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Research Article

Development of an efficient method for obtaining lactose and lactulose from whey

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Abstract

Taking into account a wide range of lactulose application in pharmaceutics, baby food production and other fields, along with the importance of technological solutions for its extraction from milk whey, the presented work was carried out to obtain lactulose in one cycle with simultaneous alkaline treatment and desalting of whey by the electromembrane method. Based on the data obtained, an effective method for obtaining a protein concentrate, lactose, and its isomer – lactulose from whey has been developed. The processes of pre-treatment and desalting of milk whey by the electromembrane method were studied and the optimal parameters for the processes implementation were determined. The curves of changes in the concentration of inorganic ions in whey in the desalination process, depending on the degree of demineralization, were plotted.

Keywords

electromembrane method, lactose, lactulose, protein

Introduction

Milk whey and its components are the most valuable agricultural raw materials for processing into foodstuffs, semi-finished products, fodder, and medications. In its natural form, whey is not suitable for direct consumption as a food product due to the high content of ash elements in it (up to 0.6–0.8%) (Lacey and Sidney 1972). However, it is a rich source of proteins, vitamins, minerals, and lactose, from which lactulose can be obtained (Javed et al. 2022). Lactose application spans the dairy, food, and pharmaceutical industries. Lactose is added as an ingredient to infant formula, pharmaceuticals, and animal feed. Additionally, lactose and lactose-based co-processed excipients (CPEs) are extensively used in the pharmaceutical industry, particularly as excipients in tablets. Approximately 60–70% of pharmaceutical preparations contains lactose, and this is one of the highest usage rates in pharmaceutical excipients (Hebbink and Dickhoff 2019; Shi et al. 2023). Lactulose is a natural powerful bifidogenic factor activating the growth of bifidobacteria in both child's and adult's intestines. The beneficial properties of lactulose are widely used in the production of baby and dietary foods, as well as in medicine. The non-absorbable disaccharide lactulose is mostly used in the treatment of various gastrointestinal disorders such as chronic constipation and hepatic encephalopathy (Prasad et al. 2007; Yao et al. 2018).

The mechanism of lactulose action remains unclear, but it elicits more than osmotic laxative effects. As a prebiotic, lactulose may act as a bifidogenic factor; it serves as a

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prebiotic rather than a laxative. In a randomized controlled multicenter trial involving 98 cirrhotic patients, there were significant differences between the lactulose and control groups in the abundance of Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria. Furthermore, lactulose has prebiotic effects that have been proven to reduce fasting and postprandial glucose and inflammation markers and improve insulin sensitivity and lipid profile in subjects with prediabetes (Barengolts 2016; Wang et al. 2019).

Based on published studies, lactulose may act as a prebiotic, which has positive effects in preventing and controlling diabetes (Snelson et al. 2021; Chu et al. 2022).

Many works have been published on methods for obtaining lactose and its isomer (Gavrilov and Gavrilov 1998; Khramtsov et al. 1999).

The method for producing crystalline lactulose is described in the work (Panesar and Kumari 2011): a water solution of lactulose with approximately the same amount of lactose is evaporated to adjust the concentration of dry matters (DM) to 70%. Then the solution is cooled to 13 °C, crystalline lactulose is added as a seed and kept at this temperature for 24 h. In biotechnological industries, solutions of biologically active substances are usually subjected to desalination by various methods: ion exchange, electrodialysis or nanofiltration. The choice of desalination method depends on the composition of the solution. These processes have been investigated for more than 20 years. Considering sustainability, both processes require quite large cumulative energy and produce large amounts of wastewater not yet fully assessed (Houldsworth 1980; Greiter et al. 2002). Despite the fact that this method is economical and structurally simple, during whey desalting the irreversible sorption of proteins on ion exchangers occurs. Adsorbed proteins block the surface of resins and with prolonged use, the ion exchange stops (Greiter et al. 2002). However, the main disadvantage of ion-exchange technology is the need to regenerate ion-exchange resins after their exchange capacity is exhausted. To demineralize whey, electrodialysis technology is also used, which has a number of advantages compared to ion exchange technology (Houldsworth 1980; Veikko 2001; Greiter et al. 2002). However, whey desalination using cation- and anion-exchange membranes faces two obstacles caused by membranes clogging with poorly soluble calcium salts and deposition of protein fractions on the surface of anion-exchange membranes (Lacey and Sidney 1972).

