

USAGE OF CHITOSAN IN DAIRY PRODUCTS PRODUCTION

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Abstract: The use of chitosan can significantly reduce energy costs in the processing of milk protein and carbohydrate raw materials, and is very promising for use in the dairy industry. In solution of serum proteins, the chitosan binds β -lactoglobulin and other proteins, thus forming an insoluble complex. The formation of complexes of proteins with the chitosan can be accompanied by both a change in the balance of forces that determine the nature of intra- and intermolecular interactions of protein globules, and formation of coacervates differing in size, shape, charge, degree of hydration. Electrostatic forces make the main contribution to the formation of the insoluble chitosan protein complex. In the study of the chitosan complexes formation conditions with the milk serum protein, the pH interaction was studied, as well as concentration of chitosan, the molecular mass of the polysaccharide, ionic strength, and other factors. These patterns of interaction of the milk serum proteins with the chitosan have formed the basis for the development of sorbents based on this polysaccharide. We studied the sorption properties of various forms of chitosan: granulated, as cryogels, as well as part of calcium tartrate gel. Using the chitosan containing sorbents allows us to select proteins from the milk serum and obtain purified preparations of β -lactoglobulin and lactoferrin. Inclusion of the chitosan in the milk beverages and dairy desserts allows us to create a functional food, where the polysaccharide acts as a technological, bactericidal and fungistatic agent.

Keywords: Chitosan, milk serum (whey), β -lactoglobulin, α -lactalbumin, bovine serum albumin, complexation of proteins

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INTRODUCTION

In recent years, intensive studies are being performed to assess the possibility of the chitosan usage in the manufacture of functional foods. Chitosan is a linear polysaccharide consisting of N acetyl 2 amino-2-deoxy-D-glucopyranose and preferably 2-amino-2-deoxy-D-glucose, which are in pyranose form and connected by 1–4 glycosidic linkages. This polysaccharide is second most abundant natural polymer after cellulose. It is biocompatible and biodegradable to conventional materials of the body, such as N-acetylglucosamine or glucosamine, has immunomodulatory, antimicrobial, fungistatic, antitumor, radioprotective, anti-inflammatory, anti-cholesteric action, and thus it has low toxicity [1, 2]. The chitosan can be attributed to

the group of parapharmaceuticals, which are natural substances with specific pharmacological activity. Many states approved its use as a dietary supplement to food [3]. The combination of safety and biological activity of the chitosan creates prerequisites of its wide use as a food supplement. Lipotropic effect of chitosan is attached particular importance as an important factor contributing the confrontation to cardiovascular disease. In studying the effect of chitosan on lipid metabolism, we observe a significant reduction of total lipids, triglycerides, serum cholesterol, and reduced serum aminotransferase activity, indicating the positive effect of chitosan on liver function [4]. The chitosan useful qualities when used for food purposes are sorption properties and the ability to restore the intestinal

microflora. Primary amino groups of chitosan mediate binding of heavy metals and radionuclides. The ability of chitosan to form polyelectrolyte complexes with anionic biopolymers can be used for binding and excretion of the different toxins [5, 6]. The chitosan action mechanism on pathogenic microbial flora is associated with the integrity violation of their outer membrane composed of lipopolysaccharides, glycoproteins, phospholipids. It is shown that the chitosan enhances the nonspecific resistance to adverse environmental factors. In addition, it has the ability to stimulate growth of bifidobacteria and beneficial intestinal flora. Long-term studies on the the chitosan usage as a food additive found no contraindications of its use, it can be limited only when acidity, acute gastritis and peptic ulcer disease. The chitosan is one of the few enterosorbents, for which extensive biomedical research of security and preventive effects has been conducted [7–9].

The chitosan is becoming more widely used in the food industry, particularly in the production of dairy products. Milk processing is developing towards increasing the share of the production of cheese and cottage cheese. In connection with this, a volume of the resulting whey is increased. According to the International Dairy Association, the whey volume, which is obtainable in the world as a byproduct of processing, has reached 140 million tons per year. The Republic of Belarus is ranked 5th in the world in the export of dairy products, where the major share is made up of cheeses. The increase of production leads to an increase in the amount of serum, which remains within the Republic, which amounts to more than 4 million tons per year [10]. The whey contains 0.55% protein, 4.8% lactose, 0.05% fat, 0.5% minerals, and is a valuable product [11]. Its further use is not significant. The whey losses and waste discharge undermine the effectiveness of milk processing, and cause economic and environmental damage [12]. An urgent task is to complete the development of technologies and rational use of whey, which has a high nutritional and biological value. Proteins are an important component of whey, and are optimally balanced by amino acid composition. For years, the whey proteins have been the subject of intensive research, which found their physicochemical properties, structure, and some biological activities [11–15].

Among all the whey proteins, the largest number accounts for β -lactoglobulin (β -Lg) with content of 60%. By its structure, β -Lg is a globular protein consisting of 162 amino acid residues. In cow milk, the protein is present in two isoforms, which differ in their physicochemical properties. The biological function of β -Lg is still not clearly established. It is believed that this protein is involved in the regulation of phosphorus metabolism in the breast. Since β -Lg is resistant to proteolytic attack, it can cause allergic reactions in infants consuming cow milk in one form or another [13, 14].

The second quantitatively whey protein is α -lactalbumin (α -La) of 20%. It also has a globular structure stabilized by four disulfide bonds and consists of 123 amino acid residues [13, 14].

Among minor whey proteins, a content of bovine serum albumin (BSA) is 7%. It consists of 582 amino acids and has 17 intramolecular disulfide bridges. Due to its size and structural features, the BSA binds with the free fatty acids, lipids, and many other hydrophobic compounds [13].

