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Empirical predicting permeate flux in skim milk microfiltration

Dmitriy S. Mamay*, Sergey P. Babenyshev, Ivan A. Evdokimov, Angelina V. Mamay, Vyacheslav A. Lisitsyn

North-Caucasus Federal University, Stavropol, Russia

* e-mail: dima-mamaj@yandex.ru

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Abstract:
Native micellar casein and whey proteins can be obtained from skim milk by microfiltration. It is a popular method that yields high-quality dairy products. The article introduces an empirical approach to predicting the permeate flux value during microfiltration of skim milk. The research objective was to produce retentates with a target ratio of protein fractions in the true protein.

The physicochemical profile of skim milk was studied by standard methods. The experimental microfiltration involved a Spectrum Labs KrosFlo® Research II TFF System.

The research revealed the optimal operating modes of microfiltration and diafiltration for 0.1 μM membranes (Vladisart, Russia): operating pressure 0.2–2.5 bar, circulation rate 65–140 mL/min, temperature 50 ± 1°C. These modes made it possible to obtain a ratio of casein to whey proteins that exceeded 95:5. At the optimal ratios of pressure, circulation rate, and temperature, the amount of casein proteins grew from 2.2 to 4.0% in relation to whey proteins.

The grid search analysis confirmed a set of similar values of $Y = f(X_1, X_2, X_3)$. Microfiltration of skim milk proved effective at different combinations of pressure, circulation rate, and temperature, depending on the production technology, target products, and target ratio of mass fractions of casein and whey proteins in the true protein.

Keywords: Filtration, separation, dairy industry, casein, whey proteins, empirical approach

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INTRODUCTION

High-quality foods based on animal proteins have always attracted consumers' attention. The ever-growing demand creates prerequisites for new, more efficient processing methods. For instance, the method of membrane separation divides milk into fractions – casein, whey proteins, lactose, etc. – to be used in functional foods [1, 2]. Microfiltration-derived micellar casein is a popular raw material in the dairy industry. Microfiltration separates permeate from enzymes and technological ingredients to use it in cheese or cottage cheese formulations. Microfiltration-derived permeate contains all native whey proteins, including *β*-lactoglobulin and *α*-lactalbumin [3, 4]. However, the permeate flux depends on the properties of raw materials and membranes, as well as on the operating parameters. As the membrane fouling increases, the equipment performance goes down while the operating cost goes up. As a result, the method of membrane filtration has serious industrial limitations. Permeate flux, or the permeate flow rate, is the volume per unit of time and membrane area. Its prediction and optimization are crucial for effective baromembrane separation.

Forecasting and optimizing involve two methodological approaches. The first one relies on optimization and planning that determine the most effective processing options and the highest yield across the entire process line [5]. The main disadvantage of this approach is that the result depends on special criteria, e.g., the physicochemical profile of the raw materials, product range, environmental safety, etc. [6–8]. As a rule, these criteria are individual for each dairy enterprise. The second approach presupposes creating optimal conditions for microfiltration and fractionation by using selected modes of

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preparatory technological operations. The physicochemical and sensory indicators of the separation products should comply with the technological requirements. The main disadvantage is that the operating microfiltration parameters have to be optimized for many variables, and their characteristics depend on the specific requirements for the target product. The physicochemical properties of raw materials and membrane materials, as well as the membrane separation modes, are to be compared for a number of quantitative and quantitative characteristics.

To calculate the future permeate flux, dairy producers use different models for membrane processes [9–11]. The models can be divided into phenomenological (concentration polarization, osmotic counterpressure, series resistance, film, etc.) and non-phenomenological ones [12–16]. Phenomenological predictions are highly variable. However, they provide information on the effect of various parameters on the permeate flux, which is very important for scaling. Non-phenomenological models can be empirical, semi-empirical, or statistical. They are more accurate, but only if the membrane and raw materials have a limited physicochemical variability.

Some researchers believe that membrane separation can be optimized for only one type of liquid high-molecular polydisperse systems [17, 18]. The Box-Jenkins model of autoregressive integrated moving average (ARIMA) predicts the permeate flux for fruit juice ultrafiltration: it provides a 99% correlation between the precalculated and experimentally obtained values [19]. However, it does not reveal the patterns between the membrane characteristics, the physicochemical parameters of the system, the hydrodynamic phenomena during filtration, and the operating parameters.

