Original scientific paper
UDC 637.344.045:004.942

COMPUTER SIMULATION OF WHEY PROTEIN β-LACTOGLOBULIN BEHAVIOR UNDER ULTRASOUND TREATMENT OF ITS SOLUTIONS AT DIFFERENT pH LEVELS

Konstantin Kostenko[1*], Andrey Bratsikhin[2], Maria Shpak[1]

[1]Institute of Engineering, North Caucasus Federal University, Kulakova Avenue 2, 355029 Stavropol, Russia
[2]Institute of Life Sciences, North Caucasus Federal University, Kulakova Avenue 2, 355029 Stavropol, Russia

[*]e-mail: kostenko.ncstu@gmail.com

Abstract

One of the important and complicated process at dry milk products reconstitution is the protein fraction hydration and its transition to a stable emulsion-colloidal state. In this regard, the aim of this work was to study the process of hydration protein fractions of dry whey using electro-activated aqueous media with different pH that are reconstituted by cavitation disintegration.

Various effects of water pH level used for whey reconstitution on whey protein (β-lactoglobulin) hydration were studied with using computer molecular modeling in the programs: visual molecular dynamics (VMD), nanoscale molecular dynamics (NAMD), and adaptive Poisson-Boltzmann solver (APBS) plugin. The β-lactoglobulin molecule was placed in a water chamber corresponding to the size of the molecule, and the needed pH level was set by the plug-in APBS. The conformational state of the protein was calculated by the molecular dynamics method in NAMD. The VMD program was used to visualize the calculation results. Cavitation effect initiated under reconstitution process was simulated used method, which consist of setting of a specially calculated temperature gradient depending on the heat capacity and density of the product according to condition that all energy produced at cavitation disintegration transmitted to the heat energy in close system.

According to the data obtained during the modeling process, it was established that the protein molecule, immersed in an alkaline medium (pH = 11), had several active centers into molecule, that provided high reactivity of the studied molecule under cavitation treatment. There were similar results in acidic medium (pH = 2) when duration of cavitation treatment was up to 90 sec.

Method of process simulation allowed to determine the more relevant regimes of reconstitution as well as optimal pH value of the liquid system used for whey proteins hydration. The most optimal conditions were provided when acidic (pH = 2), and alkaline (pH = 11) aqueous media were used under short cavitation treatment up to 30 sec.

Key words: *Whey, Reconstituted whey, Cavitational disintegration, VMD, NAMD, β-lactoglobulin, Alkaline, Acidic.*

1. Introduction

The dairy industry of the Russian Federation at the current stage of development is in the conditions of a rigid formation of prices for dairy products due to a shortage of raw milk. Rising of the financial burden of enterprises producing and processing milk, contributed by decreasing of the cattle population leads to the dairy products cost increasing. The situation on the dairy market leads to the deficit formation and causes of Russia's dependence on milk import, and reduces the quality of certain types of dairy products. This problem can be solved by increasing of the areas for application of secondary milk products like whey and buttermilk for foods manufacturing. Whey has a high nutritional value as it is consist of more than 50% of milk solids. It should be noted that in order to reduce the costs associated with the storage and processing of whey, more than 60% of all whey produced is usually dried and used for production of a numerous types of products in dried condition. But for some of the liquid types of milk products the whey must be used in native condition that means that dried whey must be reconstituted and its properties must be the same as natural one has.

In the most cases the quality of the final product directly depends on the used reconstitution process. Therefore, in addition to the traditional technology of reconstitution consisting of the intensive mechanical mixing of dry material with liquid, there are some new methods that are used separately or in their combination like mechanical, hydrodynamical and acoustic treatment of the system [3].

Cavitational disintegration as a special case of acoustic processing of liquid systems treating is one of the promising methods used in practical application for reconstitution of dried dairy products. However, the method has not been sufficient studied yet.

It is known that the reconstitution process quality is determined by effectiveness of the protein fraction hydration and its transition to a stable emulsion-colloidal state [8, 10].

