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## The effect of storage conditions on the microstructure of sterilized canned meat

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### ABSTRACT

The article presents the results of studies of changes in the microstructure of the meat system as a whole and its protein component during freezing, subsequent defrosting, and storage of canned meat. Microstructural analysis of the prototypes showed the presence of several types of destruction of muscle fibers, loosening of collagen fiber bundles, and the formation of multiple cavities due to the action of ice crystals. The main components of sterilized canned meat had new characteristics after thawing, such as decreased transverse striations of muscle tissue fibers, loosening of myofibrils, changes in the size and shape of sarcomeres, violation of sarcolemma integrity, and multiple fiber fragmentation with the formation of a fine-grained protein mass. Freezing did not lead to a decrease in the content of the high-molecular-weight protein fraction of the nitrogen system, the ratio of the peptide fraction content to the residual nitrogen remained equal to 5.2. However, the ratio of non-protein nitrogen to total nitrogen decreased by 1.8 times due to the destruction of low-molecular-weight nitrogen under the action of ice crystals. The dynamics of the eh values of control and experimental canned food samples during storage indicated the loss of oxidative stability of the protein system of the samples subjected to freezing. Based on the results, we would like to recommend that logistic organizations sort and confirm canned meat safety and quality requirements after thawing in the case of unforeseen circumstances.

**Keywords:** canned meat, model, freezing, storage, microstructure, physicochemical processes

### INTRODUCTION

The life cycle of sterilized canned meat includes a storage phase. Storage of such products is a long and complicated process, requiring conditions to maintain quality and safety. According to the regulatory documents of Russia, sterilized canned meat is stored at a temperature between 0 and 20 °C and not higher than 75% humidity. Such conditions ensure the stability of quality indicators for 1 – 5 years, depending on the technological features of obtaining canned meat. Sterilized canned meat is a closed system where external access of oxygen to the meat system is excluded. Therefore, the system exchanges only thermal energy with the environment; an important role in this exchange is assigned to the transportation temperature and storage temperature.

Histology and sensory analysis methods can effectively resolve an issue relating to the quality of raw materials and finished products. While studying the sensory characteristics of the finished product makes it possible to determine its potential consumer acceptability, histological studies make it possible to identify changes in the morphological components at the cellular level when exposed to various technological factors. Also, histological studies enable the purposeful improvement of meat processing technology and quality monitoring of finished products, including during storage. So, using microstructure analysis, [1] established that a modified gas atmosphere influences the formation of fissures and pores in ground beef during storage. Disruption of the structure of muscle fiber bundles and loosening of the endomysium were detected in beef steaks under the influence of electrohydraulic shockwaves. These microstructure changes have been detected using confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM) [2]. In a study of porcine longissimus muscle, the results of circular dichroism (CD) spectral analysis and fluorescence spectroscopy indirectly proved that thawing can cause protein cross-linking and degradation, destruction of secondary structure, exposure of non-hydrophilic domains, and conformational changes of samples [3].

Scientific data on the histological and physicochemical changes that occur in the main components of canned meat in case of violation of the normal temperature and humidity of storage conditions and their impact on quality indicators are not systematized. Such violations include freezing canned meat during its transportation to consumers in the Arctic zone. It is known that recrystallization of ice due to fluctuations in storage temperature leads to tissue cell restructuring and a change in the morphological characteristics of muscle tissue. These changes, in turn, negatively affect the quality of frozen meat [4]. Fluorescence optical microscopy and SEM have also been used to confirm the effect of the freezing temperature on the size of ice crystals in frozen samples of minced beef [5]. As for sterilized canned meat, we obtained the first results of studies on changes in the morphological components of such products under the influence of model low temperatures [6]. The microstructural analysis is one of the traditional and effective tools for analyzing past transformations through visualization of the microstructure.

This study aimed to study the destructive changes in canned meat after sterilization, model freezing, and subsequent storage.

### Scientific Hypothesis

We hypothesized that negative storage temperatures are the root cause of destructive changes in the meat sterilized system.

## MATERIAL AND METHODOLOGY

### Samples

Sterilized canned meat was selected as the object of this study. Canned food was made from chilled beef.

