BIO Web of Conferences **116**, 02015 (2024) https://doi.org/10.1051/bioconf/202411602015
EBWFF 2024

Ultrafiltration of skim milk microfiltration permeate

Dmitriy Mamay[1*], *Sergey* Babenyshev[1], *Angelina* Mamay[1], and *Vyacheslav* Lisitsyn[1]

[1]North Caucasus Federal University, 355017 Pushkin str, Russia

Abstract. The purpose of the research conducted in the laboratory is to study the basic regularities of the ultrafiltration process of microfiltration permeate of skim milk, called native whey (NW). The objects of the research are NW, its ultrafiltration process and the obtained ultrafiltration retentate, NW permeate. To create the initial information base of the research work we used the results of the analysis of literature data from the libraries of the Russian State Library, North Caucasus Federal University, as well as the global network of the Internet and so on. Physicochemical characteristics of NW, as an object of ultrafiltration, were determined according to standard methods and on certified equipment. The main operating modes of the NW ultrafiltration process were determined experimentally. A mathematical expression allowing to quantitatively determine the permeability of ultrafiltration membrane depending on the working pressure, flow rate of circulation and temperature of the separated system was obtained. Quantitative indices of casein and whey proteins content in the resulting ultrafiltration retentate and permeate were determined. The results of the experimental research stage are the basis for the preparation of recommendations on the technology of ultrafiltration of NW with the use of pilot baromembrane equipment in industrial conditions. The obtained experimental data can be used as initial information for nanofiltration of ultrafiltration permeate. The field of application of the results is dairy industry.

1 Introduction

During the initial period of growth and development of the organism, the biological functions of milk are numerous and are mainly mediated by proteins and peptides. Amino acids released during protein digestion are not only a source of nutrients, but also act as signaling agents that activate the immune and other physiological systems, especially in infants. But the unique biological functions of the main components of milk, first of all, whey proteins show only when they are in native form, which can be preserved by their ultrafiltration separation from microfiltration permeate of skim milk - native whey (NW). Accordingly, scientific work aimed at further improvement of membrane technology of their production is relevant. However, despite the great achievements in the field of ultrafiltration separation of whey proteins from NW, the efficiency of practical application

* Corresponding author: dmamai@ncfu.ru

of this process remains low in terms of margins of marketable products. This is due, firstly, to the complexity of the composition of raw materials subject to ultrafiltration, changes in its physical and chemical parameters depending, above all, on the technology of skim milk microfiltration. And, secondly, the modes of carrying out the process of ultrafiltration of NW, the type of apparatus, membrane material, requirements to the physicochemical characteristics of the resulting retentate and permeate, have a great influence on the result obtained. Limited theory of membrane processes, as well as the experience of its application in the dairy industry determine the need to conduct their own research work in this area.

The purpose of the research conducted in laboratory conditions is to study the basic regularities of the process of ultrafiltration of NW. To achieve this goal the following scientific tasks are solved:

- to study the properties of NW as an object of ultrafiltration, physical and chemical characteristics of the obtained NW retentate and permeate;
- determine the ranges of the main operating parameters of the process of ultrafiltration of NW;
- to obtain a mathematical model in the form of regression equation, which allows to quantitatively determine the membrane permeability index depending on the process parameters of ultrafiltration of NW (working pressure, flow rate of circulation and temperature of the separated system in the channel of the baromembrane apparatus);
- to determine the content of casein protein fractions in relation to whey proteins (CP/WP) in the true protein of ultrafiltration retentates at baromembrane separation of NW.

The results of the performed stage of experimental research are the basis for the continuation of scientific work and preparation of recommendations on the technology and procedure of NW ultrafiltration using pilot baromembrane equipment, as well as can be used as background information in the development of methods of nanofiltration process of ultrafiltration permeate.

2 Organization of ultrafiltration process for separation of milk whey proteins from NW

2.1 Characteristics of the baromembrane separation object and technological properties of natural whey protein concentrates

Milk contains a complex protein system, the basis of which are caseins (insoluble) and whey proteins (soluble). In accordance with the recommendations [1], in the terminological concept "whey proteins" (WP) we will include all proteins that are in milk whey after casein precipitation at pH 4.6 and 20°C. From this point of view, the main WP of milk are β-lactoglobulin (β-LG), α-lactalbumin (α-LA), bovine serum albumin (BSA), immunoglobulins (IgM, IgG), lactoferrin (LF) and proteozoic peptone fraction. It should be taken into account that whey from rennet-induced milk coagulation, as the most common source of WP, contains other serum soluble proteins [2], including those from milk fat globule membrane (MFGM). Of note, β-LG and α-LA account for 50% and 20% of WP in both whey and native whey [3]. Proteolytic peptide (PP), derived from the cleavage of κ-casein (κ-CN) by chymosin [4], is the third most important peptide component of whey.

As a source of native whey proteins, NW, which contains a residual amount of caseins and has differences compared to conventional, e.g., whey, both in nutritional properties and technological functionality, is of particular interest [5,6]. This is due to the fact that microfiltration of skim milk avoids protein denaturation by conducting the baromembrane separation process at temperatures below 20°C, which promotes increased release of β-

casein from the casein micelle, which partially penetrates the microfiltration membrane together with whey proteins [7]. In general, the industrial use of concentrates (CWP) derived from NW, similar to the use of WPC and WPI (respectively, whey protein concentrates and isolates produced from normal whey), includes use in the form of semi-finished products in the formulation of ready-to-drink beverages, yogurt, sour cream, confectionery, various baked goods, and so on. However, the high level of protein purity and the absence of cheese by-products make CWP, above all, a universal ingredient for the production of ready-to-drink protein drinks and especially infant formulae. The data on organoleptic parameters, taste properties of CWP, WPC and WPI obtained using membrane processes are still limited, the exact values of their fractional compositions and concentrations required for the production of the same protein drinks without extraneous flavors have not yet been determined.

