Original scientific paper
UDC 637.34.055:542.879.7

EFFECT OF ELECTRODIALYSIS ON DAIRY BY-PRODUCTS MICROBIOLOGICAL INDICATORS

Georgy Anisimov[1], Svetlana Ryabtseva[1*], Ivan Evdokimov[1], Andrei Khramtsov[1], Irina Kulikova[1], Maria Kosenko[1], Vitaly Kravtsov[1]

[1]Department of Applied Biotechnology, Institute of Life Sciences, North-Caucasus Federal University, Pushkin Street 1, 355009 Stavropol, Russia

[*]e-mail: ryabtseva07@mail.ru

Abstract

The microbiological aspects of membrane processing of dairy by-products have been studied mainly in relation to the formation of biofilms during ultrafiltration and reverse osmosis. Electrodialysis allows to adjust the salt content and reduce the acidity of the whey, however, it is a long process during which different groups of microorganisms in the raw material can multiply and form biofilms. The purpose of this research was to study the effect of electrodialysis temperature on microbiological indicators of natural and condensed dry solids of curd and cheese whey and skim milk permeate.

We investigated the effect of electrodialysis temperature on microbiological indicators (mesophilic aerobic plate count, spore-forming bacteria, yeast, molds, and coliforms) on natural and condensed to 20% dry solids samples of curd whey, cheese whey and skim milk permeate. Electrodialysis treatment of samples was carried out at the ED (R) -Y/50 facility of the Czech company Mega at temperatures of: 15, 22, and 30 ^{0}C. Analysis of microbiological indicators was carried out using 3M™ Petrifilm™.

Electrodialysis at 15 ^{0}C does not have a significant impact on the development of the studied groups of microorganisms in all types of raw materials. Electrodialysis at 22 ^{0}C contributes to the slow development of some microorganisms. Electrodialysis at 30 ^{0}C leads to a significant increase in the aerobic plate count of natural cheese whey (Δ lgN = 0.49), the number of yeast in natural (Δ lgN = 1.12) and condensed (Δ lgN = 0.65) curd whey, and also in natural permeate (Δ lgN = 0.50). Stimulation of the growth of yeast during electrodialysis of curd whey may be due to favorable pH, active mixing and aeration. Increasing of spore-forming bacteria during electrodialysis at 30 ^{0}C can be explained by the formation of biofilms on the membranes. The suppression of microbial growth during electrodialysis was found for mesophilic aerobic plate count in permeate, as well as for coliforms in all types of natural raw materials at 30 ^{0}C.

Electrodialysis can be carried out without significant deterioration of microbiological indicators on all the studied types of raw materials at 15 ^{0}C and 20 ^{0}C, on condensed permeate at 30 ^{0}C.

***Key words**: Dairy by-products, Electrodialysis, Temperature effect, Microbiological indicators.*

1. Introduction

The problem of dairy waste utilization is an issue of environmental, economic and social importance. The world production of whey is estimated at approximately 165 million tons. About 60% of this volume is recycled, and the rest is used for animal feed or dumped, causing serious environmental pollution [1]. Traditionally, dairy by-products (DBP) are used to produce beverages, dry products, protein concentrates, lactose, etc., [2]. The global production of skimmed milk and whey powders has increased by 32.4% and 19.7%, correspondingly, for the last seven years [11]. DBP are cheap raw materials used to produce some functional ingredients, including lactose derivatives and whey proteins. That creates new opportunities for satisfying increasing consumer interest in healthy food (health and wellness), and DBP cost-efficient processing.

In this respect, baro- and electromembrane methods of DBP processing are assuming particular importance as they ensure preservation of DBP components native properties [3]. One of the main problems of these methods is associated with the formation of protein and mineral layer on membranes. Microbial cells attach to the layer easily and reproduce quickly, causing the formation of complex self-regulating systems (biofilms) which are the sources of continuous contamination of products. That leads to reduced performance and premature equipment failure, efficiency losses in cleaning and disinfection which badly affect quality and safety of the food products [4].

According to Chamberland *et al.*, [5], the microflora of the raw materials is found to have significant influence on the composition of early microbial communities that appear on the membranes during the ultrafiltration of milk and whey [5]. It is emphasized that while developing methods for the prevention of ultrafiltration membrane biological fouling, it is necessary to take into account not only the filtered liquid properties and the feed temperature, but also the microbial environment of the milk processing plant [5]. During whey ultrafiltration, membrane physical properties affect the composition of biofilms on membranes more than their chemical properties, whereas during milk processing, properties of the casein layer formed on membranes are found the most significant [6]. The analysis of microflora by metabarcoding in combination with real-time polymerase chain reaction showed that pasteurized milk retentates contained mainly *Streptococcus*, *Pseudomonas* and *Enterobacteriaceae*, while cheese whey retentates also contained *Lactococcus* [6]. Marka and Anand, [7], demonstrated that bacilli biofilms formed during the reverse osmosis of ultrafiltration permeate are more resistant to cleaning and disinfection than those in cheese whey. It should be noted that microbiological aspects of DBP membrane treatment have been studied mainly in relation to biofilm formation during ultrafiltration and reverse osmosis [4 - 7].

