ELECTROPHYSICAL WHEY PROCESSING

***Bologa Mircea, Vrabie Elvira**

Institute of Applied Physics, Academy of Sciences of Moldova – Chişinău, Moldova

***Bologa Mircea, mbologa@asm.md**

Abstract: Electrophysical technology is the most effective method for processing secondary milk products with a subsequent recovery of a protein-mineral concentrate and simultaneous isomerisation of lactose into lactulose. The need for obtaining them is highlighted; possible mechanisms of complexation and coagulation during the electrophysical processing of secondary unboiled-milk products are explained. The possible methods for optimizing the conversion of protein fractions into a protein-mineral concentrate, as well as the fractional analysis of the minerals, are described, and their role in the complexation of the concentrate is estimated. The electrophysical parameters at different compositions of the anode liquid, and the types of separating of elements are analyzed.
Keywords: secondary milk products, protein-mineral concentrate, electro-physico-chemical activation, a-lactalbumin ß-lactoglobulin

1. INTRODUCTION

The complete and wasteless milk processing may be considered as one of the key problems. It envisages an integral utilization of raw materials, including secondary resources and processing wastes, and solution of environmental problems if to take into consideration high values of biological and chemical oxygen demands in whey [1]. Consumption of milk and dairy products is rather substantial and differs in various countries dependent on their traditions, geographical position, the degree of the development of agriculture and industry [2]. Milk processing results in huge amounts of by-products that should be used with maximal efficiency. The principles of wasteless technology were formulated by the UN Commission, which stressed its expediency and the necessity of the obligatory environment protection [3, 4]. Utilization of whey proteins (lactalbumins, lactoglobulins, immunoglobulins) that have the highest decomposition rate among whole proteins is promising. A wasteless processing cycle of whey and degreased milk, recovery of immune proteins and other valuable whey components in foodstuffs as well as creation of new products and beverages may solve the existing problems [5]. Recycling of secondary milk products remains to be an indispensable part of research, because various formulations of foodstuffs for children and biologically active additives on the basis of whey protein concentrate become more and more important. It is difficult to choose a rational direction for whey utilization because it is defined by many factors. Therefore, analysis of the role that each factor plays will allow one to make a right choice, especially taking into account that whey contains all valuable milk components, such as vitamins, proteins, mineral substances at virtually complete lack of fats [6]. Dry whey contains 71.1% of lactose, 14% of protein fraction, 7.7% of mineral substances and 0.9% of other components. Lactose is a unique carbohydrate that contains only in milk products and plays an important physiological role in human organisms. By their composition protein fractions may be referred to as the most valuable proteins of animal origin (Table 1); in addition, they are a rich source of essential amino acids [7].

Table 1. Content of main protein fractions in whey

Proteins	Content in whey, g/l	Molecular weight, kDa	Isoelectric point
a-lactalbumin	0.7	14.1	4.8
ß-lactoglobulin	3.0	18.2	4.9-5.4
Bovine serum albumin	0.3	66	4.8
Lactoferrin	0.1	78-80	8.0-8.8
Lactoperoxidase	0.04	78-80	8.6-9.6
Immonoglobulins	0.5	150-900	5.8-7.3

Therefore, electrophysical methods are of great interest for processing protein-carbohydrate milk raw materials, being based on the stimulation and new approaches to the usage of internal resources of each product. Combination of two or several methods can ensure the most effective whey processing [8].

2. RESULTS AND DISCUSSION

Taking the above into consideration, the authors used electroactivation of initial whey in a flow-type membrane electrolyser with a subsequent recovery of a protein-mineral concentrate (PMC) in the field of mass forces, as well as of deprotenized whey (DW), for further lactose processing(Fig. 1, 2). The main parameters controlling the process are: the electric current density, the composition of anodic solution, the rate of flow of a liquid into the cell, and the membrane type.

