UDC 637.147.2
DOI 10.12737/1514

METHOD OF OBTAINING MICROPARTICULATED CASEIN AND THE POSSIBILITY OF ITS APPLICATION IN THE PRODUCTION OF NONFAT FERMENTED MILK PRODUCTS

I.A. Smirnova, I.V. Romanovskaya, V.K. Shtrigul

Kemerovo Institute of Food Science and Technology,
bul'v. Stroitelei 47, Kemerovo, 650056 Russia
phone:/Fax: +7 (3842) 39-68-58, e-mail: milk@kemtipp.ru

(Received February 12, 2013; Accepted in revised form April 03, 2013)

Abstract: Reducing the calorie content of everyday food, including dairy beverages, is among the key recommendations of present-day dietitians. However, fat reduction worsens the organoleptically evaluated texture and flavor score of food. The organoleptic correction of nonfat products using fat simulants often needs additional financial inputs. Furthermore, fat simulants added to the product can be hazardous to human health.

Taking into consideration these facts, we have developed a casein microparticulation technology and have investigated the possibility of employing microparticulated casein in the production of nonfat fermented dairy beverages. Unlike the existing milk protein microparticulation technologies, the technology presented here uses casein in place of whey protein, which is difficult to produce and, as a consequence, expensive. A casein coagulation method has been devised, and the optimal processing parameters have been determined. A technology for producing nonfat lapper milk from skim milk has been developed. The new product has the same texture and flavor as its analogue containing 3.2 wt % fat.

Key words: microparticulation, casein, renneting, fat simulant, viscosity, active acidity, enzyme.

INTRODUCTION

The rapid progress in engineering and technology has markedly reduced the amount of manual labor in the present-day society. The decreased physical activity and sedentary lifestyle combined with traditional dietary patterns leads to overweight and obesity, which can cause so-called diseases of civilization, such as hypertension, coronary heart disease, insult, gallbladder diseases, osteoarthritis, dyspnea, rectal cancer, and diabetes [1].

The problem of instilling the culture of consumption in people and that of changing their dietary patterns should be solved at all society levels, including the government. Of great importance for solving the latter problem are public awareness of the principles of a healthy balanced diet and production of low-calorie foods.

A significant decrease in the calorie content of food can be achieved by reducing the amount of fat, the highest calorie component, whose energy value is more than two times higher than that of proteins and carbohydrates, the more so because milk fat is not an indispensable component of food from the standpoint of nutritional value.

However, low-fat dairy products may have some defects, for example, flat taste, too thin texture, and too coarse texture with signs of mealiness and curd grains (for protein foods) [2]. The nutritional value of food is of obvious significance, but the consumer's choice is actually governed by the organoleptic properties of food.

In practice, the organoleptic properties of nonfat foods is increasingly being corrected using fat simulants, which are low-calorie substances creating the illusion of the presence of fat. This property of fat substitutes is due to their particles falling in the size range from 0.5 to 2.0 ?m and having a spherical or near-spherical shape. This is the reason why foods containing fat substitutes provide a creamy mouthfeel when they are masticated. Particles larger than 3 ?m in aqueous dispersion are perceived as powdery, chalky, and sandy (as their size increases), and dispersed particles smaller than 0.1 ?m are perceived as a true solute [3].

Fat simulants differ in their nature and may be based on a carbohydrate, protein, or fat or on their combination.

Carbohydrate-based fat substitutes are produced from natural gums, agar, modified starch, or corn fiber. They absorb water, thus imitating the fat volume and structure, and are used in the production of pastry, convenience meat products, spreads, soups, salad seasonings, glaze, and frozen desserts [4].

Protein-based fat substitutes are produced by heating and grinding (microgranulation) of milk and egg proteins or a mixture of egg and whey proteins and xanthan gum. They are unsuitable for foods whose preparation involves high-temperature treatment, because the proteins undergo denaturation at elevated temperatures, their structure breaks down, and they lose their ability to simulate fat [5].

The method of producing microgranulated whey protein (MWP) was developed in 1984 by the Canadian inventors Norman S. Singer, Shoji Yamamoto, and Joseph Latella [6]. The microgranulated protein Simplesse®-100 is readily dispersible and dissolves rapidly without use of specialized equipment or technology; it acts as an ersatz dispersed phase in place of the fat droplets that traditionally function as the dispersed phase and simulates the creamy texture of the product [7, 8].