The aim of this work is to develop methods for more efficient obtaining protein concentrate, lactose, and lactulose from milk whey.

Materials and methods

The content of dry matters (DM) in the solution was determined on a refractometer "RL-10" (Poland). Quantitative determination of trace elements in whey was carried out using inductively coupled plasma optical emission spectrometry (Agilent 5800 VDW in ICP).

HPLC conditions for organic acids

Chromatographic analysis of organic acids was performed using an HPLC system (Waters 2695 Separation Module) equipped with a "Waters 2487" Dual λ Absorbance UV Detector (Milford, MA, USA).

The column was Agilent C18, 4.6×250 mm with a 5 μm particle size. The mobile phase was an aqueous solution of H_3PO_4, with a concentration of 0.1% at pH 3.0, containing 0.5% acetonitrile and 0.5% methanol as an organic modifier, and flowing at a rate of 0.5 ml/min. Separation and quantification of the organic acids were monitored at 210 nm, and the sample injection volume was 10 μl.

HPLC conditions for saccharides

The chromatographic separation of sugars (some mono- and disaccharides) was achieved with a KNAUER Azura HPLC system coupled to a refractive index detector (RID) in an isocratic mode. The samples were analyzed on a Nucleodur 100-5 NH_2-RP column (250×4.6 mm I.D., 5 μm). The column and RID temperatures were set at 30 °C, respectively. The mobile phase was composed of acetonitrile and water (79:21, v/v), and the flow rate was 0.5 mL/min. The injection volume was 30 μL. Peak detection and integration were done using a Clarity Chrom CDS data system (KNAUER Azura, Wissenschaftliche Geräte GmbH, Berlin, Germany).

Production of lactose and lactulose by milk whey processing

Separation of sediment and suspension from curd (or cheese) whey was carried out by centrifugation (4000 rpm, 15 min, with a 1480 separation factor). Ultrafiltration of the obtained supernatant was carried out by passing it through a separating ultrafiltration apparatus with hollow fibers AR-02M of periodic action (Russian Federation). The working mixture circulated in a closed loop at a 190–230 cm^3 /min rate.

Experiments on the desalination of whey permeate were carried out in a direct-flow circulating multi-chamber apparatus with an intermembrane distance of 3 mm manufactured by us. The chambers of the electrodialyzer were separated by an MK-40 cation-exchange membrane and an MA-40 anion-exchange membrane (Russian Federation). The working area of each of the membranes was 53 cm^2. To determine the value of limiting current density by the method of Cowan and Brown (Cowan and Brown 1959), two platinum wires were placed at the ends of the ion-exchange membranes to measure the internal potential (U_i). Through the membranes, turning off the electrodes, the voltage was increased in certain steps and the relevant values of the current strength (J) were monitored. Membrane resistance (R_m) was calculated by the ratio of $R_m = U_i$ / J; diagrams were plotted on the coordinates of the resistance and the inverse value of the current strength (1/J) (Greiter et al. 2002). On the graph, the data were

obtained in the form of two straight lines, the intersection point of which corresponded to 1.05 A^{-1}.

The ratio of the value 1/1.05 = 0.952 A to the effective surface of the membranes (53 cm^2) gives the limiting current density, which was ~ 18 mA/cm^2. Desalting and alkaline treatment of whey (when using a four-chamber electrodialyzer) were carried out at the constant value of limiting current density. The volume of circulating solutions was 500–700 cm^3, the linear velocity of the liquid flow through the chambers was 6.5 cm/s. The liquid supply rate was monitored using peristaltic pumps of the Masterflex type (USA). The content of metal cations in the solution was determined by the atomic absorption method on the device C (Germany), anions – by titration, as well as by the ion-exchange chromatographic method (Acikara 2013). The content of lactulose in the solution was calculated indirectly by measuring the glucose content in the initial whey and in the resulting product (Wilson and Turner 1992; Zimmer et al. 2017). Lactic acid was determined qualitatively by the colorimetric method with p-oxydiphenyl and by the enzymatic method using the enzyme lactate dehydrogenase (Datta et al. 2013). Citric acid and its salts were determined after their conversion to pentabromoacetone, its extraction with chloroform followed by determination of the optical density of the extract at a 245 nm wavelength as well as qualitatively. The color of the solution was determined by the photometric method (Pazouki and Panda 1998). Vacuum evaporation of solutions was carried out at a residual pressure of 0.08–0.1 MPa and a temperature of 55–60 °C on an SB-1 10CE vacuum evaporator (China). The electrical conductivity of whey during electromembrane demineralization was measured with an "OK 102/1" conductometer (Hungary). The content of dry matters (DM) in the solution was determined on a refractometer "RL-10" (Poland).

