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Original article

Modeling the kinetics of survival of *Staphylococcus aureus* in regional yogurt from goat's milk

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Abstract

The aim of this study was to determine the kinetics of the survival of the test strain of *Staphylococcus aureus* in the product investigated. Yogurt samples were contaminated with *S. aureus* to an initial level of 10^3 - 10^4 cfu/g. The samples were then stored at four temperatures: 4, 6, 20, 22°C. During storage, the number of *S. aureus* forming colonies in a gram of yogurt was determined every two hours. Based on the results of the analysis culture the curves of survival were plotted. Three primary models were selected to describe the kinetics of changes in the count of bacteria: Cole's model, a modified model of Gompertz and the model of Baranyi and Roberts. Analysis of the model fit carried out based on the average values of Pearson's correlation coefficient, between the modeled and measured values, showed that the Cole's model had the worst fit. The modified Gompertz model showed the count of *S. aureus* as a negative value. These drawbacks were not observed in the model of Baranyi and Roberts. For this reason, this model best reflects the kinetics of changes in the number of staphylococci in yogurt.

Key words: goat milk, primary model, regional food, staphylococci, yogurt

Introduction

Over the past few years there has been an increased interest in regional products from farms in the states of the European Union. This phenomenon stems from the EU policy that supports the development of production and distribution of local food (Feenstra 1997, DuPuis and Goodman 2005). Con-

sumers driven by various motivations are increasingly selecting products produced in the nearest vicinity of their households (McIntyre and Rondeau 2011). These prerequisites contributed to the establishment in 1995 of the European Network of Regional Culinary Heritage promoting the increased production and consumption of regional food. Currently, this network includes the following nine Polish regions with

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full membership status: Lower Silesia, Mazovia, Opole Voivodeship, Pomerania and Kujawy, Pomeranian Voivodeship, Świętokrzyskie Voivodeship, Warmia Mazury Powiśle, West Pomeranian Voivodeship and Wielkopolska. One of the activities registered in the region of Warmia Mazury Powiśle is the production of goat's milk products. Increasing sale of goat's milk and its products is determined by their physico-chemical, sensory and nutritional qualities (Ribeiro and Ribeiro 2010). Goat's milk, compared to cow's milk, contains smaller protein micelles and smaller fat globules. In addition, goat's milk fat has a higher amount of short- and medium-chain fatty acids, particularly caproic, caprylic and capric acids, and higher contents of unsaturated fatty acids (Faye and Konuspayeva 2012). Due to these properties and components, goat's milk and its products are easier to assimilate and digest than cow's milk products.

Regional farm products are usually produced by people involved in both milking and processing of milk, which may carry a risk of cross-contamination with environmental microorganisms. Commonly occurring microorganisms on the skin and mucous membranes of animals in the barn environment are staphylococci, which may also be detected in milk (Contreras et al. 2003, Pereira et al. 2010). The microbiological quality of goat's milk from farms is assessed on the basis of the total bacterial count (TBC) and does not take into account the number of staphylococci, similarly as in the case of the milk of other animal species. The TBC acceptable in goat's milk is higher than in cow's milk, which may be directly related to the increased number of staphylococci (Regulation 853/2004). Given these facts, we have studied the survival of *Staphylococcus aureus* in regional yogurt produced from goat's milk and stored at different temperatures. The aim of this study was to determine the kinetics of the survival of the test strain of *S. aureus* in the product investigated.

Materials and Methods

Material for the study was yogurt produced at an agro-processing farm located in Warmia and Mazury and registered with the European Network of Regional Culinary Heritage. The raw material for the production of natural yogurt was goat's milk from the farm and dairy cultures (Vital, Danisco). The product was collected from April to August, once a month and transported to the laboratory under refrigeration. Aseptically weighed 10-gram portions were then placed in sterile bags. Some of the portions were inoculated with *S. aureus*. Non-inoculated yogurt samples served as the control.

Preparation of *S. aureus* inoculum

After thawing the mixture with *S. aureus* (ATCC 25923, Mecconti, Luxemburg) and brain-heart infusion broth (BHI) (Oxoid, Basingstoke, UK), inoculating loops were used to transfer microorganisms to nutrient agar (Oxoid, Basingstoke, Hampshire, UK) and incubated for 24 h at 37°C. One colony of *S. aureus* was then suspended in BHI and incubated for another 20 h at the same temperature. The resulting culture was diluted in Maximum Recovery Diluent (Merck, Germany). The inoculum for the yogurt samples consisted of 10^3 - 10^4 cfu/g of *S. aureus*.