The particles of denaturated whey protein in milk–protein products and fermented dairy beverages participate in the formation of a casein clot: they incorporate in the protein matrix to function as the fat globules that they have replaced [9, 10].

However, the price of these additives is 670–730 RUB/kg, which is unacceptable for Russian manufacturers. In addition, MWP is not manufactured in Russia on the industrial scale, and this can make the production of MWP-based low-calorie foods import-dependent.

Our analysis of the literature concerning this problem suggests that Russia strongly needs domestic technologies for production of reduced-fat foods with good organoleptic properties using raw materials and equipment available for the existing enterprises.

The purpose of this study was to develop a protein-based low-calorie fat simulant and to see whether this simulant is usable in the production of nonfat lapper milk.

EXPERIMENTAL

The technical problem addressed here is to obtain ~1 ?m spherical protein particles that are uniformly distributable in the raw material–product colloidal system without changes occurring in their dispersion, shape, and size in all technology steps.

The objects of this study were nonfat cow's milk (Magnitogorsk Dairy Plant) and a nonfat fermented dairy beverage produced by the thermostat method involving a casein microparticulation stage.

The following reagents were used in milk coagulation:

- heat–acid coagulation: 10% acetic acid solution (VitaKhim Perm' Co., Perm, Russia);
- acid coagulation: dry starter cultures *L. lactis, Str. thermophilus,* and *L. acidophilus* (Barnaul'skaya Biofabrika Co., Barnaul, Russia);
- heat–calcium coagulation: 40% calcium chloride solution (Univerkhim Co., Chelyabinsk, Russia);
- renneting: microbial chymosin Maxiren® (DSM, Netherlands).

The medium was neutralized to pH 7.00 with 10% sodium hydroxide E524 (Kaustik Co., Volgograd, Russia).

The organoleptic, physicochemical, and rheological properties of the raw materials and products were investigated by standard methods.

The raw materials and products were sampled and prepared for physicochemical characterization in conformity with ISO 707:2008 (IDF 50: 2008) "Milk and milk products – Guidance on sampling".

Organoleptic analyses were performed in conformity with ISO 22935-2:2009 "Milk and milk products. Sensory analysis. Part 2. Recommended methods for sensory evaluation (IDT)."

Active acidity was determined potentiometrically as prescribed by the RF standard GOST R 53359-2009 (Milk and products of milk processing. Method for determination of pH). Active acidity (pH) can be measured with any potentiometer whose measurement range is pH 0–12 (14) by following the instructions for the instrument.

The viscosity of dairy raw materials and products was determined on a VPZh-4 capillary viscometer via a procedure prescribed by the RF standard GOST 33-2000. In this method, a certain volume of the liquid examined flows by gravity out of a graduated glass capillary at a constant temperature and its outflow time is measured in seconds. Kinematic viscosity is the product of the outflow time and the viscometer constant [11].

The shape of casein micelles was determined by microscopic examinations.

The size of casein micelles was determined by a turbidimetric method based on measuring the intensity of light passing through the dispersion [12]. These turbidity measurements can be carried out with a KFK-2 photoelectric colorimeter, which is intended for measuring the absorbance of colored molecular solutions. Its operation is based on the equalization, with an adjustable slit diaphragm, of two light fluxes, one passing through a cuvette with the sol analyzed and the other through a cuvette with a standard sol,. A calibration plot is constructed beforehand using, e.g., an electron microscope. The particle size is derived from colorimetric data using this plot and Geller's formulas.

RESULTS AND DISCUSSION

The scientific novelty of the technology suggested here is that particles of desired size and shape are obtained by controlled coagulation of native proteins of milk, with the process terminated once the particles have reached a size of ~1 ?m. This technology is unique and is not used by any manufacturer in the world. It differs from the existing technologies in that the main component of the fat simulant is casein, a readily available substance, rather than an expensive whey concentrate (Table 1).

