THE USE OF AGGREGATIVE STABILITY THEORY FOR ANALYSIS OF THE PROCESS OF COAGULATION STRUCTURE FORMATION BY MEAT PROTEINS

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This report includes the results of investigations which were carried out in the Moscow Technological Institute for Meat & Dairy Industry for a period of time after publishing in Proceedings of the 33rd International Meat Congress the report "Theoretical approaches to the modelling of protein spatial structures formation in the near and distant energy minimum at various stages of meat raw material processing", which is a fundamental one in this field [1, 2].

At the first stage of investigations, the results of which are performed in this report, the values of Stern's potentials were calculated on the basis of suggested by the author mathematic dependencies [2] with the use of SM-4-computer, these potentials being correspond to macromolecules of four main types of meat proteins in isoelectric field. Values of Stern's potentials determined for these protein fractions are, respectively, as follows: $\Psi\sigma_1 = -3{,}21 \cdot 10^{-3}$ V for actin; $Ys_2 = 2{,}41 \cdot 10^{-3}$ V for globulin; $Ys_3 = -2{,}00 \cdot 10^{-3}$ V for myosin and $Ys_4 = -1{,}21 \cdot 10^{-3}$ V for myogen.

Computer-modelling of the interaction energy of macromolecules of concrete meat protein fractions depending on the space between their surfaces was developed for a complex of physico-chemical parameters which correspond to mean statistical conditions of meat chopping and those of ending the cooking process of sausage products.

For sausage emulsion preparing the temperature of 287 K (14°C) and pH-value of 5,9 were selected as characteristic ones. At such conditions the Debye's radius screening of potential-carrying protein particles is characterised by the following value of parameter æ = $2{,}633 \cdot 10^6$ m^{-1}.

For ending the cooking process of sausage products the temperature of 343 K (70°C) was selected as a characteristic one. At such temperature, as it is concluded from the results of our experiment, pH value of the system is lowered in average to 0,5 pH as compared with its initial value corresponding to the temperature of 14°C, that it becomes equal to 5,4. Namely this pH value was selected as a characteristic one for ending the sausage cooking process. At such values of temperature and pH the value of parameter æ was found to be $4{,}283 \cdot 10^6$ m^{-1}.

As the values of interaction energy between particles and, therefore, the value of energy barrier which they must overcome to be mutual-fixed in a potential well, or the depth of a distant energy minimum, which is rather enough for particles mutual fixing, are proportional to their radius the process of computer-modelling was realised only for those particles whose radius was 10^{-7}m.

Dependence of total interaction energy of protein particles on the space between their surfaces which corresponds to above-mentioned conditions of sausage emulsion preparing and of ending the cooking process of sausage products is graphically represented in Fig.1. These plots are based on the results of computer-modelling.

