STUDY OF POSSIBILITY OF OBTAINING CHEMICALLY BOUND COMPLEXES OF MEAT AND VEGETABLE PROTEINS

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Introduction

A large amount of different non-meat additives is currently used in the meat industry. First of all it is proteins of soya having a high food value. Understanding the events occurring on molecular level with soya proteins being a part of a meat system subjected to heating is important for improving the use of vegetable proteins in meat industry. One of the ways of improving functional properties of soya containing meat systems is a possibility of formation of complexes of soya proteins with meat myosin on the level of their macromolecules.

In the 80s such complex was obtained by American scientists (1,2) in diluted solutions of proteins. Basis chains of soya glycinine and heavy chains of native muscular myosin take part in its formation. A basic reserve protein of peas - legumin having a similar molecular structure and aminoacid composition is a homologue of soya glycinine – 11S protein (3). It is known that 11S protein enters the process of formation of complexes with meat myosin only in denatured state, when basis chains become most accessible. For the denaturation of vegetable protein it is necessary to heat it to 90-100^{0}C (4,5,6), however such high temperature causes coagulation and aggregation of muscle proteins in the combined mincemeat system, containing soya protein which makes the process of complex formation impossible. Therefore we chose another way of carrying out denaturation of vegetable protein – acidification with subsequent neutralization.

Purpose

The purpose of this work was to determine possibilities and ways of formation of complexes of 11S protein with non-denatured meat myosin, as well as with myosin being a part of meat emulsion under the conditions of preliminary denaturation of 11S protein by acid at room temperatures.

Materials and methods

Myosin from m. Long dorsi of rabbit was isolated by the method (7). Meat emulsion was prepared by homogenizing the meat specimen (beef) trimmed from fat and connective tissue in the solution of 0.6M NaCl.

Legumin (S11) of peas was isolated from seed of peas by method of Popello et al. (8). Glycinine of soya (S11) was obtained from defatted soya meal Soprolets 8-TB-325 (Yugoslavia) by method of Bigbov T.M. et.al. (9).

HPLC was carried out on chromatograph "Yanako" using a column TSK-G 4000SW (21*300 mm). Protein detection was carried out at λ=280 nm. Protein eluation was carried out with the speed 0.9 ml/min. Microcalorimetric investigations were performed on microcalorimeter DASM-4 (Institute of biological instrument engineering of RAN, the town of Puschino) in the temperature interval 10-120^{0}C with the rate of scanning 2^0/min and gauge pressure 4 atm. The scale of heat capacity in each experiment was calibrated by the effect of Joule-Lentz. The enthalpies of denaturation were determined as areas under denaturation peaks of protein according to method (10). Concentration of protein in the solution was determined by a microbiurete method (11) using a calibration curve, constructed for glycinine of soya.

Protein denaturation was carried out as follows: solutions of peas legumin or soya glycinine were titrated by 0.1N solution of HCl up to pH=2.0 with this the vegetable protein was completely denatured, then pH value was brought to 7.2 by 0.1N solution of NaOH.

To carry out the process of complexes formation of the vegetable protein 11S with myosin, the acid denatured vegetable protein was mixed in the equiweight correlation (so that pure proteins were taken into account) with myosin in the solution, or as a part of meat emulsion or with the supernatant of the meat emulsion. The obtained suspensions were centrifuged, the concentration of protein was determined in a supernatant liquid, and the investigations were carried out.

For the investigations the following mixtures of proteins were prepared:

1. native myosin and soya glycinine as denatured by acid
2. native myosin and legumin of peas as denatured by acid
3. myosin as a part of a supernatant of meat emulsion and the individual, previously denatured by acid glycinine of soya
4. myosin as a part of a supernatant of meat emulsion and the individual, previously denatured by acid legumin of peas
5. myosin as a part of non-centrifuged meat emulsion and the individual, previously denatured by acid legumin of peas

Results and discussion

Fig. 1 shows chromatograms of the denatured soya glycinine (1), native myosin (2a) and their mixture (3). It can be seen that the areas of peaks of native myosin and denatured glycinine on the chromatogram (3) are significantly less than on chromatograms (1) and (2). On the thermogram (Fig. 2) the peak area of denaturation of myosin mixed with denatured glycinine (2) is significantly less than the peak area of denaturation of individual myosin (1). Results as obtained by two methods of analytical chemistry show that a part of myosin and glycinine, having formed the insoluble complex, remained as a sediment during centrifugation, therefore their quantities in the supernatant correspondingly become lower. Similar data were obtained for the legumin of peas as denatured by acid. The amount of myosin, bound with denatured vegetable protein into an insoluble complex was calculated from the value of areas correlation of microcalorimetric peaks of denaturation of individual myosin and its mixture with a vegetable protein. For the mixture with denatured glycinine the amount of bound myosin was 70%, and for the mixture with denatured legumin – 80%.