Table 1. Some characteristics of the product obtained in this study and analogous fat substitutes

Evaluation parameter	Our product	Simplesse	Olestra
Base	Casein (main milk protein)	Whey proteins	Sucrose polyester
Assimilability in organism	Completely assimilable	Completely assimilable, recognized as safe	Nonassimilable; no final data on safety
Thermal processibility	Intolerant to heat	Intolerant to heat	Heat-treatable
Calorie content, kcal/g	1–4	1–4	5
Price, RUB/t	60 000–80 000	690 000	450 000

Initially, we studied the main milk coagulation mechanisms in order to find the most appropriate method of obtaining particles of desired size and shape.

In the investigation of heat–acid coagulation, the coagulant was a 10% solution of acetic acid (VitaKhim Perm', Perm, Russia), with its dose varied between 0.2 and 1.0%. The acid type, concentration, and dosage were chosen on the basis of earlier data on the heat–acid coagulation of milk proteins [13]. Coagulation was performed between 25 and 85°C, with the temperature changed in 20°C steps. The coagulation process was terminated by rapidly cooling the system to 20°C.

The appropriateness of the size of the resulting casein particles was indirectly assessed by viscosity measurements after cooling (Table 2).

Table 2. Variation of the viscosity of the product with coagulation temperature at different doses of acetic acid

Dose of acetic acid solution, %	Viscosity of the mixture, mm^2/s, at the following temperatures			
	25±2°C	45±2°C	65±2°C	85±2°C
0.2	3.31	3.61	4.40	Pronounced coagulation
0.4	3.56	3.86	4.42	Pronounced coagulation
0.6	3.59	3.93	4.69	Pronounced coagulation
0.8	3.84	4.10	4.69	Pronounced coagulation
1.0	3.86	4.14	4.97	Pronounced coagulation

The results of this experiment indicate that increasing the dose of 10% acetic acid at a constant experimental temperature exerts an insignificant effect on milk coagulation, increasing the viscosity by only 6–8%. Raising the coagulation temperature turned out to be a more effective means to increase the kinematic viscosity by approximately 14–16%. This is due to the fact that the action of heat on protein molecules is the main dehydrating factor, which simultaneously causes the breaking of intramolecular bonds and protein denaturation.

Coagulation can theoretically be hampered by abruptly dropping the temperature. However, experience has demonstrated that milk, like any other colloidal system, is inertial and it takes a relatively long time to change its temperature. Because the state of the milk proteins changes very rapidly under the action of the acid and heat, it seems impossible to control the heat–acid coagulation process, particularly under industrial conditions.

Acid coagulation was examined as another possible way of obtaining casein-based fat simulants. Experiments were performed with lactic acid bacteria differing in their heat tolerance and acid production activity: *L. lactis, Str. thermophilus,* and *L. acidophilus.*

Milk coagulation was terminated by rapidly cooling the mixture to 10°C—the simplest possible method. This was done 1 h before a significant increase in viscosity took place, specifically 6, 3, and 4 h after the beginning of fermentation for *L. lactis, Str. thermophilus,* and *L. acidophilus,* respectively.

Pasteurized and cooled milk was fermented with pure cultures of lactic acid bacteria, which were introduced as a bulk starter (1% of the milk weight). Thereafter, milk samples were thermostated for a certain time at the optimum temperature for the development of the microorganisms. Next, the samples were cooled and their viscosity was measured.

As a result of this processing, the viscosity of the mixture increased to $(6.3–7.15) \cdot 10^6$ m^2/s. This was due to the aggregation of protein molecules as a result of acid coagulation occurring via the following mechanism: lactic acid accumulating in the milk owing to lactose fermentation by the lactic acid bacteria reduces the negative charge of casein micelles and brings casein into the isoelectric state (pH 4.6–4.7). At this point in time, the interparticle repulsion potential decreases and the protein macromolecules lose their solubility and stability.

But acid coagulation is a difficult-to-control process depending on many factors that are uncorrelatable under industrial conditions, including milk composition variation with the cow's breed, individual features, state of health, and lactation period, with the way of caring for the cow, with the type of feed, and with the sanitary conditions under which the milk was produced. Note that dairy plants most often receive bulk milk, and it is difficult to control its quality and to take into account all significant factors.

In addition, as the starter cultures employed in the dairy industry are transported and stored, they may undergo progressive deactivation. Different starter batches may contain bacterial strains differing in their acid formation capacity. However, the most frequent cause of the deactivation of starter microflora is the action of bacteriophages [14].

Thus, the production of protein-based fat simulants by the acid coagulation of milk is difficult to organize and control on the industrial scale.