The analysis of the results of studies performed using traditional whey as a raw material [8-11] showed that the degree and rate of denaturation of whey proteins are influenced by pH, ionic strength and mineral composition of the dispersion phase, its content of solids, lactose and casein. It is possible that this may be true for NW as well. In [12] it was shown that there is no significant change in the nativity of quantitatively defined proteins in unpasteurized milk samples skimmed at 10°C and 50°C. But industrial pasteurization (72°C, 20 seconds) leads to denaturation of whey proteins in whole and skim milk up to 4% and 11%, respectively [13-15], and according to [16] for the same conditions the degree of denaturation of whey proteins is 24%. At the same time, [17] recommended two basic modes of pasteurization of raw milk - at 72-75°C (15-20 seconds, HTST) and up to 63°C (30 minutes, LTLT).

The native concentrate (NWPC) obtained by ultrafiltration of NW, compared to WPC produced by conventional methods from normal whey, has more pronounced solubility, sensory, foaming and gelling properties [18-20]. The foaming properties of NWPC were comparable to egg white foam [21]. Foam made from buttermilk-derived WPC was less stable than NWPC foam [22]. WPC and NWPC (24-37% total protein) obtained by ultrafiltration from NW and the same milk were found [23] to have a higher WP:TP (True Protein) ratio compared to commercial WPC. According to [20] due to the absence of CMP (Caseinomacropeptide (glycosylated and non-glycosylated)) in NW, less NPN (non-protein nitrogen) was found in NWPC than in commercial WPC. It should be noted that any heat treatment of raw materials changes the protein profile of NWPC [24], and the difference in the content of mineral complex components in WPC derived from whey and NWPC is due to the lower pH value, which allows soluble calcium to transfer to the whey. As for NWPC and WPC made from the same milk using the same equipment and process, lower calcium, phosphorus and sodium contents were found in NWPC and WPC compared to whey protein concentrate derived from whey [25]. The fact that WP are soluble even at low pH makes them an interesting ingredient for protein enrichment of carbonated clear beverages. For example, NWPC remains transparent after dissolution in water and does not contain PP, which could form aggregates at low pH. However, it should be noted that for NWPC to be used in acidic beverages, its casein content should be minimal, since the solubility of such a concentrate decreases at pH≤4.6.

Regarding gelling properties, NWPC gels have a denser structure than gels made from WPC derived from whey. The presence of PP in WPC affects the rheological properties and microstructure of the gels as a function of pH: gels without PP addition had much higher strength at pH 4.0 than at pH 7.0. And gels containing CMP had similarly low gel strength regardless of pH [20].

Thus, it can be concluded that NW has little in common with milk whey, since by definition it lacks bacteria from the starter, rennet enzyme, etc. components, mechanical impurities in the form of casein dust, proteolytic peptide (PP) and proteins in denatured

form. And their mineral complex will also differ depending on the type of the target product (cheese, cottage cheese, casein), methods of its production. Exclusion of NW pasteurization determines the absence of any denaturation of its protein components without heat treatment of initial raw materials.

2.2 Theoretical aspects of the baromembrane process for the separation of milk whey proteins from NW

Since NW from unpasteurized skim milk contains less calcium, phosphorus, but more sodium in comparison with NW obtained from pasteurized skim milk [26], it gives grounds to assume that in ultrafiltration of NW, on the one hand, its physical and chemical characteristics will determine the peculiarities of practical implementation of the process. On the other hand, the effect of concentration polarization on the permeability of ultrafiltration membranes in this case may be comparable to the adsorption interaction in the system "membrane - native protein". At the same time, the phenomenon of concentration polarization is considered to be reversible [27,39,40] and in the membrane apparatus it can be influenced by changing the flow rate of circulation of the separated system, pulsation of the working pressure, imposition of ultrasound, electric field, etc. However, all these measures, as a rule, are taken on the basis of empirical expert knowledge obtained during operation, for example, ultrafiltration units at milk processing plants. And more reasonable recommendations in this area can be developed on the basis of modeling the phenomenon of concentration polarization, which determines the beginning and subsequent dynamics of the process of membrane fouling during ultrafiltration of both dairy raw materials in general and NW in particular. The main model proposed to explain the phenomenon of concentration polarization in baromembrane separation is based on the concept of gel polarization [28]. The hypothesis of this model is that when a certain value of transmembrane pressure (TMP) is exceeded, the rate of permeate passage through the membrane is limited by the resistance of the gel layer formed on the membrane surface, which increases the effective thickness of the membrane and thus reduces its hydraulic permeability. In this case, it is possible to calculate the rate of permeate transfer through the membrane (flux) based on the theory of mass transfer [29]. In this case, the gel layer should have a fixed concentration value (Cg) varying along its thickness. The permeate flux (Jv) is expressed as:

$$J_v = -D\frac{dC}{dx}\ , \qquad (1)$$

where: D - is the diffusion coefficient of dispersed phase particles in the dispersion medium; C - is the concentration of dispersed phase particles retained by the membrane, and dC/dx - is their concentration gradient.

Equation (1) can be integrated, but under given boundary conditions: the concentration of the solute on the membrane surface is fixed at the upper boundary (saturation level, Cg), and the concentration in the bulk flow is known (Cb). Then:

$$J_v = \frac{D}{\delta}\ln\left(\frac{C_g}{C_b}\right) \quad \text{or} \quad J_v = K \cdot \ln\left(\frac{C_g}{C_b}\right) \qquad (2)$$

where: δ - is the thickness of the boundary layer within which the concentration of dispersed phase particles varies.

