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The Influence and Effect of Treatment by Pressure of Gaseous Nitrogen on the Biotechnology and Microbiological Indicators of Cow's Milk.

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ABSTRACT

The article the results of studies are present about the effect of processing raw milk by pressure nitrogen gas by different modes of basic hygienic characteristics of milk cows, as well as the duration of the bactericidal phase. As a result, this method of exposure on milk can increase the variety of milk on bacterial indicators. It was also found that this treatment can improve properties of milk which will suit for cheese-making. After processing milk with pressure nitrogen gas the stable decrease of titratable acidity was observed and the increase of the bactericidal phase, which is also a positive point, especially in the summer time, and allows to increase the shelf life of milk and the time of its preservation. Comparing those data obtained after single and double treatment milk pressure, it is evident that the double treatment, during which the pressure drop occurs twice, is more efficient. However, this mode requires a larger flow of working gas - nitrogen, which further can impact on reducing the profitability of production.

Keywords: Microbiology; biotechnology; milk; treatment of milk; analysis of milk; gaseous nitrogen.

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INTRODUCTION

Dairy products, which are consumed daily by each person must play the role not only for the providers of nutrients, but must first of all be safe for consumption. In this regard, the production of quality and safe dairy products – one of the strategic objectives of producers and researchers.

There is only one way out of this situation – the creation of a qualitatively new biotechnologies which will be able to use rationally and preserve all the useful components of natural raw materials.

For one of the most important criteria to ensure the safety and quality of food products can be named microbiological parameters, the maximum indicators of which are even higher now, in comparison with the State All-Union standard, so called GOST R 52054. Beginning from May the 1st, 2014 the technical regulations of the Customs Union "On the safety of milk and milk product"(TR CU 033/2013) approved by the Board of the Eurasian Economic Commission came into force.

The quality of the raw material supplied to the milk processing enterprises depends on a combination of factors that are difficult to influence on by the tightening of regulatory requirements outlined by the Federal Law of the Russian Federation N 88 -FB "Technical regulations on milk and dairy products".

Besides that, milk, which will suit cheese coagulation by rennet, comes on to dairy factories on average about 30% [1].

In this regard, along with the sequences of measures in the production of milk on farms, there is a question arises about improving the quality of indicators of milk by various methods, including biotechnological, allowing to manage and maintain the components of natural raw materials.

The experience in different countries shows that there are real ways to mend some defects of milk to improve its microbiological and biotechnological properties, and use in the production of dairy products ecologically safe raw materials.

Practically, some operations – mechanical removal of impurities and inactivation of microflora – practiced by filtration or centrifugation, and by heat treatment – which is pasteurization and sterilization.

However, during any thermal effects on raw milk there are physical and chemical process are taking place, and biological and the colloid properties change, thereby decreasing its nutritional value.

In recent years, in the literature has appeared totally unjustified tendency to reduce the diversity of the questions of quality of milk and milk products only to the microbiological criteria. The problem of the quality of milk should be considered comprehensively. The quality of milk should be understood its composition, properties and nutritional value and digestibility, the presence in it impurities and substances.

The method of heat pasteurization today is the main but not the only way to inactivate microorganisms. The laws of modern life encourage to search for optimal solutions, allowing for less different kinds of resources to increase the effectiveness of any processes. Thus, in the literature there are described some methods of disinfecting the milk based on the effects of UV radiation, laser activation and etc. [2;3].

METHODS

One possibly, in our opinion , and promising ways to improve the quality of raw milk without heat exposure is its treatment by the pressure of nitrogen gas, followed by a sharp discharge (Patent RU 2248732 C2. Raspopov V.A.).

When processing nitrogen gas is used, which is neutral and the most secure in use, it has a small coefficient of solubility in liquids and at high pressure and displace more gas phase found in milk (such as carbon dioxide and oxygen). Also nitrogen at low temperatures reduces the growth of psihrotrofnyh microorganisms in milk.

This method is ecologically safe and does not require additional energy as the temperature corresponds to the temperature in the processing of prepared milk according to current regulatory documents. The treatment time is also not significantly prolonging the standard production processes of milk products.

The influence of milk processing by pressure of nitrogen gas in the physics-chemical, microbiological and technological properties of milk was investigated at the Chair of Technology of Milk and Dairy Products of Mari State University (Yoshkar-Ola, Russia).

