REGULATING THE NUMBER OF MICROORGANISMS IN RAW MILK

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Abstract: Producing high quality and safe milk requires developing and improving technological processes at animal farms. Violation of sanitary and technological rules and requirements (during milking, first treatment of milk, washing special equipment) causes the increase of non-specific microorganisms in milk. The authors have developed a treatment methodology for milk and milk storage tanks using electrochemically activated water, which allows to kill disease-causing microorganisms in raw milk.

Key words: safety of raw milk, quality, disease-causing microorganisms, bacteria, electrochemically activated water, electric water conditioner.

In dairy industry, microbiological safety of raw milk provides the basis for production technologies of high-quality production. High bacterial population decreases the quality of raw milk and derived products and creates a hazard for human health.

Milk is a good nutritive medium for various microorganisms. Usually lactic, coliform, butyrate, propionate and putrefactive bacteria can be found in milk [1]. The use of beneficial microorganisms in production makes it possible to get products with good organoleptic, medical and dietary properties. First of all, naturally beneficial microorganisms form quite a large group. They include, e.g., lactic bacteria which are used in production of such products as kefir, cottage cheese, sour cream, cheese. Lactic bacteria include cocci and rod bacteria which do not form spores.

All pathogenic microorganisms found in milk and causing infectious diseases of animals and humans belong to inimical bacteria. Also, nonpathogenic or opportunistic pathogenic bacteria allocating toxins in milk, e.g., staphylococcus, are harmful. Intestinal rod bacteria, hay bacillus, fluorescing bacteria, some micrococci, etc., are similarly undesirable.

To suppress the development of microorganisms various ways are used. Thanks to them, desired quality of milk and dairy products can be achieved due to the external impact on the development of microorganisms. The destruction of bacteria in milk and dairy products occurs in particular when they are exposed to some physical factors. Physical methods of conservation are based on the use of high and low temperatures, ultrasound, ultra-violet and infrared rays, ionizing radiation, etc. [9].

High temperature kills the germs that increase the product's firmness, therefore, such a way of milk preservation has been widely adopted. Depending on the purpose of dairy product manufacturing, different modes of thermal treatment of milk are used: pasteurization and sterilization. Conservation by low temperatures involves suppression of microorganism activity, decrease of enzyme activity, delay of biochemical processes [11].

Conservation by ionizing radiation allows to keep natural nutritional and biological properties of milk to the fullest and to ensure the preservation for a long time. A specific

feature of the preservation by ionizing radiation is its antisepticising effect without the necessity to increase the temperature [3]. The radiation by ultra-violet rays (UVR) with the ray length of 60-400 nanometers leads to the destruction of organisms caused by the adsorption of UVR by nucleic acids and nucleoproteins and their subsequent denaturation. Pathogenic microorganisms and putrefactive bacteria are especially sensitive to UVR [6].

Conservation by ultrasound (more than 20 kHz) causes anumber of physical, chemical and biological phenomena: inactivation of enzymes, vitamins, toxins, destruction of protista and metazoa. That is why this method is used for milk pasteurization in fermentative and non-alcoholic industry for the sterilization of canned food.

Sterilization by filtering is a process of mechanical separation of the product from damage activators by means of filters with microscopic pores, i.e., ultra filtration process. This method allows to keep the nutritional value and organoleptic properties of goods at the highest level and is applied in milk processing. It is based on the use of special asbestic or ceramic sterilizing filters through which the milk is made to pass under pressure.

Yet another method is known which assumes processing of liquids containing bacteria *(Escherichia coli* K12) with electric impulses, the intensity of the electric field not exceeding 6-20 kV/cm [7].

According to this method, external pulse electric field can induce an imposed transmembrane potential which then causes irreversible increase in membrane permeability after the electric field exceeds the critical value. This will, in its turn, lead to changes and eventual loss of the cell content, biological resorption of the cell and its irreversible destruction.

At present, there is a method of processing, which allows to improve products' shelfiife and storability, to maintain or even improve their initial characteristics through the mechanism of an inactivating effect on the microorganisms, including processing of liquids and fiowable products with impulses of an electromagnetic field [10].

When this method is used, there is a chance of suppressive impact on the control system of the cell as well as on genetic material of the microorganism cell as directly by the electromagnetic field and by the free electrons formed in a cell due to its strong impact.