It is assumed that under conditions when this model is valid, the flux through the membrane is invariant with respect to TMP and is determined only by the characteristics D, C and δ. The main drawback of this classical, and all models based on it [29, 30, 31] is that

it cannot in principle describe the whole range of flux Jv dependence on TMP. The problem is the difficulty in determining the mass transfer coefficient K [28]. But in cases where the coefficient K is determined experimentally, the validity of equation (2) is confirmed for a large number of macromolecular dissolved substances and colloidal particles. However, in our opinion, this is an unpromising way to solve the problem of theoretical description of the processes of baromembrane separation of dairy raw materials, because, firstly, it is still necessary to determine the mass transfer coefficient K only for special cases, and secondly, the physicochemical properties of NW, as an object of baromembrane separation, differ from its analogs, first of all, the presence of proteins in native form and the variability of characteristics. This gives grounds to believe that for NW the organization of the UF process and its prediction requires a fundamentally new paradigm of studying the processes of baromembrane separation: experimental studies should be directed not to the determination of intermediate data for the calculation of fluxes Jv, i.e., partial values of mass transfer coefficients K, but immediately to the formation of a database on the dependences of the main operating parameters of the process of membrane permeability Q by permeate and selectivity φ by the main components of the separated systems on external factors (TMP, V, t, τ, etc.). From the methodological point of view, this approach is fully consistent with the previous scientific paradigm in the study of membrane processes and is based on the application of the unique computational capabilities of modern computers. Data Science-based analysis of large data arrays obtained as a result of full factor experiments can provide a solution to the problem of predicting the kinetics of the ultrafiltration process of NW.

2.3 Analysis of published results of experimental studies of NW ultrafiltration

In a study [32], it was shown that only about 40% of whey proteins can be isolated from skim milk by microfiltration using polymeric membranes and a total of 60% when double volume of diafiltration water is added to the resulting MF retentate. Subsequent ultrafiltration of the permeate obtained from this process can produce concentrates of native whey proteins, α-lactalbumin and protein ingredients enriched with β-lactoglobulin. In this case, the TMP value is the main factor determining the kinetics of the NW ultrafiltration process. However, although the increase in TMP leads to a higher initial permeate flow rate, it also causes an increase in the level of concentration polarization, which contributes to accelerated blocking of the membrane pore space [33]. At the same time, the possibility of increasing the flow rate of circulation of the separated systems in the circuit of plants using roll-type membrane apparatuses, which causes a decrease in the level of concentration polarization in the submembrane zone, is very limited, compared to the option of using ceramic membranes. In [34] the results of comparative studies of the complex influence of temperature t and TMP on the permeability Q of permeate and water of three polymeric membranes (100 kDa, made of polysulfonamide, 50 kDa and 10 kDa of polyethersulfone) during ultrafiltration of NW are presented. For water, the high permeability of the membrane with a "cutoff" index by molecular weight of 100 kDa was established by the authors at t = 30 ° C and TMP = 2 bar. At the same time for permeate permeability index FMS max = 24.82 l/m^{2}h at t = 40 °C and TMP = 3 bar. And the lowest value of FMS_{min} = 14.17 l/m^{2}hour is at 5°C and 1 bar. Regression equations were also obtained (correlation coefficients respectively 0.95 and 0.91 results are significant ($p<0.05$)):

$$FMS_1 = 21.25 + 1.05X_1 + 3.65X_2 \quad (3)$$

and $$FMS_2 = 17.02 + 0.64X_1 + 1.64X_2 \quad (4)$$

where: $FMS_{1,2}$ - membrane permeability by permeate, X_1 - temperature (°C) and X_2 - pressure (TMP).

From the analysis of the presented data, it follows that the permeability of the membrane on permeate linearly increases in proportion to the parameters TMP and t. The influence of TMP and t parameters on the permeability of 50 kDa and 10 kDa membranes was investigated in the selected experimental data for 100 kDa membrane (TMP=2 bar and t=10°C). The authors found that during the first 20 minutes of the process, a rapid and very sharp drop in the permeate permeate permeability index was observed. Between 20 and 60 minutes, a further decrease in flow was observed. And after 90 minutes from the beginning of the process the permeate flux decreased by more than 50% (from 11.12 l/m2h to 5.47 l/m2h). In terms of average permeability, the 50 kDa membrane provided a flow rate of 7.19 L/m2hour, which was 60% lower than that of the 100 kDa membrane under the same conditions (TMP=2 bar and t=10°C). Table 1 shows the physicochemical characteristics of NW, retentate and permeate, as well as the selectivity values for the main components of the separated system obtained using the 10 kDa membrane at TMP=2 bar and t=10°C at 10°C and 2 bar.

Table 1. Selectivity for NW components, characteristics of its retentate and permeate obtained by UF method using a 10 kDa membrane at t=10°C and TMP=2 bar [34].

Components	NW	Retentate	Permeate	Selectivity (%)
Protein (g/100ml^{-1})	0.82±0.03	1.46±0.03	0.16±0.02	80.48
Dry solids (g/100ml^{-1})	5.81±0.03	6.78±0.02	4.15±0.09	28.57
Lactose (g/100ml^{-1})	4.96±0.01	4.41±0.08	4.16±0.01	16.12
Mineral (g/100ml^{-1})	0.57±0.04	0.48±0.03	0.49±0.02	14.04
Electrical conductivity, (μScm^{-1})	5.68±0.04	5.61±0.06	5.77±0.09	-
Acidity, T°	12.67±0.57	13.33±0.57	13.00±0.5	-
pH	6.51±0.01	6.55±0.01	6.58±0.02	-

The authors have shown that the 10 kDa membrane has a protein selectivity of about 80%, which is higher by about 10% compared to the 50 kDa membrane. Of particular interest in the present work are the electrophoresis data of proteins in retentate and permeate (Figure 1) obtained using the 10 kDa membrane. Based on the fact that the permeate sample shows weak signals for β-lactoglobulin (18.3 kDa) and α-lactalbumin (14.2 kDa) in permeate, it is concluded that these two proteins are in retentate. The band of BSA (69 kDa) is absent for the permeate, which may indicate its complete retention by the membrane. In addition, the presence of other proteins and fractions with different molecular masses in retentate and permeate is noted.

