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Development of a laboratory method for determination of the quality and freshness of frozen poultry meat

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ABSTRACT

Traceability of poultry meat quality imported into Kazakhstan is an urgent task. To increase their benefits, some suppliers resort to falsification – they misrepresent thawed meat for chilled raw materials or carry out several cycles of freezing-thawing meat. The objective of these studies is to develop reliable methods for determining the quality and freshness of frozen natural semi-finished poultry meat, including the number of cycles of freezing and thawing meat. Dressed broiler chickens developed by the manufacturer from the Russian Federation using gas and electric stunning of poultry were selected as the research objects. A synchronous analysis device was used for thermal analysis in the heating-cooling process. Histomorphological studies were carried out on a microscope with an eyepiece magnification of x7. Histological examination revealed alterations in the structure of re-frozen and thawing lean tissue. The differential scanning calorimetry (DSC) analysis showed that the specific heat of thawing broiler fillets stunned by electric current during the first thawing is 218.8 J/g, at the second 209.5 J/g, and the third 201.4 J/g, and the specific heat of thawing broiler fillets stunned by gas during the first thawing is 176.5 J/g, at the second 171.9 J/g, and the third 162.6 J/g. Samples of broiler fillets stunned by electric current lost about 2.5% at each thawing stage, and with gas stunning 3% of moisture. The obtained results and research methods can be used to establish the falsification of the thermal state of broiler chicken meat by its undeclared freezing-thawing.

Keywords: poultry stunning, broiler chicken meat, freezing, thawing, morphological changes

INTRODUCTION

Poultry meat production is increasing its volumes in Kazakhstan and abroad due to the availability and continuous improvement of technical support at all stages of the technological process **[1]**, **[4]**. The technological processing of poultry meat includes several interrelated stages that significantly affect the final quality indicators of meat and, consequently, of finished ready-to-serve foods **[2]**. The quality of the food product largely depends on the chemical, physical and structural changes that occur in the muscles during autolytic transformations after poultry slaughter **[3]**. One of the main stages of technological processing of dressed poultry is the stunning process, where the electric current of certain parameters is used in most cases of foreign and domestic practice. An alternative method of stunning is a regulated gas medium used in many European enterprises **[4]**, **[7]**. The advantages of stun technology in a controlled gas environment are to improve the quality of meat: there are no bruises, the colour and taste of meat are significantly improved, due to more intensive bleeding, the carcass and liver have a better appearance **[5]**, **[8]**. Currently, the stunning birds in a gaseous atmosphere are practically not used in Kazakhstan. This technology has found its application in Europe only recently, and in Kazakhstan, it has hardly been studied, and in this regard, it is of great scientific and practical interest **[22]**. Despite the increasing rates of poultry meat production in Kazakhstan, the share of imported meat is still high. Over the past 2020, the main suppliers of poultry meat to Kazakhstan were Russia, Ukraine, the USA, and Belarus (Figure 1) **[1]**. Traceability of the quality of meat raw materials imported into the republic is an urgent task. Some suppliers resort to falsification to increase their benefits – they misrepresent thawed meat for chilled raw materials or carry out several freezing-thawing cycles **[6]**.

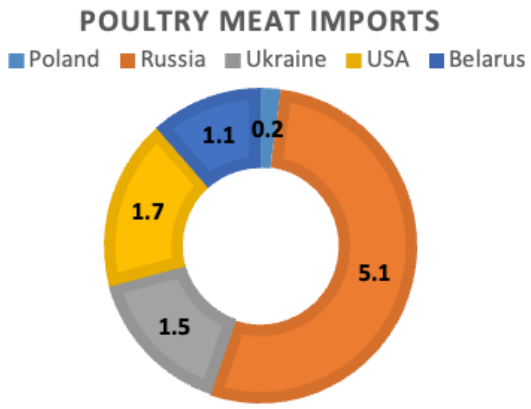


Figure 1 Structure of poultry meat imports to Kazakhstan, million tons.

Scientific Hypothesis

Studied the dependence of storage method on moisture bonding and histomorphological characteristics of chicken meat.

