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OPTICAL-SPECTROSCOPIC ANALYSIS OF COLORIMETRIC CHANGES IN MEAT DURING ITS STORAGE

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Abstract
The colorimetric and spectral characteristics of meat and their changes during the period of storage were researched. It was shown that spectral methods of analysis can potentially be used to assess the properties of meat during its storage in order to define the degree of autolytic changes which occur in the meat along with its histological-structural and proteomic changes. The work studies the quality characteristics of meat on the base of an array of its parameters and a correlation between them. Considerable attention is paid to the determination of colorimetric and spectral characteristics of autolytic and other changes in meat during its storage. The possibility of using the method of optical spectrometry for assessing the quality of meat is considered. The data obtained by processing the absorption spectra of aqueous extracts from muscle tissue confirm the promising prospects of using this method in a comprehensive study of the raw meat materials properties. The work proves possibility of classifying raw meat according to the degree of its autolysis for further assessment of its colorimetric characteristics, the value of extinction coefficients and the relative area of peaks at the wavelength λ_{415}, λ_{525}, λ_{542}, λ_{555}, *u* λ_{582}.

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Introduction

Color evaluation is an integral part of meat quality research, development of a high-quality and safe food product and elimination of errors during its processing. Tomashevich I. B. has noted that the improvement in color stability of meat and meat products is closely related to their shelf life. It is desirable to increase the period during which the meat is still visually acceptable to the consumers in retail trade. Measuring the color of meat with a colorimeter or computer vision system allows evaluating its suitability for the processing, the quality of the finished food product, the correctness of the technological processes, etc. [1,2]

It is known that the optical properties of meat play an important role in color formation. The color of meat dramatically depends on its pH [3], the chemical condition and amino acid sequence of myoglobin [4,5], redox processes and interactions between muscle pigments [6,7]. However, studies [8,9,10] showed that, in addition to myoglobin, other compounds also influence the final formation of meat color, the most significant of which are the endogenous pigments (chromoproteids). Among meat chromoproteids, hemoglobinogenic pigments (ferritin, hemosiderin, bilirubin), proteinogenic pigments (melanin, adrenochrome) and lipopigments (lipofuscin, ceroid, lipochromes) are denoted.

The content of chromoproteids in meat and its optical characteristics depend on many factors, for example, the type of animal, its genetic characteristics, its diet, post-slaughter changes in muscles, mode of refrigeration, storage time, way of packaging, etc. [11].

As shown in the work [12], the optical properties and color of meat can be assessed by two main methods: chemical and physical. For an objective measurement of the color of food products by chemical methods, the pigments from the samples are extracted and their concentration is measured by a spectrophotometric method. Physical methods are based on the interaction of light with the object of study — its reflective, absorbing or transmissive capacity.

The optical properties of meat and meat products are also determined by the complexity of their microstructure and physicochemical properties. Absorption and scattering of radiation are determined by four main processes: resonant absorption of radiation by dry matter molecules, as well as molecules of structural and bound moisture; scattering of radiation due to fluctuations in the density of a substance, as well as scattering on molecules of pro-

teins, polysaccharides, ions, etc.; scattering of radiation on suspended colloidal particles, cells, pigment particles, etc., as well as scattering of radiation on optical inhomogeneities — capillaries, pores [13].

Thus, the correct visual and instrumental study of the optical properties and color of meat can be powerful and beneficial for the meat processing industry. However, this study must be run with the help of thoroughly designed techniques to avoid artifacts or incorrect data.

Currently, there are many options for instrumental analysis of the optical properties and color of meat. The most common color measuring instruments are colorimeters and spectrophotometers [14].

Colorimeters determine the proportions of primary additive light sources that match the color reflected from the sample or transmitted by the sample.

Spectrophotometers measure the amount of light of various wavelengths reflected from the sample or transmitted through the sample, resulting in a reflectance, absorption, or transmission spectrum. The transmission or absorption spectrum of the sample can be used together with the standard CIE observer function and the relative spectral energy distribution of the light source to calculate tricolor CIE XYZ values for the given sample with the selected light source.

As shown by Chernousova O. V., Rudakov O. B. [15], spectrophotometric methods for studying the optical properties and color of meat are very promising, as they provide accuracy and high speed in obtaining results and are often characterized by ease of their application. Currently, there is an active introduction of instruments and devices based on the spectroscopy principles.

The principle of operation of the devices described above serves as basis in various portable analyzers of the raw meat materials quality. Thus, the authors of the work [16] described a device for the integrated measurement of physical-chemical parameters and food color, which provides fast measurements in the standard CIELAB format.

The paper [17] presents the results of the development of a portable color analyzer for assessing the qualitative characteristics of poultry meat. The optical methods allowed establishing a relationship between the change in the dominant wavelength and duration of the carcasses storage.

The papers [18,19] proved the prospects of using optical spectroscopy for colorimetry of cognac products to detect the fact of product adulteration.

Pochitskaya I. M. proposed to evaluate the color characteristics of food products using the Adobe Photoshop program in the RGB (red-green-blue) coordinate system, which makes it possible to predict the color of the resulting finished product in order to create new food products with pre-defined color and taste characteristics [20].

