Journal of Microbiology (2014) Vol. 52, No. 2, pp. 169–178
DOI 10.1007/s12275-014-3045-0

Predictive Modelling of *Lactobacillus casei* KN291 Survival in Fermented Soy Beverage

Zielińska Dorota[1*], Kołożyn-Krajewska Danuta[1], Goryl Antoni[2], and Ilona Motyl[3]

[1]*Department of Food Gastronomy and Food Hygiene, Faculty of Human Nutrition and Consumer Sciences, Warsaw University of Life Sciences – SGGW, ul. Nowoursynowska 159c, 02-776 Warsaw, Poland*
[2]*Department of Econometrics and Operational Research, Cracow University of Economics ul. Rakowicka 27, 31-510 Cracow, Poland*
[3]*Institute of Fermentation Technology and Microbiology, Technical University in Łódź, ul. Wólczańska 171/173, Poland*

(Received Jan 21, 2013 / Revised Jul 18, 2013 /Accepted Aug 19, 2013)

The aim of the study was to construct and verify predictive growth and survival models of a potentially probiotic bacteria in fermented soy beverage. The research material included natural soy beverage (Polgrunt, Poland) and the strain of lactic acid bacteria (LAB) – *Lactobacillus casei* KN291. To construct predictive models for the growth and survival of *L. casei* KN291 bacteria in the fermented soy beverage we design an experiment which allowed the collection of CFU data. Fermented soy beverage samples were stored at various temperature conditions (5, 10, 15, and 20°C) for 28 days. On the basis of obtained data concerning the survival of *L. casei* KN291 bacteria in soy beverage at different temperature and time conditions, two non-linear models (r^2= 0.68–0.93) and two surface models (r^2=0.76–0.79) were constructed; these models described the behaviour of the bacteria in the product to a satisfactory extent. Verification of the surface models was carried out utilizing the validation data - at 7°C during 28 days. It was found that applied models were well fitted and charged with small systematic errors, which is evidenced by accuracy factor - *Af,* bias factor - *Bf* and mean squared error - *MSE*. The constructed microbiological growth and survival models of *L. casei* KN291 in fermented soy beverage enable the estimation of products shelf life period, which in this case is defined by the requirement for the level of the bacteria to be above 10^6 CFU/cm^3. The constructed models may be useful as a tool for the manufacture of probiotic foods to estimate of their shelf life period.

***Keywords*:** probiotic bacteria, predictive model, validation

Introduction

Predictive microbiology is a sub-discipline of food microbiology dealing with the development of mathematical models describing the responses of microorganisms to specific environmental conditions, and verifying their use in predicting the growth, survival and inactivation of microorganisms in food. Predictive microbiology is based upon the premise that the response of population of microorganisms to environmental factors are reproducible, and that by characterizing environments in terms of identifiable dominating constraints it is possible, from past observations, to predict the responses of those microorganisms in other, similar, environments. (Ross and McMeekin, 1994; Van Impe *et al*., 2005). It combines elements of microbiology, mathematics and statistics to supply information on the behaviour of microorganisms in food (McMeekin *et al*., 2008).

A number of models have been developed to represent and predict microbial growth or inactivation in food. Examples of primary models, that are also used for lactic acid bacteria growth include the exponential model, lag-exponential model and modifications of Gompertz model (Chowdhury *et al*., 2007; Slongo *et al*., 2009). On the other hand, among the models of bacteria inactivation the log-linear inactivation model and the Weibull model are distinguished (Avsaroglu *et al*., 2006). Examples of secondary growth models for one variable include the Arrhenius or Ratkowsky models; the Bigelow model is an example of the a secondary model of inactivation for variable temperature (Geeraerd *et al*., 2005; Le Marc *et al*., 2009). The multinomial model and gamma hypothesis model are examples of models for more than one variable (Zwietering *et al*., 1992; Biesta-Peters *et al*., 2010). Tertiary models include combinations of primary and secondary models of growth, acid production, and buffer capacity describing lactic acid fermentation (Zwietering, 2005).

Nowadays study of biokinetic reactions in beneficial food-grade microorganisms, such as lactic acid bacteria, is increasing. Modelling techniques are applied to predict the outcome of fermentation processes under particular circumstances and/or to investigate the application potential of starter cultures producing bacteriocins, and to assess the effects of environmental conditions on growth. Such models can be very useful in food technology and processing. In the food industry, lactic acid bacteria are intentionally added as starter cultures to basic products such as milk, meat, vegetables and cereals, in order to obtain a stable and safe final product with unique sensory properties (Leroy *et al*., 2002; McMeekin *et al*., 2008; Jaworska *et al*., 2011).

