Prediction of the properties of food biopolymer gels at the molecular-atomic level

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Abstract. The analysis of the main regularities, conditions and factors influencing the mechanism of structural changes in food biopolymer molecules during gelation using the laws of thermodynamics is carried out. A computational method for predicting the properties of gel-like food systems is considered. It makes it possible to establish the dependence of the characteristic viscosity of aqueous solutions of hydrocolloids and the hydrodynamic radius of biopolymer molecules on the charge of their molecular structures and the pH of the active acidity of the medium. On the example of gelatin, calculations are presented and it is shown that, in comparison with a neutral medium, the hydrodynamic radius of protein molecules increases approximately to (120–123) % both at high and low pH values, which makes it possible to predict the water-binding capacity of protein molecules and determine the optimal modes of technological processes.

1. Introduction

Theoretical and practical issues of gelation of food biopolymers of both animal and plant origin in a systemic form are described in the works of various authors [1–7]. However, for a criterial assessment of the regularities of the gelation process, a quantitative (calculated) analysis of its parameters is required. It should be based on an accessible mechanism for revealing the features of the phase transitions and processes occurring at the level of molecular structures of hydrocolloids, taking into account the degree of participation of their most active functional groups in the interaction with the dispersion medium.

2. Materials and methods

In a solvent subjected to gelation, according to the laws of thermodynamics, under the influence of external influences and internal factors, the process of structuring the solid phase of the hydrocolloid proceeds with a simultaneous redistribution of moisture until the system reaches thermodynamic equilibrium. With its high plasticity, the two-phase layer “dispersed phase – dispersion medium” withstands significant “deformation” until a single continuous phase is structured. This process proceeds due to the presence of weak interconnections between the boundary monomolecular layer of water and the gelling agent.

By varying the composition of the components, their quantity and parameters of technological modes, gelsystems with the given structural and mechanical properties are obtained. For example, gels
that are less durable and with more noticeable thixotropic properties are obtained under intense heat treatment conditions followed by cooling.

The mechanism of gelation of most hydrocolloids is characterized with the pattern of “unity of continuity and jump” [1–3]. Similar processes in the fundamental sciences are terminologically designated as continuous (gradual) and phase (abrupt, abrupt) [8]. During continuous conformational changes in hydrocolloid systems, for example, proteins, as typical representatives of food biopolymers, simultaneously and for a long time can exist in the initial and final conformational states and smoothly transition from one to another. In this case, the rheological parameters and physicochemical properties of their gels are in direct proportion to the specified process parameters. The jumps during gelation are accompanied with the absorption or release of energy, as well as jump-like changes in some properties of proteins. In this case, a change in one significant factor leads to a fundamentally new character of the investigated quantity.

According to modern concepts, the mechanism of gelation directly depends on three main reasons: the nature of the biopolymer, the composition and properties of the solvent (dispersion medium), and the process parameters. All other things being equal, it was established [6] that the presence of one set of cause-and-effect relationships can determine the formation process and the specificity of the properties of the gel-like system of a food product.

Biopolymer molecules must have some minimum energy in order to overcome the energy barrier and begin to react with each other. The activation energy is considered in thermodynamics as one of the quantitatively significant characteristics of the ability of substances to react. Proceeding from the fact that the interaction of molecules obeys the Arrhenius equation (1), it can be assumed that their activation ultimately occurs due to thermal energy and in the course of the reaction the equilibrium energy distribution over the degrees of freedom of the reacting particles should not be violated [3].

\[ K = A \exp\left(-\frac{E_A}{RT}\right) \]  

Where \( K \) – the rate constant of a chemical reaction; \( T \) – temperature; \( A \) – quantity characterizing the collision frequency of reacting molecules; \( E_A \) – activation energy; \( R \) – universal gas constant.

The activation energy (modification of molecules during the phase transition - gelation) can be determined with the formula

\[ E_A = \Delta H^\circ = (\ln K_1 - \ln K_2) \times (R \cdot T_1 \cdot T_2)/(R \cdot T_1 - T_2) \]  

Where \( E_A \) – activation energy; \( \Delta H^\circ \) – enthalpy change during gelation; \( K_1, K_2 \) – respectively, the rate constants at temperature \( T_1 \) and \( T_2 \).

The rate of interaction of molecules can be represented with the rate of a chemical reaction, and the constant of gelation is determined with the equations:

\[ K = \lg C - \frac{\Delta H^\circ}{(2.3 \cdot R \cdot T_g)} \text{, at } M = \text{const} \]  

\[ K = \lg M - \frac{\Delta H^\circ}{(16 \cdot R \cdot T_g)} \text{, at } C = \text{const} \]  

Where \( M \) – molecular weight of gelling agent; \( C \) – concentration of macromolecules; \( T_g \) – gelation temperature.