Milk Treatment was carried out in a laboratory. The temperature of the milk before the exposure of a pressure was 4±2ºC. This is, firstly, meet the requirements for milk at acceptance. Secondly, under mechanical action increases the activity of native milk lipases.

The main reason for the induced lipolysis is intensive mixing of the milk with air (air is partly destabilizing the shell of ball of fat). This is because any processing, causing a strong agitation or shaking (the nature of the effects of other gases such as nitrogen, same as air). To reduce the induced lipolysis, it is important that the temperature should be as low as possible. Cold milk (temperature 5°C) is more resistant to mechanical stress.

We have selected two processing mode. First Mode – treated milk by single exposure of nitrogen gas at pressure P = 1.4 MPa for 5 minutes followed by abrupt release of pressure (less than 1 second), the second mode – a double pressure P = 1.4 MPa for 5 minutes (pressure release was carried out at intervals of 2,5 minutes). Thus, the exposure time of nitrogen gas in both parties remained unchanged and changed the multiplicity of impacts and pressure relief.

RESULTS

The samples of the control party and processed milk immediately subjected to organoleptic, physics-chemical and microbiological analysis

As the result of the studies in control samples of raw milk and processed milk samples, both single and double treatment, there were no significant changes in organoleptic properties mentioned. Color, texture, smell, tastes were the same for all the parties and all the milk sample on the milk organoleptic meet the requirements of regulatory and technical documentation.

High nutritional value of milk due to the optimal content in it necessary for human consumption of fats, proteins, carbohydrates, minerals and vitamins, as well as favorable, almost perfect ratio of them in which these substances are completely absorbed.

The content of the basic biochemical milk components after exposure of a pressure of nitrogen gas is shown in Table 1.

Table 1: Composition of milk depending on the type of treatment

Indicator	Milk		
	Raw (Control)	Single Proceeding	Double Proceeding
Mass fraction of fat, %	3,25±0,14	3,27±0,13	3,27±0,14
Mass fraction of dry non-fat milk solids, %	8,24±0,05	8,23±0,04	8,24±0,04
Mass fraction of proteins, %	2,86±0,02	2,85±0,02	2,87±0,02
Mass fraction of lactose, %	4,33±0,03	4,31±0,02	4,30±0,03
The content of vitamin C in milligrams,%	2,34±0,27	2,34±0,26	2,33±0,25

It is thus apparent that the quantitative content of the main components of milk, namely the mass proportion of fat, protein, lactose, dry non-fat milk solids, and vitamin C in treated and control parties does not change, hence this treatment did not change the nutritional value of milk, which is sufficiently positive factor.

Mechanical effects on milk are often accompanied by changes in the degree of dispersion and stability of the fat phase. It may also change the structure and properties of casein and whey proteins of milk.

The effect of exposure of gaseous nitrogen on dispersed phase of milk is shown in Table 2.

Table 2: The change in particle size of the disperse phase depending on the processing mode

Indicator	Milk		
	Raw (Control)	Single Proceeding	Double Proceeding
The average micelle diameter casein, nm	69,01±2,4	71,03±2,34	72,05±2,08
The average diameter of fat globules, micron	5,83±0,70	5,20±0,59	4,63±0,45

Milk Treatment pressurized nitrogen gas resulted in a slight increase in the mean diameter of the micelles of casein, wherein the single effect – on to 2.02 nm (3.0%) and double – in 3.04 nm (4.4%).

Probably resizing casein micelles was caused by exposure of hydromechanical forces while casein micelles are destroyed into fragments and sub-micelles, their surface is loosened, and the hydrophobic regions preferably laid bare. As a result, there was a spontaneous recovery of a large casein micelles.

In the study of the size of the fat globules in the treated and control parties traced a decrease in the average diameter. In control parties of milk fat globules average diameter was 5,83 microns, single treatment of pressure led to a decrease in the average diameter of the fat globules on the 11% (5,20mkm), but doubled – on 20% (4,63 microns).

This can be explained as follows: – when a sharp pressure drop in the reservoir fracture subjected largest fat globules, as it is obvious that the efficiency of crushing the fat globules was affected by the multiplicity of processing milk.

Physics-chemical properties of milk as a single polydisperse system are caused by the concentration, the degree of dispersion of its components and the interactions between them. Consequently, changes in the content and state of the dispersed phases of the system can cause changes in the physicochemical properties [4].

The results of studies of the effect of milk processing by pressure of nitrogen gas at different processing modes on the density, viscosity and titraded acidity is given in Table 3.