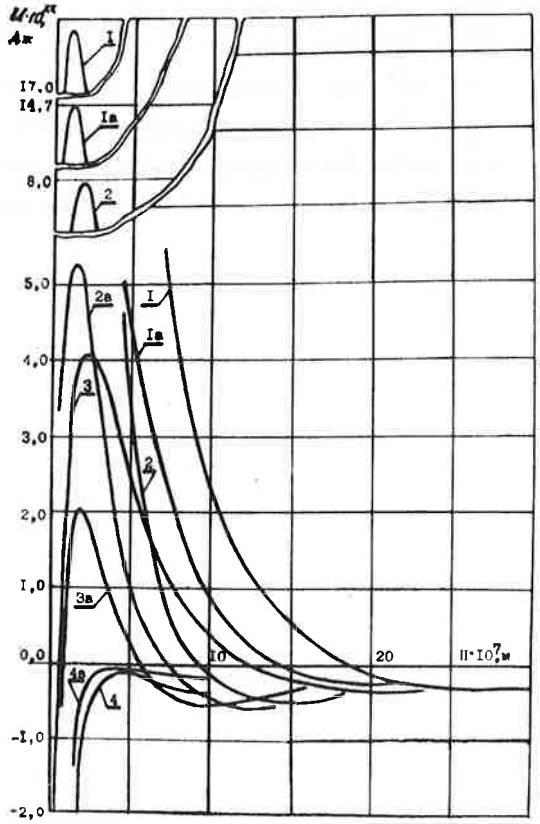

Fig. 1. Dependence of interaction energy of two protein particles on the space between their surfaces at conditions coresponding to meat raw material chopping (T=287 K, pH 5,9): 1 - actin; 2 - globulin; 3 - myosin; 4 - myogen; and at conditions corresponding to cooking sausage products (t=343 K, pH 5,4): 1a - actin; 2a - globulin; 3a - myosin; 4a - myogen.

Analysis of modelling results shows that for such protein fractions as actin, globulin, myosin the value of an energy barrier corresponding to the conditions of ending the cooking process of sausage products seems to be significantly lower as compared with the value of an energy barrier corresponding to the conditions of chopping process. It should be noted that this lowering of the value of an energy barrier is only due to decreasing of the value of energy maximum, as the depth of a distant energy minimum corresponding to the conditions of ending the cooking process for all the protein fractions above-mentioned seems to be higher than that one corresponding to the conditions of chopping process. The reason for decreasing the value of an energy maximum corresponding to the conditions of ending the cooking process as compared with conditions of the chopping process is an increase of hydrogen ion concentration in a disperse medium which results in increasing the value of parameter æ and decreasing the electrostatic component (repulsive energy) of total interaction energy of particles.

Qualitative analysis of interaction curves allows to conclude that thermocoagulation structures corresponding to the conditions of ending the sausage cooking process which are able to be formed from protein particles in a distant energy minimum are more compact as compared with analogous

structures corresponding to the conditions of the chopping process, as spaces between surfaces of concrete protein fractions, which secure the presence of a distant energy minimum corresponding to the conditions of ending the cooking process, seem to be two times less than analogous spaces corresponding to the conditions of the chopping process.

Another qualitative conclusion resulting from the analysis of these curves and allowing for that in the meat raw material protein fractions to be considered, particularly actin and myosin, are placed in native bio-organic above-molecular structure formations, the mechanical dispersion degree of which is really limited, is based on the fact that decreasing the value of an energy barrier at conditions corresponding to the cooking process as compared with conditions taking place in the chopping process has to allow the possibility of including a considerably greater number of dispersion products of native protein structures of meat in the process of coagulation mutual fixation of particles in a potential well. This, in its turn, must be and is accompanied by that thermo-processed fine-comminuted emulsions acquire qualitatively new physical (structural) properties, these being developed in combining solidity with elasticity, plasticity and an absence of isothermal thixotropy, as in a potential well the energy of molecular attractive forces, which makes particles to be mutual fixed, considerably (for two or more orders) exceeds the energy of mutual particle fixation in a distant energy minimum, even without taking into account a possibility of rising in it of chemical bonds between particles.

Quantitative analysis of the results of computer-modelling allows to determine the following values of "depths" of distant energy minimums for particles of actin, globulin, myosin with the radius $\alpha = 10^{-7}$m at conditions corresponding to chopping process: $U_{min1} = -2{,}71 \cdot 10^{-23}$ j; $U_{min2} = -3{,}01 \cdot 10^{-23}$ j; $U_{min3} = -3{,}26 \cdot 10^{-23}$ j:as well as at conditions corresponding to cooking process: $U_{min1} = -4{,}81 \cdot 10^{-23}$ j; $U_{min2} = -5{,}43 \cdot 10^{-23}$ j; $U_{min3} = -5{,}50 \cdot 10^{-23}$ j, respectively. On the basis of the formula / /, taking into account that at conditions corresponding to chopping process the energy of Brownian motion of particles is equal to $kT = 39{,}63 \cdot 10^{-22}$ j, it is not difficult to calculate that for this case in a distant energy minimum those particles are able to be mutual fixed which consist of actin with the radius $\alpha_1 = 14{,}63 \cdot 10^{-6}$m, globulin with the radius $\alpha_2 = 13{,}21 \cdot 10^{-6}$ m and myosin with the radius $\alpha_3 = 12{,}15 \cdot 10^{-6}$ m. At conditions corresponding to cooking process of sausage products the energy of Brownian motion of particles is equal to $kT = 47{,}37 \cdot 10^{-22}$ j. Calculations made for this case show that for mutual fixation in a distant energy minimum particles of actin must be characterised by the radius $\alpha_1 = 9{,}85 \cdot 10^{-6}$m, of globulin - by the radius $\alpha_3 = 8{,}72 \cdot 10^{-6}$m and myosin - by radius $\alpha_3 = 8{,}61 \cdot 10^{-6}$m.

