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Processing Quality of Milk-Protein Concentrates Obtained by Ultrafiltration.

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ABSTRACT

The article deals with the study of processing quality of milk-protein concentrates obtained by ultrafiltration. Creaming ability of milk-protein concentrates obtained by ultrafiltration is inevitably associated with the presence of rehydrated proteins in its composition. With increasing temperature, the ability of proteins to absorb on the plasma-gas interface is reduced, as evidenced by a decrease in the creaming ability. This factor also leads to an increase in the difference of creaming activity of milk protein concentrates obtained by ultrafiltration technique or with the use of various starter cultures. It is shown that with decreasing temperature the consistency index increases, however, even at the temperature close to that of cryoscopic point of milk, it was impossible to achieve in the experiments absolute stability of the suspension system of milk-protein concentrates obtained by ultrafiltration. Despite the relatively higher values of creaming ability of milk protein concentrates, obtained from lactic acid clot fermented by complex starter of mesophilic and thermophilic streptococci, produced froths are characterized by the higher values of consistency index. This implies that not only the interface area but also the composition of concentrates as well as their processing quality determine the kinetic behavior of the froths in time.

Keywords: milk protein concentrates, ultrafiltration, creaming ability.

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INTRODUCTION

The use of milk protein concentrates obtained by ultrafiltration (MPC-UF) is possible only based on a detailed knowledge of their composition and properties [1]. However, studies of their functional properties are determined not only by proteins, contained in the concentrates as well as other nutritional components and microorganisms, but a variety of indicators that affect the quality of finished products in the processing of raw materials and semi-finished products [2]. Requirements to the processing quality of the proteins contained in milk protein concentrates differ depending on their process flow parameters as well as the principles and directions of further use [3].

The most important feature of protein systems is their ability to participate in surface phenomena. They are widely used in various food processing technologies [4, 5]. Surface phenomena occurring in protein systems are most widely used for obtaining products based on frothed and emulsion systems [6]. Some of the features of the formed interfacial layers of the dispersions depend on the composition and properties of the concentrates obtained by fundamentally the same technology, though with some particular features [7, 8].

OBJECTS AND METHODS

At different stages of the current research we studied the following products: cow's raw milk not lower than grade II (GOST 13264); cream, obtained by separation of cow's milk (GOST R 52054); skim cow's milk without foreign tastes and odors with acidity not more than 20°T produced by separation of cow's raw milk of the appropriate grade; skimmed milk powder (GOST 10970); and freeze-dried culture for direct inoculation FD-DVS CH-N-19. We used also porcine pepsin (Moscow Rennet Enzyme Factory); drinking water (GOST 2874); hard, semi-hard, soft and brine cheeses according to the current reference documentation; cheeses and cheese curds for melting (TU 9225-113-04610209); low-fat cheeses for melting (TU 9225-107-04610209); cheeses and curds for melting (TU 9225-113-04610209); cheese curd for melting (TU 9220-001-83196489); cheeses covered by GOST R 52686 and semi-hard cheeses (GOST R 52972); butter (GOST R 52969); butter and butter paste (GOST R 52253); butter oil and milk fat (GOST R 52971); whey butter (TU 9221-112-04600209); sour cream (according to GOST R 52092 and other current regulatory documents); drinking pasteurized cream (TU 9222-366-00419785); albumin curd from cheese whey (TU 9229-062-04610209); cottage cheese (according to GOST R 52096 or other types of cottage cheese approved by the current reference documentation); milk whey powder (according to TU 9229-123-04610209 or domestic/foreign reference documentation approved by the bodies of Rospotrebnadzor of the Russian Federation and having the appropriate safety data sheet (SDS); and cheese powder (according to TU 10-02-02-0274 or received on imports and approved for use by the bodies of Rospotrebnadzor of the Russian Federation and having the appropriate SDS) [9].

When carrying out the work we used conventional, standard and original research methods.

Sampling of milk and dairy products as well as their preparation for analysis was carried out according to GOST 26809-86, while sampling for microbiological studies was conducted according to GOST 9225-84.

