В даний час встановлено, що штучні нейронні мережі (ШНМ) забезпечують краще моделювання та прогноз щодо кількості мікроорганізмів в сировини та харчових продуктах. Тому їх можна використати для контролю безпечності харчових продуктів та оцінки мікробіологічного ризику. В цьому випадку ШНМ можуть бути використані як інформативні, швидкі та економічно ефективні засоби. Відповідно до Європейських вимог щодо безпечності харчових продуктів, основними мікробіологічними показниками є встановленя загальної кількості мікроорганізмів і кількості бактерій родини Enterobacteriaceae, так як вони найчастіше пов'язані з харчовими захворюваннями та отруєннями. Метою роботи було розроблення методу прогнозування кількості бактерій родини Enterobacteriaceae в сирому молоці при його зберіганні охолодженим та оцінити прогностичну спроможність ШНМ. Розробка методу складалася з 4-х етапів. На першому етапі проводили вивчення кількості ентеробактерій в залежності від фізико-хімічного складу сирого молока, температури і часу зберігання в умовах холодильника. На другому етапі формували базу експериментальних даних, отриманих в дослідних моделях. На наступному етапі вводили отриману базу даних до ШНМ. І на останньому етапі проводили оцінку ефективності способу прогнозування. Створена ШНМ складається з трьох шарів: вхідний шар (5 параметрів: температура зберігання молока (4; 6; 8 і 10 °С); період зберігання молока (від 1 до 48 годин); кислотність молока (17–20 %), вміст жиру (3,2; 3,6; 4.0; 4,5 %) і вмісту білка (2,9; 3,0; 3,3 %) у молоці, прихованих шарів (з 30 нейронами) і вихідного шару (спрогнозоване число бактерій). Для навчання та оптимізації ШНМ використали 1200 експериментальних даних, які показали, що прогнозування має найбільший показник відхилення – 2,497 % (або 370 бактеріальних клітин в 1 мл). Таким чином, розроблений метод прогнозування може бути використано для прогнозування кількості бактерій з врахуванням комплексу перемінних умов навколишнього середовища в різних харчових продуктах. Також даний підхід, в якості штучного інтелекту, може бути використаний при оцінці мікробіологічних ризиків та для швидкого контролю за безпечністю харчових продуктів

Ключові слова: Enterobacteriaceae, сире молоко, штучні нейронні мережі, прогнозування кількості бактерій

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CONSTRUCTION OF A METHOD FOR PREDICTING THE NUMBER OF ENTEROBACTERIA IN MILK USING ARTIFICAL NEURAL NETWORKS

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1. Introduction

At present, all countries face an important challenge to ensure food security [1, 2]. The most significant risks for human health associated with food products are the microbiological hazards. Since the microbiological risks are the most important among other dangers, such as chemical and physical, they are paid special attention to in the regulation of food safety in each country [3–5]. It is commonly known that microorganisms can change their behavior under the influence of environmental factors, including technological parameters. Microorganisms become more resilient, demonstrate the properties not previously observed, and their number may vary, even increase [6]. In this connection, it is necessary to constantly study the properties of microorganisms, especially the pathogenic bacteria and their behavior (growth, survival, resilience, and death) and quantitative changes in a technological process. In this case, is very

important to apply modern research methods or methodologies such as predicting microbiology. Therefore, studying and understanding the behavior of microorganisms in raw materials and ready-made food products, which are affected by environmental factors, is the basis of control over microbiological safety of food. Moreover, a better understanding of this is very important to devise effective strategies for the prevention of food poisoning and diseases associated with food products in each country [6–8].

Contamination of raw materials and products with microorganisms occurs mainly because of the lack of proper hygienic practices in the process of their production. The microbial contamination of raw milk may happen from three main sources: from the udder, from the surface of the skin of the udder, and from dairy equipment. The lack of sanitary-and-hygienic conditions during milking increases the amount of bacteria in raw milk [9–11]. *Enterobacteriaceae* is a family of the gram-negative, non-sporous bacteria, which ferment lactose with the formation of acid and gas. Representatives of the Enterobacteriaceae family are associated with diseases that are transmitted by food, or poisoning, while some of them can also cause food spoilage. This family includes a series of important food pathogens such as *Salmonella, Yersinia enterocolitica*, pathogenic *Escherichia coli* (*E. coli* O157:H7), *Shigella spp.* and *Cronobacter spp.* [12]. Raw milk may be the source of the following food pathogens from the *Enterobacteriaceae* family: *Salmonella spp.*, Shiga toxin-producing *Escherichia coli* [13, 14]. That is why bacteria from the *Enterobacteriaceae* family are the indicative microorganisms for raw materials and food products, and are, accordingly, used to control the level of hygienic and sanitary practices in the process of food production [15, 16].

