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Research of The Influence of Technological Factors On Concentration of Milk Proteins by Ultrafiltration

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ABSTRACT

The main advantage of membrane methods, along with comparable energy cost, is the ability to obtain target products with the controlled composition and properties. It is known that the use of a membrane processing allows fractionating and concentrating the component parts of raw milk preserving their nutritional, biological value and technological properties. Taking into account that the "Membrane technology" is included in the list of critical technologies of the Russian Federation, approved by the President, were carried out studies on the use of membrane methods of treatment of milk (in particular ultrafiltration) to obtain milk protein concentrates.

Keywords: ultrafiltration, structure producers, proteins of milk, pasteurization, acid coagulation of casein.

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INTRODUCTION

In ultrafiltration is required a careful preliminary preparation of the milk, which is complicated by the fact that it is a complex biological system in which are dissolved mineral salts, carbohydrates, lipids, nitrogenous substances and other components. They constitute the disperse system with suspended colloidal and emulsion particles. In addition, microorganisms that are containing in milk can greatly reduce the quality of the concentrate and the permeate [1].

An analysis of published data allowed formulating the requirements for pre-treatment of milk before ultrafiltration: maximum destruction of microorganisms and regulation of the state of the casein phase with the aim of maximum extraction while minimizing the phenomenon of concentration polarization [2]. It is possible to ensure these requirements by pasteurizing the milk source, raw material, and give such size for the casein micelles that would be sufficient for their extraction (the size of the particles of fatty phase sufficiently meets the specifications of the ultrafiltration, and the lactose molecules are too small and in a small extent transferred to the concentrate during ultrafiltration processing) [3].

OBJECTS AND METHODS

The objects of a research were: cow's raw milk of a second grade and higher according to the State Standard - GOST 13264; cream, obtained by separation of cow's milk (GOST R 52054); skim cow's milk, without foreign tastes and odors, acidity not more than 20; the lyophilized culture of direct applying FD-DVS CH-N-19 (consisting of *Lactococcus lactis spb. cremoris, Lactococcus lactis spb. lactis, Leuconostoc mesenteroidis spb. cremoris, Lactococcus lactis spb. lactis biovar diacetylactis*) и EZAL U-D MYE 96 (consisting of *Streptococcus termophilus, Lactobacterium delbrueckii spb. bulgaricus*); starter cultures of direct application, developed specially for the Russian market a series of "DELVO-YOG" (name: CY-346/347; FVV-21; CY DSL; FVV-31); "AiBi" (name: LbS 22.11(R4); LbS 22.11(R2); LbS 22.11(Y3); LbS 22.11(Y2)) and "Lactoferm" (names: KEFIR-30; YO-441; MSO-11; RENNET; PROTEK) [4].

Were used structure producers (stabilisers): carrageenan, gum carob, guar gum, xanthan gum, distarchphosphat and (or) modified starches domestic or imported, and also produced by «BK Giulini», Germany ("Turrisin", "Becaplus"), authorized for use by the bodies of Rospotrebnadzor of the Russian Federation; some other auxiliary raw materials, and materials that meet the requirements of current normative and technical documentation.

In carrying out the work were used conventional, standard and original methods.

Selection of milk and dairy products, preparation for analysis were carried out according to GOST 26809-86. Sampling for microbiological studies was carried out according to GOST 9225-84 [5].

Titratable acidity was controlled according to GOST 3524-92. The method is based on the neutralization of acids and their salts contained in the product by the solution of caustic alkali in the presence of phenolphthalein indicator. Active acidity was measured on the potentiometric analyzer according to GOST 26781-85 [6].

Were used modern instrumental, physical, physico-chemical and rheological methods. Below are the basics of the research methods that were used in the work, which helped to receive the most essential characteristics of a structured cheese product.

To study the composition of structure stabilizers were used the analyzing station JEOL JED-2300. It helped to obtain spectrometric profiles by the method of x-ray spectral microanalysis, allowing determining the chemical composition of the structure stabilizers [7].