Results

In curd whey taken from the Arzni milk processing plant of the Republic of Armenia, trace elements present in the whey with inductively coupled plasma were determined by optical emission photometric method. Trace elements present in the whey were determined. The data obtained are given in Table 1.

Table 1. Composition of macro and microelements in curd whey.

Label	Solution Concentration	Conc. SD	Conc. % RSD	Intensity (c/s)	Calculated Concentration
Ag(328.068 nm)	0.0024	0.1322	54.68	57.8788	0.2418
Al(396.152 nm)	0.0037	0.2322	62.98	379.0015	0.3687
Cr(267.716 nm)	0.0056	0.5328	94.63	269.5044	0.5630
Cu(223.009 nm)	0.1531	6.5968	43.09	757.6550	15.3090
Mg(280.270 nm)	0.9100	76.3960	83.95	119612.8165	91.008
Mo(281.615 nm)	0.0059	0.0843	14.20	99.4759	0.5937
Sr(421.552 nm)	0.0025	0.1706	67.59	4526.3869	0.2524
TI(276.789 nm)	0.0112 u	1.0790	96.64	26.2014	1.1165 u
V(292.401 nm)	0.0002 u	0.0427	>100.00	24.0337	0.0237 u
Zn(213.857 nm)	0.0245	1.8861	76.98	2697.9616	2.4500

The study conducted by the chosen method allowed determining with high accuracy 23 trace elements in curd whey.

The HPLC method revealed the presence of organic acids in the serum

The quantitative and qualitative content of organic acids in cheese (1) and curd (2) whey is presented in Table 2.

The selected method revealed lactic, malic, oxalic, and citric acids present in the cheese-curd whey of the Arzni dairy plants of the Republic of Armenia. Studies have made it possible to clarify that the above organic acids are present in the whey of cheese and cottage cheese with high rates. However, there was not such a big difference in the quantitative ratio of ingredients in the cheese-curd whey of the dairies of Armenia.

Table 2. Composition of organic acids in Cheese (1) and Curd (2) whey.

| Compound name	Cheese whey, mg /L	Curd whey, mg/ L
Oxalic acid	–	0.223
Malic acid	0.549	0.072
Lactic acid	0.409	0.807
Citric acid	2.33	1.793
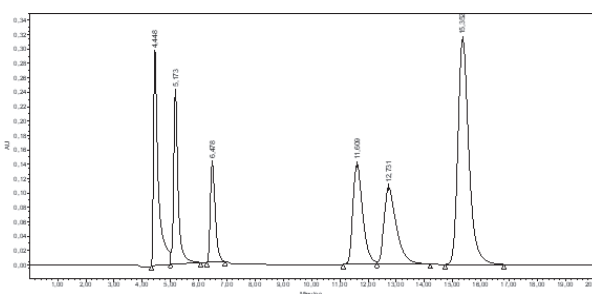
Figure 1. HPLC chromatogram of organic acids standard: 4.448 – Oxalic acid, 5.173 – Tartaric acid, 6.478 – Malic acid, 11.609 – Citric acid, 12.731 – Succinic acid, 15.352 – Fumaric acid.
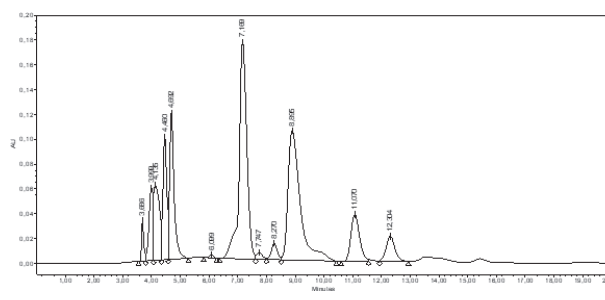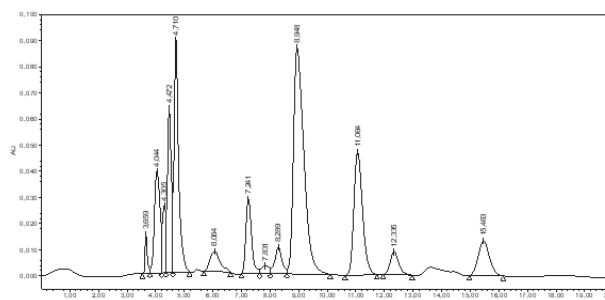
Figure 2. Chromatograpms of organic acids in Cheese (1) and Curd (2) whey.