It is also very important to know that the number of micro-organisms, including the number of enterobacteria, may vary at all stages of milk processing as milk is an excellent medium for bacterial growth [17, 18].

The main stage in the processing of milk, which can potentially change the number of *Enterobacteriaceae,* is the refrigeration storage of raw milk. Typically, raw milk is kept at a temperature of 4–6 °C. Previous studies noted the microbial growth and behavior (growth, survival, resilience, and death) in refrigerated milk during storage in a refrigerator [17, 19]. The behavior of microorganisms in milk is determined by its properties (pH, water activity, content of NaCl) and the conditions of storage (temperature and freezing, relative humidity, the presence of other microorganisms). The effect of these properties can be predicted by applying the mathematical models derived from the quantitative research on a microbe population. That is necessary to develop improved strategies for the prevention and control of food pathogens and human disease [20].

2. Literature review and problem statement

There is a growing trend towards the application of artificial intelligence in the food microbiology and food safety. Predicting microbiology is a new and important scientific approach, which includes the knowledge on microbial growth in response to environmental factors, generalized in the form of quantitative or mathematical models [6, 7, 21]. In general, the predicting food microbiology is a prognosis of microbial behavior in food products. Papers [21–23] described the development, validation, and application of the principles of predicting microbiology in the United Kingdom, the United States, Canada, and Australia over the past two decades. The literature sources [22, 24] stated that the mathematical models that quantitatively describe the combined effect of parameters of the environment could be used to predict the growth, survival, or inactivation of microorganisms. Hence, the prediction of the growth of microorganisms in raw materials and food products that are processed provides important information to ensure their microbial safety.

Recently, there has been a growing trend in scientific research to use artificial intelligence, namely, modeling and prediction, employing computer applications, specifically artificial neural networks (ANN). ANN is a mathematical model that simulates the structure and function of the biological nervous system; it has been discovered that it offers a better modeling and predicting approach in addressing the uncertainties and fluctuations that are often associated with microbial growth. Currently, a given approach that employs ANN is applied in economics, politics, technology, ecology, and other biological sciences [24–26]. In addition, the scientific literature cites more researchers from different countries that devise methods for predicting the behavior of microorganisms in raw materials and food products [24, 25, 27]. Predicting modeling is a modern direction to control microbiological hazards in food raw materials and food products, using mathematical models and statistical methods. This is a new approach to the establishment and prediction of changes in the qualitative state of microorganisms (growth, reproduction, or death) in raw materials or products under the influence of various parameters of the environment. These environmental factors are temperature, humidity, the presence of other organisms, the ratio of salts, acids, or other chemicals [17, 19, 27]. The principles and methods of predicting modeling are used in the analysis of microbiological risks within the HACCP system, as well as in the implementation of new technologies for the production of food [6, 24, 25, 28].

In some countries, this direction in the microbiology of foods has only started to develop. Therefore, the design of methods for predicting the number of microorganisms in food products, as well as the construction of high-speed methods to control microbiological indicators using artificial intelligence, become more relevant [29].

Most scientific papers [7, 19, 24, 26] report results on predicting the number of certain types of microorganisms in milk, rather than groups of microorganisms. For instance, a group of conditionally pathogenic bacteria. The reason for this is the difficulty associated with creating a database on experimental research, whose acquisition is quite labor-intensive. The expediency of conducting our study relates to the use of rapid microbiological control over raw materials and food products of animal origin using modern computer software. That in turn will contribute to the implementation of one of the basic measures for the prevention of diseases associated with consumption of food products. This would also contribute to the development of predicting methods for other micro-organisms, while the use of computer software would contribute to the improvement of food safety and consumer health.

3. The aim and objectives of the study

The aim of this work was to devise a method for predicting the number of bacteria from the *Enterobacteriaceae*

family in raw milk stored under refrigeration conditions using ANN.