The whey specific feature is a high content of mineral salts which should removed during whey processing, especially if whey or whey ingredient powders for infant food are produced. There are several ways of DBP desalting: ion exchange, electrodialysis, or nanofiltration. Electrodialysis has some advantages as it enables dissolved substance ions transfer through a membrane in an electric field, the electric potential gradient being a driving force [8].

Electrodialysis ensures salt content regulation and raw material acidity reduction. However, electrodialysis is a long process during which different groups of microorganisms can reproduce in DBP, and little research has been undertaken on the effect of the electric field on the microflora growth. In addition, whey proteins and sparingly soluble calcium salts can precipitate on electrodialysis membranes. This layer is a suitable basis for the attachment and growth of DBP microflora. To prevent microbial contamination, electrodialysis equipment manufacturers recommend processing at low temperatures (10 - 15 ^{0}C), which results in its reduced performance [9]. The membrane process temperature increase may affect the microorganism growth rate, and, as a result, badly affect the product quality [4, 5, 6, and 10].

The study of the pasteurized curd whey electrodialysis by Kosenko *et al.*, [12], demonstrates that the increase of the process temperature from 15 ^{0}C to 30 ^{0}C leads to a significant increase in thermophilic microorganisms and yeast count, but it does not affect mesophilic or psychrophilic microflora considerably. No significant effect of electrodialysis on mesophilic, psychrophilic or thermophilic aerobic microorganisms in the skimmed milk permeate is noted by Anisimov *et al.*, [13].

Thus the comparative study of different microorganisms' growth in different types of DBP during electrodialysis treatment at different temperatures is of interest. The main microbiological indicators of dairy product quality and safety are the number of mesophilic aerobic microorganisms, yeasts and molds, and coliform bacteria. The purpose of this research was to study the effect of electrodialysis temperature on microbiological indicators of natural and condensed dry solids of curd and cheese whey and skim milk permeate.

2. Materials and Methods

The dairy by-products (DBP) were provided by Molochny Kombinat Stavropolsky JSC (Stavropol, Russia). The following types of industrial DBP were used in the experiments: the curd whey obtained during the low-fat curd production using mesophilic lactococcal starter culture; the cheese whey obtained during the production of cheese «Rossiiskii» using mesophilic lactococcal starter culture; the skim milk permeate obtained by ultrafiltration through 10 kDa PES spiral-wound membranes HFK131 (Koch Membrane Systems, Wilmington, USA). The DBP were vacuum-concentrated in a batch mode using an industrial double-effect evaporator. The temperatures in the first and the second tanks were maintained as high as 67 and 50 ^{0}C, respectively. The obtained DBP were stored at 6 - 8 ^{0}C for no longer than 24 hours.

The electrodialysis of the samples was carried out using the ED(R)-Y/50 unit (Mega a.s., the Czech Republic) at: 15 ± 1 ^{0}C, 22 ± 1 ^{0}C, and 30 ± 1 ^{0}C. Each electrodialysis run was conducted in a batch mode for four hours and corresponded to the demineralization level (82 ± 8%). The polarity of membrane module was reversed after each run, i.e. the anode was swapped with

the cathode, resulting in the change of direction of ion migration in the membrane module. Seven electrodialysis runs were conducted in total.

DBP samples had been taken before the electrodialysis, and in four hours after the start; the control samples were thermostated for 4 hours at an appropriate temperature. The total solid content was determined by the refractometer PalS (Atago Co., Ltd., Japan). pH was measured using portable pH meter Multi 3620 IDS equipped with Sentix 940 pH electrode (WTW, Germany).

The analysis of microbiological parameters of DBP was conducted using Petri films in accordance with GOST 32901-2014 [14], GOST 10444.12-2013 [15], and Methodical Guidelines 4.2.2884-11 [16]. Preliminary tenfold dilutions were prepared in phosphate buffer solutions. 1.0 ± 0.1 cm^3 of the sample or its dilution was applied on a dry nutrient medium in the center of the base of the Petri film under the upper transparent film. Then, the top film was gently lowered and the sample was distributed over an area of about 20 cm^2 of the surface of the nutrient medium using a plastic spreader. The Petri films were left for 1 - 2 minutes to freeze the gel, and then incubated at a certain temperature for a certain time depending on the microbiological parameter. After that, typical colonies of microorganisms grown on Petri films with a certain allowable number of colonies were counted.

Mesophilic aerobic and facultative anaerobic microorganisms were determined using 3M Petri film - aerobic count plates (AC), the incubation was carried out at 30 ± 1 ^{0}C for 72 ± 3 hours; the red colonies count were carried out on Petri films with the number of colonies from 15 to 300. This standard indicator characterizes general contamination of dairy products in the regulatory documents of the Russian Federation and the Customs Union [17]; it is equal to the aerobic plate count (APC) in the international standards [18].

The spores of mesophilic aerobic and facultative anaerobic microorganisms were determined using 3M Petri film aerobic count plates (AC). The samples were preheated in a water bath at 88 ± 2 ^{0}C for 12 ± 2 minutes and then cooled to the temperature of 23 ± 1 ^{0}C; the incubation was carried out at 30 ± 1 ^{0}C for 48 - 72 hours; the red colonies count was carried out on the Petri films with the number of colonies from 5 to 150.

