

Comparison of the experimentally obtained growth model of *Listeria monocytogenes* on cucumber and zucchini with existing model generated by ComBase Predictor

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Abstract The quality and microbiological safety of produced and stored food is a challenge for its producers and official control. Numerous examples have shown how important it is for them to have access to precise data about the growth time and rate of microorganisms in stored products, especially in the case of those which are the etiology of digestive system diseases such as *Listeria monocytogenes*. It is one of the most virulent foodborne pathogens, showing a mortality rate of approximately 20–30 %, which is the highest among food microbiota. In order to determine the microbial growth kinetics in time on food matrices, so-called growth modeling is used. It allows to determine the growth of microorganisms in time and depending on the temperature, pH, water activity (a_w), or content of acids. In this paper, we verified the growth model of *Listeria monocytogenes* on cucumber and zucchini with an existing model in the ComBase Predictor.

Keywords *Listeria monocytogenes* · Cucumber · Zucchini · Growth modeling

Introduction

Focusing on the microbiological safety of produced and stored food products is a priority for the institutions

controlling and producing them. Events from a few years ago caused by *Listeria monocytogenes* detected on cantaloupes, which caused 32 deaths and 1 miscarriage [16] and the recent case of suspected contamination by the same bacteria of sliced apples and withdrawal of entire batches of the product in November 2013 [4] showed how important it is for food producers to have access to precise information about the growth time and growth rate of microorganisms in stored products.

Listeria monocytogenes is one of 19 species of the genus *Listeria* and the second, next to *Listeria ivanovii*, showing pathogenic properties [15]. It is a Gram-positive bacillus, which lives intracellularly and is the etiological factor of listeriosis. Bacteria belonging to this species have the highest virulence of all foodborne pathogens (20–30 % of infections are fatal), where the most pathogenic properties were noted for serotype 4b [18]. An example of this can be the USA—approximately 2500 cases and 500 deaths per year [5], thereby causing a higher mortality rate than *Salmonella* sp. or *Clostridium botulinum*. The main symptoms of listeriosis are septicemia, meningitis, encephalitis, corneal ulcer, pneumonia, and infections of the uterus in pregnant women, which can lead to a miscarriage or intrauterine fetal death [6, 9, 10, 13, 22].

Determining the bacterial growth, including *Listeria monocytogenes*, on different matrices, both fresh and processed, is the role of predictive microbiology. Within it, so-called growth models are formed allowing to determine the growth rate of microorganisms depending on the environmental conditions [20]. The information from modeling is used in the creation of databases, such as the ComBase Predictor (combined database for predictive microbiology), allowing to generate a model based on set variables that have an influence on bacterial growth, such as the pH, a_w , temperature, concentration of NaCl [8, 14].

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The goal of this work was the validation of an existing model in the ComBase Predictor with data obtained experimentally from cucumber and zucchini. The comparison of those results with the predictions allows to verify the reliability of the latter, especially to check whether they retain the necessary safety margin required from the point of view of food producers.

Materials

The matrices were fresh products of plant origin—zucchini and cucumber, with a pH of 6.44 and 6.20, respectively.

For the fortification of the samples, a strain CCIM 1171 of *Listeria monocytogenes* 4b from the culture collection of industrial microorganisms was used.

In this work, *Listeria* Agar according to Ottaviani and Agosti (ALOA) (Bio-Rad) was used as the culture media, and as the diluent buffered peptone water (BWP) (bioMérieux) and saline with peptone [12].

The growth modeling of *Listeria monocytogenes* was prepared with DMFit version 2.1., an Excel add-in (Institute of Food Research, Norwich, UK), created by using an algorithm developed by Baranyi and Roberts [1, 2].

Growth predictions of the tested microorganisms were generated and downloaded from the ComBase Predictor [3], created by the Food Safety Centre at the Institute of Food Research in the UK.

Methods

The method of modeling was based on an algorithm made by Baranyi and Roberts [1, 2], whereas the cultures on ISO 11290-2:1998/AM1:2004 [12].

All products were thoroughly washed with water and detergent, in order to mechanically remove any contaminants and microorganisms existing on the surface of the vegetables. Next, the products were immersed in a 70 % aqueous solution of isopropanol for 30 min, taken out and left to dry in a sterile environment.

The vegetables, after the skin had been removed using a sterilized knife, were cut into $10 \text{ g} \pm 1 \text{ g}$ pieces, and then, they were fortified with 0.1 ml of inoculum that contained 10^5 CFU/ml of *Listeria monocytogenes*, the final number amounted to 10^3 CFU/g in the sample. Incubation was carried out at a temperature of 4, 25, and 37 °C.

The test was carried out in two repeats and with two dilutions. The number of bacteria in 1 g or 1 ml was counted according to ISO 7218:2008 [11] in Microsoft Excel.