To accomplish the aim, the following tasks have been set:

– to examine the total number of bacteria and the number of bacteria from the *Enterobacteriaceae* family, depending on the composition of raw milk, as well as its temperature and storage conditions, in a refrigerator;

– to build a database of results from experimental studies on the number of the above-specified microorganisms, derived from models involving the samples of raw milk;

– to introduce the resulting database of experimental data to an artificial network;

– to determine the efficacy of the method for predicting the number of microorganisms in raw milk during storage under refrigeration conditions.

4. Materials and research methods to devise a method for predicting the number of bacteria from the *Enterobacteriaceae* family

4. 1. Taking samples

We took 290 samples of milk from dairy tanks about 300 ml each from 5 dairy farms in Sumy oblast (Ukraine) over the period from 2015 to 2018. Under laboratory conditions, each sample of the raw milk was divided into two parts. One part of the sample was used for a microbiological study (10 ml), and the other part – for the experiment (250 ml). All the samples of raw milk for the experiment were grouped based on the content of fat (from 3.2 to 4.5 %), protein (from 2.9 to 3.3 %), and acidity (17–20 °T). To identify these parameters of milk, we used the ultrasonic analyzer "Ekomilk-Ultra" (Labtime ltd., Bulgaria).

4. 2. Microbiological methods for studying the samples

All these samples were analyzed by standard methods for calculating the total number of bacteria and the number of bacteria from the *Enterobacteriaceae* family. At the beginning, we diluted each sample of milk to the concentration of 10^3 in peptone water. Then we seeded them on petrifilms to calculate the number of aerobic microorganisms (Petrifilm for Aerobic Plate Count (AC), made by "3M Microbiology, Maplewood, MN"). To calculate the number of *Enterobacteriaceae* bacteria, we used other petrifilms (Petrifilm for *Enterobacteriaceae* Count (EB), made by "3M Microbiology, Maplewood, MN"). All seeds were then incubated at 35±1 °C for 24±2 hours. To determine the quality of milk, we applied the number of aerobic microorganisms. The study involved the samples of milk with a total number of aerobic microorganisms of up to 100,000 CFU/ml, which corresponds to the "extra" quality of milk.

Upon incubation, we interpreted the results. On petrifilms for enterobacteria, a characteristic growth for the representatives of the *Enterobacteriaceae* family was believed to be associated with red colonies with yellow zones and/or red colonies with gas bubbles or without yellow zones. The colonies without gas bubbles (a distance that is greater than the diameter of the colony between the colony and a gas bubble) and not related to the yellow zone were not considered typical for *Enterobacteriaceae*. We counted all the typical colonies on both types of petrifilms.

4. 3. Design of experimental models and building a database on the dynamics of bacteria number

The number of bacteria from the *Enterobacteriaceae* family in each sample (part of samples for the experiment, 250 ml) was assessed under different temperature regimes and milk composition.

Models for the experiments were closer to practical conditions of production and storage of raw milk. The models involved the samples of raw milk with a different content of fat, protein, and acidity, stored at different parameters for temperature and duration. Each sample of milk was examined after 1, 2, 4, 6, 8, 10, 12, 16, 20, 24, 36, and 48 hours of storage at a storing temperature of 4, 6, 8 and 10 °C. The effect of milk acidity on the number of bacteria from the *Enterobacteriaceae* family was determined at the following values – 16, 17, 18, 19, 20 °T. The content of fat in the milk changed as follows: 3.2; 3.6; 4; 4.5 (%), the protein content – 2.9; 3.0; 3.3 (%).

The design of new ANNs was used to establish the relationship between the input and output layers. The network consists of three layers: an input layer (5 input parameters: milk storage temperature (4, 6, 8, and 10 °C), duration of milk storage (from 2 to 48 hours), the content of fat and protein in milk (%), the acidity of milk (°T), the hidden layers (with 30 neurons), and an output layer (the predicted number of bacteria from the *Enterobacteriaceae* family), as shown in Fig. 1.

We established the effectiveness of the method for predicting the quantity of bacteria from the *Enterobacteriaceae* family in chilled milk using ANN during its storage by comparing experimental data to the data obtained from computer forecast.