Storage of inoculated yogurt samples

The inoculated samples were stored at four temperatures: two refrigerating temperatures of 4 and 6°C, and two room temperatures of 20 and 22°C. During storage, a 10-gram portion was collected every two h, and assayed for the number of *S. aureus* forming colonies in a gram of yogurt at each of these temperatures.

Culture analysis

The number of colony forming units was calculated on the basis of the procedures described in the EN ISO 6888-1:1999 standard. The detection limit of the plate counts method was 10 colonies of coagulase positive staphylococci per milliliter. Culture analysis was carried out in five replicates at each of the above-listed temperatures. Non-inoculated yogurt samples were stored and tested for the presence of *S. aureus* in the same manner as the inoculated samples.

Measurement of pH

The pH measurements were conducted using an HI model 9224 pH-meter (Hanna Instruments, USA). The first measurement was made after inoculation (0 h), and subsequent measurements were carried out during the storage of samples every 2 h. As with the culture analysis, pH measurements were performed five times for each series.

Data analysis

Three primary models were selected to describe the kinetics of changes in the count of bacteria: Cole's model, a modified model of Gompertz and the model

of Baranyi and Roberts. These models are among the most commonly used mathematical models for modeling the survival of bacteria on the nonlinear nature of these changes (Xiong et al. 1999, McKellar and Lu 2004, Huang 2009, Lobacz et al. 2013).

The non-linear Cole's model is described by equation (1) (McKellar and Lu 2004):

$$X = \alpha + \frac{\omega - \alpha}{1 + e^{\frac{4\sigma(\tau - t)}{\omega - \alpha}}} \quad (1)$$

t – time log [h]
 X – number of cells [log cfu ml⁻¹]
 α – upper asymptote [log cfu ml⁻¹]
 ω – lower asymptote [log cfu ml⁻¹]
 τ – position of maximum slope [h]
 σ – maximum slope [log cfu ml⁻¹ h⁻¹]

The coefficients in the Cole's model were: α, ω, τ, σ. It was assumed that these coefficients are only empirical in order to obtain the best fit of the model to the experimental values.

The modified Gompertz's survival model is described by equation (2) (Xiong et al. 1999):

$$X = Ce^{-e^{B(t-M)}} - Ce^{-e^{BM}} + X_0 \quad (2)$$

X – number of cells [log cfu ml⁻¹]
 t – time [h]
 C – the difference between the upper and lower asymptote
 B – relative growth rate at M
 M – the time at which the absolute maximum growth rate is observed [h]

Subscripts:

0 – initial value

B, C, M, X₀ were the coefficients calculated in model (2). Each measured value was subject to a measurement error: therefore, it was assumed that the value of X₀ can be erroneous by ±0.5 log cfu ml⁻¹.

The cell death model of Baranyi and Roberts is expressed as a set of equations (3) (McKellar and Lu 2004).

$$\left\{ \begin{array}{l} \frac{dX}{dt} = \frac{q}{q+1} \cdot \mu_{\max} \cdot \left(1 - \left(\frac{X}{X_{\min}}\right)^m\right) X \\ \frac{dq}{dt} = \mu_{\max} \cdot q \end{array} \right. \quad (3)$$

X – number of cells [log cfu ml⁻¹]
 m – empirical coefficient in the model
 t – time [h]
 q – concentration of limiting substrate (virtual)
 μ_{max} – constant of growth [h⁻¹]

Subscripts:

min – minimum value

0 – initial value

In the model of Baranyi and Roberts the following coefficients were calculated: q₀, X₀, μ_{max}, m.

The values of the coefficients were determined using knitro specialized optimization software (Zien Optimization LLC, USA) (the procedure imposes constraints on the values of the coefficients), compatible with MATLAB. Pearson's correlation coefficient *r* was used as the statistical fit of the model to the experimental values and was determined using MATLAB procedure.

Results

Table 1 shows the descriptive statistics (mean, standard deviation, minimum and maximum) from the pH measurements at temperatures of 4, 6, 20 and 22°C. On the basis of the presented standard deviations, the minimum and maximum can be seen that in each of the temperature pH changed to a very small range, of up to about 0.17 at 4°C, with an average value of 4.12 at 22°C to 4.23 at 4°C. In the studied temperatures, pH were not involve a permanent trend changes.

