Detection of myoglobin in somatic muscle tissue and analysis of its content in meat and meat products

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Abstract - **The aim of this study was to develop the methodology for the hystochemical detection of myoglobin in meat and meat products. Refrigerated and frozen meat, blood and cooked sausages, tendons and cardiac muscle were used as the subjects of research. Myoglobin was determined by the modified Verbolovich method. For the application of the image analysis system with the Bouin's reagent the contrast of the preparations was enhanced, after that the differentiated elements were seen more clearly. The results showed that myoglobin was released from muscle fibre into intercellular space through sarcolemma in meat that has undergone freezing. In refrigerated meat in the absence of pronounced autolysis myoglobin release from sarcoplasma into intercellular space was not observed. In meat products myoglobin was detected in association with the components including starch, soybean, collagen fibres and flour. It was concluded that the established fact of myoglobin release from muscle fibres in frozen meat allowed to differentiate initial meat thermal condition and, therefore, to distinguish meat that has undergone freezing from refrigerated meat at the early stages of process. The methodology for the histochemical detection of myoglobin was developed. The analysis of the obtained data showed that this method is semiquantitative.**

Keywords: **myoglobin, muscle tissue, microstructure**

I. INTRODUCTION

The methodology for the histochemical detection of myoglobin in somatic muscle tissue was developed and the comparative analysis of its content in meat raw material and finished products was carried out. Analysis of the obtained results of the experiments on the myoglobin detection demonstrated that this method can not be used in the development of the methodology for quantitative detection of muscle tissue content because it is difficult to detect the precise quantity of myoglobin and correlate it with the quantity of muscle tissue. In this connection, this method is semiquantitative. It was found that myoglobin was released from muscle fibre through sarcolemma into intercellular space in frozen meat raw material. This did not occur in refrigerated meat. This phenomenon allows to detect the thermal condition of meat and to distinguish frozen meat from refrigerated meat at the early stages of production.

II. MATERIALS AND METHODS

The first stage of the development of the methodology for microstructural detection of myoglobin in somatic muscle tissue and in raw material and finished meat products was the identification of the differences in the configuration of myoglobin and haemoglobin. It is known from the literature that myoglobin is stained brown in colour and has a pattern of granular deposits; haemoglobin, in its turn, is revealed as brown needle-shaped formations.

The second stage was the conduction of the examinations of muscle tissue by the histochemical method of myoglobin detection in cardiac muscle according to Verbolovich taken as a basis. This method comprises fixation of specimens in Baker's fluid for three days, preparation of sections using freezing microtome, staining of sections with benzidine reagent for myoglobin.

Conducting these investigations we found that fixation with Baker's fluid used in this method was not appropriate for study of preparations in the image analysis system because the necessary background contrast of a preparation for clear distinction of elements was absent. In connection with this, the colourless Baker's fluid was replaced by Bouin's fluid, which stained a section yellow in colour, and in reaction with benzidine reagent imparted a section yellow-green hue; on this background, elements stained brown were perfectly seen.

III. RESULTS AND DISCUSSIONS

In studies of refrigerated beef (*semitendinosus* muscle) on myoglobin, we observed the occurrence of brown particles only on the muscle fibre; this reaction was not seen in the intercellular space (Fig. 1).

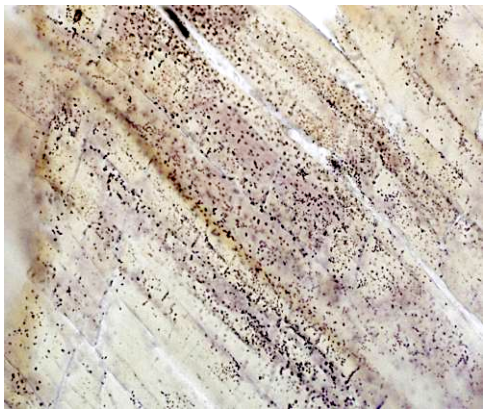

Fig. 1 refrigerated beef, for myoglobin, with Bouin's reagent and benzidine (40x)

In studies of frozen pork (*longissimus dorsi* muscle) on myoglobin, the occurence of brown particles was observed not only on the muscle fibre, but also in the intercellular space, which gave evidence of early disruption of sarcolemma and release of muscle proteins into intercellular space. Also, in the intercellular space needles and stars were found; this can be explained by the presence of haemoglobin in muscle tissue due to the presence of numerous capillaries in proximity to muscle fibres, which were damaged during freezing of muscle tissue, and haemoglobin was distributed through meat structure. The development of stars can be explained by imposition of needles and sticks (Fig. 2).

The study of the sausage 'Doctorskaya' using Bouin's reagent and benzidine showed that myoglobin was present in ground meat basic structure in the form of granules. On the envelope, a lot of stars with short thick arms and a small number of stars with long thin arms were seen; long needles were in vacuoles; granules were observed on the ground meat structure. The presence of stars with short arms and short sticks was revealed at the other end of the specimen.

Nails and short sticks were seen in vacuoles. Granules were found on a muscle fibre. In the area of vegetable flour location a reaction did not take place and the formation of a dye was not observed (Fig. 3).