Thus, obtained data were entered into DMFit, which generated a primary growth model and two parameters

validating it: R^2 is the coefficient of determination, indicating how well data fit the model, and SE(fit), the standard error, is the standard deviation of observed independent variables and growth rate.

To generate the predictive models of *Listeria monocytogenes*, the ComBase Predictor application was used (Food Safety Centre at the Institute of Food Research, UK).

The growth rate was used to receive the mean square error (MSE).

This value is received from the formula:

$$\text{MSE} = \frac{\text{RSS}}{n} = \frac{\sum (\mu_{\text{obs}} - \mu_{\text{pred}})^2}{n}$$

where RSS is the residual sum of squares; n is the number of degrees of freedom/observations; μ_{obs} is the observed growth rate (h^{-1}); μ_{pred} is the predicted growth rate (h^{-1}).

The lower the value of the MSE, the better the fit of the model [21].

Additionally, graphic validation was used, comprised of a comparison of the predicted and observed growth rate, which allows to preliminarily evaluate the observed results. Their deviation from the so-called equivalence line (eq line) in the direction of the predicted growth indicates that the prediction has a higher growth rate value than the observed growth rate.

Then, the secondary Ratkowsky model was prepared, showing the relationship between the growth rate and temperature. The model was validated with two variables—the bias factor (B_f) and accuracy factor (A_f).

The bias factor (B_f) informs how much a point is deviated from the equivalence line and whether it is above or below it.

$$B_f = \exp \left[\frac{\sum (\ln \mu_{\text{pred}} - \ln \mu_{\text{obs}})}{n} \right]$$

where n , μ_{obs} , μ_{pred} —as above.

When $B_f = 1$, it indicates a perfect fit of the model; when $B_f < 1$, the observed growth rate is lower than the predicted one, $B_f > 1$ indicates a situation recognized as unsafe—the observed growth of microorganisms is faster than the predicted one.

The accuracy factor (A_f) indicates the average distance between every observed point and the eq line [21].

$$A_f = \exp \left[\sqrt{\frac{\sum (\ln \mu_{\text{pred}} - \ln \mu_{\text{obs}})^2}{n}} \right]$$

where n , μ_{obs} , μ_{pred} —as above.

When the value of the A_f factor is equal to 2, it indicates that the observed value differs on average from the prediction by 2 units [21].

Results and discussion

The most effective growth of *Listeria monocytogenes* was noticed after 24 h of incubation at 4, 25, and 37 °C on zucchini (Table 1). A similar amount after 24 h at 20 °C was received on papaya by Penteado and Leitao [17]. However, the same authors obtained higher values on watermelon and melon (around 6 log CFU/g and 4 log CFU/g, respectively). At 30 °C, they achieved approximately 7 log CFU/g on papaya and 9 log CFU/g on watermelon and melon, the naturally created fruit acid did not obstruct the growth of bacteria. The growth of different serotypes of *Listeria monocytogenes* on cantaloupe was researched by Fang et al. [7]. Serotype 4b at 37 °C reached a value of 9 log CFU/g.

The results for modeling at 37 °C for cucumber and zucchini are shown in Figs. 1 and 2.

Based on the analysis of R^2 , it was noted that the most fitted is the value obtained for cucumber at 25 °C amounting to 0.992838, and the least is the one for zucchini at 25 °C—0.609912. For cucumber at 4 °C, R^2 could not be determined. Similar values, in the range of 0.92–0.96, were obtained by Kowalik et al. [13] and 0.97 by Danyluk et al. [6].

Table 1 Number of *Listeria monocytogenes* in log CFU/g on zucchini and cucumber after 24 h of incubation at 4, 25, and 37 °C

Matrices	Temperature		
	4 °C	25 °C	37 °C
Zucchini	2.90	3.64	6.27
Cucumber	2.88	3.19	5.02

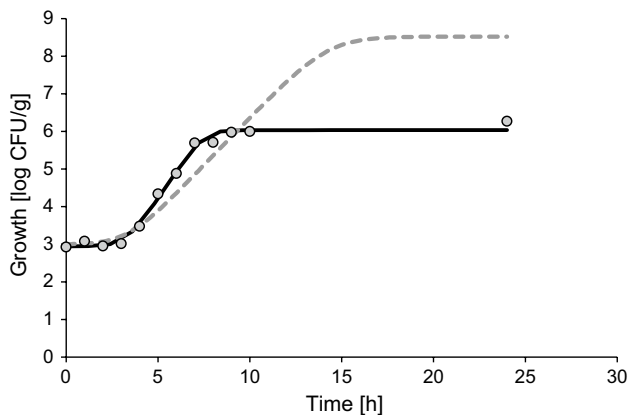


Fig. 1 Results for primary modeling at 37 °C for zucchini. Circles data obtained experimentally; black line data fitted into a model of Baranyi and Roberts [1, 2]; dotted line data generated by ComBase Predictor

