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Effect of temperature on the growth kinetics of *Salmonella* Enteritidis in cooked ham

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Abstract

The aim of the study was to determine a growth rate of *Salmonella* Enteritidis in cooked ham stored under different temperatures and to compare usefulness of the mathematical models for describing the microbiological data. The samples of cooked pork ham were inoculated with the mixture of three *Salmonella* Enteritidis strains and stored at 5°C, 10°C, 15°C for 21 d, and at 20°C and 25°C for 5 d. The number of salmonellae was determined at 10 periods of storage at each temperature. From each sample a series of decimal dilutions were prepared and plated onto Brilliant Green Agar. The plates were incubated at 37°C for 24-48 h under aerobic conditions. The colonies grown on culture media were counted, bacterial counts were multiplied by the appropriate dilutions, and number of bacteria (colony-forming units) was calculated. The bacterial counts were transformed into logarithms and analysed using IBM SPSS Statistics 20. The experiment was performed in five replicates. The obtained growth curves of bacteria were fitted to primary growth models, namely Gompertz, logistic, and Baranyi models. The goodness-of-fit test was evaluated by calculating mean square error and Akaike's criterion. Growth kinetics values from the modified Gompertz and logistic equations were calculated. It was found that in samples of ham stored at 5°C and 10°C for 21 d, the number of bacteria remained almost at the same level during storage. In samples stored at 15°C, 20°C, and 25°C growth of salmonellae was observed. It was found that logistic model gave in most cases the best fit to obtained microbiological data describing the behaviour of *S.* Enteritidis in cooked ham. The growth kinetics values calculated in this study from logistic equations can be used to predict potential *S.* Enteritidis growth in cooked ham stored at 15°C, 20°C, and 25°C.

Key words: ham, *Salmonella* Enteritidis, growth, predictive microbiology.

Introduction

For many years *Salmonella* has been the most commonly reported cause of food-borne outbreaks in Europe. In 2011, most of the reported outbreaks were caused by *Salmonella* bacterial toxins, *Campylobacter*, and viruses (3). Salmonellosis in humans was still the second most commonly reported zoonotic disease in 2011. Among 95 548 confirmed cases of salmonellosis in European Union, 8400 were reported in Poland. Twenty six Member States (MSs) and three non-MSs provided data on the presence of *Salmonella* in foodstuffs. *Salmonella* was most often detected in meat and products thereof. Most MSs reported data on *Salmonella* concerns food of animal origin, primarily poultry meat, porcine meat, and bovine meat. In 2011, the highest levels of non-compliance with *Salmonella* criteria occurred in foods of meat origin (3).

S. Enteritidis from pigs and porcine meat is among the most frequently isolated serovars in the EU and non-MSs as mentioned in reports for 2004-2011 (3). Recontamination of ready-to-eat products, such as cooked ham, during post-processing may be the cause of food-borne outbreaks (8).

According to General Food Law Regulation (EC) No 178/2002 (Art. 17) food and feed business operators at all stages of production, processing, and distribution within the businesses under their control should ensure that foods or feeds fulfil the requirements of food law, which are relevant to their activities, and should verify that such requirements are met. Thus, the determination of shelf-life and storage temperature is the duty of food

manufacturers, who take a whole responsibility for the final product. National legislation in Poland does not contain specific requirements in this regard. Most of the cooked ham producers provide the information on the label, that product should be stored at 2-7°C no longer then 14-30 d. In one of the studies on cold chain maintaining in food trade, temperature conditions in cooling appliances for storage of 1688 perishable food products were measured (12). It was found that in most of the cases, the temperatures measured differed from the labelled on the packages, even for up to 10°C. Temperatures in home refrigerators are also in many cases much higher than required (6, 11). It means that meat products, including cooked ham, are very often stored under temperature abuse. Predictive microbiology seems to be a useful tool for evaluation of bacterial behaviour in improperly stored food.

In recent years, progress in predictive microbiology has been impressive, and microbial models are increasingly used by food producers and food inspectors in their routine work. One of the reasons for that are changes in European food law, particularly the obligatory introduction of HACCP, risk analysis, and microbiological criteria for food. Predictive microbiology has been an important supporting tool in food chain risk management (17).

The aim of the study was to determine and compare the growth rate of *Salmonella* Enteritidis in cooked ham stored at different temperatures, to evaluate usefulness of the mathematical models of Gompertz, logistic, and Baranyi models for describing the microbiological data, and to calculate growth kinetics of *S*. Enteritidis in cooked ham as to predict its behaviour under storage at temperature abuse.