Water binding, fat emulsion and fat holding ability of MPC-UV (milk-protein concentrate) was studied by traditional methods that are given in the workshop on colloid chemistry [8].

Rheological characteristics of gels based on whey and on the product samples were determined viscosimetric on a rotational viscosimeter “Reotest-2” [9].

Microphotographs of gels were obtained by the electron microscope according to the methods developed at KSC SB RAS and tracing by L. Y. Gradus.

Mathematical processing of the obtained microphotographs of structure stabilizers was in the determining of content of micro hollows using the program Corel Photo Paint X3. Was used a creation of masks by selecting of elements by color, a transfer of photos into a binary image and a determination of target items, using histograms [10]. The reliability of experimental data was determined by the method of mathematical statistics on the IBM PC. Optimization was performed by the Simplex method of Nedler Meade.

Results and discussion. Table 1 shows the influence of the modes of pasteurization on its effectiveness and the diameter change of the micelles of casein in the milk raw material.

Table 1: The effect of thermal pasteurization on bacterial contamination of milk

Temperature, °C	The efficiency of pasteurization (%) by duration of exposure, sec					
	0	30	60	180	240	300
72±2	98,521	98,550	98,569	98,629	98,668	98,674
82±2	99,728	99,821	99,843	99,967	99,968	99,969
92±2	99,943	99,950	99,961	99,983	99,975	99,990
97±2	99,965	99,974	99,986	99,997	99,998	99,999

Analysis of the results showed that with the increase of temperature and duration of thermal influence the efficiency of pasteurization is increased. This means that increases the ratio of the number of killed bacterial cells to the number of bacterial cells in raw milk.

The required efficiency of pasteurization (not less than 99.99%) differs with the following thermal conditions:

- temperature 92±2°C, not less than 300 sec;
- temperature 97±2°C, for at least 180 sec.

The parameters of pasteurization inevitably lead to a change in caseinate-calsium-phosphate complex and the native state of serum proteins: the diameter of the micelles of casein changes, there is a partial disruption of solvation of molecules, is disrupted a relationship in the structure of the formed nitrogenous substances. The complex of these phenomena, ultimately, will affect patterns of a membrane concentration. In this regard, let`s consider the effect of pasteurization on the changes in the protein phase of the milk.

It is established that with the increasing of temperature of pasteurization, the dispersion of casein micelles is increased: in unpasteurized milk 51.3% of all micelle have a diameter of less than 60 nm. The corresponding figure for milk that is pasteurized at a temperature of 92±2°C for 300 sec is 72,0%, and at a temperature of 97±2°C within 180 sec is around 88%. However, the aggregation of micellar casein in the particles with a diameter of more than 80 nm at pasteurization is not observed, that is probably related to the inability of small submicelles absorb on the interface of a large micellar aggregates. This fact is probably associated with redistribution of the ratio between the individual forms of protein molecules in the milk.

To establish the peculiarities of the changes in protein molecules during pasteurization were conducted a series of experiments. The results are given in table 2.

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Table 2: The effect of pasteurization on the changes of nitrogen forms in the milk

Characteristic	Samples (pasteurization)		
	Control	92±2°C, 300 sec	97±2°C, 180 sec
Mass fraction of total nitrogen, %	0,49	0,49	0,49
Mass fraction of protein nitrogen, %, including: - nitrogen casein fractions - nitrogen fractions of the whey proteins	0,47	0,43	0,41
	0,36	0,35	0,35
	0,11	0,08	0,06
Mass fraction of non-protein nitrogen, %	0,02	0,06	0,08

It was revealed that pasteurization leads to a change in the fractional composition of nitrogenous substances in milk, however, the change of the fractions of casein proteins is minimized (at 2.8%, which is less than the permissible error of measurement). These data confirm the heat resistance properties of proteins casein fractions.