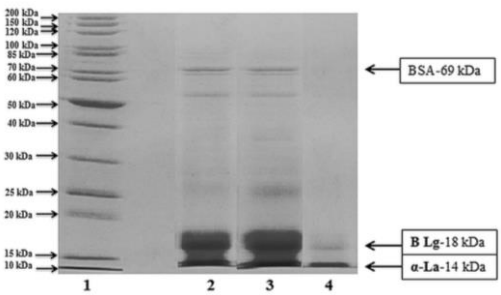


Fig. 1. SDS-PAGE gel electrophoresis image for UF retentate and permeate samples obtained using a 10 kDa membrane. Column 1 - molar mass marker; Column 2 - NW; Column 3 - NW retentate after UF; Column 4 - NW permeate after UF [34].

However, the above experimental data, in our opinion, can be used only as indicative data for our own studies. This is due to the fact that the flow rate of circulation of the separated system, as well as interfactor interaction can have a significant effect on the kinetics of the process. The protein selectivity values established by the authors for 100 kDa membranes - 55%, and for 10 kDa - 80% - require experimental verification.

2.4 Purpose and objectives of experimental research in laboratory conditions

The purpose of experimental studies in laboratory conditions is to study the basic regularities of the process of ultrafiltration separation of NW. To achieve the goal it is necessary to solve the following tasks:

1. To determine the ranges of the main operating parameters of ultrafiltration NW (including diafiltration).

2. To obtain and carry out theoretical analysis of the mathematical model of the process of ultrafiltration separation of NW (in the form of regression equation), allowing to quantitatively determine the permeability of ultrafiltration membrane depending on the main operating parameters of the separation process - TMP, flow rate of circulation V and temperature τ in the channel of the baromembrane apparatus.

3. to determine the content (mass fraction in %) of casein and whey proteins in the UF-retentate (UF-permeate) of NW.

3 Materials and Methods

3.1 Procedure of experimental study of the ultrafiltration process of NW

Baromembrane separation processes of liquid polydisperse high molecular weight systems, including ultrafiltration of NW, is a complex of interrelated and proceeding, as a rule, in interdependence of physical and chemical events. Accurate description of such processes in most cases difficult (or impossible) to reliably determine the regularities of their individual stages, and / or mutual influence on each other. Accordingly, taking into account the variability in time, all membrane processes are referred to the class of stochastic processes, in which the value of output parameters is not in unambiguous correspondence with the input ones. Statistical and probabilistic methods are usually used to describe such processes [35, 36]. One of the most widespread methods that has proven itself in solving such problems from the point of view of practical application of research results is considered to be the method of complete factorial experiment (CFE). From the analysis of our own experience in conducting studies of the process of baromembrane separation of secondary dairy raw materials, it was established that the main external factors that have a predominant effect on the permeability of permeate Q of polymeric membranes in the separation of NW should be considered: the value of working pressure TMP, the flow rate of circulation of the flow of the separated system V and its temperature t. Analysis of data from open sources of information has shown that for baromembrane separation of NW it is necessary to use polymeric membrane with "cut-off" index by molecular weight of retained particles equal to 10 kDa (pH of washing solution - 2-12, working temperature - up to 5-15 ˚ C, TMP_{max}=5.0 bar) in baromembrane unit Spectrum Labs KrosFlo Research II TFF System with cassette-type apparatus Novaset-LS-LHV SS316. In accordance with the technical characteristics of this experimental laboratory equipment, we introduced the following limitations: TMP, ΔP_{max} = 4.5 bar, V = 140 ml/minute (units of measurement - in accordance with the tariff scales of measuring devices of the experimental unit). The NW temperature is limited to the range $t_{max} \leq 15°C$. The value of the concentration factor is

accepted CF ≤ 4.5, and the duration of the ultrafiltration process is limited to τ ≤ 60 minutes, after which the membrane should be washed. Depending on the results of analysis of the obtained experimental data, further step-by-step increase of the parameter τ up to 6-8 hours and more, CF over 4.5 is possible. Experimental work - according to the plan of CFE 2^3 with threefold repetition of each experiment. The order of execution of each experiment provided the following sequence of basic operations:

1- Determination of NW temperature, titratable acidity, mass fraction of dry solids, selection, freezing and storage of raw material sample for Kjeldahl protein.

2. Checking the water permeability Qv of the membrane (initial value of Qv = 34-36 ml/min.).

3. Filling the working tank of the unit with raw materials in the amount of 450 ml, controlling the temperature of the process.

4. Bringing the plant to the operating parameters of the process set by the plan of the current experiment.

5. Bringing the control and measuring equipment into working position and the beginning of experimental work.

6. Control and fixation of the received data, their operative analysis (correction, if necessary, of the working parameters of the process), selection, freezing and sending for storage of samples of retentate and permeate for Kjeldahl protein.

7. Depending on the set operating parameters of the process of ultrafiltration NW sampling permeate is carried out after τ = 3÷30 minutes, but not less than 6-8 points.

8. Completion of the process and washing the membrane to 90-95% recovery of the index from the initial Qv.

9. On the basis of analysis of the experimental data processed on the computer, discussion of the results, formulation of working conclusions, hypotheses and formation of material for the working plan of the next stage of research.

3.2 Experimental equipment and specialized instrumentation

To create the information base of the research the results of the literature data analysis from the libraries of the Russian State Library as well as the global Internet, etc. were used. Physicochemical characteristics of NW as an object of ultrafiltration were determined according to traditional methods using standard instruments:

- laboratory refractometer IRF-454B2M - determination of dry matter content in the separated systems;
- UDK-149 VELP protein analyzer - determination of protein fractions by Kjeldahl method;
- electronic titrator Brand Titrette - determination of titratable acidity.

Reproducibility and reliability of the results of experimental studies are conditioned by the use of arbitration, standard and generally accepted methods of research (Table 2).