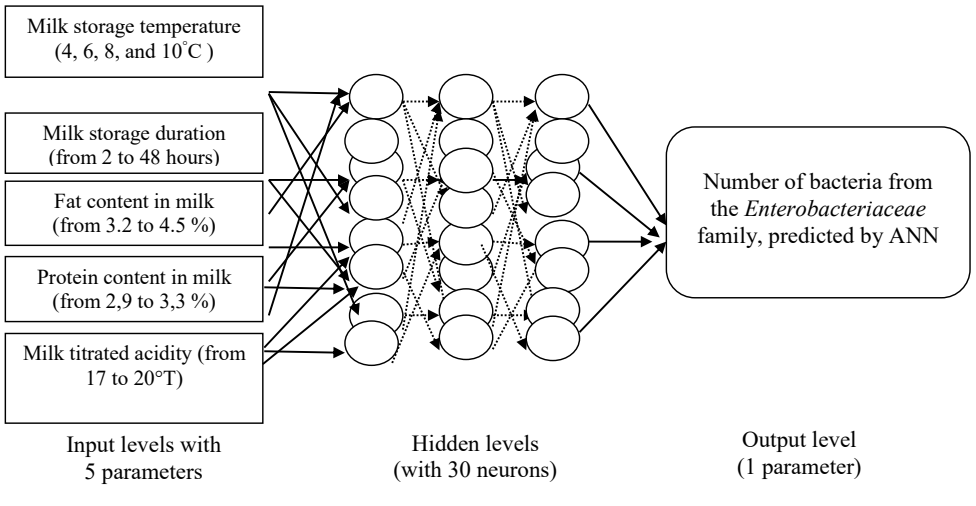

Fig. 1. Schematic representation of the new artificial neural network for predicting the number of bacteria from the *Enterobacteriaceae* family in raw milk when stored chilled

5. Results of studying the effectiveness of a predicting technique

When storing raw milk in the chilled state, there are many different external (temperature and duration of storage) and internal (chemical composition of milk) factors that may affect the number of microorganisms in it. The study began with studying the initial number of bacteria from the *Enterobacteriaceae* family in the samples of raw milk received from dairy farms (Table 1).

Table 1

Initial number of bacteria from the *Enterobacteriaceae* family in raw milk samples (n=290)

Number of investigated samples	Total number of bacteria, CFU/ml	Number of bacteria from the *Enterobacteriaceae* family, CFU/ml	Percentage of bacteria from the *Enterobacteriaceae* family, %
75	$58\pm5.7\times10^3$	$7.91\pm2.5\times10^3$	13.64
125	$90\pm12.8\times10^3$	$11.23\pm1.3\times10^3$	12.48
90	$115\pm12.1\times10^3$	$17.3\pm1.3\times10^3$	15.04

The total number of bacteria in the raw milk samples varied from $58\pm5.7\times10^3$ to $115\pm12.1\times10^3$ CFU/ml, the average value was $90\pm12.8\times10^3$ CFU/ml. It was found that 69 % of 290 samples of milk contain the least total number of microorganisms 100×10^3 CFU/ml. The initial level of bacteria from the *Enterobacteriaceae* family was from $7.91\pm2.5\times10^3$ to $17.3\pm1.3\times10^3$ CFU/ml, the average value was $11.23\pm1.3\times10^3$ CFU/ml. The characteristic growth of enterobacteria on petrifilms is shown in Fig. 2.

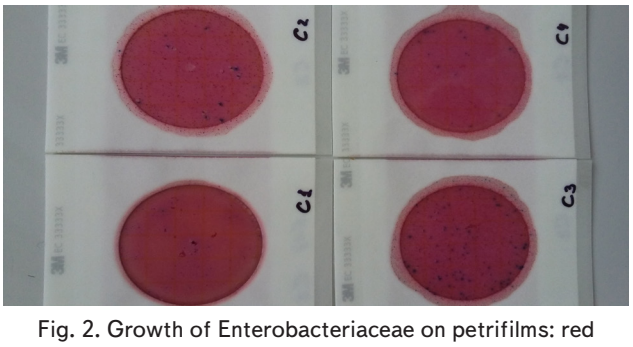

Fig. 2. Growth of Enterobacteriaceae on petrifilms: red colonies with gas bubbles, with or without yellow zones

According to the study objectives, the first step was forming a database on the dynamics of number of bacteria from the *Enterobacteriaceae* family in raw milk during storage under refrigerating conditions. The number of these bacteria in raw chilled milk depended on the complex of variables effects: temperature and duration of storage, the content of fats, proteins, and the titrated acidity of raw milk.

