DOI: https://doi.org/10.21323/2414-438X-2024-9-4-323-334
Received 16.09.2024
Accepted in revised 18.11.2024
Accepted for publication 27.11.2024
Available online at https://www.meatjournal.ru/jour
Original scientific article
Open Access

GAS DISCHARGE VISUALIZATION AS A PROMISING TOOL FOR MEAT ANALYSIS DURING ITS STORAGE

Aleh U. Shkabrou[1,*], Irina M. Chernukha[2], Viktar D. Raznichenka[3,4], Lyubou U. Lazovikava[4], Irina I. Andreeva[4], Sviatlana N. Baitava[4]
[1] Ministry of Agriculture and Food of the Republic of Belarus, Minsk, Republic of Belarus
[2] V. M. Gorbatov Federal Research Center for Food Systems, Moscow, Russia
[3] Slutsk Meat Processing Plant JSC, Slutsk, Republic of Belarus
[4] Belarusian State University of Food and Chemical Technologies, Mogilev, Republic of Belarus

Keywords: *pork, meat storage, gas discharge, GDV, electrobioluminescence*

Abstract
Recently a lot of analytical methods have been developed, however, only a few of them have found application in the meat industry, since they do not provide sufficient understanding of the processes that occur in meat during its storage. The use of the GDV method for analyzing the food products quality has got a number of advantages, since it allows for quick and non-invasive getting of information on the food product condition, which capability may be quite promising for meat analysis. The study described in this paper is based on the GDV method application for analyzing the condition of the chilled pork at various stages of its storage and for detecting the peculiar changes in its quality.
The study analyzed samples of Sus scrofa m. longissimus dorsi stored at a temperature of 0 to 4 °C for five days. Using the software ImageJ to analyze the gas-discharge glow of meat, its main parameters were obtained, such as an area, average radius of glow and color characteristics. The most significant characteristics of the gas-discharge glow were determined, among which the glow area, shape factor, uncertainty and dispersion were focused on. It was shown that synchronously with the development of rigor mortis, there was a decrease in the area of the gas-discharge glow, and with its resolution and further storage of meat — a noticeable increase. The dispersion of the radius of meat glow by the end of the storage period increased by 2.03 times in comparison with the original value, and the gas discharge was unstable and featured a large number of streamer branches.
The influence of histostructural changes and fractional composition of proteins on the properties of the electromagnetic field during GDV of meat has been proven. It has been shown that the method of gas discharge visualization, along with histological studies, can be used to analyze meat during its storage and defining the depth of autolytic changes that take place in the meat.

For citation: Shkabrou, A. U., Chernukha, I. M., Raznichenka, V. D., Lazovikava, L. U., Andreeva, I. I., Baitava, S. N. (2024). Gas discharge visualization as a promising tool for meat analysis during its storage. *Theory and Practice of Meat Processing,* 9(4), 323–334. https://doi.org/10.21323/2414-438X-2024-9-4-323-334

Introduction

The analysis of meat quality via traditional physical (mechanical) and chemical methods is still reliable and still serves as "reference standard", but most of the methods are labor-consuming and possess a row of disadvantages, such as the necessity to violate the integrity of the sample as well as the pretty considerable duration of the analysis execution, which is not always acceptable in case of express monitoring or online monitoring. Therefore, to improve the efficiency of analysis and to minimize the associated losses, modern methods are being developed taking into account the reasons of speed, accuracy and non-invasiveness [1,2].

In most cases there are two main methods used for objective assessment of meat quality: subjective and objective ones. Subjective methods are usually based on sensory assessment, which includes visual analysis and taste sensations. The main disadvantage of these methods is their strong dependence on the expert's personal experience, low reproducibility and difficulties with quantitative assessment. Objective assessment methods traditionally include laboratory tests targeted at analyzing the physical and chemical properties of meat, as well as detecting the available microorganisms [3]. These methods provide high accuracy of results; however, they can vary in wide range due to the extreme complexity of the structure and composition of meat, as well as its biological origin, animal's growing conditions, transportation, duration of storage, etc. [4].

The issue of obtaining the reliable information on the meat quality and properties throughout the entire production process is one of the main problems that the meat industry encounters. Therefore, one of the tasks of food quality control is the development of reliable express methods of analysis that allow detecting the adulteration or quality reduction, which analysis, in its turn, can be implemented by electrophysical properties measuring. For example, measuring the specific electrical conductivity of meat allows for a high-precision assessment of its functional and

technological properties and detect the adulteration of the thermal condition [5], and application of electrical impedance spectroscopy allows determining the degree of freshness [6], predict the course of meat maturation and curing [7] and assess the influence of various defrosting methods on its quality [8]. In this regard the development of new methods for meat quality assessing through measuring its electrophysical properties is highly relevant and has high potential for its using in arbitration express-test of meat and meat products quality.

As is known [9,10], electric discharge is widely used to study the microrelief of a surface, the heterogeneity of the dielectric constant and studying the degradation of insulating materials. In emission spectroscopy, an electric discharge allows for the qualitative and quantitative determination of the composition of the sample under study [11]. Thus, one of the possible and appropriate electrophysical methods for analyzing meat quality may be the electrobioluminescence or gas-discharge visualization (GDV) method, which is based on the effect of air breakdown in result of the electric current impact on the object.

The research of the biological objects properties based on the analysis of the characteristics of gas discharge glow has a certain prospect. Absolute values of the parameters of gas discharge glow allow evaluation the properties of liquid-phase and solid-phase organic and inorganic objects (blood, water, plant crops, minerals, etc.) in order to perform quality control of plant materials, including fruits, seeds and grain crops, as well as the impact of various food products on the human health condition [12].

The GDV method is based on quantum biophysics and is exercised via Kirlian effect principle. During the process of GDV of the biological object being analyzed, a complex interaction of the applied pulsed electric field and the formed gas discharge takes places. The state of the surface structures and the electrical resistance of the internal structures of the object being analyzed determine the minimum value of the electrical breakdown voltage. Due to the heterogeneity, surface and volume properties of the object under analysis, the electromagnetic field is modulated, which influences on the gas discharge parameters, based on which a conclusion is made about the condition of the object. The processed GDV-gram (discharge image) allows analyzing a number of parameters which indicate the state of the object, and on this basis it is possible to make certain conclusions. The most important GDV parameters are intensity, perimeter, glow area, shape factor and entropy [13].

The scope of GDV method application is quite wide and nowadays there is a growing interest in its application in various areas of science and life [14], which interest is also proven by the growing number of publications on this topic, according to Science Direct, Google Scholar and PubMed.

As of today the hundreds of practical modifications of the GDV method have been developed worldwide depending on the geometric shape, parameters and physical properties of the studied objects of animate and inanimate nature. This method has shown a fairly high sensitivity and information content for assessing the physiological condition of plants and their antioxidant status [15], for finding pathologies in biological tissues [16], for analyzing blood and determining the properties of precious materials [17]. GDV analysis allows identifying the differences in the glow of electrolyte solutions of different concentrations and compositions, as well as to differentiate natural and synthetic essential oils with identical chemical composition [18].

In the works [19,20] it is shown that the gas-discharge visualization method in combination with automatic analysis of digital gas-discharge images can serve as an efficient additional tool for the prompt assessment of the heterogeneity and hidden defects of wheat seeds. The results presented in these works do not contradict to Kolesnikov et al. [21], who proved that GRV of soft wheat allows obtaining more complete characteristic of biological and economic suitability of the seed material. The results obtained by the authors allow prediction of field germination of the seeds and their potential yield, allow identifying the main defects of seeds, prediction of diseases development and probable changes of plant resistance to diseases.

It should be noted that despite the variety of areas of application of this method, it has found its greatest reflection in biology and medicine for diagnostics of various diseases [13], for assessment of the body functional state [22], for analysis of the antigen-antibody reaction [23], etc., since the GDV method combines non-invasiveness, safety, methodological simplicity, simplicity of use and high throughput [22]. However, the application of the GDV method in medicine still remains highly controversial, as the GDV glow characteristics have high variability and/or little information [24]. This suggests that the literature related to this area of research should be reviewed and questioned [25].