In contrast, treatment of milk at a temperature of 92±2°C for 300 s is the reason for the decries in protein nitrogen by 8.5%, raising of a temperature to 97±2°C leads to a decrease of the discussed index by 12.8%. Such changes occur as a result of denaturation of the whey proteins (by 27.3 and 45.5%, respectively, and a transition their fractions in various forms of non-protein nitrogen). Table 3 shows the effect of pasteurization on the content of whey proteins in milk.

These data indicate that the whey proteins are heat-labile and partially denature during pasteurization; is observed their transition into a non-protein form.

In a series of conducted experiments was also found that not all the whey proteins are presented by immunoglobulins, 〈-lactalbumines and ®-lactoglobulines.

Table 3: The effect of pasteurization on the content of whey proteins in milk

Characteristic	Samples (pasteurization)		
	Control	92±2°C, 300 sec	97±2°C, 180 sec
Mass fraction of nitrogen, %: - immunoglobulins - 〈-lactalbumins - ®-lactoglobulins	 0,02 0,03 0,04	 0,01 0,02 0,03	 0,01 0,01 0,03

Comparing the results given in table 3 it can be assumed that denatured whey proteins are likely to form complexes with the casein micelles, which increase their size. However, it is assumed that at the same time the water binding capacity of the main fraction of milk proteins changes and the other important functional characteristics: the viscosity properties, the ability to gelation, surface activity, biological value. In this regard, the increasing of the pasteurization temperature of over 92±2°C is impractical, because it is necessary and sufficient for ensuring the microbiological reliability of milk, which will be further subjected to ultrafiltration.

Minor changes of caseins in pasteurization are a prerequisite to their further use by means of ultrafiltration processing of milk. However, some studies show that a low content of micellar casein with a diameter of micelles less than 80-100 nm is a deteriorating factor of the process of membrane concentration.

In this regard, it is useful to consider the process of aggregation of micelles via acid coagulation.

The structure of the experiment on the use of leaven prepared on pure cultures of microorganisms is given in table 4. Temperature parameters were selected based on the existing recommendations; a combination of starter cultures was carried out to enable the regulation of the composition and properties of the resulting protein clots.

Table 4: The scheme of experiments on the acid coagulation of casein

Series	The type of a starter culture	The temperature of the fermentation, °C
I	Mesophilic lactic acid streptococci (*Lactococcus lactis spb. cremoris, Lactococcus lactis spb. lactis, Leuconostoc mesenteroidis spb. cremoris, Lactococcus lactis spb. lactis biovar diacetylactis*)	28±2
II	Thermophilic lactic streptococci and sticks (*Streptococcus termophilus, Lactobacterium delbrueckii spb. bulgaricus*)	43±2
III	Mesophilic lactic streptococci, thermophilic lactic streptococci and sticks (for experiments I and II)	33±2

Ferments were added into the milk in an amount of 3-7%, and were kept at a predetermined temperature. Were controlled pH values and the diameter of the micelles of casein.

In a series of experiments I it was found that mesophilic starter cultures are not the intense acids producers. pH values close to the isoelectric point of milk proteins (4,6-4,8) were obtained after 10-12 hours of ripening. The increase in the average diameter of casein micelles from 84 nm to 200-250 nm occurred in 8-10 hours. However, the use of the lactic acid microorganisms of Lactococcus genus in a production environment will be constrained by a long process of mixture fermentation, prepared for the ultrafiltration.

Analysis of the results showed that thermophilic cultures act in an intense acid formation, because they help to lower the pH to 5.0 and below after 3-4 hours. The figure clearly shows the initial phase of development of the microflora and the ability of milk proteins do not dramatically change the active acidity at the initial stage of biotechnological process as a result of buffer properties. Essential for technological processing (ultrafiltration) the growth of micelles of casein (more than 200-250 nm) was observed in an active acidity of milk at 5.0-4.8, and the dose of the leaven of 7% is insignificantly intensify the process of fermentation of milk compared to the dose of leaven 5%. Use of integrated starter consisting of mesophilic lactic streptococci, thermophilic lactic streptococci and sticks allows intensifying the process of increasing the acidity, stimulating the increasing of the micelles of casein in a diameter for ultra-filtration processing, and providing the specified physical and chemical characteristics of the mixture in 8-9 hours.

