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Construction of predictive models of growth of microorganisms in salted and cured meat products

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Abstract

The aim of the research was to produce mathematical models for the growth of natural microflora (as total plate count, TPC) in the model salted and cured meat products representing a group of products made of minced meat. Research material comprised meat products in the form of meat balls prepared under the laboratory conditions. Microbiological analyses were performed in the raw product, after roasting (day 0) and after 4, 8, 12 and 16 days of storage at the temperature of 5, 10 and 15 °C. Total plate count (TPC) (cfu/g) was determined on a nutrient agar (Oxoid). On the basis of data obtained in the performed experiments parameters of non-linear Gompertz models and logistic of the total plate count growth log (cfu/g) in meat products stored at various temperatures were matched in a satisfactory way. The addition of $NaNO_2$ at the level of 60 ppm affected the inhibition of the number of microorganisms in a statistically significant way. At the level of 2% NaCl no inhibitory effect on bacterial growth was observed.

Keywords: Predictive microbiology; Cured beef meat; Gompertz function; Logistic function

Industrial relevance: Predictive modelling of microbial growth is highly essential for food safety and regulatory reasons. The present work describes the development of a mathematical model of the growth of total plate count – natural microflora in minced meat model products. The ultimate aim of the ongoing efforts will be the development of a computer programme including the effects of various environmental factors – including preservatives – on growth and inactivation of microorganisms.

1. Introduction

An alternative to time consuming and expensive investigations can be the microbiological prediction. Mathematical models are mainly used for predicting the growth, death and survival of microorganisms or toxin production. They include the effect of factors such as temperature, a_w, pH, nitrite content, gaseous atmosphere, the content of organic acids or other preserving substances. The process of modelling usually begins with the first order models which are mathematical formulas describing microorganisms growth or survival curves. The answer may be expressed by a total plate count, toxin production, the level of substrates or the level of metabolites. Due to the first order models it is possible to follow the changes in time of the number of microorganisms and obtaining information about the generation time, lag phase, exponential growth and the maximum population density (McDonald & Sun, 1999; McMeekin, Olley, Ratkowsky, & Ross, 2002; Whiting, 1995; Whiting & Buchanan, 1993, 1994). The second order models describe the effect of factors on the environmental conditions. The obtained algorithm is included to computer programming in order to construct third order model (McMeekin et al., 2002).

2. The aim of the work

The aim of the research was working out mathematical models of the growth of the total plate count (TPC) — natural microflora in the model meat products representing products from the minced meat. The model parameters were time and the temperature of the storage of products as well as the addition of NaCl and $NaNO_2$.

The investigations presented in this paper are a continuation of research carried out by the scientists of the Food Hygiene Group, Warsaw Agricultural University — SGGW on

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Table 1
Parameters of growth of the total plate count (TPC) estimated on the basis of the Gompertz (G) and logistic (L) functions in salted and cured meat products stored at the temperature of 5 °C

additive		Functions	μ	λ	N	GT	R^2	Adj R^2	RMSE
NaCl (%)	$NaNO_2$ (ppm)								
0	0	G	0.15*	5.96	4.77	1.93	0.998	0.991	0.042
		L	0.15*	5.56*	2.34	1.35	0.995	0.980	0.063
0	60	G	0.04*	2.03	0.36*	8.02	0.997	0.987	0.012
		L	0.04*	2.56*	0.34*	5.15	0.996	0.985	0.013
0	120	G	0.02	−0.21	0.34	13.60	0.936	0.746	0.042
		L	0.02	0.08	0.32	9.30	0.915	0.660	0.049
1	0	G	0.27*	8.09*	1.53*	1.11	0.999	0.998	0.023
		L	0.32*	8.80*	1.46*	0.64	0.999	0.998	0.021
1	60	G	909.22	67.04	86792.61	0.00	0.975	0.898	0.072
		L	193.84	32.92	2203.28	0.00	0.978	0.913	0.067
1	120	G	0.01	1.52	0.13*	19.3	0.979	0.917	0.011
		L	0.02	1.90	0.12*	12.28	0.971	0.886	0.013
2	0	G	0.18*	6.46*	1.67*	1.67	0.993	0.973	0.072
		L	0.19*	6.78*	1.52*	1.09	0.997	0.990	0.043
2	60	G	0.09	6.07	3.73	3.46	0.978	0.910	0.071
		L	0.08	4.81	1.64	2.60	0.973	0.893	0.077
2	120	G	–	–	–	–	–	–	–
		L	–	–	–	–	–	–	–

