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CRITERIA TO DETERMINE THE FRESHNESS OF CHICKEN MEAT USING BIOPHYSICAL AND MORPHOLOGICAL METHODS

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The article is dedicated to the experimental impedancemetry of fresh chicken meat (fillet) after storage in the refrigerator and the meat with signs of damage. It was determined that passing an alternating current of different frequencies through pieces of meat of different freshness results in the change of its overall electrical resistance. It was experimentally proved that the resistance dispersion curve for fresh chicken meat is in the range from 754.4 to 3720.0 Om, for the meat with signs of aging (damage) — in the range from 141.3 to 156.1 Om, which testifies to the dependence of the tissue electrical impedance module from its physiological condition. Along with the impedancemetry histological studies of the selected samples of chicken fillet and studies of surface structure of meat by scanning electron microscopy to confirm the effectiveness of the proposed method to determine the freshness of chicken meat was conducted.

Histologically, the fresh muscle fibers were located densely, under sarcolema numerous elongated-oval nuclei of dark blue colour appeared. In the chicken meat, which is being stored in the refrigerator for 6 days, in contrast to the fresh one, a stratification and local destruction of sarcolemma, the swelling of muscle fibers, the destruction of connective tissue, the deformation and ruptures of deep layers of muscle tissue were observed. Electronic microscopic study of lengthwise superficial muscle tissues detected pressed in muscle clusters and separate fibers, sarcolemma exfoliates from the sarcoplasm, protein mass accumulated in intercluster layers.

The histological examination of chicken meat samples with signs of damage, indicated a strong swelling of muscle fibers, loss of transverse and longitudinal streakiness, lysis of most nuclei, between several muscle fragments the colonies of microorganisms were revealed. The scanning electron microscopy of the medicinal products showed the thickening of internal layer with densely located fibers, the formation of small number of bags where the colonies of microorganisms are likely to multiply.

Key words: IMPEDANCE SPECTROSCOPY, CHICKEN MEAT FRESHNESS, BIOLOGICAL MEMBRANES, RESISTANCE DISPERSION

КРИТЕРІЇ ОЦІНЮВАННЯ СВІЖОСТІ КУРЯЧОГО М'ЯСА З ВИКОРИСТАННЯМ БІОФІЗИЧНОГО, ГІСТОЛОГІЧНОГО ТА ЕЛЕКТРОННОМІКРОСКОПІЧНОГО МЕТОДІВ

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У статті подається експериментальна імпедансометрія свіжого курячого м'яса (філе куряче), м'яса після зберігання у холодильнику та м'яса з ознаками псування. Встановлено, що в результаті пропускання змінного струму різної частоти через шматочки м'яса різної свіжості, змінюється його комплексний електроопір. Експериментально доведено, що крива дисперсії електроопору свіжого курячого м'яса знаходиться в межах від 754,4 до 3720,0 Ом, м'яса з ознаками старіння (псування) ¾

в межах від 141,3 до 156,1 Ом, що показує на залежність модуля електричного імпедансу тканини від її фізіологічного стану. Одночасно з імпедансометрією проведено гістологіче дослідження відібраних взірців курячого філе з метою підтвердження ефективності запропонованого методу для визначення свіжості курячого м'яса.

Гістологічно свіжі м'язові волокна були компактно розміщені одні біля одних, під сарколемою добре проглядались численні, видовжено-овальної форми темно-синього кольору ядра. У м'ясі, яке зберігалось у холодильнику, на відміну від свіжого, відзначали локальну деструкцію сарколеми, набухання м'язових волокон, руйнування сполучної тканини, утворення порожнин між пучками м'язових волокон, деформацію та розриви глибоких шарів м'язової тканини. При електронно-мікроскопічному дослідженні поздовжніх поверхневих м'язових тканин виявляли вдавлені м'язові пучки і окремі волокна, сарколема відшарована від саркоплазми, у міжпучкових прошарках нагромаджувалась білкова маса.