It can be stated that the selected dose of the leaven of from 3 to 7% of thermophilic and mesophilic microorganisms in equal proportions in this case is rational, because they allow intensifying the process of acid and regulating the properties of the clot, subjected to ultrafiltration. The increased doses of the leaven to 7% reduce a process duration by 20-30%, while providing the required acidity of the clot.

Summarizing the obtained data it can be stated that it is possible to prepare the bunch for ultra-filtration processing by the prior pasteurization of the mixture to inactivate the microflora with a subsequent fermentation of the mixture to increase the average diameter of the casein micelles, which are necessary and sufficient for effective ultrafiltration processing. However, evaluating the possibility of the process of extracting a protein fraction from the fermented clot by different types is only possible by setting up of special experiments.

It is known that the efficiency of the membrane concentration depends on a number of factors: the temperature and acidity of the active process, physico-chemical and rheological properties of processed biological systems, pore diameter, surface area of membrane, concentration factor and others. These technological factors affect the specific productivity of the membrane device, the yield of target components and their residual content in permeate.

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To establish the application possibilities of use of the ultrafiltration device were carried out a series of experiments. Figure 1 shows the specific productivity of the ultrafiltration unit according to the temperature and the used starter culture (pH 4,8-5,0; dose of leaven 5%).

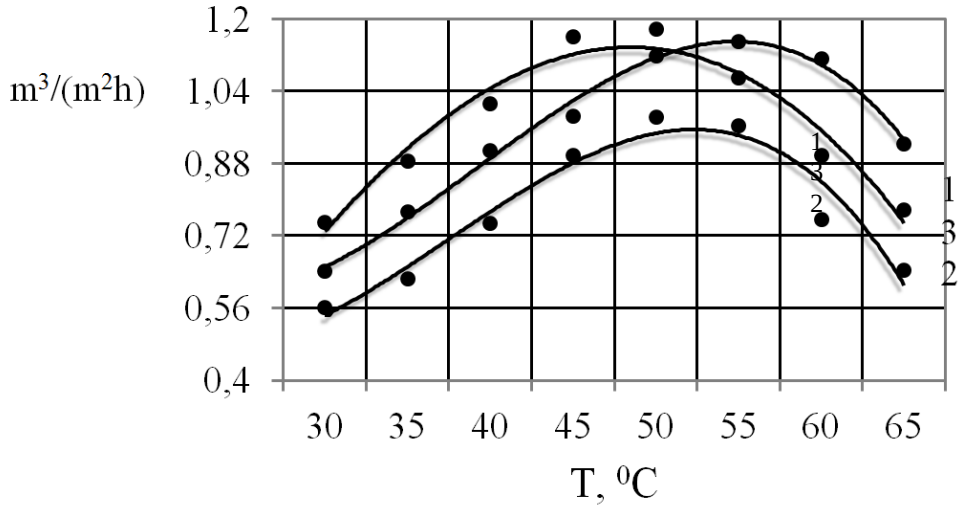

Figure 1. The effect of temperature on a specific productivity of the ultrafiltration device: 1 - a series of experiments I; 2 - series of experiments II; 3 - series of experiments III.

Based on the analysis of the research it can be stated that the temperature is a significant factor in the process of ultrafiltration. Regardless of the specific characteristics of used starter cultures a rational ultrafiltration temperature is 50±5. This fact is probably linked to the required viscosity of milk-protein clot, the mobility of coagulation-condensation structures of the formed dispersed structures, as well as the ability to provide the required diameter of the micelles of casein, sufficient for ultrafiltration processing.