MATERIAL AND METHODOLOGY

Samples

To maintain the experimental integrity, dressed broiler chickens bred in the conditions of the agro-industrial complex of BEPFC of "Belgrankorm" (Belgorod region, Russia) using gas and electric stunning were selected as research objects.

Fresh frozen broiler chicken fillet stored before freezing at a temperature of 0 – 4 °C for a day after slaughter and broiler chicken meat subjected to several freeze-thawing cycles were used for research.

Instruments

A synchronous thermal analysis device STA 449 F3, Jupter by NETZSCH was used for thermal analysis during heating-cooling. The device simultaneously records the curves of differential scanning calorimetry (DSC) and mass loss (ML). Histomorphological research was carried out were studied on a Bio lamP 1 U4 2 microscope under lenses 3,2; 10; 40 with an eyepiece magnification of 7×.

Laboratory Methods

Histomorphology research methods were carried out according to GOST 19496-2013 **[12]**. The thermal analysis method was used to determine the forms of moisture-binding in raw materials **[21]**.

Description of the Experiment

Sample preparation: The thermal analysis method was used to determine the forms of moisture-binding in raw materials. To perform thermal analysis, a piece of cloth was cut out with a blade and placed in a crucible, then covered with an aluminum lid. A sample of lean tissue weighing 24 mg was taken for analysis.

The device simultaneously records the curves of differential scanning calorimetry (DSC) and mass loss (ML). The analysis of the broiler sample was carried out in a copper furnace connected to a Dewar flask in oxidized aluminium crucibles in a helium atmosphere. The accuracy of the temperature measurement was ± 0.3 °C.

The meat sample was continuously cooled – heated at a rate of 5K/min, according to the developed temperature program shown in Table 1.

Table 1 Temperature research program.

No.	Process	Process conditions and modes	
		initial temperature, °C	resultant temperature, °C
1	Freezing of fresh meat	25 in a closed crucible	-30, nitrogen cooling
2	Heating (Thawing)	–30 in a closed crucible	25
3	Freezing	25 in a closed crucible	-30, nitrogen cooling
4	Heating (Thawing)	–30 in a closed crucible	25
5	Freezing	25 in a closed crucible	-30, nitrogen cooling
6	Heating	–30 in an open crucible	250

Measuring and processing the output information in calorimeters is controlled from an IBM-compatible personal computer using a special software package, "NETZSCH-Proteus". Programmatically, calorimeters are

configured, modes are selected, experimental parameters are set, calorimeters are graduated based on measurements of the properties of standard samples, parameters are optimized, operation control, output information processing, printing and storing analysis results.

During the measurement process, the display of the personal computer is displayed in the mode of real-time heat flux values [мW] – (Y axis) as a function of temperature [t, °C or K] or time [τ, min or sec]. Upon completion of the experiment, the desired temperature of phase or structural transformation (T, °C or K), specific heat of phase or structural transformation (ΔH, J/kg), and specific heat (C, J/kg.K) are calculated using a special software section.

For histomorphological research, samples were prepared: scraps of 2 x 3 mm meat were cut off from the dressed poultry fixed in 10% neutral formalin for seven days. The samples were dehydrated in alcohols of ascending concentration starting from 50% and ending with absolutely anhydrous (100% concentration) with an interval of 4 – 6% and the duration of each stage of 24 hours. Dehydrated samples were filled with paraffin. Sections with 7 – 10 microns were made from paraffin blocks on a sledge microtome, stained with hematoxylin-eosin. Boehmer's alum hematoxylin was used as a nuclear dye, and alcohol eosin was used as the main one.

For histomorphological methods of broiler meat studies, samples intended for research were fixed in 10% neutral formalin.

The slices were obtained on the OMT 0228 microtome cooler mounted based on a sledge microtome. The hematoxylin-eosin method was used for staining, Ehrlich's alum hematoxylin was used as a nuclear dye, and eosin was used as the main one.

Number of analyzed: 12
Number of repeated analyses: 5
Number of experiment replication: 5

Statistical Analysis

Fundamental statistical analysis was carried out using the Statistica-6.1 software package (Sentinel System 7.5.7, V6.1). The Student's t-test determined the probability of similarity between the average values of the samples.