Until now, predictive modeling has been employed on a few species of lactic acid bacteria, considered to be undesirable microorganisms (Devlieghere *et al*., 1998; Rodrigo *et al*., 2003; Gómez *et al*., 2005; Kilimann *et al*., 2005; Altieri *et al*., 2008). In the last 10 years, there has been an increasing interest in

*For correspondence. E-mail: dorota_zielinska@sggw.pl; Tel.: +48-0-225 937068; Fax: +48-0-225937068

modeling the kinetics of lactic acid bacteria as a beneficial microorganisms in food systems. Studies were conducted on interactions of lactic acid bacteria and pathogenic bacteria (Breidt and Fleming, 1998; Malakar *et al*., 1999; Martens *et al*., 1999; Vereecken *et al*., 2003; Le Marc *et al*., 2009). These were related to the possibility of producing certain bacteriostatic and bactericidal substances by lactic acid bacteria (LAB) (Vázquez and Murado, 2008). Fewer models have been described for beneficial bacteria including probiotic ones, which are intentionally added to fermented food products in order to shape sensory and health-promoting properties (Gianotti *et al*., 1997; Gomes *et al*., 1998; Jayamanne and Adams, 2009; Nualkaekul and Charalampopoulos, 2011).

Probiotic bacteria reveal a health-promoting effect via, inter alia: by producing important digestive enzymes and antibacterial substances, stimulating the immunological system, and lowering the activity of fecal enzymes responsible for the reduction of pro-carcinogens to carcinogens (Nagpal *et al*., 2012).

The emphasis for prolonged survival of probiotics in the food matrix has resulted in the alteration in the functionality and efficacy of the food product. The number of living bacteria introduced into the host is very important in terms of obtaining a probiotic effect. According to FAO/WHO (2002) reports, that in order to exert health benefits, probiotic bacteria must remain viable in the food carriers and survive the harsh condition of GI tract, with a minimum count of 10^6 CFU/g (Nagpal *et al*., 2012). Considering these facts, predicting the behaviour of probiotic bacteria in the product during its shelf life period seems to be purposeful.

Probiotic bacteria are primarily used to produce yoghurts, but they are also added to cold cuts or juice. Soy yoghurt is an example of an interesting functional product - it is produced from soy beverage, namely from the aqueous extract of soy beans that are rich in proteins of high nutritional values, without cholesterol or lactose, and with only a small amount of saturated fatty acids (Wang *et al*., 2003).

Recently attempts have been made to ferment soy beverage with strains with probiotic properties (Wang *et al*., 2002, 2003, 2006; Farnworth *et al*., 2007; Ewe *et al*., 2010; Bao *et al*., 2012). This type of product is an excellent alternative for people with milk protein allergy or lactose intolerance, or for vegetarians. The process of soy fermentation leads to the release of amino acids and synthesis of vitamins from the B group. Anti-nutritive factors such as trypsin inhibitors, phytates, hemaglutinins and oligosaccharides are deactivated by high temperatures (trypsin inhibitors) or by enzymes (phytates, oligosaccharides) (Messina *et al*., 2002). Soy fermentation also results in the elimination of the undesired 'beanie taste', which is mostly due to the presence of *n*-hexanal and pentanal (Scalabrini *et al*., 1998). It has been observed that soy products lose their allergenic properties after fermentation (Hefle *et al*., 2005); moreover, the absorption of genistein and isoflavones in their free form, namely aglycones, is increased (Fitzpatrick, 2003).

The ability to predict the response to a new situation is proof of the model's quality. In food microbiology, a model developed in the laboratory should precisely specify the state of the microorganism found in the product during the technological process, or during storage or distribution. The models should be developed and verified in practice (McMeekin and Ross, 2002). Therefore objectives measures to assess model performance have been proposed (Ross, 1996), and criteria values of these measures suggested as criteria for acceptance of models. Many models currently available to satisfy these criteria when compared to appropriate observations.

The aim of the work was to construct and verify predictive models for the growth and survival of *Lactobacillus casei* KN291 bacteria in the fermented soy beverage, as a functional food product.

Materials and Methods

Materials for research

The material for the studies consisted of natural soy beverage (commercial product, producer: Polgrunt, Poland), fermented with a species of lactic acid bacteria *Lactobacillus casei* KN291, obtained from the Institute of Fermentation Technology and Microbiology, Technical University in Łódź, Poland.

The *Lb. casei* KN291 strain was selected in earlier studies (Zielińska, 2005) from 10 strains of lactic acid bacteria (5 species of *Lactobacillus acidophilus* and 5 species of *Lactobacillus casei*) The sensory quality of the products received was a criterion for selecting a strain for the fermentation of soy beverage. The bacteria strains used in the study came from the collection of the Institute of Fermentation Technology and Microbiology, Lodz University of Technology, and during in vitro test they exhibit potentially probiotic properties (Moneta and Libudzisz, 2000; Motyl, 2002).

Lactobacillus casei KN291 exhibits potentially probiotic properties and was deposited as LOCK 0904 in the Pure Collection of the Technical University, Lodz (LOCK). The strain adheres to the Caco-2 epithelial cell line. The survival of this strain after incubation at pH 2.5 did not change markedly, and remained at 82% (10^7 CFU/ml). This strain exhibited a high survival rate at pH 3.5 (>90%), whereas pH 1.5 was lethal. The incubation with 2% bile salt solution resulted in a survival rate of 92% (10^8 CFU/ml), whereas after incubation in 4% solution it was 84% (i.e. 10^7 CFU/ml). The test strain showed antagonistic activity against all the pathogenic bacteria used (*E. coli* 018, *E. coli* LOCK 105, *E. coli* ATCC 25922 *Listeria monocytogenes*, *Listeria innocua*, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis*, *Salmonella enterica* ssp. *enterica* sv. Enteritidis, *Salmonella enterica* sv. Typhimurium), but no antagonistic properties towards the other *Lactobacillus* strains observed. An analysis of 16S rDNA sequences demonstrated that the strain studied belongs to the genus *Lactobacillus*, and more precisely to the group of closely related species including *L. casei*, *L. rhamnosus*, and *L. paracasei*. The strain showed 98% similarity to the sequence ATCC 8530 (*L. rhamnosus*) BLASTN 2.2.287 (Motyl, 2002).