In a series of experiments was established an interesting feature characterizing pecularities of the ultrafiltration of lactic acid mixtures obtained in different modes of fermentation: the most effective (on ensuring of a specific performance of the membranes) is the use of mesophilic lactic acid organisms, both on its own as a starter, and in combination with thermophilic bacillus.

Maximum specific productivity of the device at the level of 1.17 $\frac{m^3}{m^2*hour}$ was observed in the case of integrated use of mesophilic lactic streptococci, thermophilic lactic streptococci and sticks (for experiments I and II), with the fermentation temperature of 35±2°C and a subsequent ultrafiltration at 50°C. The increasing of the temperature to 55-60°C reduces the specific productivity of the device on 8.6-23.2%; on the contrary, a temperature decrease from 50 to 40°C also provides the reduction of a specific performance of membrane unit by 13.4%.

Fermentation of a mixture by the mesophilic lactic streptococci and fermenting at a temperature of 28±2°C do not provide a reliable decrease in the specific productivity of the membrane device, however, an optimum of temperatures from 50°C enters the area of 55-60°C. This fact can be explained in terms of physico-chemical properties of the clots formed in this case. In particular, at visual assessment they are coarser, calle (fr. - *caille*) is characterized by a greater strength. These features are to some extent offset by an increase in temperature ultrafiltration on five-ten degrees, clots according to their properties are close to those obtained in the case of complex fermentation under option III.

The use of lactic *Streptococcus thermophiles* and sticks *Lactobacterium delbrueckii spb. bulgaricus* allows to obtain a fermented mixture, which cannot be effectively subjected to fractionation even at the optimum temperature (the device capacity is lower in average on 15.9%). Deviation from the optimum temperature leads to the unreasonable performance degradation, which reaches at extreme temperature ranges of ultrafiltration 30°C and 65°C average 51.9 and 44.6%, respectively.

With the aim of obtaining objective data, showing the relationship of a specific device performance

and active acidity were conducted a series of experiments, the results of which are shown in Figure 2 (temperature 50±5°C, a dose of leaven 5%).

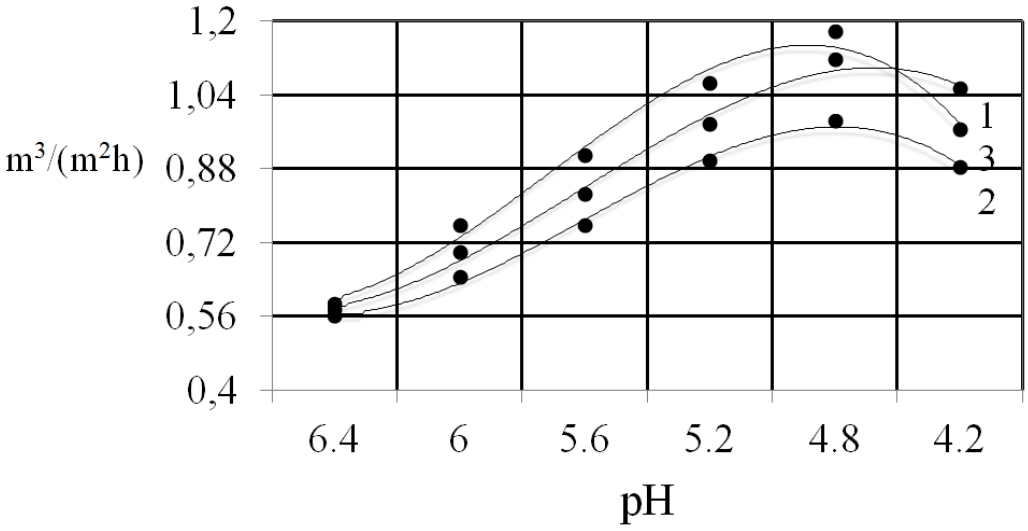

Figure 2. The influence of active acidity for specific performance of the ultrafiltration device: 1 - series of experiments I; 2 - series of experiments II; 3 - series of experiments III

The dependencies shown in Figure 2 are of the same nature as the results of the experiments shown in Figure 1: a maximum specific capacity of the unit identified in the narrow sense of the changed factors (at the level of active acidity 4,8±0,2), which corresponds to the isoelectric point (IEP) of the majority of milk proteins, particularly caseins. To the best ultrafiltration is subjected a mixture which consists of the leaven of mesophilic lactic streptococci.