Material and Methods

Organisms and inoculum preparation. The following *Salmonella enterica* subsp. *enterica* serovar Enteritidis strains were used in the experiments: strain No. 1592/08 isolated from turkey, strain No. 2419/07 isolated from poultry (obtained from the National Veterinary Research Institute in Pulawy, Poland), and ATCC strain No. 13076. To prepare the inoculum, the organisms were cultured in 10 mL nutrient broth at 37°C for 24 h. Cell suspension from each strain was mixed together, and the mixture was diluted with sterile dilution fluid to achieve a population of approximately 10^5 CFU/mL for use as inoculum. The cell count of each inoculum was determined by spread-plating of 0.5 mL of diluted cell mixture on Brilliant Green Agar (Merck®).

Material. Cooked ham was purchased from a local producer (Meat processing plant, Kozienice, Poland). Technology of the ham included wet-brine cure, light smoking, and cooking (to internal temperature of 70°C). Composition of the ham declared by the producer was as follows: boneless ham; pig hind leg (75%), water, natural spices and their extracts, sodium chloride, E-250, E-316, E-407, E-452, and E-621. Chemical and microbiological requirements to be met for the ham are given in Polish Standard (15).

Inoculation and storage of samples. Samples (10 g) of the ham were placed in Stomacher bags and inoculated with 0.1 cm^3 of suspension containing the three-strain *Salmonella* mixture. Immediately after inoculation, ham samples were placed in incubators and stored at 5°C, 10°C, 15°C for 504 h, and at 20°C and 25°C for 108 h.

Examination of samples. Number of salmonellae was determined at 10 periods of storage at each temperature. After homogenisation (3 min) ten-fold dilution series were prepared followed by plating (0.5 mL) on BGA (Merck®). The plates were incubated at 37°C for 24 h under aerobic conditions.

The colonies grown on culture media were counted, bacterial counts were multiplied by the appropriate dilutions, and numbers of bacteria (colony-forming units) were calculated. Surface plating was done using three plates for each dilution. The experiment was done in five replicates.

Statistical calculations. Bacterial counts, transformed into logarithms, were used for calculations using the General Linear Models supplied through IBM SPSS Statistics 20.

Curve fitting. Obtained growth curves of bacteria were fitted to modified Gompertz, logistic, and Baranyi models. Modified Gompertz model (Eq. 1) and logistic model (Eq. 2) are the most frequently used to describe the bacterial growth in foods (4, 10, 13, 14).

$x(t) = C + A \exp(-\exp(-B(t-M)))$, (1)

$x(t) = C + A / (1+\exp(-B(t-M))$,(2)

where $x(t)$ is $\log_{10}$ (CFU/g) of cell concentration at time t; C is the value of lower asymptote in units of $\log_{10}$ (cfu/g); A is equal to $\log_{10}(x_{max}/x_0)$; x_0 is the initial population density; x_{max} is the maximum population density; B is the maximum relative growth rate at M in 1/h; M is the time at which the absolute growth rate is maximum in hours. Baranyi and Roberts (1) introduced a mechanistic model that describes sigmoidal bacterial growth curves referred as Baranyi model:

$$y(t) = y_0 + \mu_{max} F(t) - \ln\left(1 + \frac{e^{\mu_{max} F(t)} - 1}{e^{(y_{max} - y_0)}}\right) \quad (3)$$

$$F(t) = t + \frac{1}{v}\ln(e^{-vt} + e^{-h_0} - e^{(-vt-h_0)}) \quad (4)$$

where: $y(t)$ – cells concentration at time t (ln CFU/g), y_0 – initial cell concentration (ln CFU/g), y_{max} – maximum cell concentration (ln CFU/g), μ_{max} - maximum specific growth rate (h^{-1}), v – rate of increase in the limiting substrate, h_0 – is a product of $\mu_{max}\lambda$.

The modified Gompertz and logistic equations parameters (A, B, C, M) were subsequently used to calculate: lag phase duration (h) = M - (1/B), generation time (h) = (log 2e)/BC, exponential growth

rate [(log cfu/g)/h] = BC/e, maximum population density (log cfu/g) = A + C (4, 21, 22).

The goodness-of-fit was evaluated by calculating mean square error (MSE) and Akaike's criterion (AIC) for each of the studied models according to following equations:

- the mean square error (*MSE*):

$$MSE = \frac{RSE}{df} \quad (5)$$

where: *RSE* – residual sum of errors, *df* – degrees of freedom.

