Modeling the growth of *Listeria monocytogenes* in mold-ripened cheeses

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ABSTRACT

This study presents possible applications of predictive microbiology to model the safety of mold-ripened cheeses with respect to bacteria of the species *Listeria monocytogenes* during (1) the ripening of Camembert cheese, (2) cold storage of Camembert cheese at temperatures ranging from 3 to 15°C, and (3) cold storage of blue cheese at temperatures ranging from 3 to 15°C. The primary models used in this study, such as the Baranyi model and modified Gompertz function, were fitted to growth curves. The Baranyi model yielded the most accurate goodness of fit and the growth rates generated by this model were used for secondary modeling (Ratkowsky simple square root and polynomial models). The polynomial model more accurately predicted the influence of temperature on the growth rate, reaching the adjusted coefficients of multiple determination 0.97 and 0.92 for Camembert and blue cheese, respectively. The observed growth rates of *L. monocytogenes* in mold-ripened cheeses were compared with simulations run with the Pathogen Modeling Program (PMP 7.0, USDA, Wyndmoor, PA) and ComBase Predictor (Institute of Food Research, Norwich, UK). However, the latter predictions proved to be consistently overestimated and contained a significant error level. In addition, a validation process using independent data generated in dairy products from the ComBase database (www.combase.cc) was performed. In conclusion, it was found that *L. monocytogenes* grows much faster in Camembert than in blue cheese. Both the Baranyi and Gompertz models described this phenomenon accurately, although the Baranyi model contained a smaller error. Secondary modeling and further validation of the generated models highlighted the issue of usability and applicability of predictive models in the food processing industry by elaborating models targeted at a specific product or a group of similar products.

Key words: *Listeria monocytogenes*, predictive microbiology, mold-ripened cheeses

INTRODUCTION

Listeriosis is a serious foodborne disease caused by rods from the *Listeria* genus, especially the *Listeria monocytogenes* species. According to The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial Resistance and Foodborne Outbreaks in the European Union (EFSA, 2007), in 2006 a total of 1,588 cases of listeriosis were confirmed by laboratory tests in all EU countries. The incidence of the disease in 2006 was 0.3 cases per 100,000 people and increased compared with the previous years. In developed countries, infections caused by *L. monocytogenes* are the most common cause of death among foodborne diseases (Swaminathan and Gerner-Smith, 2007). *Listeria monocytogenes* is not a thermoresistant bacterium, and therefore does not survive pasteurization. The presence of *L. monocytogenes* in pasteurized food is typically due to contamination after the heat treatment process. It is a psychrotrophic bacterium, able to grow and multiply during cold storage, and even a few cells can multiply to a level that is dangerous to consumers (Posfay-Barbe and Wald, 2009). As about 50% of the outbreaks of listeriosis in Europe have been associated with dairy products, special control measures are needed to eliminate the presence of *L. monocytogenes* in dairy foodstuffs (Lundén et al., 2004).

Due to a generally weaker hurdle effect of soft cheeses compared with hard ones, soft cheeses demonstrate a higher frequency of bacterial contamination and a greater potential for the growth and survival of *L. monocytogenes*. In addition, soft cheeses have been involved in most of the fatal cases of listeriosis (Rogga et al., 2005). For these reasons, ready-to-eat dairy products, especially mold-ripened cheeses such as Camembert and blue cheese, are products that need a special control; especially after the last Austrian outbreak, which accounted for 14 cases in 2009, including 4 fatal cases (Fretz et al., 2010).

The growth of bacteria depends on external factors (parameters of the environment) and the internal characteristics of food products. Temperature is the most important environmental parameter governing microbial growth (Juneja et al., 2009). Determination of the influence of temperature on the behavior of pathogenic

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bacteria is one of the tools that enables us to control the growth of pathogens in food. The discipline of science that deals with predicting the response of microorganisms, especially pathogenic ones, to environmental conditions (including temperature, water activity, pH, and so on) is known as predictive microbiology. Predictive microbiology relies on the assumption that the response of microorganisms to environmental factors is reproducible. By defining the parameters that have the strongest effect on the growth of microorganisms, it is possible to predict the future response of microorganisms based on the performed observations (Devlieghere et al., 2006; Black and Davidson, 2008).