Despite the existing numerous studies, the deep aspects of the mechanism for regulating the strength characteristics of gelsystems have yet to be learned. At the molecular-atomic level, as the results of [3] show, the strength of the gel changes in direct proportion to the value of the electric charge parameter of the molecule, which in turn depends on the charge of the biopolymer molecules. It is noted that the ultimate shear stress of gelsystems increases with an increase in the charge of molecules, and the strength of the structures of gelatin and pectin gels as a result of a change in the charge of molecules is greatest.
This is due to the ability of hydrogen ions to block charges on the surface of the gelling agent molecule, followed with a change in the sign of the molecule charge while simultaneously enhancing the gelling properties. In practice, this means that in order to optimize the gelation process, one of the promising areas is the search, calculation, modeling and use of those factors and technological modes that lead to the achievement of the optimal charge of the gel-forming molecule and the energy of the system as a whole.

3. Results
It is known [6] that the intrinsic viscosity of aqueous solutions of hydrocolloids is minimal when the charge of its molecules is equal to zero, that is, at the isoelectric point and smoothly increases with increasing charge. For example, for gelatin it has, in all likelihood, two maxima: in the alkaline region with a molecular charge equal to minus 45–50 units and in acidic - with a molecule charge of 55–60 units [6]. The graph of the dependence of the intrinsic viscosity of gelatin molecules (0.2% solution in drinking water) on the charge (figure 1) shows that the charge of the molecules in the range of numerical values is ± 40 units almost symmetrical about their isoelectric point.

![Figure 1](image1.png)  
**Figure 1.** The dependence of the intrinsic viscosity of gelatin molecules on their charge.

![Figure 2](image2.png)  
**Figure 2.** Dependence of the charge of gelatin molecules on the indicator pH of the medium.

At present, it is quite difficult to perform a holistic mathematically adequate modeling of the gelation process, since significant factors interact with an antagonistic or synergistic effect. In this regard, in our opinion, the applied use of the dependence of the charge value of biopolymer molecules on the pH of active acidity pH (figure 2), which can be determined following the methodology [9], and go to the intrinsic viscosity of solutions of hydrocolloids (figure 1), is becoming relevant.

The calculation of the number of amino acid residues in the gelatin molecule (table 1, [9]) was carried out on the basis of its known (empirically determined) amino acid composition [10] and taking into account the accepted average weight of the gelatin molecule:

\[
M_{avgi} = \sum (A_i \cdot M_i)
\]

where \( M_{avgi} \) – average mass of a gelatin molecule (60500 Da), \( A_i \) – the number of residues of the \( i \)-th amino acid in a protein molecule; \( M_i \) – molecular weight of the \( i \)-th amino acid in a protein molecule.

Let us assume that the content of amino acids in each protein molecule is proportional to their content in the protein, determined empirically for each of them [10]. Based on theoretical concepts [8, 9] and the data in table 1, the number of positively and negatively charged ionogenic groups of gelatin in the medium with different pH levels was determined, and the calculated dependence of the charge of gelatin molecules on the pH of the medium was built (figure 2).
Table 1. Results of calculating the number of amino acid residues in a gelatin molecule.

| Amino acid   | Content in protein, % | Molecular weight \( M_A \), Da | Number of amino acid residues \( A \), pieces | \( pK_a \)^a |
|--------------|-----------------------|---------------------------------|-----------------------------------------------|-------------|
| Alanine      | 9.0                   | 89                             | 4606.60                                       | 52          |
| Glycine      | 26.0                  | 75                             | 13307.95                                      | 177         |
| Valine       | 2.5                   | 117                            | 1279.61                                       | 11          |
| Leucine      | 3.4                   | 113                            | 1740.27                                       | 15          |
| Isoleucine   | 1.8                   | 113                            | 921.32                                        | 8           |
| Proline      | 17.5                  | 115                            | 8957.28                                       | 78          |
| Phenylalanine| 2.5                   | 165                            | 1279.61                                       | 8           |
| Tyrosine     | 0.4                   | 181                            | 204.74                                        | 1           |
| Tryptophan   | 0.0                   | 204                            | 0.0                                           | 0           |
| Serine       | 3.1                   | 105                            | 1586.72                                       | 15          |
| Threonine    | 2.2                   | 119                            | 1126.06                                       | 9           |
| Cystine      | 0.0                   | 121                            | 0.0                                           | 0           |
| Methionine   | 0.6                   | 149                            | 307.11                                        | 2           |
| Arginine     | 10.0                  | 174                            | 5118.44                                       | 29          |
| Histidine    | 0.7                   | 155                            | 358.29                                        | 2           |
| Lysine       | 4.0                   | 146                            | 2047.38                                       | 14          |
| Aspartic acid| 6.7                   | 133                            | 3429.36                                       | 26          |
| Glutamic acid| 11.8                  | 147                            | 6039.76                                       | 41          |
| Oxyproline   | 14.7                  | 131                            | 7524.11                                       | 57          |
| Hydroxylysine| 1.3                   | 162.2                          | 665.4                                         | 5           |
| TOTAL        | 118.2                 | -                              | \( M_{avg} = 60500 \)                          | 550         |

^a \( pK_a = -\lg K_a \), where \( K_a \) – apparent dissociation constant [8].