- Akaike's Information Criterion with bias adjustment for small sample size (2):

$$AIC_c = -2 * \ln(likelihood) + 2 * K + \frac{2 * K * (K+1)}{n - K - 1} \quad (6)$$

where: *n* – number of observations, *K* – number of the model's parameters.

Results

All the experimental data related to ham samples stored at 5°C, 10°C, 15°C, 20°C, and 25°C, fitted into three primary growth models used in this study, are presented in Figs 1-5.

In samples of cooked ham stored at 5°C and 10°C, the number of salmonellae remained almost at the same level during storage for 504 h (Figs 1, 2). Analysis of variance showed that the effect of storage time on the number of bacteria was not statistically significant at $P < 0.01$. Mean values for log numbers of *Salmonella* in samples stored at 5°C and 10°C in subsequent periods of storage also did not differ statistically at $P < 0.01$ in Tukey's test.

In samples of ham stored at 15°C, 20°C, and 25°C, the number of test organisms significantly increased with extending storage time. The growth rate of salmonellae increased with an increase in storage temperature being the most dynamic in the samples stored at 25°C (Figs 3-5).

Growth kinetics parameters characterising the growth of *S.* Enteritidis in samples of cooked ham incubated at 15°C, 20°C, and 25°C are listed in Table 2.

The logistic model gave in most cases the best fit to obtained microbiological data describing the behaviour of *S.* Enteritidis in cooked ham (Table 1). Therefore, using the growth kinetics values calculated from logistic equations should give better predictions of potential *S.* Enteritidis growth in cooked ham stored under temperature abuse than data calculated from Gompertz equations (Table 2).

Table 1. Comparison of statistics obtained from three primary models. The goodness-of-fit was estimated by calculating the mean square error (MSE) and Akaike's criterion (AIC)

Storage temperature	Model	MSE	AIC
5°C	Modified Gompertz	0.0228*	-181.04*
	Logistic model	0.0228*	-181.04*
	Baranyi model	0.0368	-155.11
10°C	Modified Gompertz	0.0283*	-170.24*
	Logistic model	0.0283*	-170.24*
	Baranyi model	0.0460	-143.91
15°C	Modified Gompertz	0.2240	-66.80
	Logistic model	0.2152*	-68.81*
	Baranyi model	0.5393	-20.88
20°C	Modified Gompertz	0.2318*	-65.09*
	Logistic model	0.2347	-64.48
	Baranyi model	0.5396	-20.85
25°C	Modified Gompertz	0.0794	-118.67
	Logistic model	0.0778*	-119.70*
	Baranyi model	0.1945	-71.87

* The best fitted model (the lowest value of MSE and AIC)

Table 2. Growth kinetics values of *Salmonella* Enteritidis in cooked ham, calculated from the modified Gompertz and logistic equations

Storage temperature	Growth kinetics	Gompertz model	Logistic model
15°C	Lag time (h)	139.08	187.78
	Generation time (h)	17.22	12.35
	Exponential growth rate (log/mL/h)	0.02	0.02
	Max. pop. density (log/mL)	8.82	8.66
20°C	Lag time (h)	22.50	39.85
	Generation time (h)	7.86	5.62
	Exponential growth rate (log/mL/h)	0.03	0.05
	Max. pop. density (log/mL)	8.94	8.38
25°C	Lag time (h)	15.94	25.04
	Generation time (h)	3.88	3.28
	Exponential growth rate (log/mL/h)	0.07	0.08
	Max. pop. density (log/mL)	8.66	8.56