An objective estimation of the possibility of mutual fixation of particles of actin, globulin and myosin in a distant energy minimum during chopping and thermo-processing shows its very small probability, as particles with diameters more than 5 micron do not practically participate in Brownian motion, that is they cannot spontaneously be close together in distances corresponding to a distant energy minimum. Particles of actin, globulin and myosin whose diameters are less than 5 micron do spontaneously close together in distances corresponding to an energy minimum, but they cannot be mutual fixed in it, as for conditions corresponding to chopping and thermo-processing the energy of their Brownian motion exceeds the depth of a distant energy minimum.

In connection with the above-mentioned, it's of a certain interest the further analysis of results of computer-modelling dealt with quantitative estimation of a possibility of three-dimensional protein structures formation by actin, globulin and myosin particles due to their mutual fixation in a potential well. Such a mutual fixation would be to take place, it is necessary for particles above-mentioned to overcome an energy barrier rising as they are closed together in distances corresponding to a potential well. The results of computer-modelling allowed to determine that at conditions corresponding to chopping process the value of an energy barrier for actin seems to be equal to U_{Σ_1} $18{,}12 \cdot 10^{-22}$ J for globulin $U_{\Sigma 2}$ $8{,}20 \cdot 10^{-22}$ J and for myosin $U_{\Sigma 3}$ $4{,}47 \cdot 10^{-23}$ J. At conditions corresponding to cooking process the values of energy barrier are as follows: $U_{\Sigma 1}$ $15{,}17 \cdot 10^{-22}$ J for actin; $U_{\Sigma 2}$ $6{,}02 \cdot 10^{-23}$ J for globulin and $U_{\Sigma 3}$ $2{,}70 \cdot 10^{-22}$ J for myosin.

Common calculations show that for coagulation structure formation during emulsion preparing due to mutual fixation of particles in a potential well their radius must satisfy the following inequalities for actin, globulin and myosin, respectively: $\alpha_1 \leq 0{,}212 \cdot 10^{-6}$m, $\alpha_2 \leq 0{,}483 \cdot 10^{-6}$m, $\alpha_3 \leq 0{,}866 \cdot 10^{-6}$m. During thermo-processing of sausage products before the moment of achieving the characteristic conditions above-mentioned of cooking process the values of energy barrier for every protein fraction are decreased what results in increasing by 1,47 and 1,63 and 1,98 times, respectively, the size of actin, globulin and myosin particles, which are able to take part in coagulation structure formation caused by their mutual fixation in a potential well. Inequalities limiting their size are as follows: $\alpha_1 \leq 0{,}312 \cdot 10^{-6}$m for actin; $\alpha_2 \leq 0{,}789 \cdot 10^{-6}$m for globulin and $\alpha_3 \leq 1{,}734 \cdot 10^{-6}$m for myosin.