The processes of intelligent analysis and construction of a predicting model, based on neural networks, employed the multilayer perceptron. The perceptron has an optimally selected value for the number of neurons in the hidden layer, which is 30. The model operates in line with an error propagation method. In a neuron model of the perceptron, element Σ multiplies each input x, weight ω, and summarizes the weighted inputs. If this magnitude exceeds a preset threshold, then the output is equal to unity, otherwise zero is equal to zero. These systems (and many similar ones) consist of a single layer of artificial neurons associated through weight coefficients with a set of inputs, although in principle they are estimated by applying more complex systems.

We first tested the ANN for predicting the number of bacteria from the *Enterobacteriaceae* family in chilled milk, depending on the different values for the duration of milk storage (this is the main variable magnitude). In this case, the total number of bacteria from the *Enterobacteriaceae* family in the raw milk samples was 10,000 CFU/ml. That is the predicted expected magnitude. In this example, the following ratios of milk were permanent, they amounted to: storage temperature is 6 °C, fat content is 4.5 %, protein content is 3.3 %, acidity is 16 °T. Changes in the number of bacteria in the chilled milk were determined in 1, 2, 4, 6, 8, 10, 12, 16, 20, 24, 36, and 48 hours. The effectiveness of the proposed technique in a given example was determined by comparing the results from experimental studies and neural forecast. The research results are given in Table 2.

Table 2

Estimation of the method for predicting the quantity of bacteria from the *Enterobacteriaceae* family in milk samples using ANN depending on milk storage duration

Duration, hours	Number of bacteria from the *Enterobacteriaceae* family, CFU/ml		Degree of reliability	
	Actual	ANN forecast	Deviation	%
1	10,000	9,977.92	22	0.221
2	10,000	10,025.58	26	0.256
4	10,000	10,160.41	160	1.604
6	10,100	10,143.19	43	0.428
8	10,250	10,123.25	127	1.237
10	10,500	10,573.26	73	0.698
12	10,800	10,636.0	164	1.519
16	10,800	10,780.29	20	0.182
20	10,800	10,851.53	52	0.477
24	10,800	10,709.45	91	0.838
36	10,800	10,750.19	50	0.461
48	10,320	10,542.96	223	2.160

The next check for the ANN was predicting the number of bacteria from the *Enterobacteriaceae* family in chilled milk depending on the different values for the titrated acidity of milk (this is the main variable magnitude). In this case, the total number of bacteria from the *Enterobacteriaceae* family in the raw milk samples was 10,000 CFU/ml. That was the predicted expected magnitude. In a given case, the following milk indicators permanent; they amounted to: storage temperature is 8 °C, the content of fat is 4.0 %, the content of protein is 3.0 %. The effectiveness of the proposed technique in a given example was determined by comparing the results from experimental studies and neural forecast. The research results are given in Table 3.

The next test for ANN was predicting the number of bacteria from the *Enterobacteriaceae* family in chilled milk, depending on varying amounts of protein and fat in it (key variables). In this case, the total number of bacteria from the *Enterobacteriaceae* family in the raw milk samples was 10,000 CFU/ml. That was a predicted expected magnitude. In this example, the following ratios of milk were permanent; they amounted to: the titrated acidity is 20 °T, protein content is 3.0 %, storage

temperature is 8 °C. A change in the number of bacteria in the chilled milk in a given example was determined in 48 hours. The effectiveness of the proposed method in this example was determined by comparing the results from experimental studies and neural forecast. The research results are given in Tables 4, 5.

Table 3

Estimation of the method for predicting the quantity of bacteria from the *Enterobacteriaceae* family in milk samples using ANN depending on different values for the titrated acidity of milk

Acidity, °T	Number of bacteria from the *Enterobacteriaceae* family, CFU/ml		Degree of reliability	
	Actual	Deviation	Deviation	%
16	13,800	13,891.92	91.92	0.666
17	10,950	11,092.38	142.38	1.300
18	10,600	10,631.24	31.24	0.295
19	10,280	10,319.49	39.49	0.384
20	10,175	10,289.10	114.1	1.121

Table 4

Estimation of the method for predicting the quantity of bacteria from the *Enterobacteriaceae* family in milk samples using ANN depending on milk protein content