The results of the quantitative analysis of *S. aureus* survival in yogurt stored at selected temperatures are presented in Figs. 1-4, which show the model lines calculated using three methods: Cole's model, a modified model of Gompertz and the model of Baranyi and Roberts. The survival lines of *S. aureus* in yogurt stored at temperatures of 6, 20 and 22°C were very similar. The greatest differences between the lines of the model were found at 4°C (Fig. 1). Cole's model had the weakest fit. The course of the remaining model lines indicated that the model of Baranyi and Roberts was characterized by a better fit. The modified Gompertz's model provided a negative cell count in the final phase. The drawbacks of this model were not observed in the model of Baranyi and Roberts.

Table 1. Values of descriptive statistics (mean, standard deviation, minimum and maximum) of all pH measurements at temperatures of 4, 6, 20 and 22°C.

Temperature	Mean	Std	Min	Max
4°C	4.23	0.04	4.13	4.30
6°C	4.18	0.05	4.07	4.26
20°C	4.19	0.06	4.10	4.28
22°C	4.12	0.03	4.08	4.18

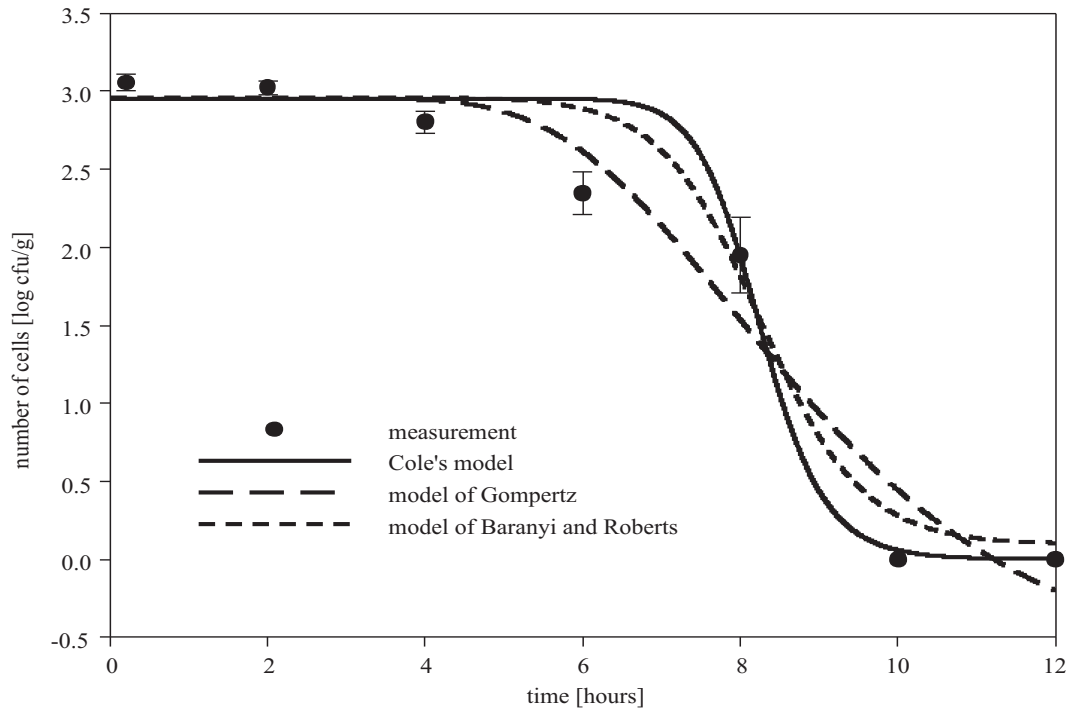


Fig. 1. Kinetics of *S. aureus* cell number in yogurt stored at 4°C and model lines. Experimental points are the average of 5 measurements with standard deviation.