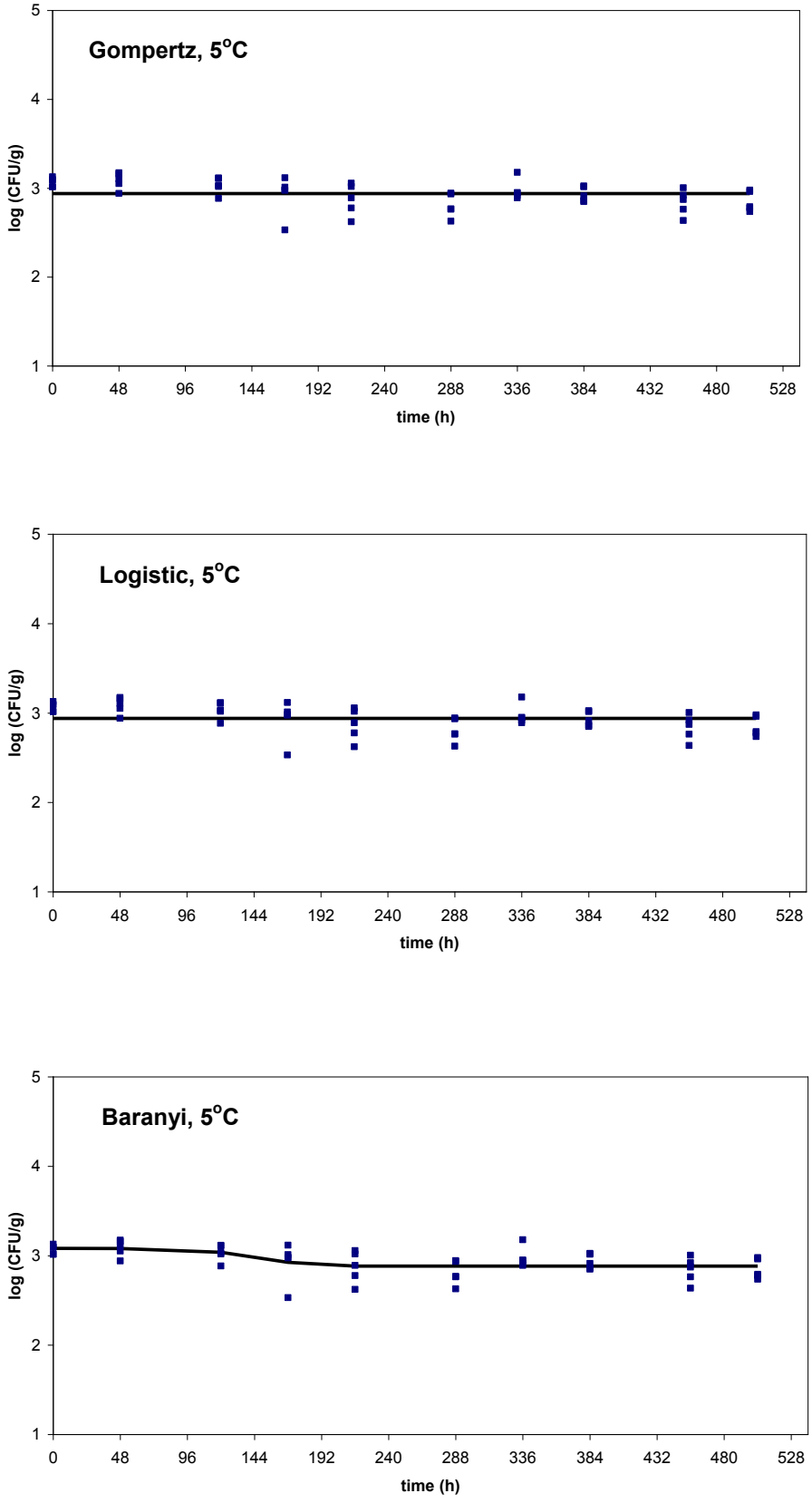
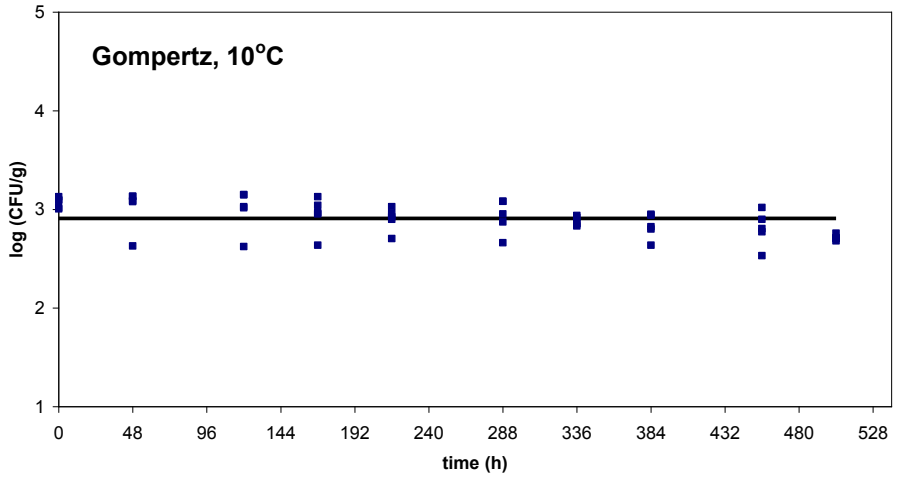
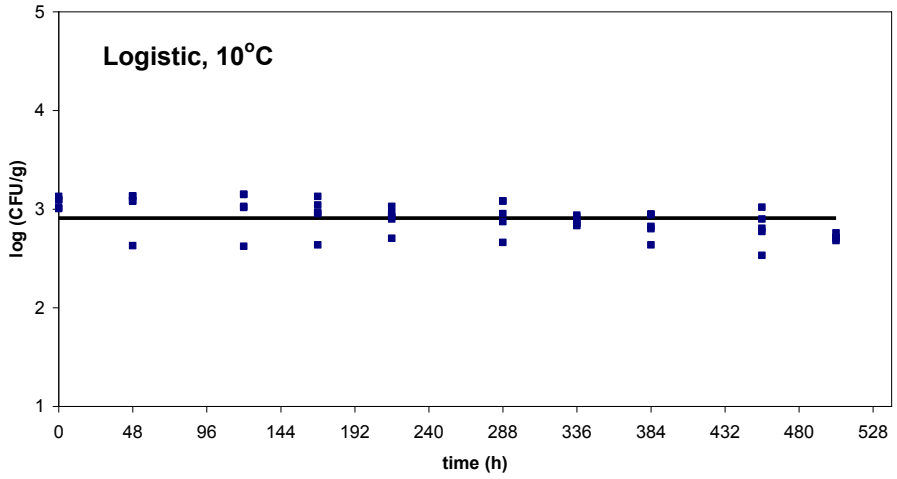


