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Temperature Effect on *Listeria monocytogenes* Growth in the Event of Contamination of Cooked Pork Products

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ABSTRACT

The aim of this study was to describe the effect of temperature on the growth of *Listeria monocytogenes* in the event of postprocess contamination of packaged pork meats. This study was carried out in two steps. In the first step, the effect of temperature on *L. monocytogenes* growth rates was determined in duplicates at 13 temperatures between 2 and 43°C by turbidimetric methods and adjusted by a quantitative secondary model. Then, seven sets of growth kinetics were collected by challenge testing in white pudding and roulade, both cooked pork products prepared according to an industrial process and stored at suboptimal temperatures ranging from 2 to 20°C. In the second step, objectives were to (i) collect direct information on the temperature effect of *L. monocytogenes* on the two pork products, (ii) compare the two products regarding *L. monocytogenes* exposure, and (iii) compare results given by modeling (step i) with results obtained independently and then evaluate the model application domain. Each kinetic was built with at least 10 experimental data and two replicates. Comparison between *L. monocytogenes* behavior at 4°C on white pudding and roulade indicated that both meat products were affected by food safety problems. Indeed, after contamination and storage for 10 days at 4°C, the bacterial population increased by 2 log CFU/g in both products. Comparison between growth kinetic simulations and experimental data obtained separately gave satisfactory conclusions; the difference between observed and predicted bacterial population values was always less than 1 log CFU/g and a bias factor of 1.18 when growth rates were compared. These results applied to *L. monocytogenes* contamination of white pudding or roulade can now be used either in the management of optimal process and distribution networks or in risk assessment (exposure assessment).

Over the last 20 years, food safety has been improved in the industry by hurdle technology to formulate products *(12)* or by rational methods such as hazard analysis critical control point (HACCP), decision support *(27),* or predictive modeling *(15).* However, in cooked pork products (e.g., ham, sausage, pâté) that are to be sliced or packed after cooking, postprocess contamination by *Listeria monocytogenes* is still possible *(1, 5, 9).* Moreover, because of its capacity to grow at low temperature, *L. monocytogenes* is considered a pathogenic bacterium of major interest *(4, 18, 24, 25).*

In the food safety domain, predictive microbiology *(15)* is a convenient way to describe the effects of environmental factors on the growth, survival, or decrease of foodborne microorganisms *(14, 17, 26).* Among environmental factors, temperature, pH, and water activity are the most significant. Quantitative models provide information to make decisions in the prediction of risk (estimation of bacterial population after a period of storage) and in product development (microbial consequences from changes in food composition). Otherwise, challenge-test procedures are used widely in the food industry to evaluate the exposure of products in the event of microbial contamination and to provide product shelf-life limitations. Challenge tests are relatively expensive (process and laboratory work) and time consuming (especially at refrigerated temperatures), whereas modeling is quick and easy to use after validation.

The aim of this paper is to describe the effect of temperature on the growth of *L. monocytogenes* Lm 111 contamination of postprocess-packaged pork meat. This study was realized with the combination of two approaches: (i) a quantitative microbiologic approach to evaluate the effect of temperature on *L. monocytogenes* Lm 111 growth rates and, consequently, to simulate the growth at various refrigerated temperatures and (ii) challenge tests conducted on food products—white pudding and roulade—to assess the accuracy of the model and then to validate its use in the pork meat industrial context.

MATERIALS AND METHODS

Organism and culture and subculture conditions. The strain used in this study was *L. monocytogenes* (Lm 111), a wild-type isolated from pork meat at Gourmets de l'Artois, France. The laboratory culture medium was brain heart infusion (Difco, Sparks, Md.).

To establish the temperature effect on growth parameters, two subcultures from frozen strains were carried out in 250-ml flasks containing 50 ml of medium, and the culture was carried out in 17-ml tubes containing 5 ml of medium. The first subculture was incubated at 30°C and the second subculture at the same temperature as the culture. Twelve temperatures were studied: 2, 3.5, 5, 7, 10, 15, 25, 30, 35, 37, 40, and 43°C.