### Chemicals

Chemicals are necessary for fixing samples during histological analysis, such as a neutral aqueous solution of formalin and gelatin, as well as substances necessary for staining samples: Ehrlich's hematoxylin and 1% aqueous-alcoholic solution of eosin were purchased from AppliChem GmbH (Germany). Other reagents and chemicals used in this study were purchased for analytical purposes from OOO TH CHIMMED and OOO LABTECH (Moscow, Russia). All chemicals obtained were of analytical grade unless otherwise indicated.

### Instruments

AxioImager A1 light microscope with AxioCam MRC 5 video camera and AxioVision 4.7.1.0 computer image analysis system (Carl Zeiss, Germany); potentiometer FE20 FiveEasy instrument (Mettler-Toledo, Switzerland); seaming-machine B4-KZK-79A (Russia); horizontal autoclave AG-1200 (Russia)

### Laboratory Methods

According to the methodology of histological analysis (Saprikin et al., 1997) for fixation, the samples were placed in a 10% neutral aqueous solution of formalin at a temperature of 21 – 23 °C for 48 h [7]. After that, the samples were washed with cold water for 4 h, after which they were impacted in gelatin. For this, the sample was first impregnated with a 12.5% gelatin solution for 6 h at 37 °C; then the impregnation was carried out in a 25% gelatin solution in a thermostat at 37 °C for 12 h, then the samples were filled with fresh 25% gelatin solution and quickly cooled in the refrigerator. After cooling, blocks with a size of 15 mm x 15 mm x 4 mm were cut out. Sections 16 µm thick were made on a microtome. Sections were stained with Ehrlich hematoxylin and a 1% aqueous-alcoholic solution of eosin and were enclosed in glycerine gelatin under a coverslip. Histological preparations were examined using the light microscope with a video camera and computer image analysis system.

The content of nitrogen fractions in the canned meat was determined by methods based on the ability of protein substances to precipitate under the influence of various reagents. Protein nitrogen was precipitated with trichloroacetic acid, followed by mineralization of the precipitate and determination of the nitrogen in it according to the Kjeldahl method (ISO 937:1978). Peptide nitrogen was determined by the difference between nitrogen precipitated with phosphotungstic acid and nitrogen precipitated with trichloroacetic acid. The amount of residual nitrogen was the difference between the amount of total nitrogen and the amount of protein and peptide.

The pH value was determined from the potential difference between the glass electrode and the reference electrode inserted into the product sample using a method authentic to the international standard ISO 2917: 1999, IDT.

The redox potential was determined on the potentiometer.

### Description of the Experiment

**Sample preparation:** To perform histological analyzes, the cans with canned food opened, pieces of meat removed, and cut across the muscle fibers into thin pieces and they used for research. Physicochemical

indicators were determined in the average sample of canned food. For this, the contents of the jar were taken out and thoroughly crushed to a homogeneous state.

**Number of samples analyzed:** 115.

**Number of repeated analyses:** 3.

**Number of experiment replication:** 3.

**Design of the experiment:** Samples of canned meat produced in an industrial plant from one batch of chilled beef weighing 1500 kg. After deboning the half carcasses and trimming the meat, the beef was chopped on a meat-cutting machine into pieces weighing 80 – 120 g. The rendered beef fat was heated to a temperature close to

70 °C. The edible salt, ground black pepper, and crushed bay leaf were thoroughly mixed. The ingredients are packaged in metal cans in the sequence: a mixture of salt, pepper and bay leaves, fat, and meat. The cans rolled on the seaming machine (Russia) and were placed in autoclave baskets. Sterilization with a water shower was carried out in the horizontal autoclave at a temperature of 120 °C and a system pressure of 0.18 – 0.22 MPa for 80 minutes. 600 pieces of canned meat were produced.

After sterilization, the entire batch of canned food was placed in a refrigerator with a temperature of minus 12 °C. After 7 days of storage, 300 cans were transferred to the defrosting chamber. Accelerated defrosting was carried out at an air temperature of 16 – 20 °C and its movement speed of 0.2 – 0.5 m/s. After defrosting with canned food, a set of studies was carried out according to the abovementioned methods.

Statistical Analysis

Microsoft Excel and XLSTAT software were used in this study for statistical analysis. All determinations were performed in triplicate. Significant differences between the average values of protein fractions were established using Student's test at  $p < 0.05$ .