It is known that the whey proteins, such as β -Lg, α -La, immunoglobulins, lactoferrin, exert their biological activity, either directly or after enzymatic hydrolysis. Antimicrobial peptides have been found in the partial hydrolyzate of the whey proteins together with antioxidant, antifungal, immunomodulatory, and other antihypertensive properties. In its natural form, they are present in fermented milk products, such as yogurt, kefir, feta cheese, and other cheeses [13, 15, 16]. Since the whey proteins have valuable biological properties, their isolation and purification are seemed to be an urgent task. To achieve it, ultrafiltration or various chromatographic techniques are used in order to provide protein preparations of high purity. However, the process of chromatography is time consuming, and needs special equipment. Simpler methods are selective precipitation of individual proteins using specific reagents. Such reagents include the chitosan and its various derivatives [17].

In view of the fact that the whey proteins are different in their structure and properties, they may have different affinities for the chitosan. In this regard, there is a great theoretical and practical interest of physical and chemical phenomena and laws of interaction of proteins with anionic and cationic polysaccharides. Such processes shall be useful for processing of various proteins, i.e. shall be "universal" and at the same time shall be simple and have manufacturability. The common proteins physicochemical nonequilibrium phenomena include complexation of proteins with anionic and cationic polysaccharides, anisotropic gels formation of two-phase systems, protein solutions concentration when establishing phase equilibrium between solutions of polysaccharides, proteins, and others [5].

It should be noted that in modern technologies of milk processing, a method for enrichment is widely used for traditional foods with dietary fiber. This approach is within the concept of functional food that provides for the development and production of products, which have not only a high nutritional value, but also are useful for human health. In this connection, in the dairy industry, the polysaccharide food additives are widely used as thickening and gelling agents. They are classified depending on the source of origin, structure of the polymer chain, the nature of the monomer residues, the charge. Depending on the charge, the polysaccharides are divided into the following types: Neutral, which are cellulose derivatives, amylopectin, galactomannans; anionic (acidic), which are alginates, carrageenans, pectins, xanthan, gum karaya, ghatti, and tragacanth, acacia, furcelleran, gellan gum; cationic, (basic), which are chitosan [18]. Among these polysaccharides, the chitosan is rarely used as a food additive. This is due to the fact that the mechanism of its interaction with the components of milk is not investigated yet.

The present author's review is devoted to the study of the chitosan interaction mechanism with the whey proteins, and its possible usage in the manufacture of dairy products.

CHITOSAN INTERACTION MECHANISM WITH MILK PROTEINS

The ability to interact with proteins is an important property of this polysaccharide, and it can form emulsions, gels, act as a stabilizer and an antioxidant [5]. In the dairy industry in the production of the series of products (lactose, syrups, beverage), removal of the proteins from the whey is necessary. The chitosan can be successfully used for the coagulation of the whey proteins. The mechanism of the whey proteins completing with chitosan is determined by the physicochemical properties of these biopolymers.

The chitosan is soluble in mineral and organic acids. At pH above 7, the amino group is deprotonated and shows nucleophilic properties. A preparation of water soluble chitosan derivatives is based on this characteristic of the chitosan, including chitosan succinate. At low pH ($pK_a < 6.5$), the amino group is protonated, chitosan is a water soluble cationic polyelectrolyte. Due to its chemical nature, it is able to various kinds of interaction forming 4 basic chelation types of connections, such as ionic, hydrogen, hydrophobic, wherein the chitosan acts as the complex nucleus. The ionic interactions occur due to the amino groups. The weak linkages (hydrogen, hydrophobic) are formed by interaction of the chitosan with a number of organic substances. This same type of interaction is observed with the introduction of excess chitosan to collagen solution [5].

The chitosan ability to form complexes is due to the presence of a lone electron pair on the nitrogen atom, and in some cases, the linkages are formed by a lone electron pair of the oxygen atom. This principle forms the formation of numerous types of chelate linkages

with metals. The chitosan can form such links under certain conditions with all metals except the alkali and alkaline earth ones [9].

In these types of interactions is a separation of raw milk for protein and protein-free fraction by introducing a colloidal solution of the chitosan as an active whey protein cationic completing agent. The polycationic nature of the polysaccharide opens up broad prospects for its use for the separation of milk proteins that have low values of isoelectric points [14].

Based on the properties of the whey protein and chitosan, we have put forward a hypothesis, which is based on the assumption that the division of the milk raw materials on the protein and protein fractions with using the chitosan is based on the formation of the chitosan and proteins complexes. Such interaction of negatively charged ions of milk serum proteins with the protonated chitosan can occur in a narrow range of pH 5.0–6.5 (Fig. 1). Among the whey proteins, the largest number amounts for β -Lg with an isoelectric point, this is 4.9–5.4. The second quantitatively whey protein is α -La with an isoelectric point of 4.6. The bovine serum albumin has an isoelectric point of 4.8 [13]. At a pH above 5.0, these proteins will have a negative charge, and up to pH 6.5, the chitosan has a positive charge. This creates the possibility of the ionic interactions.

In this regard, we have previously studied the interaction process of the chitosan with whey proteins [14, 16, 19, 20]. We added a solution of chitosan to a 1% solution of demineralized whey at pH 6.2. Binding to the proteins, the chitosan formed insoluble protein-polysaccharide complex, and for 30 min there was an increase in absorbance at $\lambda = 580$ nm. For the separation of insoluble particles, the whey protein solution, mixed with chitosan, was centrifuged for 10 min at 9 000 g. We conducted an electrophoretic analysis of the whey proteins residue, and calculated their relative amount in the insoluble complex with the polysaccharide [14, 20].

