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Development of a Microbial Model for the Combined Effect of Temperature and pH on Spoilage of Ground Meat, and Validation of the Model under Dynamic Temperature Conditions

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The changes in microbial flora and sensory characteristics of fresh ground meat (beef and pork) with pH values ranging from 5.34 to 6.13 were monitored at different isothermal storage temperatures (0 to 20°C) under aerobic conditions. At all conditions tested, pseudomonads were the predominant bacteria, followed by *Brochothrix thermosphacta*, while the other members of the microbial association (e.g., lactic acid bacteria and *Enterobacteriaceae*) remained at lower levels. The results from microbiological and sensory analysis showed that changes in pseudomonad populations followed closely sensory changes during storage and could be used as a good index for spoilage of aerobically stored ground meat. The kinetic parameters (maximum specific growth rate [μ_{max}] and the duration of lag phase [λ]) of the spoilage bacteria were modeled by using a modified Arrhenius equation for the combined effect of temperature and pH. Meat pH affected growth of all spoilage bacteria except that of lactic acid bacteria. The "adaptation work," characterized by the product of μ_{max} and $\lambda(\mu_{max} \times \lambda)$ was found to be unaffected by temperature for all tested bacteria but was affected by pH for pseudomonads and *B. thermosphacta*. For the latter bacteria, a negative linear correlation between $\ln(\mu_{max} \times \lambda)$ and meat pH was observed. The developed models were further validated under dynamic temperature conditions using different fluctuating temperatures. Graphical comparison between predicted and observed growth and the examination of the relative errors of predictions showed that the model predicted satisfactorily growth under dynamic conditions. Predicted shelf life based on pseudomonads growth was slightly shorter than shelf life observed by sensory analysis with a mean difference of 13.1%. The present study provides a "ready-to-use," well-validated model for predicting spoilage of aerobically stored ground meat. The use of the model by the meat industry can lead to effective management systems for the optimization of meat quality.

Fresh meat is a highly perishable food product and unless appropriately actions are taken, e.g., packaged, transported and stored at refrigeration temperatures, can spoil in relatively short time. Factors affecting meat spoilage include intrinsic (e.g., pH, a_w, composition, type, and extent of initial contamination) and extrinsic parameters (e.g., temperature and packaging atmosphere). Among these, temperature is considered the most important factor. Although most countries have established regulations with maximum temperature limits for refrigeration storage, in practice these are often violated. Survey studies have shown that temperature conditions higher than 10°C are not unusual during transportation, retail storage, and consumer handling (13, 15). Such temperature abuses during any stage of the chill chain may result in an unexpected loss of quality and a significant decrease of meat shelf life.

Challenge tests are the main current method used by the meat industry and academia to evaluate product's shelf life. The disadvantages of this approach are well known (30). Estimation of shelf life based on this method is valid only for the conditions tested, while any changes to these conditions require repetition of the test. Furthermore, no information is provided on the magnitude of influence of the controlling factors on microbial growth and product shelf life.

An alternative to traditional methods in estimating shelf life of foods is to use the concept of predictive microbiology. Predictive or quantitative microbiology (31) involves knowledge of microbial growth responses to environmental factors expressed in quantitative terms by mathematical equations (models). The data and models can be stored in databases and used to interpret the effect of processing, distribution, and storage conditions on microbial growth (31). This approach provides precision in estimating the shelf life of foods. In addition, the combination of data on the temperature history of the product and mathematical models may lead to "intelligent" product management systems for the optimization of food quality and safety at the time of consumption (13, 21, 22).

During the last decade a significant number of mathematical models for the growth of various spoilage bacteria, such as *Photobacterium phosphoreum*, pseudomonads, *Shewanella putrefaciens*, and *Brochothrix thermosphacta*, have been published (6, 27). Despite this progress, however, spoilage models remain a research tool rather than an effective industrial application (28). There are a number of reasons for this.