Quantitative and qualitative identification of sugars in desalinated milk whey by HPLC was also carried out. The results are shown in Fig. 3 and Table 3.
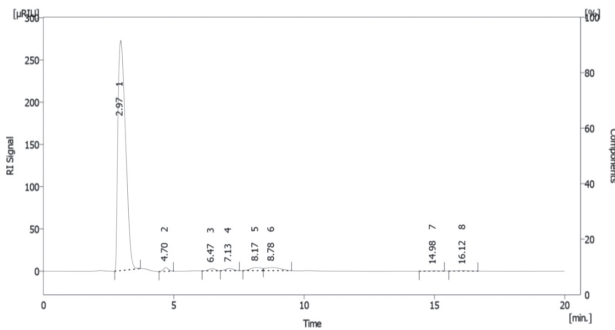
Figure 3. Chromatogram of sugars composition in milk whey.

Table 3. Result table of sugars composition in milk whey.

| N	Compound name	Retent. Time[min]	Conc. mg/l
4	Fructose	7.133	0.457
5	Glucose	8.167	0.832
6	Galactose	8.783	1.607
8	Lactose(g/l)	16.117	0.45

The quantitative and qualitative indicators of the sugar content of milk whey are presented in Table 3.

Desalting of whey by the electromembrane method to obtain lactose was carried out in a three-chamber electrodialyzer, and the simultaneous desalting and alkaline treatment of the whey to obtain lactulose were carried out in a four-chamber electrodialyzer.

Fig. 4 shows the results of desalting cheese whey evaporated to 10.5% DM in a three-chamber electrodialyzer, the middle chamber of which is separated from neighboring anion-exchange and cation-exchange membranes. In the first approximation, as is customary in the scientific literature (Greiter et al. 2002) the criterion for desalting on an electrodialyzer can be a characteristic of changes in current and electrical conductivity. As seen in Fig. 4 (curve 3), based on the electrical conductivity data, in a short time (almost 40 min), whey was desalted to ~ 65%.

Since modern anion-exchange membranes do not have the absolute ability to limit the diffusion of mobile protons (Novalic et al. 1996), after 40 min of electrodialysis, part of H^+-ions from the anode chamber diffuses into the middle one. At the end of the desalination process, it leads to a decrease in the whey pH from 7.0 to 4.5 (Fig. 4, curve 1), and the electrical conductivity does not change significantly. As is known from the literature (Polyansky et al. 2014), when whey is desalted by an electromembrane method with an increase in current density due to the conversion of lactose into lactic acid, a linear decrease in the pH value of whey occurs according to the equation: $pH = 4.5–0.2\ i$, where 4.5 is the pH of the natural initial whey. In our experiments, the pH of the initial whey during desalting by the electromembrane method is 7.0. Considering this equation, it is evident that the selected value of the limiting current density (i) is optimal (18 mA/cm²), since a decrease in the value of i below this value leads to a decrease in whey pH, and an increase in i above this value leads to the occurrence of side processes in the electrode chambers. The same Fig. 4 shows that about 40 min after the start of desalination, the process should be stopped, since further continuation of the process does not significantly affect parameters of the electrical conductivity. In the process of desalination, due to a decrease in the mineralization of the solution, the value of the limiting current density decreases. Therefore, to maintain it at a constant level, it is necessary to raise the voltage in the circuit so that at the end of the process it reaches 108 V (Fig. 4, curve 2). Fig. 5 shows the results of studying the change in the concentration of inorganic ions in the whey during its demineralization depending on the degree of desalting.