The course of the experiment

The technology of manufacturing the fermented soy beverage was developed in previous studies (Zielińska, 2005, 2006;

Zielińska and Uzarowicz, 2007).

Fifteen samples of sterile beverage were poured into sterilized conical flasks and inoculated with *L. casei* KN291 bacteria in the quantity of 4.9–5.1 log CFU/cm^3 on average, and then were subjected to fermentation at a temperature of 37°C for 6 h, and ripened at a temperature of 15°C for 48 h. The samples of the fermented beverage were transferred to an incubator with a cooling system and stored at various temperature conditions (5, 10, 15, and 20°C) for 28 days. The number of lactic acid bacteria in the beverage was examined immediately after fermentation and then, after 4, 8, 12, 16, 20, 24, and 28 days of storage.

The data obtained on survival of *L. casei* KN291 bacteria in the fermented soy beverage were utilized in the construction of predictive microbiological models. The suitability of non-linear models (with one independent variable, that is, time of storage) and the surface models (with two independent variables: time and temperature of storage) were tested.

The next stage of the study concerned verification of the constructed microbiological models for growth and survival of *L. casei* KN291 bacteria in the fermented soy beverage. In order to obtain the external data for verification, an experiment was carried out in which fermented soy beverage was stored at a temperature of 7°C. Obtained results were compared with the data estimated by the models.

Microbiological analyses

The number of *Lactobacillus casei* KN291 in the fermented soy beverage was determined by the pour plate method on the – MRS agar selective medium. Inoculation was done from 3 successive dilutions (depending on the expected result) in two replicates. The plates were incubated at 30°C for 72 h (PN-ISO: 15214:2002).

Statistical analyses – modeling

Modeling was carried out in TableCurve2D and TableCurve3D programs (Systat, 2006). A significance level of p=0.05 was adopted. Functions constructed in previous studies were adopted for modeling of probiotic bacteria growth and survival in the fermented soy beverage:

Non-linear functions (Zielińska *et al*., 2008):

$$z=4\cdot a\cdot exp[-(t-b)/c]\cdot\{1-exp[-(t-b)/c]\} \quad (1)$$

$$z=a\cdot exp\{-exp[-(t-b)/c]-(t-b)/c+1\} \quad (2)$$

where:
z – dependent variable [log CFU/cm^3]
a, b, c – parameters of predictive models
t – independent variable, time [days]

Surface models:

$$z = a + b \cdot exp\left[-exp\left(-\frac{t-c}{d}\right) - \frac{t-c}{d} + 1\right] \cdot exp\left[-exp\left(-\frac{T-e}{d}\right) - \frac{T-e}{d} + 1\right] \quad (3)$$

$$z = a + b \cdot exp\left[-exp\left(-\frac{t-c}{d}\right) - \frac{t-c}{d} + 1\right] \cdot exp\left[-exp\left(-\frac{T-e}{f}\right) - \frac{T-e}{f} + 1\right] \quad (4)$$

where:
z – dependent variable [log CFU/cm^3]
T– independent variable, temperature [°C]
t – independent variable, time [days]
a, b, c, d, e, f – parameters of predictive models

Confidence intervals were calculated using the Student t-value. The quality of the obtained models which mean fitting, was tested by the following goodness-of-fit coefficients:

r^2 – determination coefficient [0; 1] informs what part of the variation of the dependent variable is explained by the variation of the independent variable; the closer to 1, the better the model.

$Adj.r^2$ – adjusted determination coefficient [0; 1] is a coefficient of determination, corrected by the size of the sample; it is expressed by the following equation:

$$\text{Adj } r^2 = 1 - (1 - r^2)\,\frac{n-1}{n-k} \quad (5)$$

where:
n – number of observations;
k – number of estimated parameters.

MSE – Mean Squared Error is the avarage value of the error's squared, i.e. the difference between the estimator and the estimated value, and it is expressed by the following equation:

$$MSE = \frac{RSS}{n} \quad (6)$$

where:
RSS – residual sum of squares;
n – number of observations.

The lower coefficient *MSE*, the better the adequacy of the model to describe the data.

F-test – to statistically quantify the comparison between the lack of fit and the measurement error.

Verification of models

Verification of the predictive models for the growth and survival of *Lactobacillus casei* KN291 bacteria in the fermented soy beverage was carried out using the following parameters:

MSE – Mean Squared Error is the average value of the error's square, that is, the difference between the estimator and the estimated value, and it is expressed by the following equation:

$$MSE = \frac{\Sigma(\mu_{observed} - \mu_{fitted})^2}{n} \quad (7)$$

where:
n – number of observations.

The closer value of the coefficient *MSE* is to 0, the better the model is.

