Studies on the identification of frostbitten and frozen meat after defrosting by the spectrophotometric method for determining DNA in muscle tissue extracts

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Abstract. Changes in the state of meat during freezing are determined by the phase transition of water into ice and an increase in the concentration of substances dissolved in the liquid phase. The process of crystal formation leads to a change in the physical characteristics of the meat and may be accompanied by changes in its structural properties. The effect of the proportion of frozen water on the permeability of the membranes of muscle fibers of frostbitten and frozen meat has been established. The ratio of optical densities at wavelengths of 270 nm and 290 nm (R) can be used to judge the thermal state of the meat. It is shown that the value of R after defrosting frozen meat is 2 times higher than for frostbitten meat.

1 Introduction

It is known that the formation of ice crystals during freezing causes a change in the structure of muscle tissue cells, which in turn affects the thermophysical and biochemical parameters of meat [1, 2].

In early studies on cryobiology and the technology of cold meat preservation, there were hypotheses about the causes of mechanical degradation of cells due to crystal formation and an increase in the concentration of extracellular fluid and, as a result, damage to membranes. The mechanism of membrane damage was considered as consisting of two stages: an increase in the permeability of cell membranes under the action of a concentrated solution and the formation of micropores in them at the thawing stage [3].

In later studies, membrane damage was associated with the volume of the unfrozen water fraction, and not with an increase in the concentration of extracellular fluid or a change in membrane permeability upon freezing [4].

Freezing and thawing mainly affects the water fraction of meat, since water is contained within and between the muscle fibers. As water freezes, the concentration of remaining

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solutes (proteins, carbohydrates, lipids, vitamins, and minerals) increases, which in turn affects cell membranes [5-7].

In the literature on food freezing, to study changes in the permeability of muscle tissue membranes, the determination of the content of deoxyribonucleic acid (DNA) in freely flowing or pressed muscle juice is widely used.

DNA is found in the nuclei of cells and in the mitochondria of muscle tissue. Damage to cells during low-temperature treatment causes partial destruction of the cell wall of organelles and can lead to the release of their structural components.

There are a number of studies devoted to the study of the effect of freezing on the destruction of cellular structures and the degree of release of enzymes, proteins and cell DNA. These questions were considered in refrigeration technology and cryobiology in the works of Meryman H.T. 1966, Islam M. S. et al. 2017 [8, 9]. The content of enzymes, proteins and DNA in the outflowing juice during defrosting can characterize the degree of damage to muscle tissue and the methods of freezing and thawing [10, 11].

The nature of crystal formation also depends on the depth of meat autolysis. Freezing meat in the early stages of autolysis results in the formation of small ice crystals inside the muscle fiber. In all likelihood, the high hydration of fresh meat proteins and the low permeability of the sarcolemma prevent the movement of moisture from the muscle fiber. As a result, ice crystals are concentrated inside the muscle fiber [12].

Thus, the formation of ice crystals in such a complex system as meat depends on the rate of freezing and the physicochemical and structural properties of muscle tissue, which are determined by the depth and nature of autolysis. A brief theory of crystal formation during meat freezing is necessary to provide a more complete understanding of the issue of changes in the DNA content and its structure during the freezing-thawing process of meat, as well as to determine the number of nuclei released as a result of damage to the cell sarcolemma by histological method.

Currently, the amount of DNA and the nature of its damage are used to determine the falsification of meat raw materials: chilled, thawed, species, as well as in finished food products to control compliance with cooking technologies and product composition.

The works of Šimoniová A. et al. (2013), Zhao J. et al. (2018) show the possibility of differentiating chilled meat from thawed meat by identifying the nature of DNA degradation caused by the action of specific enzymes (citrate synthase and mitochondrial enzymes), which are partially released during defrosting. The influence of the shelf life of frozen beef on changes in DNA yield was also studied [13, 14].

In researches by Wei R. et al. (2017), the goal was to study the electrical properties and quality of frozen-thawed chicken breast meat and to study the relationship between these parameters at different times of frozen storage. The results showed that water-holding capacity (WHC) and protein solubility decreased, while the content of thiobarbituric acid reactive substances increased with increasing storage time [15].

Park J.H. (2000) when studying the effect of cooling, freezing and re-thawing on the change in meat, to determine the content of DNA and the nature of its damage, they used the electrophoretic method "method of comet". The data obtained from experiments with cold storage of meat for 3 to 10 days were compared with fresh meat. The differences between the values of the average “DNA tail length” were not so significant as to use these data to distinguish between the shelf life of meat [16].

A significant increase in the level of DNA damage was observed after repeated thawing, which was not observed during refrigerated storage. As a result of the study, it was shown that the determination of DNA in muscle juice can be used to differentiate chilled and thawed meat, as well as the freeze-thaw frequency (in 5 cycles).

The analysis of informational data allows us to assert that by determining the nature of DNA destruction and changing its amount in the resulting muscle juice, it is possible to

differentiate chilled and frozen meat, as well as use these data to determine the freshness of meat and the freeze-thaw frequency. The research results are of interest in conducting scientific research to determine DNA in muscle tissue extracts in order to identify frostbitten and frozen meat after defrosting.