As seen in Fig. 5, when whey is desalted by an electromembrane method, the diffusion of various ions into neighboring chambers occurs at a different rate. In the initial period of desalting, mainly monovalent ions of sodium, potassium, chlorine, which have greater mobility and most strongly affect the taste of whey, are removed. Then, as desalting proceeds, calcium ions are
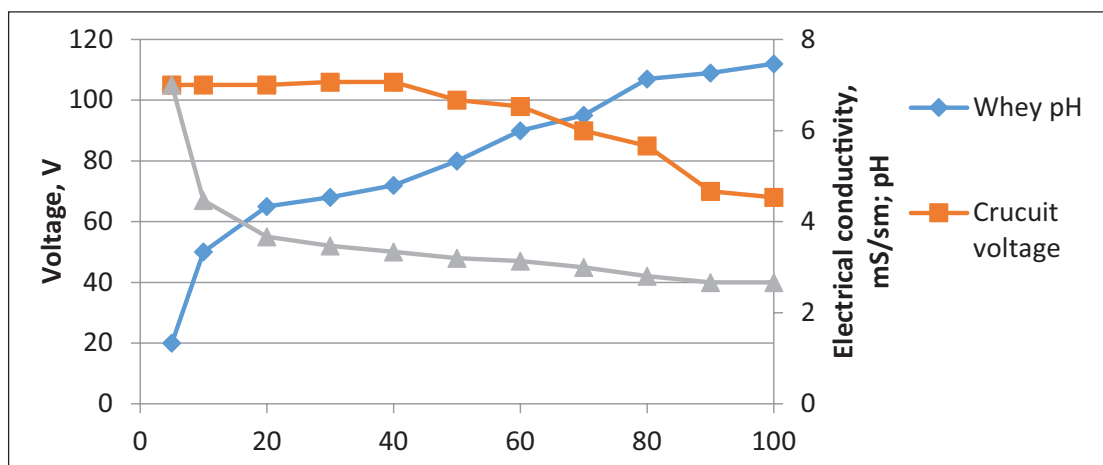
Figure 4. The desalting kinetics of evaporated cheese whey in a three-chamber electrodialyzer: 1 – whey pH; 2 – circuit voltage; 3 – electrical conductivity of whey.
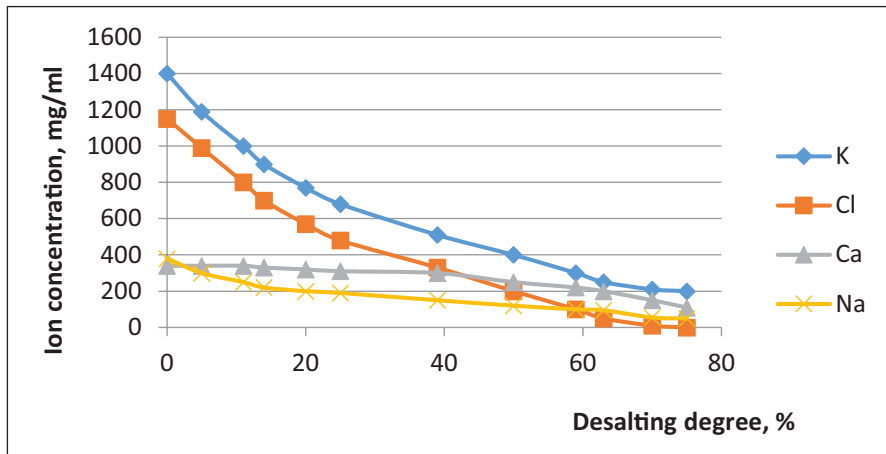
Figure 5. Change in the concentration of ions in whey in the process of desalination depending on the level of demineralization: 1- K^+; 2 – Cl^-; 3 – Ca^{2+}; 4 – Na^+.

being removed. It has been established that the transfer of bivalent ions to the adjacent chamber occurs after the removal of ~ 65–75% of monovalent ions from the whey. Technologically, it is preferable to desalt whey to 55–60%.