To determine the specific growth rate, μ, at each temperature,

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a method based on the turbidimetric measurement of a bacterial population in consecutive binary dilution tubes *(7, 16)* was used. A spectrophotometer (320-RD-constant, Prolabo, Paris, France) adapted to measure bacterial population concentration through the tubes, was employed at 550 nm. The second subculture was diluted and then inoculated into the first culture tube (D1) to obtain an initial concentration of 10^6 CFU/ml. From D1, consecutive binary dilutions were performed to obtain eight tubes containing different inoculum levels. Two replicates and one control were generated for each dilution.

To perform challenge tests on pork meat, two subcultures from frozen strains were incubated in 250-ml flasks containing 50 ml of brain heart infusion, the first at 30°C and the second at the temperature of the challenge test.

All brain heart infusion cultures and subcultures were incubated and shaken (90 rpm) in a waterbath SWB25 controlled by a cryostat DC10-K15 with a precision of ±0.1°C (Haake, Karlsruhe, Germany).

Cooked pork products. The first cooked pork product, white pudding, was prepared by ADRIANOR according to an industrial process. Ingredients used to produce white pudding include natural casing, meat and fat (Vandeville, Tilloy, France), whole egg (SOVIMO, Vimy, France), whole milk powder (Ingredia, Arras, France), glucose syrup (Roquette, Lestrem, France), ascorbic acid and polyphosphates (Vopak, Paris, France), spices (Darégal, Milly la Forêt, France), and cognac (Martel, Cognac, France). Minced meats were mixed with other ingredients in a cutter for 3 min at 2,500 rpm (cutter UMC12, Stephan, Lognes, France). Then the meat emulsion was cased in a hydraulic press (NBS, Haubourdin, France), cooked in water (25 min; 80°C), and finally cooled at the initial temperature value of the challenge test. Four sets of white pudding were used to study the effect of temperature on *L. monocytogenes* Lm 111 growth (4, 10, 20 to 10, and 10 to 2°C).

The second cooked pork product was a French cooked pork product, called roulade, with a process similar to the Frankfurt sausage. Samples of roulade were provided directly by the industrial company (Gourmets de l'Artois, Libercourt, France). Pork patties have a larger diameter (110 mm) than sausage. As in white pudding technology, ascorbic acid and polyphosphates were used as ingredients to prepare roulade, and minced meat was emulsified. After cooking, roulade was conditioned in a vacuum package. Three different sets of roulade were used to study the effect of temperature on *L. monocytogenes* Lm 111 growth (4, 8, and 20°C).

Challenge testing: pork meat inoculation and bacterial population counts. White pudding samples were inoculated by immersion in 6 liters of a 9.5 g/liter tryptone salt solution (AES, Combourg, France) containing 10 ml of the second subculture (bacterial population concentration was approximately 1.6×10^6 CFU/ml), drained off, and stocked in boxes covered by plastic film to avoid water and oxygen transfer.

Roulade samples were sliced under aseptic conditions (depth 1.5 mm, 15 g per slice). Roulade slices were inoculated with 10 spots of 10 μl of the second subculture after appropriated dilution in a 9.5 g/liter tryptone salt solution. Each inoculated slice was packaged in a high-density polyethylene box under modified atmosphere: CO_2 50%; N_2 50% (Air Liquide, Choques, France).

White pudding and roulade samples were incubated in a refrigerated incubator (Memmert 600 ICP, Schwabach, Germany). The temperature inside the sample was measured with a thermal probe with a precision of ±0.1°C (Testostor 175-1, Forbach, France).

The bacterial population was measured by counting cell colonies of *L. monocytogenes* on Oxford *Listeria* selective agar (Biokar) after appropriate dilution in a 9.5 g/liter tryptone salt solution in sterile tubes. At least, two replicates of growth curves were generated for each experimental treatment. Total mesophile microflora was determined on plate counting agar (Biokar) at the initial and final step of kinetics in both food contaminated with *L. monocytogenes* and noninoculated controls.