Table 2. Methods

№	Name	Document references
1	Acidity, T°	GOST 3624-92
2.	Solids content, % mass.	GOST 34128-2017
3.	Total Nitrogen, TN, %.	AOAC 991.20-1994
4.	Casein Nitrogen, CN, %.	AOAC 998.07 Casein Nitrogen Content of Milk Kjeldahl Method, Indirect Method
5.	Whey Protein Nitrogen, WPN/ Non-protein nitrogen, %.	Velp Scientifica Application Note F&F-K-005-2013/A1
6.	Temperature, C°	GOST 26754-85

As a result of analyzing the literature data [37,38] it was found that when studying the influence of the main parameters of the process of ultrafiltration separation of NW, first of all, it is necessary to determine the optimal value of TMP. For this purpose, before conducting experiments, a sample of NW in a clean glass container was taken in a volume of up to 450 ml, poured into the initial container of the baromembrane unit and began the separation process in accordance with the plan of the current experiment. Control samples of permeate and retentate in the volume from 10 to 50 ml were placed for short-term storage in a refrigerator. Determination of the dependence of membrane permeability Q on TMP was carried out according to the following procedure. With the help of a measuring container the volume w of permeate is measured, which is collected in a special container during a given interval of continuous operation of the unit at constant values of TMP, V and t. All collected permeate is weighed using electronic scales, and the results are used to calculate the average value of membrane permeability for a given period of time:

$$\rho = \frac{m}{w}, \quad (5)$$

$$Q = \frac{m}{s\tau}, \quad (6)$$

where: ρ - density of permeate, kg/m^3; m - mass of permeate, kg; w - volume of permeate, m^3; Q - permeability of membrane on permeate, kg/m^{2}hour; s - area of working surface of membrane, m^2, τ - duration of process, hour.

At threefold repetition of experiments and standard mathematical processing the obtained results for Q are drawn up in the form of appropriate tables and graphs using standard PC programs. When selecting the approximating curves $Q=f_1(TMP)$ it is necessary to take into account that at the initial moment of time each of them should pass through the origin of the corresponding coordinates. Thus, equations of the form $Q=f_1(TMP)$, $Q=f_2(V)$, $Q=f_3(t)$ are obtained. To obtain initial experimental data, by which it would be possible to find the dependences of membrane permeability on the time of the process, i.e. $Q=f_3(\tau)$, the volume of filtrate, which was collected in a special container was measured every 1-60 minutes of continuous operation of the unit at the given values of operating parameters of the process. It should be noted that in the experimental study of NW ultrafiltration process the analysis of the obtained data is significantly complicated by the fact that the permeability $Q=f(TMP,V,t)$ is a complex function of three variables. In turn, some of these variables also represent functional dependences. Therefore, in order to improve the efficiency and quality of scientific research, it is advisable to use mathematical methods of planning and processing of experimental data. Consequently, rational values of the process duration τ will be limited by the value of C_{max}. However, this is true only for the periodic scheme of operation of the membrane plant. If fresh NW with initial value C_{max} of dispersed phase particles is constantly added to the initial vessel, then in this case the growth of concentration of these particles slows down. Such scheme of functioning of the membrane unit can be called semi-periodic. The following assumptions concerning its realization should be made:

1. the volume of the separated system V_k in the membrane channel of the apparatus is always constant, since the filtrate flow rate V_f is always compensated by the supply ΔVΣ of the separated NW to the unit, i.e. there is a relation: $Vf=\Delta V\Sigma$.

2. Recycling of the separated system increases the content of dispersed phase particles in the initial vessel, which changes the membrane permeability in proportion to the process duration τ.

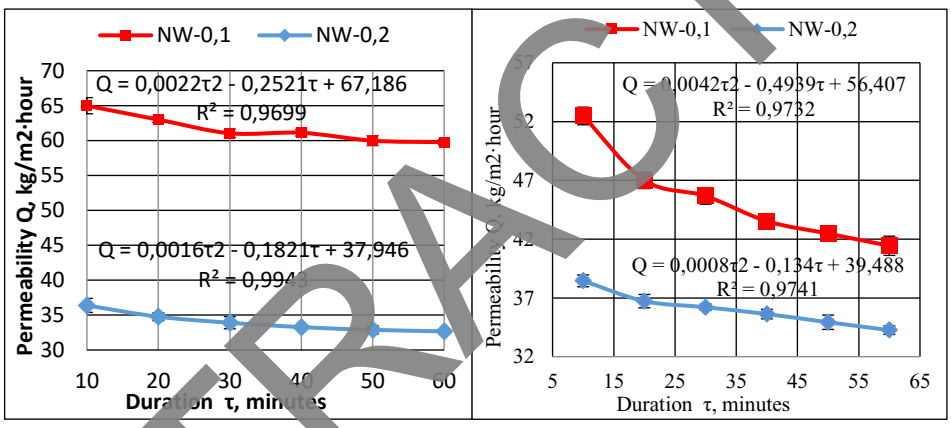
3. During the process of baromembrane separation of NW, a layer of deposits is formed on the membrane surface, which significantly changes its permeability depending on the content of dispersed phase in the separated NW.

4 Results and Discussion

NW obtained by microfiltration using membranes with conditional pore diameter (CPD) 0.1 (NW-0,1) and 0.2 μm (NW-0,2) of skim milk was used as a feedstock. Varying the main operating parameters of TMR, V and t, was carried out according to the plan of CFE 2^3 (Table 3) in ranges corresponding to data from open scientific publications on the corresponding ongoing R&D topics. The results of these preliminary studies are presented graphically in Figure 2.

Table 3. CFE Implementation Plan Matrix 2^3.