При гістологічному дослідженні курячого м'яса з ознаками псування відзначали сильне набубнявіння м'язових волокон, лізис більшості ядер, зникнення поперечної та поздовжньої посмугованості, між пучками м'язових волокон виявляли колонії мікроорганізмів. На препаратах, отриманих після скануючої електронної мікроскопії (SEM) відзначали ущільнення поверхні зовнішнього шару з компактним розміщенням волокон, утворення невеликої кількості порожнин в яких, ймовірно, і розмножуються колонії мікроорганізмів.

Ключові слова: ІМПЕДАНС, М'ЯСО КУРЯЧЕ, СВІЖІСТЬ, БІОЛОГІЧНІ МЕМБРАНИ, ОПІР ДИСПЕРСІЇ

КРИТЕРИИ ОЦЕНКИ СВЕЖЕСТИ КУРИНОГО МЯСА С ИСПОЛЬЗОВАНИЕМ БИОФИЗИЧЕСКОГО, ГИСТОЛОГИЧЕСКОГО И ЭЛЕКТРОННОМИКРОСКОПИЧЕССКОГО МЕТОДОВ

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В статье подается экспериментальная импедансометрия свежего куриного мяса (филе куриное), мяса после хранения в холодильнике и мяса с признаками порчи. Установлено, что в результате пропускания переменного тока различной частоты через кусочки мяса различной свежести меняется его комплексное электросопротивление. Экспериментально доказано, что кривая дисперсии электросопротивления свежего куриного мяса находится в пределах от 754,4 до 3720,0 Ом, мяса с признаками старения (порчи) в пределах от 141,3 до 156,1 Ом, показывающая зависимость модуля электрического импеданса ткани от ее физиологического состояния. Параллельно с импедансометрией проведено гистологическое исследование отобранных образцов куриного филе с целью подтверждения эффективности предложенного метода для определения свежести куриного мяса.

Гистологически свежие мышечные волокна были компактно размещены, под сарколеммой хорошо просматривались многочисленные, овальной формы темно-синего цвета ядра. В мясе, которое хранилось в холодильнике, отмечали локальную деструкцию сарколеммы, набухание волокон, разрушение соединительной ткани, образование полостей между пучками мышечных волокон, деформацию и разрывы глубоких слоев мышечной ткани. При электронно-микроскопическом исследовании поверхностных мышечных тканей отмечали вдавленные мышечные пучки и отдельные волокна, сарколемма отслоена от саркоплазмы, в межпучковых слоях накапливалась белковая масса.

При гистологическом исследовании куриного мяса с признаками порчи отмечали сильное набухания мышечных волокон, лизис большинства ядер, исчезновение поперечной и продольной исчерченности, между пучками мышечных волокон локализировались колонии микроорганизмов. На препаратах, полученных после сканирующей электронной микроскопии (SEM) отмечали уплотнения поверхности наружного слоя мышечных волокон с образованием небольшого количества полостей в которых, вероятно, и размножались колонии микроорганизмов.

Ключевые слова: ИМПЕДАНС, КУРИНОЕ МЯСО, СВЕЖЕСТЬ, БИОЛОГИЧЕСКИЕ МЕМБРАНЫ, СОПРОТИВЛЕНИЕ ДИСПЕРСИИ

Introduction. For several decades scientists from different countries studied the problem of morphological characteristics of meat material, changes in muscle tissue during autolysis, mechanical and physical effects of the prolonged storage. So far, veterinary and sanitary experts do not have a reliable and accurate method that would allow the detection of meat that long remained on the shelves of supermarkets, stale meat in warehouses etc. Typically, organoleptic methods do not always justify themself as the experts must be guided solely by their senses, while stale meat does not always reveal itself by color or odor, especially after special treatment. Physico-chemical methods of meat quality control are quite labor-, time-consuming and expensive.

