Biotechnology of freeze-dried sour clotted milk with pumpkin and topinambour

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I S Krasnova[1,3], V I Ganina[2], G V Semenov[1], I I Ionova[1] and Zh L Guchok[1]

[1] Department of technology and biotechnology of animal food, Moscow State University of food production, 33, Talalikhina street, Moscow, 109316, Russia
[2] Laboratory of Design and implementation of personalized food products and diets, K.G. Razumovsky Moscow State University of technologies and management (the First Cossack University), 73, Zemlyanoy Val street, Moscow, 109004, Russia

[3] E-mail: ira3891@mail.ru

Abstract. The research aimed to substantiate rational parameters of the vacuum freeze-dried sour clotted milk biotechnology. It was revealed that the use of novel starters containing cultures that can synthesize exopolysaccharides provided a symbiotic relationship of fermented milk system particles and prevented its disintegration in freeze dry products rehydration. This was confirmed by microscopic analysis of histological preparations prepared from rehydrated samples of freeze-dried sour clotted milk. It was shown that the addition of topinambour powder resulted in production of freeze-dried sour clotted milk with organoleptic parameters not meeting established requirements. Adding freeze-dried pumpkin powder to the sour clotted milk formulation contributes to increased survival of lactic acid bacteria cells during vacuum freeze drying by an order of magnitude in average. It has been shown that the combined use of novel starter cultures and prebiotics results in production of freeze-dried sour clotted milk with required quality parameters.

1. Introduction

Fermented milk products, including probiotic ones, are widespread, have a variety of useful properties and are actively consumed by the population [1,2]. It is known that health benefits of fermented milk products are largely provided by the composition and properties of starter lactic acid bacteria used [3-6]. However, a short shelf life, temperature-controlled conditions during storage and transportation do not always make it possible to deliver fermented milk products to consumers in remote regions under emergency, autonomous and other conditions. In this regard, it is important to develop and implement technologies that ensure maximum preservation of natural substances of the source raw material and production of dry fermented milk functional products with a long shelf life. One of the generally recognized innovative technologies is vacuum freeze-drying. This technology combines two processes. In the first process, the drying objects are frozen to fix their structure. In the second, frozen moisture is removed under vacuum during the “ice-vapor” phase transition, i.e. freeze-drying at pressure lower than triple-point pressure of water. Moisture migrates inside the material as steam, keeping the shape and size of the drying objects practically unchanged. A porous structure with low final moisture content and low specific gravity is formed. At all stages of drying, exposure to high temperatures is excluded, which are the main reason for the reduced quality of thermolabile materials.

To preserve the number of lactic acid bacteria cells and increase their viability during freeze-drying, cryoprotectors are recommended. It is believed that the presence of dietary fibre in a product is one of the possible factors that act as cryoprotectors to provide more gentle conditions for freezing and drying of bacterial suspensions.

In this regard, the research objective is to substantiate the biotechnology of freeze-dried sour clotted milk with the use of polysaccharides contained in topinambour and pumpkin.

2. Materials and methods

2.1. Research objects

The research objects were sour clotted milk samples produced using previously isolated, identified, researched and selected lactic acid bacteria strains (*Lactococcus lactis ssp., Streptococcus salivarius thermophilus, Lactobacillus delbrueckii subsp. bulgaricus*), having a complex of technological properties and exopolysaccharide capacity [7]. Freeze-dried powders of pumpkin (a source of pectin) and topinambour (a source of inulin) were used as prebiotics.

2.2. Sour clotted milk production

Sour clotted milk was produced using pasteurized normalized milk with 3.2% fat in dry matter. Freeze-dried topinambour or pumpkin powders were then added to the research samples in the amount of 5%. When producing research samples, starter cultures of novel lactic acid bacteria strains with functional and technological properties were used, and for control samples - typical starter cultures were used. Sour clotted milk samples were obtained in a thermostat by fermenting them at a temperature of 32±1°C. Control samples were produced in the same way, but without additives. After reaching the required titratable acidity, the sour clotted milk samples were cooled to 4±2°C and sent for freeze-drying.

