Theoretical approaches to the modelling of protein spatial structures formation in the neighbouring and distant energy minimum at various stages of meat raw material processing

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Many properties of proteins as edible components of technological raw material for the meat industry may be connected with their primary, secondary, tertiary and quaternary structures. So, the biological value of proteins which is characterized by the essential amino acids content is fully predetermined by its primary structure. Digestibility (attacking) of proteins by the proteolytic enzymes depends first of all on their conformation, i.e. on their secondary and tertiary structures. Preliminary theoretical works allow to state that a number of functional, physico-chemical and organoleptic properties of the meat systems - prepared foods and finished products - is stipulated for initial quaternary protein structures and those being formed on the different technological stages of the meat product manufacture.

Before going on, let's pay attention to that terms "structure of protein" and "protein structure", speaking strictly, are not equivalent. However, as to the quaternary structure, definitions which are accepted in the biochemistry allow to interprete these terms as to be rather identical.

One of the most wide-spread in modern biochemical literature /7,10,13/ interpretations of the concept "quaternary structure of protein" is a non-covalent interfixation of protein macromolecules-monomers in the form of spatially ordered oligomers. This interpretation allows to look at the process of quaternary structures formation by protein fractions of the meat systems from the positions of physical chemistry fundamentals.

If to consider protein macromolecules-monomers or their associators as charged disperse particles which have adsorption and diffusion layers of counter ions using approaches /1,2, 3,12/ developed on the basis of the DLFO-theory*, one can then qualitatively and quantitatively analyse the influence of disperse systems parameters on the aggregating stability of these objects and model the process of their structure-formation. If starting from these positions it is possible to introduce concepts of quaternary protein structures of the first, second and third levels which have no analogs in the biochemistry.

Under quaternary structures of the first level we'll understand associators of macromolecules-monomers which are formed in consequence of their coagulation in a potential well (in primary energy minimum) for which the value U_I of energy barrier of paired interaction at pre-determined parameters of a disperse system exceeds their Brownian-motion energy

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* theory of Deryagin-Landau-Fairway-Overbeck

and depth of the secondary energy minimum, if such takes place, is not enough for their interfixation, i.e.

$$\bar{U}_I > kT > |\min U_I| \qquad (1)$$

where k is Boltzmann's constant, J; T temperature, K.

Quaternary protein structures of the second level may be understood as periodical colloidal structures being formed as a result of a coagulation interfixation of protein associators in the secondary energy minimum. For this the following inequality must be satisfied:

$$|\min U_I| > kT \qquad (2)$$

And, finally, under quaternary protein structures of the third level we mean spatial protein carcasses consisting of chain aggregates being formed in consequence of coagulation interfixation (in the primary energy minimum) of associators of protein macromolecules-monomers. In forming such structures protein associators are able to take part for which at predetermined parameters of a disperse system the Brownian-motion energy exceeds an energy barrier value of their paired interaction. As examples of quaternary protein structures of the first level the most coarsely disperse elements of fine-grained and fibrillar mass /8/ of destructured sausage emulsions (as a result of treatment in grinder), muscle fibres and friable connective tissues (as a result of massaging or tumbling the raw material for cured meats manufacture) may serve. At the same time, structures formed by mean-statistical elements of fine-grained and partly fibrillar mass, e.g. in raw sausage emulsions, are examples of the quaternary structures of the second level. A special feature which allows to judge about their presence is an isothermic thixotropy of a number of structural-mechanical properties of sausage emulsions. During heat treatment, quaternary protein structures of the third level are formed in sausage emulsions. These structures ensure the necessary elasticity and solidity of sausages. Destruction of individual chains of the quaternary structures of the third level is accompanied by non-thixotropic changing structural-mechanical properties of macrovolumes in spite of that microvolumes which compose them are able to keep original properties of the initial product.

In principle, formulated above phenomenologically formalized concepts of the quaternary protein structures of three levels allow (on the basis of fundamental conceptions of the physical chemistry) to evaluate quantitatively the role of protein organizations (elements), corresponding to the concrete level of the quaternary structure, in such properties of meat systems as consistency of the finished product, limit shearing stress and intrinsic viscosity of masses to be agitatedand conveyed, water- and fat binding capacities, water- and fat-holding capacities, etc. Carrying out such an evaluation may serve as a necessary base for realization of the idea to regulate above-enumerated properties at the expense of purposive changing the relationship between structural elements of every level. At the same time, it should be realized that in this way there is the whole number of serious difficul-

ties connected with the absence of a necessary information of some parameters of proteins-monomers or their associators and with an insufficient development of corresponding computational algorithms which restrain the use of numerous results based on the DLFO-theory for modelling of the quaternary protein structures formation process in meat systems.