Protein content in milk, %	Number of bacteria from the *Enterobacteriaceae* family, CFU/ml		Degree of reliability	
	Actual	Deviation	Actual	Deviation
2.9	11,850	11,633.08	216.92	1.83
3.0	11,850	11,741.42	108.58	0.92
3.3	11,950	11,795.88	154.12	1.29

Table 5

Estimation of the method for predicting the quantity of bacteria from the *Enterobacteriaceae* family in milk samples using ANN depending on milk fat content

Fat content in milk, %	Number of bacteria from the *Enterobacteriaceae* family, CFU/ml		Degree of reliability	
	Actual	Deviation	Actual	Deviation
4.5	10,600	10,444.51	155.49	1.47
4.0	10,600	10,449.5	150.5	1.42
3.6	10,600	10,453.66	146.34	1.38
3.2	10,600	10,457.93	142.07	1.34

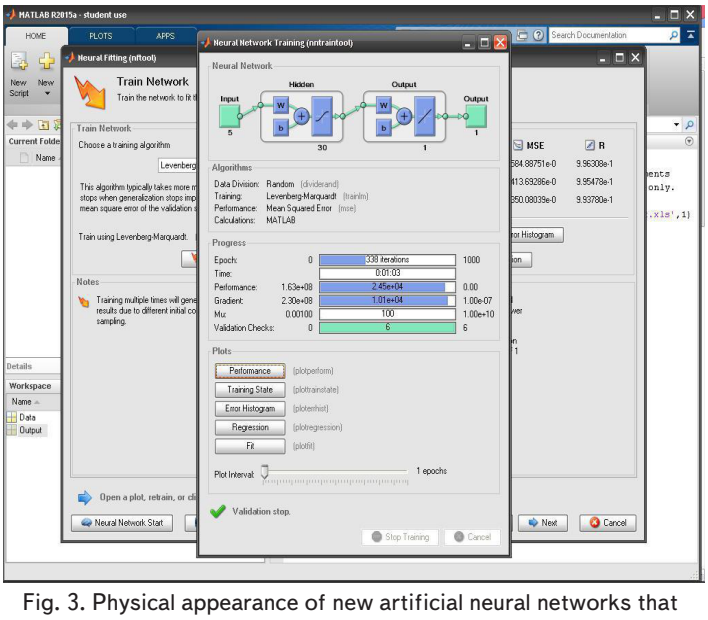

Fig. 3. Physical appearance of new artificial neural networks that show the patterns of training and the iteration epoch of logistical and hyperbolic activation functions

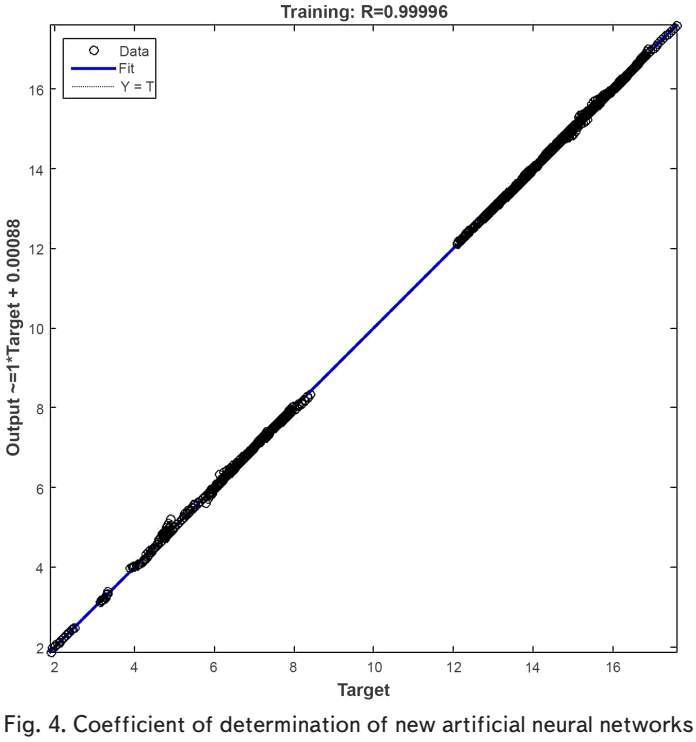

Fig. 4. Coefficient of determination of new artificial neural networks

As one can see from the above results, the proposed method for predicting the number of enterobacteria in milk samples using ANN depending on different values for the titrated acidity and its content of fat and protein demonstrates the highest degree of deviation 2.160 %, or 223 bacterial cells per 1 ml (Table 2).