The known numerical value of the intrinsic viscosity index makes it possible to determine the parameters of the molecular structure of a biopolymer in the system under study, to estimate the size and behavior of its molecule.

Using the Einstein-Sim equation [13], knowing the intrinsic viscosity and molecular weight, it is possible to calculate the hydrodynamic radius \( r \) of biopolymer molecules:

\[
r = \left( \frac{3M[\mu]}{4\pi Na} \cdot 2.5 \cdot M \right)^{1/3}
\]

(6)

where \( Na \) – Avogadro's number; \( [\mu] \) – intrinsic viscosity, \( \text{cm}^3 / \text{g} \); \( M \) – molecular mass.

Based on the analysis of equation (6) and the dependence of the intrinsic viscosity on the charge value of the molecular structures of the biopolymer (figure 1), and, therefore, on the \( pH \) value, we obtain the dependence of the hydrodynamic radius of protein molecules on the \( pH \) of the medium, i.e. \( r = f \) (\( pH \)).

Note that, from the technological point of view, the range of \( pH \) values of 2.8–3.0 units and 10.8–11.2 units are of greatest interest, in which the charge of the molecules is practically the same and is about 40 units in absolute value. In this case, the protein molecules should have a larger hydrodynamic radius than near the isoelectric point, that is, in the range of neutral \( pH \) values, and taking into account equation (6), the intrinsic viscosity of the system should have higher values.

At a given \( pH \) of the medium, knowing the value of the charge of the molecules of the structurant \( Z = f \) (\( pH \)) (figure 2), one can go to its intrinsic viscosity, obtain the numerical values of the hydrodynamic radius of protein molecules, or, conversely, from formula (6), from the known radius, calculate the characteristic system viscosity.
Taking into account the foregoing, as well as according to the data presented in figures 1 and 2, table 2 shows the results of calculating the value of the hydrodynamic radius of gelatin molecules depending on the pH of the medium.

**Table 2.** Calculated indices of globular gelatin molecules depending on the pH of the medium.

| Index | pH units | Charge, units | Intrinsic viscosity, cm³ / g | Hydrodynamic radius, nm | Surface area, nm² |
|-------|----------|---------------|----------------------------|------------------------|------------------|
| 3.0   | +40      | 14.0          | 5.12                       | 329.42                 |                  |
| 7.0   | -24      | 8.0           | 4.25                       | 226.98                 |                  |
| 11.0  | -40      | 15.0          | 5.24                       | 345.04                 |                  |

Analysis of the results of predictive calculations (table 2) shows that, compared with a neutral medium, the hydrodynamic radius of protein molecules increases by about (20–23)% both at high and low pH values. This, in turn, indicates an increase in the water-binding capacity of protein molecules, which is undoubtedly significant from a technological point of view.

**4. Conclusion**

Thus, not only is the position that, with distance from the isoelectric point, the structure of biopolymer molecules is more expanded, loose, and possibly for this reason, its core and other internal structures are more accessible to the solvent, is clearly confirmed. But it also becomes possible to fairly simply calculate the numerical values conformational characteristics of molecules, taking into account the charge of their functional groups and, most importantly, on this basis, proceed to predicting the properties of real food systems.

The results of molecular modeling of proteins in media with different pH by the methods of classical and controlled molecular dynamics implemented in the programs HyperChem Professional and NAMD (Nanoscale Molecular Dynamics), with visualization of 3D modeling data in the program VMD (Visual Molecular Dynamics), confirm the presented analytical calculations [14–17]. This indicates the far from exhausted technological possibilities of accounting for and using the features of the mechanism for regulating the properties of gels at the molecular-atomic level to obtain high-quality gelsystems in the production of various, including jelly-like food products.

Thus, knowing the magnitude of the charge, dimensional and other characteristics of food biopolymer molecules (table 2) in the conformation of a globule (coil), it is possible to predict the behavioral model of the protein under study in various physicochemical and technological processes – dissolution, determination of the hydromodule during swelling and hydration, gelation, and others.

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