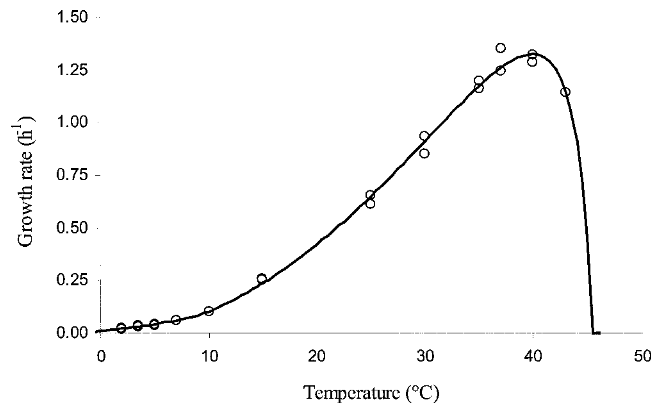

FIGURE 1. *The specific growth rate of* Listeria monocytogenes *Lm 111 versus temperature. Observed values (symbols) and secondary predictive model (solid lines). The mathematical expressions are given in equation 1; the parameters values are in Table 1.*

Statistical analyses and simulation procedures. All the statistical analyses were performed by S-plus software (AT&T Bell Laboratories, Murray Hill, N.J.). To determine the specific growth rate, μ, the statistical analyses of optical density curves were obtained by a method adapted from Cuppers and Smelt *(7)*; the detection time was computed by regression in the linear phase, and when plotted versus initial dilution values, the slope of the linear regression corresponded to $-\mu$ *(16)*. To fit the primary and secondary predictive models to the data, nonlinear regression was applied *(11)*. To homogenize the variance, the temperature-dependent model was fitted after a square root transformation of the response, μ.

To generate simulation kinetics, when the temperature was constant along all the kinetics (at 4, 8, 10, and 20°C), the modeling procedure was achieved using the equations on an Excel spreadsheet. When the temperature was decreasing from 20 to 10°C, or 10 to 2°C, the growth kinetic equations were applied under a differential form with Matlab software (The MathWorks, Inc., Natick, Mass.). However, results were compared with those obtained by a direct use of the growth kinetic under the integrated form, step by step (temperature profiles simplified as a series of small intervals [1°C], in which the temperature could be considered constant). The difference between the two methods, in our study, was less than 0.2 log CFU/g in the bacterial population estimation, which led us to prefer the integrated form because it is convenient to use in an Excel spreadsheet by industrial and technical centers such as Adrianor.

RESULTS AND DISCUSSION

Quantitative microbiology: temperature-dependent growth model. In laboratory medium, growth rates of *L. monocytogenes* Lm 111 strain were studied at different levels of temperature (from 2 to 43°C). Optical density curves (192: 8 dilutions, 12 levels, 2 replicates) were generated to obtain the temperature-dependent growth parameters. The pH and

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TABLE 1. *Model parameters (equations 1 and 2) to describe the temperature effect on* L. monocytogenes *Lm 111 growing on white pudding*

Temperature-dependent parameters[a] (°C)	
T_{min}	−5.0
T_{opt}	39.9
T_{max}	45.5
T_1	0.3
Food-dependent parameters	
N_{max} (log CFU/g)	9.2
μ'_{opt} (h^{-1})	0.90
L_{min} (h)	1.20

[a] To avoid an overparameterization, T_c was chosen at the constant value of 9°C.

water activity were not studied as growth-dependent factors because the objective of this work was to mimic microbial proliferation in pork meat packaged with constant pH and water activity values (pH 6.2, $a_w = 0.975$ at the white pudding surface; pH 6.2, $a_w = 0.980$ in roulade slices), not to predict microbial proliferation on products with pH or a_w changes.