Fig. 3 illustrates the predictive power of the newly created model of a neural network. The average coefficient of determination of new artificial neural networks R=0.99996, which is a rather insignificant indicator, given that the original data are expressed in tens of thousands (Fig. 4). By taking the square root, we obtain an average error of 171.50 CFU/ml), which is ranges from 0.99 % to 1.715 %, depending on characteristics for the respective samples of milk.

The last stage of the study was to predict the number of bacteria from the *Enterobacteriaceae* family in the samples of chilled milk, depending on the complex of variables. These variable effects included: storage temperature, fat content, protein content, milk storage duration. In this case, the initial number of bacteria from the *Enterobacteriaceae* family was 10,000 CFU/ml (the predicted magnitude). The effectiveness of the proposed technique was determined by comparing the results from experimental studies and neural forecast. The research results are given in Table 6.

As shown in Table 6, the proposed technique for predicting the quantity of bacteria from the *Enterobacteriaceae* family in milk during storage, using ANN in the form of a multilayer perceptron, depending on its acidity and its

content of fat and protein demonstrates the high degree of reliability 2.497 % (or 370 bacterial cells per 1 ml).

Table 6

Estimation of the method for predicting the quantity of bacteria from the *Enterobacteriaceae* family depending on a simultaneous effect of different physical-chemical parameters

storage temperature, °C	fat content, %	protein content, %	titrated milk acidity, °T	milk storage duration, hours	actual (experimental) result	ANN forecast	Degree of reliability	
							Deviation	%
10	4.5	2.9	20	20	10,640	10,804.85	165	1.549
8	4.5	2.9	16	36	14,800	14,430.49	370	2.497
4	4	3	20	8	6,690	6,972.05	282	4.216
6	4	3	18	24	10,600	10,659.36	59	0.560
10	3.2	3	17	48	16,650	16,727.88	78	0.468
10	3.2	3.3	19	48	15,000	15,327.69	328	2.185

6. Discussion of studying the efficiency of a predicting technique for the number of microorganisms in raw milk during storage under refrigerating conditions

This study addresses the development of a method for predicting the number of enterobacteria in raw milk during storage in a refrigerator. The priority was to justify the choice of a group of micro-organisms for the research. Following an analysis of the scientific literature, we learnt about the devised and proposed prognostic models on the behavior of various microorganisms in food products. There are examples of predicting the number of bacteria in raw milk, including pathogenic microorganisms (*Staphylococcus aureus*) and useful microflora (*Bifidobacterium bifidum* and *Lactobacillus acidophilus*) [20, 31]. Earlier research mainly focused on specific kinds of microorganisms and a group of psychrotrophic microorganisms [6, 7, 19, 27]. However, we could not find in the scientific literature any data on forecasting the number of bacteria from the *Enterobacteriaceae* family in raw milk during storage.

It was then necessary to choose software for forecasting. The principle of artificial neural networks has been selected, as it was established that they provide the best modeling and predicting approach [26, 29]. ANNs consist of many interconnected processors, called neurons, which ensure a more accurate processing of numerous data simultaneously. In addition, an important advantage of using ANNs for processing a large amount of experimental data is their capability of learning, as well as the selection of input parameters that warrant the derivation of the most precise model.

In several publications, ANNs were chosen over other programs because they produce a smaller error for the values of prediction [28, 34]. Thus, it was reported that the application of ANN to predict the behavior of *Staphylococcus aureus* in milk yielded a low value of error (10.95 %) [27]. The foregoing testifies to the substantiated application of ANN.

The development of a method for predicting the quantity of enterobacteria consisted of four stages. At the first stage, we examined the total number of bacteria and the number of bacteria from the *Enterobacteriaceae* family depending on the composition of raw milk, as well as its temperature and its storage under conditions of a refrigerator. The total number of bacteria in the raw milk samples before the start of the study was from $58\pm5.7\times10^3$ to $115\pm12.1\times10^3$ CFU/ml; the mean value was $90\pm12.8\times10^3$ CFU/ml. It was also found that the number of enterobacteria was on average $11.23\pm1.3\times10^3$ CFU/ml.