For achieving a complete picture of the process of three-dimensional structures formation by meat proteins it seemed to be logical to make an analysis of the possibility of coagulation interactions between particles of different proteins, namely actin and myosin, actin and globulin, globulin and myosin. This analysis, as well as that one above-mentioned, was based on the results of computer-modelling with taking into account that in this case particles interacted had different values of Stern's potential. As in preceding case, modelling was realised for those particles whose radius was equal to 10^{-7}m. On the basis of computer-modelling at conditions corresponding to chopping process the following values of energy barriers for interactions of types of actin + globulin, actin + myosin and globulin + myosin were received, respectively: $U_{\Sigma 1} = 12{,}07 \cdot 10^{-22}$ J, $U_{\Sigma 1,3} = 8{,}73 \cdot 10^{-22}$ J and $U_{\Sigma 2,3} = 6{,}04 \cdot 10^{-22}$ J. At conditions corresponding to cooking process the values of analogous barriers would be as follows: $U_{\Sigma 1,2} = 8{,}95 \cdot 10^{-22}$ J, $U_{\Sigma 1,3} = 6{,}53 \cdot 10^{-22}$ J and $U_{\Sigma 2,3} = 4{,}07 \cdot 10^{-22}$ J. Calculations made show that at conditions corresponding to chopping process three-dimensional structure formation due to coagulation mutual fixation in a potential well is possible when their radius is satisfied to the following inequalities: $\alpha_{1,2} \leq 0{,}328 \cdot 10^{-6}$m for interaction of actin+globulin type: $\alpha_{2,3} \leq 0{,}656 \cdot 10^{-6}$m for interaction of actin+myosin type. At conditions corresponding to cooking process these inequalities would be as follows: $\alpha_{1,2} \leq 0{,}529 \cdot 10^{-6}$m, $\alpha_{1,3} \leq 0{,}723 \cdot 10^{-6}$m and $\alpha_{2,3} \leq 1{,}164 \cdot 10^{-6}$m.

In Fig.2 a scanning electron-microscope photograph of typical hystostructure of a mass received after chopping emulsion prepared from cattle Long. dorsi. On this photograph

Fig. 2. Hystostructure of an emulsion from Long. dorsi after chopping. Scanning electron microscopy x 1000.

Fig. 3. Hystostructure of a muscle filament. Scanning electron microscopy x 1000.

large concrete particles of muscle filaments are distinctly seen, which keep their individual morphological signs. Analysis of different elements of comminuted mass which were looked through on microscope display allowed to determine that sizes of these particles are in the following limits: diameters - $(10 \div 5)\cdot10^{-6}$m, length - $(18 \div 25)\cdot10^{-6}$m. Particles of muscle filaments are placed in a grained protein substance consisting of particles whose form is rather closed to spherical. Dispersion analysis allowed to establish that the radius of a large number of spherical particles is in the limits from $0{,}45\cdot10^{-6}$m to $1{,}25\cdot10^{-6}$m, the particle radius of $\approx1{,}0\cdot10^{-6}$m (Fig.4) being predominated. Comparing the dispersion analysis results with the limited particle size above-mentioned, which predetermined the possibility of their participating in structure formation corresponding to their mutual fixation in a potential well, showed that at presence in a grained protein substance of particles of all three main meat proteins from energy positions at conditions corresponding to chopping process such interactions are possible as "myosin + myosin" and "globulin + myosin". Taking into account that myosin shares nearly 40 per cent of total mass of muscle tissue proteins and that particles with the radius of $\alpha \leq 0{,}886\cdot10^{-6}$m, at which interaction of "myosin + myosin" is possible, occupy nearly one third of total number of particles of a grained protein substance of muscle tissue comminuted it is not difficult to conclude that only 13 per cent of all particles of this substance are able to take part in structure formation during chopping process predetermined by their mutual fixation in a potential well. At conditions corresponding cooking process of sausage emulsion all myosin particles of a grained protein substance can participate in interactions of "myosin + myosin", "myosin + globulin" and "myosin + actin" types. Due to this, they additionally draw into the process of coagulation structure formation, corresponding to mutual fixation of particles in a potential well, practically all the globulin particles that remain unincluded in interaction of "globulin + globulin" (nearly 14 per cent of a grained substance) and more than one fourth (nearly 5 per cent of grained substance proteins) of actin particles which are not able at all to be mutual-fixed by type of "actin + actin". On the whole, more than 65% of protein particles of a grained substance of fine-communited

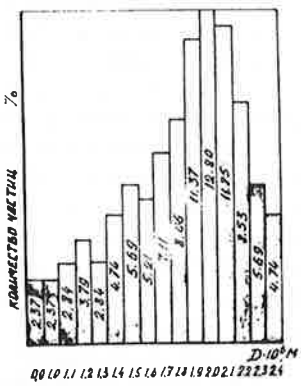

Fig. 4. Hystogramme of distribution of grained protein substance particles of meat mass prepared by chopping emulsion from muscle.