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

Evaluation of taste and smell was carried out according to GOST 28283-92.

Determination of moisture content and dry matter was carried out according to GOST 3626.
Total protein content was measured according to GOST 23327-78.

Sucrose weight fraction was determined by iodometric titration method according to GOST 3628 (arbitration method). The method is based on oxidation of reducing sugars containing aldehyde group by iodine in an alkaline medium. Sucrose weight fraction was calculated by difference between the amount of taken and unspent iodine, determined by titration [10].

We used advanced instrumental, physical, physicochemical and rheological methods. Basics of

research methods, used in the work to obtain the most essential characteristics of a textured curd product are described below.

The method to determine galactosidase activity of lactic acid bacteria is as follows: after preparing a 5% lactose solution using buffer solution with a pH of 4.2 or 7.0 and 1% enzyme solution, we determined the activity of the enzyme by cryoscopic method. For this purpose, 4 cm^3 of substrate was added to 1 cm^3 of the prepared solution and mixed. Then we took 1 cm^3 of mixture as the control sample. The remaining solution was incubated at 30°C for 30 minutes. Further we took 1 cm^3 of the test sample and measured its freezing temperature to calculate galactosidase activity.

Method to determine proteolytic activity is based on the hydrolysis of sodium caseinate with studied enzyme preparation until obtaining peptides followed by their further definition. The ability of the enzyme to convert during one minute the sodium caseinate at 30°C into condition nonprecipitating by trichloracetic acid in the amount corresponding to 1 µmol of tyrosine (GOST 20264.2-88) is taken as a unit of proteolytic activity.

Determination of nonprotein nitrogen was performed in the filtrate after precipitation of proteins by the photometric method. Fractionation of nitrogenous substances and the study of their composition and properties were carried out according to known techniques. Sample preparation included following operations: hydrolysis with hydrochloric acid at a temperature of 110±10°C and pressure of 0.8 MPa for 12-15 h, and centrifugation through a membrane with a defined pore size to remove various impurities from solution.

Fat weight fraction was determined by Gerber method according to GOST 5867. The method is based on the isolation of fat from milk and dairy products under the action of concentrated sulfuric acid and isoamyl alcohol followed by centrifugation and measurement of the volume of the released fat in the graduated part of butyrometer.

Determination of lactose, glucose, and galactose weight fractions as well as oligosaccharides was carried out by high performance liquid chromatography employing the "Color 500 M" instrument.

RESULTS AND DISCUSSION

The creaming ability of MPC-UF is inevitably associated with the presence of rehydrated proteins in its composition. Maximum values of creaming ability were observed at the gas saturation temperature of 39-40^{0}C. With increasing temperature, the ability of proteins to absorb at the plasma-gas interface is reduced as evidenced by a reduction in the creaming ability. This factor also leads to an increase in the difference in creaming activity of MPC-UF obtained using different starter cultures. Thus, at the temperature close to that of cryoscopic point this difference is uncertain (less than 2.3% in relative units) while at 10 and 50°C it increases to 9.1 and 29.4%, respectively.

In general, all MPC-UF samples are capable of formation of suspension systems (SS), however in case, if the gas saturating process will occur at a temperature above 10°C, then the preference should be given to the sample, obtained using the mixed starter consisting of mesophilic and thermophilic cultures. In case, if the gas saturation will occur at a temperature from 0 to 10°C, essentially we can use MPC-UFs obtained with the application of both mesophilic and thermophilic cultures.

It is shown that consistency index increases with decreasing temperature, however, even at a temperature close to cryoscopic point, absolute stability of SS obtained from MPC-UF cannot be achieved in the experiments. Despite the relatively higher values of creaming ability of MPC-UF obtained from lactic acid clot fermented by mix starter of mesophilic and thermophilic streptococci, produced froths show higher consistency index. This implies that not only the interface area but also the composition of concentrates, as well as their processing quality determine the kinetic behavior of the froths in time. Minimum consistency indices (less than 50% after three hours of exposure) were noted in gas-liquid dispersed systems investigated at a temperature above 30^{o}C.