Parameter	Experience number							
	1	2	3	4	5	6	7	8
Pressure, TMP	+1	-1	+1	-1	+1	-1	+1	-1
Flow rate of circulation, V	+1	+1	-1	-1	+1	+1	-1	-1
Process temperature, t	+1	+1	+1	+1	-1	-1	-1	-1



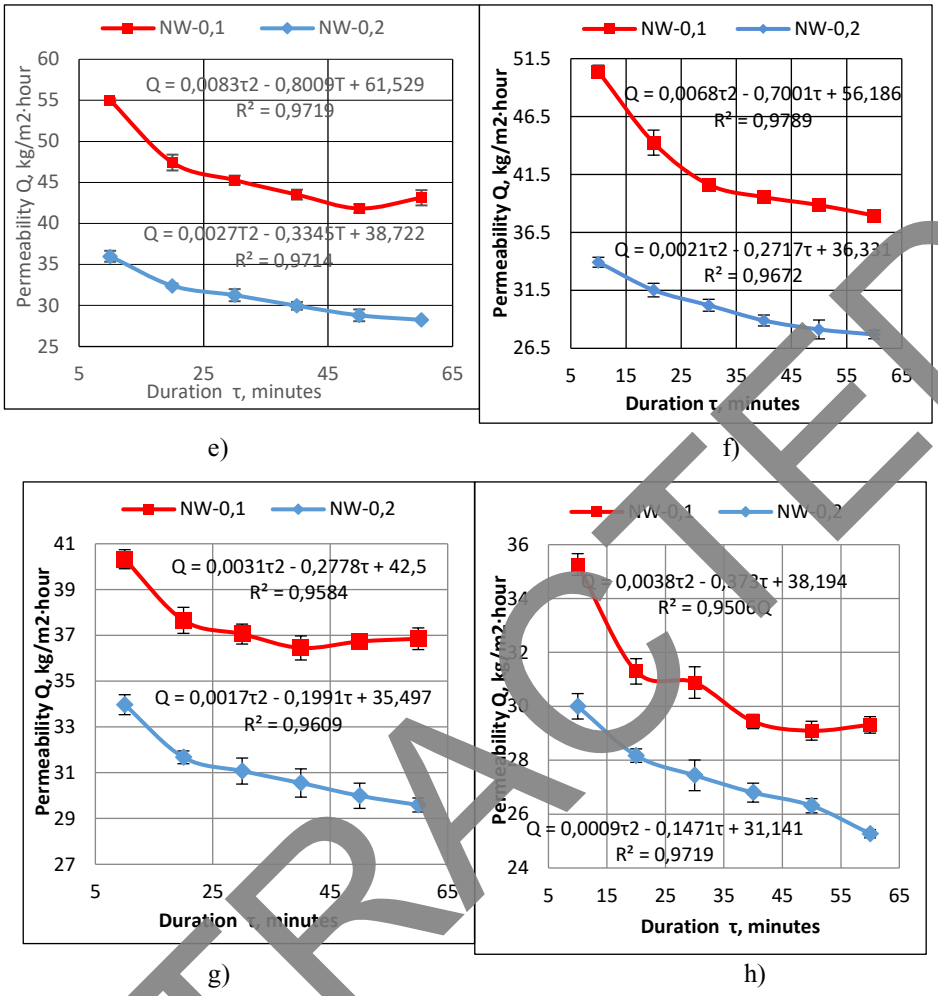


Fig. 2. Dependence of permeability of Novaset LS membrane with CPD=10 kDa on the duration of ultrafiltration process NW: a) TMP=4.5 bar, V=140 ml/min, t=15 °C; b) TMP=4.5 bar, V=140 ml/min, t=5 °C; c) TMP=4.5 bar, V=70 ml/min, t=15 °C; d) TMP=4.5 bar, V=70 ml/min, t=5 °C; e) TMP=2.5 bar, V=140 mL/min, t=15 °C; f) TMP=2.5 bar, V=140 mL/min, t=5 °C; g) TMP=2.5 bar, V=140 mL/min, t=5 °C; h) TMP=2.5 bar, V=70 mL/min, t=5 °C.

Experimental studies of membranes were carried out during 60 minutes of working time after their output to the operating mode. The choice of the process duration is due to the fact that, firstly, the permeability index of membranes on permeate Q during this time remains quite stable from the point of view of achievability of the research goal. And secondly, after the end of the first hour of membranes operation in standard washing modes the control indicators of their permeability by water are restored (up to 95-97% of the passport values). At the initial stage each membrane passed the stage of "shrinkage" when the process was carried out on distilled water in accordance with the recommendations of the manufacturer's data sheet.

The analysis of the obtained dependences of the form Q=f(TMP) showed that permeability Q on permeate of membrane with CPD=10 kDa Novaset LS at ultrafiltration NW-0,2 is lower by 20-22 % (depending on the operating modes of the process) than at separation NW-0,1. According to the process sieve model for ultrafiltration of NW-0,1 and NW-0,2, such permeability values are quite adequate because NW-0,2 has a higher total

protein content than NW-0,1. The selectivity of both membranes with respect to protein components is quite comparable, which makes it reasonable to conduct experimental studies of ultrafiltration of NW-0,1 using membranes with CPD =10 kDa according to the plan of CFE 2^3.

To study the main regularities of the ultrafiltration process of NW obtained on the polymeric membrane of MCM (Vladisart) grade with CPD=0.1 μm (NW-0,1), the influence of TMP values, flow rate of circulation V and temperature τ of NW in the membrane channel of the apparatus on the permeability Q of Novaset-LS-10 kDa polymeric membrane was determined. As a result of CFE 2^3, the desired regression equation in coded variables is obtained in the following form: $Y = 31.4 + 1.6X1 + 1.4X_1 + 1.4X_2 + 0.8X_3 + 0.6X_1X_2 - 0.4X_1X_3 - 1.0X_2X_3 - 0.3X_1X_2X_3$ *. From its analysis it follows that the greatest influence on the output parameter (membrane permeability Q by permeate) has the value of transmembrane pressure TMP in the channel of the ultrafiltration apparatus of cassette type. Then, the flow rate of circulation V of NW-0,1, compared to t, has a greater influence on the Q value. The obtained data of the experimental study agree with information from open publications. When optimizing the operating parameters of the process of ultrafiltration of MF-permeate skim milk, it is necessary to take into account the interfactorial influence on Q.