The influence of broiler age on color characteristics (lightness L*, redness a*, yellowness b*) was shown by Janisch et al [21], who found a significant difference between the values of electrical conductivity, volume of meat juice loss, shear force and color in meat of 28 and 41-days old broilers.

The authors [9,22] proved that the study of meat by optical spectroscopy methods makes it possible to differentiate the muscle tissue of wild and domestic animals depending on the processing conditions: freezing, water extraction, lyophilization, and fermentation. This method makes it possible to reveal significant differences in changes in the spectral characteristics of the main components and components of the muscle tissue and muscle fiber of pork and beef, depending on the degree of oxidation, degree of mechanical destruction, strength of myofibrils and bond of myoglobin pigment protein with them.

Holman et al. [23] used a linear model to establish the relationship between color values, volume of meat juice losses, pH values, and shear forces during storage.

The high sensitivity, resolution and analytical features of optical spectroscopy methods in the study of biological tissues of animal origin in solid and liquid state of aggregation make it possible to obtain factual information about changes in the spectral characteristics of their main components.

Optical spectroscopy makes it possible to judge the depth of destructive chemical processes at the level of all components of biological material. Thus, as a result of storing chilled meat for a week, the myoglobin doublet disappears and absorption decreases in all areas of the electromagnetic spectrum, while the spectrum of thawed muscle tissue, on the contrary, lies above the spectrum of the original sample [9, 22].

Over the past five years a lot of researches into the optical properties and color of meat have been run, but several fundamental concepts still remain unsolved. In particular, insufficient attention has been paid to the role of autolytic changes in the formation of meat color during its storage. Additional study of the fundamental relationships between protein fractionation, histological-structural changes, and optical properties of meat can help solve numerous practical color stabilization issues.

Thus, for deeper understanding of autolytic processes and changes in the spectral characteristics of meat during its storage, it is advisable to study the characteristics of meat quality as the complex of its parameters array and establish a correlation between them in order to improve the methodology for meat color measuring.

Materials and methods

When constructing the experimental plan, the factors that provide the strongest effect on the meat quality were considered. These factors include ante-mortem and post-mortem conditions and storage duration.

Meat raw materials. Chilled samples of the porcine rib eye (*Sus scrofa M. longissimus dorsi*) were used as raw materials. Meat samples were put into storage at a temperature from 0 °C to plus 4 °C. Sampling and preparation of

Table 1. Change in the color parameters of meat during its storage

Day	L*	a*	b*	λ_{dom}	Color purity	Saturation	Color shade
1	98.29 ± 0.2	0.97 ± 0.04	5.15 ± 0.30	581.17 ± 0.04	6.84 ± 0.35	5.24 ± 0.28	0.57 ± 0.03
2	96.43 ± 0.8	2.43 ± 0.04	9.74 ± 0.31	582.36 ± 0.43	13.11 ± 0.42	10.04 ± 0.29	1.44 ± 0.02
3	97.10 ± 0.4	1.88 ± 0.05	8.36 ± 0.15	581.90 ± 0.09	11.17 ± 0.23	8.57 ± 0.16	1.11 ± 0.03
4	98.08 ± 0.3	0.98 ± 0.04	5.26 ± 0.28	581.14 ± 0.04	7.00 ± 0.35	5.35 ± 0.28	0.57 ± 0.03

Note: The values in the columns are statistically significantly different ($p < 0.05$)

samples for the analysis complied with GOST 7269–2015[1] and GOST R 51447–99 (ISO 3100–1–91)[2].

Determination of color and spectral parameters of meat. The color of meat was determined in an aqueous extract of muscle tissue with the help of a spectrophotometer PE-5400VI (LLC "Ecokhim", Russia) in the visible wavelength range within 340–830 nm with an increment of 5 nm. To obtain an aqueous extract, a lump of meat samples (10.00 ± 0.02 g) was ground in a manual meat grinder of the brand "Motor Sich 1 MA-s" (manufactured by JSC "MOTOR SICH", Ukraine), placed in a 100 ml flask and extracted with distilled water in a ratio of 1:5 on a SHR-1D laboratory shaker (Daihan Scientific, South Korea) for 30 min at 20 °C and stabilized with 1% gelatin solution. The resulting extract was filtered through a folded paper filter with a pore size of 8–12 μm and exposed to spectrophotometry to determine the curves of light absorption and color. Light absorption curves were obtained in the coordinates $A = f(\lambda)$, where λ is the wavelength, nm; A is the optical density.

The absorption bands were resolved by calculating the second derivative. Noise was filtered out during the second derivative analysis by approximating the absorption spectra by non-uniform rational Bezier splines of various orders (Non-Uniform Rational Bezier Spline, NURBS,) with oversampling. In case of ambiguity in the interpretation of weak signals, Savitzky-Golay Smoothing Filters (SGSF) were used as an alternative. For a relative quantitative assessment of the certain component content in an aqueous extract, the relative peak area was used.

The color coordinates in the CIE system L* a* b*, color purity, degree of saturation, color shade and dominant wavelength λ_{dom} were calculated as described in the paper [24]. Color coordinates were represented in the CIE L* a* b* system, which were calculated using standard light source A.