The yeasts and molds were determined using 3M Petri film yeast and mold count plates (YM), the incubation was carried out at 24 ± 1 ^{0}C for 72 ± 3 h (for preliminary counting) and 120 ± 3 h (for final counting); the colonies of various colors (from pink-yellow to blue-green) of round shape with smooth edges were counted as yeasts on the Petri films with the number of colonies from 5 to 150; the colonies of different colors (black, yellow, green, blue) with a diffuse edge and a clear center were counted as molds on the Petri films with the number of colonies from 5 to 50. Additionally, the microscopic examination was carried out to differentiate the colonies of yeast and mold.

The coliforms were determined using 3M Petri film coliform count plates (CC), the incubation was carried out at 37 ± 1 ^{0}C for 24 ± 2 hours; the red colonies with gas bubbles were counted on the Petri films with the number of colonies from 15 to 150.

The significance of the difference between the groups was determined at the level of significance $\alpha = 0.05$ using ANOVA followed by Tukey HSD post-hoc test. The normal distribution within the groups of 6 - 8 values was confirmed with Shapiro - Wilks test ($\alpha = 0.05$). The graphical representation of the data was performed using Microsoft Office Excel 2010.

3. Results and Discussion

3.1 Characteristics of initial DBP

Physico-chemical and microbiological characteristics of the initial DBP samples are presented in table 1.

Natural curd whey and cheese whey were highly contaminated by mesophilic aerobic microorganisms (at the level of 10^7 CFU/cm^3 and 10^6 CFU/cm^3, respectively). This was mostly due to *Lactococcus* spp. mesophilic starter cultures which form small red colonies on AC Petri films. Permeate was less contaminated with mesophilic bacteria (at the level 10^5 CFU/cm^3), but this level was determined by extraneous microflora. The data correspond to the results of Chamberland *et al.*, research [5], in which the highest microbial count was found in fresh whey (up to 6.66 log CFU/cm^3).

The condensation of DBP has a versatile effect on the microflora. A significant part of the metabolically active cells dies at the condensing temperature (about 65 ^{0}C). On the other hand, the remaining heat-resistant microorganisms are also concentrated. This effect explains the higher dispersion of all microbiological indicators of condensed DBP rather than natural DBP (Table 1). During the concentration of curd whey, mesophilic APC was decreased by 2.83 lg N, probably affected by the reduction in pH to 4.24. The decrease of mesophilic APC in cheese whey after condensation is less (Δ lg N = - 1.92) than in curd whey. Significant increase ($p < 0.05$) of mesophilic APC was found in the concentrated permeate, which indicates high contamination with heat-resistant microorganisms. This is confirmed by the data on spore-forming bacteria, which were mostly detected in permeate rather than in the whey (Table 1).

The curd whey (natural and condensed) contained a high count of yeast (thousands of cells within ($3 - 10 \times 10^3$ CFU/cm^3), which develop better with lactococci and are resistant to acidic medium and high osmotic pressure.

Table 1. Characteristics of the initial raw materials

Indicator, unit	Value (M ± SD)* for raw materials					
	Curd whey		Cheese whey		Skimmed milk permeate	
	natural	condensed	natural	condensed	natural	condensed
Dry solids, %	5.6 ± 0.2	18.0 ± 0.2	6.0 ± 0.5	20.0 ± 0.9	4.3 ± 0.3	20.0 ± 1.1
pH	4.85 ± 0.25	4.24 ± 0.19	5.75 ± 0.17	4.76 ± 0.15	6.10 ± 0.21	5.91 ± 0.23
Mesophilic APC, lg N***	7.28 ± 0.39	4.45 ±0.60	6.04 ± 0.31	4.12 ± 0.53	5.31 ± 0.32	5.85 ± 0.45
Spore-forming bacteria, lg N	1.32 ± 0.45	2.01 ± 0.58	1.59 ± 0.48	1.66 ± 0.47	2.62 ± 0.43	2.78 ± 0.52
Yeasts, lg N	3.82 ± 0.16	3.75 ± 0.28	2.38 ± 0.11	2.31 ± 0.27	2.40 ± 0.25	2.29 ± 0.38
Molds, lg N	1.48 ± 0.64	2.30 ± 0.91	1.10 ± 0.36	ND	1.85 ± 0.33	1.55 ± 0.67
Coliforms, lg N	2.04 ± 0.22	2.80 ± 0.24	3.19 ± 0.26	3.27 ± 0.35	4.27 ± 0.26	3.94 ± 0.51

Legend: * M - average value, SD - standard deviation; ** APC - Aerobic plate count, *** N - number of microorganisms, CFU/ cm^3.

The cheese whey and permeate were less contaminated with yeasts (hundreds of cells, within (2 - 4 × 10^2 CFU/cm^3), and their count changed slightly after condensation. Mycelium fungi (molds) were not found in most DBP samples; in the contaminated samples their count was in the range from several cells to hundreds of CFU/cm^3 with a large dispersion of data.

The curd whey contained the lowest count of coliforms (tens-hundreds of CFU/cm^3), which can be explained by unfavorable conditions for their survival and development (high acidity and the presence of bacteriocins produced by *Lactococcus* spp.) A larger amount of coliforms was found in cheese whey and permeates. Condensation did not significantly affect the population of coliforms in all types of DBP.

3.2 Effect of electrodialysis on mesophilic aerobic microflora

Changes of mesophilic APC (Δ lg N = N_{fin} - N_{init}) in the samples of DBP after electrodialysis and the control samples of DBP at different temperatures are shown in Figure 1. Trend lines and describing equations with the accuracy of approximation are shown for data in natural DBP after electrodialysis (as for the most obvious changes).