Besides the mentioned regularity it was established that milk with the values of active acidity close to native (at the level of 6.4 - 6.6) does not allow to maximize the use of the ultrafiltration membrane device, despite of the rather significant average diameter of casein micelles of 84 nm. Under ideal (calculated) specific productivity of a device 1.2 $\frac{m^3}{m^2*hour}$ at the initial moment of ultrafiltration its performance is 0,562-0,589$\frac{m^3}{m^2*hour}$, which is on 50.9-53.2% lower than the ideal value, and by 49,7-51,9% lower than the specific productivity obtained in our studies. The established fact can be explained due to the presence in milk of a very large number of submicelles of casein, having a diameter smaller than 20 nm (from 16.8% to 32%), which pass into permeate. When you consider that in the total content of the micelles from 49,6% to 68% have in an average diameter of 40 nm, it is quite natural that there is the need for some "standardization" of the sizes of micellar casein to a value that enables the effective use of a membrane technology, in this case - ultrafiltration. This factor allows ensuring rational parameters of the ultrafiltration process with a minimum consumption of lactic acid clot and minor negative effects associated with concentration polarization on the membrane surface.

With the aim of establishing the characteristics of ultrafiltration from the length of working of ultrafiltration device, surveying of methods of ultrafiltration intensification, as well as the rational justification of the duration of the technological process are investigated hydrodynamic and kinetic features of ultrafiltration, including the refined nature of the process of fractionation of milk components. The parameters of the device operation were selected based on the analysis of the results of previously conducted experiments.

Table 5 shows the specific capacity of the unit according to the speed of the milk clot in the channels of the membrane apparatus (temperature 50±5 °C, the dose of the used leaven 5%, pH 4,8-5,0).

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Table 5: The influence of hydrodynamics of a process in the specific productivity of the ultrafiltration device ($\overline{X}$ ⍰m; m≤1,2)

The speed of a fermented milk clot, ×10^{-2} m/sec	The specific capacity of the product ($\frac{m^3}{m^2*hour}$) obtained in a series of experiments		
	I	II	III
1,0	0,992	0,747	0,845
1,5	1,053	0,832	0,922
2,0	1,178	0,984	1,118
2,5	0,120	0,834	0,928
3,0	0,865	0,781	0,811

It is revealed that with the increase in the velocity of lactic acid clot to 2.0×10-2 m/s the specific productivity of the ultrafiltration device reaches its maximum values regardless of the type of the used starter culture. This fact can be explained by flushing of the surface layer of the membrane and the access of particles to the interface system for the fractionation of milk components. This principle is in the basis of most methods of combating the extremely undesirable phenomenon of concentration polarization occurring on the surface of the existing membrane.

The increase of speed of lactic acid clot through the channels up to 3.0×10-2 m/s is the cause of the degradation in the specific productivity (in average of 20.6-27.5%). This can be explained by the several reasons: the hydrodynamics of the process changes, there is a transition of flow from laminar to turbulent. This is a factor for a destabilization of the casein micelles, and as a result - reduces their ability for ultrafiltration. For illustration and confirmation of the assumptions were held a series of model experiments, the results of which are shown in table 6.