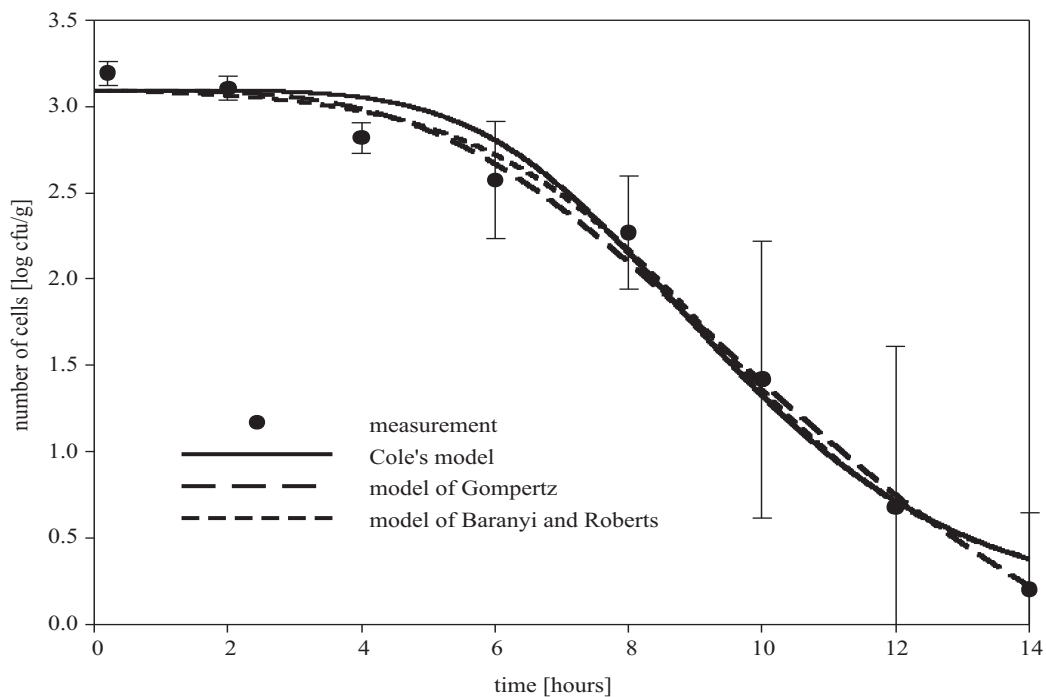


Fig. 2. Kinetics of *S. aureus* cell number in yogurt stored at 6°C and model lines. Experimental points are the average of 5 measurements with standard deviation.