Mesophilic APC changed insignificantly ($p > 0.05$) at 15 °C in all trials as only psychrotrophic bacteria could reproduce slowly at this temperature. A more active growth of microflora was observed at 22 °C, especially after electrodialysis in the condensed whey: mesophilic APC (CFU/cm^3) was 1.5 times more after electrodialysis and 1.17 times more in the control samples than in the initial samples of concentrated curd whey; 1.3 times more after electrodialysis and in the control samples than in the initial samples of natural cheese whey, 1.6 times more after electrodialysis and 1.4 times more in the control samples than in the initial samples of concentrated cheese whey; 1.2 times more after electrodialysis and in the control samples than in the initial samples of the permeate. But there was no significant difference revealed for lg N at 22 °C in almost all the trials except for concentrated curd whey.

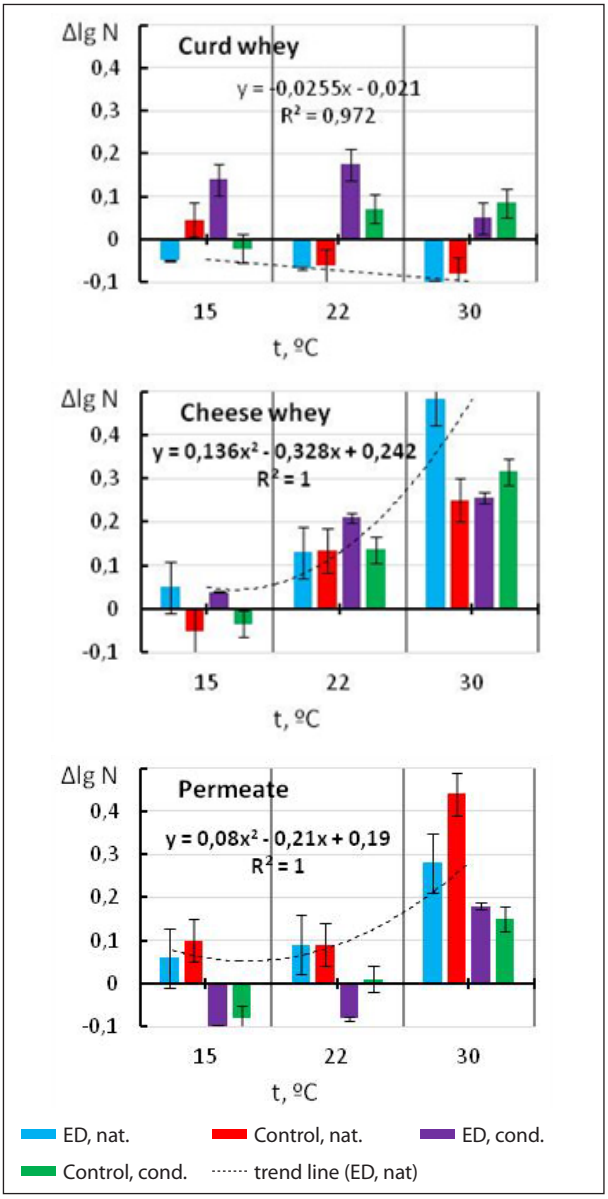

Figure 1. The effect of electrodialysis treatment temperature on mesophilic APC variation in different types of DBP (Δ lg N = N_{fin} - N_{init}; nat. - natural DBP, cond. - condensed DBP; R^2 - magnitude of the approximation; ED - electrodialysis treatment)

Mesophilic APC changed insignificantly ($p > 0.05$) at 30 ^{0}C in curd whey, but this microbiological indicator increased significantly ($p \leq 0.05$) in cheese whey and permeate. In natural cheese whey, mesophilic microflora was more active during electrodialysis (Δ lg N = 0.49) than during incubation at 30 ^{0}C (Δ lg N = 0.25). In contrast, in natural permeate mesophilic microorganisms reproduced faster under thermostatic conditions (Δ lg N = 0.44) than during electrodialysis (Δ lg N = 0.28). No significant difference ($p > 0.05$) was observed between mesophilic APC in concentrated electrodialyzed and control DBP.

The analysis of diagrams, trend lines and equations in Figure 1 shows that the patterns of mesophilic APC change during electrodialysis are significantly different for DBP different types. In the curd whey, the mesophilic population decreased, which could be due to low pH and the presence of lactococcal bacteriocins inhibiting growth of extraneous microflora. As a result, mesophilic APC in the natural curd whey was decreased after electrodialysis, which was more noticeable at 30 ^{0}C: decrease in mesophilic APC (CFU/cm^3) was fixed 1.1 times at 15 ^{0}C, 1.2 times at 22 ^{0}C, 1.3 times at 30 ^{0}C. The growth of mesophilic population was observed in all the samples of cheese whey at 22 ^{0}C and 30 ^{0}C, as well as in all the samples of permeate at 30 ^{0}C.