(i) The developed models were based on observations in a well-controlled laboratory environment with microbiological

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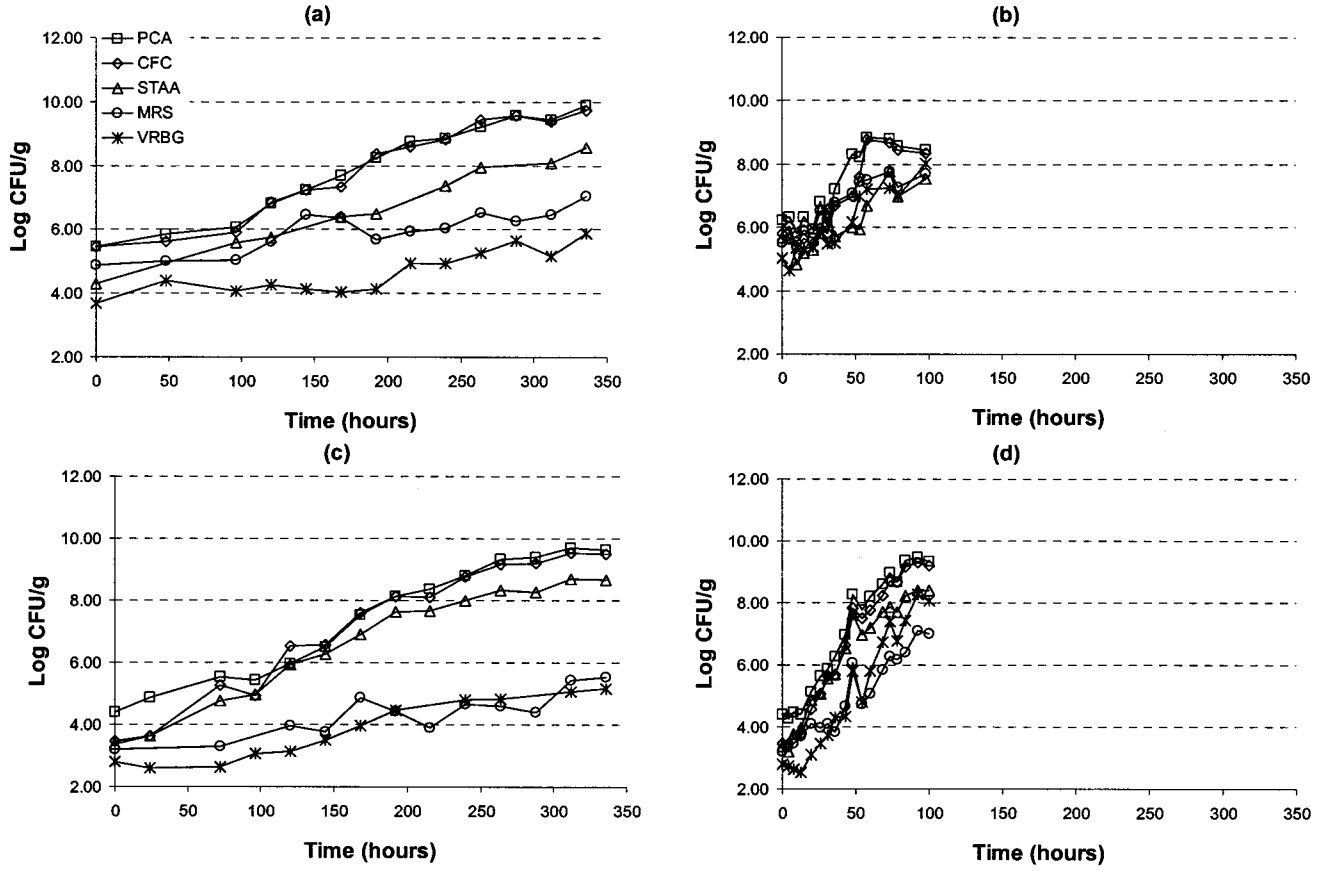

FIG. 1. Representative growth curves of the spoilage microflora on ground meat: ground beef with pH 5.34 (a and b) and ground pork with pH 6.13) (c and d) stored aerobically at 0°C (a and c) or 10°C (b and d). Media: PCA, plate count agar (total aerobic populations); CFC, cetrimide fusidin cephaloridine (pseudomonads); STAA, streptomycin-thallous acetate-actidione agar (*Brochothrix thermosphacta*); MRS, Man Rogosa Sharp (lactic acid bacteria); VRBG, violet red bile glucose agar (*Enterobacteriaceae*).

media. Predictions based on such models are not necessarily valid in complex food environments such as meat since significant factors for microbial growth such as structure of food (37, 40, 47) and interaction between microorganisms (16, 36) are not taken into account. As a result, application of the models to food products often shows low accuracy, which limit industry confidence.