Analysis of the fractional composition of proteins of the longissimus porcine muscle was carried out by one-dimensional electrophoresis in 12.5% polyacrylamide gel in the presence of sodium dodecyl sulfate in a VE-10 chamber (Helicon, USA). As a standard for electrophoresis, a marker from the company "Thermo", USA, was used, which marker is a mixture of 11 recombinant proteins (250, 150, 100, 70, 50, 40, 30, 20, 15, 10 kDa). Staining was performed by Coomassie G-250 followed by densitometric quantification.

Study of the histological structure of meat. Histological examination of the sample was carried out in accordance with GOST R 31479–2012[3] and GOST 19496–2013[4]. Slices were evaluated using a microscope "Micromed-1 var.2–20" (Micromed, Russia).

The pH value was determined by the potentiometric method according to GOST R 51478–99[5] using a pH meter "HI98163" from the company "Hanna Instruments" (USA). Its measurement range of the active acidity of the medium lies within from — 2.00 till 20.00 units with an error of ±0.01 units.

Statistical analysis of the results was performed using Excel 2019 software (Microsoft, USA). The results obtained were considered significant at $p < 0.05$. Pearson's correlation coefficients were calculated to evaluate any relationships between various factors.

Results and discussion

Table 1 shows the changes in the main colorimetric characteristics of chilled meat during its storage. On the first day of storage, the lightness value (L*) was 98.29 units. On the second day of storage the L* value of aqueous extracts from muscle tissue decreased by 1.86 units, which was clearly visible to the human eye (color difference in reference to the original sample $\Delta E_{2000} = 3.92$ units.).

The values a* and b*, that characterize the color transitions of meat, increased by 1.46 and 4.59 units on the second day of storage, respectively. The color of the extract increased by 6.3%, thereby approaching the spectral (100%), as can be seen from the changes in color purity on the second day of storage. The saturation and hue of meat extracts also changed: from 5.24 to 10.04 and from 0.57 to 1.44, respectively.

Further storage of chilled pork samples was accompanied by a monotonous increase in lightness L* almost to the initial value. Thus, the value of L* on the third day of storage was 97.10 units, and on the fourth day it was 98.08. It should be noted that as the chilled meat was stored, the

[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 April 10, 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 April 10, 2023 (In Russian)

[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 April 11, 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 April 11, 2023 (In Russian)

[5] GOST R 51478–99 "Meat and meat products. Reference method for measurement of pH". Moscow: Standartinform, 2018. Retrieved from https://docs.cntd.ru/document/1200028185 Accessed April 11, 2023 (In Russian)

color difference in aqueous extracts of muscle tissue decreased, and on the 4th day was equal to 0.15 units.

The values of a* and b* on the 4th day approached the initial values and amounted to 0.98 and 5.26 units, respectively. The purity of color, saturation and color shade of meat extracts also reached their values recorded on the first day of storage.

Such changes in colorimetric characteristics can be explained by the influence of post-slaughter changes in meat. On the first day of storage of chilled meat, *rigor mortis* occurs, accompanied by peculiar changes in the histological structure of tissues and the fractional composition of proteins [25]. With the resolution of rigor mortis and subsequent storage of meat, its colorimetric characteristics changed similarly to the ongoing biochemical processes.

In addition to chromoproteids there are three key mechanisms that affect the color of meat [11]:

1) variations in the distance between myofilament lattices, which size changes due to osmotic swelling or contraction, or due to a change of the muscle sarcomere length [26], as a result, changing the diameter of myofibrils and muscle fibers. The lightness value (L* value) of the muscles increases together with a change in the diameter of muscle fibers;
2) variations in the length of sarcomeres, if this is associated with changes in the diameter of myofilaments and myofibrils;
3) variations in the sarcoplasmic proteins distribution.

Thus, the change in the color of meat occurs as a result of the influence of post-mortem biochemical mechanisms that occur in its tissues during storage, which is confirmed by the results of studies of the histological structure of tissues and the fractional composition of proteins.

When analyzing the absorption spectra of aqueous extracts from the muscle tissue of chilled meat during storage (Figure 1), several absorption bands were found that are peculiar for the biomolecules involved in color formation. It is known from the specialized literature that 320–380 nm is the area of absorption of unsaturated fatty acids of lipid components; an intense broad band with a maximum in the region of 400–430 nm is caused by the absorption of mucopolysaccharides (glucosaminoglycans), and the muscle pigment myoglobin, which provides red color to the tissue. This band appears as a doublet within the visible area at 540–580 nm [9].

The absorption spectra of the four redox species overlap and intersect (isobestic point) at 525 nm, and spectrophotometric absorption at 525 nm was used to estimate the total concentration of Mb in aqueous meat extracts.

Among the detected absorption peaks (Table 2), the peaks characteristic of cytochromes and various redox forms of myoglobin were identified.

Throughout the entire period of storage of chilled meat, a hypsochromic shift of the λ_{415} absorption band by 5 nm was observed and a slight hyperchromic effect on the second day was observed too (Figure 1). This effect can be caused by the fact that, as a result of biochemical processes, the viscosity of the aqueous extract drops down and an increase in its optical density is also observed. Thus, the shielding level of organic compounds decreases, and they absorb more light [27].