## RESULTS AND DISCUSSION

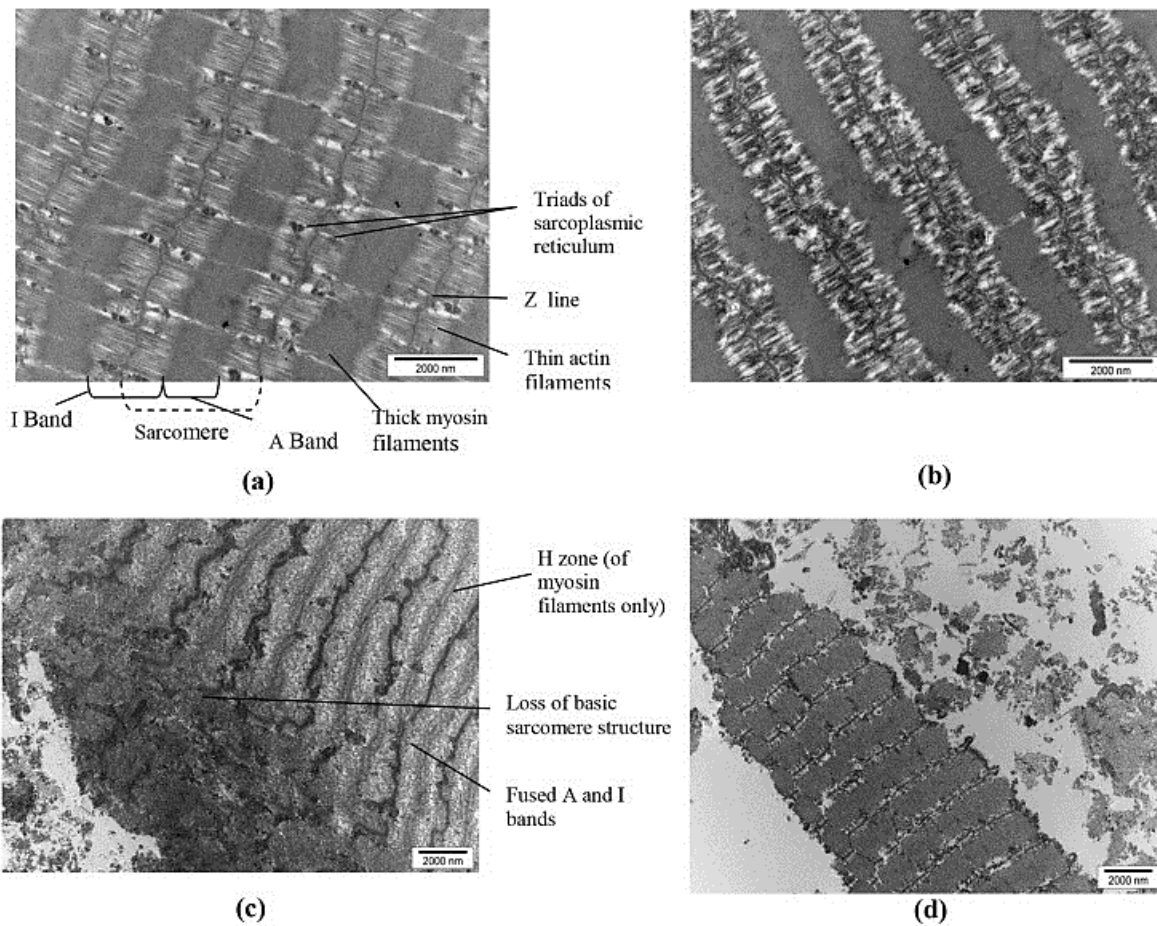
It reported changes in myofibrillar proteins and collagen of the connective tissue during heat treatment of different types of meat at the temperature range from 60 to 100 °C. The obtained results of studies of the microstructure of meat confirm the general trend of destructive changes in muscle tissue [8], [9], [10], [11], [12], [13].

Kaur et al. [14] noted that boiling beef at a temperature of 100 °C for 30 minutes led to an increase in the diameter of myofibrils by 3 – 4 times about their diameter in raw meat. After boiling, a decrease in the length of beef myofibrils by 186 – 189 nm was noted. The result is shown in Figure 1.