At the second stage of our study, it was necessary to build a base of experimental data obtained from experimental models with the samples of raw milk of various physical-chemical composition. It is known that the number of microorganisms in food is affected by the internal and external factors. The internal factors include the chemical composition of the product, water activity, and pH. The external factors include the time and temperature of product storage [7, 19]. In a given case, the chosen task was to examine the effect of different content of fat, protein, and a titrated milk acidity index, on enterobacteria. These are the basic parameters that can exert an effect on the growth and reproduction of microorganisms in milk.

To build a database of experimental data, we used the samples of raw milk with the initial number of bacteria from the *Enterobacteriaceae* family of 10,000 CFU/ml. Next, we examined changes in the number of these microorganisms at different temperatures and duration of milk chilling and its various qualitative composition. In this study, models for experiments were closer to practical conditions of production and storage of raw milk. To this end, the samples of raw milk with different content of fat, protein, and titrated acidity were stored under the following conditions: the temperature is 4, 6, 8, and 10 °C, the duration is from 2 to 48 hours. Each sample of milk was examined after 1, 2, 4, 6, 8, 10, 12, 16, 20, 24, 36, and 48 hours. The influence of milk acidity on the number of bacteria from the *Enterobacteriaceae* family was determined for the following values: 16, 17, 18, 19, 20 °T. The content of fat in milk varied as follows: 3.2, 3.6, 4.0, 4.5 %, and the protein content – 2.9, 3.0, 3.3 %. The result was the compiled base of 1,200 experimental data sets.

At the next stage, we introduced the resulting database to ANN. To construct the new ANN for predicting the number of enterobacteria, we applied a multi-layer perceptron. The new model had 5 input parameters and a single output parameter – the predicted number of bacteria from the *Enterobacteriaceae* family (Fig. 1). However, it was important to train and adapt the newly created ANN to conduct a given type of prediction using actual examples, which were obtained under laboratory conditions. Upon training and optimizing the newly built ANN, the average error obtained was 171.50 (CFU/ml), which ranges from 0.99 % to 1.715 %, depending on the characteristics of the respective milk samples (Fig. 3). As seen from the above (Tables 3–5), deviations between the actual data and the neural forecast were insignificant.

At the final stage, we estimated the effectiveness of the technique for predicting the quantity of bacteria from the *Enterobacteriaceae* family in raw milk during storage under refrigeration conditions. In a given case, the application of ANN to predict the number of enterobacteria in milk demonstrated the highest degree of deviation – 2.497 % (or 370 bacterial cells per 1 ml).

Using the described method makes it possible to improve the speed, accuracy, informativeness, and to significantly

reduce the research required for forecasting the number of microorganisms. This method will make it possible to change actual studies to mathematical (computer) models that adequately reflect the most important patterns in the examined objects. A possibility to build new predictive neural network models for a complex of influence from various external factors (time, temperature, humidity, etc.) and internal (complicated) environment on the microbiological quality of milk (or other foods) opens up important avenues for the application of such methods to control safety in the food industry.

7. Conclusions

1. Our research has found the influence of storage regimes of chilled raw milk and its chemical composition on the number of bacteria from the *Enterobacteriaceae* family that it contains. It was established that the total number of microorganisms in the raw milk samples was on average $90\pm12.8\times10^3$ CFU/ml. In this case, the initial level of bacteria from the *Enterobacteriaceae* family was on average at the level of $11.23\pm1.3\times10^3$ CFU/ml. The result of studying the influence of a complex of variables effects (temperature and duration of storage, the content of fats, proteins, and the titrated acidity of raw milk) was the compiled database of results from own experiments.

2. The built base of research results included about 1,200 experimental data sets on the number of enterobacteria derived from models that involved the samples of raw milk. Hence, one can argue that there is a certain impact of milk storage conditions, as well as its chemical composition, on the number of enterobacteria in raw chilled milk.

3. It was established that the introduction of the resulting database of experimental data to an artificial neural network makes it possible to forecast the number of micro-organisms depending on a complex of variable effects (storage temperature, fat content, protein content, milk storage duration).

4. Owing to the application of a given ANN, it was established that the efficiency of the method for predicting the number of microorganisms in raw milk during storage under refrigeration conditions has a high degree of reliability, 2.497 % (or 370 bacterial cells per 1 ml). That allows us to argue about the effectiveness of using this method under practical conditions that would make it possible to change actual laboratory studies to artificial intelligence.

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