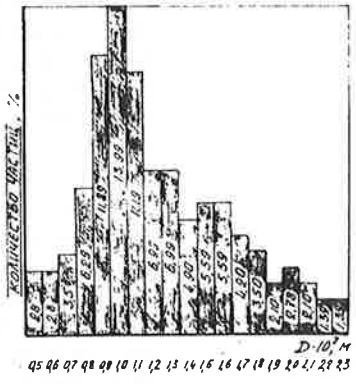

Fig. 5. Hystogramme of distribution of globular protein subunits in the muscle filament structure.

meat emulsion prove to be included due to interaction of "myosin + myosin", "myosin + globulin", "globulin + globulin", myosin + actin", globulin + actin" types in cooking process in three-dimensional structure formation as a result of mutual fixation of protein particles in a potential well. Such a quantity of proteins more than 5 times exceeds a quantity of a grained substance protein of fine-comminuted mass, which is able to take part in three-dimensional structure formation due to mutual fixation in a potential well at conditions corresponding to chopping muscle tissue. If taking into account that at conditions corresponding to cooking sausage products in such a structure formation only those particles can take part whose size is almost twice larger than that one of particles at conditions corresponding to chopping process, it is not difficult to be convinced that, if excluding even the influence of increased temperature as an initiator of possible chemical interactions between protein particles, being physico-chemically mutual-fixed in a potential well, cooking of fine-comminuted meat systems must be accompanied, as a minimum, by ten times larger energy increasing which is necessary for a destruction of structures formed in them as compared with structures corresponding to unthermo-processed systems.

In Fig.3 a photograph of a fragment of acrossed cut structure of a muscle filament being increased 10000 times is represented. A visual analysis of pictures of analogous objects on the display of scanning electron-microscope allowed to discover that an internal structure of muscle filaments was formed from a number of spherical particles. The results of scaling sizes of nearly four hundred particles which are most

distinctly looked through on the display at magnifying 10000 x of cuts of five muscle filaments in a sample of comminuted muscle tissue represented as a hystogramme are shown in Fig.5. Investigations showed that a majority of particles is characterised by diameters from $0,05 \cdot 10^{-6}$m to $0,23 \cdot 10^{-6}$m. Prevailed number of particles (more than 37%) from all looked through are characterised by diameters of $(0,09 \div 11) \cdot 10^{-6}$m. Comparing the results of evaluation of particle dispersion which form the structure of a muscle filament with above-mentioned analysis of the possibility of mutual fixation of actin and myosin macromolecules shows that in a muscle filament these macro-molecules cannot exist independently one from another, but the most advantageous distances, from energy position, of maximum removing between their surfaces are those which do not exceed 10^{-8}m, that is those which are less in an order than the most probable diameters.

CONCLUSION

As a result of computer realisation of theoretical methods for physico-chemical analysis of coagulation structure formation process by meat proteins it was established that heating of fine-comminuted meat emulsions from 287 K to 343 K in combination with predetermined by this increasing of their active acidity in 0,5 pH ensures including into the coagulation structure formation process in a potential well additionally more than 52% of protein particles of a grained substance of fine-comminuted muscle tissue as compared with 13% in the original emulsion. During coagulation structure formation process elements of a dimensional protein framework are formed which predetermine a solidity of finished sausage products. Due to formation of "myosin + myosin", "globulin + globulin", myosin + globulin", myosin + actin" and globulin + actin" chain elements of this framework it takes place strengthening of a structure that causes a traditional consistency accompanied by more than 10x energy increasing necessary for their destruction as compared with unthermoprocessed emulsions.

It was established that for actin and myosin macromolecules which form muscle filaments these macromolecules cannot exist in a unconsolidated state which is the most stable from the position of aggregative stability.

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