Fig. 1. Effect of time on the number of *S.* Enteritidis in cooked ham stored at 5°C. Experimental data fitted into three primary models. Black squares represent raw data from five replicates





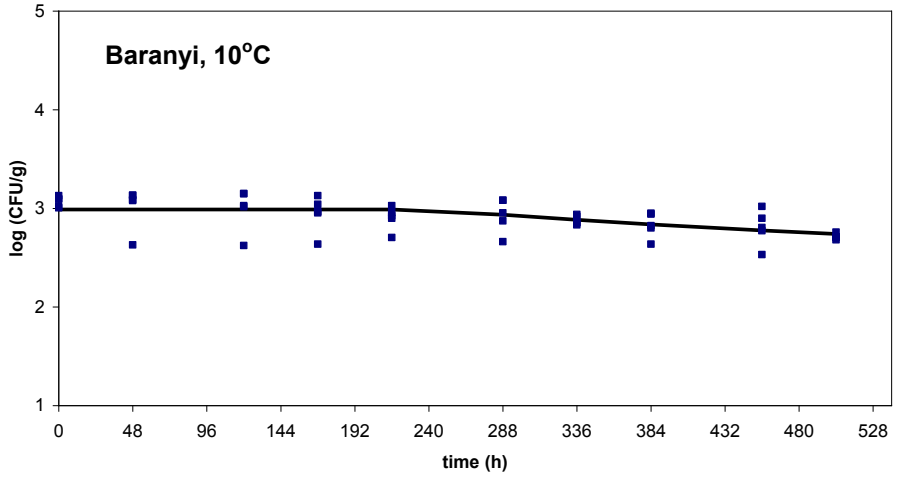


Fig. 2. Effect of time on the number of *S*. Enteritidis in cooked ham stored at 10°C. Experimental data fitted into three primary models. Black squares represent raw data from five replicates