Table 3 : Physical and chemical properties of milk depending on the processing mode

Indicator	Milk		
	Raw (Control)	Single Proceeding	Double Proceeding
Density, kilos/м^3	1028,04±0,20	1028,00±0,20	1028,02±0,19
Toughness, 10^3 Pac	2,18±0,02	2,22±0,02	2,21±0,02
Titrated acidity, °T	17,53±0,26	16,53±0,28*	16,50±0,25*
From this point and then: * P<0,05; ** P<0,01; *** P<0,001			

As a result, the density of the processing of milk has not changed, and an average of 12 samples, was 1028,00-1028,04 kg/m^3. This corresponds to the data given previously by chemical composition, which is also not changed.

Processing raw milk by pressurized nitrogen gas resulted in a slight increase in viscosity on 1.4-1.8% compared to control samples. Since the main quantitative composition of milk has not changed, apparently the change of the sizes of the dispersed phase was influenced by the amount of viscosity.

Processing raw milk by pressurized nitrogen gas, an average of 12 replicates, has led to significant reduction in titratable acidity after a single exposure to 1,00°T (P <0.05), and the processing in the case of double 1,03°T (P <0,05). The difference between single and double treatment is negligible.

This is probably due to the removal of some of the gases that are acidic in nature (oxygen and carbon dioxide) as a result of the displacement of the pressure of competing with nitrogen gas. Nitrogen has a low coefficient of solubility in liquids, so it can be assumed that as a result of the discharge gas pressure and the discharge abrupt degasification of milk occurs.

Thus, the removal of gases from milk determining acidity led to the restoration of the original values of the acidity.

Reducing the acidity of processed milk on 1ºT is rather positive factor, as the longer because it is possible to save the properties of fresh milk, which is especially important in the summer, when the acidity of raw milk is growing very fast and actual on small farms, where the accumulation of adequate amount for transportation of milk requires considerable time [5].

Studies of the effect of milk processing by pressure of nitrogen gas under different conditions showed that a sharp drop in pressure leads to a decrease in the number of microflora in comparison with the increase in raw milk and increase of milk bactericidal phase [6].

We have determined changes in the basic standardized microbiological indicators of raw milk, such as the number of mesophilic aerobic and facultative anaerobic microorganisms (NMAFAM), the presence of coliform bacteria (coliform) group, the number of yeasts and molds by conventional means.

The data obtained are the average for 10 parties are presented in Table 4.

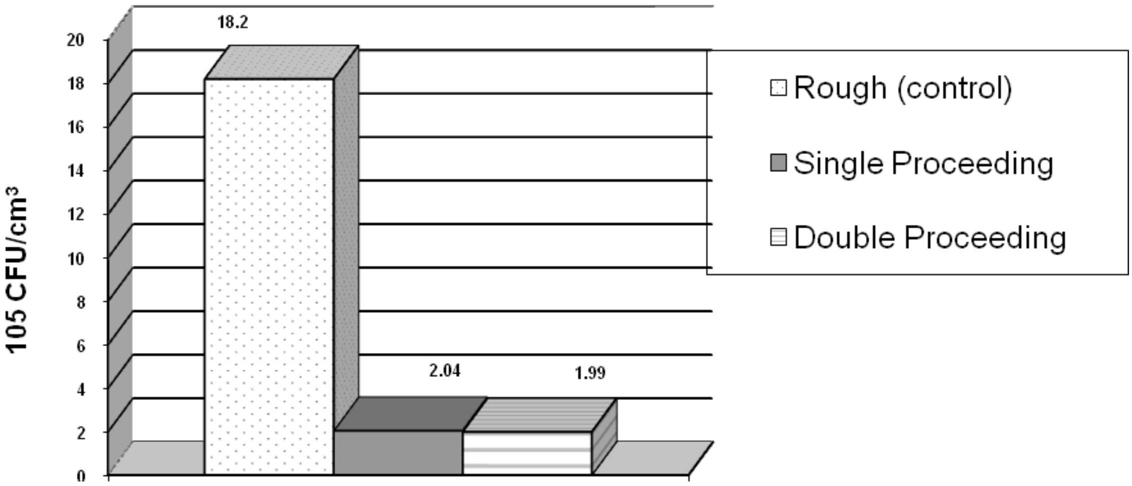
Obtained results showed that the treatment of the milk by preassure of 1.4 MPa significantly reduce the total number of colony NMAFAM practically 9-10 times (pic.1). Thus, in the amount of raw milk NMAFAM, an average of 10 samples, was $1{,}82 \bullet 10^6$ CFU/cm^3, which corresponds to the second grade of harvested milk. After a single treatment of milk NMAFAM number was $2{,}04 \bullet 10^5$ CFU/cm^3 (P <0,05), and after a double impact - $1{,}99 \bullet 10^5$ CFU/cm^3 (P <0,05).

