O.D. conceived of the presented idea. K.T. developed the theory and performed the computations.

M.M. verified the analytical methods. O.D. encouraged A.D. to investigate and supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

REFERENCES

GOST 19496-2013 "Meat and meat products. Method of histological examination". http://docs.cntd.ru/document/1200107317.

GOST 31467-2012 "Poultry meat, by-products and semi-finished products from poultry meat. Methods of sampling and preparing them for testing (Edition with amendment). http://docs.cntd.ru/document/1200096152.

GOST 31470-2012 "Poultry meat, by-products and semi-finished products from poultry meat. Methods of organoleptic and physical and chemical research". http://docs.cntd.ru/document/1200096484.

GOST 31473-2012 "Turkey meat (carcasses and parts thereof). General technical conditions (Reissue).http://docs.cntd.ru/document/1200096486.

Kalyuzhnaya TV, Orlova DA, Drozd AV, (2020). Patent for invention 2714044, Russian Federation, (52) SPK B01J 37/03 (2019.08); B01J 21/02 (2019.08); B01J 21/04 (2019.08); C01F 7/02 (2019.08). Method of manufacturing micro-preparations. Patent holder: FGBOU VO SPbGABM. - No. 2019104489; declared 02/18/2019; publ. 02/11/2020, bul. No. 5.

Kalyuzhnaya, TV, (2019). Veterinary and sanitary examination and evaluation of nutria meat at different temperature and humidity storage conditions. International Veterinary Bulletin, 2: 86-92.

Khidhir, Zaid Abdulaali, Shamaail. (2021). The impact of storage duration and conditions on the formation of biogenic amines and microbial content in poultry meat. Iraqi Journal of Veterinary Sciences, 35(1): 183-188.

Malak NML, YHA AwadAllah and HMBA Zaki, (2020). Using histological and chemical methods for detection of unauthorized tissues addition in emulsion type meat product. Int J Vet Sci, 9(3): 438-442.

Orlova D, Drozd A (2020). Using the histological method to identify the turkey meat thermal state. Adv. Anim. Vet. Sci. 8(s2): 12-17.

Orlova D, Kalyuzhnaya T, Tokarev A, & Kuznetsov Y. (2020). New method for veterinary and sanitary control of defrosted meat and fish. International Journal of Veterinary Science, 9(2): 317-319.

Orlova DA, Kalyuzhnaya TV, Drozd AV, (2019). Evaluation of microimages of native muscle tissue preparations during veterinary and sanitary examination of meat. International Veterinary Bulletin, 2: 62-67.

Ruiz-Capillas C, & Herrero AM. (2019). Impact of Biogenic Amines on Food Quality and Safety. Foods (Basel, Switzerland), 8(2): 62.

Sinanoglou VJ, Cavouras D, Xenogiannopoulos D, Proestos C, & Zoumpoulakis P. (2018). Quality assessment of pork and turkey hams using FT-IR spectroscopy, colorimetric, and image analysis. Foods, 7(9): 152.

Triki M, Herrero AM, Jiménez-Colmenero F, & Ruiz-Capillas C. (2018). Quality assessment of fresh meat from several species based on free amino acid and biogenic amine contents during chilled storage. Foods, 7(9): 132.

Wojnowski W, Kalinowska K, Majchrzak T, Płotka-Wasylka J, & Namieśnik J. (2019). Prediction of the biogenic amines index of poultry meat using an electronic nose. Sensors, 19(7): 1580.