Table 6: The relationship of the hydrodynamic conditions and the diameter of the micelles of casein

The speed of a fermented milk clot, ×10^{-2} m/sec	The Reynolds Criterion	The flow regime	The average diameter of micelles, % of the initial in the clot
1,0	Less then 1,0·10^5	Laminar	93,0-100,0
1,5	(1,0-2,0)·10^5	Laminar	93,1-97,5
2,0	(2,0-3,0)·10^5	Transition	85,0-93,1
2,5	More then 3,0·10^5	Turbulent	74,3-85,0
3,0	More then 3,0·10^5	Turbulent	68,4-74,3

Analysis of the hydrodynamic conditions of the process flow showed that with the increasing speed of milk clot occurs the transformation of the regime from laminar to turbulent, which inevitably leads to a gradual reduction of the diameter of the micelles of casein.

If to compare the data obtained on the hydrodynamics of the process and the specific productivity of the device, it can be stated that the maximum values of a device performance on products 1,178; 0,984 and

1,118 $\frac{m^3}{m^2*hour}$ were observed in the case of speed of a clot movement in the channels of 2.0×10^{-}2 m/s and a series of experiments with starter cultures I, II, III, respectively.

In this case, as an indirect value on which to focus, is the Reynolds criterion.

Its value at the level (2,0-3,0)·10^5 is not allowed to occur the irreversible changes with the clot, in particular, the transition of casein in submicellar form, the ultrafiltration, and provides the best possible performance of the device due to the sufficient diameter of the micelles and the heavy traffic of biological material in the channels of the device.

Of course, the flow regime is a derived quantity from the used pressure, since the ultrafiltration refers to the baromembrane processes. Table 7 shows a comparison of the flow regimes of the liquid and the pressure in the device.

Table 7: The dependence of the Reynolds number from the working pressure in the installation

Working pressure, MPa	0,2	0,4	0,6	0,8
The Reynolds Criterion, ×10^5	Less then 1,0	1,0-2,0	2,0-3,0	More then 3,0

After analyzing the results, it was found that the working pressure of 0.6 MPa is justified because it allows a maximum use of the device. The use of higher pressures is impractical not only because of the peculiarities of the hydrodynamics of the process (exacerbation of the phenomenon of concentration polarization), but in view of the increasing energy costs.