There are some research works dedicated to study of milk and whey protein behavior under different conditions. It was established that molecule of β-lactoglobulin, which is the dominating protein in dry whey, could improve the protein reactivity if pH of system had been changed from neutral. It was shown that anolyte (pH = 2) and catholyte (pH = 11) of water using instead of drinking water (pH = 7.6) led to intensification of reconstitution process of dry whey as well as its stability development needed for high quality products making based on reconstituted dry whey. It was noted that anolyte and catholyte using could lead to significant molecule polarization and creation of many new same-charged zones in protein molecule [4, 6]. At the same time, study of possibilities to use some methods of water and solution treating for reconstitution process development has scientific and practical interest.

In the article the theoretical study of β-lactoglobulin behavior in electroactivated aqueous medium with different pH level under its treating by cavitational disintegration has been described.

2 Materials and Methods

2.1 Materials

β-lactoglobulin is one of the base proteins of dry whey and its content in all amount of whey proteins is about 58%. This protein has a globular structure consisting of 162 amino acid residues. There are several versions of its influence on whey functional properties. One of the functions may be connected with transportation of important acid-resistant substances, due to its properties. β-lactoglobulin is resistant to pepsin and chymosin in the acidic environment of the stomach [6].

There is a proposition that it can reach the intestine indigestion due to mentioned property, and thereby can provide substances transfer. In addition, β-lactoglobulin has the ability to bind: hydrophobic and amphiphilic molecules, hexane, palmitic acid and vitamin D [6].

For this research, 3-D model of β-lactoglobulinwas downloaded from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank. That molecule had the neutral electrostatic field without noticed areas with electro-positive or electro-negative charges in vacuum (Figure 1).

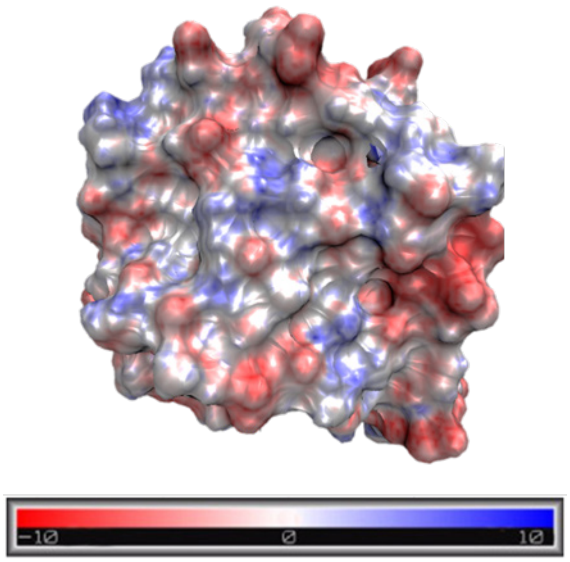

Figure 1. 3-D model of β-lactoglobulin in vacuum

2.2 Methods

Complex of programs was used for computer modeling of β-lactoglobulin that includes NAMD, VMD and special APBS plugin. Program complex NAMD based on the methods of molecular dynamic with model of parallel programming Charm++ using. The mentioned complex is recommended as highly effective tool for large bio-molecular systems [2, 5].

Program complex VMD is oriented for visualization of the modeling results made in NAMD, and could represent and calculate the electrostatic fields for studied objects that are formed at different pH-levels of the modeled system [1, 9].

Model of the studied protein was placed into the water box that had respective level of pH in relation to the size and solvent condition, which was fixed in the program. There were three systems modeled at the following pH-levels: pH = 2.0 (anolyte), pH = 7.0 (drinking water), and pH = 11.0 (catholyte). Calculations of the protein conformation condition were made by the molecular dynamic methods using [4, 7].

On the basis of the well-known modeling technique for cavitational disintegration processing described in the works of Bratsikhin A. A., a theoretical study for modeling and calculation of results of the complex effect provided by used both pH levels and ultrasonic treatment was conducted. In these studies, it was found that transfer of potential energy into thermal energy occur under cavitation effects initiation, which could be similar to the temperature increasing in the treated medium. The mentioned proposal was used for modeling of results of cavitation treatment the studied systems. Offered method concluded the calculated temperature gradient setting depending on the heat capacity and density of the product, as well as the transmitted energy of the cavitation effect calculation. According to a set duration of exposure (10, 30 and 90 seconds), the potential energy in the system was calculated as 5, 15 and 45 kJ respectively (provided that the technical parameters of the ultrasonic processor corresponded to 400 W and 22 kHz ultrasonic oscillation frequency) [4].