The relative content of cytochromes (λ_{415}) remained practically unchanged during the storage of meat, however, on the third day there was an increase in their content by 1.13 times in comparison with the initial content on the first day of storage. That was confirmed by an increase in the relative area of the λ_{415} peak from 63.93% up to 72.29% (Table 2). This effect could be associated with loosening of muscle fibers, which was confirmed by the results of histological tests (Figure 3a).

The relative content of various forms of myoglobin changed insignificantly, which could be observed within the area of the $\lambda_{540-580}$ peaks. The extinction coefficient of the solution at λ_{525} on the second day increased by 2.18 times in comparison with this level on the first day, which can be

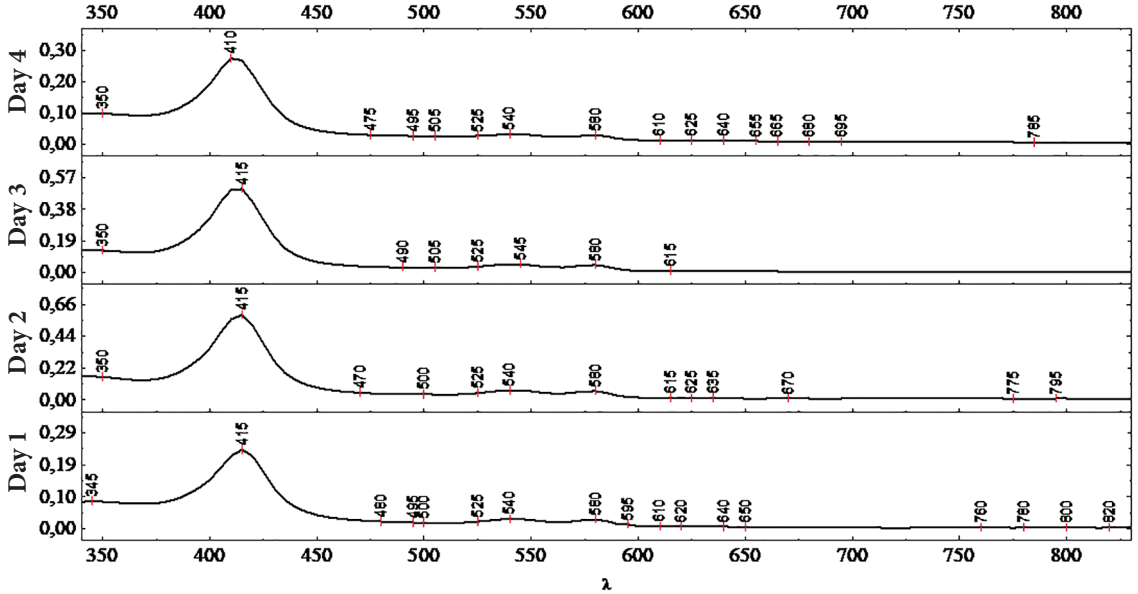


Figure 1. Absorption spectra of muscle tissue extracts

seen from the changes in the peak height from 0.022 to 0.048 (Table 2).

Table 2. Characteristics of bands of some muscle tissue extraction

1st day			2nd day		
λ_{of} the peak, nm	Relative area of the peak, %	Height of the peak	λ_{of} the peak, nm	Relative area of the peak, %	Height of the peak
345	13.78	0.084	350	7.67	0.159
415	63.93	0.238	415	65.06	0.588
480	1.80	0.023	470	2.86	0.051
495	0.56	0.020	500	1.05	0.041
500	1.64	0.019	525	2.14	0.048
525	1.16	0.022	540	5.41	0.067
540	5.37	0.030	580	4.63	0.061
580	4.52	0.029			
595	0.80	0.014			
3rd day			4th day		
λ_{of} the peak, nm	Relative area of the peak,%	Height of the peak	λ_{of} the peak, nm	Relative area of the peak,%	Height of the peak
350	6.14	0.133	350	6.97	0.097
415	72.29	0.503	410	64.15	0.273
490	0.99	0.032	475	2.67	0.029
505	0.98	0.031	495	1.23	0.025
525	1.54	0.036	505	0.61	0.025
545	5.44	0.051	525	1.83	0.026
580	3.36	0.045	540	4.89	0.032
			580	4.91	0.028

Note: The values in the columns are statistically significantly different ($p < 0.05$)

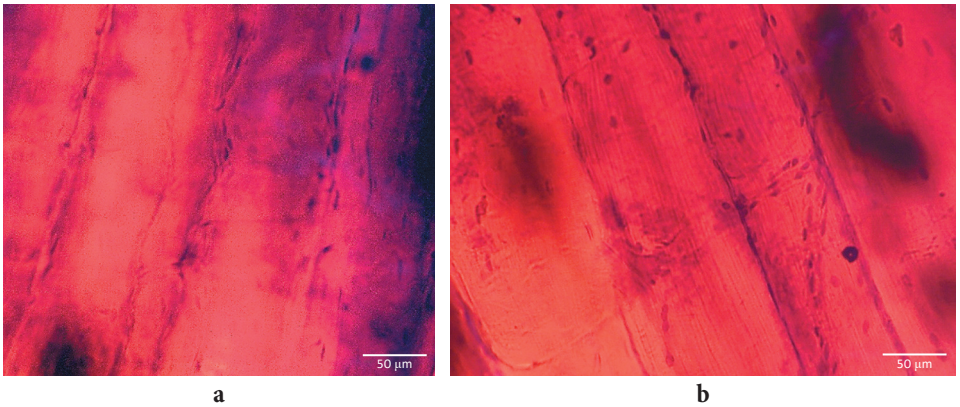
Sharp loom of the extinction coefficient on the second day at the isobestic point of various redox forms of myoglobin caused a hyperchromic effect, which was later replaced by a hypochromic effect, starting from the 3rd day. These effects characterize an increase or decrease in the intensity of absorption, which were caused by biochemical transformations in meat during its autolysis, which was confirmed by the results of the analysis of the protein composition in meat. The results obtained do not contradict to this assumption [28].