It was stated in (Lacey and Sidney 1972) that when whey is desalted by the electromembrane method, the diffusion of lactic acid from the diluate chamber into neighboring chambers occurs at the same rate as the diffusion of monovalent ions.

Thus, based on the data obtained, namely, the short duration of the electrodialysis process at low energy costs, as well as the achievement of the required degree of desalting, we can conclude that the selected method of whey processing is effective.

For the first time the process of desalting and simultaneous alkaline treatment of evaporated curd whey in a four-chamber electrodialyzer was studied by Aghajanyan et al. (2010), the disposition of the ion-exchange membranes is shown in Fig. 6. In parallel with the desalting of whey in chambers 1 and 3, its alkaline treatment takes place in chamber I, since water electrolysis occurs in chamber I, resulting in the formation of hydroxyl ions.
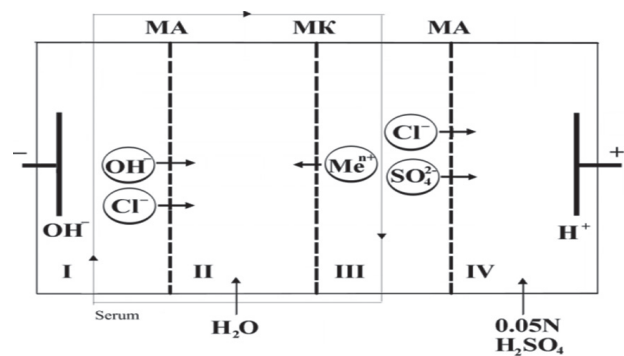
Figure 6. Scheme of arrangement of membranes in a four-chamber electrodialyzer in the process of desalting and alkaline treatment of whey.

In the electrodialyzer with the presented layout of ion-exchange membranes, the diffusion of protons into the chamber of the target product is reduced to minimum, which makes it possible to carry out whey desalting and its alkaline treatment practically without the use of chemicals.

The results of desalination and alkaline treatment of curd whey, previously evaporated to a DM content of 10.3%, in a four-chamber electrodialyzerare are shown in Fig. 7.
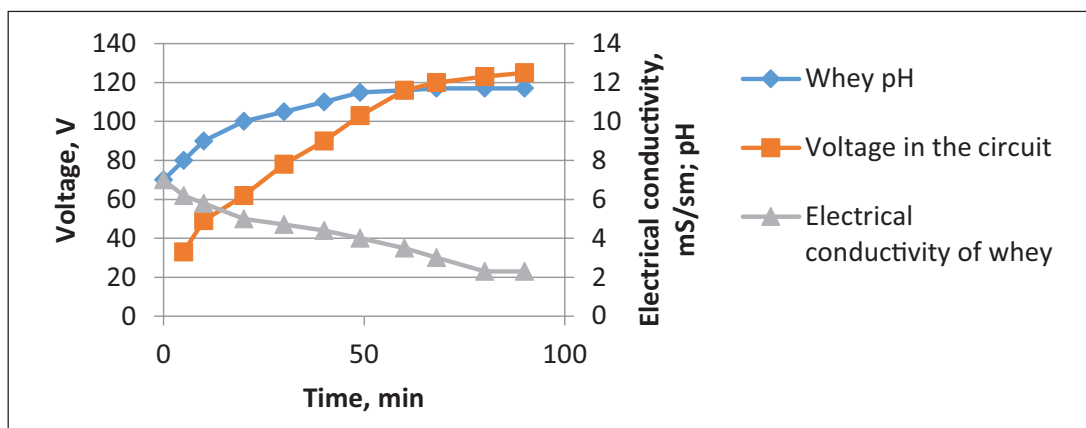
Figure 7. Kinetics of desalting and alkaline treatment of evaporated curd whey in a four-chamber electrodialyzer: 1- whey pH; 2 – voltage in the circuit; 3 – electrical conductivity of whey.