Fig. 2 frozen pork, for myoglobin, with Bouin's reagent and benzidine (63x)

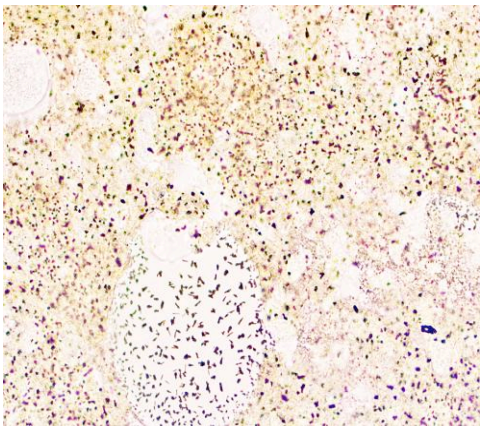

Fig. 3 sausage 'Doctorskaya' with Bouin's reagent and benzidine (20x)

The examination of the same sausage but with use of Bouin's reagent, NaOH and benzidine demonstrated the presence of granules on the sausage structure; short sticks were in vacuoles on periphery. Granules and partially short sticks were observed in the centre of the specimen; short sticks and a few needles were in vacuoles. Granules were found on a muscle fibre. In the area of vegetable flour location a reaction did not take place and the formation of a dye was not observed (Fig. 4).

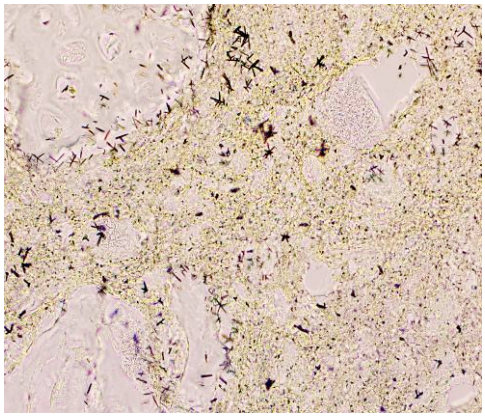

Fig. 4 sausage ‘Doctorskaya’ with Bouin's reagent, NaOH and benzidine (20x)

The investigation of blood sausage ‘Krovjanaja’ on myoglobin with Bouin's reagent and benzidine showed that myoglobin was located on the surface of the muscle fibre section in the form of granules. Stars with arms in the form of needles were found in the rest of ground meat structure. Mainly thin, short, small sticks and sticks in the form of needles occurred in sausage vacuoles (Fig. 5).

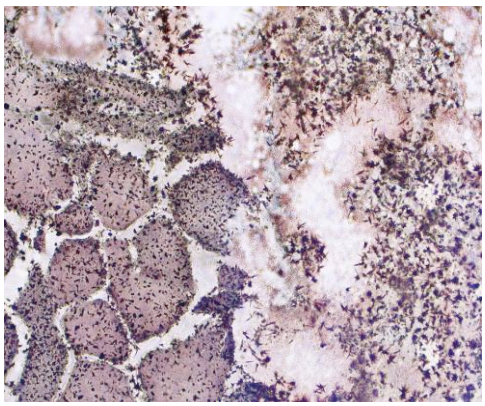

Fig. 5 sausage ‘Krovjanaja’ with Bouin's reagent and benzidine (40x)

In study of the same sausage with NaOH, Bouin's reagent and benzidine, the localization of myoglobin on the surface of meat fibre in the form of granules was revealed. Thick rod-shaped and star-shaped formations were found in the rest of the ground meat structure. Mainly thin, short, small sticks were seen in sausage vacuoles.

In study of meat products, the detection of myoglobin on all components including vegetable can be explained by the structure of the examined products resulting from strong mechanical grinding and temperature effect.

The examination of the pork skin preparation with Bouin's reagent and benzidine showed the complete absence of dye on the collagen and elastin fibres, as well as on the inclusions of fat tissue. Thus, the elements oxidizing benzidine were absent (Fig. 6).

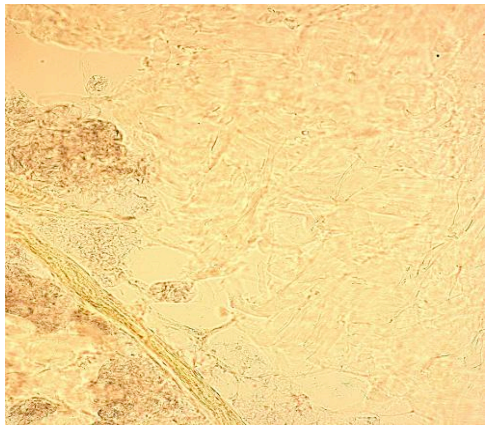

Fig. 6 pork skin with Bouin's reagent and benzidine (40x)

IV. CONCLUSIONS

As a result of this research, the appropriate fixing fluid was selected so as to obtain more contrast sections. The final methodology for myoglobin detection in muscle tissue and meat products includes the following: treatment with Baker's fluid and benzidine reagent according to Verbolovich.

The analysis of the obtained results of the experiments on the myoglobin detection demonstrated that this method can be used for the determination of different thermal conditions of meat. It is possible to distinguish frozen meat from refrigerated meat at the early stages of production. In studies of frozen muscle tissue the release of myoglobin from muscle fibre thorough sarcolemma into intercellular space was established, which gave evidence of primary disruption of cell membrane. The examination of refrigerated meat raw material has not revealed the release of myoglobin into intercellular space, which suggests sarcolemma integrity.