The temperature-dependent growth term used in this study was based on the secondary predictive model *(23)* adapted to *Listeria (13)* to take the specific behavior of *L. monocytogenes* at low temperatures into account *(2)* (equation 1).

$$\text{if } T > T_{max} \quad \text{then } \gamma 1(T) = 0$$
$$\text{if } T < T_{min} \quad \text{then } \gamma 1(T) = 0$$
$$\text{if } T_{min} \leq T \leq T_{max} \quad \text{then:}$$
$$\text{if } T < T_c \quad \text{then } \gamma 1(T) = \gamma 1'(T)$$
$$\text{if } T \geq T_c \quad \text{then } \gamma 1(T) = \gamma 1''(T)$$

$$\gamma 1'(T) = \langle [(T_c - T_{max})(T_c - T_1)^2] \div \{(T_{opt} - T_1) \times [(T_{opt} - T_1)(T_c - T_{opt}) - (T_{opt} - T_{max})(T_{opt} + T_1 - 2T_c)]\}\rangle \times \left[\frac{(T - T_{min})}{(T_c - T_{min})}\right]^2$$

$$\gamma 1''(T) = [(T - T_1)^2(T - T_{max})] \div \{(T_{opt} - T_1) \times [(T_{opt} - T_1)(T - T_{opt}) - (T_{opt} - T_{max})(T_{opt} + T_1 - 2T)]\} \quad (1)$$

T is the temperature and T_{min}, T_{opt}, and T_{max} are the estimated values of the minimal, optimal, and maximal temperatures, respectively, in which growth is observed. In addition, T_c corresponds to the estimated value of the critical temperature under which the usual secondary model *(23)* no longer fits *L. monocytogenes* growth rates. When the temperature is above T_c, T_1 corresponds to the apparent minimal temperature (equivalent to T_{min} in the $\gamma 1''[T]$ expression).

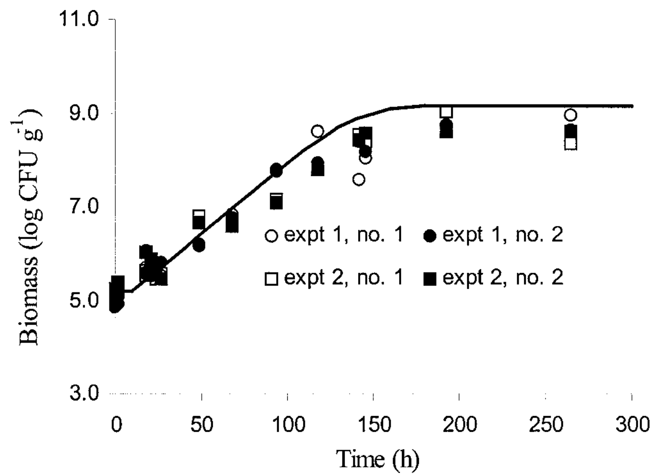

FIGURE 2. *Growth kinetics of* Listeria monocytogenes *on white pudding surface. Validation of predictive models with a set of data obtained independently at 10°C. Symbols, observed values; solid lines, predicted values.*

L. monocytogenes growth rate versus temperature was accurately fitted by equation 1 (Fig. 1). The parameter values are given in Table 1. To avoid an overparameterization, T_c was chosen at 9°C, which is consistent with literature data *(3)*.

Experiments on cooked pork products (white pudding and roulade). The second part of the study concerned the bacterial behavior after cooking in food products to be sliced or packed. Cooked pork products were prepared, packaged, and stored following an industrial and commercial process. Seven sets of growth kinetics were established with food to obtain direct information of the temperature effect on *L. monocytogenes* contamination of two cooked pork products—white pudding and roulade—and to compare the results given by modeling with kinetics established with food.

On white pudding surfaces, data were generated at a constant temperature of 10°C (Fig. 2) and at decreasing temperature values from 20 to 10°C (Fig. 3) and from 10 to 2°C (Fig. 4). These two dynamic profiles were obtained to mimic cooling processes observed after the cooking and packaging steps of this product.