We also investigated the applicability of the calcium chloride coagulation of milk proteins to the production of casein-based fat simulants. Calcium chloride was introduced as its 40% solution, with the solution dose varied between 0.5 and 1.25% in 0.25% steps. As in the case of heat–acid coagulation, the milk coagulation temperature was varied between 25 and 85°C in 20°C steps. The coagulation process was terminated by rapid cooling. The viscosity of the medium was chosen to be the response parameter (Table 3).

An analysis of these experimental data demonstrated that an increase in the dose of 40% calcium chloride at a fixed temperature exerts only a slight effect on milk coagulation, increasing the viscosity of the medium by only 1–3% on the average. A more significant factor here is coagulation temperature: as the temperature is elevated from 25 to 65°C, the viscosity increases by 16–79%, depending on the calcium chloride dose.

The increase in milk viscosity is explained by the growth of protein particles. The introduction of calcium chloride into fresh milk reduces the stability of the colloidal dispersion of the calcium caseinate–calcium phosphate complex. This is accompanied by the exchange of H ions of the casein complex for Ca ions from the calcium chloride solution. As a result of this

cation exchange, the calcium caseinate–calcium phosphate complex is further enriched with calcium owing to the liberation of H ions. This acidifies the milk, bringing it from pH 6.5 to pH 5.0, and causes the aggregation of particles of the complex. Because of the specific physicochemical properties of milk, it is impossible to quickly terminate its coagulation by lowering its temperature.

Table 3. Calcium chloride dose and temperature effects on the viscosity of the milk mixture in calcium chloride coagulation

Dose of 40% $CaCl_2$, %	Viscosity of the mixture, mm^2/s, at the following temperatures			
	25±2°C	45±2°C	65±2°C	85±2°C
0.25	3.05	3.35	4.21	Pronounced coagulation
0.50	3.11	3.42	4.71	Pronounced coagulation
0.75	3.15	3.50	4.98	Pronounced coagulation
1.00	3.26	3.55	5.50	Pronounced coagulation
1.25	3.32	3.61	6.82	Pronounced coagulation

Thus, the calcium chloride coagulation of milk is difficult to monitor and control. Cooling only slows down the process slightly but does not terminate it. A significant drawback of the process is the unpleasant taste of its product that appears even in the presence of a small amount of calcium chloride.

In order to form protein particles of desired size and shape, we investigated milk renneting, in which the main coagulant is a milk-clotting enzyme. The action of milk-clotting enzymes can be corrected by varying the processing temperature, the ionic strength of the medium, the $CaCl_2$ concentration, and the processing time.

Investigation of the milk-clotting activity (capacity for rapidly hydrolyzing χ-casein) and total proteolytic activity (capacity for cleaving other bonds in proteins) demonstrated an obvious advantage of chymosin over the other enzymes: chymosin shows the lowest proteolytic activity relative to milk-clotting activity.

All of the milk-clotting enzymes commonly used in the dairy industry are acid proteases, which exhibit their maximum activity in acid media. The optimum active acidity for χ-casein hydrolysis with chymosin is pH 6.0, and the reaction rate varies only slightly in the pH 5.6–6.4 range. A common property of the milk-clotting enzymes is that their total proteolytic activity declines when pH is above its optimum level. We suggest that this property be used to terminate the coagulation process.

Milk-clotting enzymes vary in terms of the temperature dependence of their activity; for chymosin, the optimum temperature is 45°C.

The necessary amount of enzyme depends considerably on the physicochemical properties of the milk to be processed, but usually 2.5 g of an enzyme preparation with an activity of 100 000 U is added to 100 kg of milk. In order to check the effect of a dose of rennet enzyme and the duration of its coagulating action on milk, we carried experiments in which the rennet enzyme dose was reduced so as to prevent pronounced coagulation of milk proteins.

An enzyme solution (0.02–0.1% of the mixture volume, 0.04% increments) was introduced into milk at 40–42°C for a higher effectiveness of the enzyme. The increasing size of casein globules was estimated as the viscosity of the medium 10, 20, and 30 min after the introduction of all reagents (Table 4).