The average value of the trait before the experiment is 201.333 ±8.200 (m = ±4.734).
The average value of the trait after the experiment is 209.900 ±8.707 (m = ±5.027).
The number of degrees of freedom (f) is 2.
The Student's paired t-test is equal to 23.079.
The critical value of the Student's t-test for a given number of degrees of freedom is 4.303.
tnabl > tkrit, changes in the trait are statistically significant ($p = 0.002$).

RESULTS AND DISCUSSION

Histological examination of fresh broiler chicken meat showed a clear division of muscle tissue into peculiar slices **[14]**. Myocytes had a polygonal shape with rounded corners. Almost all cells had a uniform colour of the cytoplasm **[20]**. The cores were visualized clearly and had a rich colour. The striation of the fibers was distinguishable (Figure 2).

After a single cycle of freezing and thawing, the studied muscle tissue had a very similar structure to the cooled one; however, it was noticeable that the intercellular space was edematous, which is noticeable why the cells were located less densely and looked dormant. On the longitudinal sections of the fibers, their somewhat uneven colouring was visible, the nuclear apparatus was preserved, and the striation was weakly expressed (Figure 3).

Defects in muscle tissue that appeared due to thawing meat when injured by ice crystals are partially expressed **[16]**, **[19]**. Histological examination revealed alterations in the structure of re-frozen and thawing lean tissue **[7]**, **[20]**. The distinctive features were local layering of myocytes on top of each other, swelling of the cellular space, collapsed and destroyed connective tissue, and dormant myocytes **[8]**, **[10]**. At the same time, the striation and nuclear apparatus of the muscle fibers also changed: the pattern of the fibers was fuzzy, and the nuclei were destroyed. Muscle tissue frozen more than once is represented by a typical microstructure of highly fragmented muscle tissue after low-temperature treatment **[17]**, **[19]**. The pattern of transverse striation of muscle fibers was not visible. The nuclei of muscle cells were absent. Multiple "fractures" of muscle cells specific to low-temperature exposure were detected (Figure 4). Moreover, these signs were specific to these methods of stunning.