2.3. Cell number determination

The number of lactic acid bacteria was determined by the plate method by inoculation of the analyzed object dilutions on MRS agar medium, as well as inoculation in sterile skim milk, followed by cultivation and counting the most probable number of cells. The plates were incubated anaerobically and the cultures in sterile milk in test tubes were incubated under aerobic conditions at 37°C for 72 hours. [8].

2.4. Freezing

Sour clotted milk was frozen under conditions of forced convection at minus 20°C for 5-7 hours. Then the trays with the frozen product were placed in the lab scale freeze dryer [9].

2.5. Vacuum Freeze-Drying

Freeze-drying was carried out at a primary temperature of minus 25+20C. The secondary drying temperature was 40+2°C. The total duration of the freeze-drying process was 14 hours. The final moisture content of the dried sour clotted milk samples was 3.8-4.2%.

2.6. Microstructure analysis

The microstructure of specially prepared sections of curds of research and control samples of sour clotted milk restored from a dry state was researched and photographed using a light microscope Yenaval (Germany) with an image analysis system VideoTesT and Morfo-4.0 software. [10].

2.7. Sensory evaluation of sour clotted milk

The sensory evaluation included colour, aroma, taste, texture and overall acceptance. The panelists evaluated each attribute using a five-point scale to compare traditional yoghurt without fruits. Colour, taste, odour, consistency, texture and general perception of sour clotted milk samples were estimated

before and after freeze-drying. The panelists evaluated each attribute on a five-point scale. The control was traditional sour clotted milk produced with standard ferments without prebiotics.

2.8. Statistical Analysis
All experiments were performed in 5 replicates and the average value was calculated.

3. Results and discussion

3.1. Sour clotted milk production
In all researches, model samples of sour clotted milk with additives formed a curd after 6.0±0.25 hours, and control samples - after 7.5±0.5 hours. After reaching titratable acidity in model samples of sour clotted milk (85 ±3) ^{0}T and control ones (90 ±5) ^{0}T, they were cooled. The earlier curd formation in the model samples of sour clotted milk was due to introduction of topinambour or pumpkin powder, which was involved in the destabilization of calcium caseinate phosphate complex in milk and contributed to gel formation at a higher level of active acidity (lower titratable acidity) compared to control samples.

3.2. Cell number
The number of lactic acid bacteria cells after fermentation in research and control samples of sour clotted milk differed insignificantly and was as follows: in the control sample – $2.5*10^8$ CFU in 1 cm^3; in the sample with topinambour – $5.0*10^8$ CFU in 1 cm^3; in the sample with pumpkin – $6.0*10^8$ CFU in 1 cm^3. The number of lactic acid bacteria cells after freeze-drying was as follows: in the control sample – $6.0*10^8$ CFU in 1 cm^3; in the sample with topinambour – $3.0*10^9$ CFU in 1 cm^3; in the sample with pumpkin – $7.0*10^9$ CFU in 1 cm^3. The results obtained allow for the conclusion that lactic acid bacteria were more likely to survive in the samples with topinambour or pumpkin powders in the formulation as compared to the control one. Therefore, these additives acted as cryoprotectors.

3.3. Microstructure
Freeze-dried sour clotted milk samples restored their structure well as compared to the control samples, which was due to novel starter cultures synthesizing exopolysaccharides, and use of topinambour containing pectin and inulin, and pumpkin (a source of pectin). This was evidenced by microstructure of the control and research samples restored after vacuum freeze-drying (figures 1-3).

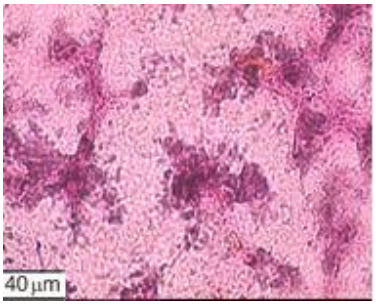

Figure 1. Microstructure of the control sample of sour clotted milk with a typical starter culture.