*p-value < 0.05.
– — unsuccessful functions estimate.
μ — maximum specific growth rate [log (cfu/g)], λ — lag time [h], N — maximum density of population [log (cfu/g)], GT— generation time [h], R^2 — determination coefficient, Adj R^2 — revised determination coefficient, RMSE — root mean square error.

microbiological predictive concerning meat products. Up to date, models for growth and survival of various groups of saprophytic and pathogenic bacteria in the model meat products representing a group of products of minced meat have been developed. These in future will assist in the preparation of a computer programme, which will include, the effect of various environmental factors (including preserving substances) on the growth and inactivation of microorganisms.

3. Research material

3.1. Material for research

Research material comprised meat products in the form of meat balls prepared under the laboratory conditions. The products were made of boneless round of beef (minced), bread crumbs (100 g per 1000 g meat), UHT milk 2% fat (100 ml per 1000 g meat), onion (100 g per 1000 g meat), NaCl (0%, 1% and 2% of meat weight), $NaNO_2$ (0, 60 and 120 ppm); pH = 6.2–6.5.

3.2. The course of the experiment

Before the beginning of work, the bowl, cutting board and knives were disinfected with the agent Incidin Plus (Henkel-Ecolab GmbH and Co.), hands were disinfected with Spitaderm (Henkel-Ecolab GmbH and Co.). Connective tissue was removed from the meat before it was minced in a meat grinder. Equipment parts that come in contact with the meat had been sterilized previously with hot air in the dryer (160 °C for 2 h). Then NaCl and $NaNO_2$ were added to the meat to cure it (temperature 4 °C time 2 h). After curing the remaining components were added to the meat and it was thoroughly mixed. Meat balls of the weight 100 ± 2 g were formed and placed in baking trays lining with aluminium foil and roasted in

Table 2
Parameters of growth of the total plate count (TPC) estimated on the basis of the Gompertz (G) and logistic (L) functions in salted and cured meat products stored at the temperature of 10 °C

additive		Functions	μ	λ	N	GT	R^2	Adj R^2	RMSE
NaCl (%)	$NaNO_2$ (ppm)								
0	0	G	0.15	−0.57	2.50	1.98	0.942	0.768	0.280
		L	0.15	−0.09	2.32	1.32	0.927	0.710	0.314
0	60	G	0.13	−0.16	1.49*	2.28	0.971	0.885	0.137
		L	0.13	0.02	1.44*	1.60	0.952	0.808	0.177
0	120	G	0.18*	5.73*	3.54	1.68	0.990	0.961	0.109
		L	0.19*	6.29*	2.41	1.05	0.984	0.938	0.136
1	0	G	0.17	1.87	4.95	1.76	0.951	0.803	0.292
		L	0.18	2.47	3.23	1.12	0.948	0.791	0.301
1	60	G	0.26	8.82*	7.03	1.13	0.999	0.994	0.043
		L	0.24*	7.96*	2.90*	0.85	0.997	0.988	0.063
1	120	G	0.23*	7.80*	2.15*	1.30	1.000	1.000	0.003
		L	0.28*	8.50*	1.80*	0.74	0.9999	0.9998	0.008
2	0	G	0.20	−0.65	2.64*	1.46	0.940	0.761	0.337
		L	0.19	−0.62	2.56*	1.06	0.915	0.661	0.402
2	60	G	0.17*	3.93*	1.49*	1.76	0.999	0.997	0.026
		L	0.18*	4.36*	1.39*	1.11	0.994	0.974	0.071
2	120	G	0.43	6.27	1.27*	0.69	0.991	0.963	0.091
		L	1.30	7.43	1.26*	0.16	0.991	0.963	0.091

*p-value < 0.05.
μ — maximum specific growth rate [log (cfu/g)], λ — lag time [h], N — maximum density of population [log (cfu/g)], GT — generation time [h], R^2 — determination coefficient, Adj R^2 — revised determination coefficient, RMSE — root mean square error.