The commonly accepted classification of predictive microbiology models, suggested by Whiting and Buchanan (1993), distinguishes primary, secondary, and tertiary models (McMeekin and Ross, 2002; Baranyi and Pin, 2004). Primary models determine the basic characteristics of bacterial growth kinetics (lag phase duration, growth rate) under constant environmental conditions. A modified Gompertz function and Baranyi and Roberts model are used at this stage. Secondary models describe the influence of environmental factors on the parameters of primary models; among the widely used models in this group are square root models. Tertiary models are a combination of the 2 former groups, included in an easily applicable computer interface [e.g., Pathogen Modeling Program (PMP 7.0, USDA, Wyndmoor, PA), ComBase Predictor (Institute of Food Research, Norwich, UK)].

Models predicting the growth of bacteria are often generated in microbial culture media, under strictly defined and controlled environmental conditions (Ölmez and Aran, 2005; Xanthiakos et al., 2006). Predictions generated on the basis of such models often diverge from the actual growth of bacteria in food. To increase the applicability of predictive models in the food industry, it has been suggested to base them on microbiological data obtained from food assays (Dalgaard and Jørgensen, 1998). At this stage, a validation process of predictive models is necessary to assess the accuracy of predictions generated by the tested models. Bias (B_f) and accuracy (A_f) factors, proposed by Ross (1996) and corrected by Baranyi et al. (1999), are commonly used for this purpose.

The objective of this paper was to discuss the applicability of predictive microbiology in the modeling of microbial safety of mold-ripened cheeses with respect to *L. monocytogenes* during (1) the ripening of Camembert cheese, (2) cold storage of Camembert cheese at 3 to 15°C, and (3) cold storage of blue cheese at 3 to 15°C. Another aim was to evaluate and compare the accuracy of the primary models, such as a modified Gompertz function and the Baranyi-Roberts model, in terms of 2

factors, namely, mean square error (MSE) and Akaike's information criterion (AIC). To describe the influence of temperature on the growth rate of *L. monocytogenes* in mold-ripened cheeses, the polynomial and square root models were generated. The observed growth of *L. monocytogenes* in cheeses was compared with the simulations obtained from 2 tertiary models (the Pathogen Modeling Program and ComBase Predictor). Moreover, the validation process according to external data from the ComBase database was performed, which enabled us to determine the usability of the generated models for *L. monocytogenes* in Camembert and blue cheeses for other dairy products.

MATERIALS AND METHODS

Isolation and Preparation of the *L. monocytogenes* Culture

A single strain of *L. monocytogenes* was isolated from raw milk at the Chair of Industrial Microbiology, Faculty of Food Sciences, University of Warmia and Mazury in Olsztyn, Poland, and was registered in the Collection of Strains of the Chair of Industrial and Food Microbiology as *L. monocytogenes* 38. The isolation of the strain was carried out in compliance with the standard PN-EN ISO 11290-2:2000 (ISO, 2000).

The stock cultures of *L. monocytogenes* were maintained on tryptose soy agar (Merck, Warsaw, Poland) slopes incubated at 30°C for 48 h, subsequently stored at 2°C and subcultured monthly. Prior to use for the tests, the bacteria were grown in tryptone soy broth (Merck) at 37°C for 18 h. The count of *L. monocytogenes* after passage was ~ 9.2 log cfu/mL.

Preparation of Cheese Samples for Tests, Inoculation, and Determination of the *L. monocytogenes* Count

Samples of cheeses used for the study were taken from the manufacturer either immediately after they had been produced [Camembert cheese (Hochland Polska Sp. z o.o., Baranowo, Poland) before ripening] or after ripening [Camembert and blue cheese (S.M. Lazur, Nowe Skalmierzyce, Poland)]. The core of immature Camembert cheese before aging was inoculated with *L. monocytogenes* cells by injecting a culture with sterile needles and syringes to obtain the concentration of *L. monocytogenes* $\sim 10^3$ cfu/g. The cheese was then stored according to the maturation parameters set by the manufacturer, at 13°C for 10 d. The relative humidity was set at 95%.