One of the methods, which make it possible to distinguish fresh meat from the one stored in the refrigerator for several days or even months or with signs of damage, is impedance spectroscopy. This method is known in electrochemistry for a long time [11, 14]. It is also used to diagnose the state of accumulators [21]. A number of papers is dedicated to the study of electrical impedance of tissues [15, 9] and organs of plants [16, 19], to the study of the cytotoxicity of metal oxides ZnO, CuO and TiO_2 [25], the cytotoxicity of anticancer drugs [30, 31] and effects of cytotoxic agents on single cells using an impedance spectroscopy [22], skin cancer identification [1], detection of cervical intraepithelial neoplasia [6], detection of basal cell carcinoma [12, 7] and melanoma [1], diagnosis of bladder pathology [18]. The application of the method was proposed to identify fish freshness [24].

It is known that all biological tissues are capable of conducting direct and alternating currents, and create appropriate resistance. The passage of an alternating current through a tissue (cell) is followed by the occurrence of the total (complex) resistance, which is called impedance (Z), consisting of active (R) and reactive (capacitive (Rc)) component. Its active component R is associated with the internal conductivity of liquid media — electrolytes, and the reactive component Rc is determined by the capacitive properties of studied tissues, particularly the capacity of biological membranes. The capacitive impedance occurs as a result of polarization effects on the surface of cell membranes. Differently charged ions on the surface membrane are redistributed in such a way that the generated electric field is directed opposite to the applied external electric field. Polarization phenomenon is peculiar only to living, intact cells and tissues. Thus, the essence of the method of impedancemetry is to determine the values of polarization parameters, which characterize the type of biological tissues reflected as physical values of conductivity and dielectric permeability, which in turn determines its structural and physiological state.

In the laboratory of morphological studies of State Scientific Research Control Institute of Veterinary Medicinal Products and Feed Additives, together with experts from the Department of the technology of biologically active substances, pharmacy and biotechnology of Lviv Polytechnic National University the experimental studies on the determination of meat freshness by impedance spectroscopy are conducted.

Materials and methods

1. Histological study. Test samples of meat were fixed in 10% neutral formalin solution for 2 days, followed by rinsing in tap water. After rinsing the material was carried through an upward series of alcohols. The sample was kept in each of the alcohol solutions for 24 h. At that a process of dehydration occurred. The next stage was the compaction of test samples in two portions of chloroform (up to 3 h each). Then the sample was transferred to a chloroform-paraffin and kept in a thermostat at

37 °C for 1-2 h. While in thermostat, the sample was twice transferred into paraffin, using a new portion each time, and kept in each portion for 2 h at 56 °C. After that, the studied samples were poured into special forms with paraffin. Using the sledge microtome, sections 5-15 mcm thick were made and stained with hematoxylin and eosin [23].

2. Scanning electron microscopy study. Qualitative indicators of meat were evaluated based on the studies of its surface grains using scanning electron microscope (SEM) JEOL-T220A. Magnification of 50-350 times was used for photographing. Preparation of samples for SEM research included two procedures-fixation and metallization of the sample. Fixation is used when the surface of living tissues in samples is studied. Metallization is a procedure of spatter of thin metallic film (thickness 5–10 nm) of gold, carbon, copper etc. on the surface of the samples. Metallization of muscle tissue samples was performed in vacuum spattering apparatus VUP-5 by thermal sputter of copper. As a result of the SEM, photographing images of the studied muscle fibers were obtained, the state of the surface fibers, damage, etc. were assessed.

3. Impedance spectroscopy. The study was conducted on 10 samples of chilled fresh chicken meat (fillet), kept in the refrigerator for more than 6 days and 10 samples of chicken meat with signs of damage. All studied samples had weight of 60 g and during the experiment were in the same storage conditions, at a constant temperature and humidity. Indicators of frequency dependences of complex electrical resistance and conductivity, capacitive and inductive characteristics were determined sequentially in six parallels.