Table 3
Parameters of growth of the total plate count (TPC) estimated on the basis of the Gompertz (G) and logistic (L) functions in salted and cured meat products stored at the temperature of 15 °C

additive		Functions	μ	λ	N	GT	R^2	Adj R^2	RMSE
NaCl (%)	$NaNO_2$ (ppm)								
0	0	G	0.53	−0.05	4.57*	0.57	0.953	0.812	0.575
		L	0.56	0.27	4.35*	0.36	0.928	0.714	0.709
0	60	G	0.65*	0.48	4.71*	0.46	0.993	0.973	0.228
		L	0.68	0.74	4.57*	0.30	0.978	0.913	0.411
0	120	G	0.44	0.12	4.26*	0.68	0.982	0.927	0.324
		L	0.43	0.28	4.15*	0.48	0.964	0.855	0.457
1	0	G	0.48	0.11	5.03*	0.62	0.972	0.888	0.469
		L	0.48	0.37	4.79*	0.42	0.952	0.807	0.615
1	60	G	0.66	0.23	4.21*	0.46	0.978	0.913	0.371
		L	0.90	1.15	4.04*	0.23	0.961	0.846	0.494
1	120	G	0.66*	0.63	4.48*	0.46	0.997	0.987	0.154
		L	0.71	0.97	4.35*	0.29	0.984	0.937	0.334
2	0	G	0.37*	0.71	4.77*	0.82	0.991	0.966	0.227
		L	0.38*	1.23	4.39*	0.53	0.979	0.917	0.355
2	60	G	0.56*	0.45	4.34*	0.54	0.991	0.965	0.239
		L	0.58	0.73	4.19*	0.35	0.975	0.901	0.399
2	120	G	0.54	0.24	4.61*	0.55	0.984	0.937	0.334
		L	0.52	0.36	4.50*	0.39	0.966	0.864	0.490

*p-value < 0.05.
μ — maximum specific growth rate [log (cfu/g)], λ — lag time [h], N — maximum density of population [log (cfu/g)], GT — generation time [h], R^2 — determination coefficient, Adj R^2 — revised determination coefficient, RMSE — root mean square error.

an electric oven at 150 °C until they reached the inside temperature of 75 °C. The roasted products were covered with aluminium foil and cooled to the room temperature for 2 h. Then the products were packed in polyethylene bags (0.66 mm in thickness, water steam permeability 8.96 g/m^2/24 h±0.28, oxygen permeability 888 cm^3/m^2/24 h), closed by pressure welding and stored in a microbiological incubator with extra cooling (Prebatem) at the temperature of 5, 10 and 15 °C up to 16 days. Nine series of replications were done for products stored at various temperatures.

3.3. Microbiological analyses

Microbiological analyses were performed in the raw product, after roasting (day 0) and after 4, 8, 12 and 16 days of storage at the temperature of 5, 10 and 15 °C. Total plate count (TPC) (cfu/g) was determined by pour plates method on nutrient agar (Oxoid). Inoculation was done from 3 successive dilutions (depending on the expected result) in two replications. Plates were poured over with the nutrient agar (Oxoid) cooled to the temperature of 45 °C, gently stirred and left for incubation at the temperature of 37 °C. After 48 h incubation such plate, which contain 15 to 300 colonies on a plate, were chosen for counting.

3.4. Statistical analyses — modelling

Statistical analyses were performed using the statistical packet Statgraphic Plus for Windows, ver. 4.1, Statistical Graphics Corp. and Microsoft Excel. The obtained microbiological results were converted to $\log_{10}$ values. Results included in the 95% confidence intervals were used for further analyses. On the basis of the Shapiro–Wilk test it was noted that the samples did not show the normal distribution thus the nonparametric equivalent of variance analysis, i.e. the Kruskal–Wallis test was used for statistical analyses (Stanisz, 1998). The differences were tested at the significance level p-value = 0.05.

To work out the non-linear predictive models the sigmoidal Gompertz function (1) and (2) logistic function were used with the parameterization proposed by Zwietering, Jongenburger, Rombouts, and Van't Riet (1990) where parameters have a direct microbiological interpretation.

$$\mathrm{TPC} = A \cdot \exp\{-\exp[\mu \cdot \exp(1) \cdot (\lambda - t)/A + 1]\} \quad (1)$$

$$\mathrm{TPC} = A/\{1 + \exp[4 \cdot \mu \cdot (\lambda - t)/A + 2]\} \quad (2)$$

Directly from the function formula the duration time of lag phase (λ) was calculated as well as the exponential growth rate