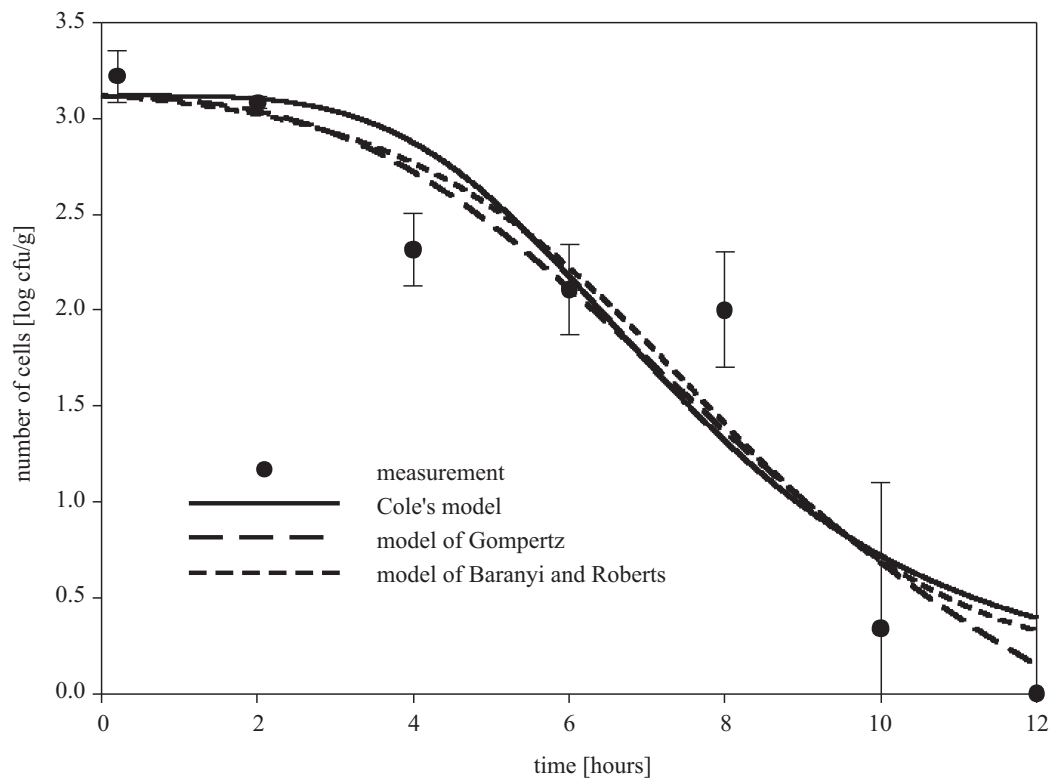


Fig. 3. Kinetics of *S. aureus* cell number in yogurt stored at 20°C and model lines. Experimental points are the average of 5 measurements with standard deviation.

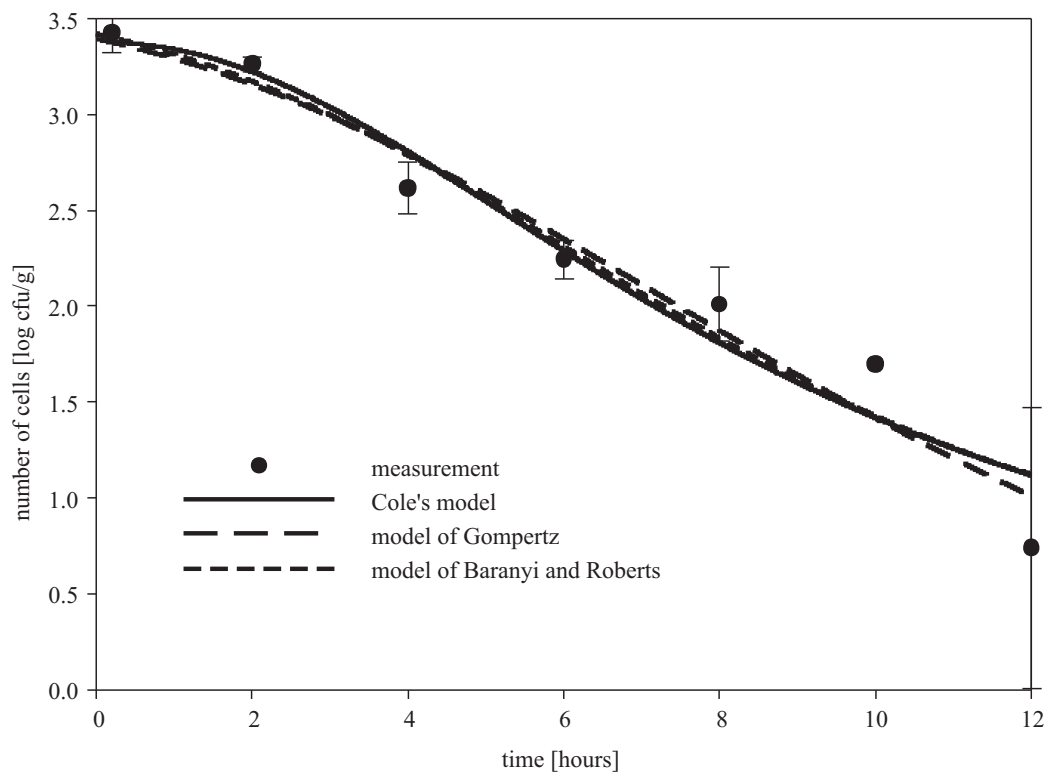


Fig. 4. Kinetics of *S. aureus* cell number in yogurt stored at 22°C and model lines. Experimental points are the average of 5 measurements with standard deviation.

Table 2. Values of coefficients for Cole's model, a modified model of Gompertz and the model of Baranyi and Roberts (p – values for testing the hypothesis of no correlation, RLO – lower boundary for a 95% confidence interval, RUP – upper boundary for a 95% confidence interval).

