10.63550/ICEIP.2026.22.17.068

Protein Products Isolated From Whey

Ustinova Yulia Vladislavovna[1][a], Donya Denis Viktorovich[2][b] and Prosin Maksim Valerievich [3][c]
[1,2,3] K.A. Timiryazev Moscow State Agricultural Academy, Moscow, Russia
yul48888048@yandex.ru; doniadv@rambler.ru; prosinmv@yandex.ru

Keywords: protein products, whey, chitosan, modification, glycine, ultrasound, protein, lactose.

Abstract: The results of studies on the isolation of proteins from whey are presented. The initial and modified natural polyelectrolyte chitosan was used to isolate whey proteins. For different ratios of components in the milk base, the zones of the most effective complexation have been identified and the main factors influencing the intensity of the separation process and the properties of the protein-chitosan complex have been established. It was found that pH = 4.5-4.6 is the optimal value for the isolation of whey components using chitosan. The largest mass of protein precipitate is observed when using chitosan modified with glycine, prepared in water pretreated with ultrasound for 5 seconds.

1 INTRODUCTION

Complex processing of dairy raw materials is currently the main and unresolved problem. First of all, it is the irrational use of secondary dairy raw materials, in particular, whey, which leads to the loss of valuable components, the most important of which are whey proteins (Lebedeva, Shchekotova, Atlasova, Ayusheeva, Khamagaeva, Kotova, 2025).

Whey is a by-product of cheese production, containing protein, lactose, vitamins, minerals and accounts for 80-90% of the total volume of milk supplied to the technological process. In the production of hard, semi–hard or soft cheese, the whey has a pH of 5.9 - 6.6 (Khamagaeva, Shchekotova, Khamaganova, 2017).

Membrane filtration is the process of separating serum at low temperature, which uses porous membranes. Due to the different pore sizes, the membranes are able to destroy bacteria, degrease whey, pass carbohydrates and minerals, and retain whey protein (Shishackij, Belozercev, Barbashin, Nikel, 2019).

Whey proteins are well known for their universal functional properties and high nutritional value in food products. About 700,000 whey proteins are available as valuable food ingredients, according to estimates of global whey production. Nutritional and functional characteristics of whey proteins are related to their structure and biological functions.

The serum contains a heterogeneous mixture of proteins with a wide range of functional properties and is used not only in the food industry, but also in the chemical, pharmaceutical and cosmetic industries. The main fraction of whey protein consists of a mixture of β-LG, α-LA, immunoglobulins, serum albumin and lactoferrin. The development of new processes for isolating certain types of whey protein with constant and well-defined properties allows using whey in a new way (Ustinova, 2010; Serkova, Glazova, Zainkova, Bunyatyan, 2017).

Chitosan, a polysaccharide consisting of copolymers of glucosamine and N-acetylglucosamine, has great potential for the food industry and biotechnology. As a non-toxic, biocompatible and biodegradable polymer, chitosan can be used in the dairy industry. Chitosan has been studied to be a coagulating agent in wastewater treatment and the recovery of lipids and proteins from food waste, including wastewater from the dairy industry (Zilbergleit, Markhel, Loban, Maevskaya, Maslennikov, Zhdanovich, 2019).

In the dairy industry, chitosan is used to remove milk fat, proteins, and peptides from whey.

Non-covalent and covalent interactions occur to form protein-chitosan systems. Non-covalent interactions include electrostatic, hydrophobic

[a] https://orcid.org/0000-0002-1649-889X
[b] https://orcid.org/0000-0002-5818-0804
[c] https://orcid.org/0000-0002-4615-5628

interactions, hydrogen bonds, and Van der Waals forces, which are formed by mixing substances and can contribute to the formation of stabilizers, emulsions, and gels. Most non-covalent interactions are weak, reversible, and can be changed by adjusting factors such as pH, ionic strength, and temperature (Zilbergleit, Markhel, Loban, Maevskaya, Maslennikov, Zhdanovich, 2019). Covalent interactions are processes that occur through Maillard reactions, enzyme-catalyzed reactions, and chemical crosslinking reactions that make protein-chitosan complexes more stable. However, conditions such as pH, temperature, ionic strength, and time must be adjusted to achieve the desired reaction. The stages of protein and chitosan binding are physical copolymerization, chemical crosslinking, enzymatic glycosylation, and Maillard reaction.

The authors Kablov V.F., Ishchenko Yu.P. developed a technology for isolating protein from whey using a colloidal chitosan solution and obtaining functionally active protein-polysaccharide polymer complexes based on it.