Due to the described properties, froths formed from MPC-UF can be used to create aerated products. This is evidenced by the presence of frothing properties of milk and low values of dispersion. At that frothy

mass obtained at a temperature below 10°C are monodisperse (Table 1) because from 84.6 to 95% of the dispersed phase particles have a predominant size less than 1 mm.

The dispersion increases with increasing temperature that leads to appearance of fractions with a larger size. It is established that other conditions being equal the SS of MPC-UF, obtained using mesophilic cultures (option I), contain gas bubbles of the largest size. For example, at the frothing temperature of 0-2°C in the samples of SS of MPC-UF (options II and III) there were no particles of disperse phase greater than 5 mm (in the option I – up to 2.3%). The increase in the frothing temperature to 10-12°C leads to the appearance of bubbles larger than 5 mm in the options II and III up to 9.4 and 6.3%, respectively (in the option I – 18.6%).

Table 1 Disperse composition of SS obtained from MPC-UF

Tempe-rature, °C	Relative distribution (%) of air bubbles obtained from MPC-UF in various options											
	I				II				III			
	less than 1 mm	1-3 mm	3-5 mm	more than 5 mm	less than 1 mm	1-3 mm	3-5 mm	more than 5 mm	less than 1 mm	1-3 mm	3-5 mm	more than 5 mm
0-2	84.6	8.0	5.1	2.3	92.6	7.4	0.0	0.0	95.0	5.0	0.0	0.0
10-12	11.6	57.3	18.6	12.5	17.5	59.6	13.5	9.4	73.4	11.2	9.1	6.3
20-22	9.9	46.1	34.5	21.5	5.1	25.6	46.9	16.2	51.6	23.5	17.6	7.3
30-32	5.0	56.1	15.2	45.9	6.7	28.3	33.5	31.5	25.2	23.3	29.5	21.9
40-42	0.0	0.0	14.9	85.1	7.6	11.1	18.6	62.7	12.7	16.4	16.6	54.3

In the suspension system obtained from MPC-UF at 40-42°C for option I, all the particles of the gas phase had a size of more than 3 mm, at that the proportion of air bubbles with a diameter of 3-5 mm was 5.7 times less than those with a diameter greater than 5 mm that indicates intensive processes of froth destruction. The similar parameters for experiments designated as II and III are as follows: the total number of particles with diameter greater than 3 mm – 81.3 and 70.8%, respectively; the ratio of particles with diameter of 3-5 mm and greater than 5 mm – 1:3.8 and 1:3.3%, respectively.

Table 2 Water retention capacity of MPC-UF

Temperature, °C	Water retention capacity of MPC-UF (g/g) obtained in various options		
	I	II	III
0-2	3.81	5.14	4.62
10-12	3.67	4.72	4.24
20-22	3.24	4.03	3.67
30-32	2.56	3.21	2.86
40-42	2.48	3.11	2.78

After receiving satisfactory results on the ability of MPC-UF to form gas-liquid dispersed systems, we have further considered the water retention capacity (WRC), fat binding capacity (FBC) and fat emulsifying

properties (FEP) of MPC-UF. The results of these studies are presented in Tables 2-4.

The WRC evaluation results presented in Table 2 indicate that MPC-UF, produced from lactic acid clot fermented with thermophilic cultures *Str. termophilus* and *Lbm. delbrueckii spb. Bulgaricus* possess the maximum ability to retain water. With increasing temperature from 0-2 to 10-12, 20-22, 30-32 and 40-42°C this indicator decreased by 8.2, 21.6, 37.6 and 39.5%, respectively.

