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COMPUTER MODELING OF WHEY PROTEIN Β-LACTOGLOBULIN BEHAVIOR IN THE ACTIVATED LIQUID SYSTEMS

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Abstract

There is a problem of raw milk deficit in East and Central Europe countries that can be solved by effective using of the secondary raw materials such as milk whey, which is produced in the enormous volumes. The most popular method of whey reservation that could prolong its shelf-life is drying by the different ways, which is used for treating 60% of the produced whey. Dry whey could be reconstituted and then using for some kinds of technologies. Reconstituted whey in that case must have the same properties as a natural one such as high stability without any chemical stabilizers using, and have the physical and chemical properties appropriated for its using as an ingredient for respective foodstuffs production. In that way, behavior of the dry whey in the different solvent types reconstituted by existed methods is one of the actual task.

Different pH-level influence on the whey protein (β-lactoglobulin) hydration was studied by using of the computer molecular modeling method in the special programs Visual Molecular Dynamics (VMD), Nanoscale Molecular Dynamics (NAMD) and Adaptive Poisson-Boltzmann Solver (APBS) plugin. β-lactogloguline molecule was placed into the water box corresponded with the molecule size, and the pH-level was set by the APBS plugin. The conformation state of the protein was calculated by molecular dynamic method using that was made in NAMD. VMD program was used for visualization of the calculation results.

The results of modeling showed that the shape and size of protein molecules in alkaline environment at the level pH = 11 was comparable to the conformational state of molecule in acidic medium at pH = 2. The model of protein had branched fragments and poles of globules at the level pH = 2. These fragments could be the active centers of molecule due to formed charges of them, and could be involved into intermolecular bonds formation.

It was recommended to use the alkaline (catholyte) and acidic medium (anolyte) at the level pH = 11.0 and pH = 2.0 respectively for reaction ability increasing as well as intensification of the hydration and stabilization processes of the systems contained the whey protein.

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

1. Introduction

Modern effects of globalization have formed the deficit of raw milk in some countries of East and Central Europe that have led to the cost of the milk product increasing. At the same time it has provided the consumer demand reduction on the expensive milk products as well as consumers orientation on the plain and cheaper foodstuffs [1, 2].

Developing of the technology of the dry milk products (dry milk, whey, etc.) using instead of the natural ones is one of the ways of that problem solution. The methods of dry milk products reconstitution and high quality of the reconstituted systems providing, which properties could be corresponded to the natural ones would have the important meaning for the food science. There are many research works dedicated to the reconstitution that are described internal processes and their regimes as well as the machinery supporting schemes of such kinds of technology [3, 4, 5, 6, 7, and 8].

Dairy industry is produced enormous volumes of milk whey and one of the ways of its treatment is drying. This treatment is used for 60% of produced whey. Dry whey can be preserved for one year and may be used for large scale of different foodstuffs production. In some kinds of foodstuffs like beverages and milk deserts the liquid state of the whey must be used. It means that dry milk whey must be reconstituted and its properties should be corresponded to the natural whey. The reconstituted whey must have high stability, and its physical and chemical properties should be appropriate for high quality foodstuffs production without any chemical additives using.

It is known that properties of the solvent influence on the effectiveness of the reconstitution process and different pH-level and redox potential as well as mineral content of water could lead to the different ways of the process realization [9, 10]. Regulation of that parameters could manage the solubility of water that is used for whey reconstitution. One of the methods that can regulate the water properties is the electrochemical treatment of water, which may be used for production of catholyte with pH = 11.0 and anolyte with pH = 2.0 [11, 12, 13, 14, 15, and 16]. Both of them are safe and could be used instead of water for whey reconstitution. One of the important characteristics of the suspension and emulsion is protein condition in the systems, which provides stability and other properties of reconstituted solutions.

It is known that optimal pH-level can provide more effective hydration of the protein and reaction abilities of such kinds of systems increases. Virtual experiments made on the special computer programs that can be used for visualization and calculations could allow the suspension or emulsion behavior under its treatment predicting.

The goal of the research was to establish an optimal condition of solvent used for whey reconstitution by molecular modeling of the whey protein in professional programs: VMD, NAMD and APBS plugin.

2 Materials and Methods

2.1 Materials

β-lactoglobulin is one of the base protein of dry whey, and its content in all kinds of whey protein is about 58%. 3-D model of β-lactoglobulin was downloaded from the RCSB Protein Data Bank and used for molecular modeling and calculations. That molecule had the neutral electrostatic field without noticed areas with electro-positive or electro-negative charges in vacuum (Figure 1).

2.2. Methods

Complex of programs were used for computer modeling of β-lactoglobulin that includes: NAMD, VMD and special APBS plugin.