Table 4. Variation of viscosity with the rennet enzyme dose and fermentation time

Holding time, min	Dose of rennet enzyme (as a solution with an activity of 100–150 U), %		
	0.02	0.06	0.1
	Viscosity, mm^2/s		
10	3.07	3.47	4.07
20	3.29	4.46	8.87
30	3.53	5.67	Pronounced coagulation
Total clotting time, min	70	60	30

Note that the smaller the rennet enzyme dose the longer the clotting time. Based on experimental data, we set the enzyme dose to be 0.1%, since only with this dose did we obtain the desired casein particle sizes in a short time.

To study the active acidity effect on casein coagulation with chymosin, we carried out a series of experiments in which active acidity was varied between pH 5.80 and pH 7.00 in 0.40 steps by adding a 10% acetic acid solution. The enzyme dose was fixed. The response signal was the kinematic viscosity of the medium measured aster fermentation (Table 5).

Table 5. Dependence of the viscosity of the fermentation mixture on the dose of rennet enzyme (as a solution with an activity of 100–150 U) and on the active acidity of the medium

Dose of rennet enzyme (as a solution with an activity of 100–150 U), %	Viscosity, mm^2/s			
	pH 5.80	pH 6.20	pH 6.60	pH 7.00
0.02	7.03	6.95	6.72	6.24
0.06	8.15	7.52	7.25	6.65
0.1	9.05	8.92	8.72	7.85

These experiments demonstrated that, as the active acidity decreases, the viscosity of the mixture increases. This is quite natural: at pH 5.80, the system approaches the isoelectric point, at which acid coagulation dominates. Serious drawbacks of this coagulation process were revealed. This pH range is rather risky for the technology suggested. For this reason, pH 6.20 was taken to be the optimum acidity for the formation of particles of desired sizes and shapes.

We studied the effect of calcium chloride ($CaCl_2$) on the rennet coagulability of milk with the aim of gaining control over the growth rate of casein particles. Calcium is uninvolved in the enzymatic stage of renneting;

however, variation of its concentration in the medium can change the physicochemical conditions.

A calcium chloride dose recommended for the dairy industry (40 g of anhydrous salt as a 40% solution per 100 kg of milk) was introduced into the sample to be examined. Next, different amounts of the chymosin solution were added at pH 6.20. The same amount of enzyme was added to the reference sample at the same active acidity of the medium, but $CaCl_2$ was not. The viscosity data obtained in this experiment are presented in Table 6.

Table 6. Effect of calcium chloride on the viscosity of the mixture in milk renneting

Dose of rennet enzyme (as a solution with an activity of 100–150 U), %	Viscosity, mm^2/s	
	pH 6.20	
	$CaCl_2$	reference sample
0.02	6.95	6.97
0.06	7.52	7.48
0.1	8.92	8.87

Clearly, the viscosity of the milk samples into which $CaCl_2$ was introduced differs little (within the experimental error) from the viscosity of the samples obtained without adding calcium chloride. This finding confirms the data earlier published on the effect of calcium chloride on milk renneting: $CaCl_2$ exerts an effect on the first stage of coagulation only by changing the pH of the medium. Because the pH value in our experiment was maintained constant, optimal for the functioning of the rennet enzyme, $CaCl_2$ had no significant effect on the viscosity of the milk mixture.

No preliminary pasteurization was performed in this experiment. Because of this, the entire calcium remained in solution, and its amount was sufficient for milk renneting. However, dairy plants receive bulk milk (including slow-renneting milk) and it is difficult to check its renneting capacity. For this reason, it is suggested that the minimum recommended dose of calcium chloride be added into milk at the casein microparticulation stage in order to avoid spoilage losses.

By analyzing the results and parameters of this study, we established that the most rational coagulation method for obtaining the desired particle sizes and shapes is renneting. The following requirements were taken into consideration in the selection of a coagulation method for this purpose:

- acceptable organoleptic properties of the resulting mixture;
- low cost of the coagulant;
- use of standard equipment;
- controllability of the coagulation process;
- safety of the coagulant.

The renneting process is easy to control and needs no specialized equipment. A stable system can be obtained by renneting using technological manipulations. The reagents used in milk renneting do not impart a foreign smell or taste to the product. All milk-clotting enzymes are absolutely nonhazardous to human health.

Based on our studies, we have developed a technology for producing microparticulated proteins that can be used as fat simulants in dairy products.