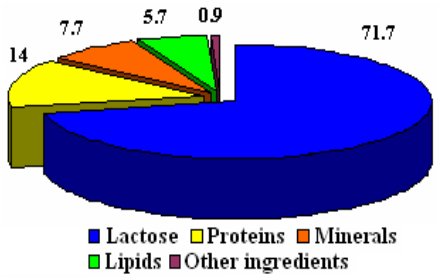

Fig. 1. Dry initial whey (IW) composition

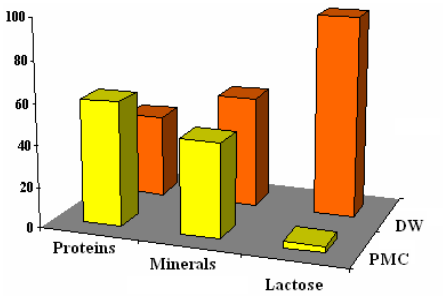

Fig. 2. Quantity of main substances transferred into PMC (percentage of IW) and remained in DW (percentage of IW)

Combination of those factors that defines the degree of temperature increase and the active acidity in the cathode cell, as well as the membrane state and electric voltage, influence the quantity and composition of the produced concentrate. The found regimes allowed the transfer of 60-65% of proteins and of 94-96% of calcium- and phosphorus-containing ions into the PMC. At least 90% of carbohydrates and almost all potassium and sodium ions remain in the DW.

Investigation of the quantity and quality of protein fractions in PMC has shown that the optimum parameters are: pH in the range of =8.0–10.0 and amount of proteins at about 60% at the current density of 0.019–0.021 A/cm^2 (Fig. 3*)*.

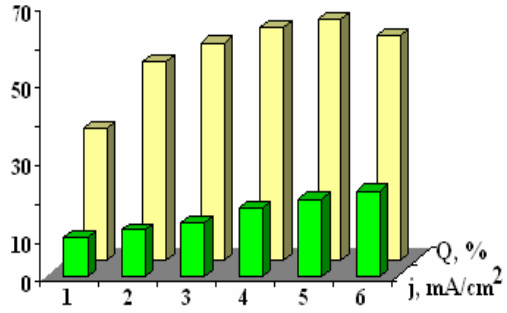

Fig. 3. Protein recovery in PMC at different values of current density: 1 – 8; 2 – 10; 3 – 14; 4 – 18; 5 – 20; 6 – 22 mA/cm^2.

The proteins in whey mainly have a globular structure. A protein globular is formed so that most of the polar hydrophilic amino-acid residues turn out to be outside and contact with a solvent, and most of the non-polar (hydrophobic) residues are inside and isolated from the interaction with water. The ionogenic R-groups (radicals) of the amino-acid residues that are on the surface manifest acid-base properties determining the amphotericity and the charge of the protein molecules. The proteins in the solution have a negative or positive charge depending on the medium reaction and the ratio between the acid and base amino acids. A protein molecule in the solution is surrounded by a hydrate (solvate) shell, i.e., water dipoles oriented around the polar groups. The proteins in the solution are kept in the native state owing to the factors of the stability including the charge of a molecule and the hydrate shell around it, which prevents the protein molecules from adhesion. The removal of these factors results in the coagulation of the proteins and their precipitation.The destruction of the hydrate shell of the MW proteins resulting from the electrochemical activation (ECA) of the water molecule decomposition in the near-cathode area leads to their coagulation and ensures the protein extraction into the concentrate from the first minutes of processing. In addition, the rupture of non-covalent bonds supporting the globular protein structure under the action of an electric current and the activation of the ionogenic R-groups of the amino-acid residues can result in the formation of new bonds and, consequently, the aggregation of the protein molecules. A significant role in extracting whey proteins is likely to be played by the R-groups of cysteine residues representing the reaction-capable sulfhydryl (thiol) groups. The oxidation of the sulfhydryl groups of two cysteine residues and the formation of a (covalent) disulfide bond (-S—S-) in proteins yields cystine, which is a dimer of cysteine, thus supporting the spatial structure of the protein molecules along with the hydrogen, ion, and hydrophilic bonds. Intermolecular bridges between cysteine radicals dissociate either as a consequence of the ECA or during the increase in pH up to 8.3, respectively. The participation of cysteine residues in the complexation of whey proteins in such treatment is confirmed by the results of blocking of sulfhydryl groups by sodium iodine acetate. The introduction of the latter in the initial whey decreases the protein yield because the aggregation of proteins is excluded according to this mechanism [9]. The degree to which the R-groups of the aminoacid residues are ionized

depends on the pH of the medium. In an acid medium, the increase in the proton concentration results in suppressing the dissociation of carboxyl radicals and decreases the negative charge of the proteins. In an alkaline medium, it leads to binding of the hydroxyl excess with the proteins formed during the dissociation of NH_3^+ -groups with the formation of water, thus resulting in a decrease of the positive charge of proteins. The pH value, at which the numbers of positively and negatively charged groups are equal, that is, the protein acquires a summary zero charge, is called the "isoelectric point" (pI). If there is a zero charge of the protein, the hydrate shell is destroyed, since there naturally can be no interaction between the water dipoles and an electrically neutral protein molecule. Separate molecules are joined thus forming large aggregates that are not able to stay in the solution and precipitate. The active acidity of the deproteinized and DW increases during the process from pH 4.55 to 11.60. The pI of the MW proteins are in this pH range (Table 2) [10, 11].