With roulade, data were generated at constant temperatures of 4, 8, and 20°C to obtain information on *L. monocytogenes* contamination when stored under satisfactory conditions (4°C, Fig. 5), high-limit refrigerated conditions (8°C, Fig. 6), or even ambient environmental temperatures (20°C, Fig. 7).

To simulate bacterial kinetics, a primary (differential form written as equation 2, *(21)*) and a secondary model (equation 1) were combined.

$$\frac{dN}{dt} = \begin{cases} 0 & \text{if } t < L \\ \mu N\left(1 - \frac{N}{N_{max}}\right) & \text{if } t \geq L \end{cases}$$
$$\mu = \mu'_{opt}\gamma 1(T) \quad (2)$$

In equation 2, t is time (h) and N is the bacterial population

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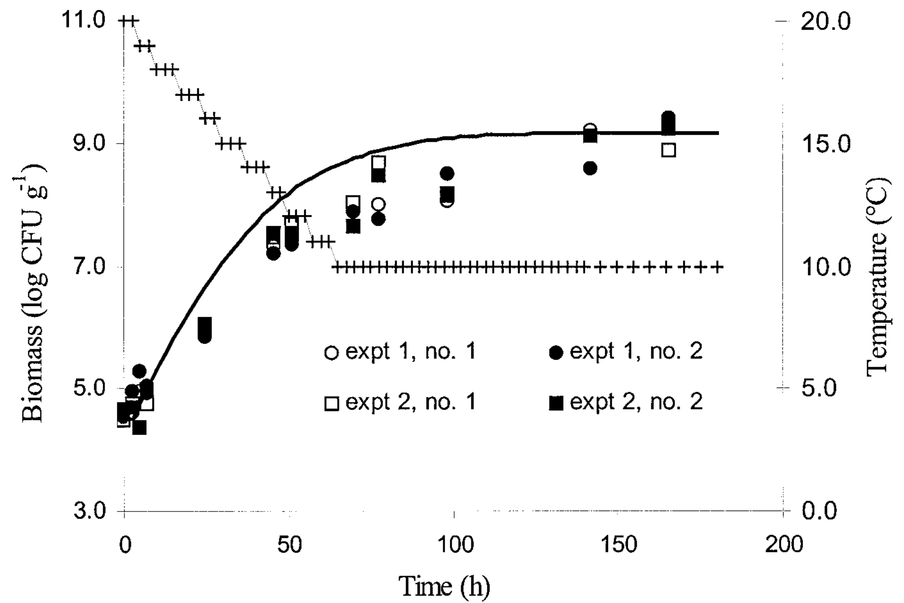

FIGURE 3. *Growth kinetics of* Listeria monocytogenes *on white pudding surface. Validation of predictive models with a set of data obtained independently at temperatures decreasing from 20 to 10°C (+). Symbols, observed values; solid lines, predicted values.*

concentration (CFU/g). This modeling approach *(22)* suggested that the bacterial growth rates, μ, depend both on environmental conditions (only the temperature in this study, through the term $\gamma 1(t)$ described by equation 1) and on the food matrix through the parameter μ'_{opt}.

The maximum bacterial population concentration N_{max} (CFU/g) was considered representative of the growth of *L. monocytogenes* Lm 111 on the food product. After integration of equation 2, the initial bacterial population concentration was written N_0 (CFU/g). This parameter was considered representative of the contamination scenario and dependent on the experimental design in the challenge test.