Figure 1 schematizes the renneting process and the way in which it is terminated.

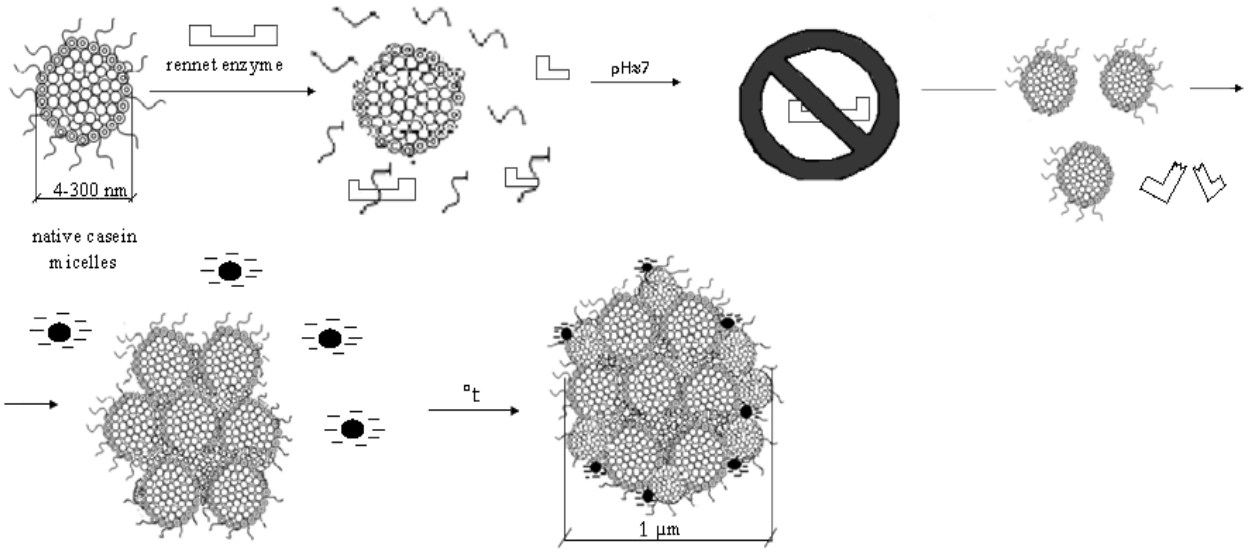

Fig. 1. Casein renneting and its termination.

For obtaining the desired particle size and shape, the renneting process should be started and terminated at a point in time such that the size of the protein particles have already increased, but no clot has formed. It is necessary here that a stable system be formed.

The average size of native casein micelles is about 40–300 nm. Provided that the optimum conditions are established, glycomacropeptide is eliminated from the surface of the micelles and hydrophobic areas appear under the action of the rennet enzyme (temperature,

calcium salts, active acidity), causing the micelles to stick together.

The process was terminated by neutralizing the medium to pH 7.00 by adding alkali solutions permitted in the food industry. This slowed down the action of the film-clotting enzyme. After heat treatment at 96 ± 2°C, the enzyme was completely inactivated and denaturated whey proteins deposited on the casein globules obtained. These proteins made the microparticles resistant to sedimentation and sticking by increasing the negative charge on their surface. This is how the desired particle size (~1 ?m) was obtained.

Using the above casein microparticulation technology, we produced experimental batches of fermented milk products, including lapper milk. The production of nonfat lapper milk containing microparticulated casein is illustrated by Fig. 2.