Based on the known fact about the ability of the GDV method to detect minor changes in the physicochemical characteristics of materials and organic and inorganic substances solutions, some researchers have tried to study the possibility of using the GDV to assess the quality of food products and meat. Thus, the authors [26] have established that the corona discharge of leaves and fruits provides useful information on the stressed state of plants and defines the variety of the plant. Laurent et al. in their study [27] presented the possibility of using the GDV to assess the impact of the fattening method on the quality of rabbit meat, and in [28] the effect of preliminary exposure of chicken meat to a structured aqueous solution of fructose on the organoleptic properties and parameters of the GDV was shown. The authors showed that after processing poultry meat in an aqueous solution of fructose, the number of photons increased and the energy of GDV luminescence rose up.

Despite the data obtained by the authors on the study of the GDV parameters of meat and food products, today the application of this method in the food industry is quite

limited due to insufficient study, the lack of scientifically substantiated information on the relation between the parameters of the GDV glow of meat and the physicochemical and other processes occurring there. It should also be noted that the lack of a standardized methodology for studying the meat properties via the GDV method leads to gross mistakes that cause errors and significant deviations of numerical values from the average, which makes this area of research deviant.

For this reason, further detailed studies are required to assess the influence of various factors such as storage duration, pH, fractional composition of proteins, the state of muscle tissue microstructures, fat content, etc. on the electrophysical properties of meat, with subsequent development of the standardized GDV method.

Thus, the purpose of this study is the adaptation of the GDV method for meat quality research, as well as the scientific substantiation of the influence of physicochemical processes in the meat during its storage on the GDV parameters.

Materials and methods

The chilled samples of the rib-eye — i. e. the longest back muscle of pork (*Sus scrofa M. longissimus dorsi*) 24 hours after slaughter were used as the objects of the study. Meat samples were taken from three different half-carcasses of two-breed crossbreed pork (large white × landrace) at Mogilev Meat-Processing Plant OJSC, Republic of Belarus, and were delivered to the laboratory in an isothermal bag within one hour from the time of collection. The meat was packed in polyethylene bags and stored at a temperature of 0 to plus 4 °C for 5 days. Sampling and sample preparation for testing were done in accordance with GOST 7269-2015[1] and GOST R 51447-99 (ISO 3100-1-91)[2]. Every day the samples of *longissimus dorsi* pork muscle were analyzed according to the following methods for 5 days of storage.

GDV of meat

From the meat pre-warmed to 20 °C the sample was cut out with a cylindrical knife so that the muscle fibers lay across the blade of the knife. The diameter of the samples was 20 mm, and the thickness was 5 mm.

Pieces of meat were carefully arranged on a clean glass electrode, which consisted of two 1 mm thick glass plates air-tightly sealed at a distance of 1.5 mm from each other, the space between which was filled with 3M of KCl solution. Grounding electrode was affixed to the meat, the GDV camera, which operating principle is shown in Figure 1, was covered to prevent access of light. To create the electrobioluminescence effect, high-frequency current and voltage generator was used, which included a signal

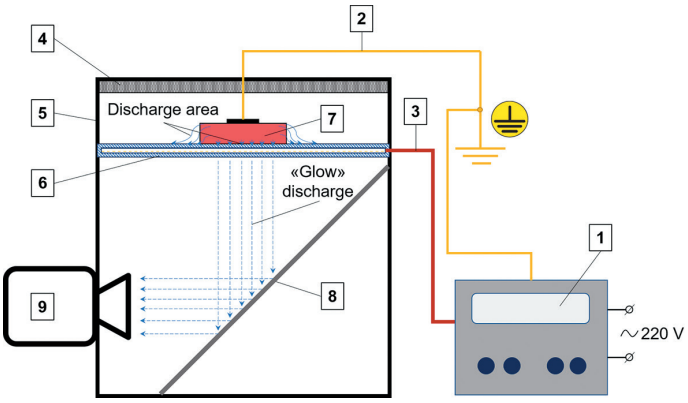


Figure 1. Schematic diagram of the GDV device for meat: 1 — generator of high-frequency current and voltage, 2 — grounding electrode, 3 — high-voltage wire, 4 — GDV camera cover, 5 –body of the GDV camera, 6 — glass electrode, 7 — object of study, 8 — mirror, 9 — photo camera

generator Power Pulse Modulator PWM -OCXi v3 (RM-Cybernetics LTD, UK) and ignition coil NGK 48342 (NGK, Germany), connected to high-voltage wire that was wired to the glass electrode. When the generator was turned on, the sample was exposed to high voltage (20–30 kV) and frequency (250–400 Hz) pulses, with a duty cycle of 80%. The total pulse exposure time was 30 s.

During the GDV of meat, a series of photographs were taken using a camera Canon SLR EOS400 D (Canon, Japan). The lens of the camera was directed at the special mirror (Figure 1). The shooting frequency was 1 frame per second. The images were analyzed with the software ImageJ.

To determine the measurement error and assess the degree of environmental factors influence on the GDV characteristics, a reference object was used which properties did not change throughout the experiment, unlike the object of study. For that a steel cylinder with a diameter of 20 mm and a height of 5 mm was used as the reference object.

To highlight various visual features of the obtained photo images, to reduce information redundancy, to provide contrast and better visualization of the discharges, the obtained images were pseudo-colored with the help of the software ImageJ, which process is based on dividing the brightness spectrum of the image into several K_i parts of equal area. Each section is assigned to a specific color $S_k(b) = \text{const}$, in result of which all points which luminescence intensity lies within the defined interval are displayed on the screen in the same color.

As a result of the analysis of the obtained images (GDV-grams), the following parameters were calculated:

1) the luminescence area (S), which was determined using the software ImageJ;
2) the average radius of the glow (R), which was determined using the software ImageJ as the arithmetic mean of the glow radii, which values are equal to the distance between the first and last points of non-zero intensity lying on the beam from the center of the glow

[1] GOST 7269-2015 "Meat. Methods of sampling and organoleptic methods of freshness test." Moscow: Standartinform, 2019. Retrieved from https://docs.cntd.ru/document/1200133105 Accessed August 20, 2023 (In Russian)
[2] GOST R 51447-99 "Meat and meat products. Methods of primary sampling." Moscow: Standartinform, 2018. Retrieved from https://docs.cntd.ru/document/1200028183 Accessed August 19, 2023 (In Russian)

at an angle $\alpha \in [0;2 \cdot \pi)$ to the vertical axis;

3) the shape coefficient (K) (dimensionless value equal to 4π for a circle and increasing along with rising complexity of the figure shape), which was determined by formula (1)

$$K = L / 2\pi R, \quad (1)$$

where L is the length of the outer glow contour perimeter;

4) color parameters of luminescence in the CIE coordinate system L*a*b*, which was determined using the software ImageJ;
5) uncertainty (N) associated with the estimate is the experimental standard deviation of the mean value, and is equal to the positive square root of the experimental dispersion of the mean value.

The uncertainty $N(x_i)$ for the measurement result $x_i = \overline{x}_i$, calculated as the arithmetic mean, was determined using the following formula (2)

$$N(x_i) = u_A(x_i) = \sqrt{\frac{1}{n(n-1)} \sum_{g=1}^{n} \left(x_{ig} - \overline{x_i}\right)^2}. \quad (2)$$

6) the dispersion of the glow radius (D) was calculated as the arithmetic mean deviation of the squares of the difference in the glow radii of the general totality from their mean value R.

The deviation of environmental parameters did not exceed 8%, so their influence was neglected in order to simplify the calculations.

