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Original scientific paper

THE STUDY OF THERMAL DENATURATION OF BEEF, PORK, CHICKEN AND TURKEY MUSCLE PROTEINS USING DIFFERENTIAL SCANNING CALORIMETRY

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In the temperature range from 45 °C to 90 °C the process of thermal denaturation of a whole complex of muscle proteins in meat takes place. An effective mode to register the thermal denaturation process is the method of differential scanning calorimetry (DSC). As a result of studies the differences during the process of thermal denaturation of muscle proteins of pork, beef, chicken and turkey were defined by the appearance of endothermic peaks in DSC thermograms. The main variances are associated with the process of denaturation of myosin and sacroplasmic proteins and indicate indirectly their quantitative ratio in meat. The values of effective specific heat capacity in the temperature range from 20 °C to 90 °C are obtained as well as those of heat spent on the denaturation process.

At reheating, the values of specific heat capacity increased by $0.1 J/(g^*K)$ on the average, and peaks of thermal denaturation were not detected, that certifies the irreversibility of the denaturation process and the decrease in the bound moisture proportion in meat after thermal processing. Knowledge of the nature of protein thermal denaturation of each kind of meat product is one of the necessary tools for developing the technology of meat product thermal processing.

Introduction

During thermal processing reversible and irreversible physical and chemical processes occur in meat products, due to that they acquire necessary taste and food digestibility increases [1]. But under the impact of high temperatures the negative changes take place too, such as contraction and compaction of muscle fibers, juice release that lead to loss of vitamins and precious microelements [2].

A lot of scientific works of domestic and foreign scientists are devoted to the study of thermal protein denaturation [2,3,4,5,6,7,8]. The major methods applied are differential scanning calorimetry and electrophoresis. The differential scanning calorimetry (DSC) is the most straightforward procedure and effective method to register the process of protein denaturation [8]. This method fits well for analyzing food systems often subjected to heating and cooling during technological processing [6].

But the initial DSC studies of denaturation of different types of muscle protein gave discrepant results [9,10,11]. It was due to one or combination of next variables: pH, muscle type, connective tissue, thermostability of muscle proteins. Post hoc, the attempts were made to normalize the variations by detailed study of the impact of each factor. Stabursvik and Martens [12] have developed a method to obtain reproducible curves by pH correction and removing sarcoplasmic and connecting proteins without the need to separate individual proteins for analysis.

From literature data it is known [5] that the thermal denaturation of animal muscular tissue protein begins at temperature above 40 °C and lasts step-by-step up to 125 °C. Herewith, three major transitions are defined that are reflected in the form of peaks in the DSC thermograms: in the temperature range from 50 °C to 65 °C the transition is associated with myosin denaturation; from 60 °C to 75 °C with denaturation of sarcoplasmic proteins and connected tissue; with denaturation of different forms of actin in the temperature range from 71 °C to 83 °C) [5,7,9,12].

In the work [13] the studies of the influence of the thermal processing temperature on the degree of denaturation of myofibrillar and sarcoplasmic protein fractions of pork of NOR and PSE qualitative groups were carried out using electrophoresis. A marked dependence of the intensity of denaturation of sarcoplasmic proteins on the pork meat belonging to one or another qualitative group was revealed. At the same time the nature of denaturation of myofibrillar proteins of NOR and PSE pork was identical.

The addition of various recipe ingredients in meat raw materials also affects the process of thermal denaturation of proteins. DSC studies of the effect of mono- and divalent salts on the protein thermal stability are given in works [14,15,16,17]. The studies of each work were carried out using meat produce of certain species of animals, mainly pork and chicken.

This paper reports the results of studies of muscular tissue of pork and beef meat, chicken and turkey meat performed using DSC method in order to compare the behavior of thermal denaturation process in the connection with belonging of meat to one or another animal species. The thermograms of muscular tissue of different animal meat and poultry were compared; the changes of specific heat capacity before and after thermal processing were analyzed as well.

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Establishing the relations between biochemical factors and the character of thermograms of raw materials at different protein content is promising. Today, there is still a lack of researches in this field. To provide a high quality meat products it is needed to define the individual thermal processing conditions on the basis of knowledge of thermal denaturation of each kind of meat raw material. That will provide quality increasing of an output product.

Materials and methods

The samples of muscular tissue of the longissimus dorsi muscle of pork and beef, sternum muscle of chicken and turkey were selected for studying. Three samples of each meat were selected; they were muscular tissues without skin, streaks of fat and bones. Sample mass was from 200 to 300 grams. The muscular tissue samples were purchased in big department stores of known trade networks.