Model	Temp. [°C]	r	p	RLO	RUP	Coefficients			
						α	ω	τ	σ
Cole's	4	0.985	0.000	0.895	0.998	2.95	8.68E-10	0.917	-34.55
	6	0.991	0.000	0.951	0.998	3.09	4.30E-10	0.975	-8.93
	20	0.944	0.001	0.659	0.992	3.12	1.63E-10	0.869	-7.18
	22	0.970	0.000	0.807	0.996	3.38	1.20E-09	0.933	-4.06
Modified Gompertz's						C	B	M	X_0
	4	0.970	0.000	0.808	0.996	3.000	0.500	7.214	2.954
	6	0.989	0.000	0.936	0.998	3.000	0.369	8.236	3.094
	20	0.944	0.001	0.661	0.992	3.000	0.352	6.022	3.119
	22	0.972	0.000	0.816	0.996	3.000	0.227	6.044	3.355
Baranyi and Roberts						μ_{max}	m	q_0	X_0
	4	0.984	0.000	0.896	0.998	1.622	1.166	3.07E-08	2.963
	6	0.995	0.000	0.970	0.999	0.567	0.934	0.000398	3.094
	20	0.959	0.000	0.740	0.994	0.531	0.745	0.001431	3.119
	22	0.972	0.000	0.815	0.996	0.417	2.754	0.000958	3.420

Discussion

Many prognostic models have been developed which estimate the safety of food products. There is a need to develop models and to express in a quantitative manner the behavior of microbial pathogens in regional food, including dairy products. The present study applied standard primary models to describe the kinetics of *S. aureus* behavior in a regional goat yogurt.

The highest differences between the plotted primary models of survival of *S. aureus* were observed at 4°C. The analysis of the model fit at 4°C based on the Pearson's correlation coefficient (r), between the modeled and measured values (Table 2), showed that the Cole's model presented the most accurate fit, because of the highest value of the correlation coefficient. However, this conclusion is based on a small number of measurement points (7), and while the fit in the points is better, the shape of the model line demonstrated worse fitting at the end of the experiment. Mean r values of Pearson's coefficients for all storage temperatures indicated the worst fit of the modified Gompertz's model, while they were comparable for the Cole's as well as Baranyi and Roberts models, where differences were significant in temperatures 4 and 6°C to two decimal points. In contrast, at 20 and 22°C values were comparable to the Cole's and slightly inferior to the Baranyi and Roberts

model. The plotted models allow the conclusion that the model of Baranyi and Roberts most appropriately reflected the kinetics of changes in the number of staphylococci in yogurt.

The survival of *S. aureus* in yogurt can be affected by the internal and external conditions (Bassett 2010). One of the most important extrinsic factors limiting the growth and survival of microorganisms is the storage temperature of the product (Lobacz et al. 2013). Mesophilic microorganisms, including *S. aureus*, show a lack of growth at temperatures of 4–6°C. Growth temperatures of *S. aureus* were determined in the range of 7–48.5°C (Valero et al. 2009). Although the temperature range of *S. aureus* growth comprises room temperature, no growth of staphylococci was observed in the regional yogurt produced from goat's milk. Storing the product at room and refrigerating temperatures causes the death of *S. aureus*. Moreover, at temperatures of 20 and 22°C, a slight decrease was recorded in the number of staphylococci after 2 h, as opposed to 4 and 6°C.

Another environmental factor influencing the growth, survival and death of microorganisms in the food products is pH. A previous study determined that *S. aureus* can grow in a pH range from 4 to 10 (Valero et al. 2009). Our study found that the value of this parameter showed very low variability. Despite the difference in the storage temperature of samples, the pH values of the product were very similar to each

other. Pzakova (1997) showed that the reduction of the number of staphylococci in the yogurt may be associated with low pH of the product. The authors describe the effect of metabolites produced by the fermentation cultures as a major factor inhibiting pathogenic microbes (Dahiya and Speck 1968, Pulusani et al. 1979, Pzakova et al. 1997). Therefore, it can be assumed that the substances produced by the strains used for the production of yogurt, i.e., *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, may affect the necrosis of staphylococci in the product.

In our study the results demonstrated that the number of *S. aureus* in yogurt during storage at room temperatures significantly reduced as, after 6 hours, the level of contamination decreased to less than 2 log cfu/ml. Clear differences can be found in *S. aureus* behavior when comparing the results of staphylococci cultures in yogurt and broth medium, which is an optimal medium for the growth of the pathogen. During storage of staphylococci in broth (pH = 4) at 25°C, a decrease in the number of cells was obtained only after 167.25 h from the initial logarithmic level of 3.56 to 2.1, whereas the presence of *S. aureus* was not detected after 192.3 h (ComeBase).

Conclusion

Summing up the results of the current work, it can be concluded that the model of Baranyi and Roberts describes most accurately the kinetics of survival of *S. aureus* in a regional yogurt produced from goat's milk.

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