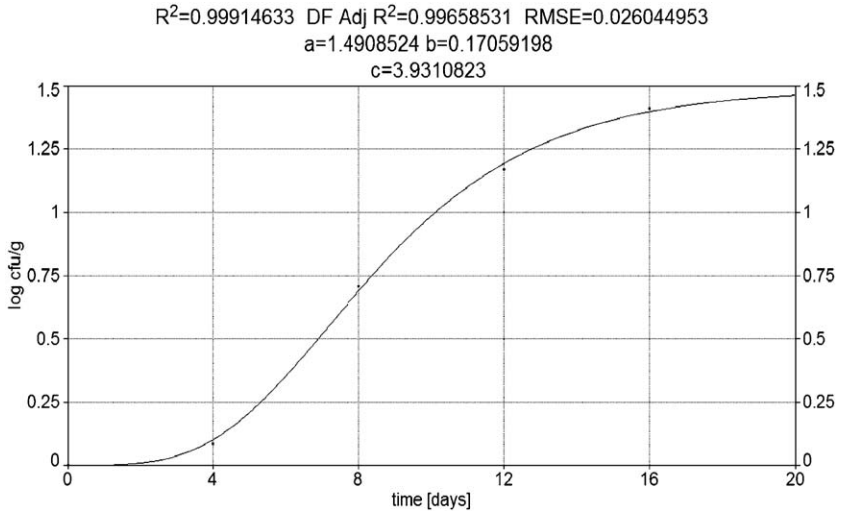

Fig. 1. Gompertz total plate count model (log cfu/g) in products containing 2% NaCl, 60 ppm $NaNO_2$ stored at the temperature of 10 °C.

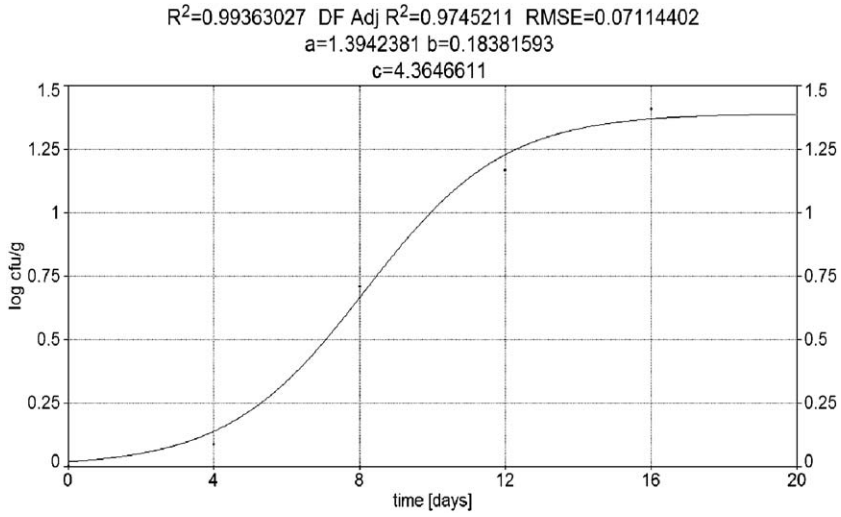

Fig. 2. Logistic total plate count model (log cfu/g) in products containing 2% NaCl, 60 ppm $NaNO_2$ stored at the temperature of 10 °C.

(μ). "A" corresponds to the maximum population size (N), "t" is the time of storage.

Generation time (GT) is calculated from the formula:

$$GT = (\log 2)/\mu. \tag{3}$$

Parameters were estimated using the iterative procedures determining the least value of the square error sums between the empirical and theoretical values. For this estimation the Marquardt algorithm was used which realizes the non-linear value of the least squares. Calculations were done in the programme TableCurve 2D for Windows, AISN Software Inc. Also the response surface models were constructed where the log cfu/g (z) was expressed in the time function (x) and storage temperature (y) for each product at various levels of the NaCl and $NaNO_2$ additive. They were the models of the Gompertz response surface and logistic TPC in meat products stored at various temperatures.

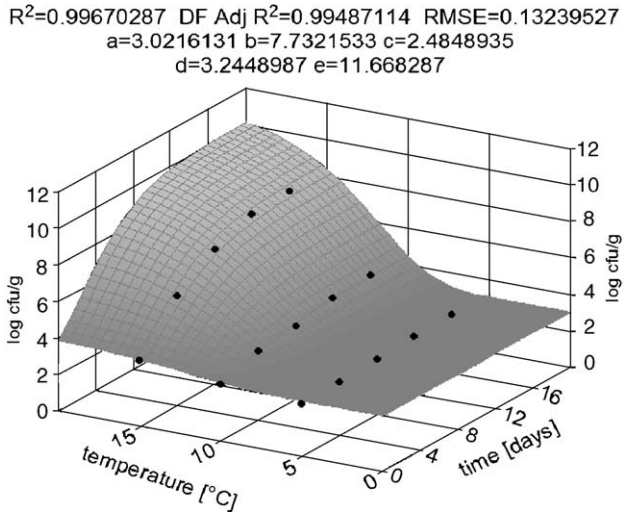

Fig. 3. Gompertz response surface model total plate count (log cfu/g) in products containing 0% NaCl, 60 ppm $NaNO_2$ stored at different temperatures.