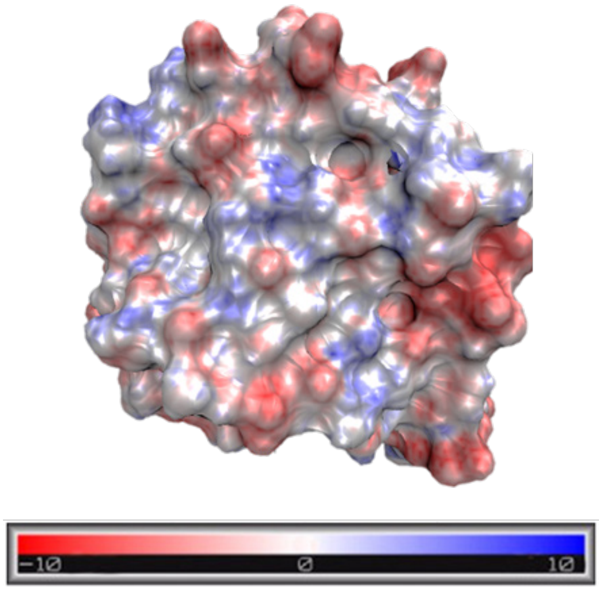


Figure 1. 3-D model of β-lactoglobulin in vacuum

Program complex NAMD based on the methods of molecular dynamic with model of parallel programming Charm++ was used. The mentioned complex is recommended as highly effective tool for large bio-molecular systems.

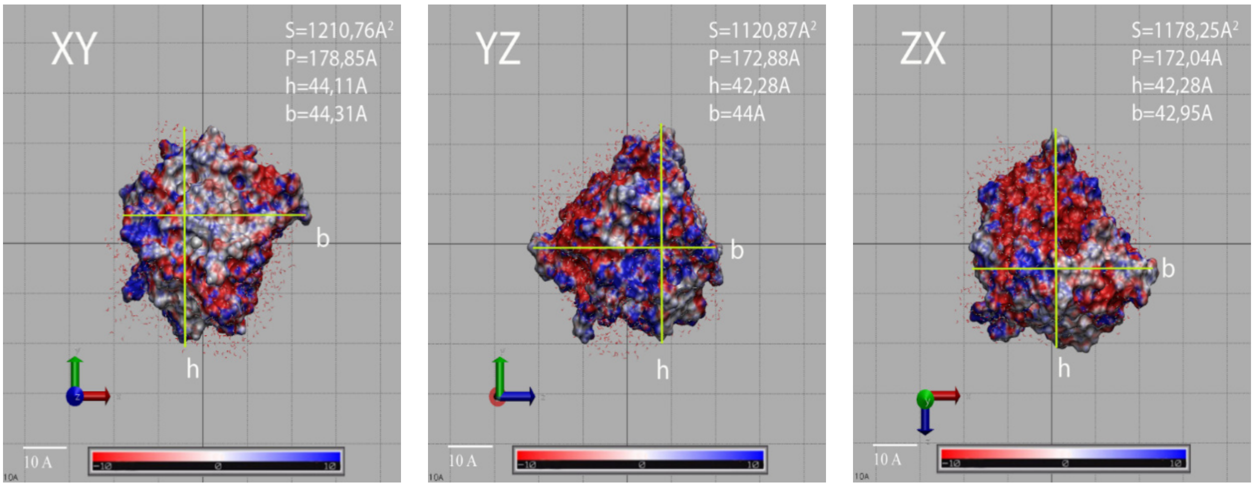
Program complex VMD is oriented for visualization of the modeling results made in NAMD, and could represent and calculate the electrostatic fields for studied objects that are formed at different pH-levels of the modeled system [17, 18].

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 next pH-level: pH = 2.0 (anolyte), pH = 7.0 (drinking water), pH = 11.0 (catholyte). Calculations of the protein conformation condition were made by using the molecular dynamic methods [19, 20].