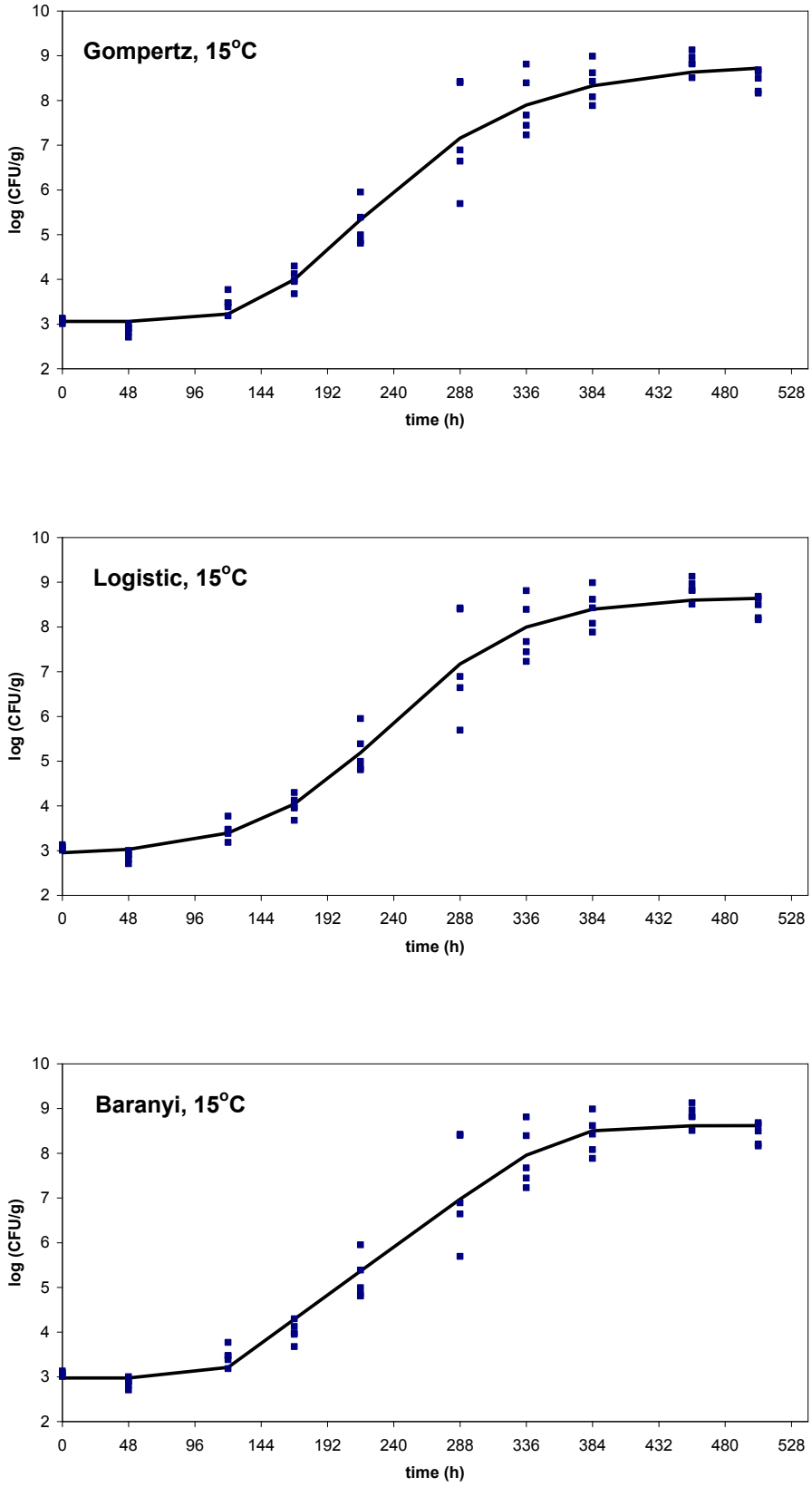


Fig. 3. Growth of *S*. Enteritidis in cooked ham stored at 15°C. Experimental data fitted into three primary models. Black squares represent raw data from five replicates