The Gompertz synergic surface with the constant (4) and synergic logistic surface with the constant (5).

General formulas of the response surface are:

$$z = a + [b \cdot \exp(-\exp(-(x-c)/d))] \cdot [\exp(-\exp(-(y-e)/d))] \tag{4}$$

and

$$z = a + [b/(1+\exp(-(x-c)/d))] \cdot [1/(1+\exp(-(y-e)/d))] \tag{5}$$

where:

z	log CFU/g
x	time of storage [day]
y	temperature of storage [°C]
a–e	parameters of the model.

In the response surface models particular growth characteristics are calculated from the formulas:maximum specific growth rate (μ):

$$\mu = B \cdot D/\exp(1) \quad \text{and} \quad \mu = B \cdot D/4 \tag{6}$$

generation time (GT):

$$GT = \log(2)/\mu \tag{7}$$

lag time (λ):

$$\lambda = M - 1/B - (z(0,y) - A)/\mu \quad \text{and} \quad \lambda = M - 2/B - (z(0,y) - A)/\mu \tag{8}$$

maximum density of population (N):

$$N = A + D. \tag{9}$$

4. Results and discussion

4.1. Non-linear Gompertz and logistic models

On the basis of the data 27 Gompertz models were estimated (for 9 types of products stored at three different temperatures)

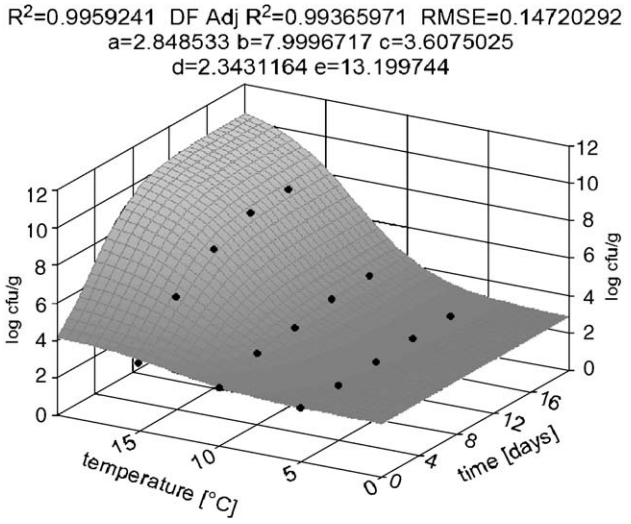

Fig. 4. Logistic response surface model total plate count (log cfu/g) in products containing 0% NaCl, 60 ppm $NaNO_2$ stored at different temperature.

and 27 logistic OLD models characterized by a high determination coefficient R^2 and adj. R^2 (revised R^2 coefficient which includes the number of the degrees of freedom) and small RMSE (root mean square error). Results are presented in Tables 1–3. Example models are presented in Figs. 1 and 2. No satisfactory matching was done of the sigmoidal TPC function in the products containing 2% NaCl and 120 ppm $NaNO_2$ stored at the temperature of 5 °C.

The application of the Gompertz function for the description of bacterial growth is widely used and described in literature. The fault of this method is the lack of a straight line in the segment of exponential growth. Instead it has clear curves at the point of inflexion, which results in a higher than could be expected maximum specific rate of bacterial growth. Because the slope of a curve cannot equal 0, the lower asymptote may be below the level of the initial number of bacteria and thus can cause the estimation of the negative lag phase (Baranyi, McClure, Sutherland, & Roberts, 1993).

High R^2 values result from a small number of degree of freedom. In such a situation a very good matching of a model to the data is not surprising (McMeekin & Ross, 2002). However, it seems that the growth parameters of microorganisms naturally occurring in salted and cured model meat products were estimated relatively well (Tables 1–3). Most of them are statistically significant at the level of p-value = 0.05. Due to the method of calculation (formula (3)), statistical significance of the estimated generation time (GT) cannot be determined.