***Table 2**. The mineral content of main ions of (DW)*

№	pH	Ca	P	Na	K
ZI	4.65	10.11	6.64	27.60	36.29
1	5.65	9.75	5.58	31.60	43.30
2	6.50	9.71	4.75	33.80	49.70
3	7.05	1.64	1.70	23.50	29.27
4	8.05	2.84	0.32	22.20	28.13
5	10.00	1.99	0.01	35.00	57.02
6	11.00	1.88	0.02	45.30	76.06
7	11.30	2.05	0.07	30.05	47.85
8	11.45	1.37	0.05	46.70	79.86
9	11.50	1.74	0-04	41.80	66.64
10	11.60	1.03	0.09	50.90	83.99
AC	2.90	5.07	2.88	17.90	14.68
CC	10.65	3.50	0.20	28.40	40.05

AC – anode cell, CC – cathode cell

However, it is very difficult to monitor the dependence of proteins precipitation on the pH of the DW (Fig. 4, 5). The DW accumulates for some time interval, which already averages its parameters. The change in the active acidity in the volume of the electrolyzer chamber does not take place in the same way and is determined by its width as well as the income of the initial solutions under the stream processing mode, which prolongs the time of the process of reaching the pI by the proteins of one fraction. In addition, the protein extraction by this method of processing is due to several mechanisms of their complexation and coagulation acting simultaneously. The predominant significance of calcium for the protein extraction by the suggested method is evidenced, on the one hand, by the decrease in its quantity in the DW during the process (table 2) and, on the other hand, by the predominance in the mineral composition of the PMC. Phosphor is the second ash component by the content in the concentrate, since the almost complete phosphate-ion depletion of the DW (table 2) is due to the phosphate ions only partially passing into the concentrate.

A significant part of these ions migrates to the anode chamber. According to the data of our X-ray analysis, the Ca : P ratio in the PMC is 2.23; meanwhile, according to the

literary sources [7], this ratio in the MW is about 1.09. To precipitate proteins, the salting-out reaction is widely used based on the phenomenon of the proteins solubility with an increase in the concentration of neutral salts. The physico-chemical basis of salting out has not been completely revealed; the destruction of a bond between a polymer and the solvent is known to be primary in this mechanism. When a salt is introduced, a part of the solvent molecules that were in the solvate bond with the polymer and solvate the molecules of the introduced salt. When a protein is salted out, the molecules are dehydrated and the charge is removed.

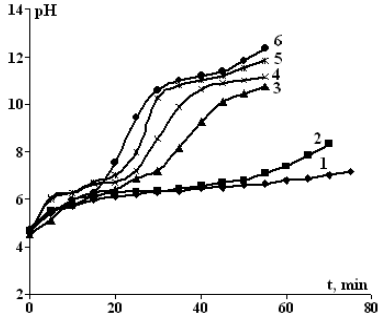

Fig. 4. Change in pH of the DW (the canvas membrane) at different current densities: 1 – 8; 2 – 10; 3 – 12; 4 – 14; 5 – 18; 6 – 20 mA/cm^2

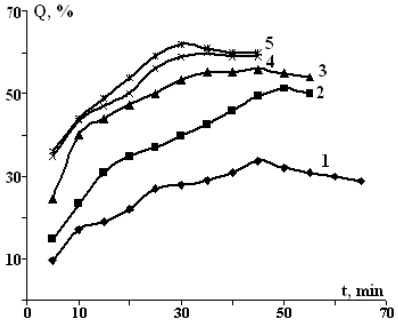

Fig. 5. Degree of the protein extraction into the PMC (the canvas membrane), Q—protein passing into the PMC (in % of its content in the initial MW) at different current densities: 1 – 8; 2 – 10; 3 – 14; 4 – 18; 5 – 20 mA/cm^2

The process is affected by the relative molecular weight, charge, and hydrophilicity of the protein. There is a direct dependence between the size of the water shell of the protein molecules and the salt concentration: the smaller the hydrate shell, the smaller the amount of salts is required. Thus, large and heavy molecules that have a small water shell precipitate if the salt saturation of a solution is incomplete, and smaller molecules surrounded by a large water shell precipitate if the saturation is complete. The neutral salts of alkaline and alkaline-earth metals are used to salt out proteins. When whey is processed, the electrolysis of the salts contained in it and the electrodialysis result in the fact that the ion concentration in the near-electrode zones can multiply (by orders of magnitude) exceed the latter in the initial solution, creating conditions ensuring the effect of the protein salting out.