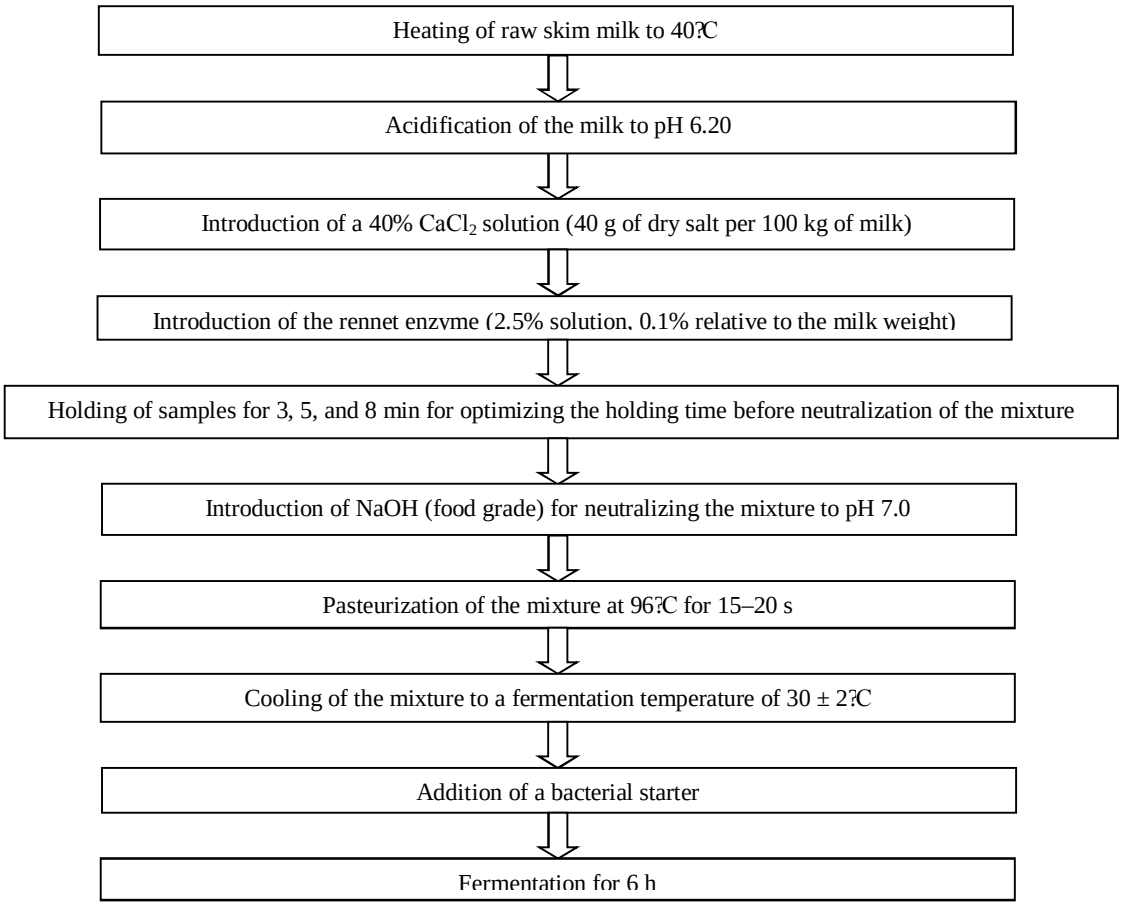

Fig. 2. Production of nonfat lapper milk containing microparticulated casein (MPC).

The reference samples were lapper milk produced using a conventional technology from nonfat milk and lapper milk with a fat content of 3.2 wt %. The quality criteria for the fermented dairy beverages were rheological properties (viscosity) and organoleptic characteristics (taste, texture). The viscosity data are listed in Table 7.

These experiments demonstrated that the viscosity of the finished product depends on the fermentation time at the microparticulation stage: as the holing time is lengthened, the viscosity of the fermented milk drink increases.

In terms of viscosity, the sample fermented for 5 min is most similar to the sample containing 3.2 wt % fat. The taste and texture of the products were evaluated in terms of the creamy mouthfeel criterion (creamy taste and thickness), which was measured on a five-point scale. The creamy mouthfeel evaluation data for the lapper milk samples are presented as a histogram in Fig. 3.

Table 7. Viscosity of the fermented milk products obtained using the casein microparticulation stage and without microparticulation

Lapper milk viscosity, mm^2/s				
nonfat	**fat content of 3.2 wt %**	**with microparticulated casein; fermentation for the following lengths of time, min**		
		3	5	8
8.45	11.3	10.5	28.7	32.2

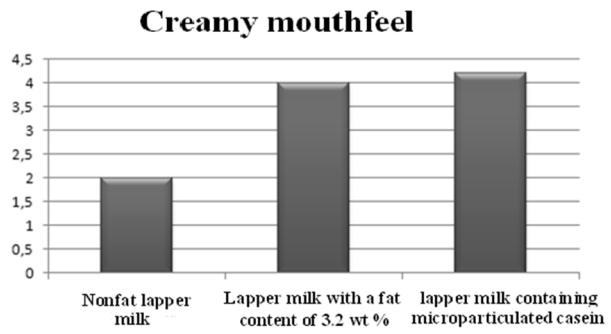

Fig. 3. Creamy mouthfeel data for lapper milk: nonfat sample and samples containing 3.2 wt % fat and microparticulated casein.