Mature Camembert and blue cheese were divided into samples weighing 25 g. The culture of *L. monocytogenes*

was injected in the cheese core to reach a contamination level of 10^3 cfu/g. Contaminated samples of cheeses were incubated at 3, 6, 9, 12, and 15°C to reach full growth curves of *L. monocytogenes*.

At appropriate time intervals, depending on the applied temperature, the samples were assayed to determine the count of *L. monocytogenes* using a selective medium Chromocult agar (Merck), selective for *Listeria* according to Ottaviani and Agosti (Merck). The mean from 3 platings of each sampling point was used to establish the growth kinetics. The experiment was conducted in 3 replicates for each incubation temperature.

Mathematical Analysis

The mathematical analysis comprised elaboration of the primary and secondary models as well as a simulation of the growth of *L. monocytogenes* based on the tertiary models and, finally, validation of the generated models according to data from ComBase database (www.combase.cc).

Elaboration and Evaluation of the Primary Models

To elaborate the primary models, the following mathematical functions were used.

- A modified Gompertz function (Gibson et al., 1987),

$$x(t) = x_0 + (x_{\max} - x_0) \exp \{-\exp[-B(t - M)]\}, \quad [1]$$

where $x(t)$ = cell concentration (log cfu/mL) at time t ; x_0 = initial concentration (log cfu/mL); $x_{\max}$ = maximum cell concentration (log cfu/mL); B = maximum relative growth rate at $t = M$ (h^{-1}); and M is the time at which the absolute growth rate is maximum.

Parameter B can be used to determine the specific growth rate of the isothermal growth $\mu_{\max}$ and lag phase duration λ according to the following formula:

$$\mu_{\max} = \frac{x_{\max} - x_{\min}}{e} B, \quad [2]$$

$$\lambda = M - \frac{1}{B}, \quad [3]$$

where $\mu_{\max}$ = specific growth rate ($\log \text{cfu/h}^{-1}$); and $x_{\min}$ = minimum cell concentration (log cfu/mL; Gibson et al., 1987).

- Baranyi and Roberts function (Baranyi and Roberts, 1994),

$$y(t) = y_0 + \mu_{\max} F(t) - \ln \left(1 + \frac{e^{\mu_{\max} F(t)} - 1}{e^{(y_{\max} - y_0)}} \right), \quad [4]$$

$$F(t) = t + \frac{1}{v} \ln \left(e^{-vt} + e^{-h_0} - e^{(-vt - h_0)} \right), \quad [5]$$

where $y(t)$ = cell concentration at time t (ln cfu/g); y_0 = initial cell concentration (ln cfu/g); $y_{\max}$ = maximum cell concentration (ln cfu/g); $\mu_{\max}$ = maximum specific growth rate (h^{-1}); v = rate of increase of the limiting substrate; and h_0 = is a product of $\mu_{\max} \lambda$.

Fitting of primary models to the observed data was performed using an application of Microsoft Office (Warsaw, Poland), called Excel Solver. It involves the nonlinear optimization generalized reduced gradient. The goodness of fit of the fitted primary models was evaluated, including the calculation of the following values.

- Mean square error:

$$MSE = \frac{RSE}{n}, \quad [6]$$

where RSE = residual sum of errors; and n = the number of degrees of freedom (Sutherland et al., 1994).

- Akaike's information criterion, with bias adjustment for small sample size,

$$AIC_c = -2 \times \ln(\text{likelihood}) + 2 \times K + \frac{2 \times K \times (K + 1)}{(n - K - 1)}, \quad [7]$$

where n = number of observations; and K = number of the model's parameters. The smaller the value of AIC, the better the description of observed data by the mathematical model (Burnham and Anderson, 2002).