It was found that the change in the colorimetric characteristics of aqueous extracts of muscle tissue occurred synchronously with the change in the microstructure of the meat, as evidenced by the data obtained from the histological analysis of chilled pork samples.

Thus, on the first day of meat storage, an asynchronous contraction of muscle fibers was detected with a weakening of the transverse striation and intensification of the longitudinal striation, along with the formation of oval contraction nodes (refer Figure 2a). Muscle fibers had an irregular shape due to deformation changes during *rigor mortis*. In muscle fibers, due to the uneven development of postmortem rigidity, both rod-shaped and round-shaped nuclei were observed. The different shape of the nuclei was caused by the fact that the fibers either were not completely contracted or were relaxed, or they were in a contracted state. The color of the cross-section was uniform. The nuclei were clearly colored. The muscle fiber diameter was 80±20 μm. The fibers were arranged in a wavy pattern, adjoining to each other pretty tightly.

On the second day of storage of meat histological sections, some signs of resolution of rigor mortis were observed. The rigidity resolution signs were accompanied by a hyperchromic effect at λ_{415} and λ_{525} wavelength, a significant lightening and an increase in the color saturation of muscle tissue extracts were observed too. During this period, relaxation of the muscle fibers was observed, although some fibers were found in a contracted state (Figure 2b). Most of the nuclei were elongated and well colored. Restoration of transverse striation was found. Longitudinal striation was clearly visible. In some places, in the nodes of contraction, ruptures of the sarcolemma of muscle fibers were found with the preservation of the fiber contents and its internal structure. The diameter of muscle fibers decreased by 18.7% and amounted to 65±15 μm. The fibers were arranged evenly, adjoining to each other pretty tightly.

On the third day of meat storage, signs of resolution of rigor mortis were clearly expressed, which indicated the beginning of the stage of meat maturation (refer to the Figure 3a). Histological tissue changes were characterized by development of destructive processes in the meat. The onset of fiber fragmentation and loosening of connective tissue fibrous elements with their detachment from



a b

Figure 2. Microstructure of meat on the first (a) and second (b) days of storage (vol. 40×)

muscle fibers was observed. No signs of the muscle fiber contracture were found. The fiber diameter was 40 ± 10 μm. In cross-sections an enlargement of interfiber space and increasing of microcracks number were noticeable. The transverse and longitudinal striations were clearly visible. In some cases, the disintegration of the sarcolemma and its granular disintegration were found. In some places, microflora foci in the form of separate diffuse overlays were detected. Condensation and margination of chromatin were observed in the nuclei; chromatin was built up under the nuclear membrane in the shape of small lumps.

On the fourth day of storage the destructive changes in muscle tissue were more pronounced than histological changes on the third day of pork storage. Muscle fibers were arranged loosely and unevenly colored. Localized lysis was observed in some places. The number of transverse slit-like ruptures of the muscle fibers integrity increased along with partial preservation of the structure of the nuclei, of transverse and longitudinal striation in fragments. Homogeneous nuclei with signs of karyolysis were found in places (Figure 3b). Due to the disintegration of chromatin, the nuclei acquired a shadowy color of varying intensity. The transverse striation was less pronounced. The fibers slightly increased in diameter up to 55 ± 15 μm. In the structure of the fibers, granular and granular inclusions were locally detected. The sections revealed the presence of coccal and rod-shaped microflora in the shape of foci and diffuse overlays. The staining of most sections was basophilic.

Morphological changes in muscle tissue during its storage were accompanied by a change in colorimetric characteristics. With the resolution of rigor mortis, a slight hypsochromic shift and hypochromic effect of some peaks, a decrease in the number of absorption bands, and discoloration of aqueous extracts, which was expressed as a decrease in redness a* and yellowness b*, were observed (Table 1).

The data obtained indirectly pointed to the proteolytic destruction of protein structures, as a result of which muscle fibers got loosened and fragmented, and muscle proteins extracted into the solution shifted the color coordinates. It is known [29] that during autolysis meat proteins undergo a line of changes, like aggregation and partial decomposition, which was confirmed by the results of studies of the proteins fractional composition.

Identification of the protein composition of raw materials is a very important aspect, since it allows you to directly determine the qualitative composition of the finished product. The use of the electrophoretic method at the initial stage of research makes it possible to evaluate the quantitative and qualitative distribution of structural and tissue-specific protein molecules; evaluate the influence of autolytic processes, etc.

As a result of the analysis of 1D electropherograms of meat proteins (Figure 4), differences in the fractional composition of proteins and their concentration, determined by the color intensity of the protein zones, were found.