The estimation of the growth parameters of microorganisms in meat products with the content of 2% NaCl and 60 ppm $NaNO_2$ stored at the temperature 5 °C was not satisfactory (Table 1). Parameters of the estimated model are not statistically significant. In products stored at 5 °C one can best see that with the increase of the $NaNO_2$ addition, the μ values are lower while the GT values higher. In the case of higher temperatures of storage these tendencies are not that clear (Tables 1 and 2). However, it seems that quite clear differences occur between parameters of growth characteristics of microorganisms in products without the addition of $NaNO_2$ and in products with the addition of $NaNO_2$ irrespective of the additive level. With the level of the added NaCl within the range 0–2% no inhibitory effect of NaCl on bacterial growth was observed. Maximum addition of NaCl was established in reference to the sensory acceptance of the product. It results from the literature data that the addition of 4% NaCl causes the decrease of a_w from 0.99 to 0.97 and inhibits the growth of bacteria (Borch, Kant-Muermans, & Blixt, 1996).

It is quite clearly visible that with the increase of the temperature of the storage of products the μ increases and the λ and

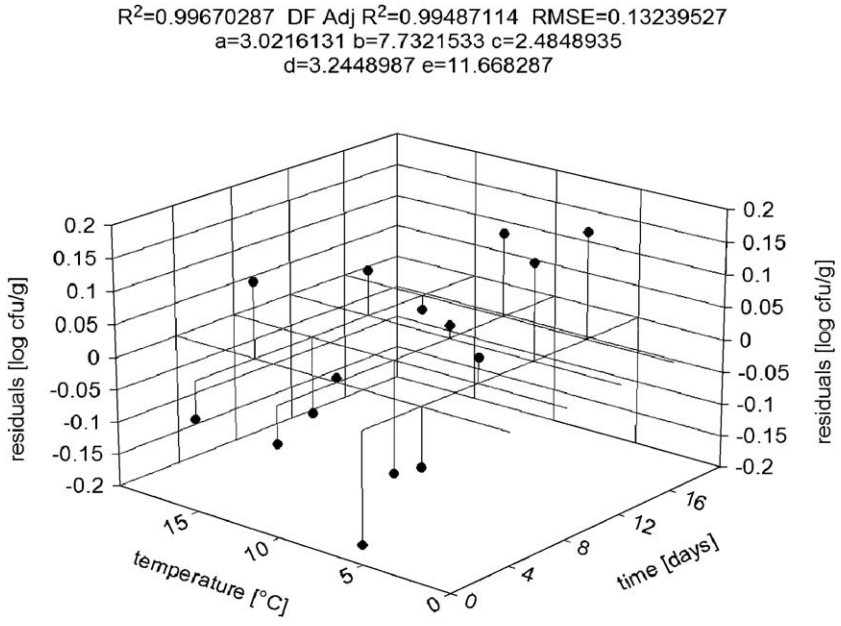

Fig. 5. Differences for the Gompertz response surface model of the total plate count (log cfu/g) in meat products containing 0% NaCl, 60 ppm $NaNO_2$ stored at various temperatures.

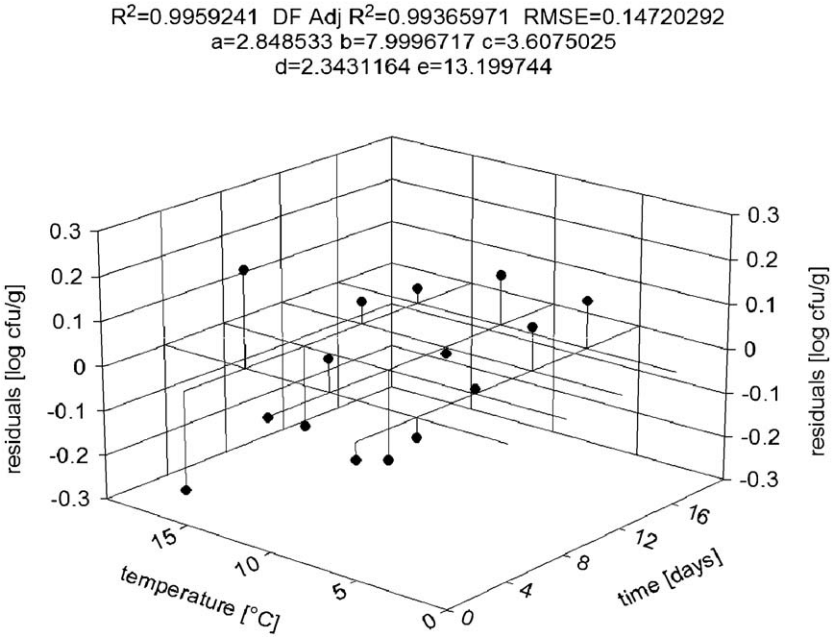

Fig. 6. Differences for the logistic response surface model of the total plate count (log cfu/g) in meat products containing 0% NaCl, 60 ppm $NaNO_2$ stored at various temperatures.