In less than 0.1 second, short impulses of the strong electromagnetic field can irreversibly destroy a variety of bonds which are weak, but important for normal functioning of the cell control system and disrupt DNA synthesis that finally will kill the cell. Thus, the initial qualities of products remain unchanged and the extent of sterilization in comparison with the traditional thermal treatment is increased. Investigations showed that changing parameters of impulses leads to full or selective suppression of microorganisms' activity in milk [8].

To implement this method for obtaining milk and dairy products of desired quality, there is a device equipped with a current impulse generator where a control panel regulates the duration of impulses, pauses and the output tension of an impulse; additionally it is supplied with a solenoid-shaped milk collecting tank [4], and also with a device in which the source of electromagnetic field impulses is placed in the milk collecting tank [5].

Main results

In order to improve the quality of milk by suppressing the development of microorganisms, the testing laboratory of milk quality at RSAU-MAA named in honour of K. A. Timiryazev conducted research on the influence of various methods of milk processing by electrochemically activated water on the development of milk bacterial microflora. The investigations took place from 2010 to 2012.

Electrochemical activation of water is induced by the electric current and aimed at the deviation of the activity of electrons in the environment from the equilibrium condition with the subsequent relaxation of the environmental disturbance. The most extensively studied method is the electrochemical activation of water which is a version of other activation processes, such as fast freezing and defrosting or mechanical spraying [2].

The principle of electrochemical activation of water is that when dilute solutions of mineral salts processed with an electric current receive and give electrons, they get into the meta-stable condition. This state is characterized by abnormal physical and chemical activity that gradually decreases in time, i.e., relaxes.

Electrochemical activation of water changes its oxidation-reduction (redox) potential, which is a measure of free energy in a redox reaction and is expressed by a version of potentials which arise in the real redox system. Besides, the process of electrochemical activation of water is accompanied by a thousandfold decrease in the quantity of bacteria and viruses.

To study the influence of electrochemically activated water on the microbiological composition of milk, an electric conditioner was used. Its work is based on the principles of electrically governed sorption. The device helps to decrease the concentration of microparticles of minerals, humus and other foreign materials, microorganisms and toxic components in water.

An important feature of electric conditioners is that, unlike filters of many other types, they substantially diminish the redox potential of water that makes the latter more favorable for the use for nutrimental purposes.

The device is a flow-type electrochemical reactor with fittings for the supply and removal of conditioned water. The flasks of reactors are equipped with chemically inert electrodes of a special type. The space between plates of electrodes is filled with granulated material which in electric field gains properties necessary for water purification. At the onset of water intake, the power supply block automatically impresses voltage between the electrodes, thus creating an electric field in the space between them.

Electrodes of the device receive the operating voltage, the water subject to cleaning passes through a layer of granulated nozzles and gets cleared of harmful components and moves on further to the user's water suction faucet. Polluting substances and microorganisms collapse under the influence of the electric field due to electrochemical processes or are stopped by the granulated nozzle. In the rinsing procedure, the pollutants withheld by the nozzle are removed by the water flow in the absence of the electric field.

To investigate into the influence of the water processed in the electric conditioner by various methods and the addition of various additives to the water on the microbiological composition of cow's milk, the following research has been conducted.

In the first experiment, tanks for milk storage were processed with specially prepared solutions:

- № 1 — water processed in the electric conditioner (cathode);
- № 2 — water processed in the electric conditioner (anode);
- № 3 — water processed in the electric conditioner (cathode + anode).

In the second experiment, water in the amount of 5% from the total amount of milk was added to milk:

- № 1 — water processed in the electric conditioner (cathode);
- № 2 — water processed in the electric conditioner (cathode + anode), with the addition of polyhexamethylen guanidine hydrochloride concentration of 10-3%;
- № 3 — water processed in the electric conditioner (anode);

- № 4 — water processed in the electric conditioner (cathode + anode), with polyhexamethylen guanidine hydrochloride added in 10^2%-concentration;

- № 5 — water processed in the electric conditioner (cathode + anode).

Polyhexamethylen guanidine hydrochloride which is used in medicine for disinfection as well as to protect various industrial products from microbial damage and crops from diseases, and also in the industry for water purification, was used in our experiments as an additive.

The total bacterial count of studied samples is presented in Table 1. It clearly demonstrates that the control sample had high bacterial count in milk, but under the effect of water processed in the electric conditioner, the total bacterial count decreased considerably. The most substantial decrease, nearly complete demolition of bacteria, occurred when milk storage tank was processed with solution of water received in a mix from anode and cathode.