In milk (pH 6.47-6.67), calcium salts are mainly represented by phosphates that have a small solubility and an insignificant degree of dissociation. Only a small part of them is contained in the form of a true solution, and a greater part is contained in the form of a colloid solution. Colloid calcium phosphate joined with casein is contained in milk in the form micelles or of the so-called "calcium caseinate-phosphate" complex (CCPC). The composition of the colloid calcium phosphate that is present in the CCPC and the character of its bond with casein are still unknown. Calcium caseinate is formed when calcium ions interact with the carboxyl and serine phosphate groups of casein. This being the case, calcium can react with two closely located —COOH and —OH-groups, forming intermolecular calcium bridges: —R—Ca—R. It is believed that hydrophosphate ions —

R—Ca—HPO_4—Ca—R or —R—Ca—HPO4—Ca—HPO4—Ca—R can also take part in forming cross-linking bridges (between two phosphoserine radicals) [11, 12].

Lactic acid formed due to the activity of the lactic-acid microflora transfers the calcium milk salts from the colloid state to the ion-molecule state. Under the action of the acid, the structure of the CCPC is destroyed—inorganic and organic calcium phosphate (of phosphoserine) are both detached from it. Lactic acid inhibits the dissociation of the free carboxyl groups and acidic groups of casein phosphate: the COO- groups transform into COOH, and PO_3^{-2} , into PO_3H_2. In whey (at pH 4.6—4.7), calcium hydrophosphates (mono- and predominately dihydrophosphates) are soluble; that is, they are electrolytically dissociated. They attain an equilibrium whose shift depends on the pH of the medium (the whey's pH): as the pH increases, dihydrophosphates transform into monohydrophosphates, reacting with hydroxylion. It is supposed that, under the conditions of electro-physical whey processing at a pH of more than 6, bonds analogous to the bridges between casein molecules entering into the composition of the CCPC are formed between the ionized acetate groups and the phosphoserine residues of both the phosphopeptides (proteose-peptones) and whey proteins, particularly those that are conformationally unchanged [9]. As the active acidity grows, the calcium orthophosphates weakly associated in the initial whey pass into the molecular-dispersed state completely and can get into the PMC together with protein. To make the protein extraction process more intensive, experiments differing mainly in the heightened concentration of calcium ions in the processed whey were conducted according to these mechanisms. Calcium chloride, which is a well-soluble salt, was used as an additionally introduced electrolyte. To avoid the formation of chlorine-bearing organic compounds in the CC and to increase the conductivity of the system, calcium chloride was introduced into the anode liquid (AL). The salt (an electrolyte) was dissolved either in MW or in (distillated) water. Three types of membranes were used: a canvas membrane, an MK-40 ion-selective membrane, and an ultrafiltration membrane. The current density (20 mA/cm^2) and the rate of the liquids incoming into the electrolyzer chambers (5 ml/min) are analogous in the presented variants of the experiments. Using 5% calcium chloride in the MW as the AL and canvas as a separating element increases the protein extraction into the PMC by almost 10% as compared to using only MW (Fig. 6, variants 1, 2). A lower protein yield in variant 2 compared to that in variant 1 is explained by the lower initial acidity of the processed whey. The usage of various membranes and of the liquid anode solutions depending on the maximum amount of proteins recovered is presented in Fig. 7.

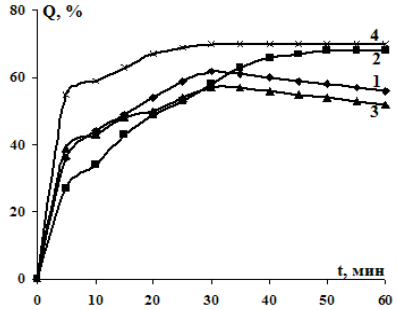

Fig. 6. Dependence of the degree of protein extraction into PMC on the composition of AL and the type of membrane. Variants of the composition of AL: (1) MW (canvas membrane); (2) sol.5% $CaCl_2$ in MW (canvas membrane); (3) MW (Ultrafiltration membrane); (4) sol. 2% $CaCl_2$ in water (MK-40 ion_selective membrane); Q—the protein passage into PMC (in % of its content in the initial MW).