Different changes of mesophilic APC during electrodialysis of DBP various types could be explained not only by different pH levels, but also by different microflora composition, as well as the possibility of biofilm formation on electrodialysis membranes. This is in agreement with the data obtained by Chamberland *et al.*, [5], who highlight that milk and whey had different bacterial composition which was influenced by various factors (temperature, oxidative conditions, availability of nutrients, competition with the starter culture). Chamberland *et al.*, [6], reported that *Lactococcus* spp. was the dominant (more than 95%) microflora in fresh and pasteurized cheese whey, while a permeate may contain many classes of bacteria [6], including α- (11%), β- (11%), and γ-proteobacteria (24%), bacilli (18%), flavobacteria (9 %) and actinobacteria (6%).

The comparison between the data for pasteurized curd whey reported by Kosenko *et al.*, [12], and our data for unpasteurized curd whey showed that a more significant growth of mesophilic APC was observed during electrodialysis in the pasteurized curd whey. This could be due to heat resistant bacteria remaining viable in whey, while starter cultures die after pasteurization. This is in agreement with the assumption of Chamberland *et al.*, [5], that filtration of unpasteurized whey at 10 ^{0}C can be an interesting compromise between the efficiency of the membrane process and the quality of obtained products.

Bacteria that can survive heat treatment represent an important challenge for the dairy industry. Aerobic heat-resistant spore-forming rods of the genera *Bacillus* spp. and *Geobacillus* spp. (B. *licheniformis, B. coagulans, B. cereus, B. rumilus, G. stearothermophilus*, *etc.*) are capable of initiating the formation of biofilms on hard-to-reach parts of equipment. The research carried out by Gopal *et al.*, [19], showed that spores attached easier than vegetative cells to the surface of stainless steel because they have relatively high hydrophobicity and they are resistant to heat and chemicals. Other spore-forming bacteria belonging to the genera *Porosarcina*, *Paenisporosarcina*, *Brevibacillus*, *Paenibacillus* can also participate in the formation of biofilms [19].

Table 2. Spore-forming mesophilic microorganisms counting results

Processing conditions		Spore-forming mesophilic aerobic bacteria, lg N** Value (M ± SD) for dairy by-products					
ED* or control	t, ^{0}C	Curd whey		Cheese whey		Skimmed milk permeate	
		natural	condensed	natural	condensed	natural	condensed
Initial	15	1.12 ± 0.40	2.05 ± 0.48	1.48 ± 0.39	1.71 ± 0.47	2.84 ± 0.23	2.57 ± 0.34
ED		1.09 ± 0.39	1.93 ± 0.57	1.51 ± 0.37	1.82 ± 0.15	2.83 ± 0.40	2.56 ± 0.43
Control		1.11 ± 0.18	2.11 ± 0.42	1.45 ± 0.60	1.75 ± 0.50	2.88 ± 0.66	2.61 ± 0.45
Significance		ns***	ns	ns	ns	ns	ns
Initial	22	1.50 ± 0.51	2.11 ± 0.69	1.89 ± 0.45	1.95 ± 0.61	2.59 ± 0.58	3.21 ± 0.52
ED		1.57 ± 0.41	2.08 ± 0.18	1.94 ± 0.61	2.04 ± 0.47	2.71 ± 0.34	3.28 ± 0.21
Control		1.42 ± 0.30	1.97 ±0.37	1.78 ± 0.42	1.90 ± 0.36	2.63 ± 0.76	3.15 ± 0.24
Significance		ns	ns	ns	ns	ns	ns
Initial	30	1.34^a ± 0.33	1.88^a ± 0.38	1.39^a ± 0.40	1.32^a ± 0.34	2.44^a ± 0.29	2.55^a ± 0.67
ED		1.55^b ± 0.19	2.13^b ± 0.50	1.67^b ± 0.28	1.56^b ± 0.31	2.72^b ± 0.50	2.68^b ± 0.72
Control		1.31^a ± 0.27	2.15^c ± 0.24	1.41^a ± 0.34	1.49^b ± 0.23	2.48^a ± 0.31	2.57^a ± 0.40
Significance		s***	s	s	s	s	s

Legend: ED* - electrodialysis treatment, N** - number of microorganisms, CFU/ cm^3, ns*** - non significant differences, s - significant differences ($p \leq 0.05$); different letters (a, b, c) show significant differences ($p \leq 0.05$) between means in the same column for the same temperature.

The results of spore-forming mesophilic microorganisms' estimation in the samples of DBP after electrodialysis and the control samples of DBP at different temperatures are shown in Table 2.

The analysis of the data in Table 2 shows that the number of spore-forming mesophilic microorganisms varies from tens to hundreds of CFU/cm^3 in the initial and treated samples of whey; from hundreds to thousands of CFU/cm^3 in the initial and treated samples of permeate. No significant differences ($p > 0.05$) were observed between spore-forming mesophilic APC in the initial, electrodialyzed and control DBP at 15 and 22 ^{0}C. It could be explained by the fact that electrodialysis treatment cycle wasn't long enough for activation and germination of spores at these temperatures, as well as for the formation of mature contaminating biofilm, which is confirmed by the data obtained by Chamberland *et al.*, [6].

An increase in the number of spores was observed in the natural and condensed DBP after electrodialysis at 30 ^{0}C. It is possible that not only spore-forming, but also heat-resistant vegetative forms of microorganisms, such as, mesophilic *Micrococcus* spp., affect the changes of this indicator. In addition, the high contamination of some samples of DBP at 30 ^{0}C may be due to the formation of hardly washable biofilms containing spores on electrodialysis membranes.