To measure the above mentioned biophysical parameters of muscle tissues in alternating current with frequency 1-100 kHz a unit, consisting of the object table, to which the studied sample was fixed and the measurement system based on chip AD5933, which is an integral transducer of measured parameters into a digital code, was used [3, 4, 5, 8, 17, 26]. Two copper wires coated with a thin layer of silver which were applied to the sample within 20 mm of each other, served as electrodes. An alternating current of different frequencies was passed through a meat sample. Then the complex resistance Z (Ohm) and the angle j (degrees), which is defined by the ratio of reactive component to active component Z, were measured. According to the obtained experimental data, the graphs of frequency response where designed using Microsoft Excel for Windows. Histological and electron microscopy study was performed using standard methods [23, 13].

Results and discussion

The presence of capacitive component in the membranes of living systems leads to the dependence of the resistance of tissues (cells) on the frequency (ω) of alternating current. It has been experimentally proved that the complex electrical resistance of tissues (Z) decreases with the increase of frequency. The curve of the dependence of resistance on frequency is called resistance dispersion. Resistance dispersion is a very important characteristic of living systems, since the functional changes in the object, result in a decrease of the slope of resistance dispersion curve. This criterion may be used as a test for the determination of tissue viability. It was shown that the functional changes of muscle fibers in the aging process significantly decreased the resistance dispersion. Thus, the dispersion curve for fresh chicken meat was in the range from 754.4 to 3720.0 Ohm (*Fig. 1*, curve 1), the meat after 6 hours storage in the refrigerator ¾ in the range from 374.6 to 326.3 Ohm (*Fig. 1*, curve 2) and for meat with signs of damage ¾ in the range from 156.1 to 141.3 Ohm (*Fig. 1*, curve 3).

Determination of polarization for evaluation of dispersion slope resistance.

A simpler evaluation of the slope of resistance dispersion curve was given by Tarusov [10, 20, 27], which is determined by the polarization coefficient (K_p):

$$K_p=Z(10^4)/Z(10^5),$$

where $Z(10^4)$ — the resistance at frequency 10^4 Hz, $Z(10^5)$ — resistance at

frequency 10^5 Hz. According to the above mentioned, the K_p in fresh intact tissues must be greater than 1. K_p of the dead tissue, or of that, which has undergone the autolysis processes, is approaching or equals 1.

As can be seen from the results of our research, Gain fresh chicken is:

K_p = 2223/754.4 = 2.95.

K_p of chicken fillet after storage in a refrigerator = 336.5/327.6 = 1.03.

K_p of chicken fillet with signs of damage = 145.2/142.5 = 1.02.

The peculiarities of time-varying conductivity of biological tissues allow the observation of shifts of physiological functions accompanying the aging processes of the objects. For dense muscular tissues the conductivity dispersion is stipulated by the oblong cell shape, peculiarities of the state of membranes, intracellular and extracellular fluids. In dead cells the violations of cell membranes and fluid loss is observed. While mostly displacement current passed through the tissue when the cell membranes were preserved, after the destruction of the cell membranes the part of conduction current in the total current increases. Thus, the dispersion of the dielectric permittivity of tissue is high enough, while the one of the dead tissue ¾ is almost absent.

It should be noted that, when maintaining the integrity of the lipid cells membranes of fresh muscle tissue, effective dielectric constant is high enough. When aging or damage of muscle tissue occurs the permeability of membranes, performing a kind of barrier function, changes, the cell membranes lose their selective permeability. In this case, when an electric current passes the ions easily penetrate the cell membranes, the polarization at the cellular level decreases, the electric capacity of tissue samples is also reduced, the curve Z(f) in the frequency range fmin ÷ fmax smoothes out. Therefore, the difference $DZ = Z(f_{min}) — Z(f_{max})$ can be used for the analysis of cell membranes. If the difference DZ is small, it is an evidence of a violation of the cell membrane.

DZ of fresh chicken fillet = 3720-754.4 = 2965.6 Hz = 2.97 kHz.