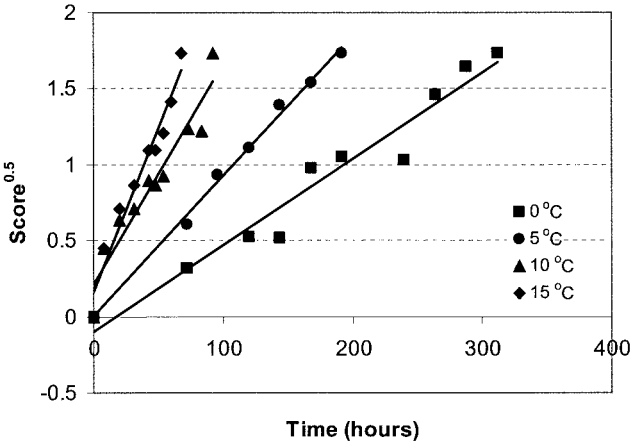

FIG. 2. Square root of sensory score values of ground pork with pH 6.13 during aerobic storage at 0, 5, 10, and 15°C.

(ii) The development of the majority of models has been focused on the effect of the environmental factors on the maximum specific growth rate without taking into account the lag phase. It has been shown however, that the lag phase duration of the "specific specific organisms" (SSO; the fraction of the total microflora which is considered responsible for spoilage) can be a significant part of the total shelf life of foods (19, 20). Ignoring lag phase may lead to underestimated shelf life predictions, with significant economic losses for the food industry.

(iii) Most models are developed and validated under static temperature conditions. In practice, however, temperature fluctuations occur often, especially during storage and distribution of foods. Thus, validation at changing (dynamic) temperatures is of great importance for evaluating the performance of the model in predicting shelf life under real chill chain conditions.

(iv) Finally, but not least important, is the lack of information required for the application of models for predicting the shelf life of specific food products (e.g., the identification of SSO, their spoilage domain, and the spoilage level) (6, 18).

The objective of the present study was to develop an accurate, "ready-to-use" microbial spoilage model targeted to

TABLE 1. Representative values for the shelf life of ground pork (pH 6.02 to 6.13) stored at different storage temperatures and levels of the different spoilage bacteria at the time of organoleptic rejection[a]

Temp (°C)	Mean ± SD				
	Shelf life (h)	CFC (log_{10} CFU/g)	STAA (log_{10} CFU/g)	MRS (log_{10} CFU/g)	VRBG (log_{10} CFU/g)
0	267.2 ± 5.1	9.2 ± 0.2	8.4 ± 0.2	4.9 ± 0.1	5.1 ± 0.1
5	146.7 ± 9.7	9.1 ± 0.3	8.2 ± 0.1	5.5 ± 0.1	6.4 ± 0.2
10	79.4 ± 3.4	8.8 ± 0.3	8.0 ± 0.2	6.0 ± 0.4	7.0 ± 0.3
15	53.7 ± 6.0	9.0 ± 0.2	8.1 ± 0.1	7.1 ± 0.3	8.0 ± 0.4

[a] CFC, pseudomonads; STAA, *Brochothrix thermosphacta*; MRS, lactic acid bacteria; VRBG, *Enterobacteriaceae*; CFC, cetrimide fusidin cephaloridene; STAA, streptomycin-thallous acetate-actidione agar; VRBG, violet red bile glucose agar.

ground meat. The model was developed by using data from commercially available products in order to take into account the effects of structure (47) and microbial interactions. Shelf life predictions were based on mathematical models for the kinetic response of pseudomonads, which were found to be a good spoilage index for aerobically stored ground meat. The model was further validated at dynamic temperature conditions using four different changing temperature profiles. The results showed that the developed model could satisfactorily predict microbial growth and shelf life of ground meat at conditions simulating meat chill chain.