Elaboration and Evaluation of the Secondary Models

Secondary modeling was analyzed according to two formulas. First, the square root model described by Ratkowsky et al. (1982):

$$\sqrt{\mu_{\max}} = b(T - T_{\min}), \quad [8]$$

where $\mu_{\max}$ = maximum growth rate (h^{-1}); b = coefficient determined during the modeling process; T =

temperature (°C); and $T_{\min}$ = determined minimum temperature for the growth of microorganisms (°C). Second, the polynomial model [9],

$$\ln y = a_1 + a_2 x_1 + a_3 x_1^2, \quad [9]$$

where y = response of microorganisms [e.g., specific growth rate (h^{-1})]; a_1 , a_2 , and a_3 = adjustment factors; and x_1 = temperature (°C). For modeling purposes, the following software packages were used: MS Excel (Microsoft), Statistica 9 (StatSoft, Krakow, Poland), Nonlinear Regression Analysis Program (NRLE; Philip H. Sherrod, Nashville, TN; 1991–2001).

Mathematical Validation of Generated Predictive Models with Independent ComBase Data

Generated secondary models were validated according to data obtained from ComBase database. The following criteria were used for data selection.

- Data describing behavior of *L. monocytogenes* were generated in dairy products without the addition of any preservatives, organic acids, and so on.
- Data were obtained using the traditional plate counts method.
- Data were obtained in the temperature range from 3 to 15°C (interpolation region of generated secondary models).

The mathematical validation was performed by calculating A_f and B_f factors, as suggested by Ross (1996),

$$A_f = 10^{\left(\sum \log \mu_{\text{predicted}} / \mu_{\text{observed}} / n\right)}, \quad [10]$$

$$B_f = 10^{\left(\sum \log \left(\frac{\mu_{\text{predicted}}}{\mu_{\text{observed}}}\right) / n\right)}, \quad [11]$$

where n = number of observations; $\mu_{\text{predicted}}$ = predicted specific growth rate; and μ_{observed} = observed specific growth rate. The B_f shows consistent over- and under-prediction, whereas the coefficient of accuracy, A_f , indicates the average difference between the predicted and observed values (Dalgaard and Jorgensen, 1998).

Simulations of the Growth of *L. monocytogenes* Using Tertiary Models

Simulations of the growth of *L. monocytogenes* were carried out using the software Pathogen Modeling Program 7.0 and ComBase Predictor. In both cases, the

following initial values were entered according to the information given by the manufacturer.

- Camembert, mold-ripened cheese covered with mold, pH 5.1, NaCl 1.7%.
- Blue cheese, mold-ripened cheese veined with mold, pH 5.9, NaCl 4%.

When ComBase Predictor was used, the simulation was conducted for the temperatures identical to the ones applied in the microbiological assays (3, 6, 9, 12, and 15°C); but with the Pathogen Modeling Program, the simulations began from 4°C, which was due to the limited temperature range this program includes.

RESULTS

Behavior of *L. monocytogenes* During Aging and Cold Storage of Camembert and Blue Cheeses

The behavior of *L. monocytogenes* during the aging of Camembert cheese at 13°C for 10 d is shown in Figure 1. During that time, no increase in the count of the pathogenic cells occurred, so the final count of the bacteria was identical to the inoculated amount (i.e., ~3.5 log cfu/g of cheese).

During cold storage of Camembert cheese, *L. monocytogenes* multiplied intensively, depending on the applied temperature (Figure 2). As the temperature increased, the duration of the lag phase shortened, reaching values 146, 112, 66, 50, and 33 h for temperatures 3, 6, 9, 12, and 15°C, respectively. The maximum density of the cells did not exceed the concentration of 8 log cfu/g and was, on average, 7.54 log cfu/g (6.7–7.9 cfu/g). In

Figure 1. Behavior of *Listeria monocytogenes* during the ripening of Camembert cheese at 13°C for 10 d (3 replicates).

Figure 2. Primary modeling results describing the growth of *Listeria monocytogenes* in Camembert cheese (3 replicates) at (a) 3, (b) 6, (c) 9, (d) 12, and (e) 15°C. Dotted line = Baranyi model; dashed line = modified Gompertz model.

Figure 3. Primary modeling results describing the growth of *Listeria monocytogenes* in blue cheese (3 replicates) at (a) 3, (b) 6, (c) 9, (d) 12, and (e) 15°C. Dotted line = Baranyi model; dashed line = modified Gompertz model.