Analysis of molecular weight distribution of the protein fractions by one-dimensional electrophoresis method

100 mg of sample was taken and 2000 µl lysing solution (9M urea, 5% β- mercaptoethanol, 2% triton X-100, 2% ampholines with a pH of 3–10) was added. The resulting homogenate was clarified by centrifugation at 14,000 rpm for 20 minutes. After that, the supernatant was separated and protein buffer was added to it in a ratio 1:1. The protein buffer was prepared by 1 ml of sodium dodecyl sulfate (SDS) 10%, 250 µl of concentrated β-mercaptoethanol, 625 µl of Tris-HCl 0.5 M, 1.5 g of urea, added to Eppendorf tubes, then bromophenol blue was added until reaching a dark color and brought to a volume of 5 ml with water, and then the samples were heated in a boiling water bath for 5 minutes.

To perform vertical gel electrophoresis, a VE-20 chamber (Helikon, Russia) was used and filled with 12.5% polyacrylamide gel. 6% gel was poured over its top, and the wells were made in its surface for nesting the samples. The sample to be studied was added in amount of 10 µl. Solution containing 25 mM of tris-HCl, 192 mM of glycine and 0.1% SDS was used as a buffer. Electrophoresis was run under the following parameters: the first 30 minutes — at 60 V, and then at 120 V until the dye front (bromophenol blue) reached the lower edge of the gel plates.

The proteins were dyed with Coomassie G-250 in a solution of the following composition: 10% acetic acid, 25% isopropanol, 0.05% Coomassie G-250. To remove the unbound dye, 10% acetic acid was used.

For computer densitometry, one-dimensional electropherograms in a wet state were used. Their full digital images were obtained with scanner Bio-5000 Plus (Serva, Germany) in 600 ppi 2D-RGB mode. The obtained digital images were edited in the graphic editor ImageJ.

Histological examination of meat

Histological examination was implemented in accordance with GOST R31479-2012[3] and GOST 19496-2013[4]. The sections were evaluated with the microscope Micromed-1 var.2-20 (Micromed, Russia). The muscle fiber diameter were measured with the software ImageJ.

Determination of shear force

The shear force values were obtained via Warner-Bratzler method according to [29].

Statistical analysis

Statistical analysis of the results was run using software Excel 2019 (Microsoft, USA). The results were considered significant at $p < 0.05$. To assess any correlations between various factors, r-Pearson correlation coefficients were calculated.

Results and discussion

Analysis of meat GDV-grams

As a result of the analysis of the gas discharge glow of the chilled meat and the reference object, significant differences were detected in the structure of their corona discharge (Figure 2). Glow of the reference object (Figure 2a) showed the uniform distribution of the streamers5 and the absence of their branching. The corona discharge was stable throughout the entire period of exposure to electrical pulses.

In turn, the figures formed during electrobioluminescence of meat, in comparison with the reference standard, featured more chaotic character, which changed synchronously with the lengthening of the samples storage duration (Figure 2b and 2c).

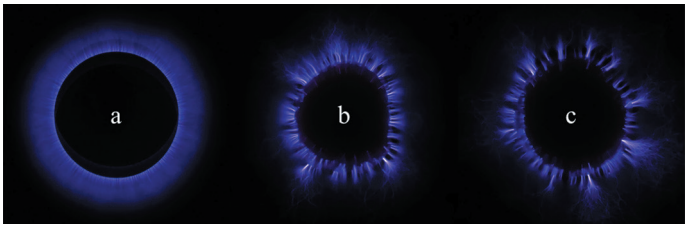


Figure 2. Gas discharge glow of the reference object (a), meat in the first day (b) and meat in the fifth (c) day of storage

[3] GOST R 31479-2012 "Meat and meat products. Method of histological identification of composition." Moscow: Standartinform, 2019. Retrieved from https://docs.cntd.ru/document/1200097485 Accessed August 19, 2023 (In Russian)

[4] GOST 19496-2013 "Meat and meat products. The method of histological investigation." Moscow: Standartinform, 2019. Retrieved from https://docs.cntd.ru/document/1200107317 Accessed August 20, 2023 (In English)

[5] Streamer is a set of thin branched channels through which electrons and ionized gas atoms move, being organized in peculiar streams.

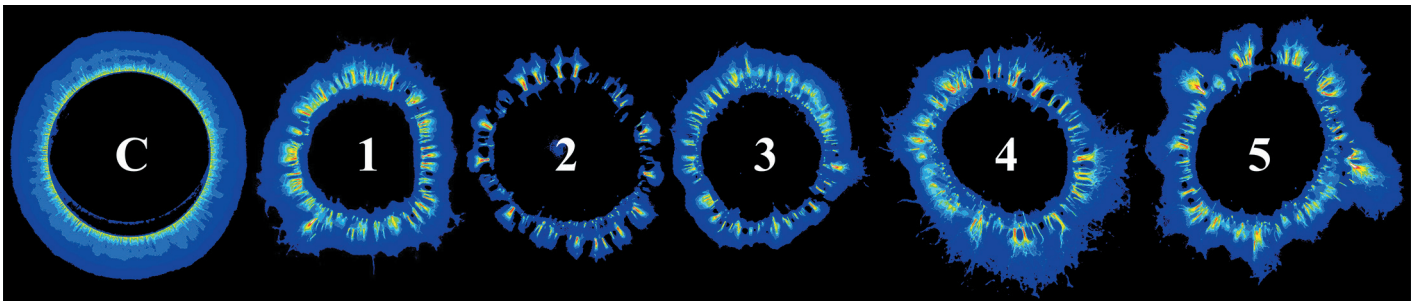


Figure 3. GDV-grams after pseudo-staining: reference object (C); chilled meat on: the 1st day of storage (1); the 2nd day of storage (2); the 3rd day of storage (3); the 4th day of storage (4); the 5th day of storage (5)

As can be seen from Figure 2, the glow structure formed by sliding and avalanche discharges is more symmetrical and uniform in the meat at the initial stage of storage. During further storage of meat, sharp spikes and more complex Lichtenberg figures were observed, the gas discharge glow became more chaotic and non-uniform, which was expressed in a change in the corona discharge geometry, formation of breaks and thickened streamers of various lengths (Figure 3).

During the first day of meat storage, when the meat was placed into a high-intensity and high-frequency electric field, its gas-discharge glow was characterized by a stable, uniform, and bright corona structure, without large breaks and defects in the inner and outer contour lines. The gas discharge was represented by closely spaced streamers with a dense and clearly defined structure. The zone with the highest discharge density was distributed in the meat evenly and continuously. The streamer channels did not exceed 7 mm in length and were stable for 30 s.

On the second day of meat storage, its gas-discharge glow was stable and without sharp bursts. However, in the GDV glow the clear signs of changes in the discharge geometry were found. As can be seen from the Figure 4, the glow area of the samples on the second day decreased down by 15.3% in comparison with the initial value. Increase of the dispersion values in the glow radius by 9.6% indicated the formation of heterogeneity and chaos in the discharge. Streamer channels became thicker and shorter by 20.8%, which was detected by the average glow radius decrease. The location of the discharge excitation points was uniformly distributed, but their number significantly decreased in comparison with the same parameter on the first day of storage, which is visible in the Figure 3.

As can be seen from the Table 1, the spectral characteristics of the discharge changed synchronously with the change in the geometry. The gas-discharge glow of meat on the second day of storage became dimmer in comparison with its glow on the first day, which clearly shows the decrease in the lightness L* and saturation *C* values * of the corona discharge color by 48.5% and 53.2%, respectively.

When analyzing the shape factor (Table 1), which value indirectly characterizes the density and uniformity of the streamers distribution, it was defined that intensity of this parameter insignificantly changed during storage, and practically did not go beyond the limits of reliable difference ($p < 0.05$). On the second day of storage, this parameter increased by 7.7%, and on the third it decreased down by 14.7% in comparison with the initial value on the first day. In its turn, on the third day of chilled pork storage, a partial restoration of the corona discharge structure up to its original condition was observed, while the area of the gas discharge glow S and the luminosity of the gas discharge L* were 1.2 times less in comparison with the first day, which evidenced a lower intensity of the glow.