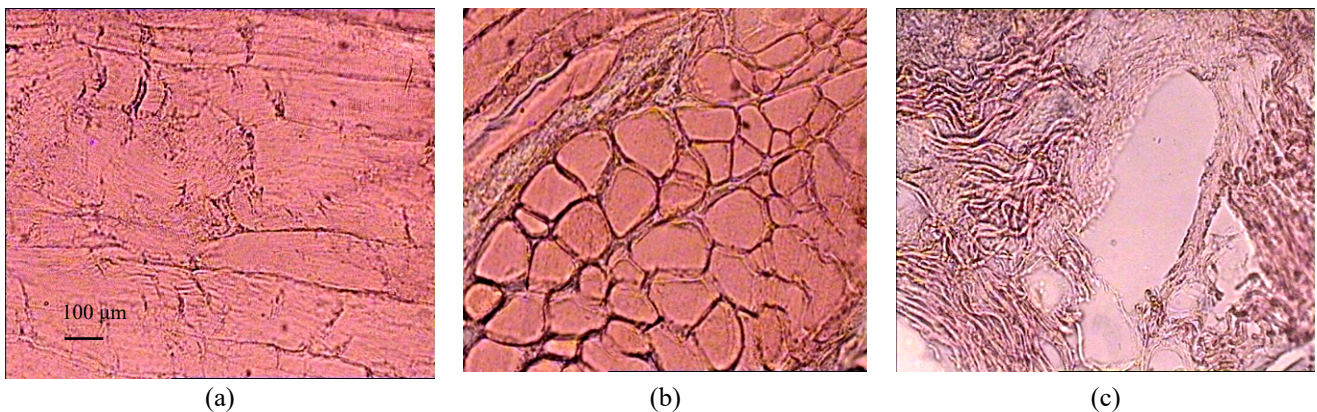
Industrial modes of sterilization of canned meat are in the temperature range 115 – 120 °C. Therefore, destructive changes in muscle proteins will be more significant, confirmed by our results.

The muscle tissue microstructure after sterilization is shown in Figure 2. Figure 2(a) shows that muscle tissue microstructure after sterilization is characterized by straight muscle fibers, the boundaries between them distinguishable. The transverse striation of the fibers was clearly defined; the length of the sarcomeres was 2.7 – 2.9 µm. Destructive changes were detected in the form of micro-fissures or isolated narrow transverse fissures.

The sarcolemma of the fibers was swollen and detached in the main part of the muscle fibers. A slight increase in fine-grained protein mass was visualized under the detached sarcolemma and between the fibers. The reason for its formation was the transition of sarcoplasmic and myofibrillar proteins into the inter-fiber spaces of muscle tissue during heat treatment of the canned meat.



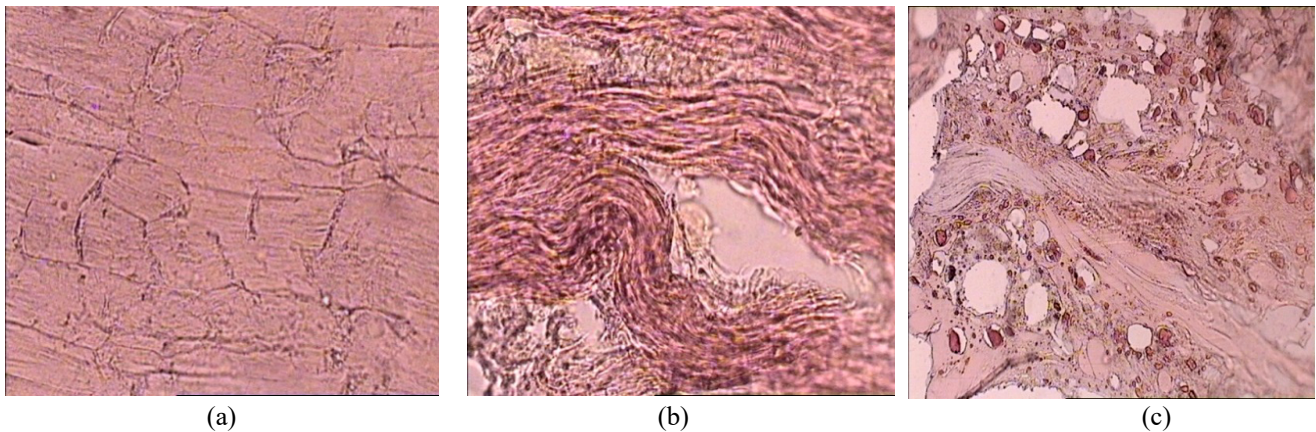
**Figure 1** TEM micrographs of (left) raw and (right) cooked (100 °C for 30 min) beef meat myofibril showing sarcomere structural detail (a – b); myofibrils after 30 (c – d) min.