Because the experimental conditions of the second subculture and the culture were at similar temperatures, the simplified hypothesis $L = (\mu'_{opt} \cdot L_{min})/\mu$ was assumed *(10).* This hypothesis was consistent with literature results concerning the temperature change effect: the smaller the temperature discrepancy between subculture and culture, the shorter the lag time observed *(6, 25).*

To determine the food-dependent growth parameters, μ'_{opt}, L_{min}, and N_{max}, the equations were adjusted to one additional experimental data set obtained at 4°C on white pudding (Fig. 8). The parameter values are given in Table 1. The choice of temperature (4°C) used to fit the primary model to cooked pork products was made, taking into account the possible additional quantitative microbiology application to pork meat products stored at refrigerated temperatures (mainly in a range from 0 to 8°C).

Accuracy and application domain of the model. Although white pudding was chosen to obtain the food-dependent growth parameters, the challenge tests performed on white pudding at different temperatures (10, 20 to 10, and 10 to 2°C) were used to compare the model results with the observed kinetics and thus to validate the temperature-dependent model (equation 1).

On the other hand, the challenge tests carried out on the second cooked pork product, roulade (at temperatures of 4, 8, and 20°C), were used to evaluate whether a model built on one food product could be extended to another product that is relatively similar (both pork meat ingredients, emulsified and cooked). Moreover, because the same

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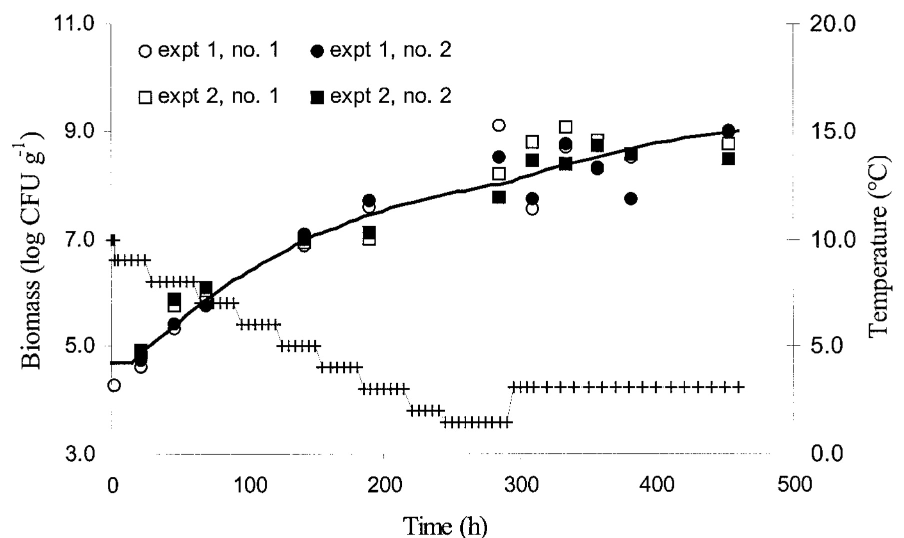

FIGURE 4. *Growth kinetics of* Listeria monocytogenes *on white pudding surface. Validation of predictive models with a set of data obtained independently at temperatures decreasing from 10 to 2°C (+). Symbols, observed values; solid lines, predicted values.*

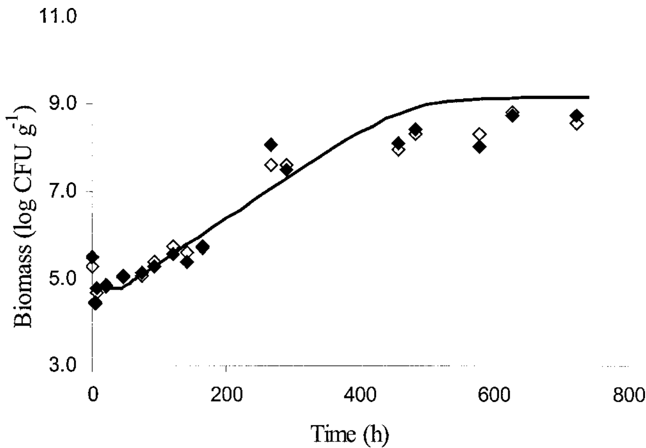

FIGURE 5. *Growth kinetics of* Listeria monocytogenes *on roulade. Application of predictive models with a set of data obtained independently at 4°C. Symbols, observed values; solid lines, predicted values.*