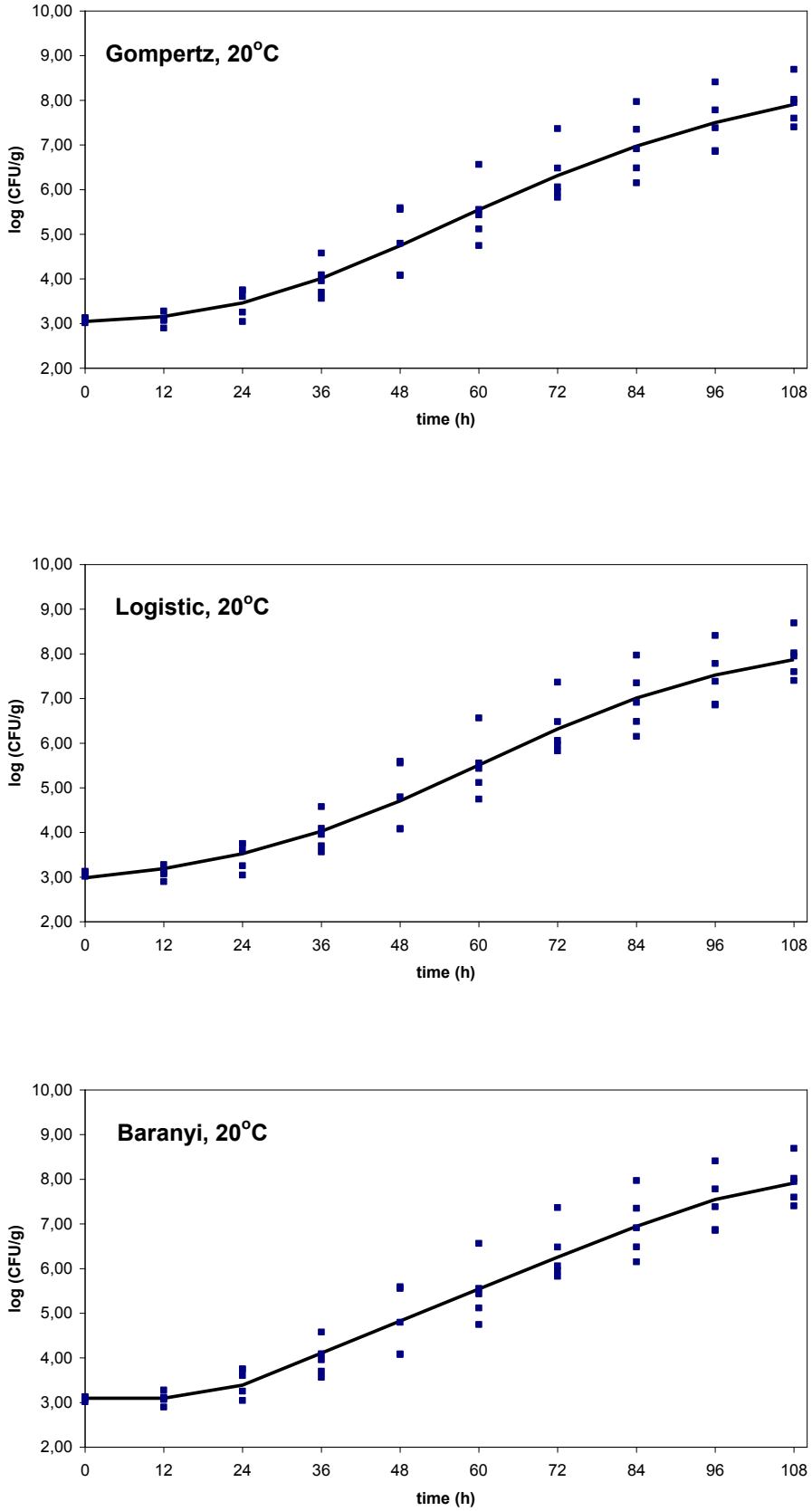


Fig. 4. Growth of *S.* Enteritidis in cooked ham stored at 20°C. Experimental data fitted into three primary models. Black squares represent raw data from five replicates