Sprinchan E.G. optimized the process of protein isolation by combining electrophysical treatment of whey using a chemical reagent in a two-chamber electrolyzer (Sprinchan, 2010).

In the work of A. Hernandez and F. M. Harte, the effect of various salts and salt concentrations on the release of casein micelles from cow's raw skimmed milk was studied (Kurbanova, Avetisyan, 2010).

The traditional methods of concentration of whey proteins, which affect the productivity of the process, the nutritional properties of products and their biological activity, are considered. The highest content of true protein, 40-53% (w/w), is found in products obtained by ultrafiltration and precipitation by adding salts. In terms of electrophoresis results and protein solubility, these methods have the fundamental advantage of maintaining proteins in their natural state. The products showed significant antioxidant activity, but the antimicrobial activity was influenced by separation methods (Varivoda, 2020; Donskaya, Drozhzhin, Bryzgalina, 2018).

The use of modified nanomaterials to isolate whey proteins, with the possibility of their further use in the production of a variety of products with preventive and curative properties in animal and poultry feed (Vikulin, Vikulina, 2019).

When ultrasound is used with a frequency above 20 kHz for water treatment, a sonochemical effect occurs based on the propagation of deformations in the water due to periodic pulses. Cavitation occurs, thereby disinfection of water occurs, which is based on the effect of ultrasound on cellular metabolism and enzyme systems of cells (Igdirova, Dzheykhunova, Kulayaeva, Pirmedova, 2024).

Free electrons are formed in ultrasound-treated water and the following reactions occur:

$$H_2O \xrightarrow{ó \quad ç} H_2O^+ \mp e$$
$$H_2O \xrightarrow{ó \quad ç} H^\bullet + OH^- + \bar{e}$$
$$H_2O \xrightarrow{ó \quad ç} OH^\bullet + H^- + \bar{e}$$
$$OH^\bullet + OH^\bullet \rightarrow H_2O_2;\ H^\bullet + H^\bullet \rightarrow H_2$$

2 MATERIALS AND METHODS

The objects of research in the work were whey, with the characteristics shown in Table 1.

Table 1: General characteristics of whey.

Naming of the indicator	Units of measurement	Result
1. Protein content	%	0,6-0,8
2. Fat content	%	0,11
3. Carbohydrate content	%	4,3
4. Ash	%	0,5
5. pH	-	4,5
6. Density at 15^0 C	г/см^3	1,021-1,024

The initial and modified natural polyelectrolyte chitosan was used to isolate whey proteins. The physico-chemical properties of chitosan are shown in Table 2.

Table 2: Physico-chemical properties of natural polyelectrolyte – chitosan (Suleymanova, 2023).

Name	Chitosan
Molecular weight	270
Mass fraction, %	12
Moisture content	0,4
Relative viscosity	22,0
Degree of deacetylation, %	80,4

To change the properties and dissolve natural polyelectrolytes, a modifier, glycine (α-aminoacetic acid), is introduced into its macromolecules to form a complex compound due to ionic bonds between positively charged chitosan groups and glycine carboxyl groups. The concentration of the modifier was 0.05%.

The main characteristics of the modifier are shown in Table 3.

Water was subjected to ultrasonic treatment to prepare chitosan modified with amino acetic acid, to change the molecular weight and structure.

Parameters of ultrasonic water treatment: frequency 30 kHz, ultrasound intensity 460 W/cm^2.

The initial or modified chitosan, in an amount of 0.02%, is added to the whey (pH = 4.2-4.6) at a temperature of 20-25^{0}C.

Table 3: Characteristics of the modifier [16].

The modifier	The structural formula	Functional group	Macromolecule sizes		Organoleptic properties
			length, oA	width, oA	
Glycine (amino acetic acid)	NH_2-CH_2 -COOH	Amino group and carboxyl group	5,07	2,20	A colorless liquid with a peculiar odor

3 DISCUSSION OF THE RESULTS

The effect of ultrasonic radiation time on the pH of water has been established (Figure 1).

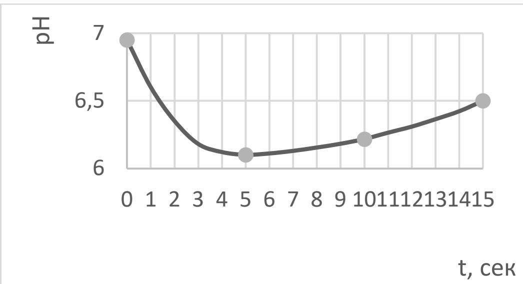

Figure 1: The effect of ultrasonic radiation time on water pH changes.