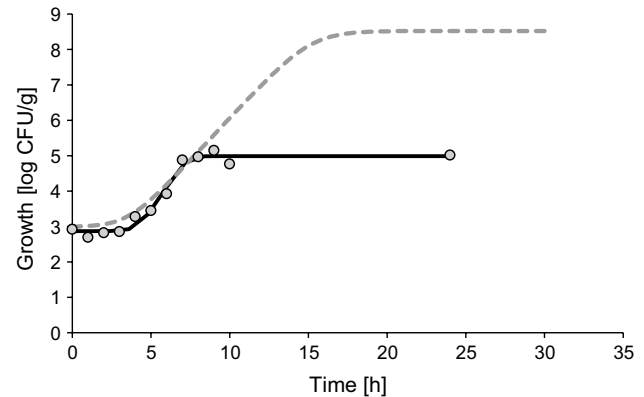


Fig. 2 Results for primary modeling at 37 °C for cucumber. Circles data obtained experimentally; black line data fitted into a model of Baranyi and Roberts [1, 2]; dotted line data generated by ComBase Predictor

In the case of SE(fit), the smallest deviation was noted for cucumber at 25 °C—0.07268, and the highest for zucchini at 25 °C—0.274805 and cucumber at 4 °C—0.265699. The highest values were similar to those obtained by Kowalik et al. [13].

The growth rate was the highest for zucchini at 37 °C—0.732795 (h^{-1}), whereas the lowest for cucumber at 4 °C—0.000999 (h^{-1}). The direct connection between the increase in growth rate and the increase in storage temperature was observed, which was confirmed by Kowalik et al. [13], Rodriguez et al. [19].

The results are shown in the Table 2.

The obtained values of growth rate allowed to calculate the MSE for both matrices. The MSE results are as follows: for zucchini—0.45687 and cucumber—0.031971.

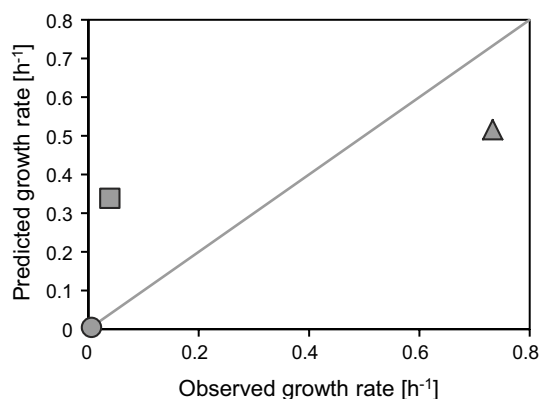
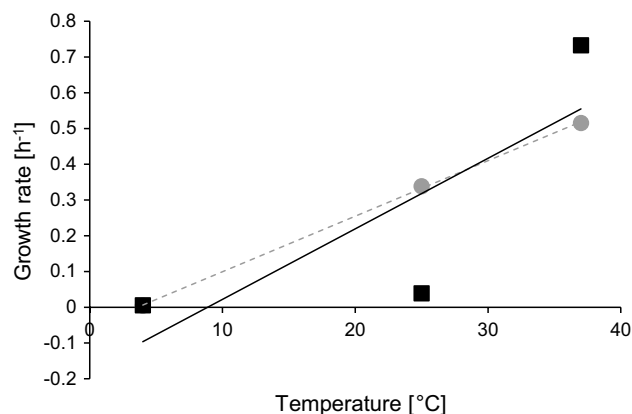
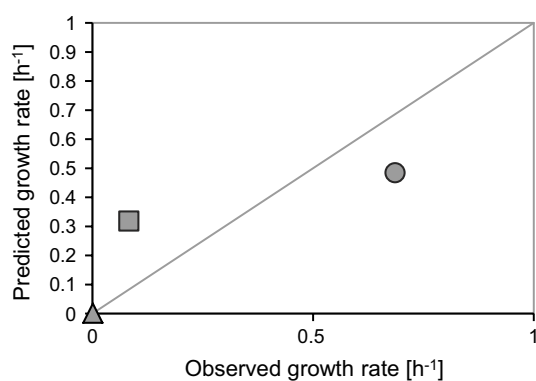
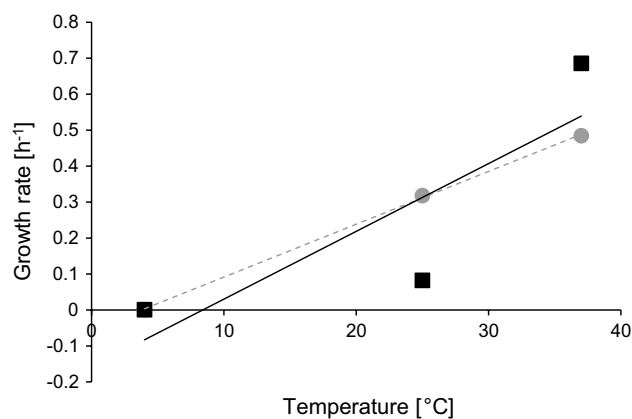
The visual representation of the observed growth rate with the predicted growth rate is shown in Figs. 3 and 4.