Thus, the milk after treatment was in line with the first class on the total bacterial contamination set for raw milk on the regulatory requirements of the Federal Law N 88-FB "Technical regulations on milk and dairy products."

Table 4: Changes in the microbiological indicators of milk

Indicator	Milk		
	Raw (Control)	Single Proceeding	Double Proceeding
NMAFAM, cm^3	$(1{,}82 \pm 0{,}61) \cdot 10^6$	$(2{,}04 \pm 0{,}64) \cdot 10^5$*	$(1{,}99 \pm 0{,}98) \cdot 10^5$*
Yeast, CFU (colony-forming unit)/ cm^3	$(5{,}73 \pm 1{,}00) \cdot 10^3$	$(14{,}5 \pm 3{,}87) \cdot 10^2$***	$(10{,}1 \pm 2{,}96) \cdot 10^2$***
Fungus, CFU (colony-forming unit)/ / cm^3	$(5{,}80 \pm 3{,}11) \cdot 10^2$	$(0{,}64 \pm 0{,}38) \cdot 10^2$	$(0{,}48 \pm 0{,}27) \cdot 10^2$
Bactericidal phase, h	6,75±0,73	11,33±0,88**	11,67±0,83**
From this point and then: * P<0,05; ** P<0,01; *** P<0,001			

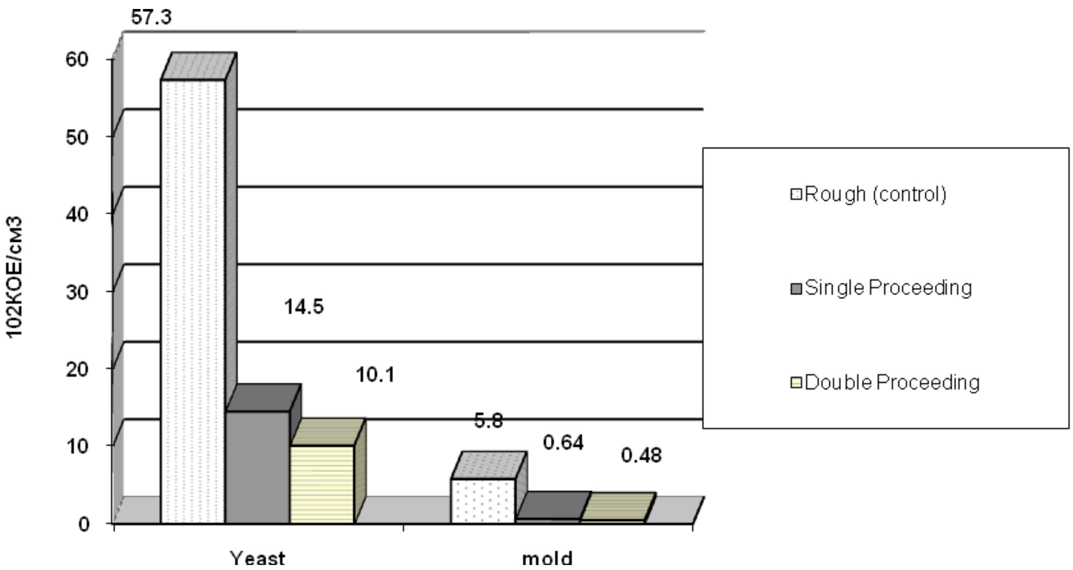
Reduced indicators of bacterial contamination occurred as a result of destruction of microorganisms under heavy pressure drop. Abrupt release of pressure results in a decrease in 14 times. The cells of microorganisms are gas bubbles which, when the pressure drops sharply increase its volume, breaking the cell wall or by stretching it, which leads to structural damage of the cell. As a result, it can be stated quite substantial reduction in the overall level of microbiological contamination of milk.



Picture 1: Change the number of NMAFAM of milk depending on the type of treatment.

The crops of control parties of milk on Saburo enviroment gave a solid growth of yeasts and molds, while after the pressure given treatment only single colonies of yeast colonies became the same type and point and were observed.