On the fourth day of pork samples storage, sharp change in the corona discharge outlines was observed. When high-voltage and high-frequency currents passed through muscle tissue, the resulting gas discharge turned to be unstable, with sharp surges and breaks. The streamers became thicker and longer, and the corona discharge outlines were more chaotic and unstable, as evidenced by a statistically significant increase in the dispersion of the glow radius by 1.57 times and uncertainty by 1.23 times. At the same time, the area of meat glow became the same as it was on the first day of the meat storage (Figure 4).

On the fifth day of storage, an increase in the average rate of change in the dispersion of the glow radius was found from 52 units / day (on the first day of storage) to 247 units / day. The gas-discharge glow of chilled meat was unstable and had a large number of streamer branches (Figure 3), while the glow area changed insignificantly in

Table 1. Changes in the parameters of GDV-grams of the meat during its storage

Shelf life, days	L*	a*	b*	*C**	*K*
1	3.01 ± 0.12	6.44 ± 0.26	–22.15 ± 0.88	23.07 ± 0.92	6.65 ± 0.30
2	1.55 ± 0.06[a]	2.70 ± 0.10[a]	–10.45 ± 0.38[a]	10.80 ± 0.39[a]	7.16 ± 0.29
3	2.48 ± 0.09[a]	5.28 ± 0.19[a]	–18.87 ± 0.68[a]	19.59 ± 0.71[a]	5.67 ± 0.23[a]
4	2.67 ± 0.10	5.59 ± 0.21	–20.12 ± 0.75	20.88 ± 0.78	5.91 ± 0.25
5	2.79 ± 0.13	5.81 ± 0.27	–21.89 ± 1.02	21.66 ± 1.01	6.35 ± 0.30

L*, a*, b* — color parameters; *C** — saturation; *K* — form factor;
[a] — the mark of the values that are statistically significantly different ($p < 0.05$) from the previous one.

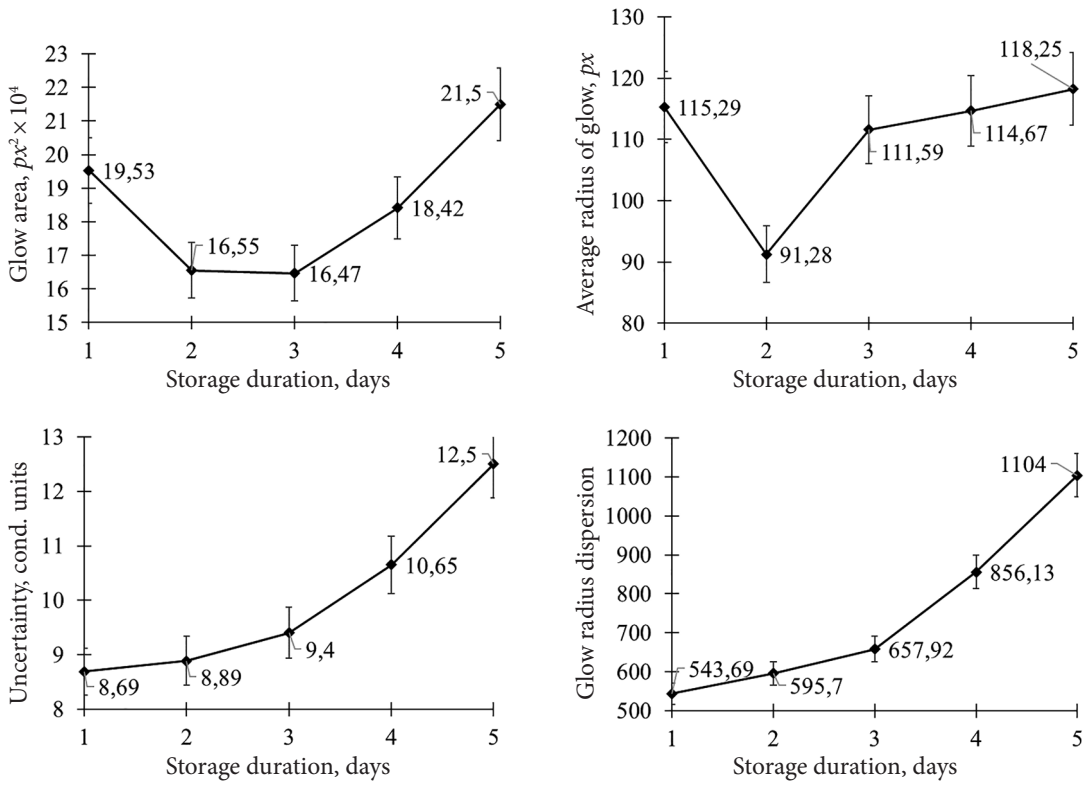


Figure 4. Dynamics of geometric parameters changes of GDV glow of chilled meat during its storage

comparison with that on the first day of storage. The uncertainty and dispersion of the glow radius of meat, indicating the heterogeneity of the glow contour, reached the maximum value (Figure 4).

It should also be noted that the uncertainty and dispersion of the luminescence of the metal reference standard throughout all studies did not exceed 2 ± 0.4 units and 50 ± 15 units, respectively, depending on environmental conditions.

Based on the obtained data, a hypothesis was offered that biochemical and microstructural changes in meat that take place during storage [30] have a strong influence on the structure and geometry of the gas discharge.

To confirm or decline this hypothesis, simultaneously with the study of gas discharge glow of meat, the studies were conducted on the fractional composition of proteins and the histostructure of muscle tissue of chilled pork during its storage, and the correlation analysis was conducted.

Analysis of the fractional composition of proteins

Analysis of 1D electropherograms of meat proteins during the storage allowed detecting the significant differences in the fractional composition of proteins and their concentration, determined by the intensity of protein zones staining.

The proteins contained in the identified zones, based on the database [31], had different origins and were identified as: connective tissue, myofibrillar and metabolic proteins.

Based on the densitometric analysis of 1D electropherograms (Figure 5), it is visible that during the first day of storage, the relative content of the high-molecular fraction of proteins within the range of 291–51 kDa and the medium-molecular fraction within 51–42 kDa increased against the background of the fraction decrease within the range 42–20 kDa.

These changes evidenced that autolysis processes took place in the meat, thus resulting in parallel aggregation and partial disintegration of various protein structures.

Based on the color intensity changes in the protein zones with a molecular weight of 239–248 kDa and 205–213 kDa, it is possible to assume changes in myosin fractions. Thus,

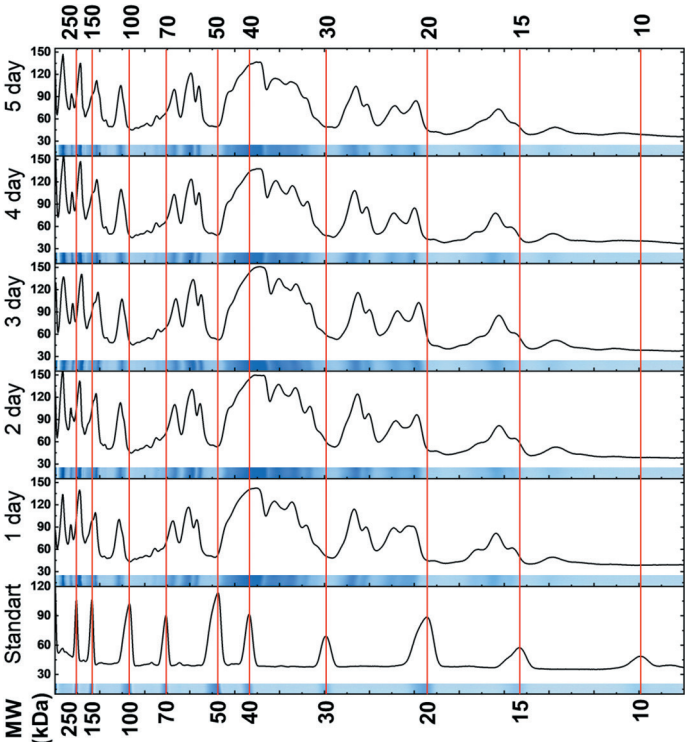


Figure 5. Results of densitometry of 1D electropherograms of *Sus scrofa M. longissimus dorsi* proteins during storage

in result of autolytic processes, on the second day of storage a decrease was observed in the relative amount of the myosin fraction by 13.9%. This could be caused by the development of *rigor mortis* and the formation of complexes between F-actin and myosin, which process is peculiar for *rigor mortis*. The following increase of relative content of low-molecular protein fractions up to the fourth day obviously indicated the destruction of actomyosin molecules and myosin aggregates down to its heavy (200–223 kDa) and light (16–20 kDa) chains.