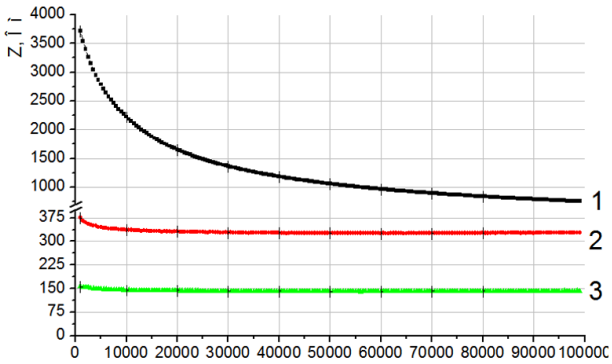

Fig. 1. Dependance of the complex resistance Z from the frequency of the measured electrical current ω: 1 — fresh chicken fillet; 2 — chicken fillet after 6 days storage in refrigerator; 3 — chicken fillet with the signs of damage

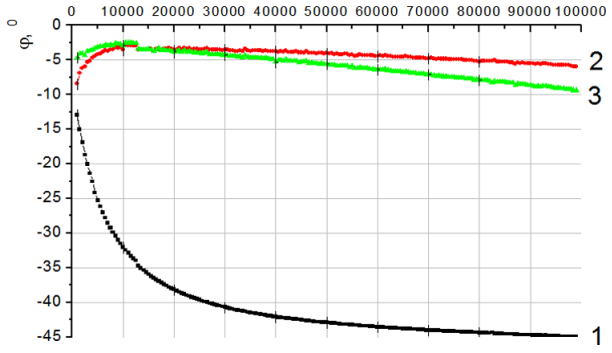

Fig. 2. Phase shift of the angle j: 1 — fresh chicken meat; 2 — chicken meat after storage in refrigerator; 3 — chicken meat with the signs of damage

D Z of chicken after storage in a refrigerator = 374.6-326.3 = 48.3 Hz.

D Z chicken with signs of damage = 156.1–141.3 = 14.8 Hz.

Thus, the polarization electric capacity of meat with the signs of aging is dramatically reduced as a result of the corruption of the integrity of cell membranes, which also reduces the complex resistance.

Angle j indicator.

Another important index of electroimpedansemetry is the value of angle j, which is defined as the ratio of the reactive component to the active component of impedance (*Fig. 2*). According to research the value of the angle j, obtained at a frequency of 1 kHz for human skin is –55 °, for rabbit muscle –65 ° [29]. The results of our study revealed that the angle j for fresh chicken fillet at a frequency 1 kHz is –32 ° (*Fig. 2*, curve 1), while the

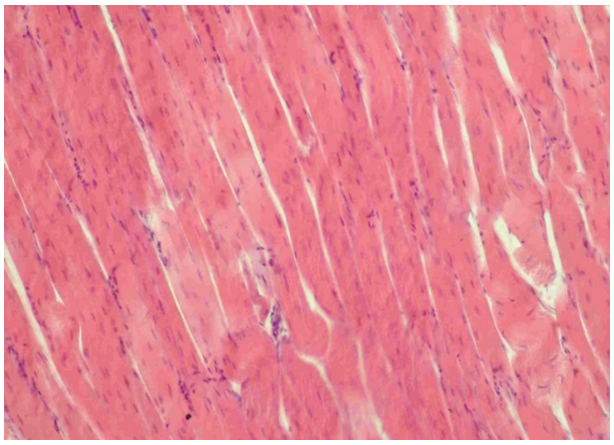

Fig. 3. Longitudinal section of fresh chicken muscles. Compact disposition of muscle fibers. Haematoxilin and eosin. ×200