3.3 Effect of electrodialysis on yeasts and molds

Yeasts are well-known spoilage microorganisms. Yeast count is an important microbiological indicator that can influence the quality and yield of product from DBP, including lactose. The results of determination of this group of microorganisms are presented in Figure 2.

The analysis of the data in Figure 2 shows that the growth of yeast in curd whey significantly depends on the temperature and processing conditions. Yeast did not develop in the natural curd whey at 15 ^{0}C. A significant ($p \leq 0.05$) increase of yeast count was observed after electrodialysis in the condensed curd whey at 15 ^{0}C (Δ lg N = 0.31) as well as in the natural (Δ lg N = 0.45) and condensed (Δ lg N = 0.33) curd whey at 22 ^{0}C. A rapid growth of yeast was detected at 30 ^{0}C in all the samples: Δ lg N = 1.12 in the natural curd whey after electrodialysis, Δ lg N = 0.83 in the control; lg N = 0.65 in the condensed samples after electrodialysis, Δ lg N = 0.39 in the control. The direct relation of the yeast count to the temperature of electrodialysis in the natural curd whey was described by a linear equation with high accuracy ($R^2 = 0.99$), as shown in Figure 2. Yeasts developed in the condensed curd whey more slowly than in the natural curd whey at 22 and 30 ^{0}C, but their growth was significant ($p \leq 0.05$). It could be assumed that the initial curd whey was contaminated with mesophilic yeasts of the genus *Kluyveromyces*, for which the conditions of curd whey (pH 4.2 - 4.9) are favorable; in addition, these yeasts are characterized by increased resistance to osmotic pressure [20].

The growth of yeast was slower in the control than in the demineralized samples of curd whey in all trials. The stimulating effect of electrodialysis conditions on the development of yeasts could be explained by the combined effect of optimum temperature, acidity and circulation, at which active mixing and aeration of the feed occur. Moreover, some mineral salts and low molecular weight metabolic products of microorganisms that inhibit the development of yeast could be removed during electrodialysis treatment. Stimulating the yeast growth during the electrodialysis of curd

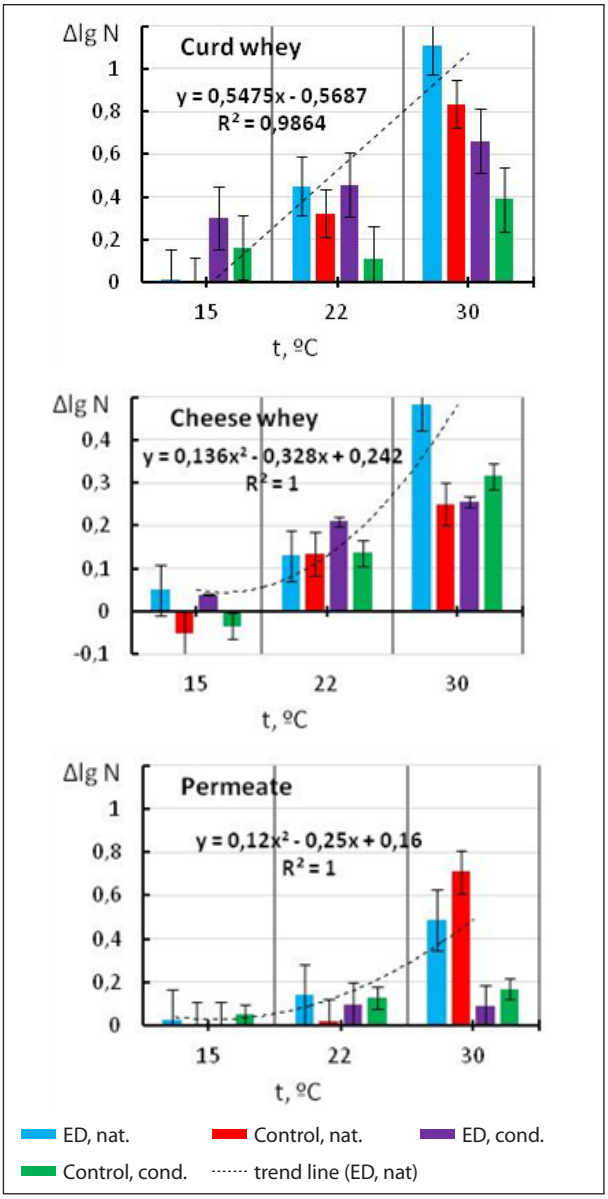

Figure 2. The effect of electrodialysis treatment temperature on the yeast count in different types of DBP (N - number of microorganisms, CFU/ cm^3, nat. - natural, cond. - condensed, R^2 - magnitude of the approximation)

whey is undesirable in terms of microbiological DBP indicators. However, it could be used to combine the processes of yeast cultivation and curd whey demineralization in biotechnology.

For the cheese whey, there was no significant change ($p > 0.05$) in yeast count in all trials. Electrodialysis temperature did not affect the growth of yeast in this type of DBP (Figure 2). Possibly, these facts were due to the lower initial contamination of cheese whey with yeasts than those of curd whey, the differences between species composition, as well as a less favorable pH level for yeast development in the cheese whey (pH 4.8 - 5.8) compared to the curd whey (4.2 - 4.9).