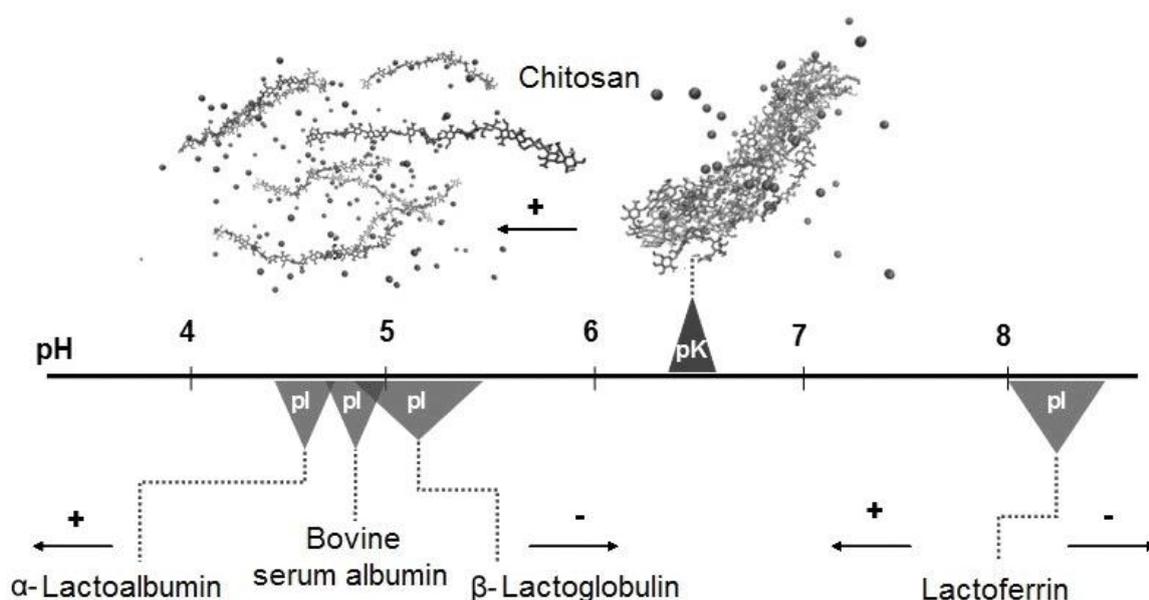


Fig. 1. pH Effect on chitosan and whey proteins charge.

The results of the authors' study showed that the major protein associated with the chitosan are α -Lg, α -La, BSA (Fig. 2). The lactoferrin and several minor proteins do not participate in the complex formation. These data suggest that maximal binding β -Lg and other serum proteins with the chitosan are observed in the content of polysaccharide in the reaction environment of 0.5 mg/ml. In this case, more than 90% of the whey protein is transferred in the precipitate. 100% of β -Lg, 20% of α -La and 10% of BSA are removed therefrom. With further increase of the chitosan content, an amount of generated sludge is considerably reduced in the solution (Fig. 2).

Thus, the chitosan is reacted with the whey at a pH above their isoelectric points, forms an insoluble complex. The Milk Protein – Chitosan Interaction comes under the influence of opposite charges in the pH range and a certain ratio of the reagents. A phenomenon of coacervation is fully observed in the system, i.e. decay into two phases, in one of which the chitosan

coacervates and whey proteins are concentrated representing protein + chitosan complex. The system second phase is an equilibrium fluid comprising lactose, whey protein residues, and chitosan. In case where the amount of chitosan in a 1% solution of the whey protein is more than 3 mg/ml, the insoluble chitosan-protein complex is formed.

In the study of the chitosan-protein complexes formation, the pH interaction was studied, as well as the influence of ionic strength, concentration of chitosan with different molecular mass, temperature, and other factors.

The pH factor is very important for the implementation of many of intermolecular interactions, as it affects the ionization of certain functional groups of polymer compounds. Our study of the interaction process of the chitosan with a molecular mass of 200 kDa, 86% DM and β -Lg was carried out in the pH range from 5.2 to 6.2. The results of the experiment are shown in Fig. 3.

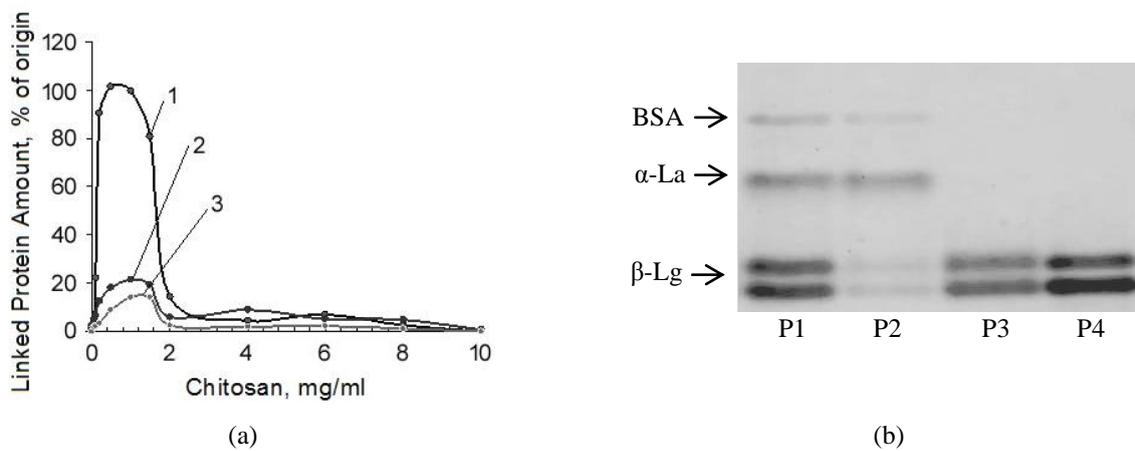


Fig. 2. Dependence of the relative content of serum proteins in the precipitate on the chitosan content in the milk serum (a) and electrophoretogram (b) of WPC solution to (P1), after (P2) treating with the chitosan, complex proteins with the chitosan (P3), the standard β -Lg (P4).