To predict the kinetics of the process of ultrafiltration separation of NW-0,1, it is necessary to establish the area of maximum values of permeability of Novaset LS membrane with a "cutoff" index by molecular weight of 10 kDa. Accordingly, the extremum of function (*) was determined for this purpose. The interior point is $M_0(X10,X20,X30)$, δ is the neighborhood of this point, which is a ball centered at the point M_0 of radius $\delta>0$. The necessary conditions for the existence of the extremum are: $dY/(dX_i) = 0$; i=1,2,3. As a result of solving the corresponding system of equations, we obtain that $M_0(2.0;4.0;2.5)$ is a stationary point. We check the sufficiency of the extremum condition by calculating the values of the partial derivatives of the 2nd order of the function (*) at the point M_0 by making the Hesse matrix. The calculation of the angular minors leads to the following result: $\delta_1=0$; $\delta_2<0$; $\delta_3>0$; Since $\delta_3\neq0$, therefore, the point M_0 belongs to the saddle point. Obviously, for the function $Y=f(X_1, X_2, X_3)$ any clear three-dimensional geometric interpretation of the obtained result is basically excluded. At the same time, by the fact of presence of the saddle point we can conclude that there is a set of combinations of values of variables TMP, V and t, at which the parameter Q takes values close to the maximum. It follows that at practical realization of ultrafiltration process NW-0,1 selection of rational values of TMP, V and t should be carried out on the basis of analysis of empirical data of protein fraction ratio in retentate and permeate (Table 4).

Table 4. Quantitative composition of protein fractions in NW-0,1 (p=0.95).

Sample name (experiment number)	Total protein, %	Casein protein, %	Whey protein, %	Non-casein protein, %	Non-protein, %
	Retentate, 10 kDa membrane				
1	0.59	0.20	0.28	0.39	0.10
2	0.69	0.27	0.30	0.42	0.12
3	0.55	0.18	0.30	0.38	0.07
4	0.70	0.24	0.31	0.46	0.15
5	0.76	0.30	0.33	0.47	0.14
6	0.61	0.23	0.28	0.39	0.10
7	0.87	0.32	0.41	0.55	0.14
8	0.82	0.33	0.39	0.49	0.10

As a result of the analysis of the obtained experimental data it was established that at ultrafiltration of microfiltration permeate the following average values of mass fractions can be obtained: casein fraction of proteins in NW-0,1 - 0,26 %, total protein in retentate R-0,1 - 0,69, whey proteins in permeate P-0,1 - 0,33 %.

5 Conclusion

1. Using Novaset-LS membrane with a CPD of 10 kDa, the operating modes of ultrafiltration of native whey NW-0,1 were determined experimentally: TMP=4.5 bar, V=70 ml/min., t=15 °C or TMP=3.5 bar, V=105 ml/min., t=10 °C at target ratios of casein/whey proteins in retentate of 37/63 and 35/65, respectively.

2. The regression equation of the form Q=f(TMP,V,t) was obtained, which allows (after its translation into natural values) to quantitatively determine the value of permeability by permeate of ultrafiltration membrane depending on the operating parameters of the separation process - TMP, V and temperature t NW in the channel of the baromembrane apparatus.

3 On the basis of theoretical analysis of the mathematical model of the process of ultrafiltration of NW, obtained as a regression equation by performing CFE 3^2, it is established that there are many combinations of values of variables X_1(TMP), X_2(V) and X_3(t), at which the parameter Y(Q) takes values close to the maximum in a given area of experimental study.

4. It was found that when using a membrane with a CPD of 10 kDa, a high content of casein protein fractions relative to whey proteins (35/65) is observed in the true protein of UF-retentates.

Acknowledgement

The work was carried out in the North-Caucasus Federal University with the financial support of the Ministry of Science and Higher Education of the Russian Federation within the framework of the implementation of a complex project to create a high-tech production facility "Creation of the first in Russia high-tech production of lactulose prebiotic and functional dairy ingredients for import substitution in medicine, veterinary medicine, infant formulae, production of therapeutic and prophylactic products for humans and animals" (Agreement on the provision of a subsidy from the federal budget for the development of cooperation between a state scientific institution and a real sector organization for the implementation of a complex project to create a high-tech production №075-11-2022-021 dated April 7, 2022) under the Resolution of the Government of the Russian Federation of April 9, 2010 No. 218.

References

1. Fox P. F. et al. Milk proteins //Dairy chemistry and biochemistry. 145-239 (2015) doi:10.1007
2. Cebo C. et al. //Journal of dairy science. **93**, 868-876 (2010) doi:10.3168/jds.2009-2638
3. Deeth H., Bansal N. Whey proteins: An overview //Whey proteins. 1-50 (2019) doi:10.1016/B978-0-12-812124-5.00001-1