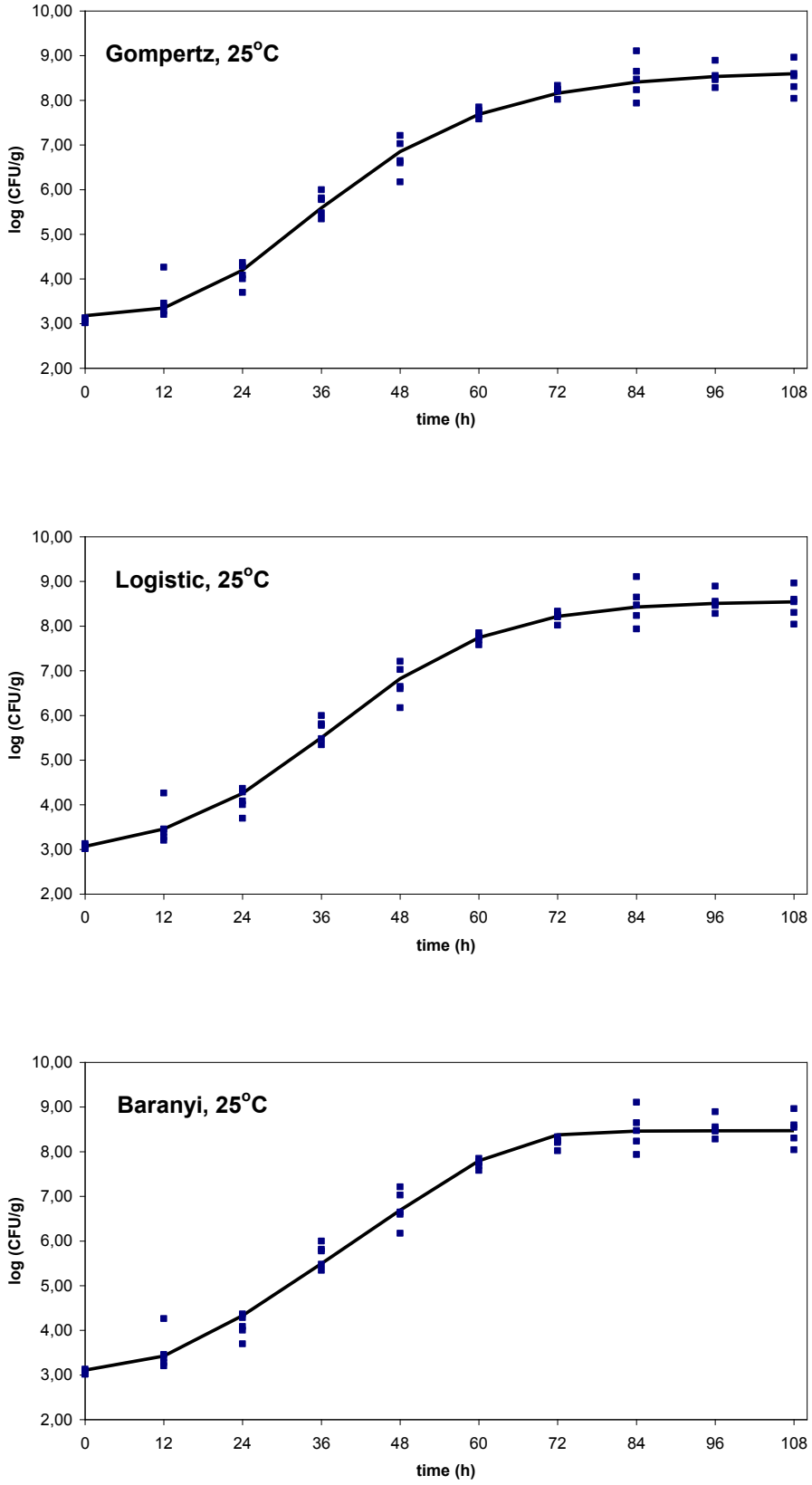


Fig. 5. Growth of *S*. Enteritidis in cooked ham stored at 25°C. Experimental data fitted into three primary models. Black squares represent raw data from five replicates

Discussion

This study demonstrated that no growth of *Salmonella* occurred at the lowest storage temperatures of 5°C and 10°C. Lack of growth of test microorganisms at 5°C could be expected, because most *Salmonella* serotypes fail to grow in food stored below 7°C (20). However, some authors observed growth of salmonellae in cooked ham stored at 10°C (7) and at 8°C (5).

The results graphically presented in Figs 1-5 indicate that all three primary models selected for this study can be used to fit the obtained experimental microbiological data.

The results of calculation presented in Table 1 enabled more detailed comparison of the models. Both MSE and AIC indicated that logistic model gave the best fit to experimental data describing the behaviour of *S.* Enteritidis in ham stored at 15°C and 25°C, whereas modified Gompertz model gave the best fit to data related to the samples stored at 20°C. In all groups of samples the Baranyi model provided the worst fit.

It should be emphasised that the differences in the goodness-of-fit between logistic and modified Gompertz model are very low (Table 1). In similar studies related to modelling the effect of temperature on growth of *Salmonella* in chicken, the modified Gompertz model provided the best fit for growth data, followed by Baranyi model, and the logistic model (10).

Juneja *et al*. (9) fitted growth data of *Salmonella* in raw ground beef at nine different temperatures into primary models, namely the logistic, modified Gompertz, Baranyi, and Huang models. Performances of these models were evaluated by using various statistical criteria. All the chosen models fitted well to the growth data based on these criteria. The results of statistical analysis showed that there was no significant difference in the performances of the four primary models, suggesting that the models were equally suitable for describing isothermal bacterial growth (9).

The data presented in Table 2 for the samples stored at 15°C, 20°C, and 25°C for 108 h clearly demonstrated that together with increasing storage temperature, a decrease in lag time and generation time, as well as an increase in exponential growth rate of tested microorganisms regularly occurred. Similar patterns were observed in our previous studies (18, 19) and studies of other authors (16).

There is not much information available on the fate of *S.* Enteritidis in cooked ham stored at temperatures used in the conducted experiments, therefore, a direct comparison of our results with other studies is difficult. However, a growth rate of *Salmonella* found in our study at 15°C was similar to the data given by Hwang *et al.* (7).

Generally, the obtained results confirmed that the storage of food of animal origin at low temperatures is one of the main factors limiting the number of foodborne illness outbreaks caused by *Salmonella.* Insufficient heat treatment or recontamination of meat products with *Salmonella* after cooking, and subsequent storage at abuse temperatures can cause a significant risk to consumer's health.

The growth kinetics values calculated in this study from logistic equations can be used to predict potential *S.* Enteritidis growth in cooked ham stored at 15°C, 20°C, and 25°C.

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