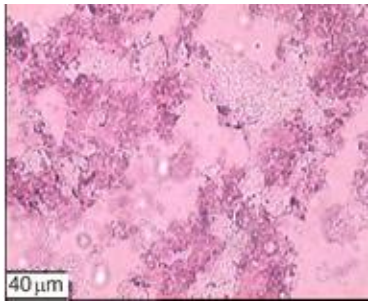

Figure 2. Microstructure of research sample of sour clotted milk with topinambour and new starter culture.

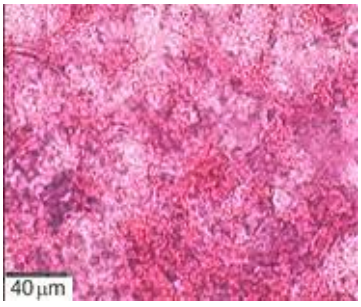

Figure 3. Microstructure of research sample of sour clotted milk with pumpkin and new starter culture.

Microscopic analysis of histological preparations of the clotted milk control sample revealed granular complexes of milk protein stained in pink-brown colour, uncolored fatty elements and basophilic cells of the starter culture microflora. Milk protein was grouped mainly in rather large unevenly distributed complexes. Between the protein complexes there were fat droplets and individual smaller particles of a

protein nature. The main part of microflora was associated with the surface of milk protein aggregates, contrasting in some places with their outer border. No large colonies of microflora were observed.

When researching the microstructure of the sour clotted milk sample with topinambour (figure 2) a mixed picture of milk protein complexes aggregation and distribution of starter microflora in histological preparations was observed. The bulk of microflora in the sour clotted milk sample with topinambour was diffusely distributed inside protein particles, however, bacterial cells were found in some places on their surface. The distribution of microflora bacterial cells on the surface of aggregated milk protein particles was significantly less intense compared to the control sample. The total mass of the system was more uniform in density and in the size of protein aggregates compared to the control sample, however, it was inferior in these parameters to the sour clotted milk with pumpkin (figure 3). In the sour clotted milk with pumpkin produced with the novel starter, a more homogeneous structure with evenly distributed small aggregates of milk protein was noted. At the same time, as compared to the control sample, an increase in the amount of smaller protein complexes was observed. Fat droplets were more evenly distributed between protein complexes evenly distributed over the mass of sour clotted milk, which is clearly seen in figure3.

Thus, in the research samples of the rehydrated sour clotted milk after freeze-drying, a large symbiotic relationship of protein complexes, bacterial cells and fat particles was observed, which led to the formation of a more uniform system. In this case, it can be said that a smaller exceptional volume has formed, which to a greater extent prevents the disintegration of the system. Therefore, we can conclude that the novel starters containing EPS cultures and the use of topinambour or pumpkin powders in the formulation contributed to the symbiotic relationship between the particles of the system and prevented its disintegration. The data obtained correlated with the sensory evaluation of the sour clotted milk samples after rehydration

3.4. Sensory evaluation of sour clotted milk

The sensitive parameters of the research samples of rehydrated sour clotted milk differed from the control samples in consistency, colour, and taste. In the research samples of sour clotted milk, the consistency was thicker and more homogeneous without visible separation of whey compared to control samples. The colour and taste of the obtained sour clotted milk samples were due to the additives. The use of freeze-dried pumpkin powder gave the sour clotted milk a slightly orange colour uniform throughout the mass and a sour milk taste with a pumpkin flavor. The use of topinambour resulted in production of grey sour clotted milk with specific taste not typical of a fermented milk product. The topinambour test samples along with samples before drying received a lower color and taste rating, and were excluded from further research.

4. Conclusion

The research results made it possible to substantiate the parameters of the biotechnology of freeze-dried sour clotted milk produced with the use of lactic acid bacteria synthesizing exopolysaccharides and pumpkin powder. The positive effect of freeze-dried pumpkin powder on the microstructure and preservation of lactic acid bacteria cells in the technology of freeze-dried functional fermented milk products has been shown.

The developed samples of freeze-dried sour clotted milk can be recommended for dietary nutrition, nutrition of people with high nervous stress and athletes, as well as for other groups of the population.

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