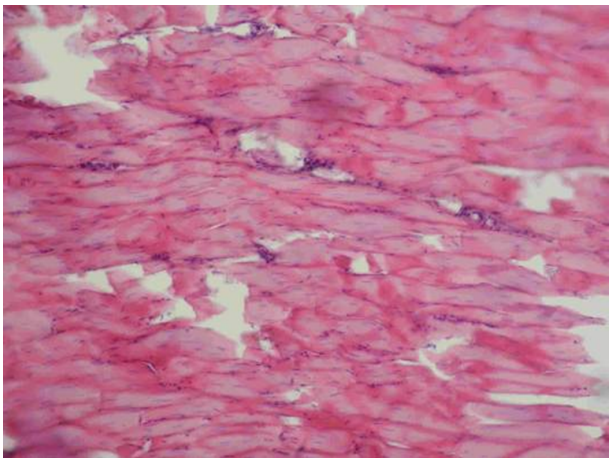

Fig. 4. Longitudinal section of muscles of chicken fillet after 6 days storage in refrigerator. Loss of longitudinal streakiness, induration of muscle fibers, in several cases destruction of sarcoplasmic membrane. Haematoxilin and eosin. ×100

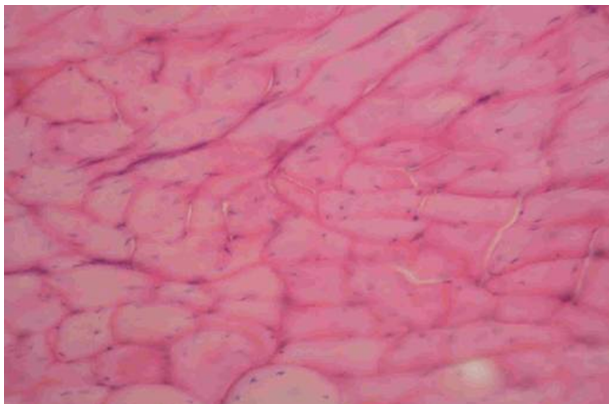

Fig. 5. Longitudinal section of muscles of chicken fillet with signs of damage. Strong swelling of muscle fibers. Nuclei are homogenous, separate ones are not observed. Haematoxilin and eosin. ×400

angle j for chicken fillet which was stored in the refrigerator for 6 days is –35 °, the angle for the meat with signs of damage is –2.9 °.

The impedance spectroscopy results are consistent with data obtained from microstructural studies.

The histological examination of the fresh chicken meat has determined that the numerous, elongated-oval dark blue kernels, which were located predominantly peripherally, can be seen on the longitudinal fiber cut under sarcolemma (*Fig. 3*). The studied muscles of chicken fillet belong to the group of white muscle fibers that are slightly thicker than the red ones, contain less myoglobin, mitochondria, small amounts of sarcoplasm and lipid inclusions and dense myofibrils. In cross sections the muscle fibers have polygonal shape, their nuclei are placed under sarcolemma.

In the chicken meat, which is being stored in the refrigerator for 6 days, in contrast to the fresh one, a stratification and local destruction of sarcolemma occurs. The fibers swell and overlap one another (*Fig. 4*). Connective tissue is destroyed and transformed into an amorphous mass.

Histological examination of chicken meat samples with signs of damage, indicated a strong swelling of muscle fibers, loss of transverse and longitudinal streakiness, lysis of most nuclei (*Fig. 5*). Between several muscle fragments the colonies of microorganisms were revealed (*Fig. 6*).

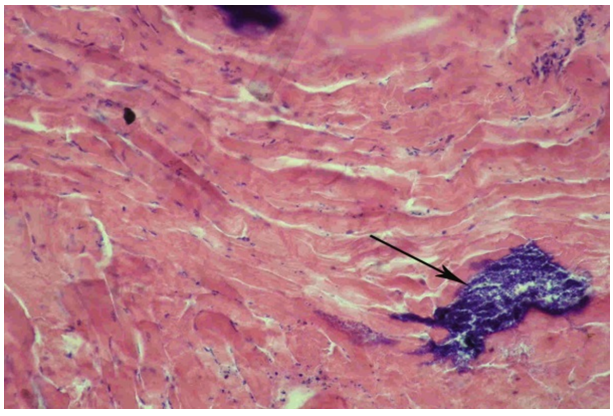

Fig. 6. Longitudinal section of muscles of chicken fillet with signs of damage. Colonies of microorganisms between muscle fibers (indicated by the arrow). Haematoxilin and eosin. ×200