The choice of microfiltration method for fractionating skim milk, i.e., technology, design, and processing conditions, relies on expert knowledge and empirical data [20–27]. Modeling also relies on the analysis of experimental data and is usually limited by the design of the baromembrane device and/or the operational properties of the membranes [28]. During skim milk microfiltration, the effect of each operating parameter on the permeate flux depends on the type of membrane [27]. As a result, no general theoretical model of skim milk microfiltration has been obtained so far to satisfy the needs of practical engineering.

The baromembrane separation of liquid polydisperse high-molecular systems is a complex of interrelated physical and chemical phenomena. Their accurate mathematical description is complicated by the complexity or impossibility of determining the patterns for individual stages and their interrelations. Considering the time-to-time variability, all membrane processes are stochastic, which means that the output values do not reliably correlate with the input ones.

Such processes are usually described by means of probabilistic statistics. Full factorial experiment is the most popular method applied to baromembrane separation of skim milk. It determines the effect of the main external deterministic factors on a certain optimization parameter. This method reveals the optimal permeability (Q) of microfiltration membranes for particular values of operating variables, e.g., pressure (ΔP) and the circulation rate of the separated system (V). Our own experience [29, 30] in experimental baromembrane separation of various dairy raw materials shows that the effect of pressure or circulation rate on the optimal permeability depends on the temperature of the separated system.

To predict the permeate flux during skim milk microfiltration, we used experimentally-obtained regression equations combined with grid search calculations. This approach makes it possible to justify the use of microfiltration separation at various combinations of variables, i.e., pressure, circulation rate, and temperature. In addition, our method also reveals the permeability values for microfiltration membranes.

The protocol was as follows:

– the determination of the operating parameters for the skim milk microfiltration, at which the true protein in the microfiltration retentate has at least a 95:5 mass fraction ratio of casein and whey proteins;

– the establishment of the conditions and modes for the skim milk microfiltration/diafiltration process to obtain a 97:3 ratio of casein to whey proteins in the true protein of the microfiltration retentate.

STUDY OBJECTS AND METHODS

The research involved skim milk supplied by a Stavropol milk factory (MK Stavropolsky LLC, Russia), pretreated as in State Standard GOST 31449-2013: Raw cow's milk. Technical conditions (Table 1).

The physicochemical profile of skim milk to be subjected to microfiltration was described using standard methods. An IRF-454B2M universal laboratory refractometer served to reveal the solids in the separated systems. A UDK-149 VELP protein analyzer determined the protein fractions in line with the Kjeldahl method. A Brand® Titrette® electronic titrator measured the titratable acidity.

All experiments were replicated 3–5 times; the standard research methods also contributed to the reliability and reproducibility (Table 2).

The microfiltration involved polymer membrane elements with an average pore diameter of 0.1 μM [29–34].

The separation process relied on a Spectrum Labs KrosFlo® Research II TFF System with a Novaset-LS-LHV SS316 cassette device (Fig. 1). The device

Table 1 Composition and physicochemical profile: Whole vs. skim milk ($p = 0.95$)

Indicator	Whole milk	Skim milk
Protein, %	3.0	3.0
Fat, %	3.80	0.02
Non-protein nitrogen, %	0.1	0.1
Lactose, %	4.7	4.7
Minerals, %	0.7	0.7
Titratable acidity, °T	17	17
Density, kg/m^3	1,029	1,034

Table 2 Research methods

Indicator	Source
Titratable acidity, °T	State Standard GOST 3624-92: Milk and dairy products. Titrimetric methods for acidity determination
Solids, %	State Standard GOST 34128-2017: Juice products. Refractometric method for determining the mass fraction of soluble solids
Total nitrogen, %	AOAC 991.20-1994 Nitrogen (total) in milk. Kjeldahl method
Casein nitrogen, %	AOAC 998.07 Casein nitrogen content of milk. Kjeldahl method, Indirect method
Whey protein nitrogen, %	Velp Scientifica Application Note F&F-K-005-2013/A1: Whey protein determination in milk according to the Kjeldahl method
Temperature, °C	State Standard GOST 26754-85: Milk. Methods of temperature measurement
Protein composition	State Standard GOST P 53761-2009: Milk. Identification of protein composition by electrophoresis in polyakrilamide gel