During experiments three measurements of each meat were carried out.

The NETZSCH DSC204 F1 device (NETZSCH-Geratebau GmbH, Germany, 54912-13 number in the State register of approved types of measuring apparatuses) was used for measurements. According to instrument rating a relative error was no more than $\pm 2.5\%$ and $\pm 3.0\%$ respectively at definition of specific isobar heat capacity and phase transition enthalpy.

The calorimeter was calibrated for temperature and sensitivity on the basis of the set of standard substances "from minus 64.5 to +476 °C" (according to producer's instructions). The set includes C10H16 (minus 64.5 °C), In (156.6 °C), Sn (231.9 °C), Bi (271.4 °C), Zn (419.5 °C), CsCl (476.0 °C). Also the additional distilled water temperature point (0 °C) was used. Calibration points were reproduced at accuracy of ± 0.1 °C.

During studies the discs at mass of 20-30 mg, corresponding to the inner diameter of a standard aluminum crucible (V = $25 \mu l$, d = 6 mm), were cut from the muscular tissue samples.

Then, the sample was placed in the pre-weighted crucible, covered with a lid, pressed in and reweighted. The weighting was carried out using a laboratory balance MB 210 — A. According to instrument rating the root-mean-square deviation of balance reading was 0.03 mg.

The DSC temperature research program, developed by the authors, includes the following stages: cooling to minus 5 °C followed by maintaining during 4 minutes to stabilize the temperature of the measuring cell and the test sample; dynamic stage of heating from minus 5 °C to 90 °C at 10 K/min speed and the final stage — cooling to a room temperature.

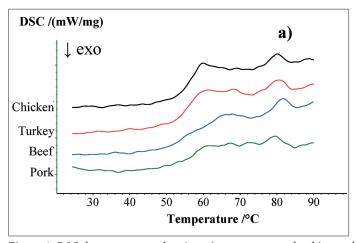
The study of one sample was carried out twice consecutively in the same crucible. Thus, we obtained the DSC thermograms and the values of the effective heat capacity of muscular tissue of animals and poultry (pork, beef, chicken, turkey) in raw form and after heating, calculated by the method of relations [19,20].

Results and discussion

When heating the samples of raw muscular tissue of pork, beef, chicken and turkey the strongly pronounced endothermic peaks are observed that reflect the heat absorption during protein thermal denaturation (Figure 1a). In previously published works [5] the generalized data are usually given without specifying the species of animals and muscle groups. Figure 1 clearly shows that the thermograms of each animal species have their own unique character due to meat composition and structure. At reheating the DSC curves are of increasing character without strongly pronounced jumps that is due to irreversibility of the denaturation process (Figure 1b).

Figure 2 presents the diagram with generalized data on the temperature range of denaturation of main muscle protein types obtained from published works [1,5,7,14,16,17,20]. The diagram shows that in the temperature range from 50 °C to 70 °C the denaturation of six protein types takes place simultaneously that is demonstrated in the DSC thermogram as a sum of peaks. For all meat samples the most pronounced peak is the last one, reaching a maximal value at 80 ± 2 °C temperature and showing the process of actin and globulin-X denaturation.

Calculated by the method of relations the values of effective specific capacity of raw muscular tissue of test samples and their values at reheating are shown in Figures 3, 4, 5, 6.



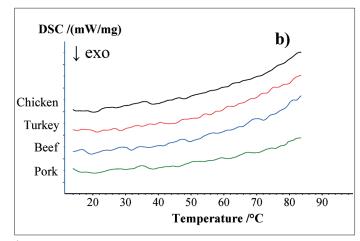


Figure 1. DSC thermograms at heating: a) raw meat samples; b) sample reheating

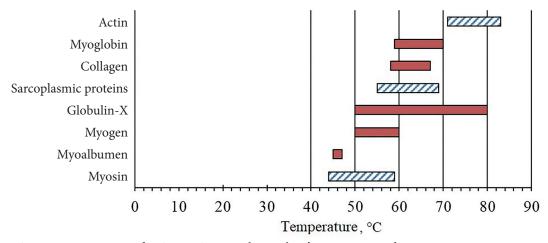


Figure 2. Denaturation temperature range of main proteins according to data [1,5,7,14,16,17,20]

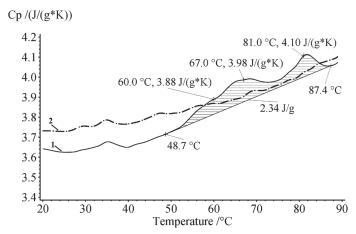


Figure 3. Beef specific heat capacity in native (1) and denatured form (2)