It can be seen from the graph that the value of the hydrogen index increases with an increase in the time of ultrasound exposure to water. A water molecule breaks down into H^+ and OH^- ions under the influence of ultrasound.

H_3O compounds are formed due to an increase in the number of OH^- ions, which provide an alkaline reaction of the medium. At the same time, hydrogen cations interact with water molecules.

When the covalent bonds between the chain links are broken, the hydrogen index changes in water.

Compared to traditional approaches, ultrasonic treatment makes it possible to intensify chemical transformations.

To precipitate protein from whey, the dependence of the hydrogen index (pH) on the mass of the protein precipitate was studied (Figure 2).

It was found that pH = 4.5-4.6 is the optimal value for the isolation of whey components using chitosan.

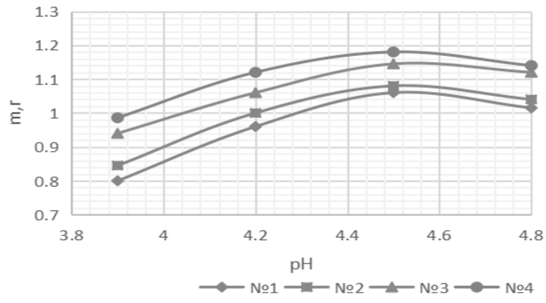

Figure 2: The effect of pH on the release of protein products from whey № 1 -sample 1; № 2-sample 3; № 3-sample 4; № 4 - sample 2

Chitosan is soluble in an acidic medium, it is a cationic polysaccharide with positively charged amino groups ($-NH3^+$), where its amino groups are protonated and positively charged, which makes it effective for interacting with proteins with an isoelectric pH point <7.

The isoelectric point is one of the key parameters that help profile biologics in addition to stability, aggregation, and particle size.

At pH<7 of the serum, the protein surface is predominantly negatively charged, and therefore similarly charged molecules will exhibit repulsive forces. The biggest disadvantage of isoelectric deposition is irreversible denaturation.

The results of protein isolation from whey are presented in Table 4.

Table 4: Results of protein extraction from whey

Sample number	Chitosan concentration, %	The modifier	Water treatment time UZ, sec	Consumption per 50 ml of serum, %	The mass of the released protein, g
№ 1	0,02	-	-	0,1	1,090
№ 2	0,02	Glycine	5	0,03	0,190
№ 3	0,02	Glycine	10	0,07	1,095
№ 4	0,02	Glycine	15	0,05	0,175

As can be seen from the table, the largest mass of protein precipitate is observed when using chitosan modified with glycine, prepared in water pretreated with ultrasound for 5 seconds. The effect of cavitation occurs when ultrasound is applied to water, due to cavitation bubbles formed as a result of irradiation of the liquid (Figure 3).

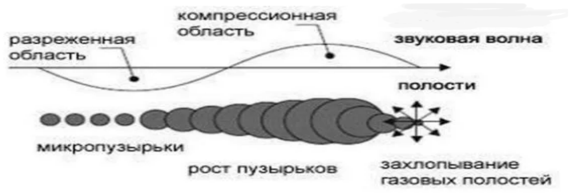

Figure 3: The effect of ultrasound on water properties.

Hydrogen bonds are formed between the hydroxyl groups of chitosan and the amino/carboxyl groups of whey proteins. Hydrophobic interactions occur when hydrophobic regions of whey proteins bind to each other and to chitosan. Van der Waals interactions are weaker, which also contribute to the overall attraction between the two molecules.

Figure 4 shows the process of protein extraction from whey. The flake-like precipitate is filtered and dried to a constant mass at a temperature of 50-60 ° C, then weighed.

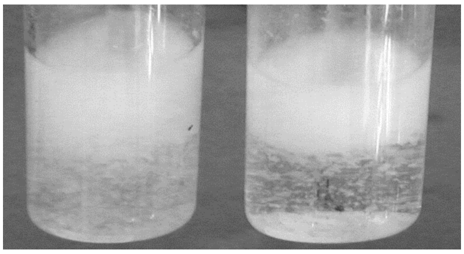

Figure 4: The process of protein extraction from whey.