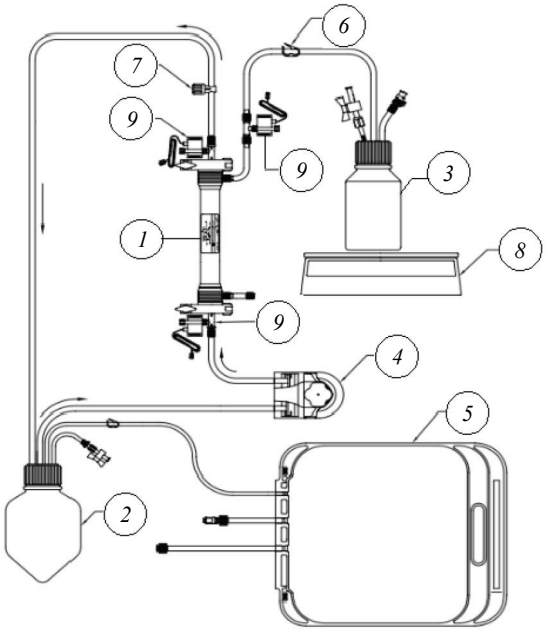


Figure 1 KrosFlo® Research IIi TFF tangential filtration system: *1* – membrane module, *2* – skim milk tank, *3* – permeate tank, *4* – peristaltic pump, *5* – control unit, *6* – permeate flow throttle, *7* – retentate flow throttle, *8* – permeate flow scale, *9* – pressure sensors

had 0.1 μM industrial polyethersulfone microfiltration membranes (Vladisart, Russia). The membranes worked at 40–55°C of skim milk and required 0.1–3.5 bar of operating pressure.

The mode limits were in line with the technical characteristics of the laboratory equipment:

– ΔP_{max} = 2.5 bar (Spectrum Labs KrosFlo® Research II TFF System);

– V_{max} = 140 mL/min (Spectrum Labs KrosFlo® Research II TFF System);

– $t_{max} \leq 50$°C.

Each experimental run lasted for 7–8 h; the permeate and the retentate returned to the initial tank after each processing circle. To measure the effect of pressure (ΔP) and circulation rate (V) on the optimal membrane permeability (Q), Stage 1 followed a 2^2 full factorial experiment (Table 3) [35]. We defined permeate flux J by weighing the permeate on a digital scale. The Eq. (1) was as follows:

$$J = \frac{W_p}{\tau \times A_p} \quad (1)$$

where W_p is the weight of the permeate that went through the membrane per unit of time τ; A_p is the operating area of the membrane surface.

The amount of permeate was checked every 5 min with an accuracy of ± 0.1 g at a constant temperature of the separated system t_1 = 50 ± 1°C.

Stage 2 yielded a matrix for regression equation with various pressures (ΔP), circulation rates (V), and temperatures (t) at a constant solids mass fraction in the separated system (Table 4).

The laboratory work was divided into the following stages to obtain retentate with a 95:5 ratio of casein to whey proteins:

1. We filtered skim milk through 0.1 μM membranes in a closed cycle for 7–8 h to achieve the maximal value of membrane selectivity for casein. After that, we continuously removed permeate at a concentration factor of 3–6. The main operating parameters corresponded to 2^2 or 2^3 full factorial experiments.

2. The microfiltered retentate and permeate were tested for mass fractions of total protein, casein, and whey proteins in the true protein. The mass fraction of true protein in each sample was the difference in the mass fractions of total and non-protein nitrogen.

3. The mass fractions of total, casein, and whey proteins in the true protein in the retentates and permeates were summarized in tables.

To obtain a microfiltration retentate with a 96:4 ratio of casein to whey proteins in the true protein, we planned and performed the following stages:

1. We microfiltered the skim milk in a closed cycle for 7–8 h to reach the maximal value of the membrane selectivity index for casein. Then, we continued to remove the permeate at a concentration factor of 3–6. The operating parameters followed a 2^3 full factorial experiment.