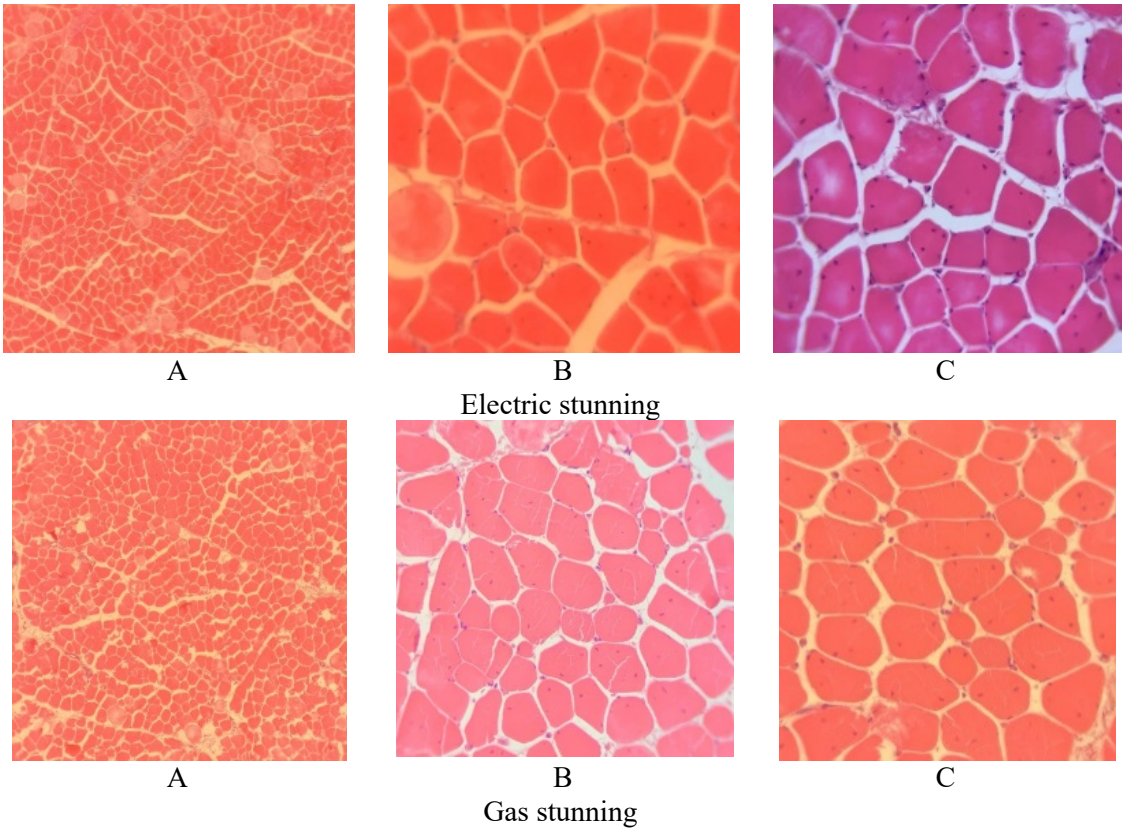


Figure 2 Histological structure of cooled muscle tissue of broiler chicken. A (mag. x100); B, C (mag. x400).

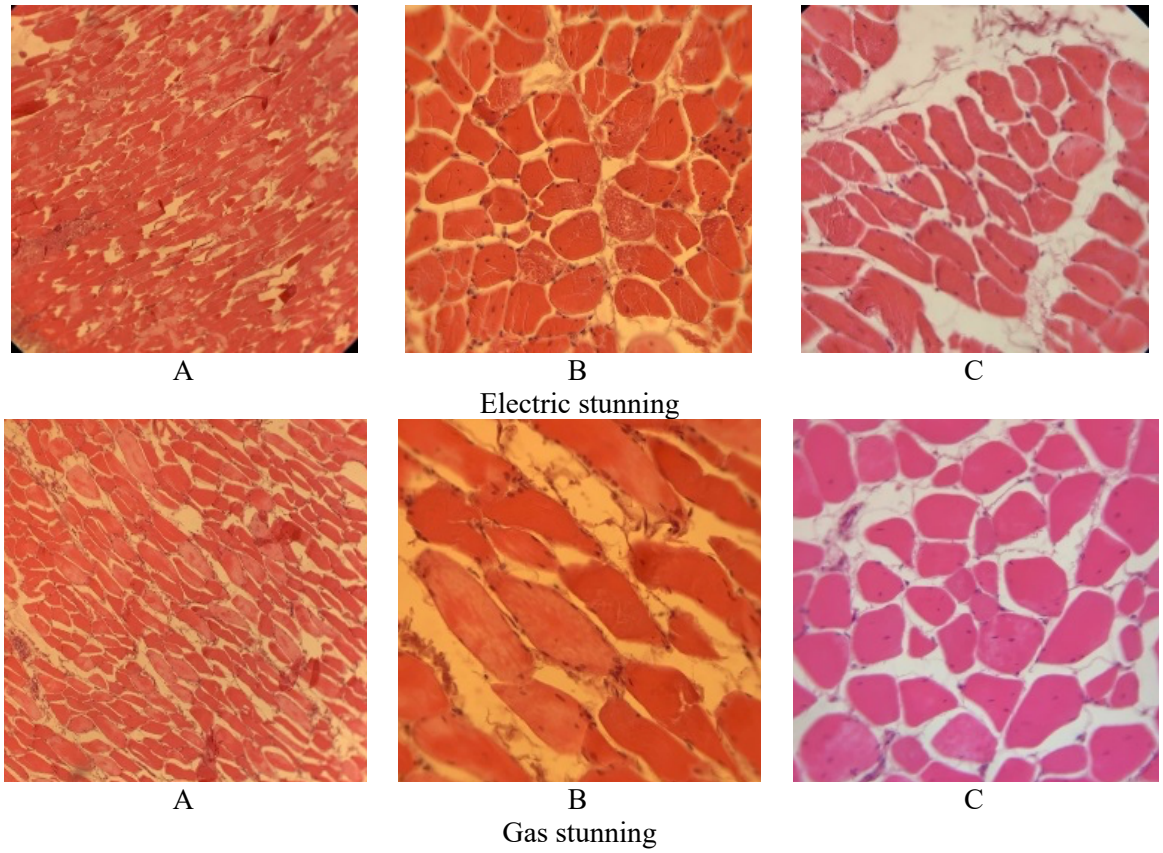
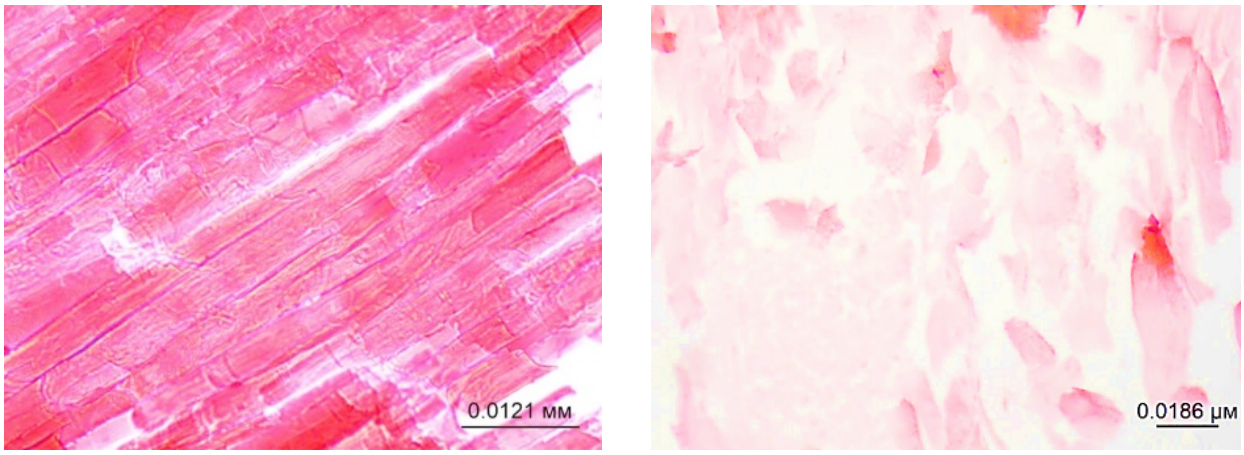