Table 1. Akaike's information criterion (AIC) and mean square error (MSE) values characterizing the goodness of fit of primary models by cheese type and temperature

Product	Temperature (°C)	AIC		MSE	
		Baranyi	Gompertz	Baranyi	Gompertz
Camembert	3	-124.46	-107.18	0.07	0.09
	6	-128.01	-123.56	0.05	0.06
	9	-83.29	-70.99	0.10	0.14
	12	-51.41	-42.44	0.11	0.15
	15	-51.41	-52.21	0.06	0.05
Blue cheese	3	-140.70	-150.47	0.04	0.03
	6	-53.28	-51.46	0.30	0.30
	9	-55.62	-54.20	0.25	0.25
	12	-67.33	-66.89	0.22	0.22
	15	-89.00	-83.60	0.03	0.03

the case of blue cheese (Figure 3), the lag phase was prolonged compared to Camembert cheese and reached 400, 350, 313, 257, and 133 h for temperatures of 3, 6, 9, 12, and 15°C, respectively. The maximum density of bacterial cells increased as the incubation temperature rose. At 3°C, the maximum count of cells in the stationary phase reached 5.8 log cfu/g of cheese, but at 15°C it was 7.3 log cfu/g.

Primary Model Fitting

During the aging of Camembert cheese (Figure 1), no growth in the population of *L. monocytogenes* occurred, therefore the data from this stage of the experiment were excluded from the mathematical modeling. The Baranyi model and modified Gompertz function were fitted to the observed data concerning the growth of *L. monocytogenes* in Camembert and blue cheese (Figures 2a–2e and 3a–3e). All of the tested models described the observed growth with satisfactory accuracy. In most of the analyzed cases (80%), the lowest AIC and MSE values, as well as the best goodness of fit, were obtained using the Baranyi and Roberts function (Table 1); therefore, the growth rates generated by this model were used for the secondary modeling purposes (Table 2).

Parameter h_0 , which describes the physiological state of the cells, should be similar in case of the identical history of the cells (Baranyi and Roberts, 1994). The product of $\mu_{\max}\lambda$ as a function of storage temperature for *L. monocytogenes* in Camembert and blue cheese is shown in Figure 4.

Secondary Modeling

For the secondary modeling, the square root and polynomial models were used to determine the effect of temperature on the growth rate of *L. monocytogenes* in Camembert and blue cheese. Figures 5 and 6 illustrate

the graphical version of the elaborated models. The parameters of the generated mathematical equations are shown in Table 3. It was noted that $\mu_{\max}$ followed a parabolic trend with storage temperature and, for this reason, the polynomial model better described the effect of temperature on the growth of the pathogen, reaching the values of $R^2 = 0.97$ and 0.92 for Camembert and blue cheese, respectively. Xanthiakos et al. (2006) explained this phenomenon as an effect of competitive microflora, which had a significant antagonistic effect toward *L. monocytogenes*.

Figure 4. Effect of applied storage temperature on the h_0 parameter for Camembert (▲) and blue cheese (□); $\mu_{\max}$ = maximum specific growth rate, λ = lag phase duration.

Figure 5. Secondary modeling results describing the effect of temperature on the growth of *Listeria monocytogenes* in Camembert cheese (μ) according to the square root and polynomial models.

Mathematical Validation with Independent ComBase Data and Comparison with Existing Tertiary Models

The last stage of work was validation of the generated Ratkowsky and polynomial models for Camembert and blue cheese on the basis of independent data obtained from the ComBase database, as well as comparison with simulations from 2 tertiary models (the Pathogen Modeling Program and ComBase Predictor). Data from ComBase were divided into 2 groups: liquid dairy products (skimmed, semiskimmed and whole milk, chocolate milk, reconstituted nonfat milk solids, whey, UHT milk) and cheeses (Camembert, whey from the manufacture of Camembert cheese, custard, mozzarella, stilton, queso fresco). The results of graphical validation using ComBase database are shown in

Figure 7 and the mathematical validation is given in Table 4. It should be noted that the polynomial models generated for Camembert cheese are more accurate in predicting the behavior of *L. monocytogenes* in liquid dairy products and cheeses (bold data in Table 4), although the A_f value averaged 1.6, indicating that the predicted growth rates were, on average, 60% different from the observed ones. The validation process of the square root model for blue cheese did not give satisfactory results, and the predicted values were significantly different from the observed growth. It was found that the generated square root and polynomial models described the behavior of *L. monocytogenes* accurately in Camembert and stilton cheeses (points are close to the equality line in Figure 7b), but failed with other types of cheese.