**Figure 2** Muscle tissue microstructure after sterilization: (a) longitudinal section: destructive changes in the form of micro-fissures and isolated narrow transverse fissures; (b) cross-section of a muscle fibre; (c) elastic fibres of the connective tissue. Note: Magnification 260×.

In cross-section, the muscle fibers were polygonal; the average fiber diameter was 42.6 µm (Figure 2(b)). The destructive effect of sterilization also affected the connective tissue. Connective tissue layers displayed varying degrees of destruction: large bundles of collagen fibers were swollen, broken into separate collagen fibers; smaller ones were a homogeneous basophilic mass of heat-treated collagen. In the structure of the interlayers, elastic fibers are clearly defined (Figure 2(c)). Heat-treated collagen mass is homogeneous, penetrated by micro-capillaries with clearly defined edges.





**Figure 3** Muscle tissue microstructure after freezing: (a) longitudinal section; (b) loosening of bundles of elastic fibers; (c) multiple cavities – areas of ice crystal localization. Note: Magnification 260 $\times$ .

The northern territories of our country are huge and differ in climatic conditions. Suppose we assume that during transportation in a zone of high negative temperatures, the equipment of the railway transport that supports the necessary conditions of transportation is out of order. In that case, the canned food will be frozen for, for example, 7 days.

The question was how deep is the effect of negative temperatures on the microstructure of muscle tissue [15], [16]. It has been reported that the sarcomeres of raw meat can shrink by 50% after freezing or subsequent thawing [17]. Freezing and thawing meat lead to a deterioration in its quality [18], [19]. During meat storage in a frozen state, the protein undergoes several changes. With an increase in the freezing time, the heavy chains of myosin and actin degraded to varying degrees [20]. We also wanted to see how the destructive processes would affect sterilized canned meat storage capacity in normative storage conditions.

The muscle tissue microstructure after freezing is shown in Figure 3. Straight or slightly wavy fibers characterize muscle tissue microstructure after sterilization; their boundaries are distinguishable. The transverse striation was shallow and close; the length of the sarcomeres was 1.5 – 1.7  $\mu\text{m}$ . Destructive changes in muscle fibers were of varying degrees in different parts of the canned meat.

In some areas, there were multiple changes, in the form of narrow transverse fissures with partial fragmentation of fibers and the violation of sarcolemma integrity. There was less destruction of muscle fibers, and it was defined mainly by micro-fissures and transverse fissures without the violation of sarcolemma integrity.