3 Results and Discussion

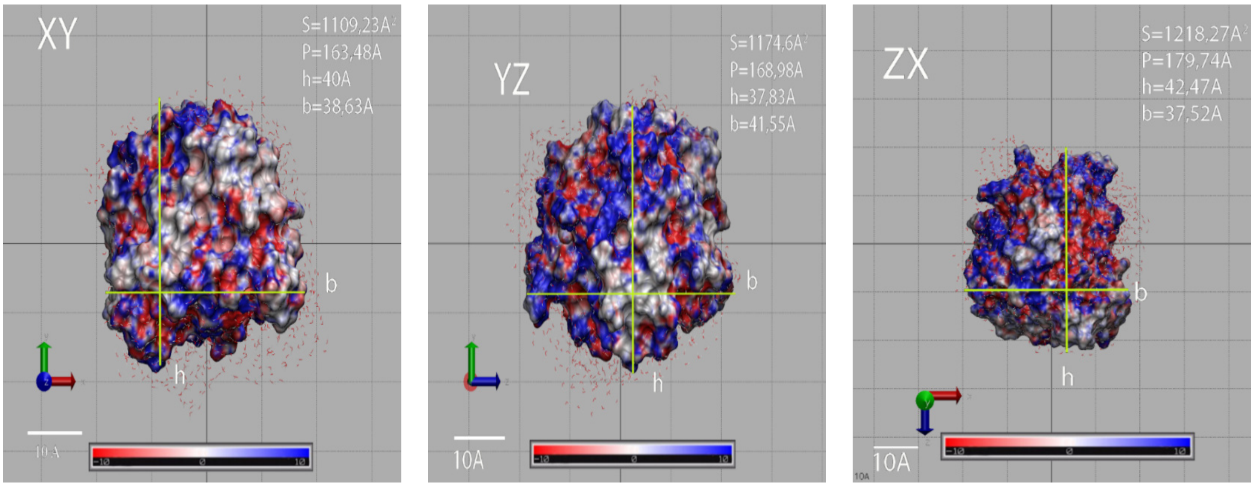
There are three projection views of β-lactoglobulin molecule placed into the systems that had pH = 2.0, which are represented on Figure 2.

Molecule placed into the systems of pH = 2.0 had changed its shape and dimensions in comparison with its condition in vacuum as well as the charges of protein had been also redistributed. Acidic medium led to the electropositive and electronegative centers creation. Active centers were formed around some kinds of amino-acid residues like: lysine, aspartic and glutamic acids. It was established that active centers created around lysine would have the positive charges, and such kind of centers around aspartic and glutamic acids would be charged negatively.



a) b) c)

Figure 2. Projections of the β-lactoglobulin molecule placed into the systems that had pH = 2.0 after conformation condition calculation: a) XY projection; b) YZ projection; c) ZX projection



a) b) c)

Figure 3. Projections of the β-lactoglobulin molecule placed into the water that had pH = 7.0 after conformation condition calculation: a) XY projection; b) YZ projection; c) ZX projection

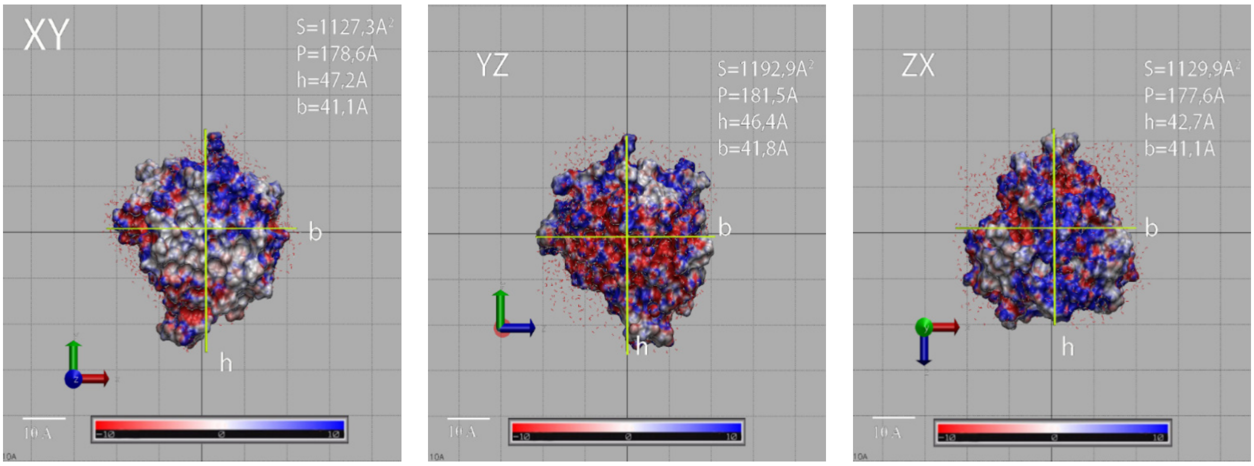
Studying of protein electrostatic fields on all of the given projections could resume that total charge characteristic of the field would have the negative value. The most negatively charged area of the protein could be viewed on ZX-plane. That area would be the major center of protein activity at the modeled condition.

There are tree projection views of the protein placed into the neutral solvent (pH = 7.0) shown on Figure 3.

Molecule dimensions had approximately the same values as in anolyte, but the molecule had not visible amino-acids fragments branching and its shape looked like a regular globule. Redistribution in the charges was also noted, but there were no visible charged areas with the same charge. Molecule of protein was more balanced in charges and had the areas of positive and negative charges that distributed in approximately equal ratio in the molecule volume. It means that molecule in the neutral medium would have low reactivity.