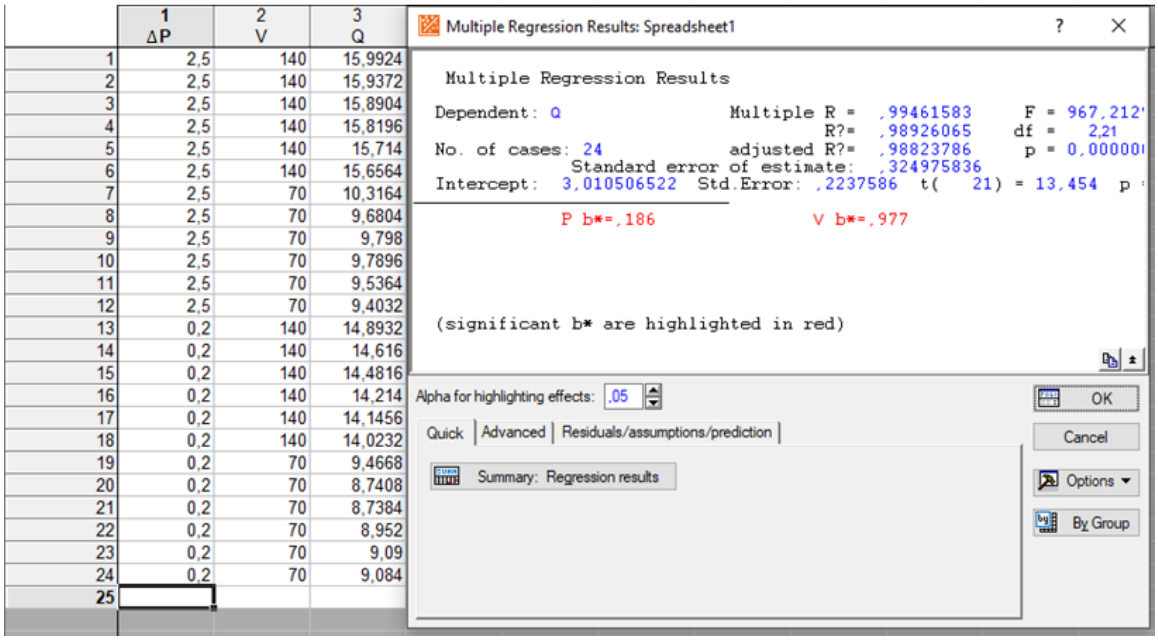
2. Upon completion, we mixed the microfiltered retentate with deionized water at a ratio of 1.0:1.0–3.0. The diafiltration involved the same basic parameters as microfiltration: the permeate was removed until the concentration factor was 3–6.

Table 3 Matrix for 2^2 full factorial experiment

Indicators		Basic level	Variability interval	Bottom level	Top level	Variable
Pressure, bar	Natural value	1.6	1.4	0.2	2.5	X_1
	Encoded value, X_1	0	–	–1	+1	
Circulation rate, mL/min	Natural value	105	35	70	140	X_2
	Encoded value, X_2	0	–	–1	+1	

Table 4 Matrix for 2^3 full factorial experiment

Indicators		Basic level	Variability interval	Bottom level	Top level	Variable	Conversion formula
Pressure (ΔP), bar	Natural value	1.6	1.4	0.2	2.5	X_1	$=\frac{\Delta P-1.6}{1.4}$
	Encoded value, X_1	0	–	–1	+1		
Circulation rate (V), mL/min	Natural value	105	35	70	140	X_2	$=\frac{V-105}{35}$
	Encoded value, X_2	0	–	–1	+1		
Temperature (t), °C	Natural value	40	10	30	50	X_3	$=\frac{t-50}{10}$
	Encoded value, X_3	0	–	–1	+1		