In accordance with described method, the temperature gradients were calculated using the formula 1.

$$Q=C_w \cdot V \cdot \rho \Delta t, \text{ kJ}, \quad (1)$$

Legend: $\Delta t = Q/(c_w \cdot V \cdot \rho) = 5{,}0 \text{ kJ} /(4{,}082 \cdot 0{,}001 \cdot 1023) = 1{,}1974\ ^0C$,
C_w is the heat capacity of the milk whey (4,082 kJ/kg ·K);
V - A constant volume of liquid, was equal to1 liter.

Based on the results of calculating the amount of energy transferred to the system during cavitation disintegration was set, as well as the different pH levels of environment. The β-lactoglobulin conformational state was performed by setting the heating temperature and pH environment.

At the next step, the results of different duration of cavitational disintegration at mentioned pH levels of systems were compared one with another based on the projections of the electrostatic field of the protein molecule at the set conditions.

Table 1. Values of temperature gradients for different duration of the cavitational disintegration of dry whey solutions

Cavitation potential energy, kJ	Δt, ⁰C	Processing time, s
5	1,1974	10
15	3,5921	30
45	10,776	90

It was studied the differences in properties of dry whey solutions made under the cavitational disintegration at the different level of system pH. There were projection maps of electrostatic potential and electronegativity coefficient (k) compared to study the protein activity under different conditions. The electronegativity coefficient was calculated as a square of electronegative field of molecule related to the area occupied by electropositive field. Physically, the electronegativity coefficient (k) shows the charge ratio inside of the protein molecule. If calculated parameter $k > 1$, the molecule is electronegative, and if $k < 1$ it means that molecule is electropositive, and if $k = 1$, the molecule is neutral. Intermolecular bonds formation would be more stable and effective as much the electronegativity coefficient as far in value from neutral one.

3. Results and Discussion

The calculations and projection maps of electrostatic potential analysis (Figure 1) shown that simulation of processing duration at 10 and 90 s for pH = 2.0 provided to coefficient forming up to 2.39 and 2.253, respectively, whereas the simulation of processing at 30 sec. led to the low electronegativity forming (k = 1.637). It meant that in acidic medium the electronegativity coefficient increased at both the short and long periods of treating by 20 and 37% respectively. At these conditions the most electronegatively charged parts of molecule could be formed that led to many intermolecular bonds formation between the water dipoles and active centers of protein molecule located on the free and highly charged amino acids residuals.

In terms of the use of cavitational disintegration processing for neutral medium (pH = 7) (Figure 2) it was found that the highest values of the electronegativity coefficients were observed when the treatment duration was equal to 10 and 90 sec. (calculated electronegativity coefficient was 1.459 and 1.655, respectively).

It should be noted that calculated values of the electronegativity coefficient were significantly lower for the neutral medium (pH = 7.0) in comparison with the same time of treating at acidic and alkaline environment.

Therefore, neutral medium using would be less preferable for cavitational reconstitution of dry whey as the protein molecule would have a lower reactivity than at acidic and alkaline environment.

Projection maps of the β-lactoglobulin protein model made for alkaline medium shown of the Figure 3. It was established the maximum value of electronegativity coefficient (up to 2.94) could be formed at 10 seconds of treatment if initial pH of medium would be equal to 11.0. That coefficient had the maximum value for all of studied mediums (neutral, alkaline and acid). It was noted that