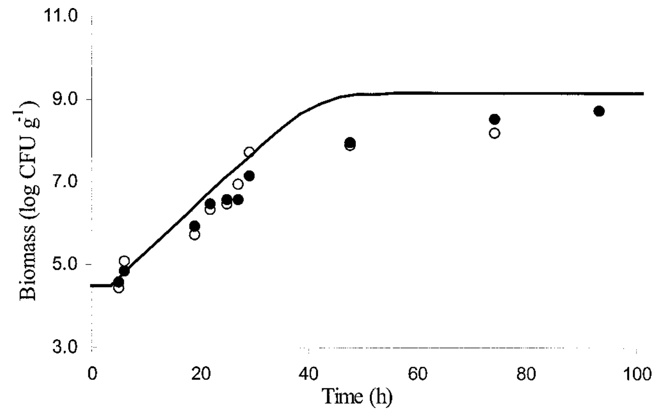

FIGURE 7. *Growth kinetics of* Listeria monocytogenes *on roulade. Application of predictive models with a set of data obtained independently at 20°C. Symbols, observed values; solid lines, predicted values.*

strain was used throughout the study, this part of the work led us to evaluate the performance of the complete methodology, including the temperature-dependent (equation 1) and food-dependent models (equation 2). In fact, the difference between predicted and observed values is first the result of the lack of fit due to the model approach. By this mechanism, the set of data obtained with the roulade product was used to calculate the bias factor by a comparison between predicted and observed growth rates *(20)*. Moreover, a comparison with Pathogen Modelling Program (PMP *(19)*) results was made, even though the software was built on the Gompertz equation as the primary model, which can generate an overestimation of growth rates and bias the comparison.

Whatever the temperature profile was between 2 and 20°C for both cooked pork products, the comparison between growth kinetic simulations and experimental data obtained separately gave satisfactory conclusions. Indeed, the difference between observed and predicted values (Figs. 2 through 7) was always less than 1 log CFU/g. In our study, the growth rate was computed with the specific model adapted to *Listeria* behavior at low temperatures (equation 1), which confirmed the relevance of this approach *(2, 12)*.

Furthermore, to evaluate the performance of the complete methodology, including the temperature-dependent model (equation 1) and food-dependent model (equation 2), the bias factor *(20)* on growth rates (bias factor >1 means a fail-safe model) of kinetics obtained on roulade at 4, 8, and 20°C was calculated. The bias factor was found to be 1.18 with our approach (equations 1 and 2), whereas the comparison between observed growth rates and PMP results gave a bias factor of 2.05 (temperatures of 4, 8, and 20°C; pH 6.2; a_w = 0.98; *L. monocytogenes*). This result is in agreement with validation studies of predictive models of *L. monocytogenes* on cold-smoked salmon *(8)*: a general model without a parameter introducing the specific food effect overestimated the growth rates. In addition, our model with parameters determined for one food product could be extended to another product, thus simplifying future challenge-test procedures.

For the lag phase, the simplified hypothesis *(10)* was good enough because short lag time values were observed in relation to kinetic duration. For instance, the lag time at

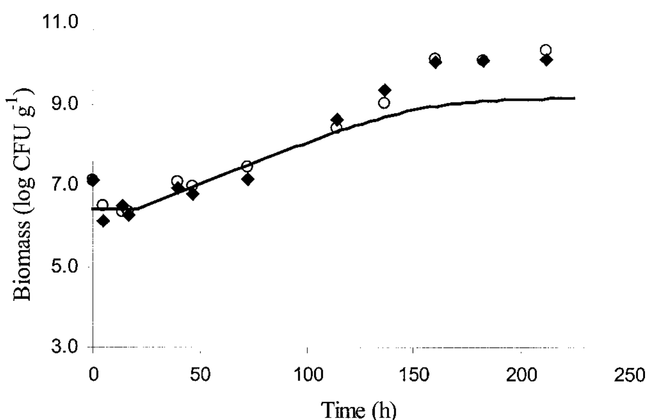

FIGURE 6. *Growth kinetics of* Listeria monocytogenes *on roulade. Application of predictive models with a set of data obtained independently at 8°C. Symbols, observed values; solid lines, predicted values.*