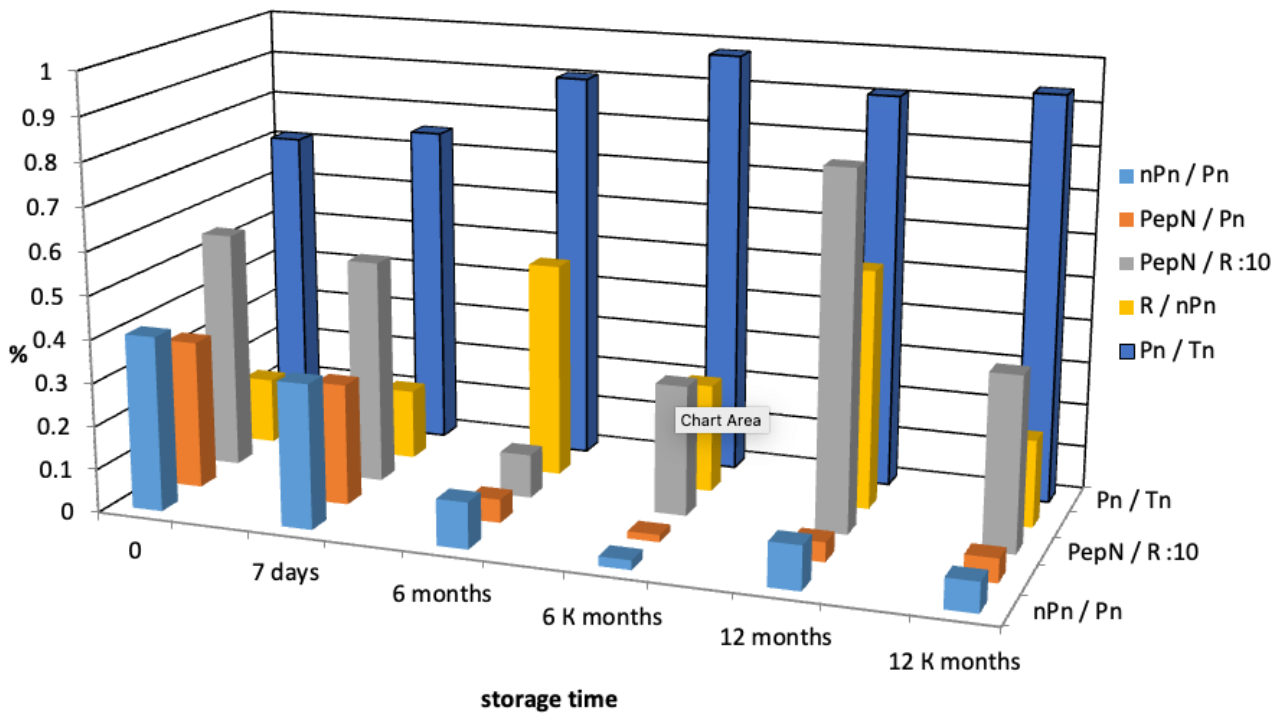
As shown in Figures 3(b) and 3(c), multiple cavities of indefinite shape – areas of ice crystal localization – were identified; elastic fibers were loosened.

In industrial production or at home, meat products are subjected to various types of processing, most of which cause destructive changes in protein [21]. Therefore, at low cooking temperatures, a slow rate of denaturation of myofibrillar proteins was observed [22], [23]. During thermal long-term low-temperature treatment, denaturation of the connective tissue of beef was noted at temperatures below 68  $^{\circ}\text{C}$  [24]. Destructive changes in pork connective tissue proteins are reported to begin at temperatures between 57  $^{\circ}\text{C}$  to 60  $^{\circ}\text{C}$  [25], [26].

The issues of destruction of sarcoplasmic, myofibrillar proteins [27], [28] and proteins of connective tissue [29] under traditional heat treatment modes are studied deeply and comprehensively, much attention is paid to prolonged low-temperature heat treatment [30], in particular its effect on the destruction of the protein system, changes in the color of meat [31] and tenderness of muscle tissue, due to the activity of cathepsins [32]. However, due to the peculiarities of the processes occurring in a closed system – a hermetically sealed can – under the influence of a temperature of 120  $^{\circ}\text{C}$  and a pressure of up to 0.22 MPa, it became difficult to compare the results obtained with the results of other scientists. Therefore, we focused our attention on studying the dynamics of the nitrogen fraction of the meat system. For this reason, as far as the authors know, destructive changes in the protein of canned meat, depending on the duration of their storage in a frozen state, have not yet been studied.

The effect of storing canned food in frozen form on the degree of change in the nitrogen forms of canned food before freezing is shown in Figure 4. After thawing, canned goods were stored for 12 months under normative temperature and humidity conditions. It was noted that freezing and subsequent storage of thawed canned food for 6 months caused an increase in the proportion of non-protein nitrogen fraction by 4.9 times compared with the control sample; after 12 months of storage, this ratio decreased to 1.5 times. The growth of the non-protein