Let's consider some of these difficulties and outline trends for their elimination. One of the instruments for modelling we are interested in is a functional dependence of total interaction energy of disperse particles on the set of concrete parameters of a disperse system and protein macromolecules or their associators which form its disperse phase:

$$U_\Sigma = U(a, H, \psi_\sigma, \varepsilon, A, \varkappa) \qquad (3)$$

Indicators-arguments of this function may be united into the following groups:
- the characteristic linear dimension a of protein macromolecules or their associators and the distance H between their surfaces are independent variables, m;
- Stern's potential ψ_σ of protein macromolecules or their associators is a conditionally constant value, which indirectly characterizes their electric charge, V;
- dielectric constant ε and constant A of molecular attraction forces are conditionally constant values, characterizing the disperse phase properties, Fm^{-1} & J, respectively;
- Debye's parameter $\varkappa$ is a design variable characterizing the intensity of potential falling in a diffuse layer of counter ions, m^{-1}.

At present, there is a sufficient quantity of theoretical and applied works in which the concrete forms of the functional dependence (3) are given and limits of their use are based. On the basis of this information analysis from the standpoint of its applicability for modelling of the quaternary protein structures formation process in meat systems, proceeding on the assumption that protein macromolecules considered or their associators in the first approximation may be recognized as having a spherical shape, identical dimensions and a fractional composition, the dependence showed in the work /2/ was selected as a basis. After its arguments that correspond to the international system of units /SI/ the next formula /6/ was developed:

$$U_\Sigma = 6{,}288\, a\varepsilon\psi_\sigma^2 \ln\{1+\exp(-\varkappa H)\} - \frac{aA}{12H} \qquad (4)$$

In connection with that in literature available we didn't find any information of Stern's potential values for proteins of the meat industry raw material which are necessary for calculations by formula (4), a computation method for ψ_σ based on the known characteristics was developed /5/. For this purpose, approaches to the derivation of dependence equatics was developed /5/. For this purpose, approaches to the derivation of dependence equation of a threshold concentration of coagulating ions on the disperse system parameters including ψ_σ were borrowed from the work /2/ and this equation was solved for ψ_σ. As a result the next mathematical expression was received:

$$\psi_\sigma = \frac{2kT}{e} \ln \left\{ \frac{1+\left(\dfrac{10^{(22-pH_{кр})} A^2 e^6}{0{,}872\varepsilon^3 k^5 T^5_{кр}}\right)^{0,25}}{1-\left(\dfrac{10^{(22-pH_{кр})} A^2 e^6}{0{,}872\varepsilon^3 k^5 T^5_{кр}}\right)^{0,25}} \right\} \qquad (5)$$

where $e = -1{,}602 \cdot 10^{-19}$ is the electron charge, Cl; $k = 1{,}381 \cdot 10^{-23}$ Boltzmann's constant, J/K; pH_{cr} the active acidity corresponding to the threshold of protein coagulation of fraction composition considered; T_{cr} the temperature corresponding to the coagulation threshold, K; $\varepsilon = \varepsilon_{H_2O} = 7{,}083 \cdot 10^{-10}$, F/m; $A = 10^{-20}$, J.

Formula (3) was used for calculations of for caseins /5/. The result received differed not more than 3 per cent from the value of a sense-analogous potential determined by means of other approaches /9/. On the basis of averaging literature data /4,9,10/ with pH_{cr} and T_{cr} values of ψ_σ were calculated for the wide group of protein fractions of the meat industry raw material. Some of them are cited lower: myogen - $\psi_\sigma = -1{,}2 \cdot 10^{-3}$, V; myosin - $\psi_\sigma = -2{,}0 \cdot 10^{-3}$, V; fibrinogen - $\psi_\sigma = -2{,}1 \cdot 10^{-3}$, V; serum albumin - $\psi_\sigma = -2{,}9 \cdot 10^{-3}$, V.

For calculations of values $\varkappa$ corresponding to the structure formation conditions considered the known /3,11/ formula was used which after reducing the dimensions of quantities entering into it to the international system of units /SI/ and after some modifying may be written in the following form:

$$\varkappa = \left\{ \frac{e^2 \cdot 6{,}022 \cdot \sum \left(10^{(26-pI_i)} Z_i^2\right)}{\varepsilon kT} \right\}^{0,5} \qquad (6)$$

where $pI_i = -\lg$ (of a molar concentration of coagulating ions of the i-th sort).

The graphs illustrating the results of a specific example of modelling the paired interaction of protein elements (with a radius $a = 100 \cdot 10^{-9}$, m) of three fractions are shown in Fig.1. The curve I corresponds to myosin, the curve II to fibrinogen and the curve III to serum albumin. The disperse medium temperature is assumed to be equal to 288K, pH 5,8. Concentration of the rest counter ions in a system is such that Debye's parameter value calculated by the formula (6) was proved to be equal to $5{,}55 \cdot 10^8$, m^{-1}. The analysis of modelling results shows that curves of "interaction energy" are of two-extremum* character and the secondary energy minimums corresponding to myosin, fibrinogen and serum albumin are not clearly marked, equal to $\min U_1 = -8{,}3 \cdot 10^{-23}$, J; $\min U_2 = -8{,}11 \cdot 10^{-23}$, J; $\min U_3 = -6{,}87 \cdot 10^{-23}$, J, respectively, and are reached at $H_1 = 750 \cdot 10^{-9}$, m; $H_2 = 800 \cdot 10^{-9}$, m;