Shape and dimensions of studied protein in catholyte at pH = 11.0 are represented on the Figure 4 and associated with conformation condition of β-lactoglobulin in anolyte at pH = 2.0. The derived fragments on the molecule poles were also noted. These fragments are molecule active centers that could be involved into the new intermolecular bonds creation.

Redistribution of the charges was forced by catholyte pH-level and led to some enormous active centers creation with positive and negative charges, which could be the new reactive zones of molecule for its intensive hydration.



a) b) c)

Figure 4. Projections of the β-lactoglobulin molecule placed into catholyte that had pH = 11.0 after conformation condition calculation: a) XY projection; b) YZ projection; c) ZX projection

4 Conclusions

- Results of β-lactoglobulin molecular modeling shown that anolyte and catholyte using could improve reactivity of whey protein that could lead to intensification of reconstitution process of dry whey as well as its stability development for using of reconstituted whey in foodstuffs technology as an ingredient instead of the natural whey.

- It was established that anolyte and catholyte using could lead to significant molecule polarization and creation of many new same-charged zones in molecule.

- It was noted that catholyte using would be more preferable than other medium as could improve the quality of the reconstituted whey that can be predicted by noted effect of more large and valuable charged areas creation under pH = 11.0 as well as sensory properties of the obtained systems would effective associated to the desirable taste of final foodstuffs.

5 References

[1] National Union of Milk Producers - Soyuzmoloko. *Dairy industry in Russia: current status and development prospects* (in Rusian). <URL: http://www.souzmoloko.ru/analitika-rinka-moloka. Accessed 29 April 2017.

[2] Marketing researches of the market. *According to UNIMILK estimates, the review of the Russian dairy marketi* (in Russian). <URL: http://expert-rating.ru/marketing_research/index.php?productID=992. Accessed 15 April 2017.

[3] [3] Zueva E. V. (2003). *Development of the technology for the production of boiled condensed milk with sugar* (in Russian). PhD thesis, St. Petersburg State University of Refrigeration and Food Engineering, St. Petersburg, Russia. pp. 176.

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

[5] Popova N. V. (2014). *Provision of quality of reconstituted milk processing products and intensification of their production on the basis of ultrasound exposure* (in Rusian). PhD thesis, South Ural State University, Chelyabinsk, Russia. pp. 168.

[6] Trubetskov D. M. (2012). *Method of recovery of dried milk* (in Russian). Patent of the Russian Federation No. 2452186.

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

[8] Grimley H. J., Grandison A. S., and Lewis M. J. (2010). *The effect of calcium removal from milk on casein micelle stability and structure.* Milchwissenschaft, 65, (2), pp.151-154.

[9] Golubeva, L. V. (2005). *Influence of the degree of purification of drinking water on the parameters of reconstituted skim milk* (in Russian). <URL: http: //www.ecodoma.ru/info/inf6. Accessed 15 April 2017.

[10] Artemova Ya. 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.

[11] Bakhir V. M. (1998). *Medico-technical systems and technologies for the synthesis of electrochemically activated solutions* (in Russian). VNIIIMT, Moscow, Russia. pp. 66.

[12] Bakhir, V. M. (1988). *Electrochemical activation of aqueous solutions and its technological application in the food industry: overview information* (in Russian). Gruz-NIINTI. Tbilisi, Georgia. pp.80.

[13] Borisenko A. A. (2002). *Theoretical and practical aspects of multifunctional use of electroactivated liquids in technological processes for the production of meat products* (in Rusian). Doctoral thesis, North Caucasus Federal University, Stavropol, Russia, pp. 505.

[14] Borisenko A. A., Chichko A. A., Borisenko L. A., Zubkov N. V. (2001). *Regulation of the pH of protein-containing systems by activated solutions* (in Russian). NCSTU, Stavropol, Russia, pp. 147-148.

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

[16] Shamanaeva E. A. (2005). *Research of the process of obtaining activated liquid systems and their use for the production of poultry meat products* (in Russian). PhD thesis. North Caucasus Federal University, Stavropol, Russia. pp. 200.

[17] APBS electrostatics in VMD. <URL: https://dasher.wustl.edu/chem478/labs/lab-08/tutorial-vmd.pdf. Accessed 15 April 2017.

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

[19] Kimberlee K. J. (2012). *Whey protein heat stability*. U.S. Dairy Export Council, pp. 1-8.

[20] NAMD. *Virtual Laboratory*. <URL: http://ru.vlab.wikia.com/wiki/NAMD. Accessed 15 April 2017.