As a result of bioinformatics analysis, it was established that the desmin fraction (50–53 kDa) went through strong destruction starting from the third day of storage, which coincides with the beginning of the *rigor mortis* resolution, which was confirmed by the results of histological analysis. On the third day after slaughter, the mass content of protein substances with a molecular weight of 51–52 kDa reached its maximum value of 3.33%. With further storage the mass content of this fraction decreased down to 3.12% on the fifth day. As a result of the weakening of the myofibril structure due to the degradation of desmin and intramuscular connective tissue under the action of calcium ions, the meat structure got looser and softer, as evidenced by the shear force decrease.

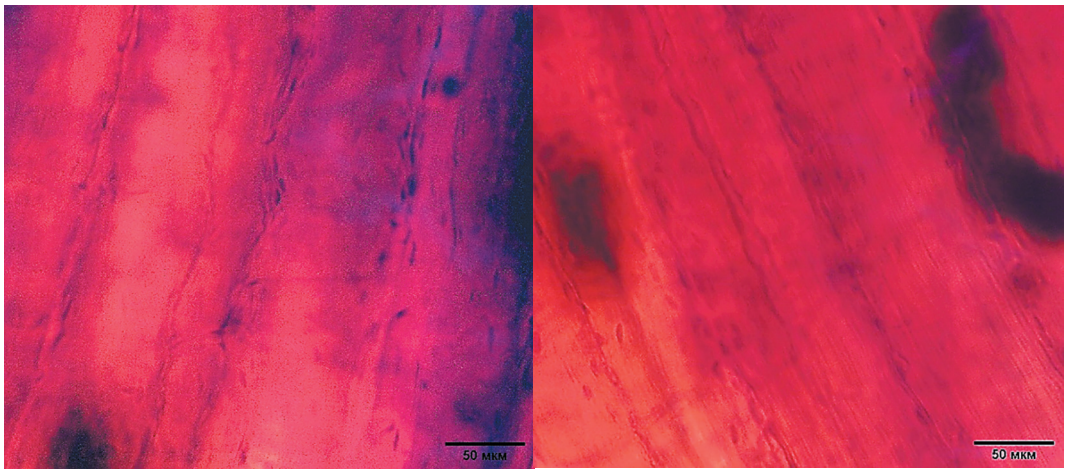
During meat storage, there was an increase in the amount of low-molecular proteins, as indicated by intensity changes of protein zones of the fraction with a molecular weight within the range of 10–18 kDa. Thus, from the data presented above, it is clear that during storage, high-molecular protein substances partially or completely decomposed with the formation of medium- and low-molecular fractions, which is also consistent with the data presented in the review of Warner et. al. [32]. The above-indicated changes in the proteins fractional composition entailed the relevant histostructural changes described below.

Study of meat histostructure

As it was already noted, the formation of high-molecular protein complexes in the first two days of meat storage was the main cause of microstructural changes in muscle tissue. Thus, in the first day of storage, asynchronous contraction of muscle fibers was observed with weakening of transverse striation and enhancing of longitudinal striation with oval contraction nodes formation (Figure 6). The muscle fibers featured an irregular shape due to deformation changes during *rigor mortis*. The fibers were wavy, tightly adjacent to each other. In some places partial relaxation of muscle fibers and restoration of transverse striation were detected. In the contraction nodes, there were ruptures in the muscle fibers sarcolemma with preservation of the fiber content and its internal structure. The diameter of muscle fibers in the first day was 80 ± 20 μm. The fibers were wavy, tightly adjacent to each other. The shear force was equal to 47.2 ± 0.11 N. On the second day of storage, the diameter of muscle fibers decreased by 18.7% and was 65 ± 15 μm, and the shear force decreased by 1.63 times in comparison with the initial value.

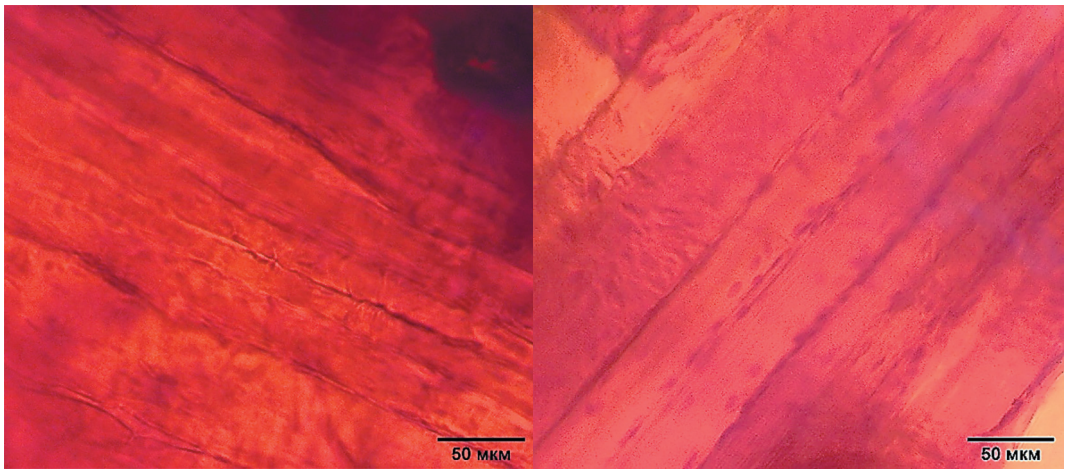
On the third day of meat storage, the histological sections of muscle tissue showed *rigor mortis* resolution and the beginning of meat maturation (Figure 7a). Histological changes in muscle tissue were expressed by the destructive processes development in meat, which became more and more intense depending on the storage time of the meat. The beginning of fiber fragmentation and loosening of connective tissue fibrous elements with their further detachment from muscle fibers were observed. No signs that evidenced the presence of contracted muscle fiber were detected. Widening of the interfiber space and increasing number of microcracks was noticeable in the sections. In some cases, separation of the sarcolemma fibers and its granular disintegration were observed. The fiber diameter was 40 ± 10 μm, and the shear force was 19.9 ± 0.9 N.

On the fourth day of storage the muscle fibers were loose and unevenly stained (Figure 7b). Local lysis was observed in some spots. The number of transverse-slit-like breaks of the muscle fibers integrity increased, while the structure of nuclei, transverse and longitudinal striations in the fragments still partially preserved. The fibers slightly increased in diameter up to 55 ± 15 μm. The shear force was 16.9 ± 0.9 N.



(a) (b)

Figure 6. Microstructure of meat on the first day (a) and second day (b) of storage (40× magnification)



(a) (b)

Figure 7. Microstructure of the meat in the third day (a) and fourth day (b) of storage (40× magnification)

On the fifth day, the muscle fibers became more fragmented, the number and size of transverse-slit-like lesions observed in the sections rose up (Figure 8). The sections got a basophilic color. Granular disintegration of individual fragments, fibers separation and local destruction of the sarcolemma were observed. Longitudinal striation was slightly distinguishable, and transverse striation was practically absent. The fiber diameter and shear force, in comparison with the meat samples on the 4th day of storage, remained almost the same and amounted to 57 ± 15 μm and 16.7 ± 0.9 N, respectively.