The properties of the fermented dairy beverages containing microparticulated casein demonstrated that casein microparticulation stage can be used to improve the organoleptic characteristics of nonfat fermented milk products. Microparticulated casein makes the texture and taste of the products more pleasant for the consumer. It provides means to significantly decrease the calorie content of the products via fat reduction, without affecting the amounts of other components (proteins, vitamins, microelements). The milk products thus retain their nutritional value and wholesomeness.

The low-calorie fermented milk beverage production technology involving a casein microparticulation stage offers the following advantages:

(1) It does not need involvement of any additional raw materials in the dairy industry.

(2) It widens the range of low-calorie dairy beverages, imparts a rich taste and smooth and creamy texture to them, and thus increases the consumer demand for these foods.

(3) The new products have a high nutritional and biological value combined with their calorie content reduced by a factor larger than 2 and are thus functionally specialized.

REFERENCES

1. Vasilevskaya, L.S. and Okhnyanskaya, L.G., Fiziologicheskie osnovy problemy pitaniya (Physiological foundations of the nutrition problem), *Voprosy pitaniya* (Topics in nutrition), 2002, no. 2, pp. 19–20.

2. Shidlovskaya, V.P., *Organolepticheskie svoistva moloka i molochnykh produktov: Spravochnik* (Organoleptic Properties of Milk and Dairy Products: A Handbook), Moscow: Kolos, 2000.

3. Singer, N. S. and Moser, R.H., Microparticulated proteins as fat substitutes, in *Low Calorie Foods Handbook*, Altschul, A.M., Ed., New York: Marcel Dekker, 1993, ch. 9.

4. Akoh, C.C., Fat replacers, *Food Technology*, 1998, vol. 52, no. 3, pp. 47–53.

5. Gershoff, S. N., Nutrition evaluation of dietary fat substitutes, *Nutrition Reviews*, 1995, no. 11. http://www.findarticles.com/p/articles/mi_qa3624/is_199511.html

6. US Patent 4734287, 1988.

7. Queguiner, C., et al., Microcoagulation of a whey protein isolate by extrusion cooking at acid pH, *Journal of Food Science*, 1992, vol. 57, no. 3, pp. 610–616.

8. Schirle-Keller, J.-P., Chang, H.H., and Reineccius, G.A., Interaction of flavor compounds with microparticulated proteins, *Journal of Food Science*, 1992, vol. 57, no. 6, pp. 1448–1451.

9. Tamime, A.Y., et al., The microstructure of set-style, natural yogurt made by substituting microparticulate whey protein for milk fat, *International Journal of Dairy Technology*, 1995, vol. 48, no. 4, pp. 107–111.

10. Manylov, S.V., Issledovanie vliyaniya denaturirovannykh syvorotochnykh belkov na svoistva nizkokaloriinykh molochno-belkovykh produktov (Effect of denaturated whey proteins on the properties of low-calorie dairy protein products), *Cand. Sci. (Eng.) Dissertation*, Moscow, 2009.

11. Krus', G.N., Shalygina, A.M., and Volokitina, Z.V., *Metody issledovaniya moloka i molochnykh produktov* (Methods of Examination of Milk and Dairy Products), Moscow: Kolos, 2004.

12. Frolov, Yu.G., *Kurs kolloidnoi khimii: Poverkhnostnye yavleniya i dispersnye sistemy* (Colloid Chemistry: Surface Phenomena and Dispersions), Moscow: Khimiya, 1982.

13. Smirnova, I.A., *Biotekhnologicheskie aspekty proizvodstva termo-kislotnykh syrov* (Biotechnological Aspects of the Production of Heat–Acid Precipitated Cheese), Kemerovo: KTIPP, 2002.

14. Gudkov, A.V., *Syrodelie: Tekhnologicheskie, biologicheskie i fiziko-khimicheskie aspekty* (Cheese making: Technological, Biological, and Physicochemical Aspects), Moscow: DeLi Print, 2004.