Figure 3 Histological structure of poultry muscle tissue after a single cycle of freezing and thawing A (mag. x100); B, C (mag.x400).



A B

Figure 4 Microstructure of the muscle tissue of a broiler chicken after several freeze-thawing cycles. Mag. x200. A – two cycles, B – three cycles.

Thus, by the type of histological sections of muscle tissue, it can be concluded that the multiplicity of freezing-thawing of broiler chicken meat and the terms and the change in the amount of moisture contained in muscle fibers during freezing **[21]**, **[22]**. And thawing can also be traced using thermal analysis by differential scanning calorimetry **[18]**, **[20]**.

Table 2 presents the results of thermal analysis of raw meat subjected to several cycles of freezing and thawing by differential scanning calorimetry.

Table 2 Results of differential scanning calorimetry of chicken fillet samples of various methods of stunning freezing cycles.

Stun method	Parameters	1 thaw	2 thaws	3 thaws
Electrical stunning	Geometric beginning °C	-3.3	-3.3	-3.4
	Area, J/g	218.8	209.5	201.4
	Shrinkage, %	2.51	2.54	2.52
Gas stunning	Beginning, °C	-3.3	-3.5	-3.6
	Area, J/g	176.5	171.9	162.6
	Shrinkage, %	3.35	3	3.15