2 Materials and methods

The object of the study was the meat of cattle – the muscles of Longissimus Dorsi with a mass of 0.2-0.3 kg. The meat was sorted into quality groups according to the values of the active acidity of the medium (pH) and cryoscopic temperature.

The active acidity of the medium was determined by directly measuring it in the thickness of the L. Dorsi muscles using a Testo-205 pH meter, entered in the State Register of Measuring Instruments of the Russian Federation under No. 30759-05. The combination of a penetrating pH sensor and a temperature probe guarantees high detection accuracy and fast temperature compensation regardless of environmental conditions.

The cryoscopic temperature was determined by thermographic analysis by the stabilization temperature on the freezing curve, characteristic of the phase transformation of water into ice, using a precision meat temperature meter MIT-2.05M at a temperature of -(20 ± 1.0) °C. The limit of permissible basic error of the MIT-2.05M ºC ± (0.015+10-5*T) is included in the State Register of Measurements of the Russian Federation under No. 46432-1.

Extraction and quantitative determination of DNA was carried out according to the method of Spirin A.S. in the modification of Severin S.E .:

- meat sample was weighed on an analytical balance (1 g of meat);
- crushed into 10 pieces, transferred to a measuring cylinder (20 ml);
- 9 ml of 0.6N HCIO4 solution was added and stirred using a magnetic stirrer for 20 minutes;
- further centrifugation was carried out for 10 minutes at 2000 rpm;
- 1 ml of the solution was taken from the supernatant and diluted 1:10 with distilled water;
- 3 ml was taken from the resulting solution into a quartz cuvette and photometrically measured at wavelengths of 270 and 290 nm.

A 0.6N solution of perchloric acid was used as a control sample.

The determination of the optical density of the studied DNA extracts was carried out using a Spekol-1500 AnalutikJENA spectrophotometer in the ultraviolet region.

Statistical data processing was carried out using MS Excel.

3 Results and discussion

It has been experimentally established that the DNA content in muscle tissue extracts of frostbitten and frozen meat after thawing differ significantly (Table 1).

Table 1. DNA values in frostbitten and frozen meat extracts after thawing

Thermal state of meat	λ_{250}	λ_{270}	λ_{290}	R=270/290
Frostbitten-thawed beef	0.954	0.497	0.130	3.83
	0.976	0.505	0.130	3.88
	0.969	0.498	0.130	3.83
x	0.966	0.500	0.130	3.85

±s	0.011	0.004	0.000	0.03
Frozen-thawed beef	0.942	0.412	0.052	7.92
	0.946	0.415	0.057	7.28
	0.948	0.416	0.055	7.56
x	0.945	0.414	0.055	7.59
±s	0.003	0.002	0.003	0.32

It can be seen from the data that the R index – the ratio of the optical density at a wavelength of 270 nm to the optical density – at 290 nm in extracts of 0.6 N HCIO4 solution for frozen-thawed meat is 2 times higher than for frostbitten-thawed meat. It has been proved that the R index can serve as an objective criterion for distinguishing between frozen and frostbitten meat after defrosting.

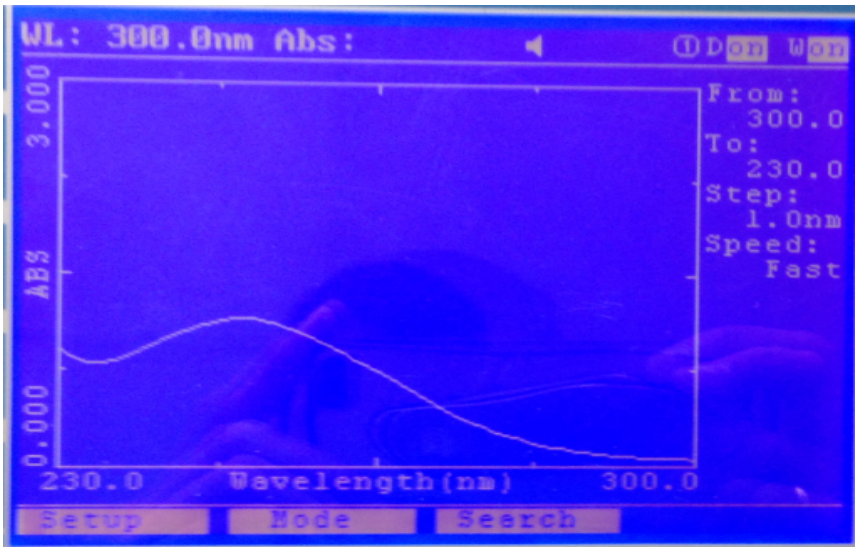

Fig. 1. – Extraction absorption spectrum of frostbitten meat after defrosting

4 Conclusion

On the basis of the obtained experimental data on the content of DNA in the muscle tissue extract, the effect of the proportion of frozen water on the permeability of the membranes of muscle fibers of frostbitten and frozen meat after defrosting was established. The ratio of optical densities at wavelengths of 270 nm and 290 nm (R) can be used to judge the thermal state of the meat. It is shown that the value of R after defrosting frozen meat is 2 times higher than for frostbitten meat.

Determination of DNA content by spectrophotometric method in extracts of muscle tissue can be used to identify frozen and frostbitten meat after defrosting.

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