On the 1D electrophoregram (Figure 4), the areas were marked in yellow where changes in the protein structures and their relative content during storage were assessed by changing the color staining intensity of the protein zones.

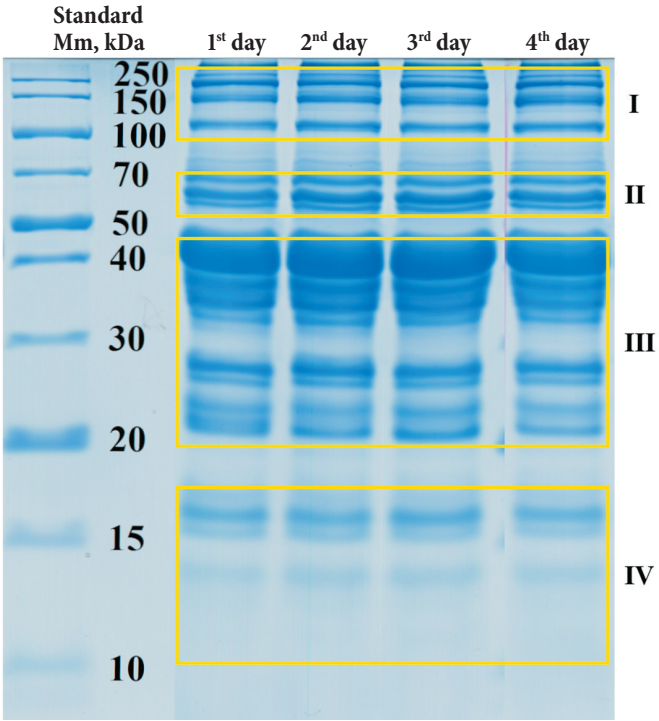
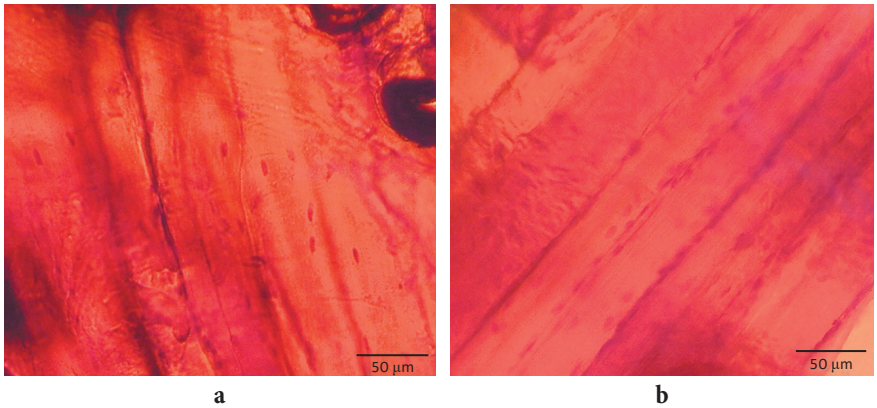


Figure 4. Electrophoregram of *Sus scrofa M. longissimus dorsi* proteins during their storage



a **b**

Figure 3. Microstructure of meat on the third (a) and fourth (b) days of storage (vol. 40×)

The proteins of the identified zones had a different origin according to the database [30], and were identified as connective tissue, myofibrillar proteins and metabolic proteins.

As can be seen from the Figure 5 above, the relative content of protein fractions with a molecular weight in the area of 110–291 kDa (area I on the electropherogram, Figure 4), 51–67 kDa (area II on the electropherogram, Figure 4), 20–42 kDa (area III on electrophoregram, Figure 4) and 10–18 kDa (area IV on the electrophoregram, Figure 4) changed in different degree, depending on the duration of meat storage.

It was revealed that in the process of rigor mortis, evidenced by the results of histological studies (Figure 2), on the first day of storage the relative content of high-molecular fractions I and II of proteins increased along with the decrease in the average molecular fraction III (Figure 5).

During the further storage, the amount of macromolecular structures of fraction I increased from 13.26% to 15.27%. In fraction III, an inverse dependence was observed. It was noted that during the storage of meat, the amount of low molecular weight proteins increased, as it was confirmed by changes in the intensity of the protein zones of the fraction with a molecular weight within the area of 10–18 kDa (region IV on the electropherogram, Figure 4).

These changes indicated the processes of autolysis that occurred in the meat, as a result of which changes in various protein structures occurred in parallel, such as aggregation and partial decay. Autolytic changes in the meat were confirmed by the results of histological studies.

Based on changes in the color intensity of protein bands with a molecular mass of 239–248 kDa and 205–213 kDa, it is possible to come to assumption about changes in the myosin fractions, which does not contradict to the researches [31, 32]. Thus, as a result of autolytic processes, a decrease in the relative amount of the myosin fraction by 13.9% was observed on the second day of storage. This could be caused by the development of rigor mortis and the formation of complexes between F-actin and myosin that characterizes it. The subsequent increase in the relative content of protein fractions II and IV up to the fourth day of storage clearly evidenced the destruction of actomyosin molecules and myosin aggregates down to their heavy chains (200–223 kDa) and light chains (16–20 kDa).