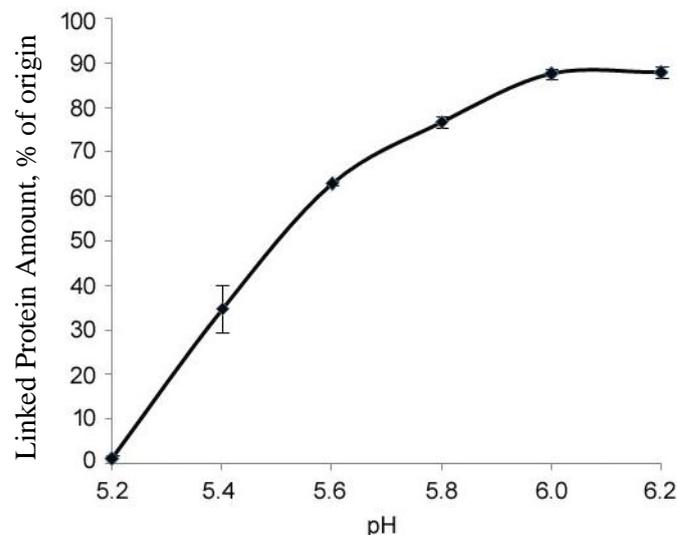


Fig. 3. pH Effect on the interaction effectiveness of β -Lg with chitosan.

At pH 5.2, there is no formation of a precipitate, indicating the formation of a complex between the protein and chitosan. The most effective complex formation occurs at pH 6.2. The latter value is close to the pH of the cheese whey, obtained by enzymatic treatment of cow milk. It should be noted that within the studied pH range, the chitosan is in the protonated form. At pH 5.2, β -Lg does not react with the chitosan as the pH value is close to the isoelectric point of 4.9–5.4, and its net charge is zero. With the increase of the pH value above the isoelectric point, the protein acquires a negative charge, which facilitates efficient ion interaction with the chitosan. Our findings suggest an important role of ionic groups in the interaction of the β -Lg with chitosan. Furthermore, these results indicate the impossibility of isolating the protein from "acid whey" obtained by isoelectric casein coagulation. Despite the important role of electrostatic interactions, one can not exclude the existence of other types of links arising

from the formation of the protein- polysaccharide complex.

Our hypothesis of the ionic groups participation in the insoluble β -Lg and chitosan complex formation was confirmed by the change of the solution ionic strength. For this, the reaction is carried out in MES NaOH buffer at pH 6.2 containing sodium chloride at a concentration of from 0.01 M to 0.2 M. It was found that best the chitosan binds to the protein when the solution ionic strength is minimal, whereas at the NaCl concentrations of 0.2 M, the interaction does not occur (Fig. 4). These data support the role of electrostatic interactions in the formation of an insoluble complex between the chitosan and β -Lg.

The whey contains ions of various metals in its structure, including calcium, which is one of the major mineral components of the whey. We studied the effect of calcium ion concentration in the demineralized whey for formation of the insoluble chitosan-protein complex (Fig. 5).

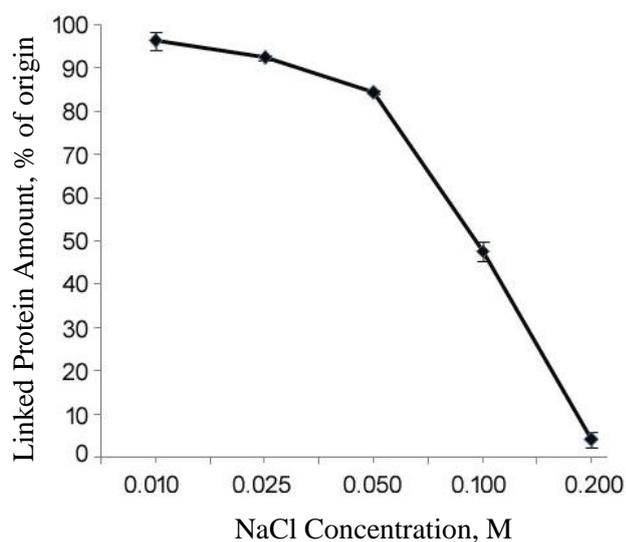


Fig. 4. Ionic strength effect on insoluble chitosan-protein complex formation.

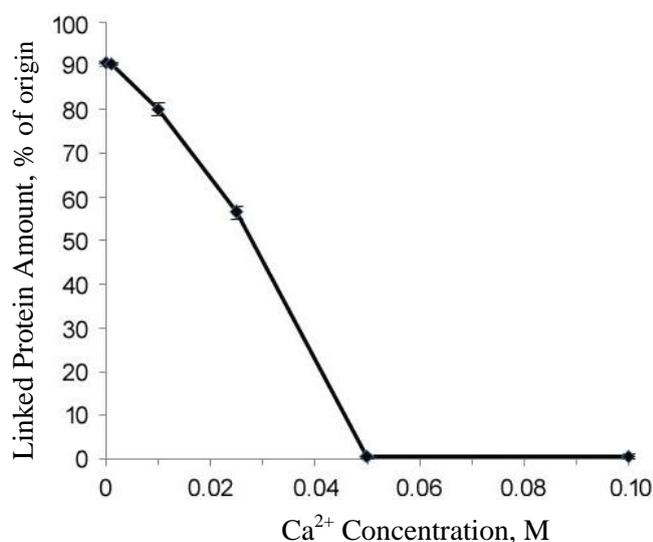


Fig. 5. Calcium ion concentration effect on insoluble chitosan-protein complex formation.

Upon reaching the calcium ion concentration of 0.05 M, the insoluble complex formation practically ceases.