Table 1

Total bacterial count in the samples under investigation*

Water processing method	Microbial count, CFU/ml
Control water, no treatment	Too numerous to count
Processed in the electric conditioner (cathode)	13×10^3
Processed in the electric conditioner (anode)	14.5×10^3
Processed in the electric conditioner (cathode + anode)	No growth

milk storage tanks were processed with water (here and in Tables 2-4).

Table 2 shows the count of colonies of yeast-like fungi and microfungi whose activity causes damage of the product. Complete demolition of bacteria was observed in milk, when the storage tank was processed with water obtained in the electric conditioner from anode and cathode.

Table 2

Count of yeast-like fungal colony *(Candida albicans* circle of affinity) and microscopic mold fungi from the genera *Penicillium* and *Aspergillus**

Water processing method	Microbial counts, CFU/ml
Control water, no treatment	Heavy growth
Processed in the electric conditioner (cathode)	6×10^2
Processed in the electric conditioner (anode)	$1{,}3 \times 10^3$
Processed in the electric conditioner (cathode + anode).	No growth

Table 3 shows the counts of *Staphylococcus aureus* colonies, where it is evident that the studied solutions made a considerable impact on the development of *S. aureus* and reduced the count by 2.6-5.2 times in comparison with the control sample.

Table 3

Count of S. *aureus* colonies in studied samples *

Water processing method	Microbial count, CFU/ml
Control water, no treatment	1.3×10^2
Processed in the electric conditioner (cathode)	0.25×10^2
Processed in the electric conditioner (anode)	0.32×10^2
Processed in the electric conditioner (cathode + anode).	0.5×10^2

In Table 4, the counts of bacteria *E. coli* are shown. An essential impact on the development of *E. coli* was made by all studied water solutions.

Table 4

Count of *E. coli* colonies *

Water processing method	Microbial count, CFU/ml
Control water, no treatment	7.5×10^2
Processed in the electric conditioner (cathode)	No growth
Processed in the electric conditioner (anode)	No growth
Processed in the electric conditioner (cathode + anode)	No growth

Table 5

Total bacterial count*

Water processing method	Microbial population, CFU/ml
Control water, no treatment	20×10^3
Processed in the electric conditioner (cathode)	No growth
Processed in the electric conditioner (cathode + anode), with addition of polyhexamethylen guanidine hydrochloride, concentration 10-3%	30×10^3
Processed in the electric conditioner (anode)	10×10^3
Processed in the electric conditioner (cathode + anode), with addition of polyhexamethylen guanidine hydrochloride, concentration of 10-2%	10×10^3
Processed in the electric conditioner (cathode + anode)	10×10^3

in Tables 5-8, water in the amount of 5% from the total amount of milk was added to milk.

Microbiological composition of milk also underwent changes after electrochemically activated water was added into milk. Table 5 presents the total bacterial count in milk after adding water processed with different methods. It has been established that water

processed in the electric conditioner from the cathode and added into milk rendered the greatest disinfecting effect in comparison with other samples. Other samples practically did not differ from the control.

Table 6 presents the general count of yeast-like fungi and micro fungi in 1 ml of milk. It is evident that complete elimination of fungi occurred after water processed in the electrolysis tank (cathode) and water processed in the electrolysis tank (the anode + the cathode) was added into milk. In samples numbered 2,4 and 5, the decrease of fungal count was by 1.3-4.1 times.

Table 6

Count of yeast-like fungal colony (C. *albicans* circle of affinity) and microscopic mold fungi from the genera *Penicillium* and *Aspergillus

Water processing method	Microbial count, CFU/ml
Control water, no treatment	4.5×10^3
Processed in the electric conditioner (cathode)	3.4×10^3
Processed in the electric conditioner (cathode + anode), with addition of polyhexamethylen guanidine hydrochloride, concentration of 10^{-3}%	No growth
Processed in the electric conditioner (anode)	1.1×10^3
Processed in the electric conditioner (cathode + anode), with addition of polyhexamethylen guanidine hydrochloride, concentration of 10^{-2}%	2.2×10^3
Processed in the electric conditioner (cathode + anode).	No growth

Table 7 shows data of *S. aureus* count in studied milk samples. The analyzed solutions did not show any result because no colonies of *S. aureus* were revealed in the control sample.