It was noted that the change in the relative content of various protein fractions was accompanied by an increase in redness a*, yellowness b*, in purity, color saturation and extinction coefficient at the isosbestic point of various redox forms of myoglobin by almost 2 times.

As the relative content of fraction II increased on the third day of storage (10.92%) at the same moment the number of absorption bands in aqueous extracts of muscle tissue dropped down from 17 to 8.

An increase in the color intensity of the protein zones with a molecular weight of 52–67 kDa and concomitant changes in the color intensity of the protein zones of the

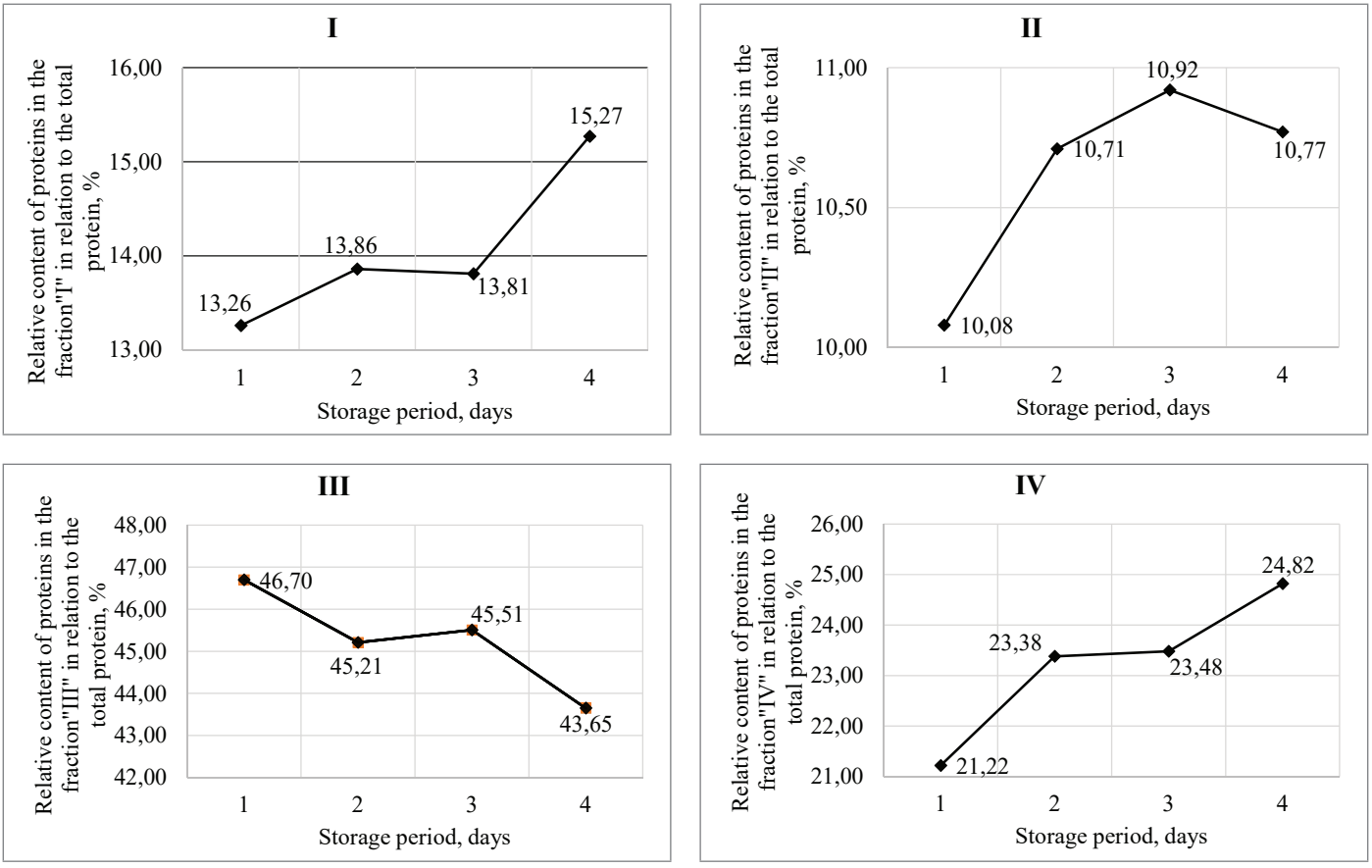


Figure 5. Change in the relative content of proteins in the various fractions:
I — 110–291 kDa, II — 51–67 kDa, III — 20–42 kDa, IV — 10–18 kDa

fraction with a molecular weight in the area of 27–33 kDa could indicate changes with desmin (53 kDa), vimentin (54 kDa), glucose-6-phosphate isomerase (63 kDa) and tropomyosin (33–28 kDa). The change in color intensity of zones with a molecular weight within 30–33 kDa and 17–19 kDa evidence the troponin changes.

The dynamics of changes in fractions III and IV (Figure 5), which is represented by troponins and cytochromes, had a strong correlation with the change in the extinction coefficient of the muscle tissue extract at λ410–415 (R = 0.94).

Softening and loosening of meat during the beginning of the ripening stage on the third day is caused by a weakening of the structure of myofibrils due to the degradation of desmin (50–53 kDa), and intramuscular connective tissue under the action of calcium ions [25].