GT of the estimated models — decreases. Temperature is responsible in a significant way for the kinetics of bacterial growth. A bigger effect of temperature, a_w and pH on the rate of bacterial growth was observed and a smaller role of nitrites and storage atmosphere (McMeekin & Ross, 1993). From the obtained models relative low N values were obtained. At the highest temperature of storage, i.e. 15 °C (Table 3) N values do not exceed 5 log (cfu/g).

The biggest problems were to be faced while estimating λ — its value is estimated with the biggest error. In some cases even a negative time of lag phase length was estimated. Data from literature confirm that the estimation of the lag phase is not easy. Time length of lag phase varies and its estimation on the basis of a predictive model is less precise than the estimation of the generation time (McMeekin et al., 2002; Robinson, Ocio, Kaloti, & Mackey, 1998).

Difficulties in estimating the length of lag phase do not result from the lack of proper models but from the lack of complex knowledge on the physiological phase of bacterial growth. A population may include cells at the stage of active growth, in the lag phase, they may be damages or repairing damages but also they may be at the stage of dying from too extensive damages (McMeekin et al., 1997).

4.2. Models of Gompertz response surface and logistic

Nine models of Gompertz response surface and nine logistic surface (for each meat product) were constructed. An example of Gompertz TPC response surface (log cfu/g) for products containing 0% NaCl, 60 ppm $NaNO_2$ is presented in Fig. 3, it is characterized by a high $R^2=0.997$ (adj. $R^2=0.975$) and small RMSE=0.132. In the case of logistic surface (Fig. 4) these values are similar: $R^2=0.996$, (adj. $R^2=0.994$), RMSE=0.147. All values of the parameters (a–e) of the obtained models are statistically significant. Very good matching of both models of the response surface affects relatively low values of the differences between the TPC values (log cfu/g) assessed experimentally and

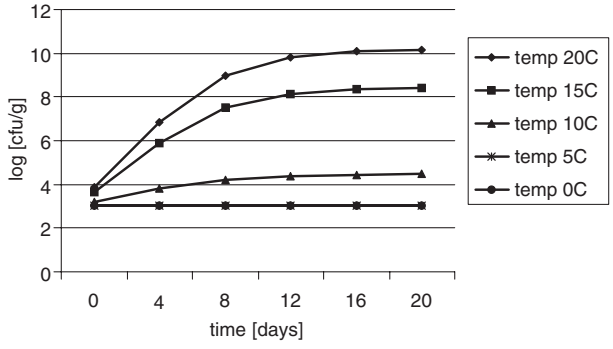

Fig. 7. Total plate count (log cfu/g) in meat products containing 0% NaCl, 60 ppm $NaNO_2$ estimated on the basis of Gompertz response surface (values extrapolated to the 20th day of storage and up to the temperature of 20 °C).

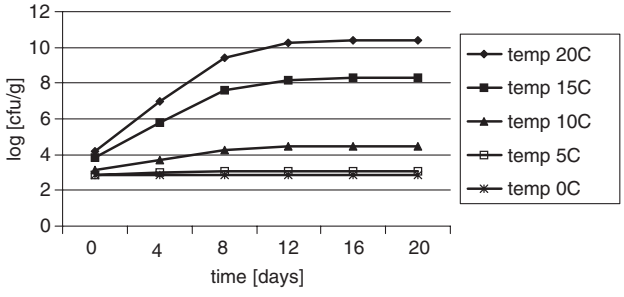

Fig. 8. Total plate count (log cfu/g) in meat products containing 0% NaCl, 60 ppm $NaNO_2$ estimated on the basis of logistic response surface (values extrapolated to the 20th day of storage and up to the temperature of 20 °C).