Af – accuracy factor; the value of the factor closer to 1, the better the model is. It measures the effectiveness of the model prognosing and informs about the degree of the fit of the model to the microorganism survival curve. The accuracy factor averages the distance between each point and

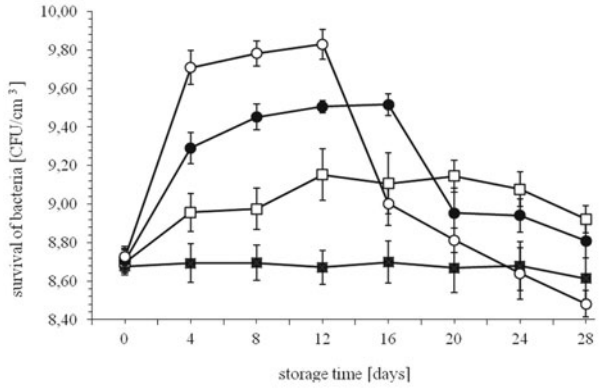

Fig. 1. Survival of *L. casei* KN291 in fermented soy beverage at temperature of 5, 10, 15, and 20°C for 28 days. Storage temperatures: (■) 5°C, (□) 10°C, (●) 15°C and (○) 20°C.

the line of equivalence as a measure of how close, on average, predictions are to observations. It is expressed by the following equation (Ross, 1996):

$$Af = 10^{\frac{1}{n}\sum\left|Log\frac{\mu_{fitted}}{\mu_{observed}}\right|} \quad (8)$$

where:
n – number of observations.
Bf – bias factor inform about, on average, the observed values lie above or below the line of equivalence. It indicates also by how much, on average, a model overpredicts (bias factor > 1) or underpredicts (bias factor < 1) the observed data. It is expressed by the following equation (Ross, 1996):

$$Bf = 10^{\frac{1}{n}\sum\left|Log\frac{\mu_{fitted}}{\mu_{observed}}\right|} \quad (9)$$

where:
n – number of observations.
The bias and accuracy factors introduced by Ross (1996) originally were used to compare models of growth rates or generation times. Usefulness of these parameters for models of bacteria number was checked in this paper.

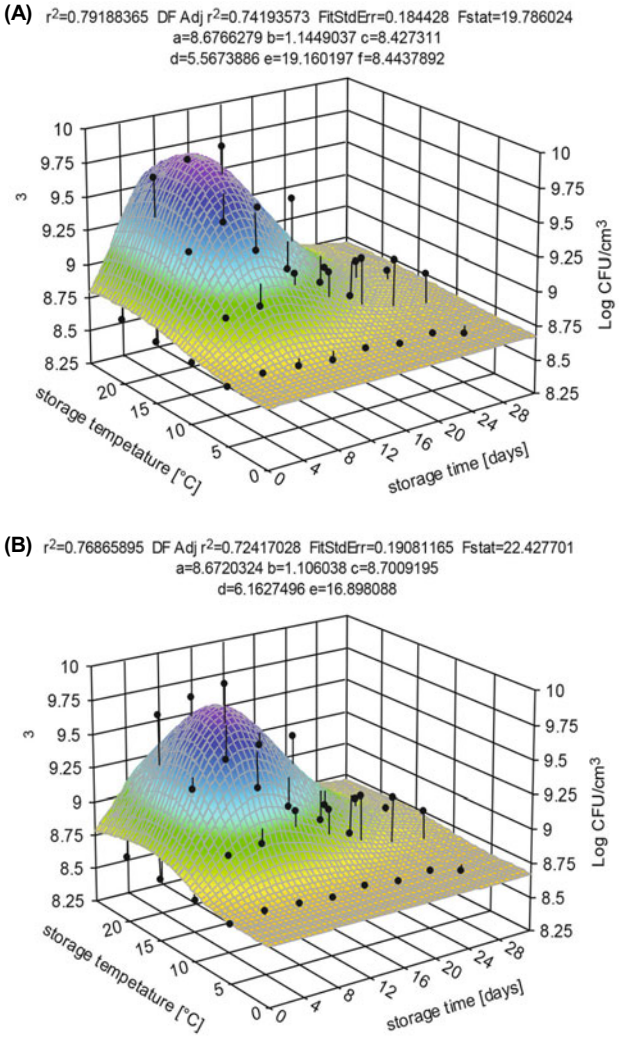

Fig. 2. Surface models according to equation (3) – (A) concerning the changes in the number of *L. casei* KN291 in fermented soy beverage during storage and surface models according to equation (4) – (B) concerning the changes in the number of *Lactobacillus casei* KN291 in fermented soy beverage during storage.