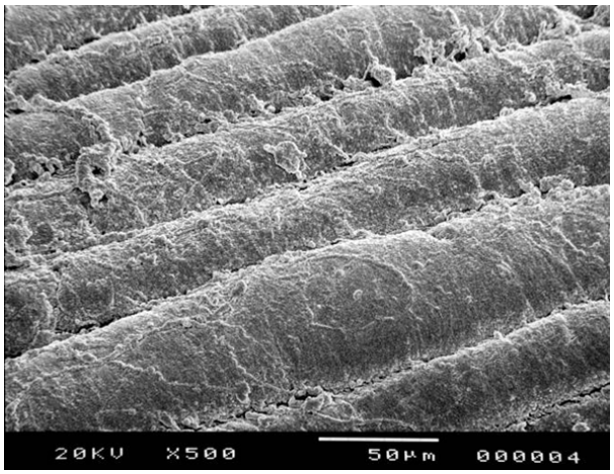

Fig. 7. Muscle fiber surface without damage of integrity. SEM. ×500

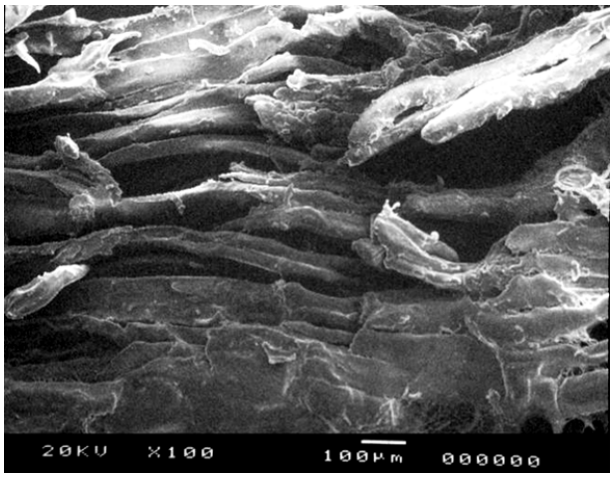

Fig. 8. Deformation and breaks of muscle fibers as a result of formation of ice crystals. SEM. ×100

The study of the surface structure of fresh chicken meat using scanning electron microscopy has revealed that the cell membrane in most parts of the muscle tissue has no structural damage. Muscle fiber surface is uneven, its integrity is not corrupted, in addition a small amount of connective tissue fibers was observed on the surface of muscle fibers (*Fig. 7*).

When storing the meat at low temperatures a dehydration of the surface layer occurs, leading to a decrease in the volume of muscle fibers, increase in their density and deformation. In the deeper layers of muscle fibers, abrupt deformation occurs resulting from the formation of ice crystals both between individual fibers and within them, under sarcolemma (*Fig. 8*). On cross-section the muscle fibers are irregularly shaped, the cavities, formed in place of ice crystals are often combined together to form multiple cracks located mainly between the bundles of muscle fibers and, less commonly, between individual muscle fibers. At that, the muscle tissue is significantly loosened in almost entire volume of muscle. The differences in the structure of the superficial and deep layers of the meat are more obvious. Sarcolemma exfoliates from the sarcoplasm. In the deep layers of meat the muscle fibers were deformed as well, but to a lesser extent than on the surface. Between the fibers the large cavities are found, but muscle fibers retain their polygonal shape (*Fig. 9*).