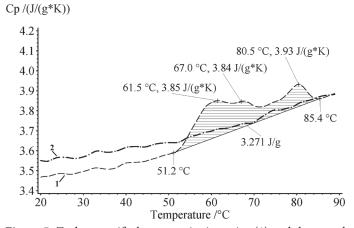


Figure 5. Turkey specific heat capacity in native (1) and denatured

According to the data obtained previously [14] three peaks of denaturation are distinguished (myosin, sarcoplasmic protein, actin); however, the first beef muscular tissue peak at 60 °C is latent and joins the next, that is maximal at 67 °C. For pork four equivalently pronounced peaks are typical at 60 °C, 67 °C, 72 °C and 80 °C temperatures respectively. For chicken and turkey samples the first and the last peaks are more pronounced with maximum values at 60 ± 2 °C and 80 ± 1 °C.

The data of chicken meat thermograms are well correlated with the results of work [17], in which a highest evidence

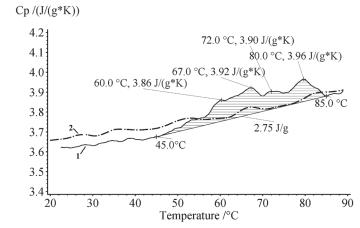


Figure 4. Pork specific heat capacity in native (1) and denatured form (2)

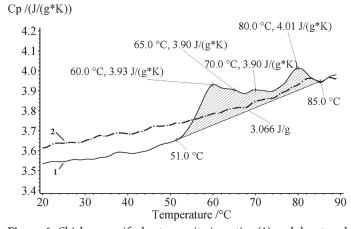


Figure 6. Chicken specific heat capacity in native (1) and denatured

of the first and the last peaks of denaturation as well as less pronounced intermediate ones are shown.

Also it was defined that the denaturation process of pork and beef samples began at lower temperatures (45±1°C and 48±1°C respectively) in comparison with chicken and turkey meat (51±1 °C). The process ended at 85 ± 1 °C temperature in all kind of meat, except beef meat, the denaturation of which occurred at 87 ± 1 °C temperature.

The data obtained certify the denaturation process uniqueness for each kind of meat raw material. As shown in works [5,14], the denaturation peaks shift in the presence of salts in meat. There are sufficient reasons for extending researches as regards the influence of different additives on various animal meats. The accumulation of data on denaturation process behavior is needed for technology development as well as for improving the control of thermal treatment processes.

At samples reheating the denaturation peaks are not detected as it is seen in diagrams 3, 4, 5, 6, and the values of the sample specific heat capacity obtained in denatured form are higher by 0.1 J/(g*K) as compared with those in native form. It is due to:

- irreversible changes of muscle fiber structure provoking fiber contraction and meat juice release that result in decrease of the proportion of bound moisture;
- in accordance with the experimental research program, the juice released remained with the meat sample in a sealed crucible at reheating. It allowed comparing the values of the sample specific heat capacity in native form with those of the denatured one at identical values of moisture and mass content.

The thermal properties of meat raw materials are needed as the initial data for calculation and modeling the processes of thermal treatment. In reference sources [18,19] the values of specific heat capacity of meat raw materials are presented fragmentarily, and as a rule, at temperatures above freezing they are accepted as constants and averaged ones. It advisable to take into account an increasing character of changes

of specific heat capacity values as well as the need to spend additional energy on the process of muscle protein denaturation at temperatures above 45 °C.

The maximal values of effective specific heat capacity corresponding to the peaks of protein denaturation in the temperature range from 45 °C to 88 °C for all samples were from 3.85 J/(g*K) to 4.1 J/(g *K).

The heat spent on the denaturation process ranged from 2.3 J/g to 3.3 J/g. Herewith, the higher values were obtained for chicken and turkey samples, but the lowest for beef ones.

Conclusion

At meat thermal processing at temperature above 45 °C an irreversible protein denaturation process takes place. Herewith, simultaneously a complex of different proteins is denatured.

Using the DSC method we showed obviously an individual character of the denaturation process behavior in muscular tissue of various animals and poultry.

The accumulation of knowledge on the nature of thermal protein denaturation of various meat raw materials will make it possible to develop thermal processing conditions providing minimization of the quality loss of a ready meat product.

To decode the peaks of protein denaturation it is necessary to conduct complex researches combining the analysis of DSC thermograms with identification of meat protein composition.

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