An important factor in the formation of the chitosan-protein complex is the chitosan molecular mass, depending on which the effectiveness of its formation may change. Our study found that, regardless of the used chitosan molecular mass, the most complete extraction of the β -Lg occurs when the contents of polysaccharide in the reaction mixture was 0.5 mg/ml. The efficiency of the chitosan and β -Lg insoluble complex increases with increasing the chitosan molecular mass from 15 to 200 kDa (Fig. 6) [14]. Probably, this effect is due to the fact that in the use of chitosan with a higher molecular mass, a sedimentation capability of the complex formed becomes higher than when using a low molecular mass of the polysaccharide.

The size and morphology of the particles formed from the chitosan and β -Lg complex were determined by atomic force microscopy. It was shown that at the reaction with β -Lg of the chitosan with a molecular mass of 200 kDa, insoluble particles of (170 ± 50) nm are formed, followed by formation of larger aggregates (Fig. 7b), while the size of particles produced by using the chitosan with a molecular mass of 21 kDa does not exceed 100 nm (Fig. 7a) [14, 20].

This effect is due to the fact that in the use of chitosan with a higher molecular mass, a

sedimentation capability of the complex formed becomes higher due to the formation of the larger aggregates than when using a low molecular mass polysaccharide. Thus, the high molecular mass chitosan binds most efficiently β -Lg and other milk serum proteins.

As a result of the complex formation between the chitosan and β -Lg, the solution became opalescent that is connected with the complex formation. It was shown that the formation of insoluble chitosan-protein complex occurred only on addition of 0.5 mg/ml of polysaccharide (Fig. 8).

It was found that the maximum protein fluorescence was observed in Zona II at 336 nm at 24°C, pH 6.2 in 0.05 M MES-NaOH buffer. By increasing the chitosan content in the protein solution (Zona III, Fig. 8), the protein fluorescence was reducing (Fig. 9). This phenomenon can be explained by intermolecular interaction of the chitosan and β -Lg, which leads to a change in the protein spatial conformation [14, 20, 21].

Using the method of isothermal calorimetric titration, we observed that in Zona I (Fig. 8), there is a change in enthalpy of $\Delta H = -2.1$ kcal/mol per the chitosan unit link, whereas the subsequent addition of the chitosan in the protein solution (Zona II, Fig. 8) did not lead to a change in the energy component of the process. This may indicate that the complex dissociation and formation of new interaction types do not occur [22, 23].

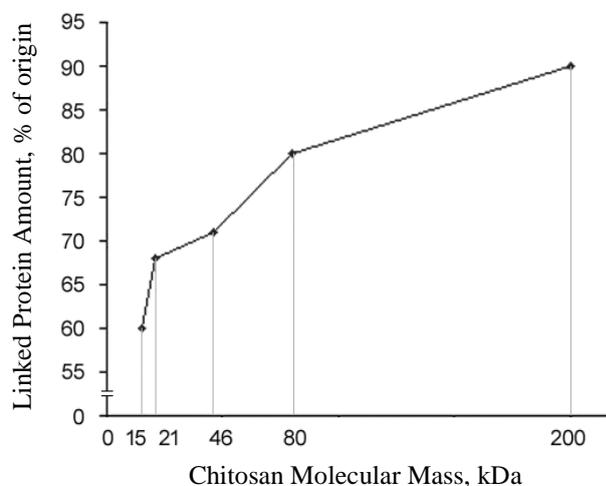


Fig. 6. Efficiency of binding β -Lg depending on the chitosan molecular mass.

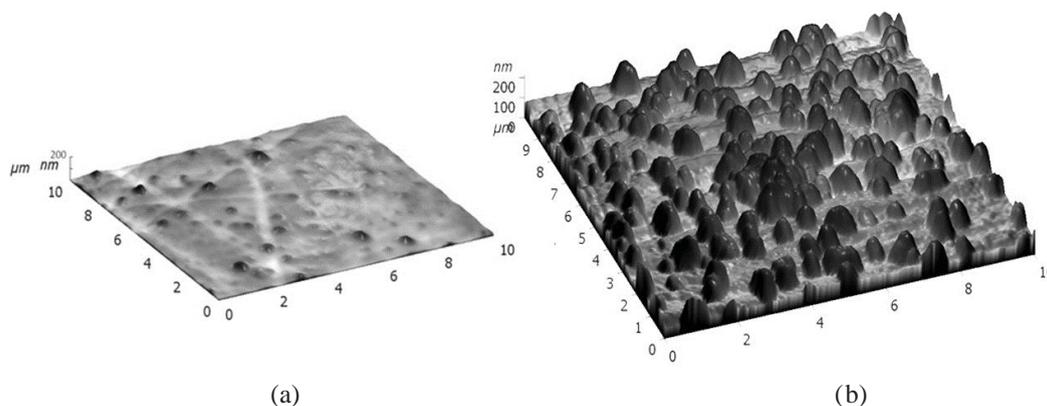


Fig. 7. Image of insoluble complex of β -Lg with 21 kDa chitosan (a), and 200 kDa chitosan (b) obtained by atomic force microscopy.

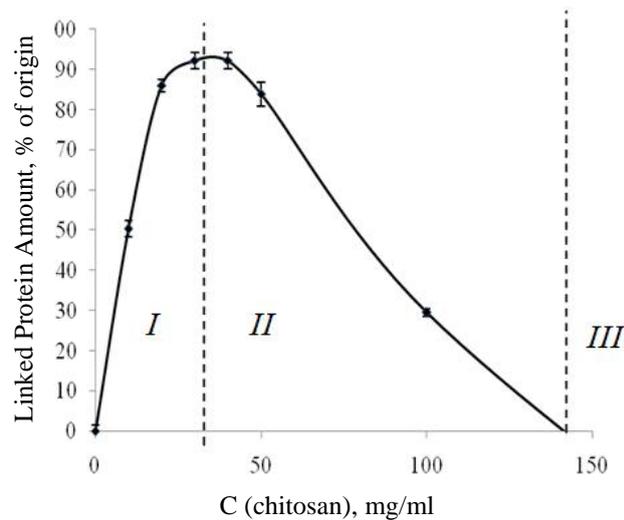


Fig. 8. Dependence of bound β -Lg amount on 200 kDa chitosan content.