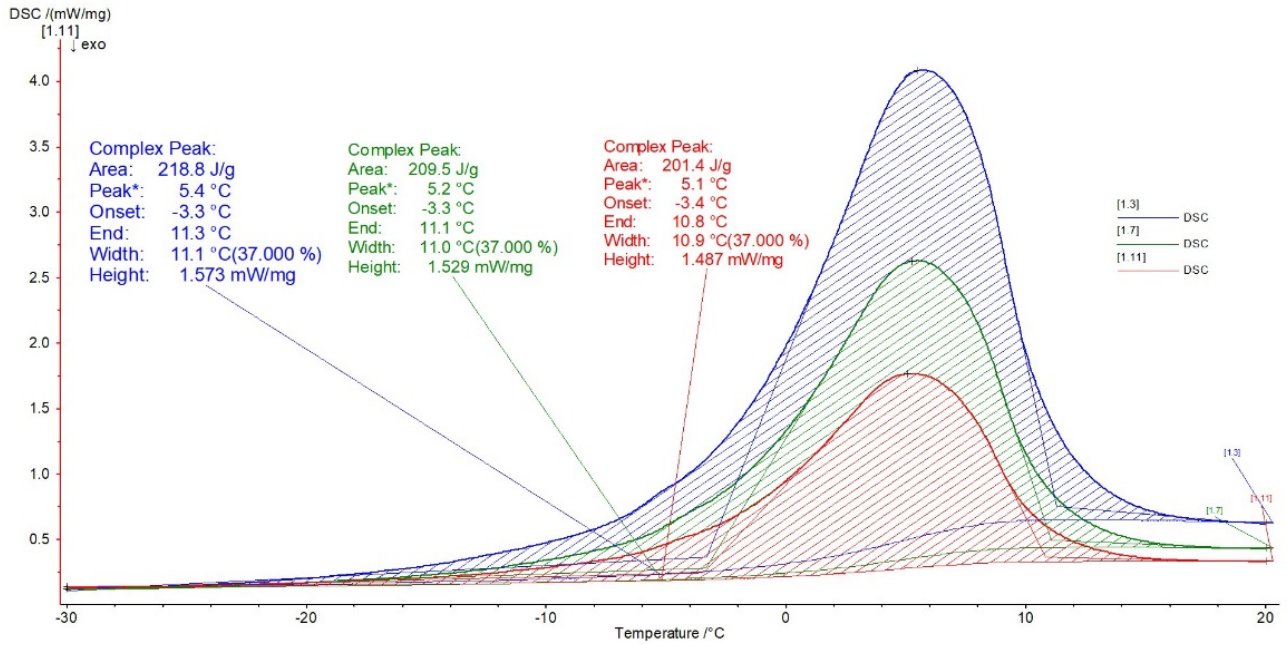


Figure 5 DSC of chicken fillet stunned by electric current (1, 2, 3 thaws).

The geometric beginning of melting moisture in broiler fillet stunned electrically is in the range from -3.3 to -3.4 °C, and in the broiler, fillet stunned by gas from -3.3 to -3.6. The specific heat of thawing of broiler fillets stunned electrically during the first thawing is 218.8 J/g, at the second 209.5 J/g, and at the third 201.4 J/g (Figure 5).

The specific heat of thawing broiler fillets stunned by gas during the first thawing is 176.5 J/g, the second 171.9 J/g, and the third 162.6 J/g (Figure 6).