As seen in Fig. 7, whey alkylation results in its simultaneous desalting, which leads to a decrease in electrical conductivity from 7.0 ms to 3.0 ms (curve 3). A relatively weak decrease in the whey electrical conductivity is caused by the fact that along with desalting, the simultaneous formation of OH$^-$ ions in the cathode chamber takes place, which is confirmed by the gradual increase in the whey pH to a value of 12. Desalting is due to the diffusion of dissociated ions from chambers 1 and 3 to neighboring chambers 2 and 4, as well as to the water electrolysis. Since the electrical conductivity decreases during desalination, it is necessary to increase the voltage in the circuit to 130 V to maintain the current density at the limiting level (Fig. 4, curve 2). At the end of the process, the degree of desalting of curd whey is 60–65%.

Based on the data obtained from the use of two types of electrodialyzers for whey desalination in order to obtain lactose and lactulose, as well as a protein concentrate and casein dust, an effective technological scheme for the complex processing of whey has been developed, which consists of the following steps:

1. The process of extracting casein dust and fat.

Curd and cheese whey contains up to 0.5% of suspended protein particles (casein dust) and up to 0.4% of fat. The literature data (Aghajanyan et al. 2011), as well as our experiments, prove that the most accessible way to extract these particles is whey centrifugation at 4000 rpm (separation factor 1480) for 15 min.

2. Extracting whey proteins.

The extraction of proteins from the supernatant obtained after whey centrifugation, was carried out by the coagulation method. Studies have shown that to coagulate proteins from curd or cheese whey, it is necessary to increase pH from 3.6–4.0 to 6.8–7.0. After that, to destroy the solvate shell of protein granules, the solution was subjected to heat treatment at 65–68 °C for 10 min. The suspension was then cooled to 18–20 °C, centrifuged, the formed protein fraction was separated from the transparent supernatant and dried at 55–60 °C by blowing hot air. The protein content in the dried product was 30–37%. The remaining components of the dry product were lactose, lactate and sodium citrate, residual casein dust, etc., which are of great nutritional value. The resulting product can be used in the food industry, in particular in the production of confectionery, sports products, desserts and ice cream, food additives, dairy products, baby food, etc. (Bazinet 2005).

3. Obtaining lactose.

To remove high molecular weight components and residual suspensions, the supernatant containing up to 5% of lactose, was subjected to ultrafiltration. The salt composition of the resulting permeate was 0.15–0.18 g-mol/ L. The permeate was desalted by the elec-tromembrane method in a three-chamber electrodialyzer according to the above-mentioned scheme at a current density of 18 mA/cm^2 and a liquid flow rate of 6.5 cm/s through the chamber. The saline composition of whey permeate after desalination was 0.05–0.06 g-mol/L. Under these conditions, the degree of whey desalination was 60–63%. To obtain lactose from the desalted whey, the solution was subjected to vacuum evaporation to the DM content of 65–67%, and then lactose crystals were isolated by the method of isohydric crystallization. The obtained crystals were subjected to recrystallization and after drying, pure lactose crystals were obtained.

4. Obtaining lactulose.

The isomerization of whey lactose to lactulose was carried out as follows. According to the above-described scheme (Fig. 3), whey permeate in a four-chamber electrodialyzer was subjected simultaneously to both desalting and alkaline treatment by the electromembrane method. Further, whey permeate demineralized to ~ 60% (pH 11.6–11.8) was subjected to heat treatment at 65 °C for 20 min. This resulted in lactose isomerization and its conversion into lactulose (Khramtsov et al. 1999). Due to the fact that in an alkaline medium at a temperature of 65–70 °C coloring matters are formed and an odor appears, to remove them, the lactulose solution neutralized with acid whey (>200 °T) was decolorized with activated carbon grade OU-V at 55–60 °C for 30 min. Carbon consumption was 1.5–2.0% of the solution amount. The decolorized filtrate was subjected to vacuum evaporation to the DM content of 60–65%, then residual non-isomerized lactose crystals were isolated from it by the method of isohydric crystallization. Then the filtrate was evaporated to the DM content of 70–75%. The lactulose content in the evaporated whey syrup was 10.6%, the residual whey proteins in the alkaline medium were subjected to hydrolysis by 40–50%. The remaining components were residual lactose, protein or products of its hydrolysis, lactate and citrate ions, residual mineral salts, as well as non-protein nitrogenous compounds, vitamins, hormones, immune bodies, and other compounds.