Table 7

Count of S. *aureus* colonies*

Water processing method	Microbial count, CFU/ml
Control water, no treatment	No growth
Processed in the electric conditioner (cathode)	No growth
Processed in the electric conditioner (cathode + anode), with addition of polyhexamethylen guanidine hydrochloride concentration of 10-3%	No growth
Processed in the electric conditioner (anode)	No growth
Processed in the electric conditioner (cathode + anode), with addition of polyhexamethylen guanidine hydrochloride concentration of 10-2%	No growth
Processed in the electric conditioner (cathode + anode).	No growth

Data on the counts of *E. coli* are presented in Table 8. As the Table shows, 1 ml of the control sample of milk contained 6,5 x 10^2 CFU of *E. coli* bacteria. All samples under investigation demonstrated completely suppressed growth *ofE. coli.*

Table 8

Count of *E. coli* colonies*

Water processing method	Microbial count, CFU/ml
Control water, no treatment	6.5 x 10^2
Processed in the electric conditioner (cathode)	No growth
Processed in the electric conditioner (cathode + anode), with addition of polyhexamethylen guanidine hydrochloride concentration of 10 ^{3}%	No growth
Processed in the electric conditioner (anode)	No growth
Processed in the electric conditioner (cathode + anode), with addition of polyhexamethylen guanidine hydrochloride concentration of 10'^{2}%	No growth
Processed in the electric conditioner (cathode + anode)	No growth

Thus, it has been established that the total bacterial count in milk was mainly influenced after the water processed in the electric conditioner (cathode) was added to the milk and after the milk storage tank has been washed with water processed in the electric conditioner (cathode + anode).

Conclusions

1. All studied solutions of water suppressed the growth of the bacterium *E. coli* irrespective of milk processing method.

2. Due to the fact that in the initial raw milk after adding the working solutions into milk there were no bacteria of the species *S. aureus,* it is impossible to make a conclusion about influence of this method on their development. At the same time, as it was noted above, the studied solutions made a considerable impact on *S. aureus* development after washing the tanks for milk storage and reduced their quantity by 2.6-5.2 times in comparison with the test sample.

3. Regardless of the method of milk processing the water processed in the electric conditioner (cathode + anode) has the greatest impact on the growth and development of yeast-like fungi *C. albicans* and microscopic mold fungi of the general *Pemcillium* and *Aspergillus.*

4. In order to decrease the bacterial count in milk and preserve its other qualitative and quantitative characteristics it is recommended to wash the tanks for storage and transportation of milk with water processed in the electric conditioner (cathode + anode).

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РЕГУЛИРОВАНИЕ ЧИСЛЕННОСТИ МИКРООРГАНИЗМОВ В МОЛОКЕ-СЫРЬЕ

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Аннотация: повышенная бактериальная обсемененность ухудшает качество сырого молока и продуктов его переработки и создает определенный фактор риска для здоровья людей. С целью подавления развития микроорганизмов известны различные способы, например, термическое воздействие. Известны также способы получения молока заданного качества за счет химического воздействия на микроорганизмы. Для изучения влияния на развитие микроорганизмов молока предлагается метод получения молока и молочных продуктов заданного качества, включающий обработку молока электрохимически активированной водой. Процесс электрохимической активации воды сопровождается значительным уменьшением количества бактерий и вирусов. Для получения электрохимически активированной воды был использован электрокондиционер, который работает на принципах электроуправляемой сорбции и способствует снижению концентраций в воде микрочастиц минералов, гумуса и иных инородных материалов, микроорганизмов и токсичных компонентов. Установлено, что на общую бактериальную обсемененность молока наибольшее воздействие оказывает добавление в молоко воды, обработанной на электрокондиционере (катод), и при промывке емкости для хранения молока водой, обработанной на электрокондиционере (катод+анод). Все изучаемые рабочие растворы воды подавили рост бактерий Escherichia coli. Исследуемые растворы оказали значительное воздействие на развитие Staphylococcus aureus при промывке емкости для хранения молока и снизили их количество в 2,6-5,2 раза. На рост и развитие дрожжеподобных грибов из вида Candida albicans и микроскопических плесне-

вых грибов из родов Penicillium и Aspergillus наибольшее влияние оказала вода, обработанная на электрокондиционере (катод+анод). С целью снижения бактериальной обсемененности молока авторы рекомендуют промывку емкости для хранения и транспортировки молока водой, обработанной на электрокондиционере (катод+анод).

Ключевые слова: безопасность молока-сырья, качество, патогенные микроорганизмы, бактерии, электрохимически активированная вода, электрокондиционер воды.

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