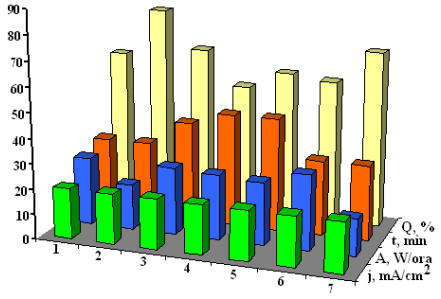

Fig. 7. Usage of various membranes and liquids anode solutions depending on the maximum amount of proteins recovered. Flow rate in both cells – 5 ml/min: variant 1 – MW, (canvas membrane); 2 – sol. 10% $CaCl_2$ in DW, (canvas membrane); 3 – sol. 5% $CaCl_2$ in MW(canvas membrane); 4 – sol. 1% $CaCl_2$ in water, (canvas membrane); 5 – sol. 1% $CaCl_2$ in water (canvas membrane); 6 – ZI, (Ultrafiltration membrane); 7 – sol 2% $CaCl_2$ in water (MK-40 ion_selective membrane).

Meanwhile, the voltage (Fig. 8, variants 1, 2) and power inputs (Fig. 9, curves 1, 2) decrease, and, respectively, the profitability of the process rises. However, after 25 working cycles, the canvas diaphragm is observed to be gradually choked up [13]. The efficiency of the device concerning the protein yield into the PMC falls by a factor of two, which is accompanied by an abrupt increase in the voltage and results in a heightened temperature of the processed whey. It is evident that the area of the separating element involves some of the supposed processes of the precipitation of the proteins migrating through the canvas diaphragm in both directions due to the difference in the charges of the molecules. The concentrate formed in the near-membrane area is held up on its surface on the side of both cathode and anode chambers. A low protein yield is observed when using the ultrafiltration membrane with the properties caused by its destination. The increase in the voltage consumption (Fig. 8, variant 3) and, respectively, the growth in the power inputs (Fig. 9, variant 3) speak for the increase in the resistance owing to the membrane's pores being choked up by the protein substances aspiring to migration. This hampers the displacement of the ions and charged molecules from one chamber into another, including the calcium cations that are important for the aggregation of the whey proteins. The choice of the ion-selective membrane (MK-40) as a separating element meets all the requirements of the method presented.

The membrane is not choked up, which promotes a decrease in the voltage and power inputs (Figs. 8, 9; curve 4). Intensive foaming in almost the entire volume of the working chamber is observed. Using 2% calcium chloride in distillated water as the AL raises the protein yield in comparison with variant 3 (Fig. 6, variants 3, 4) by almost 13%, which enabled the 70% extraction of the whey proteins. Although the content of calcium chloride is much lower (by a factor of almost three) than in the AL representing the 5% solution of this salt in the MW, the protein extraction into the concentrate is observed to be almost equal (Fig. 6, variants 2, 4).

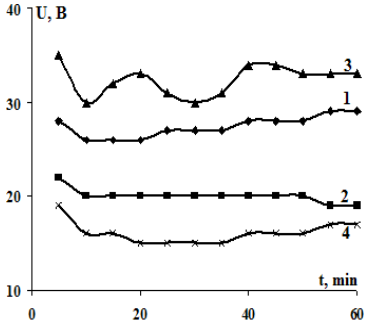

Fig. 8. Change in voltage depending on the composition of AL and the type of membrane. Variants of the composition of AL: (1) MW (canvas membrane); (2) sol.5% $CaCl_2$ in MW (canvas membrane); (3) MW (ultrafiltration membrane); (4) sol.2% $CaCl_2$ in water (MK-40 ion_selective membrane).

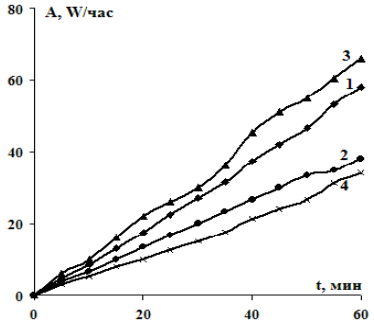

Fig. 9. Change in the power inputs depending on the composition of AL and the type of membrane: (1) MW (canvas membrane); (2) sol.5% $CaCl_2$ in MW (canvas membrane); (3) MW (ultrafiltration membrane);(4) sol. 2% $CaCl_2$ in water (MK-40 ion-selective membrane).