The MPC-UF samples, obtained with the use of mesophilic cultures *Lac. lactis spb. cremoris*, *Lac. lactis spb. lactis, Leu. mesenteroidis spb. cremoris* and *Lac. lactis spb. lactis biovar diacetylactis*, are characterized by minimum water retention capacities, while MPC-UF samples, obtained with the use of fermented mixed starter, show intermediate water retention capacities. The temperature in these cases has the same effect on the WRC indicators; at that, the reduction in the decline rate of the value under study with increasing temperature above 30-32°C was identified at a level of 3.1 and 2.8%, respectively. In this regard, it is fair to assume that to enhance the ultrafiltration process we can use partially dehydrated clot. Fat binding capacity of MPC-UF varies according to consistent patterns other than those for WRC. This is evidenced by the experimental results presented in Table 3. It is obvious that with increasing temperature from 0-2 to 40-42°C, the FBC increases by 1.52, 1.50 and 1.58 times for the samples in options I, II and III, respectively. This fact is probably associated not only with the properties of the proteins contained in MPC-UF, but also with change of the adhesive properties of the fatty phase at increasing temperature (the adhesive properties increase with regard to the milk proteins). Maximum values of FBC (at a level of 5.6 g of fat per gram of protein) correspond to MPC-UF samples obtained using the mesophilic starter culture.

Table 3 Fat binding capacity of MPC-UF

Temperature, °C	Fat binding capacity of MPC-UF (g/g) obtained in various options		
	I	II	III
0-2	3.68	3.31	3.45
10-12	4.04	3.63	3.85
20-22	4.53	4.08	4.29
30-32	4.99	4.21	4.61
40-42	5.60	5.01	5.45

The presented data correlate with the results of the evaluation of WRC: the higher the WRC, the lower the FBC values. Similar trend was noted when evaluating fat emulsifying properties of MPC-UF (Table 4).

Table 4 Fat emulsifying properties of MPC-UF

Temperature, °C	Fat emulsifying properties of MPC-UF (g/g) obtained in various options		
	I	II	III
0-2	47.84	43.11	38.58
10-12	52.91	47.32	43.14
20-22	57.51	53.21	47.87
30-32	62.96	58.34	52.04
40-42	71.51	62.85	57.85

The minimum indicators of FEP (38.58- 47.84 g of fat per gram of protein) were detected in the MPC-UF samples regardless of the method of their production at a temperature of 0-2°C. Temperature increase to 10-12, 20-22, 30-32 and 40-42°C causes the increase in FEP by 1.1, 1.2, 1.4 and 1.5 times, respectively. Data given in this section complement the available information on the subject under discussion and clearly demonstrate the possibility of using milk protein concentrates obtained by ultrafiltration for production of dispersed systems of dairy products with frothy and emulsion texture.

It is believed that the rheological properties are responsible for the quality of the products and largely determine the properties of biological systems at various stages of the production process. As previously shown, the capabilities of UV-treatment of lactic acid clot allows achieving the concentration of milk components, which in native form have some properties of Newtonian liquids. In the course of in-bulk process (during fermentation by starter population) the particles of the dispersed phase grow (micelles coagulate) losing their thermodynamic stability, whereas at ultrafiltration their concentration per unit volume increases. It is this effect that leads to the transformation of rheological properties.

Using the full factorial experiment N^3, we have investigated the two most significant characteristics, which are able to indicate the change of the dispersed system properties in time. These are the yield value (Y_1, *Pa*) and the adhesion, expressed in terms of the adhesive pressure indicator (Y_2, *Pa*) to steel St3 (contact area - 0.001 m^2, thickness - 0.001 m, and the velocity for separation - 0.005 m/s).

The duration of ultrafiltration (X_1, h), fat weight fraction (X_2, %), and the average diameter of micelles (X_3, nm) in MPC-UF were used as variables, while the normalization was performed after conducting concentration by membrane.

The adequacy of the mathematical models and the reliability of the results were checked against the known Fisher and Student criteria. The data scattering of 3-5% was quite satisfactory for this kind experiments used in the food industry.

According to the obtained results we constructed the response surface sections corresponding to certain values of resulting criteria. We present the analysis of the obtained results.

With the increase in both the duration of ultrafiltration and fat weight fraction, the yield values in MPC-UF rise. This fact is associated with the increase of the dry solids weight ratio, which largely determines the strength of ultrafiltration fermented milk clot.