Since these investigations allowed us to find the way of complex formation between pure plant protein 11S (peas legumin or soya glycinine) and individual myosin of meat in the solution, the following step of the work was the attempt to find the conditions of obtaining the complex of 11S protein with meat myosin in the meat emulsion. As can be seen from Fig. 3, the area of the denaturation peak of the mixture of soya glycinine denatured by acid with the supernatant of the meat emulsion – the curve (2) is less than the area of the denaturation peak of the pure supernatant of the meat emulsion – curve (1). The experimental data obtained demonstrate a formation of the insoluble complex between the plant protein and muscle myosin in the supernatant of the meat emulsion, because under the conditions of the expected complex formation a part of myosin and glycinine, binding into the complex,

remained during centrifugation as a sediment, therefore their amounts in a supernatant liquid decreased, and correspondingly specific enthalpy of myosin denaturation in the mixture reduced as compared to the specific enthalpy of denaturation of the pure meat emulsion. Similar data were obtained for the system of the denatured by acid peas legumin with the supernatant of the meat emulsion (Fig. 3, curve 3), as well as for the mixture of denatured by acid legumin of peas with meat myosin in the meat emulsion without preliminary centrifuging of meat emulsion (Fig. 4, curve 2). In both experiments the areas of denaturation peaks of mixtures were significantly less than those of denaturation peaks of the meat emulsion or its supernatant.

Conclusions

In this work a possibility of complexes formation of reserve protein of soya and peas (glycinine and legumin) with myosin of muscular tissue in the solution, as well as in the meat emulsion or its supernatant at an ambient temperature (18-20^{0}C) is shown.

References

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DSC data for myosin (individual and in the meat emulsion), 11S proteins and their mixtures

Sample	DSC data		
	$Tmax_1$, ^{0}C	$Tmax_2$, ^{0}C	ΔdH, J/g
Myosin (individual)	47	56	8.0
Mixture of myosin with denatured soya glycinine	45	56	2.4
Mixture of myosin with denatured peas legumin	45	-	1.0
Supernatant of meat emulsion	58	69	18.0
Mixture of supernatant of meat emulsion with soya glycinine (denatured by acid)	58	68	9.4
Mixture of supernatant of meat emulsion with peas legumin denatured by acid	63	69	8.6
Mixture of non-filtered meat emulsion with peas legumin denatured by acid	62	69	11.2

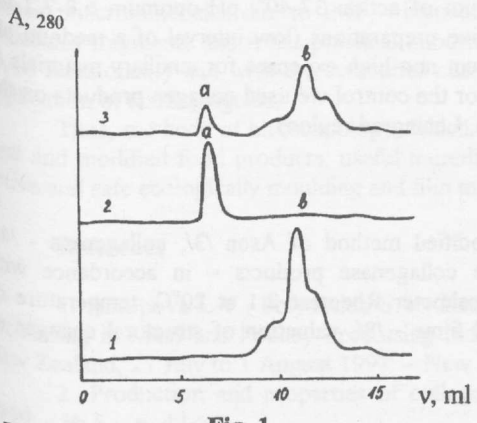

Fig. 1.
Chromatograms of protein solutions.

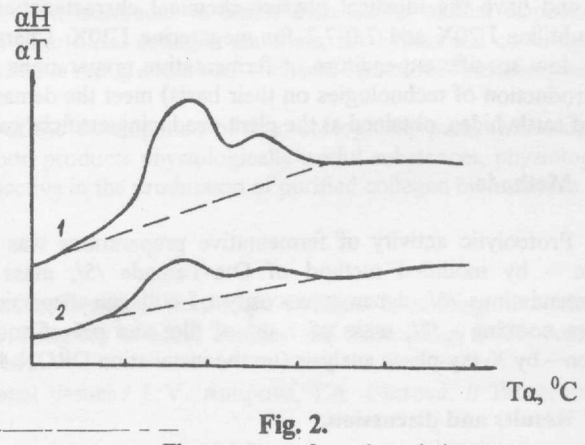

Fig. 2.
Thermograms of proteins solutions.

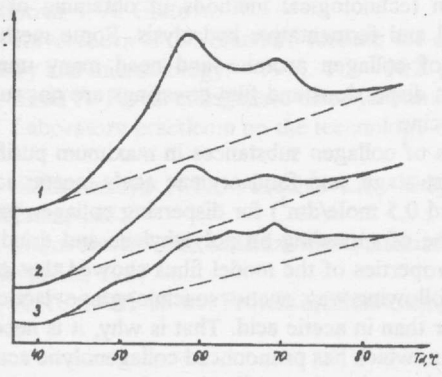

Fig. 3
Thermograms of proteins solutions.

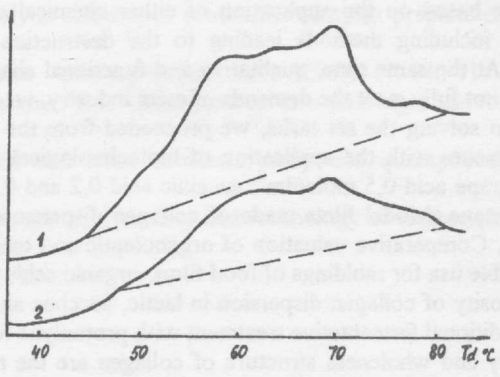

Fig. 4.
Thermograms of proteins solutions.