MATERIALS AND METHODS

Preparation of samples. Fresh (<12 h after slaughter) ground meat (beef and pork), bought from central market, butcher shop or provided by a Greek meat industry, was used for the study. Eight different meat batches with initial pH from 5.34 to 6.13 (see Fig. 3) were tested. Meat was transported to the laboratory within 1 h of purchase and held at 1°C for 1 to 2 h. Each batch was further divided into portions of 100 g, placed on each end of meat retail foam trays, and over-wrapped with air-permeable polyethylene plastic film. Packaged meat was stored under controlled isothermal conditions (0, 5, 10, 15, and 20°C) or programmed changing temperature conditions in high-precision (±0.2°C) low-temperature incubators (model MIR 153; Sanyo Electric Co., Ora-Gun, Gunma, Japan). The temperature of samples was monitored during the storage period by using electronic temperature monitoring devices (Cox Tracer; Cox Technologies, Belmont, NC). Duplicate packages from each storage temperature were taken at appropriate time intervals to allow for efficient kinetic analysis of microbial growth and sensory characteristics.

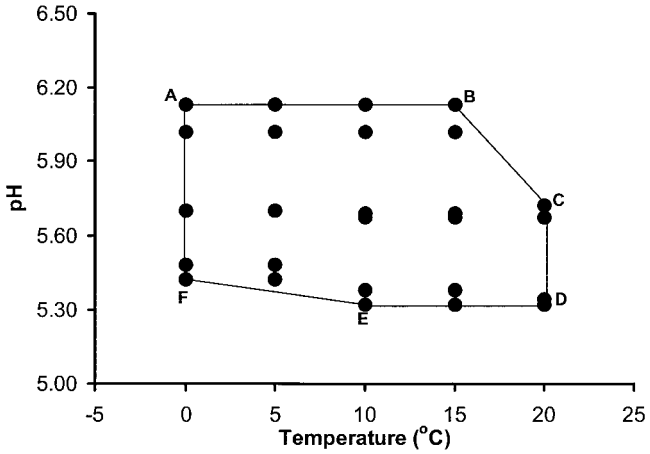

FIG. 3. Experimental conditions tested to generate the models. Area enclosed by the ground of ABCDEF illustrates the interpolation region of the model.

TABLE 2. Parameters and statistics of the Arrhenius model (equation 1) for the combined effect of temperature and pH on the maximum specific growth rate (μ_{max}) of the different spoilage bacteria grown in ground meat

Organism and parameter	Estimated value	Lower 95% CL[b]	Upper 95% CL	r^2
Pseudomonads				
μ_{ref} (h^{-1})	0.056	0.053	0.060	0.983
$E_{A\mu}$ (kJ/mol)	69.3	65.5	73.1	
d_μ	0.451	0.312	0.590	
B. thermosphacta				
μ_{ref} (h^{-1})	0.045	0.041	0.049	0.968
$E_{A\mu}$ (kJ/mol)	69.5	64.3	74.7	
d_μ	0.583	0.392	0.775	
Lactic acid bacteria				
μ_{ref} (h^{-1})	0.020	0.017	0.022	0.970
$E_{A\mu}$ (kJ/mol)	99.6	92.6	107	
d_μ	NS[a]	NS	NS	
Enterobacteriaceae				
μ_{ref} (h^{-1})	0.026	0.023	0.030	0.960
$E_{A\mu}$ (kJ/mol)	95.8	87.7	104	
d_μ	0.535	0.238	0.833	

[a] NS, parameter not significant ($P > 0.05$).
[b] CL, confidence limits.

Microbiological analysis. Ground meat (25 g) was transferred to a stomacher bag (Seward, London, United Kingdom), 225 ml of Ringer's solution (catalog no. 1.15525.0001; Merck, Darmstadt, Germany) was added, and the mixture was homogenized for 60 s with a stomacher (Lab Blender 400; Seward Medical, London, United Kingdom). Samples (0.1 ml) of the appropriate 10-fold serial dilutions were spread on the surface of the appropriate media in petri dishes for enumeration of (i) total aerobic viable count on plate count agar (Merck 1.05463) incubated at 25°C for 72 h, (ii) pseudomonads on cetrimide fusidin cephaloridine agar (CM559 [Oxoid, Basingstoke, United Kingdom] supplemented with selective supplement SR 103E) and incubated at 25°C for 48 h (32), (iii) *Brochothrix thermosphacta* on streptomycin-thallous acetate-actidione agar (the medium was made from basic ingredients in the laboratory) incubated at 20°C for 72 h (12), (iv) *Enterobacteriaceae*, and (v) lactic acid bacteria. For *Enterobacteriaceae* and lactic acid bacteria, 1.0 ml was inoculated into 10 ml of molten (45°C) violet red bile dextrose agar (Merck 1.10275) and Man Rogosa Sharpe agar (MRS; Merck 1.10660), respectively. After setting, a 10-ml overlay of molten medium was added. For the *Enterobacteriaceae*, incubation was at 30°C for 24 h. The large colonies with purple haloes were counted (33). MRS plates were incubated at 25°C for 96 h.