Since curd and cheese whey do not differ much by the component composition, the developed technological approach for whey processing can be applied to both types of whey.

The technological scheme of the complex processing of whey (obtaining casein dust, protein concentrate, lactose, lactulose) is shown in Fig. 8. Thus, we have studied the process of whey desalting by the electromembrane method, determined the parameters of the process, and presented a new technological approach for obtaining lactulose from whey. The results obtained formed the basis for the development of technological approaches for the production of a number of biologically active substances from whey, which are of great practical value.
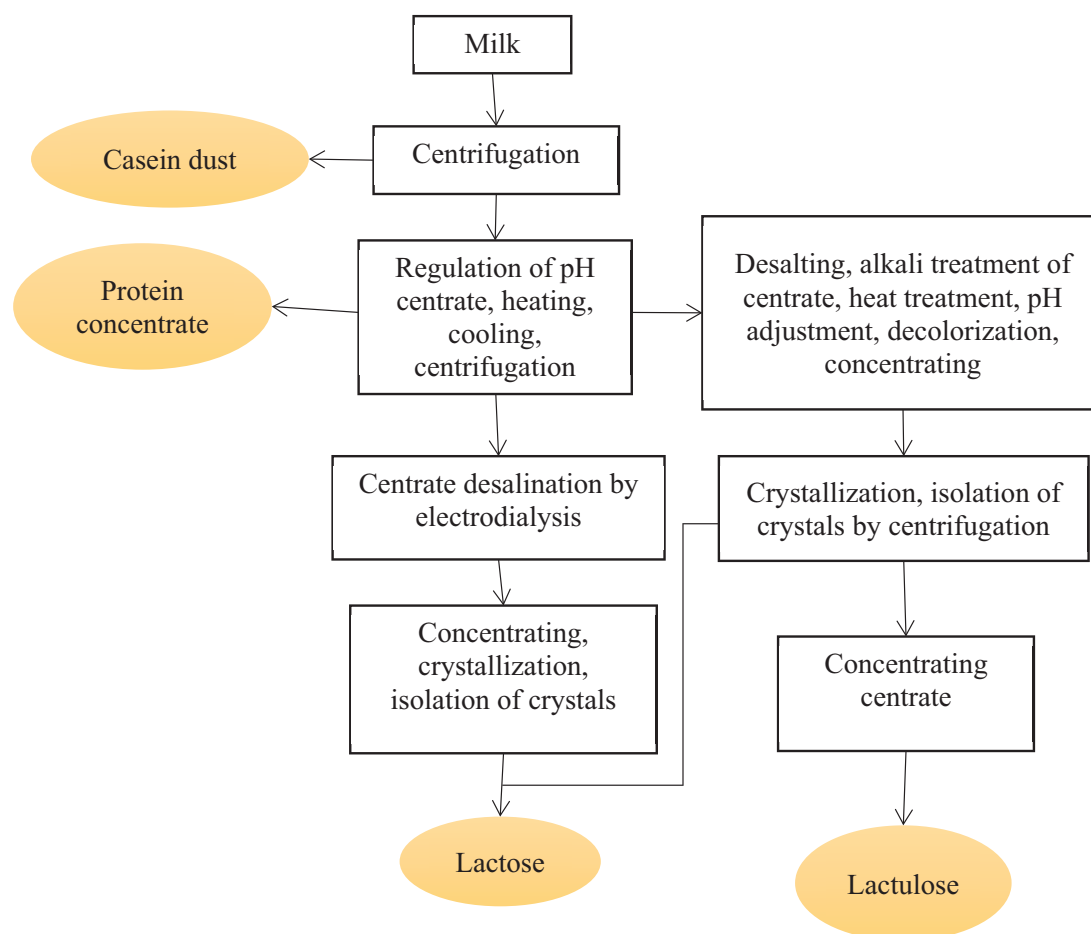
Figure 8. Technological scheme of complex processing of whey.

Conclusion

The development of an accessible technology for desalting milk whey, lactose, and lactulose, which are considered as a production waste in milk factories of Armenia, was implemented. Using modern analytical methods of high accuracy, whey has been shown to be rich in microelements, organic acids, sugars, and amino acids, which is of an important nutritional and pharmaceutical value.

Acknowledgement

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