	1 ΔP	2 V	3 Q
1	2,5	140	15,9924
2	2,5	140	15,9372
3	2,5	140	15,8904
4	2,5	140	15,8196
5	2,5	140	15,714
6	2,5	140	15,6564
7	2,5	70	10,3164
8	2,5	70	9,6804
9	2,5	70	9,798
10	2,5	70	9,7896
11	2,5	70	9,5364
12	2,5	70	9,4032
13	0,2	140	14,8932
14	0,2	140	14,616
15	0,2	140	14,4816
16	0,2	140	14,214
17	0,2	140	14,1456
18	0,2	140	14,0232
19	0,2	70	9,4668
20	0,2	70	8,7408
21	0,2	70	8,7384
22	0,2	70	8,952
23	0,2	70	9,09
24	0,2	70	9,084
25			

Figure 2 Multiple regression for a 0.1-μM membrane

3. The resulting mass fractions of total, casein, and whey proteins in the true protein in the retentates and permeates were summarized in tables.

RESULTS AND DISCUSSION

The 2^2 full factorial experiment was represented as a data set matrix (n × k) of four observations and two factors, i.e., pressure ΔP and circulation rate V, at constant temperature of the separated system $t_1 = 50 \pm 1$°C. Stage 1, which involved microfiltration with 0.1 μM membranes, yielded the necessary experimental data. The membrane permeability values and the mass fractions of casein and whey proteins in the retentate were determined every 10 min. Statistica 10.0 provided regression equations (Eq. (2), (3)) and their response surfaces (Figs 3 and 4). The method of multiple linear regression analysis (Fig. 2) made it possible to verify the regression equations with determination coefficient $R^2 = 0.989$.

To facilitate the prediction of permeate flux, we used the most visual numerical method, i.e., the trial-and-error method with target function grid search in Python.

The data obtained for a 0.1 μM membrane as a result of the 2^2 full factorial experiment (Eq. (2), (3)) and response surfaces (Fig. 3) showed that the value of the transmembrane pressure was the main factor that affected the 0.1 μM and 0.2 μM membrane permeability:

$$Q = 3.0105 + 0.4742\Delta P + 0.0819V \quad (2)$$

$$K = 21.7978 - 3.7391\Delta P + 0.0043V \quad (3)$$

where Q is the optimal permeability; V is circulation rate; K is a ratio of the mass fractions of casein and whey proteins in the retentate.

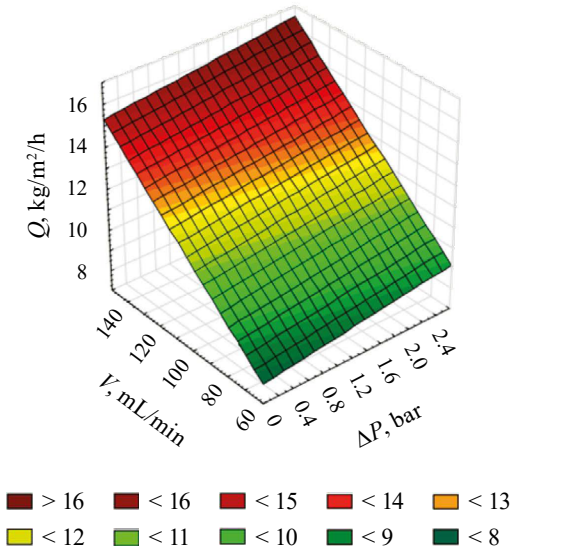


Figure 3 Effect of operating pressure (ΔP) and circulation rate (V) on permeability (Q) of 0.1 μm membranes

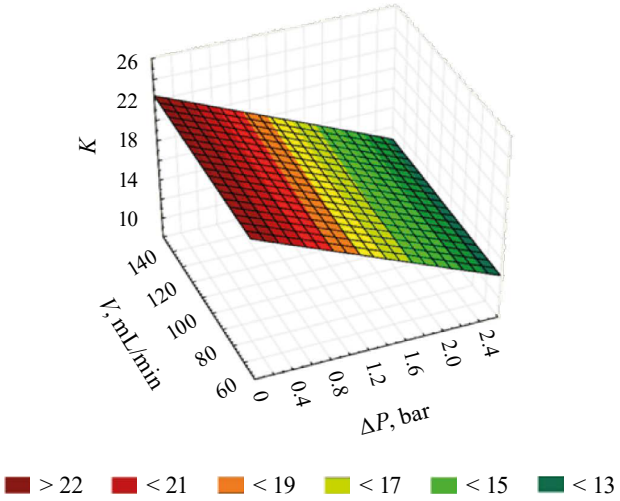


Figure 4 Effect of operating pressure (ΔP) and circulation rate (V) on the ratio of casein vs. whey proteins in microfiltration retentate (K) for 0.1 μM membranes