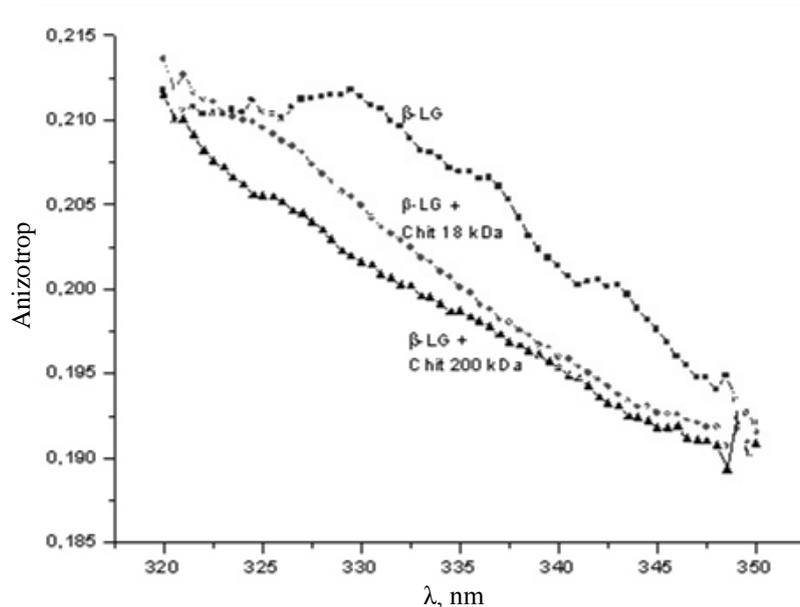


Fig. 9. β -Lg и β -Lg with chitosan fluorescence anisotropy.

Based on these data, we suggest that in the chitosan excess addition to the β -Lg solution, not all the polysaccharide functional groups are involved in the protein complex formation, which is why the ability to the complex sedimentation decreases, and the chitosan-protein complex remains in solution. The water-soluble complexes of globular proteins with chitosan are far from protein saturation and presented by an equilibrium colloid. The whey protein complexes formation with the chitosan can be accompanied by a change of their tertiary structure.

According to the hypothesis proposed, at the molecular level, the electrostatic complexes formation can be considered as the sequential addition of ligands, i.e. whey proteins macroions to the complex nucleus, i.e. the chitosan macroion. The protein can be considered as a ligand on the ground that a large number of smaller negatively charged protein macroion can bind with one

chitosan macroion. The polyion complex charge decreases with each subsequent joining of the ligand. The result is the formation of electrically neutral chitosan whey protein complex. The electrically neutral complexes aggregation leads to their isolation in a complex coacervate. The complex coacervate phase composition is defined by stoichiometry of the electroneutral insoluble complex, and depends on the charge ratio of whey proteins. The aggregation of the complex particles is due to their hydrophobic interactions and hydrogen bonding. The insoluble complexes of globular whey proteins with the chitosan contain a relative surplus of proteins.

The findings suggest to consider the chitosan usage be promising for the milk processing enterprises in the treatment of liquid industrial waste containing serum proteins, and the use of these systems for the isolation of proteins [20].

CHITOSAN USAGE FOR MILK PROTEINS EXTRACTION

These patterns of interaction of the milk serum proteins with the chitosan solution have formed the basis for the development of a sorbent based on this polysaccharide. This sorbent is to have a high whey proteins sorption capacity, and differs for use in a column chromatography. To do this, we developed a technology for producing a chitosan hydrogel, modified with cross-linking agent, which is glutardialdehyde [17].

A reactive amino group in the chitosan anhydroxyronific monomer unit allows to apply this biopolymer in order to obtain a covalently crosslinked hydrogels [2, 17]. The process of the system gelation, "solvent (water) – polymer (chitosan) – crosslinker (glutardialdehyde)", is possible not only for positive temperature values, but also in the frozen conditions, up to several tens of degrees below the crystallization point of the pure solvent. In such conditions, a frozen preparation, although it looks macroscopically solid, is microscopically heterogeneous, because it consists of polycrystalline of a chilled solvent, i.e. water, and so-called unfrozen liquid microphase, i.e. chitosan and glutardialdehyde. In this microphase, components of these substances are concentrated, and the reaction products, i.e. chemical processes occurring in it, are inherently liquid phase. Due to the effects of such cryotic concentration, we observe an apparent reduction of the critical gelling concentration, when the polymer gel, so-called cryogel may be formed at substantially lower concentrations of the starting precursors, at a temperature higher than the freezing point of the system [2].

Cryogel based on the chitosan was obtained by the ratio, which is 5/1 for the ammin groups of chitosan/ aldehyde groups of glutardialdehyde. The aqueous solution of the polymer and the crosslinking agent was placed in a chromatography column and frozen at 18°C. The gelation reaction was carried out by treating the frozen solution in a microwave oven for 1 min. The water crystals did not pass into the liquid phase state, whereby the polymer chitosan gel was synthesized around them. We obtained the chitosan macroporous form using cryotechnology [16, 17, 19]; this form had a degree of the gel swelling of 250.5% and held up water of up to 3000 %. Its use as a sorbent for

separation of proteins by column chromatography, allows us to perform the process at a high speed of up to 200 ml/ hour. To analyze the efficiency of the purified proteins adsorption, such as the BSA, β -Lg, α -La, α -casein (1S), β -casein, κ -casein, we passed individual proteins through a column of cryogel, after which the sorbent was washed with 0.01 M acetate buffer with pH 6.18, and eluted the protein bound to the chitosan using the linear gradient of 0–0.5 M NaCl in 0.01 M acetate buffer, pH 6.18. The protein content in the eluate was analyzed using denaturing electrophoresis and reverse phase HPLC. The results are given in Table 1.