Table 4
Values of parameters of the model and growth characteristic of total plate count (log cfu/g) in meat products containing 0% NaCl, 60 ppm $NaNO_2$ estimated on the basis of the Gompertz response surface

Temp (°C)	*A*	*B*	*C*	*M*	μ	GT	λ	*N*
0	3.02	0.31	0.00	2.48	0.000	2,317,226,532,708,880.00	−1,654,997.78	3.022
5	3.02	0.31	0.00	2.48	0.000	843.904	0.267	3.025
10	3.02	0.31	1.45	2.48	0.165	1.828	0.267	4.474
15	3.02	0.31	5.40	2.48	0.613	0.491	0.267	8.426
20	3.02	0.31	7.16	2.48	0.812	0.371	0.267	10.183

A, *B*, *C*, *M* — the Gompertz parameters represent the inoculum, relative maximum growth rate, population growth and time of maximum growth rate, respectively, μ — maximum specific growth rate [log (cfu/g)], GT — generation time [h], λ — lag time [h], *N* — maximum density of population [log (cfu/g)].

determined by the model (Figs. 5 and 6). Maximum values of the differences in the case of the Gompertz response surface amount to 0.18 (log cfu/g) and the logistic surface 0.22 (log cfu/g).

On the basis of the matched response surface of bacterial growth the growth of microorganisms in given conditions within the scope of experimental investigation as well as beyond it may be predicted (Baranyi et al., 1993).

The values of growth prediction of the TPC log (cfu/g) determined by models in the described our own investigations seem to be probable (Figs. 7 and 8). Estimating the TPC on the basis of extrapolation of the model of Gompertz response surface points to the lack of bacterial growth at 0 °C (Fig. 7). Growth prediction reveals a minimum growth of the TPC on the basis of the logistic model (Fig. 8). A slow rate of growth can be also pointed to by low values of μ (Tables 4 and 5) amounting to 0.000 log (cfu/g) and 0.003 log (cfu/g), respectively. On the 20th day of storage at 20 °C the TPC in products were estimated to reach the value of about 10 log (cfu/g). From the point of view of both the food producer and consumer overestimating of the growth of microorganisms or the occurrence of risk is undesirable because such product will not be acceptable (McMeekin & Ross, 2002).

If the response surface will be cut with a surface along the established temperature the values of the model parameters: *A*, *C*, *B*, *M* will be obtained as well as the values of growth characteristics TPC (log cfu/g) (Tables 4 and 5). The estimated on the basis of both models of response surface values of μ and *N* increase while GT decreases with the growth of temperature. The λ value remains the same. In the presented models of the response surface the TPC (log cfu/g) contrary to one dimensional models the estimated *N* values seem to be more probable. The approximated *N* values for products containing 0% NaCl, 60 ppm $NaNO_2$ stored at 5 °C 3 (log cfu/g), 10 °C 4 (log cfu/g) and in 15 °C 8 (log cfu/g) (Tables 4 and 5). The values estimated from one dimensional models are clearly lower amounting to 0.35 (log cfu/g), 1.47 (log cfu/g) and 4.64 (log cfu/g), respectively.

Table 5
Values of parameters of the model and growth characteristic of total plate count (log cfu/g) in meat products containing 0% NaCl, 60 ppm $NaNO_2$ estimated on the basis of the logistic response surface

Temp (°C)	*A*	*B*	*C*	*M*	μ	GT	λ	*N*
0	2.85	0.43	0.03	3.61	0.003	98.970	0.576	2.877
5	2.85	0.43	0.23	3.61	0.025	12.026	0.576	3.083
10	2.85	0.43	1.63	3.61	0.174	1.735	0.576	4.475
15	2.85	0.43	5.47	3.61	0.583	0.516	0.576	8.314
20	2.85	0.43	7.58	3.61	0.809	0.372	0.576	10.432

A, *B*, *C*, *M* — the Gompertz parameters represent the inoculum (*A*), relative maximum growth rate (*B*), population growth (*C*) and time of maximum growth rate (*M*), μ — maximum specific growth rate [log (cfu/g)], GT — generation time [h], λ — lag time [h], *N* — maximum density of population [log (cfu/g)].

5. Conclusions

- On the basis of data obtained in the performed experiments parameters of non-linear Gompertz models and logistic of the total plate count growth log (cfu/g) in meat products stored at various temperatures were matched in a satisfactory way.
- The addition of $NaNO_2$ at the level of 60 ppm affected the inhibition of the number of microorganisms in a statistically significant way. At the level of 2% NaCl no inhibitory effect on bacterial growth was observed.

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