Table 2. Growth rates ($\mu_{\max}$) and lag times of *Listeria monocytogenes* in Camembert and blue cheeses during storage at different temperatures

Temperature (°C)	Camembert		Blue cheese	
	$\mu_{\max}$ (h ⁻¹)	lag time (h)	$\mu_{\max}$ (h ⁻¹)	lag time (h)
3	0.03	146.4	0.01	398.8
6	0.04	112.5	0.01	348.5
9	0.06	66.5	0.01	313.1
12	0.08	50.7	0.02	257.6
15	0.12	33.3	0.03	133.3

Table 3. Parameters of generated square root and polynomial models

Model	Parameter ¹	Camembert	Blue cheese
Polynomial	a	0.0089	0.0049
	a_1	-0.0011	-0.0002
	a_2	0.0003	0.0000
Square root	b	2.2936	-7.2967
	$T_{\min}$	0.0041	0.0004

¹ a , a_1 , and a_2 = adjustment factors; b = coefficient determined during the modeling process; $T_{\min}$ = determined minimum temperature for the growth of microorganisms (°C).

Comparison of the observed data with simulations produced by the general tertiary models did not provide satisfactory results (Table 5). In comparing the A_f and B_f values for Camembert and blue cheese, it should be noted that square root model developed for Camembert cheese was burdened with a smaller error in comparison with model for blue cheese. However, the model still differs from simulations by approximately 25%.

DISCUSSION

Camembert cheese ripens relatively quickly, as the moisture content is high and the growth of surface layer of mold is intensive (Schlesser et al., 1992). Dur-

ing this stage in cheese processing many chemical and microbiological changes take place; *L. monocytogenes* is not capable of growing during the ripening of Camembert cheese. In a study conducted by Ryser and Marth (1987), strains of *L. monocytogenes* started to grow after 18 d of cheese ripening, reaching the maximum count of cells (1×10^6 to 5×10^7 cfu/g) after 65 d of cheese ripening. The behavior of the pathogen at this stage was most likely affected by the low pH of cheese immediately after production (~4.4), which continued to rise during the ripening up to ~6.5. Another probable factor influencing the growth was the Jameson effect (i.e., competition between particular groups of microorganisms to obtain nutrients necessary for their growth; Back et al., 1993; Murphy et al., 1996; Irlinger and Mounier, 2009).

Linton et al. (2008) demonstrated that *L. monocytogenes* is able to survive the production process of Camembert cheese. In their study, milk for cheese production was contaminated with up to 1.95 log cfu/g of *Listeria* cells. The count of bacteria rose to 4.52 log cfu/g in curd and remained at a high level during cheese ripening (13°C for 14 d), reaching the concentration of 3.85 log cfu/g in mature cheese.

The growth of *L. monocytogenes* in blue cheese was not as intensive as in Camembert cheese. This is

Figure 6. Secondary modeling results describing the effect of temperature on the growth of *Listeria monocytogenes* in blue cheese (μ) according to square root and polynomial models.

Figure 7. Validation of generated Ratkowsky and polynomial models for Camembert and blue cheeses according to independent data from ComBase (Institute of Food Research, Norwich, UK) obtained in (a) liquid dairy products and (b) cheeses. μ_{obs} = observed specific growth rate; μ_{pred} = predicted specific growth rate.

evidenced by a much longer lag phase duration in blue cheese compared with Camembert. Papageorgiou and Marth (1989) found that the growth of *L. monocytogenes* can also be affected by the presence of free FA, methyl ketones, and corresponding secondary alcohols, as well as an elevated content of NaCl (4%). The negative influence of a high NaCl concentration on the growth of *L. monocytogenes* has also been documented (Buchanan et al., 1998).

By analyzing the h_0 parameter, it was concluded that the applied storage temperature does not influence its value, which was, on average, 4.57 and 4.23 for Camembert and blue cheese, respectively. The independence of temperature from the physiological state of the cells has been reported in the literature, where researchers found that the product of $\mu_{\text{max}}\lambda$ is constant under different storage temperature conditions (Koutsoumanis et al., 2006).