Results

Soy beverage was inoculated with the *L. casei* KN291 bacteria in the quantity of 5.1 log CFU/cm^3 on average and subjected to fermentation, and then, ripening so that the initial count of bacteria in the fresh beverage was equal to 8.67–8.72 log CFU/cm^3. The fermented soy beverage was then stored at various temperature conditions. It was found that the number of *L. casei* KN291 bacteria in the beverage

Table 1. Goodness-of-fit of non-linear functions (1) and (2) of bacterial number in relation to time

Function	Storage temperature							
	5°C		10°C		15°C		20°C	
	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
r^2	0.6853	0.6877	0.9261	0.9304	0.8414	0.8000	0.7786	0.7223
Adj. r^2	0.4493	0.4535	0.8706	0.8782	0.7224	0.6501	0.6126	0.5140
MSE	0.0004	0.0004	0.0024	0.0023	0.0243	0.0307	0.0969	0.1215
Fit Std. Err.	0.0196	0.0195	0.0490	0.0475	0.1560	0.1751	0.3113	0.3486
F	5.4442	5.5054	31.3140	33.4169	13.2620	10.0026	8.7924	6.5028
p-value	0.0556	0.0545	0.0015	0.0013	0.0100	0.0179	0.0231	0.0406

Table 2. Results of estimation of surface model (3) predicting behaviour of *L. casei* KN291 in fermented soy beverage during the storage

r^2 Coef Det	DF Adj r^2	Fit Std Err	F-val			
0.7919	0.7419	0.1844	19.7860			
Parm	**Value**	**Std Error**	**t-value**	**95.00% Confidence limits**		**P>|t|**
A	8.6766	0.0778	111.5010	8.5167	8.8366	0.0000
B	1.1449	0.1247	9.1805	0.8886	1.4012	0.0000
C	8.4273	0.6025	13.9871	7.1888	9.6658	0.0000
D	5.5674	0.8876	6.2722	3.7428	7.3919	0.0000
E	19.1602	2.6083	7.3460	13.7989	24.5215	0.0000
F	8.4438	2.7411	3.0804	2.8094	14.0782	0.0048
Source	**Sum of Squares**	**DF**	**Mean Square**	**F Statistic**	**P>F**	
Regr	3.3650	5	0.6730	19.7860	0.0000	
Error	0.8844	26	0.0340			
Total	4.2493	31				

stored at 5°C did not significantly change during 28 days of storage (P=0.26 and P>0.05) (Fig. 1). At temperatures of 10, 15, and 20°C the number of *L. casei* KN291 bacteria was variable, initially we have noted an slight increase in bacteria number and next a slight decrease was recorded.

The search for a suitable model, describing the behaviour of the bacteria in the product, has brought about the construction of non-linear models (equation 1 and 2) with satisfactory quality of adjustment and approvable stochastic properties of the parameters' evaluation (Table 1).

As may be observed, the constructed models describe the growth and survival of bacteria in fermented soy beverage similarly. However, due to better adjustment of model (1) to the experimental data - which is confirmed by r^2 and $Adj\ r^2$ – especially at higher storage temperatures, and the somewhat better stochastic properties of the parameters, it is recommended to employ model (1) for application in predicting the number of *L. casei* KN291 in fermented soy beverage as a function of time.

On the basis of the experimental data collected, also the surface models (equation 3 and 4) of *L. casei* KN291 survival in fermented soy beverage were constructed. Due to the specificity of the growth and survival of probiotic bacteria in the product, modeling with the application of typical functions employed in predictive microbiology has not been successful (data not shown). Models were constructed which are the quotient of two Gompertz functions, increased by the additional parameter. In these functions, a part containing the variable time is multiplied by a part with the variable temperature. Due to this action, the effect of acceleration was achieved, that is the mutual "drive" of probiotic bacteria growth in respect to both variables. The constructed models differ in the number of parameters.

Fig. 2A and 2B shows the values observed and estimated by model (3) and (4) illustrating survival of *L. casei* KN291 bacteria during 28 days of storage at temperatures of 5, 10, 15, and 20°C. The axis z on the models shows the decimal logarithm of colony forming units in 1 cm^3 of fermented soy beverage as a function of time (axis x) and storage temperature (axis y). We may also observe a sigmoidal relationship between the number of bacteria and storage temperature. We should also pay attention to the fact that the number of bacteria over time varies depending on temperature. At low temperatures, the mentioned function is constant, whereas at higher ones it takes the form of two complex logistic functions. Tables 2 and 3 contain detailed results of the estimation of the constructed surface models, including measurements of the models' quality and evaluation of the parameters, estimation mean errors and t-Student statistics.

On the basis of the values of the adjusted determination coefficient ($Adj.\ r^2$) it may be stated that the both models (3) and (4) are characterized by a good degree of adjustment. This means that the models sufficiently explain the variation of the dependent variable (log CFU/cm^3) by the variation of independent variables (time and temperature of storage). The values of the *MSE* parameter which are close to

Table 3. Results of estimation of surface model (4) predicting behaviour of *L. casei* KN291 in fermented soy beverage during the storage

r^2 Coef Det	DF Adj r^2	Fit Std Err	F-val			
0.7687	0.7242	0.1908	22.4277			
Parm	**Value**	**Std Error**	**t-value**	**95,00% Confidence Limits**		**P>|t|**
A	8.6720	0.0698	124.2690	8.5288	8.8152	0.0000
B	1.1060	0.1211	9.1316	0.8575	1.3546	0.0000
C	8.7009	0.6833	12.7336	7.2989	10.1029	0.0000
D	6.1627	0.8609	7.1581	4.3962	7.9293	0.0000
E	16.8981	0.8260	20.4567	15.2032	18.5930	0.0000
Source	**Sum of Squares**	**DF**	**Mean Square**	**F Statistic**	**P>F**	
Regr	3.2663	4	0.8166	22.4277	0.0000	
Error	0.9830	27	0.0364			
Total	4.2493	31				