In the case of zucchini incubated at 4 °C, we are dealing with an ideal situation—the value of the observed growth rate matches the predicted one. There is a similar situation in the case of cucumber incubated at the same temperature. It is different in the case of zucchini and cucumber incubated at 25 °C, where a clear deviation of the value in the direction of the predicted growth rate was observed. It is a favorable situation as the observed growth of microorganisms is slower than the predicted one, leaving a margin of safety, which is preferable for the prediction. An opposite situation has place in the case of zucchini and cucumber incubated at 37 °C, where the observed growth rate is higher than the predicted one. This is a potentially unsafe situation as the microorganisms will continue to develop faster than it is predicted [21].

The growth rate values obtained via DMFit allow to obtain a secondary Ratkowsky model. As it was already mentioned, A_f and B_f factors are used in the validation of this model.

Table 2 Variables of primary growth model: R^2 , SE(fit), growth rate, and lag phase

Matrices	Temperature (°C)	R^2	SE(fit)	Growth rate (h^{-1})	Lag phase (h)
Zucchini	4	0.836178	0.16474	0.005385	–
	25	0.609912	0.274805	0.038756	–
	37	0.988129	0.149665	0.732795	3.274584
Cucumber	4	–	0.265699	0.000999	–
	25	0.992838	0.07268	0.082562	22.13034
	37	0.968914	0.175599	0.685763	4.179718

**Fig. 3** Comparison of observed and predicted growth rates of *Listeria monocytogenes* on zucchini. Circles zucchini incubated at 4 °C; square zucchini incubated at 25 °C; triangle zucchini incubated at 37 °C; gray line equivalence line**Fig. 5** Result of secondary Ratakowsky modeling for cucumber. Circle predicted growth rate; square observed growth rate**Fig. 4** Comparison of observed and predicted growth rates of *Listeria monocytogenes* on cucumber. Triangle zucchini incubated at 4 °C; square zucchini incubated at 25 °C; circles zucchini incubated at 37 °C; gray line equivalence line**Fig. 6** Results for secondary Ratakowsky modeling for zucchini. Circle predicted growth rate; square observed growth rate

The shown charts, based on the Ratkowsky model, allow to state that in the case of both matrices, the growth rate increases with the increase in temperature. At a storage temperature of 37 °C, a state noticed during the visual validation of the model is reached—the value of the observed

growth rate is higher than the predicted one, this was also observed by other authors [6, 13, 17]. Charts illustrating secondary Ratkowsky modeling are shown in Figs. 5 and 6.

The A_f and B_f values describing this model are shown in Table 3.

Table 3 Variables of secondary modeling: A_f and B_f

Matrices	A_f	B_f
Zucchini	2.485943	1.691753
Cucumber	2.027171	1.503776

In the case of the A_f factor, the lowest values are considered the most precise as this factor indicates by how much the prediction differs from the observed values, so it can be stated that despite the similar values of this factor, the best matched growth model is the cucumber model.

The B_f factor shows the overall deviation of the model. Obtained values higher than 1 are the potentially unsafe models, these also include the models shown here, which confirms the earlier visual analysis of charts.

Conclusion

Using the growth model of *Listeria monocytogenes* on zucchini and cucumber, it has been proven that the most beneficial temperature, in which they should be stored, is 4 °C. At the same time, it is suggested to minimize the possibility of their contact with temperatures above 30 °C, because of the observed rapid growth of this pathogen on tested food products.

The obtained results and models show the significance of experimental validations of predictions generated by programmes such as the ComBase Predictor. At this stage, data obtained this way may only be treated as a suggestion and not an incontestable determinant of food product storage. Based on the presented results, it was observed that the yield of *Listeria monocytogenes* at 37 °C, on both matrices, is a few times higher than the predicted one. It could become a dangerous situation in the future due to a rise in the amount of diseases caused by inaccurate predictions. It is suggested to continue research on the validation of generated data and confront it with that obtained experimentally.

Compliance with ethical standards

Conflict of interest All the authors declare that there is no conflict of interests regarding the publication of this article.

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

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