No significant difference ($p > 0.05$) was observed between the yeast count in the natural and concentrated permeate at 15 and 22 ^{0}C, as well as in the concentrated permeate at 30 ^{0}C. The yeasts count was increased significantly in the natural permeate at 30 ^{0}C after electrodialysis (Δ lg N = 0.50), and in the control samples (Δ lg N = 0.71), but the difference between the electrodialyzed and the control permeate samples was statistically insignificant ($p > 0.05$).

Molds were not detected in most of the examined samples. No significant differences ($p > 0.05$) were observed between the molds in the initial, electrodialyzed and control DBP samples contaminated by molds at 15, 22 and 30 ^{0}C. This could be explained by the low rate of growth and obligatory aerobic properties of this group of microorganisms.

3.4 Effect of electrodialysis on coliforms

The bacteria of coliform group are the main sanitary-indicative microorganisms which are normalized and controlled in all food products. The results of coliforms counting in different types of DBP after electrodialysis and the control samples at different temperatures are shown in table 3.

The analysis of the results shows that no significant difference ($p > 0.05$) was observed between the coliform count in the natural and concentrated DBP at 15 and 22 ^{0}C in all the trials, as well as in the concentrated permeate at 30 ^{0}C. A significant ($p \leq 0.05$) decrease of coliforms was detected after the treatment at 30 ^{0}C in the natural and condensed curd whey. These results could be associated with the competition between the lactic acid bacteria and the coliforms in the curd whey. A significant ($p \leq 0.05$) increase of coliforms was observed at 30 ^{0}C in the natural and condensed cheese whey and the natural permeate as well. The differences between the coliforms count in the electrodialyzed and control samples were statistically significant ($p \leq 0.05$) only for the natural DBP. The research data indicate the inhibition of the coliforms development in natural DBP during electrodialysis treatment at 30 ^{0}C. Probably, it was a result of the electrochemical process impact on the gram-negative microorganisms, but additional studies are required to explain this data.

Table 3. Coliforms counting results

Processing conditions		Coliforms Plate Count, lg N** (M ± SD)**					
Sample	t, °C	Curd whey		Cheese whey		Skimmed milk permeate	
		natural	condensed	natural	condensed	natural	condensed
Initial	15	2,05 ± 0,23	2,10 ± 0,26	3,21 ± 0,30	3,23 ± 0,49	4,45 ± 0,49	3,64 ± 0,58
ED*		2,11 ± 0,19	2,17 ± 0,31	3,25 ± 0,29	3,15 ± 0,16	4,50 ± 0,24	3,87 ± 0,60
Control		1,86 ± 0,11	2,11 ± 0,25	3,18 ± 0,17	3,18 ± 0,49	4,53 ± 0,31	4,02 ± 0,32
Significance		ns	ns	ns	ns	ns	ns
Initial	22	2,15 ± 0,32	2,08 ± 0,14	3,11 ± 0,27	3,31 ± 0,17	4,00 ± 0,10	4,21 ± 0,32
ED*		2,08 ± 0,14	2,16 ± 0,21	2,91 ± 0,22	3,24 ± 0,25	3,86 ± 0,37	4,48 ± 0,31
Control		1,99 ± 0,18	2,09 ± 0,19	3,20 ± 0,30	3,39 ± 0,26	3,94 ± 0,20	4,58 ± 0,38
Significance		ns	ns	ns	ns	ns	ns
Initial	30	$2,00^a$ ± 0,12	$2,13^a$ ± 0,29	$3,24^a$ ± 0,26	$3,27^a$ ±0,39	$4,37^a$ ± 0,18	3,99 ±0,60
ED*		$1,53^b$ ± 0,05	$1,85^b$ ± 0,15	$3,58^b$ ± 0,18	$3,46^b$ ± 0,32	$4,76^b$ ± 0,31	4,09 ± 0,62
Control		$1,73^c$ ± 0,20	$1,94^b$ ± 0,08	$3,76^c$ ± 0,15	$3,43^b$ ± 0,29	$4,97^c$ ± 0,45	4,14 ± 0,55
Significance		s	s	s	s	s	ns

Legend: ED* - electrodialysis treatment, N** - number of microorganisms, CFU/ cm^3, ns*** - non significant differences, s - significant differences ($p \leq 0.05$); different letters (a, b, c) show significant differences ($p \leq 0.05$) between means of the same DBP and treatment temperature.

4. Conclusions

- The results of this research indicate that electrodialysis can stimulate or suppress some microorganisms in DBP depending on their composition and properties as well as on the temperature of treatment.

- Electrodialysis at 15 ^{0}C did not have a significant impact on the development of the studied groups of microorganisms in all types of DBP. Electrodialysis at 22 ^{0}C contributed to the slow development of some microorganisms. Electrodialysis at 30 ^{0}C resulted in a significant increase in the mesophilic aerobic plate count of natural cheese whey (Δ lg N = 0.49), the number of yeasts in natural (Δ lg N = 1.12) and condensed (Δ lg N = 0.65) curd whey, and also in natural permeate (Δ lg N = 0.50).

- The stimulation of the yeast growth during curd whey electrodialysis may be due to a favorable pH, active mixing and aeration. The increase of spore-forming bacteria during electrodialysis at 30 ^{0}C can be explained by the formation of biofilms on the membranes.

- The suppression of microbial growth during electrodialysis was found for the mesophilic aerobic plate count in permeate, as well as for the coliforms in all types of natural DBP at 30 ^{0}C.