Thus, it was established that during the pork storage the irreversible changes in the muscle tissue microstructure occurred, caused by autolytic breakdown of myofibrils and destruction of muscle fibers, which is consistent with the materials presented in the review by Warner et. al. [32] and the results obtained by Soldatova et. al [33].

Correlation analysis and discussion of results

Correlation analysis of the characteristics of GDV-grams, meat histostructure and protein fractional composition (Figure 8) allowed establishing that the structure and geometry of the gas discharge of meat were closely related to post-slaughter changes in protein fractions, which led to the meat microstructure and cell membranes destruction. As a result of the analysis of data obtained during the study, a high correlation was established between the parameters of GDV-grams and the relative content of high-molecular and medium-molecular protein fractions. The change of color intensity in the protein zones with molecular weights of 281–291 kDa, 239–248 kDa, 130–140 kDa, 65–67 kDa, 51–42 kDa, 33–25 kDa and 17–19 kDa, which, based on the database [31] corresponded to various structural and myofibrillar proteins fractions, took place synchronously with the geometric and spectral characteristics change of the meat gas-discharge glow.

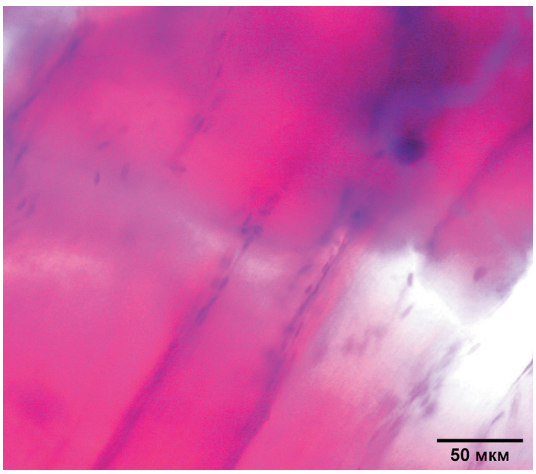


Figure 8. Microstructure of the meat in the fifth day of storage (40× magnification)

Among the most significant characteristics of meat GDV-grams presented for analysis, were the average glow radius and shape coefficient, as well as the dispersion of the glow radius and uncertainty, which reliably correlated with the change in the meat shear force during its storage.

Based on the detected changes in the structure of the meat gas discharge, it is possible to define several main stages depending on autolysis stages, which are described below.

1) During the *rigor mortis* development, the GDV-grams of meat were characterized with uniform and stable corona discharge. The more expressed microstructural changes in meat at *rigor mortis* stage were, the less intense its GDV glow was.
2) When *rigor mortis* resolved, a decrease in the area and an increase in the dispersion of the glow radius were observed, which indirectly evidenced the muscle fiber destruction onset. The significant decrease of the corona discharge brightness and saturation could also evidence the disintegration of protein structures and changes in the meat physical properties.
3) After the *rigor mortis* resolution, the corona discharge structure was partially restored, but remained less intense. This loss of intensity could be caused by complex and irreversible microstructural changes.
4) During the meat maturation and its deep autolysis, sharp changes in the corona discharge contours and uncertainty increase were observed, which happened simultaneously with decrease in the high-molecular proteins fractions and the increase in medium-molecular and low-molecular proteins fractions.

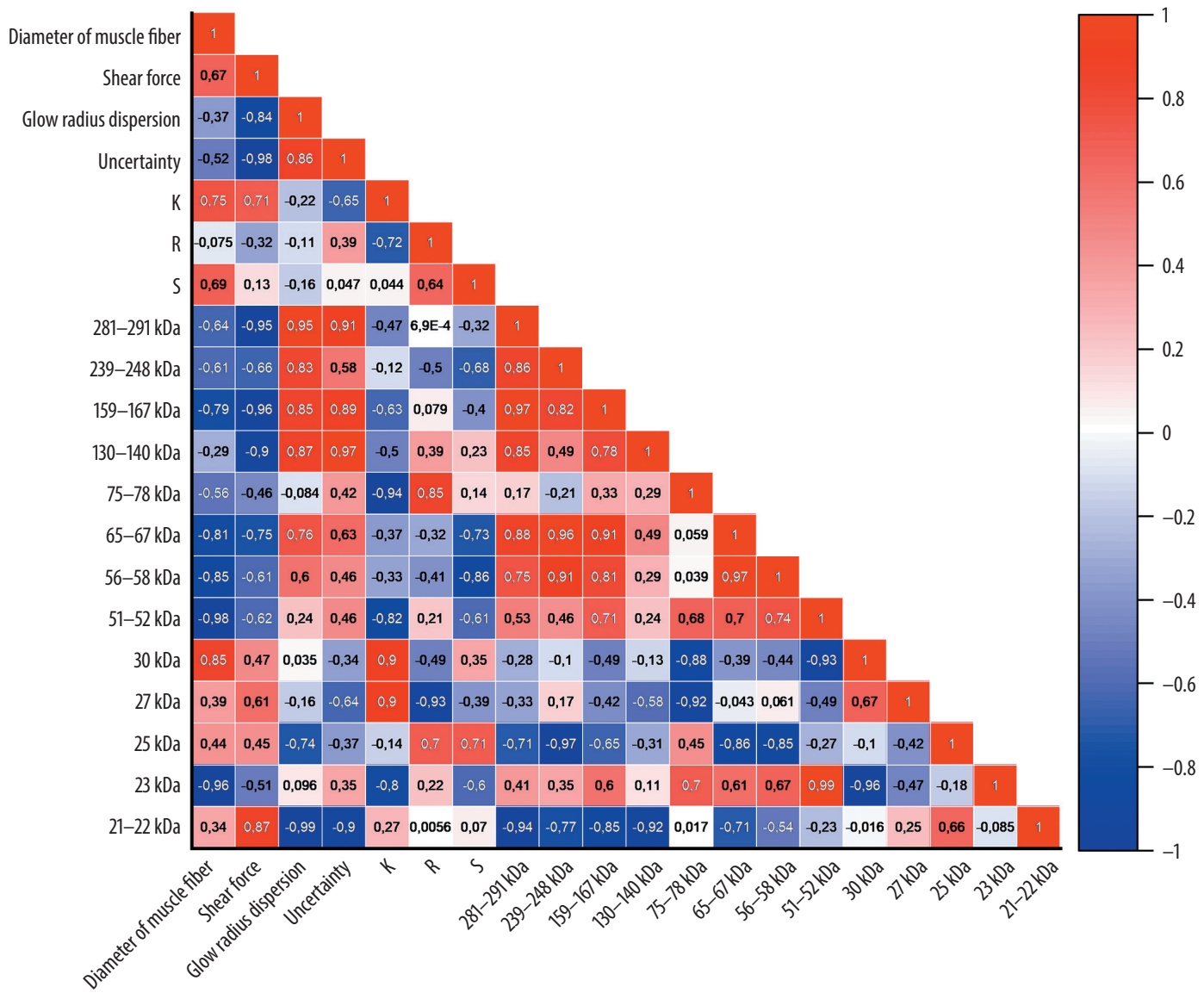


Figure 9. Correlation matrix of the characteristics of the meat GDV-grams, the results of histological studies and the molecular weight distribution of protein fractions

The high correlation between the GDV parameters and the relative content of some protein fractions confirms that changes in the fractional composition of proteins are closely related to physical changes that occur in the meat. The influence of protein fractions on the structure of the gas discharge of meat can be evaluated through several key aspects, since the proteins state plays crucial role in maintaining the physical and chemical structure of muscle tissue [34].

High-molecular proteins such as myosin and actin provide for muscle contractile activity and provide muscle contractions. Desmin maintains the structural and mechanical integrity of the cell during contraction, while helping to transmit force and withstand the longitudinal load. When they disintegrate during autolysis, the mechanical strength of the fibers decreases, the orderliness and homogeneity of muscle tissue decrease, which probably led to a more chaotic and unstable gas-discharge glow and a change in the geometry and intensity of GDV-grams.