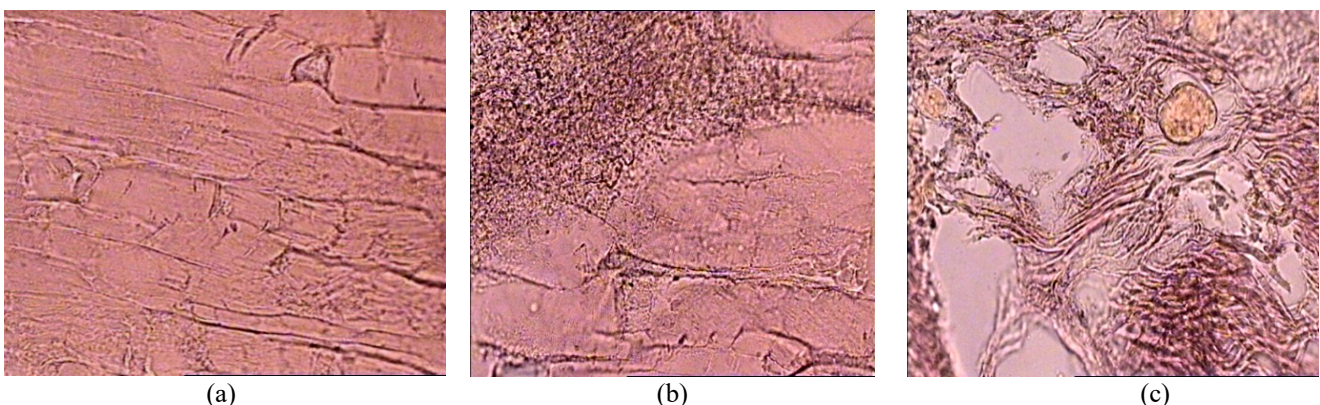
nitrogen fraction is associated with the accumulation of low-molecular-weight compounds, as evidenced by the dynamics of residual nitrogen concerning the non-protein fraction.



**Figure 4** Dynamics of the nitrogen fraction ratios during storage. Note: nPn / Pn – non-protein nitrogen / protein nitrogen, PepN / Pn – peptide nitrogen / protein nitrogen, PepN / R – peptide nitrogen / residual nitrogen, R / nPn – residual nitrogen / non-protein nitrogen, Pn / Tn – protein nitrogen / total nitrogen.

Freezing did not lead to a decrease in the content of the high-molecular-weight protein fraction of nitrogen systems. The ratio of protein nitrogen to total nitrogen tended to grow (0.71 to 0.79) and the ratio of non-protein nitrogen to total nitrogen to decrease (0.41 to 0.26), which can be explained by the destruction of low molecular weight nitrogen forms under the action of the ice crystals formed after 7 of storage. Partially relevant data was obtained in the study of pork tenderloin stored at a given negative temperature of  $-3 \pm 0.5 \text{ }^\circ\text{C}$  for 7 days: excessive protein denaturation was noted in the myofibrillar fraction with an increased storage time [33].

The muscle tissue microstructure after 12 months of storage is shown in Figure 5.



**Figure 5** Muscle tissue microstructure after 12 months of storage: (a) longitudinal section; (b) fine-grained protein mass; (c) loosening and destruction of collagen bundles in connective tissue. Note: Magnification 260 $\times$ .

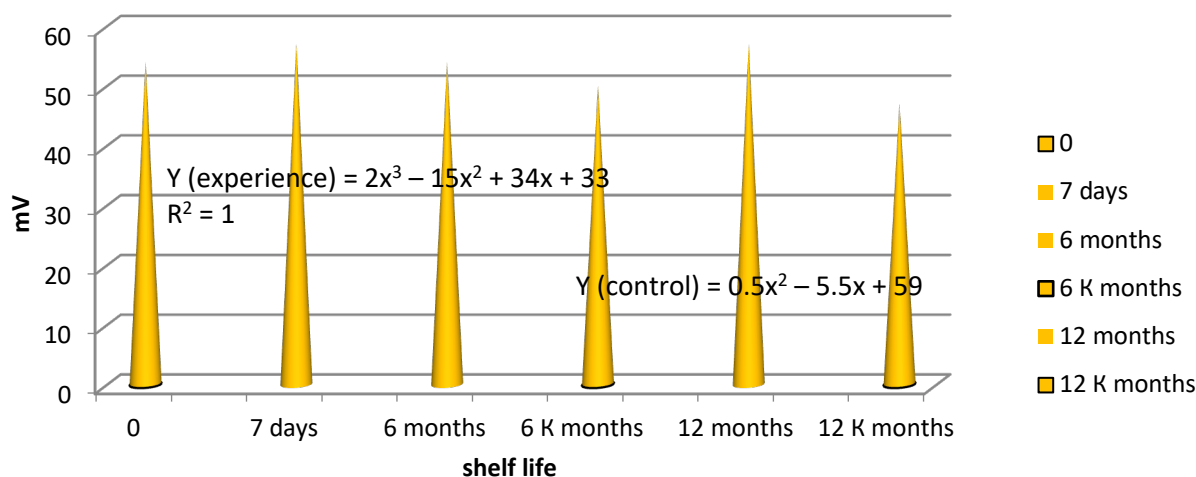
A longitudinal section (Figure 5(a)) showed that straight muscle fibers with well-defined boundaries characterized muscle tissue. The transverse striation was weak. The length of the sarcomeres was 2.7 – 2.9  $\mu\text{m}$ , the nuclei of the fibers were lysed, myofibrils were loosened, the sarcolemma of the fibers was peeled, and in some places, its integrity was violated. In the inter-fiber space, much more fine-grained protein mass was detected than unfrozen sterilized canned meat (Figure 5(b)). Destructive changes in muscle fibers were detected