Upon reaching the maximum value (10.92%) on the third day, the mass content of proteins with a molecular weight of 51–67 kDa (Figure 5) started dropping down till 10.77% on the fourth day, which coincides with the onset of the resolution of rigor mortis according to data of histological analysis (Figure 3).

As can be seen from the data presented above, during storage the high-molecular protein substances decomposed partially or completely and formed the medium-molecular and low-molecular fractions.

The longer the meat was stored, the more protein substances went into solution during water extraction. During intermolecular interaction in an aqueous solution, fractions of various proteins formed aggregated particles, which were subsequently removed during filtration, which became the main reason for the clarification of aqueous extracts of muscle tissue.

As a result of the analysis of the histological structure of meat and the fractional composition of proteins, an average and strong negative and positive correlation (R = minus 0.98 … 0.86) was found between the color intensity of protein zones with a molecular weight of 159–167 kDa, 94–97 kDa, kDa, 52–65 kDa, low molecular weight fractions of the area III and IV (Figure 5) and muscle fiber diameter. This dependence indicates that changes of muscle tissue structure are strongly influenced by changes in myofibrillar proteins, primarily myosin, troponin, desmin, calpastatin, as well as pyruvate kinase and cathepsins [31,33].

The dynamics of changes in color purity, degree of saturation, color shade, as well as the values of a* and b* possess a strong correlation with changes in the relative content of protein fractions with a molecular weight within the area of 205–213 kDa, 82–85 kDa, 56–58 kDa, 40–42 kDa and below 27 kDa, which could evidence the involvement of fractions of endoenzymes, cytochromes, myoglobin, and troponin in color transitions, which is consistent with the results of studies [34]. Thus, a change in the colorimetric characteristics of a* and b* could indicate the transformation of chromoproteids and some other proteins, which is confirmed by the results of densitometric analysis of proteins.

When storing the chilled meat, a correlation was found between lightness (L*) on the one hand, and histostructural changes and changes in fractional composition of proteins on the other hand (R = –0.81 and 0.98, respectively). It was found that a change in the relative content of proteins with a molecular weight of 205–213 kDa, 82–85 kDa, 56–58 kDa, 40–42 kDa and below 27 kDa led to a change of lightness (L*) (Table 1). This dependence evidenced to participation of other proteins in color formation, except for myoglobin. Thus, the colorimetric characteristics of aqueous extracts from muscle tissue were affected by a row of factors.

When running the experiment, it was assumed that the myoglobin protein can act as a kind of "indicator", as its color intensity can depend on pH value. In order to confirm this assumption, aqueous extracts of meat and myoglobin solutions were prepared. pH of these solutions was changed and the color difference was observed. The pH ranged from 3 to 9 units.

As a result of the experiment, it was found that when the pH value of aqueous extracts of meat and myoglobin solutions changes from 5 units up to 7 units the value of ΔE_{2000} did not exceed 0.2 units. The color difference increased only when the pH was decreased down to less than 3 and when it was increased up to more than 9 units, which, possibly, occurred as a result of conformational changes in protein molecules. The obtained results prove that changes in the optical properties of myoglobin solutions and aqueous meat extracts are affected by conformational changes in proteins rather than by the pH value.

The obtained data do not contradict to [11,32]. So the variation of the pH value is accompanied by a change in the scattering of light within the structure of the muscle fiber. The increase in light dispersion explains why the surface of the meat looks paler. Thus, the meat surface looks pale in muscle with a low pH value (pH 5.4–5.7) and looks dark in a muscle with a high pH (more than 5.8 units).

Thus, the pH value provides only an indirect effect on the meat color and extracts obtained from it. The obtained results confirm that not only chromoproteids, but also myofibrillar proteins are involved into meat color formation.

Changes in the native structure of proteins during storage affect the shielding of biomolecules, which, in its turn, affects the color of meat. Thus, the analysis of the color of meat extracts allows full evaluation of its quality characteristics and better understanding the biochemical processes.

Conclusion

In addition to the classical methods of meat quality analysis, the spectrophotometric analysis of aqueous extracts of muscle tissue is quite promising and reliable method, since the changes of optical characteristics in raw meat evidence the biochemical processes in meat. Analysis

of the absorption spectra showed that the extinction coefficients and the relative area of the peaks at λ_{415}, λ_{525}, λ_{542}, λ_{555}, and λ_{582} significantly correlated ($R > 0.8$) with histological and structural changes and the relative content of proteins like troponins, cytochromes, endoenzymes, and myoglobin.

It was determined that during the storage of raw meat, changes in optical characteristics take place along with the typical changes in the composition of proteins components and histological structure of tissues. The effect of autolytic changes on the colorimetric characteristics of meat was confirmed.

In accordance with the recommendations of the International Commission on Lighting CIE, the values of true color for chilled meat were determined in dependence to its shelf life.

The possibility of applying the method of optical spectrometry of meat to determine the quality of meat is evaluated. The data obtained by processing the absorption spectra of aqueous extracts from muscle tissue prove the promising prospects of using this method in a comprehensive research of properties of raw meat materials. It is shown that, depending on the stage of meat autolysis, characteristic changes in the absorption spectra and color of meat take place.

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