All plates were examined visually for typical colony types and morphological characteristics that were associated with each growth medium. In addition, the selectivity of each medium was checked routinely by Gram staining and microscopic examination of smears prepared from randomly selected colonies obtained from all media.

Sensory analysis. A trained sensory panel of six persons, who evaluated the color and odor of raw, and the taste and odor of cooked meat was used. Ground meat samples (100 g) were cooked, individually wrapped stem tightly in aluminum foil, at 180°C for 20 min. An adaptation of a simple three-point scoring system (18, 44) was used. Taste, color, and odor was judged and recorded in appropriate forms with descriptive terms reflecting the organoleptic evolution of quality deterioration. Rating was assigned on a continuous 0-to-3 hedonic scale (with 0 being the highest quality score and 2 being the limit of acceptance).

Data analysis. The growth data (log_{10} CFU g^{-1}) of the different spoilage bacteria of ground meat were modeled as a function of time using the model of Baranyi and Roberts (2), and the kinetic parameters (μ_{max} and λ) were estimated. For curve fitting the in-house Institute of Food Research program DMFit, kindly provided by J. Baranyi (Institute of Food Research, Norwich, United Kingdom), was used. A combined Arrhenius equation (described in detail in

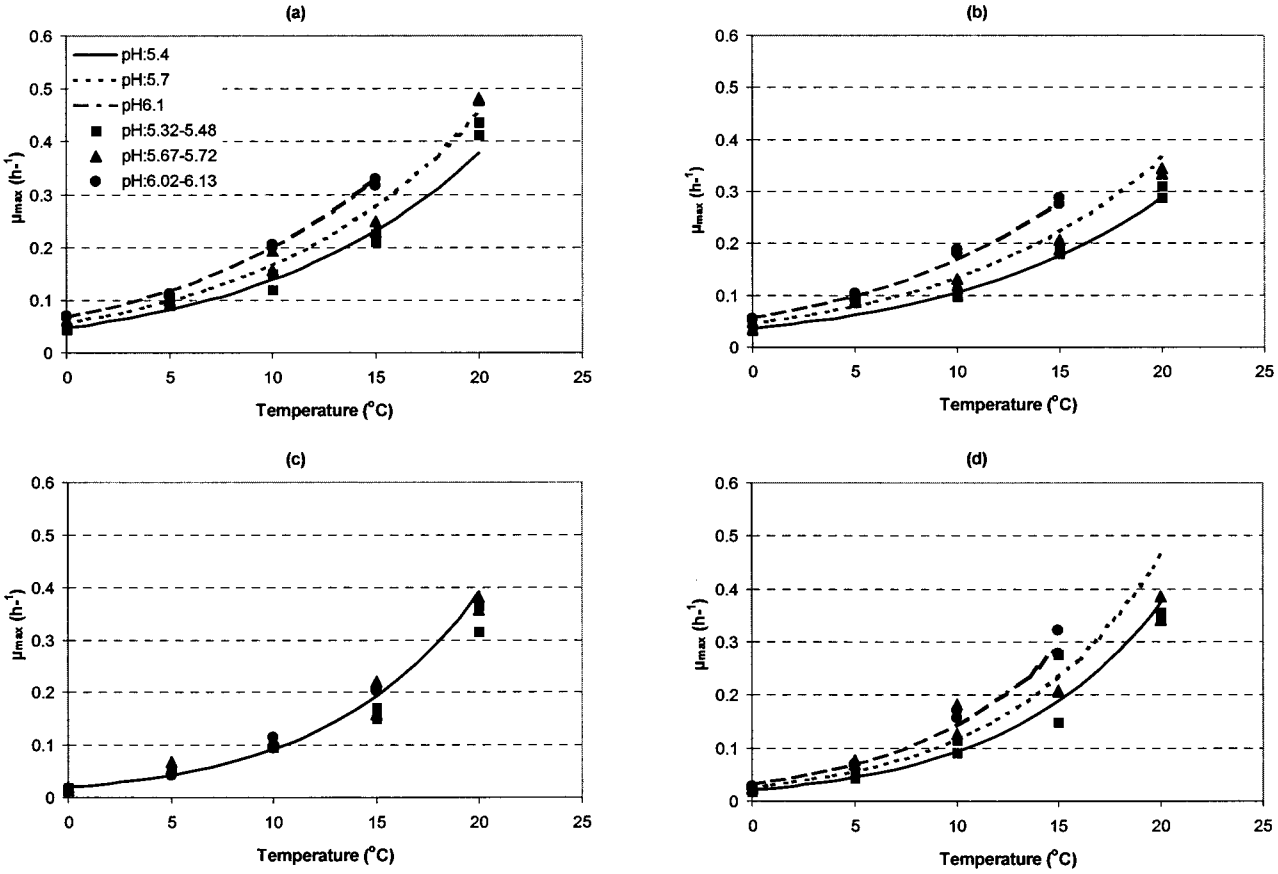

FIG. 4. Predictions of the modified Arrhenius model (equation 1) for the effect of temperature and pH on the maximum specific growth rate (μ_{max}) of the different spoilage bacteria (a, pseudomonads; b, *Brochothrix thermosphacta*; c, lactic acid bacteria; d, *Enterobacteriaceae*) on ground meat. Lines represent predictions of equation 1 at three different initial pH values of meat. Points represent observed values of μ_{max}.

Results and Discussion) was used to model the effect of pH and storage temperature on the kinetic parameters using the Microsoft Excel program.

RESULTS AND DISCUSSION