The increase in the number of microcracks and muscle fibers fragmentation, caused by autolytic changes, obviously affect the ionic composition and contribute to increase of tissue electrical conductivity [35]. This leads to variations in the electrical properties of meat and provided the decisive effect on initiation of gas discharge when pork samples are placed in a high-intensity electromagnetic field, which reason does not contradict the results obtained by Arkhipov et. al. [19]. Structural changes could also affect the degree of tissue polarization [36], which could change the electric field distribution and the pattern of the GDV [37].

Thus, along with an increase in the storage duration of the meat, changes occurred in the structure of the gas discharge glow against the background of peculiar changes in the fractional composition of proteins and the microstructure of muscle tissue. The heterogeneity of the surface and volume of muscle tissue that undergo changes during storage, provide for a significant effect on the electromagnetic field parameters, which does not contradict the data presented in the work of Priyatkin et. al. [20].

Gas discharge glow can serve as a peculiar indicator of meat quality and the autolytic changes degree. However, for deeper understanding of the processes occurring in meat during GDV, it is necessary to consider the possibility

of application of the other methods of analysis that take into account biophysical and electrochemical changes, and to evaluate the effect of various storage conditions on the meat quality.

It is worth noting that at the time of this work writing, no publications were found on the topic being studied that described the application of GDV for analyzing meat quality during its storage and the effect of autolytic changes within on the gas discharge formation. Since there was no reference point of comparison, the authors' conclusions on the work results were based on researches in the other fields of science.

These findings may be useful both for scientific researches in food processing and for their practical application in the meat industry, especially within the framework of quality control and the storage conditions optimization.

Conclusion

As the result of the implemented work, possibility of adjusting the GDV method for quality research meat was considered. The correlation between the parameters of GDV glow of meat and the development of autolytic processes and the physicochemical processes which take place in the meat during its storage was discovered and scientifically substantiated. The influence of physicochemical changes in the meat on the topographic features of the streamers distribution, the structure of the gas discharge as well as its geometric and color parameters, such as area, uncertainty, dispersion and average radius of glow were presented.

Depending on the meat autolysis stage, certain changes in the glow area and shape factor were observed. During the development of *rigor mortis*, its resolution and subsequent maturation of the meat, significant increase in the glow radius dispersion was observed. The characteristics of GDV-grams that describe changes in the state of meat raw materials during their storage are confirmed by the results of studies of histological structure and molecular mass distribution of the meat proteins.

The obtained data highlight the potential of the GDV method for monitoring the changes in the meat quality and changes in its properties related to the storage processes. However, for the effective implementation of GDV in the meat processing industry, further research is required to standardize the methodology and establish the correlation between GDV parameters and changes in the physicochemical characteristics of the meat. This will allow increasing the safety and improve the quality of meat products, which is an important issue for the food processing industry.

REFERENCES

1. Biswas, A.K., Mandal, P.K. (2020). Current perspectives of meat quality evaluation: Techniques, technologies, and challenges. Chapter in a book: Meat quality analysis. Academic Press, 2020. https://doi.org/10.1016/B978-0-12-819233-7.00001-X
2. Wu, X., Liang, X., Wang, Y., Wu, B., Sun, J. (2022). Non-destructive techniques for the analysis and evaluation of meat quality and safety: A review. *Foods*, 11(22), Article 3713. https://doi.org/10.3390/foods11223713
3. Shi, Y., Wang, X., Borhan, M. S., Young, J., Newman, D., Berg, E. et al. (2021). A Review on meat quality evaluation methods based on non-destructive computer vision and artificial intelligence technologies. *Food Science of Animal Resources*, 41(4), 563–588. https://doi.org/10.5851/kosfa.2021.e25
4. Geletu, U. S., Usmael, M. A., Mummed, Y. Y., Ibrahim, A. M. (2021). Quality of cattle meat and its compositional constituents. *Veterinary Medicine International*, 2021, Article 7340495. https://doi.org/10.1155/2021/7340495
5. Zakharov, A.N., Sus' E.B. (2013). Evaluation of the thermal state of the meat on the electrical conductivity. *Vsyo o Myase*, 4, 26–27. (In Russian)
6. Huh, S., Kim, H.-J., Lee, S., Cho, J., Jang, A., Bae, J. (2021). Utilization of electrical impedance spectroscopy and image classification for non-invasive early assessment of meat freshness. *Sensors*, 21(3), Article 1001. https://doi.org/10.3390/s21031001
7. Damez, J.-L., Clerjon, S., Abouelkaram, S., Lepetit, J. (2008). Beef meat electrical impedance spectroscopy and anisotropy sensing for non-invasive early assessment of meat ageing. *Journal of Food Engineering*, 85(1), 116–122. https://doi.org/10.1016/j.jfoodeng.2007.07.026
8. Abie, S.M., Martinsen, Ø.G., Egelandsdal, B., Hou, J., Bjerke, F., Mason, A. et al. (2021). Feasibility of using electrical impedance spectroscopy for assessing biological cell damage during freezing and thawing. *Sensors*, 21(12), Article 4129. https://doi.org/10.3390/s21124129
9. Derusova, D.A., Vavilov, V.P., Nekhoroshev, V.O., Shpil'noy, V. Yu., Zuza, D.A., Kolobova, E.N. (2024). Analysis and NDT applications of a gas discharge electroacoustic transducer. *Russian Journal of Nondestructive Testing*, 60, 119–131. https://doi.org/10.1134/S106183092470061X
10. Korotkov, K.G., Shustov, M.A. (2017). Kirlian effect — past and modernity. Saint Petersburg — Tomsk, 2017. (In Russian)
11. Gruner, H., Lorig, C. H. Mineral processing. Encyclopedia Britannica. Retrieved from https://www.britannica.com/technology/mineral-processing. Accessed November 9, 2024.
12. Priyatkin, N. S., Korotkov, K. G., Arkhipov, M. V., Gusakova, L. P. (2016). Experience of gas discharge visualization technique in terms of food safety problem solution. *Health is the Basis of Human Potential: Problems and Solutions*, 11(2), 808–809. (In Russian)
13. Oliinyk, V., Babakov, M., Lomonosov, Y., Oliinyk, V., Zinchenko, O. (2022). Modernization of gas discharge visualization for application in medical diagnostics. *Technology Audit and Production Reserves*, 4(1(66)), 21–29. https://doi.org/10.15587/2706-5448.2022.263397
14. Grozdeva, D., Dikova, T. (2018). Gas discharge visualization — historical developments, research dynamics and innovative applications. *Scripta Scientifica Salutis Publicae*, 4, 27–33. https://doi.org/10.14748/sssp.v4i0.5448
15. Maslennikov, P. V., Chupakhina, G. N., Krasnoperov, A. G. (2013). The use of a gas discharge visualization technique at estimation the antioxidant status of plants in the conditions of cadmium toxic effect. *Vestnik IKBFU. Natural and Medical Sciences*, 7, 14–21. (In Russian)