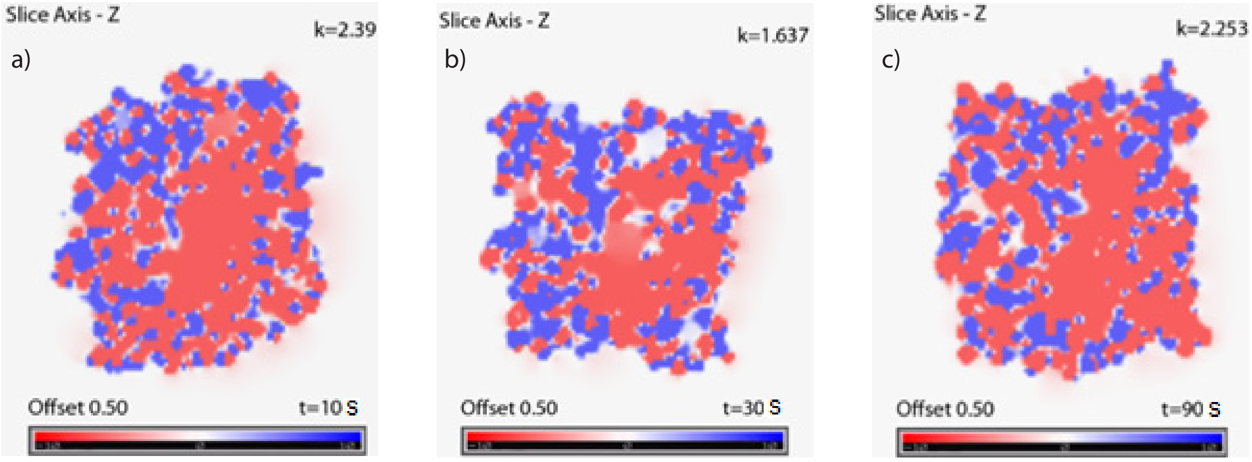

Figure 1. Projection maps of the β-lactoglobulin model at pH = 2 and processing time at: a) 10 sec.; b) 30 sec.; c) 90 sec.

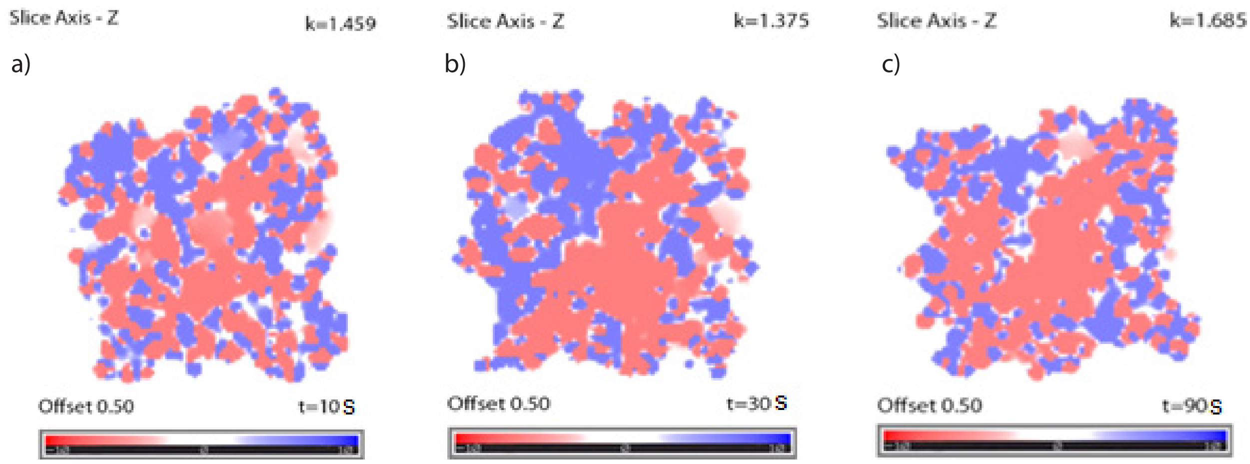

Figure 2. Projection maps of β-lactoglobulin models at pH = 7 and processing time at: a) 10 sec.; b) 30 sec.; c) 90 sec.