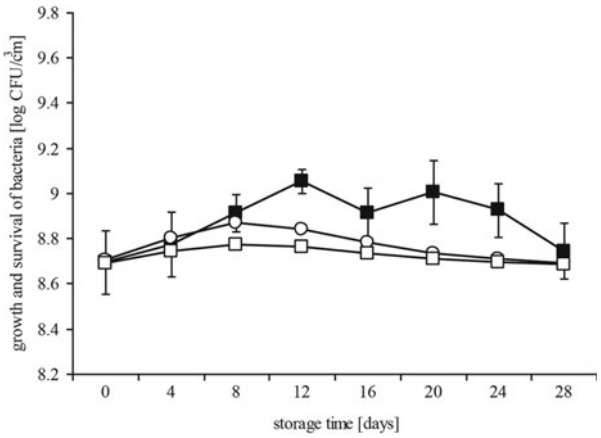

Fig. 3. Empirical data and predicted values by models (3) and (4) the number of *L. casei* KN291 bacteria in fermented soy beverage during the 28 days of storage at temperature 7°C. (■) empirical data, (○) values predicted by model (3), (□) values predicted by model (4)

0 and the low values of *Fit Std. Err* prove that the models are charged with small errors. We should also pay attention to the high values of the *F* statistics (P=0.0000 and P<0.05) and low asymptotic mean errors of the parameters' estimate allowing one to state that the parameters are well estimated and may be relied on.

In summary, two non-linear models (1) and (2) and two surface models (3) and (4) were constructed, which sufficiently describe the growth and survival of *L. casei* KN291 bacteria in fermented soy beverage.

In order to verify the effectiveness of the predictions of the constructed surface models, the following experiment was carried out. It considered a monitoring of the changes in the number of *L. casei* KN291 bacteria during the storage of fermented soy beverage at a temperature of 7°C for 28 days and comparing the obtained experimental data with the data estimated by the model (Fig. 3).

The number of *L. casei* KN291 bacteria in fermented soy beverage varied during 28 days of storage at 7°C (P<0.05). The number of bacteria increased until the12th day of storage, and a statistically significant increase of log CFU/cm^3 was recorded after 4 and 8 days of storage. Then, the number of bacteria was stabilized until the 24th day of storage; afterwards, a significant decrease of log CFU/cm^3 was observed. It was found that the increase of *L. casei* KN291 bacteria in soy beverage stored at 7°C was somewhat lower in comparison to the increase at 10°C; however, the number of bacteria varied in comparison to the behaviour of the discussed bacteria in the beverage stored at 5°C (cf. Fig. 1).

Table 4. Parameters of verification of surface models of growth and survival of *L. casei* KN291 in fermented soy beverage

Parameters	Model (3)	Model (4)
MSE	0.0237	0.0351
Af	1.0138	1.0175
Bf	0.9875	0.9827

Fig. 4A and 4B shows the dispersal of the data estimated by model (3) and model (4) in relation to the observed values. If the obtained points were situated on an equivalence line, it could be stated that the prediction is ideal. The distance between the points and the line is the measure of inaccuracy of the prediction. From an analysis of diagrams it follows that most of the points are situated on the right side of the equivalence line closer to the axis of the ordinates (the observed values). This means that the observed values differ and are higher in comparison to the estimated values.

Verification of the surface models in the present study was based on the calculations of parameters developed by Ross (1996) and *MSE* statistics (Table 4).

In both cases values of *Bf* were <1 (0.98, in average), this means that the observed values are higher than predicted ones, but the difference is small because the values of *Bf* factor are close to 1. This means that the models maintain a margin of "safety". While obtained *Af* values were close to 1 (1.01, in average), which is evidence of small differences between the observed and predicted values.

Discussion

In the present study, the growth and survival of *Lactobacillus casei* KN291 in fermented soy beverages was evaluated. We

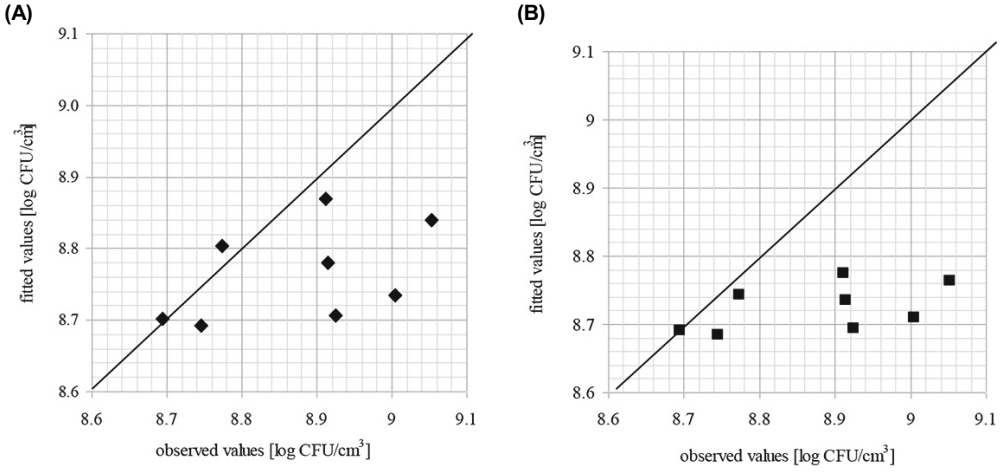

Fig. 4. Scattering of values, estimated by model (3) – (A) and model (4) – (B) in relation to the observed values.