4. Tsabouri S., Douros K., N Priftis K //Endocrine, Metabolic & Immune Disorders-Drug Targets (Formerly Current Drug Targets-Immune, Endocrine & Metabolic Disorders). **14**, 16-26 (2014)
5. Korhonen, H. Bioactive components in bovine milk. In Y. W. Park (Ed.), Bioactive components in milk and dairy products. Ames, IA, USA: Wiley-Blackwell. 13-42 (2009). doi:10.1002/9780813821504
6. Gesan-Guiziou, G. Extraction of functional food ingredients and nutraceuticals from dairy. In J. Shi (Ed.), Functional food ingredients and nutraceuticals: Processing technologies. Boca Raton, FL, USA: CRC Press. 235-267 (2015).
7. Schäfer J., Schubert T., Atamer Z. //International Dairy Journal. **97**, 222-229 (2019) doi:10.1016/j.idairyj.2019.06.009
8. Andersson I. M. et al. //International Journal of Food Science & Technology. **56**, 480-492 (2021) doi:10.1111/ijfs.14663
9. Anema, S. G. *The whey proteins in milk: Thermal denaturation, physical interactions and effects on the functional properties of milk.* In A. Thompson, M. Boland, & H. Singh (Eds.), Milk proteins: From expression to food. Amsterdam, the Netherlands: Elsevier Science. 239-282 (2009). doi:10.1016/B978-0-12-815251-5.00009-8
10. Zhang Y., Zhong Q. //Journal of Agricultural and Food Chemistry. **60**, 7526-7531 (2012) doi:10.1021/jf3021656
11. Wijayanti, H. B., Bansal, N., Deeth, H. C. Comprehensive Reviews in Food Science and Food Safety. **13**, 1235-1251 (2014). doi:10.1111 /1541-4337.12105
12. Bogahawaththa D. et al. //Innovative Food Science & Emerging Technologies. **47**, 301-308 (2018) doi:10.1016/j.ifset.2018.03.016
13. Guinee T. P. //International Dairy Journal. **121**, 10509 (2021) doi:10.1016/j.idairyj.2021.105095
14. Dissanayake M. et al. //International Dairy Journal. **31**, 93-99 (2013) doi:10.1016/j.idairyj.2013.02.002
15. Svanborg, S., Johansen, A.-G., Abrahamsen, R. K., Skeie, S. B.. International Dairy Journal. **37**, 26-30 (2014) doi:10.1016/j.idairyj.2014.02.004
16. Lorenzen, P. C., Clawin-Radecker, I., Einhoff, K., Hammer, P., Hartmann, R., Hoffmann, W., et al. International Journal of Dairy Technology. **64**, 166-178 (2011) doi:10.1111/j.1471-0307.2010.00656.x
17. Mamay D., Babenyshev S., Mamay A., Ivanets V., Bratsikhin A. *Microfiltration processing of raw materials for the fermented milk product making*/ Intelligent Biotechnologies of Natural and Synthetic Biologically Active Substances. Cham. 10-17 (2022) doi:10.1007/978-3-030-69641-6_2
18. Sanmartín B. et al. //Small Ruminant Research. **110**, 52-56 (2013) doi:10.1016/j.smallrumres.2012.11.029
19. Luck P.J., Yong Y.H., Barbano D.M. Journal of Dairy Science. **96**, 5522-5531 (2013) doi:10.3168/jds.2013-6617
20. Luck P. J. et al. //Journal of dairy science. **96**, 5522-5531 (2013) doi:10.3168/jds.2013-6617
21. S. Svanborg/ J. Dairy Sci. **98**, 5840 **(**2015) doi:10.3168/jds.2014-9039
22. S. Svanborg, K. Abrahamsen, B.Skeie. International Dairy Journal. **60**, 14-23 (2016) doi:10.1016/j.idairyj.2015.12.007

23. Evans J, Zulewska J, Newbold M, Drake MA, Barbano DM Journal of Dairy Science. **92**, 4773-4791 (2009) doi:10.3168/jds.2009-2194
24. Evans, J., Zulewska, J., Newbold, M., Drake, M. A. Journal of Dairy Science. **92**, 4773-4791 (2009) doi:10.3168/jds.2009-2194
25. Evans, J., J. Zulewska, M. Newbold, M. A. Drake, and D. M. Barbano. J. Dairy Sci. **93**, 1824–1843 (2010) doi: 10.3168/jds.2009-2723.
26. Uppu A., Chaudhuri A., Das S. P. //Desalination. **468**, 114053 (2019) doi: 10.1016/j.desal.2019.06.019
27. Mamay D., Babenyshev S., Mamay A. Membrane process for the extraction of casein and whey proteins from skim milk/ E3S WEB OF CONFERENCES. International Scientific Conference "Fundamental and Applied Scientific Research in the Development of Agriculture in the Far East" (AFE-2023). EDP Sciences. 01020 (2023) doi: 10.1051/e3sconf/202346201020
28. Halaui R. et al. //Journal of membrane science. **379**, 370-377 (2011) doi: 10.1016/j.memsci.2011.06.011
29. Mai Z. et al. //Desalination. **496**, 114094 (2019) doi: 10.1016/j.desal.2019.114094
30. A. Sagiv, A. Zhu. Journal of Membrane Science, 464, 161–172 (2014) doi: 10.1016/j.memsci.2014.04.001
31. Chen G. Q. et al. //Food Engineering Reviews. **15**, 438-465 (2023) doi: 10.1007/s12393-022-09330-2
32. Beckman S.L., Zulewska J., Newbold M., Barbano D.M. J. Dairy Sci. **93**, 4506–4517 (2010) doi: 10.3168/jds.2010-3216
33. Hurt, E.; Barbano, D.M.. J. Dairy Sci. **93**, 4928–4941 (2010) doi: 10.3168/jds.2010-3121
34. S. Iltchenco, D. Preci, C. Bonifacino. Ciência Rural. **48** (2018) doi:10.1590/jds.0103-8478
35. S. A. Ivanova, R. Sh. Garifullin, T. V. Chaplygin//Technique and technology of food production **1**, 75-79 (2011)
36. Istratova, E. E. Development and study of an ultrafiltration apparatus with combined removal of the diffusion boundary layer and membrane cleaning: specialty 05.18.12 "Processes and devices of food production" dissertation for the degree of candidate of technical sciences/Istratova Evgenia Evgenievna. - Kemerovo.**142** (2008)
37. Pavel N. et al. //Foods and Raw materials. **6**, 350-357 (2018) doi:10.21603/2308-4057-2018-2-350-357
38. Timkin V. A. et al. *Baromembrane technology of milk processing* //Dairy Industry. (2017)
39. Babenyshev S., Nesterenko P., Bratsikhin A., Zhidkov V., Mamay D., Maximenko A. / Foods and Raw Materials. **6**, 350-357 (2018) doi:10.21603/2308-4057-2018-2-350-357
40. Babenyshev S., Mamay D., Borisenko A., Mamay A., Bratsikhin A., Amanova S. / Journal of Hygienic Engineering and Design. **33**, 219-224 (2021)