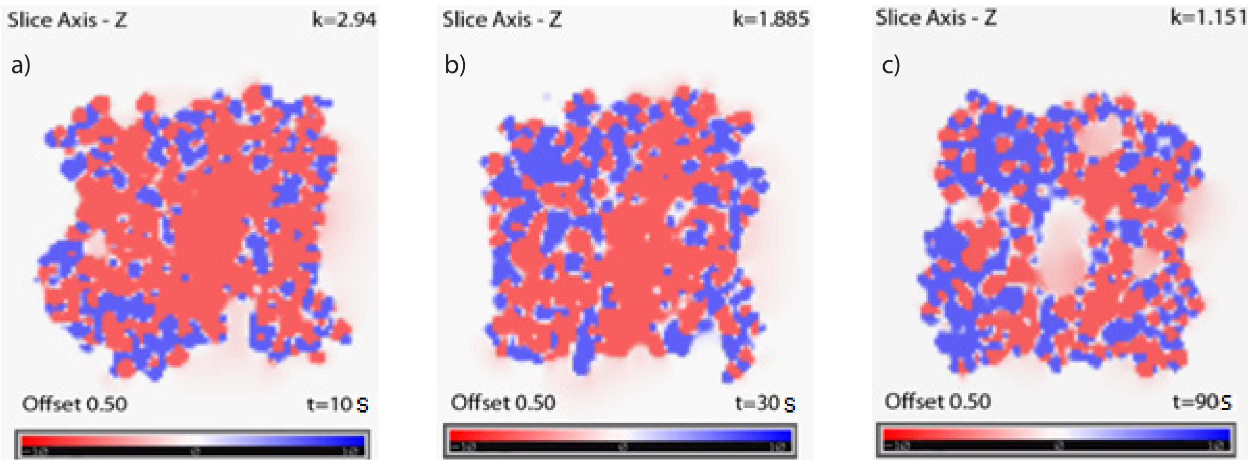

Figure 3. Projection charge maps of β-lactoglobulin at pH = 11 and processing time at: a) 10 sec.; b) 30 sec.; c) 90 sec.

extending of treatment time led to the significant electronegativity coefficient decreasing (treatment at 30 sec. formed the coefficient value up to 1.885, and cavitation disintegration at 90 sec. led to coefficient forming up to 1.151). The cavitational disintegration processing of the system at 90 sec. provided the neutral charge of the molecule forming in the center, which could reduce the molecule activity for new bonds forming (Figure 3, c).

4. Conclusions

- Methods of molecule computer modeling could predict its behavior at the real conditions. It was studied the pH level of liquid medium impact on process of new hydrogen-bonds forming.

- The optimal parameters of cavitation disintegration process were established for alkaline, neutral and acid medium that could provide the high intensify of whey protein (β-lactoglobulin) hydration at its reconstitution for the followed using in foods technology.

- Optimal parameters of cavitational disintegration were established for acidic and alkaline aqueous environment using made at the short time of cavitational disintegration (up to 10 sec.). Recommended parameters for reconstitution of dry whey under neutral pH of medium was determined at 90 sec. of treatment. At the same time, reconstituted solutions of dry whey could be made at 10 sec. of treatment and alkaline medium using instead of drinking water.

- β-lactoglobulin activity at acidic environment also was high but the final reconstituted solutions would have the low pH that would limit its using in foods technology.

5. References

[1] Aleksandr A. Borisenko, Andrey A. Bratsikhin, Ludmila A. Saricheva, Aleksey A. Borisenko, Konstantin V. Kostenko (2017). Forecasting of functional properties of the soya protein based on the molecular dynamics method. Journal of Hygienic Engineering and Design, Vol. 20, pp. 82-85.

[2] Theoretical and Computational Biophysics Group. APBS electrostatics in VMD. <URL: https://dasher.wustl.edu/chem478/labs/lab-08/tutorial-vmd.pdf. Accessed 15 January 2019.

[3] Artemova Y. A. (2011). Technology development and commodity assessment of the quality of milk drinks obtained using sonochemical water treatment (in Russian). PhD thesis, Moscow State University of Technology and Management, Moscow, Russia. pp. 136.

[4] Bratsikhin A. A. (2009) Scientific and practical aspects of the intensification of technological processes using nanoactivated liquid media in the production of meat products (in Russian). Doctoral thesis, North Caucasus Federal University, Stavropol, Russia, pp. 511.

[5] Humphrey W., Dalke A., Schulten K. (1996). VMD: Visual molecular dynamics. Journal of Molecular Graphics, 14, pp. 33-38.

[6] Khramtsov, A. G. (2011). The phenomenon of whey (in Russian), Profession Publ., St. Petersburg, Russia, pp. 804.

[7] Kostenko K., Bratsikhin A., Borisenko A., Salmanova D., Leshchenko E. (2017). Computer modeling of whey protein β-lactoglobulin behavior in the activated liquid systems. Journal of Hygienic Engineering and Design, Vol. 20, pp. 70-74

[8] Lipatov N. N. (1985). Refurbished milk (theory and practice of production of reconstituted dairy products (in Russian). Agropromizdat, Moscow, Russia, pp. 256.

[9] NAMD. Virtual Laboratory. <URL: http://ru.vlab.wikia.com/wiki/NAMD. Accessed 15 January 2019.

[10] Shestakov S. D. (2008). Method of restoring dry milk (in Russian). Patent of the Russian Federation No. 2329650.