found that, soy beverage is good medium for survive of lactic acid bacteria. Similar observations were recorded by Lin *et al.* (2004), who studied the survival of *Lactobacillus paracasei* subsp. *paracasei* and *Bifidobacterium longum* in fermented soy beverage with the addition of 25% milk and 5% juice of *Lycium chinense* Miller. The authors showed that the number of bacteria of both strains studied was maintained at a constant level (10^8–10^9 CFU/cm^3) for 14 days of storage at 4°C. Studies conducted by Donkor *et al.* (2007), in which the survival of *L. casei* in soy yogurt was examined also confirm the fact that probiotic bacteria do not reveal a high metabolic activity at refrigeration temperatures. During the storage of soy yogurt at a temperature of 4°C during 28 days, the number of *L. casei* bacteria was maintained at a constant level and was amount 9 log CFU/g. Also others authors found that soymilk is a good proliferation medium for strains of lactobacilli (Ewe *et al*., 2010; Bao *et al*., 2012). The administered strains of *Lactobacillus plantarum* were capable to grow in soymilk up to a level of 10^8–10^9 CFU/cm^3. During storage of the fermented samples of soymilk at 4°C for 28 days, viable count of this strains did not change (Bao *et al*., 2012).

In our own studies it was found that during 28 days of fermented soy beverage storage at temperatures of 10, 15, and 20°C the number of *L. casei* KN291 bacteria was variable. Just as at higher temperature the bacteria grew more quickly, the earlier and more rapidly were inactivated. Considering that changes in log numbers of *L. casei* KN291 bacteria in soy beverage become statistically significant, these dynamics were rather negligible.

In an experiment conducted by Shimakawa *et al.* (2003), it was observed that the number of probiotic bacteria *Bifidum breve* did not significantly change during 20 days of storage of fermented soy beverage at a temperature of 10°C, and it was maintained at a level of about 10^9 CFU/cm^3. On the other hand, Wang *et al.* (2002) and Chou and Hou (2000), in separate studies recorded a dramatic lowering (by 2–4 logarithmic orders) of the number of bacteria (*B. longum*, *B. infantis*, and *L. acidophilus*) during 10 days of storage of fermented soy beverage at a temperature of 25°C. The authors explain that such dramatic death of bacteria was the result of an accumulation of metabolites, e.g. organic acids, during the storage period.

It seems that the differences in the survival of bacteria in the cited studies compared to the present experiment may result from the specificity of the strain. In many cases, growth and survival of the *Bifidobacterium* bacteria is much worse in vegetable juices in comparison to bacteria from the *Lactobacillus* genus, which has been confirmed by many studies (Canganella *et al*., 2000; Wang *et al*., 2002, 2003; Garro *et al*., 2004; Saarela *et al*., 2011).

The run of growth and survival of *L. casei* KN291 bacteria in fermented soy beverage over the time differs significantly from the typical s-shape growth (see Fig. 1). Due to this reason, the primary non-linear equations universally employed for describing the growth of bacterial population in time, such as the Gompertz curve or the logistic curve, and their modifications of the discussed models did not provide for a satisfactory description of the behaviour of *L. casei* KN291 in soy beverage during 28 days of storage as a function of time.

Similarly, an atypical course of the growth curve was observed by Planas *et al.* (2004), who studied the growth of plankton (*Brachionus plicatilis*) in medium with and without probiotic bacteria. Three models were employed in the cited paper: general logistic, logistic – logistic and logistic – modification of Gompertz. The following models were the best adjusted models describing the growth of plankton: logistic – logistic (r^2=0.9946) and general logistic (r^2=0.9942).

On the other hand, in studies by Trząskowska (2006), among the primary non-linear models describing the change in the number of probiotic bacteria *L. acidophilus* in fermented carrot juice, the best description was obtained using the logistic function and the Gompertz function (r^2=0.70). Altieri *et al.* (2008) employed satisfactorily polynomial and Weibull model to modeling the survival of *Lactobacillus delbrueckii*, *Streptococcus thermophilus*, and *Bifidobacterium bifidum* cultured alone or consociated in a laboratory medium (MRS broth).

A few combined models have been constructed and presented in the predictive microbiology literature, to describe changes in a microbial population subjected to conditions that vary from the growth to inactivation ranges (Whiting and Cygnarowicz-Provost, 1992; Jones *et al*., 1994; Corradini and Peleg, 2006). In 1995 and 1996 Baranyi *et al*., developed model describing growth and inactivation of *Brochothrix thermosphacta* at changing temperature. Membre and others (1997) applied a purely empirical model to describe growth, survival, and death of *Listeria monocytogenes* at low temperatures with high concentrations of phenol and NaCl. Also a quasi-chemical model to modeling bacterial growth and/or inactivation has been presented by Taub *et al.* (2003), Doona *et al.* (2005), and Ross *et al.* (2005).