The participation of the milk proteins ionic groups by reacting with the chitosan macroporous cryogel is confirmed by chromatographic separation of the whey proteins when changing the ionic strength of the eluting solution. Eluting the proteins using the NaCl linear gradient at a salt concentration of 0.01 M to 0.5 M, there are significant differences in their binding with the chitosan cryogel. The analysis of the results presented in Table 1 indicates that the β -Lg and α -La most effectively bind with the sorbent. They are eluted out of the chitosan cryogel with a lower ionic strength.

An important result, confirming the ionic character of the interaction of the whey proteins with the chitosan cryogels is represented by the results of chromatography using the pH linear gradient of proteins elution (Fig. 10).

The resulting patterns of the whey proteins interaction with the chitosan macroporous cryogel depending on the ionic strength of the solution, the calcium ions and pH gradient allowed to use the crosslinked chitosan hydrogel based on this polysaccharide as a sorbent suitable for the α -La, β -Lg and BSA isolation and prepare up to 90% of protein from milk serum. The production technology development for the sorbent granular form from the chitosan became the consequence of the research, which showed a high separation efficiency of proteins from the whey. This sorbent is easy to use and possible to regenerate of the chitosan granular form without the use of expensive equipment [19].

Thus, using the chitosan sorbents makes it possible to remove the proteins from the whey. The resulting protein can be used in individual form, as well as an additive that improves the bioavailability of foodstuffs.

Table 1. Efficiency of binding of purified milk proteins with chitosan cryogels

Feature \ Protein	BSA	β -Lg	α -La	α -casein (1S)	β -casein	κ -casein
Molecular Mass, kDa	66.0	18.3	14.2	23.0	24.0	19.0
Isoelectrical Point, pI	4.8	5.3	5.1 (4.8)	5.1 (4.7)	5.3 (4.9)	4.1
Protein Sorption mg/mg of Cryogel	0.6	20.0	11.5	2.0	1.5	5.5
NaCl Elution Concentration, M	0.1	0.16	0.2	> 0.5	0.26	0.36

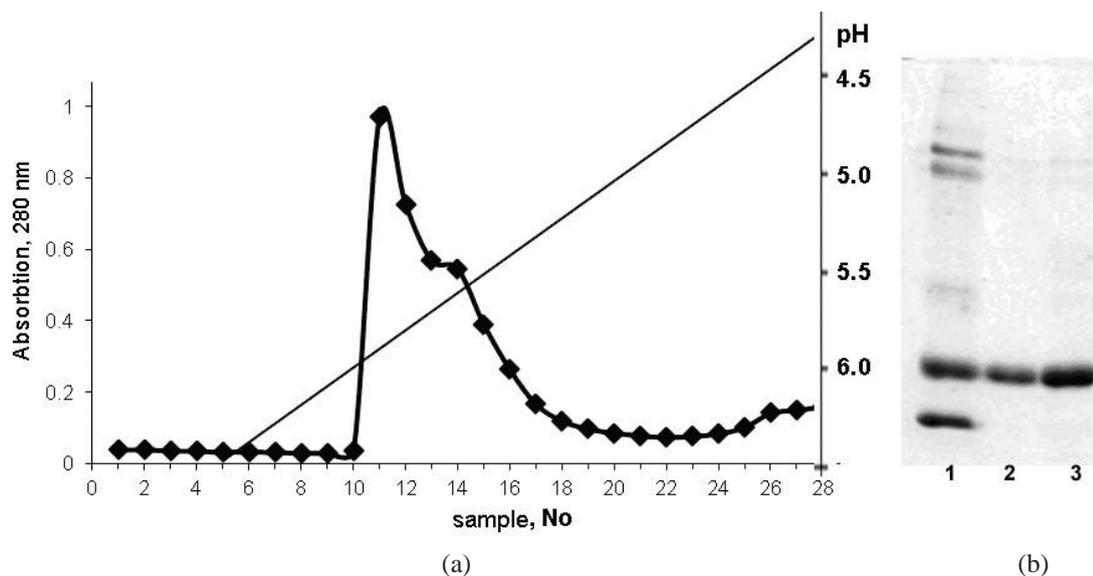


Fig. 10. β -lactoglobulin elution chromatographic profile with chitosan cryogel of pH gradient (a) and isolated protein electrophoretogram (b). B: 1 – initial whey; 2 – sample 11; 3 – sample 14.

USAGE OF CHITOSAN IN DAIRY PRODUCTS PRODUCTION

The functional products become especially popular in the area of supply that have high organoleptic properties, and also have a preventive effect. A promising direction in this area is the creation of functional foods based on milk and whey, which are sources of complete protein, vitamins, and minerals.

Since the chitosan is a structurant, emulsifier, thickener and clarifier, it has antibacterial, antifungal and antiviral properties, and is able to heal gastric mucosa; it can be assumed that its use in acidified milk beverages and dairy desserts will create a functional product, in which the polysaccharide will function as a technological, functional, bactericidal, or bacteriostatic agent.

Due to the chitosan high activity in the process of complexation with milk proteins, its using in the technology of soft drinks allows to obtain the clarified whey with low proteins and organoleptic neutral features (no serum undesirable sharp taste and smell). The technology is simple in hardware design and does not require significant capital expenditures [24].