16. Minosyants, K. A., Tumaev, E. N. (2018). Gas discharge visualization method for determining pathologies of biological tissues. *Scientific and Technical Journal of Information Technologies, Mechanics and Optics*, 18(5), 765–770. https://doi.org/10.17586/2226-1494-2018-18-5-765-770 (In Russian)
17. Korotkov, K. G., Orlov, D. V., Velichko, E. N. (2011). Application of stimulated electro-photon emission to analysis of liquids. *Journal of Instrument Engineering*, 54(12), 40–46. (In Russian)
18. Korotkov, K., Krizhanovsky, E., Borisova, M., Korotkin, D., Hayes, M., Matravers, P. et al. (2004). Time dynamics of the gas discharge around drops of liquids. *Journal of Applied Physics*, 95(7), 3334–3338. https://doi.org/10.1063/1.1651343
19. Priyatkin, N. S., Arkhipov, M. V., Gusakova, L. P., Pishchik, V. N., Kuznetsova, T. A., Karamysheva, A. V. et al. (2023). Application of gas discharge visualization technique for seeds hidden defects evaluation. *Theoretical and Applied Ecology,* 3, 37–48. https://doi.org/10.25750/1995-4301-2023-3-037-048
20. Priyatkin, N. S., Arkhipov, M. V., Shchukina, P. A., Mirskaya, G. V., Chesnokov, Yu. V. (2022). Evaluation of heterogeneity and hidden defects of wheat (*Triticum aestivum* L.) seeds by instrumental physical methods. *Agricultural Biology*, 57(5), 911–920. https://doi.org/10.15389/agrobiology.2022.5.911rus (In Russian)
21. Kolesnikov, L. E., Priyatkin, N. S., Arkhipov, M. V., Razumova, I. E., Radishevskiy, D. Yu., Kolesnikova, Yu. R. (2021). Influence of the structural and functional characteristics of the seeding material on the yield structure elements and resistance to leaf diseases of spring soft wheat. *Agronomy Research*, 19(4), 1791–1812. https://doi.org/10.15159/AR.21.152
22. Bista, S., Jasti, N., Bhargav, H., Sinha, S., Gupta, S., Ramarao, P. et al. (2023). Applications of gas discharge visualization imaging in health and disease: A systematic review. *Alternative Therapies in Health and Medicine*, 29(6), Article AT6764.
23. Stepanov, A.V., Sviridov, L.P., Korotkina, S.A., Achmeteli, G.G., Kryzhanovski, E.V. (2006). Application GDV technique in evaluation of agglutination reaction. *Journal of Instrument Engineering*, 49(2), 32–37. (In Russian)
24. Miraglia, F.E. (2024). Unreliability of the gas discharge visualization (GDV) device and the Bio-Well for biofield science: Kirlian photography revisited and investigated. Part I. *Journal of Anomalistics*, 24(1), 80–119. https://doi.org/10.23793/zfa.2024.080
25. Miraglia, F.E. (2024). Unreliability of the gas discharge visualization (GDV) device and the Bio-Well for biofield science: Kirlian photography revisited and investigated. Part II. *Journal of Anomalistics*, 24(1), 120–150. https://doi.org/10.23793/zfa.2024.120
26. Sadikov, A., Kononenko, I., Weibel, F. (2004). Analyzing coronas of fruits and leaves. Chapter in a book: Measuring Energy Fields: Current Research. Backbone Publishing Co. Fair Lawn, USA, 2004.
27. Organic Food Quality and Health Newsletter February 2007. Retrieved from https://edepot.wur.nl/115942. Accessed November 1, 2023
28. Kovalenko, V.S., Lovchev A. Yu. (2023). Studying an effect of chicken meat exposure to the fructose aqueous solution Fresh-pamyat' on the parameters of bio-photon emission of gas discharge visualization and organoleptic properties. *Vsyo o Myase.* 2, 17–21. https://doi.org/ 10.21323/2071-2499-2023-2-17-21 (In Russian)
29. Warner-Bratzler Shear Force Protocol. Retrieved from https://www.ars.usda.gov/arsuserfiles/30400510/protocols/warner-bratzlershearforceprotocol.pdf. Accessed January 19, 2023
30. Zou, X., He, J., Zhao, D., Zhang, M., Xie, Y., Dai, C. et al. (2020). Structural changes and evolution of peptides during chill storage of pork. *Frontiers in Nutrition*, 7, Article 151. https://doi.org/10.3389/fnut.2020.00151
31. Universal Protein Resource (UniProt). Retrieved from https://www.uniprot.org. Accessed March 20, 2023
32. Warner, R., Miller, R., Ha, M., Wheeler, T. L., Dunshea, F., Li, X., Vaskoska, R. et al. (2021). Meat tenderness: Underlying mechanisms, instrumental measurement, and sensory assessment. *Meat and Muscle Biology,* 4(2), Article 17. https://doi.org/10.22175/mmb.10489
33. Soldatova, S., Filatova, G. (2021). Evaluation of the freshness of mammalian meat by microstructural changes in muscle tissue. *Bulletin of Science and Practice*, 7(2), 83–88. https://doi.org/10.33619/2414-2948/63/08 (In Russian)
34. McCuller, C., Jessu, R., Callahan, A. L. (2023). Physiology, Skeletal Muscle. Chapter in a book: Treasure Island (FL): StatPearls Publishing; 2024.
35. Jukna, V., Jukna, Č., Peciulaitienė, N. (2012). Electrical conductivity of pig meat and its relation with quality. *Veterinarija ir Zootechnika*, 57(79), 18–21.
36. Rappel, W.-J., Edelstein-Keshet, L. (2017). Mechanisms of cell polarization. *Current Opinion in Systems Biology*, 3, 43–53. https://doi.org/10.1016/j.coisb.2017.03.005
37. Pushkin, A. S., Kamshilin, S. A., Drugova, E. D., Obraztsov, N. V., Dvoretskaya, S. I. (2019). The gas-discharge electrography of water and aqueous solution of chemical compound (review of literature). *Chemistry and Technology of Organic Substances*, 4(12), 51–61. https://doi.org/10.54468/25876724_2019_4_51 (In Russian)

AUTHOR INFORMATION

Aleh U. Shkabrou, Candidate of Technical Sciences, Docent, Head of the Department of Meat and Dairy Products Technologies, Ministry of Agriculture and Food of the Republic of Belarus. 15, Kirov str., Minsk, 220030, Republic of Belarus.
Tel.: +375–292–44–18–89, E-mail: olegshk@tut.by
ORCID: http://orcid.org/0000-0002-718-2237
* corresponding author

Irina M. Chernukha, Doctor of Technical Sciences, Professor, Academician of the Russian Academy of Sciences, Head of the Department for Coordination of Initiative and International Projects, Principal Investigator, Experimental Clinic-Laboratory of Biologically Active Substances of an Animal Origin, V. M. Gorbatov Federal Research Center for Food Systems. 26, Talalikhin str., 109316, Moscow, Russia. Tel: +7–495–676–95–11 (109), E-mail: imcher@inbox.ru
ORCID: https://orcid.org/0000-0003-4298-0927

Viktar D. Raznichenka, Engineer-Technologist, Department of the Chief Technologist, Slutsk Meat Processing Plant JSC, 18, Tutarinov str., Slutsk, Minsk region, 223610, Republic of Belarus. Tel.: +375–291–65–91–42, E-mail: rotcivetec@gmail.com
ORCID: http://orcid.org/0000-0002-1537-8482

Lyubou U. Lazovikava, Candidate of Technical Sciences, Docent, Docent, Department of Technology of Public Catering and Meat Products, Belarusian State University of Food and Chemical Technologies. 3, Shmidt Avenue, Mogilev, 212027, Republic of Belarus. Tel.: +375–295–45–74–27, E-mail: lyu-azarova@ yandex.by
ORCID: http://orcid.org/0009-0002-7980-9103

Irina I. Andreeva, Candidate of Technical Sciences, Docent, Docent, Department of Technology of Public Catering and Meat Products, Belarusian State University of Food and Chemical Technologies. 3, Shmidt Avenue, Mogilev, 212027, Republic of Belarus. Tel.: +375–296–41–42–77
ORCID: https://orcid.org/0009-0006-5256-9014

Sviatlana N. Baitava, Candidate of Technical Sciences, Docent, Docent, Department of Technosphere Safety and General Physics, Belarusian State University of Food and Chemical Technologies. 3, Shmidt Avenue, Mogilev, 212027, Republic of Belarus. Tel.: +375–297–43–31–94, E-mail: svetlana268@mail.ru
ORCID: https://orcid.org/0000-0003-3139-7998

All authors bear equal responsibility for the work and presented data.

All authors made an equal contribution to the work.

The authors were equally involved in writing the manuscript and bear the equal responsibility for plagiarism.

The authors declare no conflict of interest.