The qualitative (Fig. 5), quantitative (Table 5), and calculated data (Eq. (4)) for response surface (Fig. 4) showed that the highest ratio (93:7) of the mass fractions of casein and whey proteins in the retentate for a 0.1 μM membrane were obtained at $\Delta P_{min} = 0.20 \pm 0.01$ bar and $V_{min} = 70 \pm 5$ mL/min. However, a comparable ratio of 92:8 was achieved at ΔP_{min} and V_{max}, i.e., the higher the increase rate of circulation rate in the separated system, the lower the mass fraction of casein in the retentate. A higher operating pressure at V_{min} and V_{max} did not increase the mass fraction of casein either. Consequently, the ratio of 95:5 for the given operating parameters could be achieved only by changing the conditions of microfiltration and diafiltration.

Microfiltration and diafiltration made it possible to increase the content of casein fractions in the retentate.

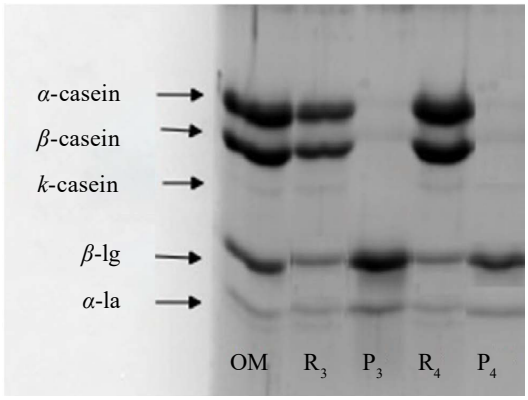


Figure 5 Electrophoresis of retentates and permeates (microfiltration, 0.1 μM membrane). OM – original skim milk, R_3 – retentate, Sample 3; P_3 – permeate, Sample 3; R_4 – retentate, Sample 4; P_4 – permeate, Sample 4 (Table 4)

Table 5 Mass fractions of casein and whey proteins in true protein of microfiltered retentate for 0.1 μM membranes

Sample	Pressure (ΔP), bar	Circulation rate (V), mL/min	Casein, %	Whey protein, %
1	2.5	140	90	10
2	2.5	70	91	9
3	0.2	140	93	4
4	0.2	70	92	8

Table 6 Mass fractions of casein and whey proteins in the true protein in retentates after defiltration

Sample	Pressure (ΔP), bar	Circulation rate (V), mL/min	Casein, %	Whey protein, %
5	0.2	140	95	5
6	0.2	70	96	4

The proportion of casein protein fractions increased from 94:6 to 95:5 in relation to whey protein fractions in the total protein (Table 6).

The results of the 2^2 full factorial experiment revealed nonlinear dependencies of the objective function Y_i on factors X_1 and X_2. The effect of the third factor, i.e., the temperature of the system after microfiltration, was highly probable for the following reasons:

– the interaction of micellar casein with whey proteins grew more intensive;

– the viscosity of the skim milk decreased as the temperature reached 50°C due to storage conditions and the permeate permeability of the membrane increased.

To raise the ratio of casein and whey protein above 95:5, the separation process had to be carried out in the microfiltrate/deionized water mode or by varying the three operating parameters, i.e., pressure, circulation rate, and temperature of the microfiltered system. As a result, the research proceeded in line with the 2^3 full factorial experiment.

The final regression equations were obtained in encoded variables for 0.1 μM membranes:

$$Y = 11.0 + 1.26X_1 + 2.46X_2 + 0.44X_3 + 0.39X_1X_2 + \\ + 0.11X_1X_3 + 0.06X_2X_3 + 0.09X_1X_2X_3 \quad (4)$$

where Y is the permeability; X_1 is the pressure; X_2 is the circulation rate; X_3 is the temperature.