* the region ($H < 50 \cdot 10^{-9}$, m) corresponding to the third extremum - potential well - is not considered in our case

$H_3 = 1000 \cdot 10^{-9}$, m. The further qualitative and quantitative pattern of the process of quaternary structures formation by protein elements of a fraction composition considered may be received on the basis of the following logical reasonings. A protein element being an associator of macromolecules of one of the fractions considered may reach a potential well, that is, interfix with an identical element at a distant corresponding to a global minimum of their paired interaction energy provided that its thermal-motion energy would be sufficient for doing work against electrostatic repulsion forces on the path from the secondary energy minimum to the primary energy maximum. Formally this condition is satisfied when

$$kT \geq |\min U_\Sigma| + \max U_\Sigma = \bar{U}_\Sigma \qquad (7)$$

As follows from the functional dependence (4), under other conditions being equal, its values are proportional to the radius a of protein elements interacted, therefore, on the basis of an elementary proportion it may be possible to set up an equation for their critical size determination a_{cr}; exceeding this size involves a change of inequality sign in the expression (7):

$$a_{cr} = \frac{akT}{\bar{U}_\Sigma(a)} \qquad (8)$$

It is not difficult to determine the value of $\bar{U}_\Sigma(a)$ proceeding from the results of modelling carried out, i.e. from graphs in Fig.1. As follows from these, the value of $\bar{U}_\Sigma$ ($100 \cdot 10^{-9}$ m) for myosin equals $19{,}1 \cdot 10^{-23}$, J; for fibrinogen and serum albumin - $24{,}1 \cdot 10^{-23}$, J, and $93{,}5 \cdot 10^{-23}$, J, respectively. Substituting these values into the formula (8) we receive that for myosin $a_{cr} = 2{,}082 \cdot 10^{-6}$, m; for fibrinogen $a_{cr} = 1{,}649 \cdot 10^{-6}$, m; for serum albumin $a_{cr} = 0{,}425 \cdot 10^{-6}$, m.

From here it follows that under conditions in a meat system considered in our example the quaternary protein structures formation of the third level by associators of myosin macromolecules is possible provided that in initial state the radius of these associators didn't exceed $2{,}08 \cdot 10^{-6}$ m. An analogous condition is possible also for fibrinogen provided that the radius of its associators shouldn't exceed $1{,}649 \cdot 10^{-6}$ m. For serum albumin the structure-formation process is possible from the standpoint of energy if the radius of protein elements doesn't exceed $0{,}425 \cdot 10^{-6}$ m.

As uncomplicated logical analysis shows, for a case when protein elements of an initial meat system are characterized by dimensions which are more than a_{cr}, two conditions may prevail: - the radius values of these elements belong to the interval $a_{cr} < a < a'_{cr}$ and elements themselves cannot interfix in the secondary energy minimum because of that their Brownian motion energy exceeds the absolute value of its depth, what allows to consider them as elements of quaternary protein structure of the first level;

- the radius values of elements of an initial system exceed a'_{cr}, what stipulates for the possibility of their interfixation in the secondary energy minimum and satisfies conditions of the formation of quaternary protein structures of the second level.

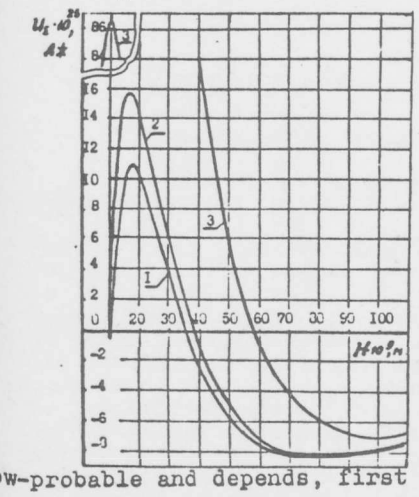

Fig.1 Graph of the paired interaction total energy as a function of the distance between surfaces of particles interacted:
1 - myosin; 2 - fibrinogen; 3 - serum albumin.

In the general case, the values of a'_{cr} may be determined by equation:

$$a'_{cr} = \frac{akT}{|\min U_\Sigma(a)|} \qquad (9)$$

For an example considered, $a'_{cr} = 4{,}79 \cdot 10^{-6}$, m, corresponds to myosin, $a'_{cr} = 4{,}21 \cdot 10^{-6}$, m, corresponds to fibrinogen and $a'_{cr} = 5{,}79 \cdot 10^{-6}$, m, corresponds to serum albumin.

As these dimensions practically exclude Brownian motion, the possibility of spontaneous formation of quaternary protein structures of the second level is low-probable and depends, first of all, on external factors.

Conclusions

1. Phenomenologically formalized concepts of three overmolecular levels of quaternary protein structures are formulated which are intended for the qualitative analysis and quantitative evaluation of meat systems properties at various stages of their technological processing.
2. Procedure of modelling the process of quaternary protein structures formation supplemented with necessary calculating formulae has been developed. It serves as an important element in developing the automated system of multicomponent meat products designing which provides for optimization of their consumer characteristics, minimization of power consumption and raw material technological losses.

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