A different pattern emerges when evaluating the participation of the micelles of casein in the formation of rheological characteristics. It is established that the increase in the average diameter of the micelles from 200 to 260 nm is a factor increasing strength. Given that in the first period of ultrafiltration, the micelles with the largest diameter undergo fractionation, we have shown that the area of the region corresponding to maximum yield values (at a level of 175 Pa) is minimal.

On the average, within limits of varying factors, the strength of the obtained concentrates changes from 155 to 165 Pa. However, in terms of the organoleptic assessment, the best samples are those with the strength changed from 160 to 170 Pa. Such characteristics can be obtained within the following values of the variable factors (in coded form): duration of ultrafiltration - from 0.13 to 1; the average diameter of micelles - from 0.35 to 0.85, and the fat weight fraction - from ….. to 0.65. This should be taken into account during processing since it is impractical to carry out the fermentation of the mixture up to micelles diameter of 302 nm (the average diameter of the micelles less than 242 nm is irrational).

It is revealed that with increasing duration of ultrafiltration the adhesion pressure increases, at that, the smaller the diameter of the micelles the greater the adhesion force between the product and the work tool.

The visual estimation revealed that the most satisfactory are the samples in which the adhesion pressure is greater than $6.0 \cdot 10^4$ Pa that meets the following process flow parameters: the duration of the ultrafiltration is more than 0.63 in coded units that corresponds to more than 3.7 hours, fat weight fraction is less than 0.3 (8.88% in its natural form), and the average micelle diameter is more than 0.8 (298 nm).

Analysis of the results obtained has shown that the strength of resultant disperse systems in the case of using thermophilic cultures is lower than that corresponding to use of mesophilic microorganisms. The maximum yield values (over 140 Pa) (corresponding to the best organoleptic estimation) are peculiar to the samples obtained through the ultrafiltration processing at a level not less than 0.58 (in coded units) and for the casein micelles with diameter of more than 0.7. Normalizing of MPC-UF (II) per the fat weight fraction to 0.3 conventional units is also a favorable factor to obtain a product with desired characteristics. However, it follows from the presented characteristic curve that in the diagram most of the yield values correspond to the strength indicators ranged from 135 to 140 Pa.

The obtained samples are characterized by high adhesion pressure. Thus, we were unable to obtain the product with the adhesion of less than $6.0 \cdot 10^4$ Pa, however, it can be assumed that the use of either longer duration of ultrafiltration or the normalization of the product per fat weight fraction (in excess of the values observed in the experiment) will allow reducing the interaction force between the product and material.

In general, it should be stated that the use of thermophilic microorganisms for fermentation is not rational, since in terms of the rheological characteristics these products are not fully satisfactory. Based on the obtained data, we can assume also that the formation of a clot on the surface of the ultrafiltration membrane will be hindered due to highly concentrated polarization conditioned by the adhesive properties of the MPC-UF (II). This does not contradict the results of the experiments that describe the specific productivity of the membrane for lactic acid clot, obtained in the series of experiments II.

Combined use of mesophilic and thermophilic starter cultures has response surfaces similar to those obtained when evaluating the consistent patterns for yield values of MPC-UF (II) with the difference that they exceed the latter on average by 6.3-6.9%. However, the samples with a strength ranged from 145 to 155 Pa are characterized by the best organoleptic properties. This extensive area of values corresponds to the following process parameters presented in coded form: the duration of ultrafiltration - from 0.2 to 0.62; the mean diameter of casein micelles - more than 0.6; and fat the weight fraction - from 0.3 to 0.81.

In terms of adhesion properties, the studied samples also require further adjustments of the process, because most of the values in the chart belong to the area, where adhesion is more than $6.0 \cdot 10^4$ Pa, while the influence of technological factors has a similar trend as in the case of use of the thermophilic starter cultures for fermentation.

CONCLUSION

In general, the experimental results presented in the current work and the conducted analysis establishes the main consistent patterns of obtaining milk protein concentrates by ultrafiltration. Identified processing quality suggests the possibility of their effective use in the production technology of protein dairy products. It is important that MPC-UF can be obtained using both mesophilic and thermophilic cultures, and it is this feature of the process that allows adjusting the composition and properties of MPC-UF.

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