A carbonated beverage production process using the chitosan is exercised as follows. Remnants of fat and casein are removed from fresh whey. The prepared serum is added a colloidal chitosan solution. After thorough mixing, the mixture is incubated, centrifuged. The clarified serum is combined with prepared blend that is then fed by the glass in the carbonated form. The best organoleptic characteristics have such drinks as "Apple", "Peach", "Orange" [25, 26].

The use of the colloidal chitosan solution as a composite structurant provides a protein complex to produce next-generation products that have curative properties. It can be used to produce enriched curd as the source of the native protein having high biological value in the production of cream cheese as the raw material, as well as the formulation of sour and milk beverages. In addition, the protein complex obtained is advisable

to apply as a protein fortifier in the production of milky beverage from serum [25, 26].

To confirm the possible role of the chitosan as the structurant, the lactic system (with 0.5% fat) properties were studied after introduction of the polysaccharide in concentration of 1–4%.

The analysis of the results shows that increasing the amount of contributed chitosan solution in the lactic system results in a slight increase of the effective viscosity of all samples. When the content of the chitosan is higher than 3%, there is a significant deterioration of the organoleptic characteristics of the product, expressed in acute unpleasant astringent aftertaste, bitter aftertaste. After 30 minutes, there is a precipitation. Thus, in future studies, we used the chitosan solution with a concentration of 3%, which provides relatively acceptable organoleptic characteristics, and does not lead to a process of complexation of the chitosan with milk proteins [26].

Given that the chitosan has sufficiently strong antibacterial and bacteriostatic properties, we studied the relationship between a chemical structure and its biological effects on microbial cells in the lactic model system. Table 2 presents the results of high molecular chitosan effect experiments on the growth of test cultures *E.coli* DSM 396 in the lactic model system.

The results showed that for three days the number of *E.coli* cells in the samples with the addition of the chitosan was reduced from 10^6 to 10^4 CFU/g, while in the control sample prepared with addition of *E.coli*, but without using the chitosan, the cell number in contrast, increased to 10^7 CFU/g. Thus, the chitosan concentration of 3% added to the lactic model system exhibits bactericidal activity against Gram-negative *E.Coli* DSM 396 microorganisms.

The research also was investigated for fungi; these data demonstrate a pronounced fungistatic action of the chitosan and allow its use as a natural preservative in production technology of unfermented dairy desserts.

Table 2. *E.coli* DSM 396 dynamics in lactic model systems

Sample	Sowing time from the samples preparation, days			
	After preparation	1st day	3rd day	8th day
	The number of detected cells (CFU/g)			
Sterilized Milk (0.5% fat) + 1% Broth with <i>E.coli</i>	$(3.8 \pm 0.2) \times 10^6$	$(6 \pm 0.2) \times 10^6$	$(1.2 \pm 0.2) \times 10^7$	$(1 \pm 0.2) \times 10^7$
Sterilized Milk (0.5% fat) + 3%, 3% chitosan solution + 1% Broth with <i>E.coli</i>	$(6.5 \pm 0.2) \times 10^6$	$(9 \pm 0.2) \times 10^5$	$(9 \pm 0.2) \times 10^4$	1×10^4
Sterilized Milk (0.5% fat)	Not detected			$(4 \pm 0.2) \times 10^1$
Sterilized Milk (0.5% fat) + 3%, 3% chitosan solution	Not detected			1×10^1

The dairy desserts were being prepared with the use of the chitosan, sodium alginate and a sweetener (fructose). It was found that the developed dairy desserts had a protective effect against erosive and ulcerative lesions caused by using aspirin gastric damage model that allows us to assign a product to a group of functional foods [25, 26].

Thus, the use of the chitosan in the manufacture of dairy products at this stage is very promising because it allows the development of new functional products possessing curative properties.

RESULTS

The modern milk processing technology is directed towards the development of functional foods that have not only a high nutritional value, but also are useful for human health. For their creation, a method of enrichment is widely used regarding the traditional foods using dietary polysaccharide fiber. Neutral, which are cellulose derivatives, amylopectin, galactomannans; anionic (acidic), which are alginates, carrageenans, pectins, xanthan, gum karaya, ghatti, and tragacanth, acacia, furcelleran, gellan gum; cationic, (basic), which are chitosan, have received widespread usage. The chitosan is the only natural cationic polysaccharide, which is used primarily because of its hypocholesteric action. It can be used in recycling of dairy industry wastes for the removal of proteins from serum. Such use of the chitosan is based on the fact that the whey proteins are characterized by low values of the isoelectric points, and at pH 4.8–6.2 have a negative charge, thereby are capable for ionic interaction with the positively charged

chitosan molecules. The proteins complexation efficacy with the chitosan depends on the pH, ionic strength, chitosan concentration, and its molecular mass. It should be noted that using one chitosan macroion you can bind a large number of smaller negatively charged protein macroions, whereby a the polyion complex charge decreases at each subsequent attachment of the ligand. This leads to the formation of electrically neutral chitosan complexes with serum proteins and their aggregation. The phenomenon of the insoluble complex formation of the globular whey proteins with the chitosan can be used in the removal of cheese production waste and recovering the valuable protein products.

The chitosan-based adsorbents are developed that permit their use for the chromatographic separation of proteins from the whey, which can be used in individual form, as well as an additive that improves the bioavailability of foodstuffs.

As dietary fiber, the chitosan is included to the biscuits, crisps, pasta, and other foods. With the use of the chitosan, dairy desserts, a variety of beverages are prepared, as well as milk-based functional products.

CONCLUSION

Nowadays, the chitosan use in the dairy industry is very promising because it allows to profitably process milk protein and carbohydrate raw materials, excluding significant energy costs. The processed products have curative properties, which make them attractive for a consumer and, as a consequence, competitive at food market.

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