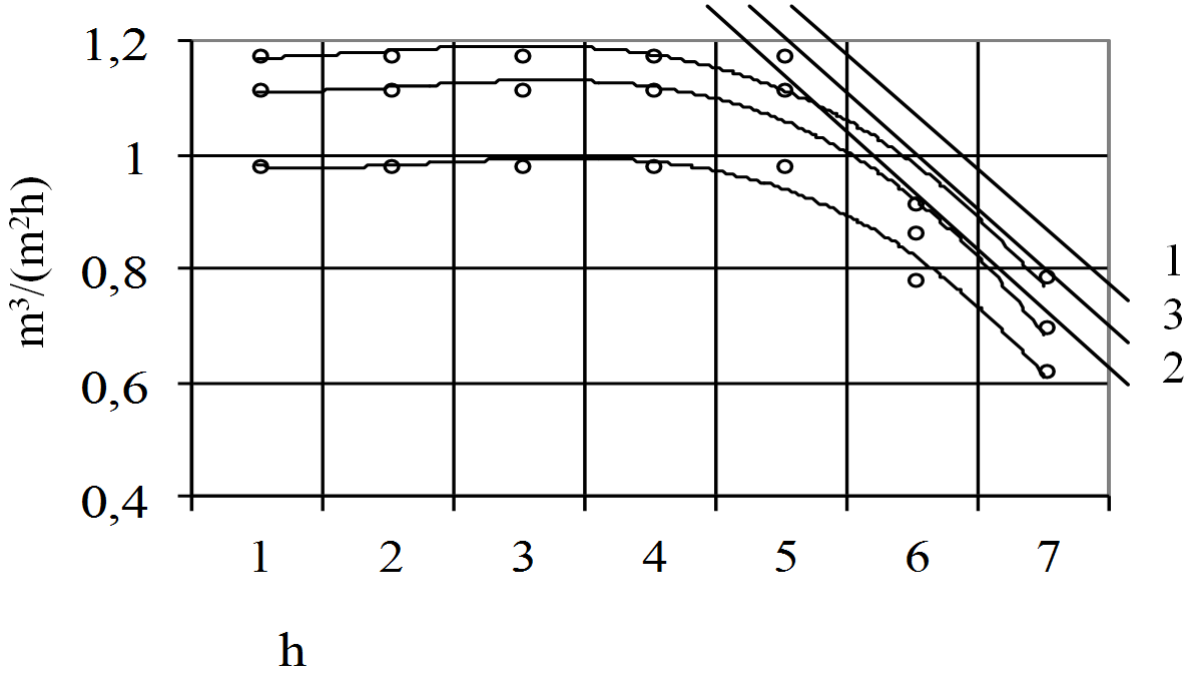

Figure 3. The effect of the duration process on the specific productivity of the ultrafiltration device: 1 - series of experiments I; 2 - series of experiments II; 3 - series of experiments III

From a practical point of view, the kinetic characteristics of the process are of the great importance, in particular the device output depends on the duration of the process of membrane concentration. These data are important in a connection with the justification of the duration of work cycles, after which regeneration of the membranes is necessary. Information reflecting the kinetic characteristics of ultrafiltration is shown in Figure 3.

On the basis of the executed researches it was established that the critical drop in the specific productivity of the membrane device is observed after 5 h of ultrafiltration process on more than 20%, and after seven hours of the process this value is 37,3% (in the case of using an integrated starter). The established feature is associated with the deposition of protein-salt complexes and the fatty phase on the membrane surface, is observed a so-called phenomenon of concentration polarization on the clogged pores). In spite of this, a sufficiently long period of use of the membranes without a regeneration (up to 5 h) is associated with the provision of transition regime of fluid flow in the channel, which allows a somewhat "flush" the particles of the concentrate from the membrane surface and, thus, to intensify the process of ultrafiltration.

Insights

In general it can be stated that the research findings help to substantiate and to develop the technology of milk-protein concentrate (MPC-UV). To assess the possibilities of its use, we studied its composition and properties.

REFERENCES

[1] Schippers J.C., Verdouw J. The modified fouling index, a method of determining the fouling characteristics of water. // Water Life. Proc. Int. Congr. Desalin. and Water Re-Use K.C. Channalasappa Mem, Nice, 1979. V. 3, p. 137-148.
[2] V. P. Dubyaga, Perepechkin L. P., Katalevsky E. E. Polymeric membranes. -M.: Chemistry, 1981.
[3] E. A. Kremenevskaya Membrane technology of desalination of water. -M.: Energoatomizdat, 1994.
[4] Prosekov, A. Y. Physico-chemical fundamentals of obtaining food products with foam structure: Monograph / A. Yu. Prosekov.- Kemerovo: Kemtipp, 2001.- p.172
[5] Prosekov, A. Y. Modern aspects of food production: Monograph / A. Y. Prosekov.- Kemerovo: KemTIPP, 2005.- p. 381
[6] Trusov, L. I. New membrane Trumem and Rusmem based on flexible ceramics. //Crete, tekhnol. Membrane. 2001. No. 9, pp. 20-27.
[7] Mulder M. Introduction to membrane technology: translation from English. -M.: Mir, 1999.
[8] Yasminov A. A., Orlov A.K., Karelin F.N. Water treatment reverse osmosis and ultrafiltration. -M.: Stroiizdat, 1978.
[9] Hillis P., Padley M.B., Powell N.I., Gallagher P.M. Effects of backwash conditions on out-to-in membrane microfiltration. // Desalination. 1998. V. 118, p. 197-204.
[10] Thompson M.A. Membrane filtration of high turbidity sources. // Proc. of the Conf. on Membranes in Drinking and Industrial Water Production (Paris, 3-6 October). 2000. V. 2, p. 329-334.