At 10–50°C of the separated system (t), the greatest effect on the output parameter Y_i belonged not to X_1, but to X_2. As for paired interactions, the combined effect of X_1 and X_3 on Y_i was important for optimizing the external microfiltration parameters. To predict the membrane permeability (Q) for permeate, we substituted the encoded variables (X_i) in Eq. (4) with natural variables (Table 4):

$$Q = 1.22 + 1.11 + 0.058V + 0.06t - 0.002 - \\ - 0.00002Vt - 0.021 + 0.0002 \quad (5)$$

The optimal result for the ratio of casein and whey proteins in the true protein of the retentate after diafiltration was 96:4. We performed a theoretical analysis of Eq. (4) and determined the extremum of the function as $Y_{0.1} = f_1(X_1, X_2, X_3)$.

The tangent point was $M_0(X_{10}, X_{20}, X_{30})$; its region ($\delta$) was a sphere centered in point M_0 of radius $\delta > 0$. After that, we analyzed the partial derivatives of the first and second orders:

$$Y_i^{'} = \frac{dY_{0.1}}{dX_i} \text{ and } Y_i^{''} = \frac{dY_{0.1}^{'}}{dX_i}$$

The analysis revealed that $\delta_1 = 0$; $\delta_2 > 0$; $\delta_3 < 0$. Since $\delta_3 \neq 0$, point $M_0(11.436, 2.419, 10.659)$ was the saddle point. Obviously, $Y_{0.1} = f(X_1, X_2, X_3)$ excluded any visual three-dimensional geometric interpretation of the obtained result. However, the mere fact of a saddle point hinted at a possibility of multiple combinations of different values of variables $X_1(\Delta P)$, $X_2(V)$, and $X_3(t)$, at which $Y(Q)$ was close to the maximum in the established region of discrete variation.

The further analysis involved grid search values of $Y(Q)$. It confirmed a set of numerically close values $Y = f(X_1, X_2, X_3)$. The skim milk microfiltration could be performed at various combinations of pressure, circulation rate, and temperature, depending on the technology requirements for target products and the required ratio of the mass fractions of casein and whey proteins in the true protein of the microfiltered retentate.

CONCLUSION

In the baromembrane separation of skim milk, the transmembrane pressure proved to be the most important variable to affect the membrane permeate for 0.1 μM membranes, if the goal was to obtain a ratio of casein to whey proteins in the true protein below 95:5.

When the goal was to obtain a ratio over 95:5, all three variables, i.e., pressure, circulation rate (V), and temperature (t), should be taken into account. In that case, the permeability of 0.1 μM membranes was affected by the circulation rate, not operating pressure.

When the experiment involved an industrial sample of a 0.1 μM microfiltration membrane (Vladisart, Russia), the operating modes were experimentally determined as follows: $\Delta P = 2.5$ bar; $V = 140$ mL/min; $t = 50 \pm 1$°C and $\Delta P = 0.20 \pm 0.01$ bar; $V = 70 \pm 5$ mL/min; $t = 50 \pm 1$°C. The resulting retentates had casein and whey protein mass fraction ratios of 96:4 and 95:5, respectively.

The regression equation of $Q = f(\Delta P, V, t)$ made it possible to calculate the permeability of the microfiltration membrane, depending on the main operating parameters in specified variation intervals.

The mathematical model of the skim milk microfiltration revealed a variety of combinations of values for variables $X_1(\Delta P)$, $X_2(V)$, and $X_3(t)$, for which the parameter $Y(Q)$ approached the maximal values in the experimentally established area.

For 0.1 μM membranes, the transition from microfiltration to diafiltration resulted in the overall efficiency of the membrane separation process that demonstrated a 2.2 to 4.0% increase in casein proteins in the retentate in relation to whey proteins.

CONTRIBUTION

All the authors contributed equally to the study and bear equal responsibility for the information published in this article.

CONFLICT OF INTEREST

The author declared no potential conflict of interest regarding the research, authorship, and/or publication of this article.

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ORCID IDs
Dmitriy S. Mamay https://orcid.org/0000-0001-6221-8230
Sergey P. Babenyshev https://orcid.org/0000-0002-1784-0837
Ivan A. Evdokimov https://orcid.org/0000-0002-5396-1548