- Condensation of DBP resulted in a decrease in most of the studied microbiological indicators except for spore-forming bacteria, and can be considered as a way to improve the economic efficiency of electrodialysis treatment and the product quality.

- Electrodialysis of all the studied types of DBP can be carried out without significant deterioration of microbiological indicators at 15 ^{0}C and 20 ^{0}C, and of condensed permeate at 30 ^{0}C.

Acknowledgement

This research work was carried out with the financial support of the Ministry of Education and Science of the Russian Federation, contract MON 03.G25.31.0241 (GRANT on the theme «Creation of high technology of lactose for pharmaceutical and food industries»).

5. References

[1] Macwan S. R., Dabhi B. K., Parmar S. C., Aparnathi K. D. (2016). *Whey and its Utilization*. Journal of Current Microbiology and Applied Sciences, 5, (8), pp. 134-155.

[2] Panghal A., Patidar R., Jaglan S., Chhikara N., Khatkar S. K., Gat Y., Sindhu N. (2018). *Whey valorization: current options and future scenario - A critical review*. Nutrition & Food Science, 48, (3), pp. 520-535.

[3] Le T. T., Cabaltica A. D., Bui V. M. (2014). *Membrane separations in dairy processing*. Journal of Food Research and Technology, 2, 1, pp.1-14.

[4] Anand S., Singh D., Avadhanula M., Marka S. (2014). *Development and control of bacterial biofilms on dairy processing membranes*. Comprehensive Reviews in Food Science and Food Safety, 13, (1), pp.18-33.

[5] Chamberland J., Lessard M. H., Doyen A., Labrie S., Pouliot Y. (2017). *Biofouling of ultrafiltration membranes by dairy fluids: Characterization of pioneer colonizer bacteria using a DNA metabarcoding approach*. Journal of Dairy Science, 100, (2), pp. 981-990.

[6] Chamberland J., Beaulieu-Carbonneau G., Lessard M. H., Labrie S. (2017). *Effect of membrane material chemistry and properties on biofouling susceptibility during milk and cheese whey ultrafiltration*. Journal of Membrane Science, 542, pp. 208-216.

[7] Marka S., Anand S. (2018). *Feed substrates influence biofilm formation on reverse osmosis membranes and their cleaning efficiency*. Journal of Dairy Science, 101, (1), pp. 84-95.

[8] Prosekov A. J., Ulrih E. V., Budrik V. G., Botina S. G., Agarkova E. J. (2013). *On ways demineralization of whey*. <URL: www.science-sd.com/452-24049. Accessed 30 October 2018.

[9] [9] Volodin D. N., Zolotoreva M. S., Topalov V. K., Novak L., Evdokimov I. A. (2015). *Demineralization of acid milk whey*. Int. Dairy Mag., 3, pp. 38-39.

[10] Luo X., Ramchandran L., Vasiljevic T. (2015). *Lower ultrafiltration temperature improves membrane performance and emulsifying properties of milk protein concentrates*. Dairy Science Technology, 95, pp. 15-31.

[11] International Dairy Federation. (2018). *The World Dairy Situation 2018*. Bulletin of the International Dairy Federation, 494, pp. 163-166.

[12] Kosenko M. E., Kulikova I. K., Donskikh A. N., Anisimov G. S., Evdokimov I. A. (2016). *Effect of the dry solids weight ratio to the development of curd whey microflora during electrodialysis*. Technology and food production, 41, (2), pp. 40-46.

[13] Anisimov G. S., Evdokimov I. A., Ryabtseva S. A., Donskikh A. N., Akhmedova V. R., Kravtsov V. A. (2018). Effect of electrodiaisis treatment temperature on skim milk permeate microflora. Dairy industry, 10, pp. 14-15.

[14] GOST. (2014). *GOST 32901-2014 Milk and milk products - Methods of microbiological analysis*. <URL: http://docs.cntd.ru/document/1200115745. Accessed 29 November 2018.

[15] GOST. (2013). *GOST 10444.12-2013 - Microbiology of food and feed for animals. Methods for identifying and counting the number of yeast and mold fungi*. <URL: http://docs.cntd.ru/document/1200107308. Accessed 29 November 2018.

[16] MUK. (2011). *MUK 4.2.2884-11 - Methods of microbiological control of environmental objects and food products using petrifilms*. <URL: http://docs.cntd.ru/document/1200089706. Accessed 29 November 2018.

[17] Technical Regulations of the Customs Union. (2013). *TR TS 033/2013 - On the safety of milk and dairy products*. <URL: http://docs.cntd.ru/document/499050562. Accessed 29.11.2018.

[18] [BAM. (2001). *BAM - Aerobic Plate Count*. <URL: https://www.fda.gov/food/foodscienceresearch/laboratorymethods/ucm063346.htm. Accessed 18 December 2018.

[19] Gopal N., Hill C., Ross P. R., Beresford T. P., Fenelon M. A., Cotter P. D. (2015). *The Prevalence and Control of Bacillus and Related Spore-Forming Bacteria in the Dairy Industry*.

<URL: https://www.ncbi.nlm.nih.gov/pubmed/26733963. Accessed 18 December 2018.

[20] Rodicio R., Heinisch J. J. (2013). *Yeast on the milky way: Genetics, physiology and biotechnology of Kluyveromyces lactis*. Yeast, 30, (5), pp. 165-177.