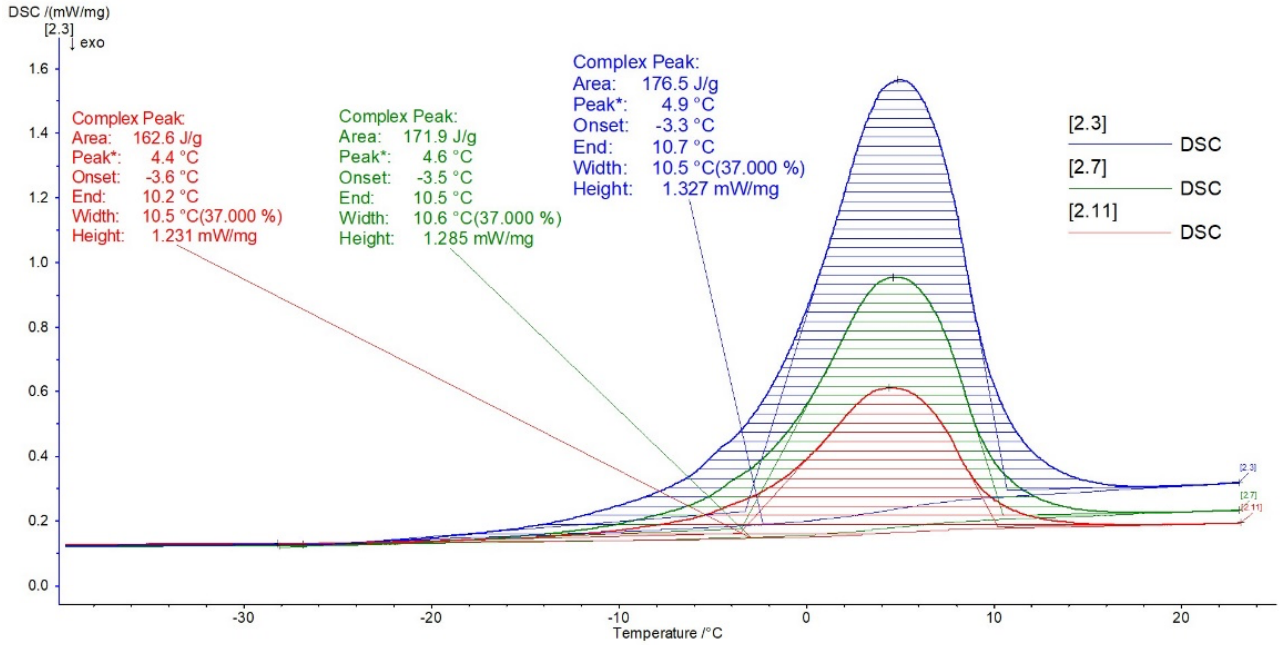


Figure 6 DSC of chicken fillet stunned with a gas mixture (1, 2, 3 thaws).

At each thawing stage, the samples lost some moisture due to evaporation. Samples of broiler fillets stunned by electric current lost about 2.5% at each thaw stage and 3% during gas stunning.

The DSC analysis curves showed that the object of study loses the amount of bound moisture with each freezing-thawing cycle, which is illustrated by a decrease in the specific heat of thawing **[17]**, **[21]**.

Temperature fluctuations cause the most noticeable damage to meat quality and its food safety. Repeated freezing and thawing of meat temperature fluctuations lead to recrystallization of ice. As a result, along with a decrease in the number of crystals, their sizes increase, violating the integrity of muscle fibers, protein denaturation, and large moisture losses **[8]**, **[10]**, **[13]**. Accordingly, the consistency of flesh becomes flabby and dry. In addition, with an increase in temperature, oxidative processes occur more actively, which is 2 – 3 times higher, for example, at a temperature of -9 °C than at a temperature of -18 °C, resulting in a rancid taste and smell **[11]**. Also, at temperatures above -10 to -12 °C moulds, some bacteria assimilate half-life products of proteins and cause the formation of ammonia, and hydrogen sulfide is in a viable state **[12]**. Repeated freeze-thawing cycles aggravate the picture: muscle fibers lose their clearly distinguishable structure and decrease volume. All of the above changes in meat can be observed during histomorphological studies **[9]**, **[15]**. The revealed difference in the stunning methods is most likely explained by the complete bleeding process at the primary processing stage of poultry meat **[7]**, **[8]**. The maximum deviation between the five measurements for each sample was no more than 0.5%.

The changes occurring during the refrigeration process are identical and reliably characterize the loss of moisture during repeated thawing and meat freezing.

CONCLUSION

Histological examination revealed alterations in the structure of thawed broiler meat tissue. The developed temperature program for conducting DSC studies of broiler meat allows using the thermoanalytical curves of differential scanning calorimetry to determine the difference in the quantitative characteristics of the processes of its one- and two-fold freezing in a closed oxidized aluminum crucible in a helium atmosphere in the temperature range from 25 to -30 °C in terms of cryoscopic temperature, peak area numerically equal to the exothermic effect of the freezing process. An urgent problem for poultry processing enterprises and trade enterprises is the objective assessment and classification of poultry meat based on the thermal state and types of

cooling, following the scheme of technological processing and logistics. The expediency of using a synchronous thermal analysis device for monitoring the storage, processing, and transportation of fresh and chilled poultry meat has been proved, and the facts of falsification of poultry meat in a thermal state in analytical laboratories have been revealed. The developed method does not require complex sample preparation, is suitable for operational or operational control of processing, and ensures the objectivity and reliability of measurement results since it allows you to identify changes in poultry meat in the surface layer and in muscle tissue. The obtained results can be used to establish the falsification of the thermal state of broiler chicken meat by its undeclared freezing-thawing.

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