At the IW electroactivation, along with PMC recovery there is a simultaneous isomerisation of lactose into lactulose by 30-35%.. Application of the combined technology of their processing increased the content of inverted lactulose up to 65% (Figure 10) and, evidently, it is not the upper limit.

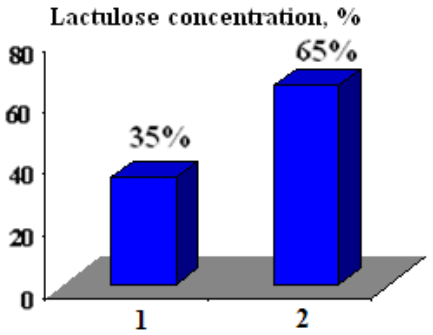

Fig.10. Lactose and lactulosa content for various technologies: (1) electroactivation,(2) combined technology

CONCLUSIONS

To conclude, using the ion-selective membrane makes it possible to increased the degree of the protein passing into the PMC, lowering the power inputs, to regulate the protein salting out, and it permits the whey to be saved (economized) only for processing in the cathode chamber.

Thus, the authors have found out that the proposed method of electrofractionation of secondary milk raw materials is a reagentless, low-temperature process that can be included in a wasteless processing of whey aimed at the simultaneous processes of recovery of protein-mineral concentrate and of isomerization of lactose into lactulose.

References

1. Sinel'nikov, B.M., *Technogenic societies and ecology: contemporary problems*, Bulletin of the North-Caucasian GTU, Ser. Foodstuffs, no. 1 (7), 2004.
2. Kravchenko, E. F., *New developments related to the efficient utilization of whey*. Program of the Int. Forum „Dairy Industry 2006"/Processing of Secondary Milk Raw Products
3. Khramtsov, A.G., and Evdokimov, I.A., *Lactosa-containing raw materials – phenomenology of the term, practice and prospects of application,* Collected works of the North-Caucasian GTU, Ser. „Foodstuffs", 2006, no. 2
4. *Agriculture and Agri-Food Canada*. Introduction to Dairy Science and Technology: *Milk History, Consumption, Production and Composition, 2006*
5. Khramtsov, A.G., Evdokimov, I.A., Nesterenco, P.G., Dubikov, D.A., and Gritsaeva, M.V., *Retrospective analysis of information concerning processing and utilization of lactose-containing raw materials,* Collected works of the North-Caucasian GTU, Ser. „Foodstuffs", 2006, no. 2.
6. Shuvaeva, V.A., Omelianciu, P.A., *Wasteless technology for fractionating degreased milk*, Bulletin of the North-Caucasian GTU, Ser. Foodstuffs, no. 1 (7), 2004.
7. Khramtsov, A. G., *Molochnaya syvorotka (Whey),* Moscow, Agropromizdat, 1990, 240 p.
8. Ionics Incorporated, Whey Membrane Filtration Applications, 2005.
9. Bologa, M.K. and Pyrgaru, Yu.M., *Processes of Electrocontact Coagulation of Whey Proteins,* Electron. Obrab. Mater., 1993, no. 6, pp. 46—50.
10. Rytchenkova, O.V and Krasnoshtanova, A.A., *Development of the Methods for Protein Extraction from Whey*, 6-ya Mezhdun. Konf. "Sotrudnichestvo dlya resheniya problemy otkhodov" (The 6-th Intern. Conf. "The Cooperation for Solving the Problem of Waste"), April 8-9, 2009, Moscow, Russia.
11. Bogatova, O.V. and Dogareva, R.G., *Khimiya i fizika moloka* (Chemistry and Physics of Milk), Orenburg: GOU OGU, 2004.
12. Gorbatova, K.K., *Khimiya i fizika belkov moloka* (Chemistry and Physics of Milk Proteins), Moscow: Kolos, 1993.
13. Sprincean, E.G. and Bologa, M.K., The Salt Composition of the Whey-Protein Concentrate Obtained by the Electrocontact Method, *Electron. Obrab. Mater,*2006, no. 6, pp. 50—55.