Table 4. Validation results of generated Ratkowsky and polynomial models describing the growth of *Listeria monocytogenes* for Camembert and blue cheeses on the basis of independent data from ComBase (Institute of Food Research, Norwich, UK)

Product	n	Model	A_f^1	B_f^2
Liquid dairy	20	Ratkowsky_Camembert	1.72	1.53
		Ratkowsky_blue cheese	4.68	4.64
		Polynomial_Camembert	1.64³	1.35
		Polynomial_blue cheese	4.85	4.75
Cheese	7	Ratkowsky_Camembert	1.60	0.87
		Ratkowsky_blue cheese	3.02	2.65
		Polynomial_Camembert	1.60	1.02
		Polynomial_blue cheese	1.78	1.67

¹ A_f = accuracy factors.

² B_f = bias factors.

³Bold cells indicate more accurate prediction from the polynomial models.

The square root model yielded satisfactory predictions regarding the effect of temperature on the growth rates of *L. monocytogenes* in Camembert and blue cheese (Figures 5 and 6), which is reflected in the adjusted coefficients of multiple determination, -0.91 and 0.85 for Camembert and blue cheese, respectively. A more accurate description of the influence of temperature on the growth rate of *L. monocytogenes* was obtained by the polynomial model, which is reflected by a higher R^2 value (0.97 and 0.92 for Camembert and blue cheese, respectively). The generated models analyze only 1 independent variable, namely, the storage temperature. Giffel and Zwietering (1999) demonstrated that models that did not include the interactions between environmental factors were, in many cases, accurate enough to predict the growth rate of *L. monocytogenes*.

Elaborated mathematical models can be successfully used to predict the behavior of *L. monocytogenes* in Camembert and blue cheese, but caution is needed when applying to other dairy products. Augustin et al. (2005) analyzed existing cardinal and square root models that describe the behavior of *L. monocytogenes* in dairy, meat, and seafood products, and suggested new cardinal models that include interactions. In their study, validation performed on external data describing the growth of *L. monocytogenes* in cheeses did not give satisfactory results, even though the B_f values were close to 1 and the A_f values were very high (3.5, on average).

The Pathogen Modeling Program and ComBase Predictor consistently over-estimated the growth of *L. monocytogenes* in both Camembert and in blue cheese (Table 5), which was most likely caused by the differences in the substrate for bacterial growth. Both tertiary models were developed on the basis of data acquired from liquid cultures, where the growth of microorganisms is generally faster than in solid matrices. Moreover, Pathogen Modeling Program and ComBase Predictor are based on pure cultures, and the effect of

inhibiting microflora on *L. monocytogenes* is not taken into account. Although both programs take into account the influence of such parameters as temperature, pH, and NaCl (%), the generated predictions contain a significant level of error. Microbial growth overestimation by the Pathogen Modeling Program in comparison with the actual behavior of microorganisms in food has been previously reported (Murphy et al., 1996; Dalggaard and Jorgensen, 1998; Augustin et al., 2005).

To improve the usability and applicability of predictive models in the food industry, generated models should be orientated toward a specific product or a group of similar products, and the microbiological data that serve to build such models should be derived from tests performed on directly contaminated food products. Throughout the present study, it was proven that models generated on the basis on microbiological data often are not accurate enough to describe microbiological behavior in real food products. Only validated mathematical models can be useful in the identification of critical control points in hazard analysis and critical control points, risk assessment, assessing the shelf-life of food products, and developing new products.

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Table 5. Comparison of the observed growth of *Listeria monocytogenes* in mold-ripened cheeses with tertiary model simulations Pathogen Modeling Program (PMP; PMP 7.0 USDA, Wyndmoor, PA) and ComBase Predictor (CP; Institute of Food Research, Norwich, UK) on the basis of accuracy (A_f) and bias (B_f) factors

Product	A_{f-CP}	B_{f-CP}	A_{f-PMP}	B_{f-PMP}
Camembert	1.24	0.81	1.02	0.87
Blue cheese	4.27	0.23	1.04	0.51

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