in the form of multiple transverse narrow fissures or transverse fissures with fibers broken into fragments. In some areas, the length of the fragments was 100 – 350 μm. The connective tissue layers were loosened, homogeneous, marked areas of collagen bundles decayed to a fine-grained protein mass (Figure 5(c)).

The redox potential (Eh) is reported to be one of the potential barriers to food safety and quality [34]. The Eh value depends on several factors, in particular, on the level of dissolved oxygen in the medium, temperature, pH, and the concentration of components capable of oxidation or reduction [35], [36], [37]. To date, certain material accumulated on the dynamics of Eh about the storage of chilled meat [38], [39] obtained data on the Eh values of cooked, raw smoked, and offal sausages, minced meat with several food additives.

To assess the quality of canned food, Eh is used in a limited number of scientific works. Therefore, the measurement of Eh has not yet entered the practice of research in canned food. We have accumulated a knowledge base on the Eh values of raw materials and canned food made from them [40], [41]. The obtained experimental data are consistent with those available in the technical literature; scientific data on the dynamics of Eh of canned food during production and storage are new today.

The dynamics of the redox potential of canned meat after thawing and during storage are shown in Figure 6. The pH value of the investigated canned food underwent minor changes within the limits of the experimental error. However, the dynamics of these changes are different. So, in the control samples, the pH values were in the range of 6.41 – 6.36 units. In experimental samples – in the range of 6.41 – 6.26 units, which correlates with the dynamics of the redox potential values and demonstrates a decrease in the stability of the meat system after freezing during further storage.



**Figure 6** Dynamics of the oxidation-reduction potential of canned meat.

The dynamics of the Eh values of control and experimental canned food samples during storage had the opposite direction. The Eh of the control samples decreased from 52 to 46 mV after 12 months of storage, which indicates the stability of oxidative reactions in the meat system. At the same time, the dynamics of Eh of canned food subjected to model freezing and thawing were unstable: a decrease in the Eh value after 6 months and a subsequent increase by 12 months of storage of canned food. This may indicate the loss of oxidative stability of the protein system of the samples subjected to freezing. The results obtained agree with the data on the dynamics of nitrogen forms in canned food samples.

## CONCLUSION

The results obtained showed that short-term freezing of sterilized canned meat led to significant changes in the histological characteristics of the main structural components of muscle and connective tissues. The formation of ice crystals led to a loosening of the structural components of muscle tissue and elastic fibers, deformation of muscle fiber bundles, and the formation of cavities. This increased the degree of destruction of muscle and connective tissues and the release of myofibrillar and sarcoplasmic proteins into the interfiber space with the formation of a fine-grained mass. Freezing did not cause a decrease in the content of the high-molecular-weight protein fraction of nitrogen in the meat system. In contrast, the destructive effect of ice crystals on low-molecular forms of nitrogen was significant. It led to a 1.8-fold decrease in the ratio of non-protein nitrogen to the total. The ratio of the values of peptide nitrogen to residual nitrogen is most indicative. The predominance of peptide nitrogen in canned food after 7 days of freezing demonstrates the preservation of canned food protein: the PepN / R ratio was 5.2.



The dynamics of Eh as a factor of the redox stability of the meat system showed that freezing and thawing of canned food led to a violation of stability. A decrease in the Eh value evidences this after 6 months and a subsequent increase in Eh after 12 months of canned food storage. The results obtained agree with the data on the dynamics of nitrogen forms in canned meat samples.

Destructive changes in the main structural components of muscle tissue became the main reason for the increased degradation of proteins during the further storage of sterilized canned meat under standard conditions. Additional physical, chemical, and organoleptic studies are needed to determine the shelf life of the product after freezing.

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