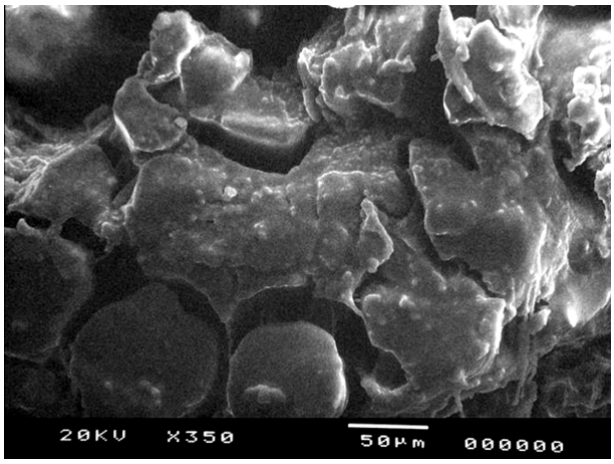

Fig. 9. Formation of cavities between muscle fibers. SEM. ×350

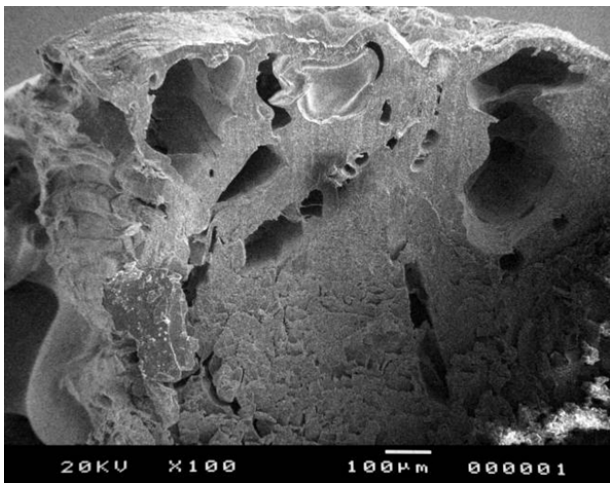

Fig. 10. Degradation and homogenization of muscle fibers under the influence of microorganisms. SEM. ×100

When studying the muscle tissue with signs of damage, it was determined that the increase in microbial contamination is taking place during the process of thawing ¾ defrostation of meat. At that the temperature on the surface of the meat rises, the muscle juice is released and thus favorable conditions for microbial growth are created. Their activity depends on the freezing method: the slow freezing results in the formation of large ice crystals, damage of muscle fibers, which, when defrosted, release considerable quantities of muscle juice and thus promote microbial growth. Rapid freezing results in formation of small ice crystals, which do not damage the muscle cells, so the released muscle juice is gradually absorbed. An important influence on the degree of microbial contamination of meat is the pH reaction, which depends on the glycogen content in muscle tissue.

In the superficial layers of muscle fibers, in the locations of ice crystals, microorganisms begin to propagate. Muscle fibers undergo degradation, fine-grained protein mass is formed between the fibers and the muscles themselves homogenize under the influence of bacteria (*Fig. 10*).

Conclusions

As a result of the conducted research, it was found that the dispersion curve of the resistance of chicken meat and polarization electric capacity of meat that was stored in the refrigerator and a meat with signs of aging, was dramatically reduced if compared with fresh chicken meat. This is because the cell membranes of fresh muscle fibers act as a barrier and hindrance to the movement of electrons and ions, while in the process of aging and damage of meat the destruction of cell membranes occurs. At that, the conductivity of meat increases, while the impedance is dramatically reduced. Accordingly, the polarization ratio for fresh chicken meat was equal to 2.95, while for the meat stored in the refrigerator and the meat with the signs of deterioration ¾ to 1.03 and 1.02, respectively. The conducted microscopic studies of chicken meat are consistent with the findings of impedance spectroscopy. Histologically, fresh chicken meat has distinct transverse and longitudinal streakiness with numerous nuclei, electron microscopy analysis detected muscle tissue cell membrane without any structural damages, while the meat, stored in the refrigerator, contained loosened muscle fibers, exfoliated sarcolemma and a large number of cavities in place of ice crystals. The chicken meat after storage and with signs of damage is characterized by strong swelling of muscle fibers, degradation and release of fine-grained protein mass between fibers, lysis of nuclei and the appearance of colonies of microorganisms.

Perspectives of future research. Since the impedance is a multicomponent index, which allows the analysis and prediction of functional condition of biological tissue, these studies should be continued both on various types of tissue and various kinds of animals.

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