In the present study two non-linear models and two surface models were constructed, which sufficiently describe the growth and survival of *L. casei* KN291 bacteria in fermented soy beverage, which is manifested by the determination the coefficients (r^2) and Mean Squared Error (*MSE*).

Similar observations were performed by Cayré *et al.* (2003), in which the Gompertz model was employed describing the growth of lactic acid bacteria in cooked meat emulsion packed in permeable film, as a function of time. A very good fit of the function in the studied temperature conditions (r^2=0.96–0.99) was obtained. On the other hand, application of the Arrhenius function and model of the square root to describe LAB growth as a function of temperature resulted in obtaining the equally satisfying adjustment (r^2 =0.93–0.94) and low *MSE* values (0.0122–0.0027).

As the adjustment of the surface models obtained in the present study was equal to 75% on average, the decision was undertaken to verify the discussed models using external data. It was observed that survival of *L. casei* KN291 bacteria in fermented soy beverage at 5°C was differed from the behaviour of the discussed bacteria at a temperature of 10°C during 28 days of storage. The so-called "refrigerator" temperature varies within the limits of 4–7°C. For technological reasons, a stable bacteria count in the product is very important, especially at the temperature at which the products will be stored by the consumer; therefore the decision was undertaken to verify the survival of probiotic bacteria in soy beverage at a higher temperature.

Verification of the surface models in the present study was based on the calculations of parameters *Af* and *Bf* developed by Ross (1996) and *MSE* statistics.

MSE statistics is a measure of residual variation which is not defined by the expected changes connected with factors such as temperature, pH and a_w. The mentioned residual variations may originate from many sources resulting from natural variation and systematic errors (Giffel and Zwietering, 1999). Low values of the *MSE* statistics (0.02–0.03) obtained in the present study provide evidence of small systematic errors.

Both the accuracy and bias factors are, in some sense, average values, but they are defined using different concepts of 'average'. The accuracy factor is based on 'mean square differences', while the bias factor is based on the 'arithmetical mean of the differences' (Baranyi *et al.*, 1999). In the present study, in both cases values of *Bf* were <1 (0.98, in average), and obtained *Af* values were close to 1. This means that the observed values are higher than predicted ones and observed values differ from the predicted ones by 13% (in case of model 3) and by 17% (in case of model 4).

Cayré *et al.* (2003) calculated accuracy factors ranging from 1.38 to 1.05 and a bias factor from 1.11 to 0.99 for the growth rate of LAB in meat emulsions packed in low permeability film. Chowdhury *et al.* (2007) applied the modified Gompertz and Logistic model to cell growth of *P. acidilactici H* in bacteriocin production. The fitted models were validated for the goodness-of-fit by *MSE* and *Bf*. They found that the bias factors were more closer to 1.0 with lower MSE values for modified Gompertz modeling. On the other hand Dalgaard and Jørgensen (1998) calculated *Af* ranging from 1.4 to 4.0 for the growth rates of *Listeria monocytogenes* in various types of seafood. However discussed models predicted growth rates can not be compare with surface models, that predicted CFU, presented in this paper. Accuracy and bias factors were originally used to predict the growth rate or generation time. In this paper, these factors were used to assess and compare the applied models (3) and (4) to one another.

As the present study demonstrates, model (3) is characterized by considerably better parameters of *Af* and *Bf* factors and of *MSE*, than model (4). Therefore the model (3) is recommended for the prediction of growth and survival of *L. casei* KN291 bacteria in fermented soy beverage as a function of time and temperature of storage.

In summary, due to the fact that microbial testing in food is expensive and time-consuming, mathematical models have become a useful tool for providing a matrix of microbial responses to a broad range of storage conditions (Chotyakul *et al.*, 2011).

Gomes *et al.* (1998) investigated the growth and survival behaviours of *Bifidobacterium lactis* and *Lactobacillus acidophilus* in a semi-hard Gouda cheese at various axial locations during 9 weeks of ripening at 13°C. The authors postulated and tested non-linear regression models, which are useful in predicting sodium chloride concentration at any time during ripening and at any position within the cheese, as well as the evolution of the viable numbers of the two probiotic strains as related to the local and instantaneous salt content. Also, Jayamanne and Adams (2009) developed the polynomial regression equations which first described the inactivation/survival of bifidobacteria during storage in fermented milk, and the first to incorporate redox potential as an environmental variable. The models are moderately conservative in their prediction, so in practice the product would always bear more organisms to the end consumer.

Meanwhile, Nualkaekul and Charalampopoulos (2011) developed models in the orange, blackcurrant and pineapple juices of survival *L. plantarum* cells during refrigerated storage. In these juices the pH and the level of organic acids were the main factors influencing cell survival. On the other hand, the model failed to predict cell survival in grapefruit and pomegranate juices.

In our study the microbiological models for growth and survival of *Lactobacillus casei* KN291 bacteria in fermented soy beverage, as constructed in the present study, enable estimation of the shelf life period of the products, depending on the storage conditions.

Verification of the constructed surface models using external data confirmed the effectiveness of their prediction what was confirmed by the *MSE*, *Af* and *Bf* parameters.

Conducted studies show that the obtained models may serve in the development of new, health-promoting products and estimation of their shelf life period.

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