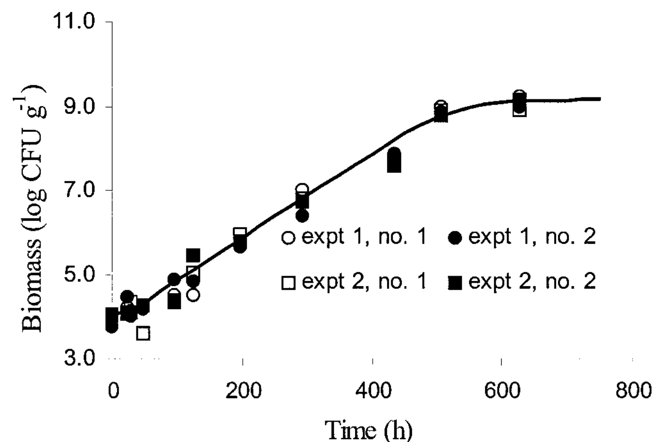

FIGURE 8. *Growth kinetics of* Listeria monocytogenes *on white pudding surface. Curves fit at 4°C. Symbols, observed values; solid lines, predicted values. The mathematical expressions are given in equation 2; the parameters values are in Table 1.*

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10°C (Fig. 2) was less than 20 h, whereas the stationary phase was achieved after about 170 h.

Comparison between *L. monocytogenes* behavior on white pudding and roulade was also performed directly with data obtained at 4°C (Figs. 8 and 5, respectively). The kinetic profiles were very similar: after contamination of the two products, the bacterial population increased 2 log CFU/g after 10 days (240 h). This similarity between the two products was a result not only of relatively similar pH and water activity values (pH 6.2 for both products, a_w = 0.975 for white pudding 0.980 for roulade), but also similar ingredients (ascorbic acid, polyphosphates) and manufacturing processes (minced meat, emulsified and cooked). Even when stored under satisfactory refrigerated conditions (4°C), these two products present food safety problems.

The application domain for the results of the white pudding and roulade study is wide: (i) to simplify challenge testing on cooked pork products at refrigerated temperature values and to assist in the management of optimal process and distribution networks; (ii) to mimic microbial proliferation in a food stored successively at 2 and 8°C, as recommended by the French Food Safety Agency, in order to evaluate the shelf-life limit *(1)*; and (iii) to assist in risk assessment (particularly in the exposure assessment step) by quantifying the effect of suboptimal temperatures on bacterial growth. However, because only a temperature-dependent growth model has been built, results cannot be extrapolated to products in which pH or a_w change with the process.

The same methodology, associating predictive microbiology and challenge testing, could be applied to other cooked pork products that are to be sliced or packed after cooking (e.g., pâté, bacon, coppa, sausage) and thus possibly contaminated by *L. monocytogenes.* In this case, results of the first quantitative microbiology step, in which the temperature-dependent growth parameters—T_{min}, T_{opt}, T_{max}, and T_1 (equation 1)—have been obtained, can be used without additional experiments. Nevertheless, challenge tests at different temperatures should be carried out to estimate the parameters μ'_{opt}, L_{min}, and N_{max} (equation 2) of bacterial growth on a food product in order to determine the application domain before simulation.

More generally, the methodology suggested in this paper could be used to study the effect of temperature on the behavior of other pathogenic bacteria in contaminated food products. Quantitative microbiology combined with experiments on foodstuffs is a helpful way of reducing challenge-test costs, making decisions concerning food safety, and teaching food industry partners about environmental factor modification consequences.

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