# THE NEW TECHNOLOGY OF COMBINED MEAT PRODUCTS ON THE BASE OF PROTEIN-PROTEIN INTERACTION BETWEEN MYOFIBRILLAR PROTEINS (MFP) AND GLYCININ-T.

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At present soybean globulins are widely used in the manufacture of processed meat products. However the possibilities of their participation in the formation of cooked sausage structure are limited. One of the reasons of it is relatively low temperatures used in the cooking sausages (from 75 to 85 °C). These temperatures are sufficient for the denaturation of meat proteins and their aggregation and gelation, that is for formation of spatial network, which assures the desirable functional and rheological properties of product.

At the same time the denaturation of soybean proteins at the above mentioned temperatures take place only partially: 2S and 7S globulins are denaturated, and 11S globulins (glycinin, the protein with molecular weight of 330 kDa, that makes up the half of soybean globulins) remains native, and therefore are not involved in the gel formation of heat-treated meat products.

It's known that the optimal temperature of soybean globulins gelation is 100 °C, which is sufficient for denaturation of all soy proteins including glycinin and explains why the attempts to obtain the complexes of glycinin with myofibrillar proteins at temperatures employed in sausage cooking were not successful. However it was established that at above mentioned (100°C) temperatures glycinin interacts with myosin , or, in other words, take part in formation of gel net at the sufficient high concentrations of proteins.

The present paper examines the mechanism of trypsin-catalyzed limited hydrolysis of glycinin, that leads in the early stages of the reaction to the formation of stable intermediate – high molecular weight residue of glycinin - glycinin-T, and the ability of glycinin-T to form complexes with myifibrillar proteins at the temperatures employed in manufacture of combined cooked sausages (not higher 75 °C).

For the detection of MFP - plant protein interaction, calorimetric measurements were carried out. Differential scanning calorimetry (DSC) can detect the heat denaturation of a protein in complex protein systems as an endothermic peak in its thermogram. Moreover, DSC technique has the advantage that it can be used to observe thermal changes and denaturation of proteins in solutions as well as in soluble suspensions and pastes. DSC is a useful technique also in the study of heat denaturation of proteins in foods such as ground meat and past-state soybean protein which are complex and concentrated protein systems.

One of the purposes of this study was to investigate, by DSC, the heat denaturation MFP, NSP (native soy protein), MSF (modified soy protein) and their mixtures and to elucidate by obtained thermodynamic parameters the protein-protein interaction between modified soybean 11S globulin – glycinin-T and MFP of bovine muscle. Thus, the heat capacity differences at 25 °C were determined. The temperatures and enthalpies of denaturation of separate proteins and their mixtures were also determined. The deviations from the additivity of separate protein contributions in the measured parameters in the case of their mixture suggests the formation of complexes.

Glycinin was isolated from defatted soybean flour Soya Fluff 200W by selective thermal denaturation and glycinin-T was prepared by the following method: trypsin was added to 5 % solutions of glycinin in 50 mM phosphate buffer containing 0,5 M NaCl (pH 7,0) in the ratio of 1:200.

The limited trypsin hydrolysis of glycinin was carried out in thermostatic cell at 37 °C and constant pH value for one hour. Enzyme activity was stopped with Kunitz soybean trypsin inhibitor at a concentration three times higher than that of the enzyme. The hydrolyzed proteins were chromatographically separated on gel-filtration column G-50.

The fraction containing high molecular weight protein (molecular weight ~ 230 kDa), was determined by means of high performance liquid chromatography on gel-filtration column TSK-G4000 was collected, dialysed against distilled water, and lyophilized.

Myofibrillar proteins (MFP) were prepared from post-rigor bovine muscle longissimus dorsi according to the method of A.W. Khan. The concentration of the MFP salt extracts were determined by evaporation at 20 °C to a constant weight. The solutions obtained were dialized via 50 mM phosphate buffer containing 0,4 KCL (pH 6,4) with the change of solvent for two days.

Protein solutions of concentration of 4,5 % in this buffer were stored at 5 °C for 10 days. By means of scanning calorimetry it was established that such storage does not change the temperature and enthalpy of denaturation of the protein.

Protein concentrations were measured with the microbiuret method, using a calibration curve based on BSA ( bovine serum albumin).

Microcalorimetric studies have been carried out on a DASM-4 differential scanning microcalorimeter in a temperature range from 20 to 100 °C and at scanning rate of 2 °C/min and excess pressure of 2 atm.
All microcalorimetric studies were performed with 0,225 % proteins solutions or their mixtures (1:1) in 50 mM phosphate buffer containing 0,4 KCL (pH 6,4). The selected pH value corresponds to that of meat (4).