The data of Table 1 shows that the amount of yeast in the treated parties of milk was significantly (P <0,005) decreased almost in 4 times in milk treated once, and 6 times in the milk after two times of processing, in comparison with the raw milk (Picture 2).



Picture 2: Changing in the number of yeasts and molds according on to the treatment of milk

Number of mold also decreased after treatment. In some samples treated milk there was observed even no growth of mold. Only three of the ten samples was detected mold, whereas in the control samples all samples mold grew. This is probably connected with the high initial colonization of raw milk, which is more than $6 \cdot 10^2$ CFU/g. In samples where the value of the initial seeding of molds was less after the treatment with pressurized nitrogen gas molds completely were inactivated.

Comparing the results obtained at different processing modes by pressure of gaseous nitrogen should be noted that during double treatment was observed greater decrease quantitative NMAFAM (2.5%), yeast (30%) and mold (25%) compared with single treatment. Thus, the tightening mode treatment led to an increase in suppression of microflora of milk.

Determining the presence of coliform bacteria in the environment by Kessler was found that the life of spores of this group of microorganisms, did no affect. In 7 out of 10 studies on the reaction of coliforms it was positive in all parties, and in 3 studies the reaction was negative. However, it should be noted that coliform bacteria are completely destroyed in any mode of pasteurization.

Reducing the colonies of microorganisms in milk after the pressure by treatment is also confirmed an increase in the bactericidal phase of milk.

The point of increasing the duration of the bactericidal phase is of great practical importance, with the extension of it milk lasts longer in the natural state. The delay period the growth of bacteria in freshly-milked milk called “bactericidal phase”. Thus, according to G.M. Sviridenko the change of titratable acidity of the milk under the influence of the microflora becomes remarkable when the content of microorganisms of more than 10^6 CFU/ml. As a rule, it is related with the development of lactic acid in milk and coliform microorganisms, under the development of which the products of metabolism accumulates in milk, mainly organic acids lead to an increase in acidity [7].

Investigation of the effect of nitrogen gas by pressure treatment on the bactericidal phase of milk were carried out at t = 20°C on average in 12 samples. Duration bactericidal phase control samples made 6,75 ± 0,73 h, in milk samples processed by the first mode - 11,33 ± 0,88 ($P < 0,01$) in samples of milk processed according to the second mode - 11 67 ± 0,83 ($P < 0,01$). In the result of a study noted an increase in the bactericidal phase of milk after a single treatment for 4.58 hours, and after the double processing of 4.92 hours.

This result can be explained by two reasons. Firstly, due to the removal of the gas phase portion of the milk, including oxygen, which is required for the growth and activity of bacteria. Secondly, the partial suppression of microflora, the number of microorganisms is reduced by about 10 times.

Also, it should be noted that after the double treatment duration bactericidal phase increased in 0.34 hours. Probably with tougher treatment regimen, ie, double exposure with a sharp discharge pressure, increases the decontamination and strongly suppress the microflora of milk.

DISCUSSION

Besides due to the lack of milk in outer chemical impurities and unwanted microflora necessary to control the technological properties of milk – is the ability to milk rennet coagulation.

To determine cheese-suitable milk for cheese production, the following parametrs should be controlled: duration of rennet coagulation, rennet-fermentation test, and also a very important indicator is the content in the milk of ionized calcium (Table 5).

After a single treatment of milk by the pressure of nitrogen gas in the average amount of ionized calcium increased by 1,17 mg /% and equal to 11,64 mg/% (close to the reliability $P < 0,05$), while after the double treatment the content of Ca^{2+} increased by 1, 33 mg/% and amounted to 11,80 mg /% ($P < 0,05$).

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Table 5: Change cheese-suitable properties of milk for cheese production

The name of indicator	Milk		
	Raw (Control)	Single Proceeding	Double Proceeding
The amount of ionized calcium in mg /%	10,47±0,43	11,64±0,45	11,80±0,44*
Test for rennet clotting, min	38,83±2,86	25,83±2,05*	23,50±1,52*
Rennet-fermental sample, class	III	II	II