Chitosan with a positive charge, when mixed, attracts oppositely charged protein particles, while forming a protein polysaccharide complex according to the following scheme:

$$Protein^{-}+Chitosan^{+}\rightarrow \text{Insoluble complex}$$

Chitosan acts as a flocculant, binding whey proteins due to electrostatic forces and converting them into an insoluble form, forming an insoluble complex between polysaccharide and proteins. Protein macroions are sequentially attached to the chitosan macroion at the molecular level and may be accompanied by a change in the balance of forces that determine the nature of intra- and intermolecular interaction of protein globules.

This makes it possible to effectively isolate the valuable protein component from the whey at minimal cost.

4 CONCLUSION

As a result of the research, conditions were found for the precipitation of protein from whey using modified and unmodified chitosan. It was found that pH = 4.5-4.6 is the optimal value for the isolation of whey components using chitosan. The largest mass of protein precipitate is observed when using chitosan modified with glycine, prepared in water pretreated with ultrasound for 5 seconds. The effect of cavitation occurs when ultrasound is applied to water, due to cavitation bubbles formed as a result of irradiation of the liquid.

REFERENCES

Lebedeva, S.N., Shchekotova, A.V., Atlasova, D.V., Ayusheeva, R.B., Khamagaeva, I.S., Kotova, T.I., 2025. Production of enriched protein concentrate based on whey. *Machinery and technology of food production.* №2. pp. 257-271.

Khamagaeva, I.S., Shchekotova, A.V., Khamaganova, I.V., 2017. Whey protein concentrate enriched with iron. *Bulletin of VSGUT.* No. 2 (65). pp. 64-69.

Shishackij, Y.I., Belozercev, A.S., Barbashin, A.M., Nikel, S.A., 2019. Thermodynamics of non-isothermal diffusion at the extraction from cheese whey by Lupin. Proceedings of the Voronezh State University of Engineering Technologies.;81(3):39-42.

Syed Mansha Rafiq and Syed Insha Rafiq (May 30th 2019). Milk By-Products Utilization [Online First], IntechOpen, DOI: 10.5772/intechopen.85533. Available from: https://www.intechopen.com/online-first/milk-by-products-utilization

Ustinova, Yu.V., 2010. Isolation of food components from milk flushing waters: dissertation ... Candidate of Technical Sciences : 05.18.04. Ustinova Yulia Vladislavovna; [Place of defense: Kemer. technol. Institute of Food Industry]. Kemerovo, 132 p.

Serkova, A.N., Glazova, N.V., Zainkova, N.V., Bunyatyan, N.D., 2017. Sorption-chromatographic isolation and purification of biologically active whey proteins. *International Journal of Applied and Fundamental Research.* No. 2-1. pp. 46-53.

Zilbergleit, M., Markhel, V., Loban, T., Maevskaya, O., Maslennikov, V., Zhdanovich, O., 2019. Chitosan - successes and challenges of commercialization. Polymer materials and technologies. 5. 6-15.

Pat. RU 2295251 Method of isolation of protein substances from dairy raw materials. Publ. 03/20/2007, Bul. No. 8.

Sprinchan, E. G., 2010. Optimization of protein release during electrophysical treatment of whey. *EOM.* No. 6 (266). pp. 81-87.

Kurbanova, M.G., Avetisyan, G.A., 2010. Investigation of the effect of the degree of hydrolysis of k-casein on the characteristics of gels. Machinery and technology of food production. No. 1 (16). pp. 58-60.

Varivoda, A.A., 2020. The use of whey protein concentrate as a structural stabilizer in food production. *Polzunovskiy vestnik.* No. 2. pp. 58-62.

Donskaya, G.A., Drozhzhin, V.M., Bryzgalina, V.V., 2018. Fermented milk drinks with a high content of whey proteins and water-soluble antioxidants. *Bulletin of the Moscow State Technical University.* No. 3. pp.471-480.

Vikulin, P.D., Vikulina, V.B., 2019. The effect of ultrasound on pH changes in water. *Water and ecology: problems and solutions.* No. 4 (80). pp. 3-8.

Igdirova, A., Dzheykhunova, S., Kulayaeva, O., Pirmedova, T., 2024. The structure and physico-chemical properties of chitin and chitosan. *Symbol of Science.* No. 10-1-2. pp. 18-21.

Suleymanova, E. I. G., 2023. Physico-chemical methods for the determination of glycine and its derivatives. *Bulletin of the Bashkir State Pedagogical University named after M. Akmulla.* No. 3 (71). pp. 142-148.