Temperature and enthalpy of glycinin denaturation were 87,2 °C and 17,1 J/g respectively. The difference of heat capacities at 25°C was 1,31 J/gK, and for glycinin-T the difference of heat capacity was 1,03 J/gK and the peak of denaturation is absent that testifies the protein denaturation even at room temperature. Based on this fact one can suppose that glycinin-T will build the complexes with MFP even at room temperature.

It was established that MFP have two peaks of denaturation with temperatures 54,3 and 64,8 °C; values of enthalpy 13,8 J/g and difference of heat capacities 1,00 J/gK. For the mixture of MFB with glycinin three peaks of denaturation were observed: two low-temperature peaks correspond to temperature and enthalpy of glycinin denaturation that suggest the absence of interaction between MFP and glycinin in their mixture. The equality of heat capacity differences for the sum of separate components and their mixture also suggests the absence of plant protein - meat protein interaction. At the same time for the mixture of MFP with glycinin- T two peaks of denaturation were observed with the denaturation temperatures 50,8 and 61,3 °C (3,5 °C less than for MFP) and enthalpy 29,5 J/g , that suggests the contribution of intermolecular interactions of MFP and glycinin-T to the measured thermodynamic parameters. The data on the differences of heat capacities of MFP, glycinin-T and their mixtures also ague for the complex formation. The value of heat capacity difference for the protein mixture is 2,51 J/gK, and for the sum of separate components is 2,03 J/gK.

It's necessary to note, that heat capacity is very sensitive test as regards to intermolecular interactions, especially if during complex formation the hydrophobic aminoacid residues of proteins are shielded from the solvent. On this reason the observed variations on the differences of heat capacities for the separate proteins and their mixtures are high and are likely connected with the shielding of aminoacid residues of denaturated polypeptide chains of glycinin-T. It is believed that cmplexes of glycinin-T with MFP are more structurally ordered than separate proteins.

On the base of this data the technology of combined cooked sausages with the stable complexes of plant and muscle proteins "Modern" was developed.

For the comparison of the quality characteristics of minced products with the change of portion of meat farce (25%) with soy protein preparations, it was chosen the cooked sausage "Stolovaya" the formula of which doesn't include any plant protein preparations. On the base of this formula three samples of combined farces were studied: 1- without the change of meat portion; 2- with the suspension of native soy flour (NSF); 3- with the suspension of modified soy flour (MSF).

The change of 25 % of meat stock to the protein preparations significantly decreases the fat content, and at the same time the content of protein and water in the samples is raised.

Farces that include NSF and MSF have the identical chemical composition on the score that limited enzyme hydrolysis doesn't change the chemical composition of substrate.

The introduction of MSF into farce instead of portion of meat stock leads to the increase of pH value and water – binding capacity. The yield of the ready product is increased. The increase in the sausage yield with the increase in the change of meat stock is correlated with the values of water holding capacities of farces and is conditioned by the retention of high hydration value of proteins after heat treatment.

The study of technological characteristics (breaking stress and strain, water-binding capacity, yield and mass losses)of raw and cooked samples of farces showed that the functional properties of the samples containing modified soybean flour (MSF) were comparable to the standard sample, whereas the samples with NSF had slightly worse characteristics.

The fact, that limited proteolysis leaves the primary protein structure or aminoacid sequence intact, explains why the nutritive and biological value of the raw and cooked farces are not reduced by replacement of the portion of meat from the sausage recipe by MSF and NSF. Aminoacid composition of the proteins of the examined combined recipes fully meet the requirements of FAO/WHO, that could be explained by well-balanced aminoacid composition of soy proteins. Furthermore aminoacid composition of combined sausages containing MSF and NMS was not only in no way inferior to the standard, but even exceeded it in some respect.

**Conclusions.**

In the present paper the conditions of interaction of modified 11S globulins of soybean flour with MFP on the macromolecular level in the model (solutions of low concentrations) and in meat-plant systems were found.

The experimentally obtained results of the study of limited proteolysis of soy glycinin can be applied in the modification of 11S globulins of other grain cultures dure to the homogeneity of their proteins with their subsequent incorporation in meat-soy systems.

It was established that the incorporation of the modified soy flour into a heated meat system leads to the improvement of its functional and rheological properties and doesn't change the aminoacid composition.

The good consumer properties of combined cooked sausages produced in terms of interaction by meat and plant proteins allow the use of the limited proteolysis of plant proteins in the manufacture of processed meat products.

**Literature.**

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