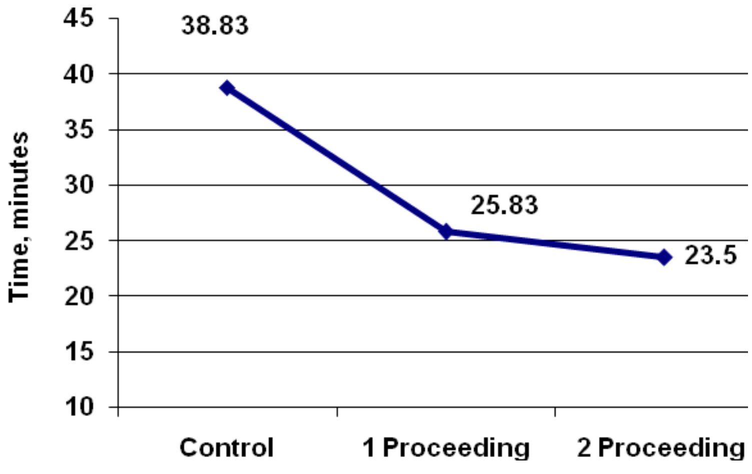
It is known that the content of ionized calcium and the amount of casein micelles are in their direct proportion. Calcium in the casein micelles are contained in two forms: one are attached to the carboxyl groups phosphate casein, others are part of the colloidal calcium phosphate and calcium citrate [8, 9].

Thus, it can be assumed that some rearrangement submicelles of casein resulted in the release of small amounts of phosphate and colloidal calcium citrate from the micelles, and further to its ionization [10].

Calcium ions play an important role as a binder bridges in the protein structure during the formation of rennet curd. Therefore, it can be assumed that the increasing of the amount of calcium ions and the average diameter of the casein micelles resulted in increasing speed rennet coagulation, which is confirmed by the data in Table 5.

Thus, the clotting time was significantly reduced from 38.83 minutes in the control samples up to 25.83 min ($P < 0,05$) at single and up to 23.5 minutes ($P < 0,05$) in the double treatment.

Time clot formation in the control samples corresponded to milk type III (more than 35 min), such a milk we can say that this milk-rennet is sluggish. However, after forming the clotting time of milk decreased in both modes, milk refers to the type II (25-35 min) and is optimal for cheese producing (Picture 3).



Picture 3: Changing the rennet coagulation depending on the mode of treatment of milk

Improving of cheese–suiting milk for cheese production is also noted in the results of rennet-fermentation tests. On average, after the effects of single and double pressure the class of milk according to rennet fermentation sample improved by one unit and corresponded to class II, and thus meet the requirements of TC (technical conditions) 9811-153-0461-0209-2004 “Milk raw material for cheese”, while raw milk, according to the rennet fermentation sample corresponded to Class III, which is classified as a sluggish milk-rennet.

Besides that, we investigated clots resulting from rennet-fermentation tests, on a five-point scale (modification by G.S. Inikhov).

As it is clearly shown in Picture 4, the clot obtained from a control sample of milk is torn, not solid, which corresponds to group 3 and is worth 3 points. The curds from milk treated singly and doubly by nitrogen gas pressure, characterized as normal with smooth surface, resilient in the touch, and evaluated in 5 points.

Picture 4: The nature of clot formation depending on the processing (where K (R) - raw milk, O_1 - single exposure, O_2 - double action)

Thus, the method of treating milk with pressurized nitrogen gas with its sharp discharge can allow to reduce the number of microorganisms in raw milk, improve the grade of raw milk, without changing its organoleptic properties and chemical composition.

After processing milk with pressure nitrogen gas the stable decrease of titratable acidity was observed and the increase of the bactericidal phase, which is also a positive point, especially in the summer time, and allows to increase the shelf life of milk and the time of its preservation.

CONCLUSION

The improving of cheese-suitable milk for cheese production, in our opinion, one of the most significant results of the study. The part of the milk, suitable for cheese production in the total amount of his pre-made pieces, is still quite low, and even with all the veterinary measures aimed at the production of milk, you can not get raw materials technology meets the requirements of cheesemaking. Therefore, increasing the quality of milk which will suit for cheese-making using quite a safe way, without changing the composition and application of chemical components, is clearly promising and certainly a positive factor.

Comparing those data obtained after single and double treatment milk pressure, it is evident that the double treatment, during which the pressure drop occurs twice, is more efficient. However, this mode requires a larger flow of working gas - nitrogen, which further can impact on reducing the profitability of production.

All of this allows us to recommend a single treatment of milk by pressure with nitrogen gas by above mentioned regime for all cheese-making enterprises of the dairy industry applied to raw materials less than 1 grade, a bacterial contamination of more than $5 \cdot 10^5$ CFU/cm^3 immediately after acceptance before the reservation.

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