The changes in microbial flora of fresh ground meat (beef and pork) with pH ranging from 5.34 to 6.13 were monitored at different isothermal storage temperatures (0 to 20°C). At all conditions tested, pseudomonads were the dominant bacteria, followed by *B. thermosphacta*. The remaining members of the microbial association (lactic acid bacteria and *Enterobacteriaceae*) remained at lower levels (Fig. 1). The microbial profile described above has also been reported in other studies on aerobically stored chilled meat (14, 24, 35, 42, 43). The results of the study showed that the development of microbial association during storage was identical for all pH and temperature conditions tested, with pseudomonads dominating the microbial populations.

In the present study the sensory evaluation of ground meat was performed in parallel with the microbiological analysis. The square root of sensory score was linearly related to time (Fig. 2). The shelf life was estimated as the time at which score reached the value of 2 ($\sqrt{2}$ in Fig. 2), which was the rejection score of the method. The level of the members of the microbial association spoilage bacteria at the end of shelf life was estimated using the primary growth model and setting time equal to shelf life. Representative values of the bacterial levels at the end of shelf life for ground meat are shown in Table 1. At all conditions tested, the level of pseudomonads at the end of shelf life was constantly close to 10^9 CFU/g. The level of *B. thermosphacta* was also relatively constant but always at least 1 log CFU/g lower than pseudomonads. Populations of the rest spoilage bacteria at the end of shelf life ranged from 10^4 to 10^8 CFU/g, depending on the storage temperature. The observation that pseudomonads were the dominant organisms at the end of shelf life with a constant population level can lead to their characterization as a good spoilage index for aerobic stored ground meat. Other studies have reported that spoilage of aerobically stored chilled meat cuts occurs when pseudomonads reach 10^7 to 10^8 CFU per cm^2 or per g (14). In spoilage experiments with aerobically stored meat cuts performed in our laboratory we found that the level of pseudomonads at the end of shelf life was close to 10^7 CFU/g (data not shown). The increased spoilage level of pseudomonads in ground meat compared to meat cuts could be attributed to the higher surface/weight ratio of the former. Based on the observation that no spoilage (i.e., according to sensory analysis) was observed before pseudomonads reach 10^9 CFU/g, any value

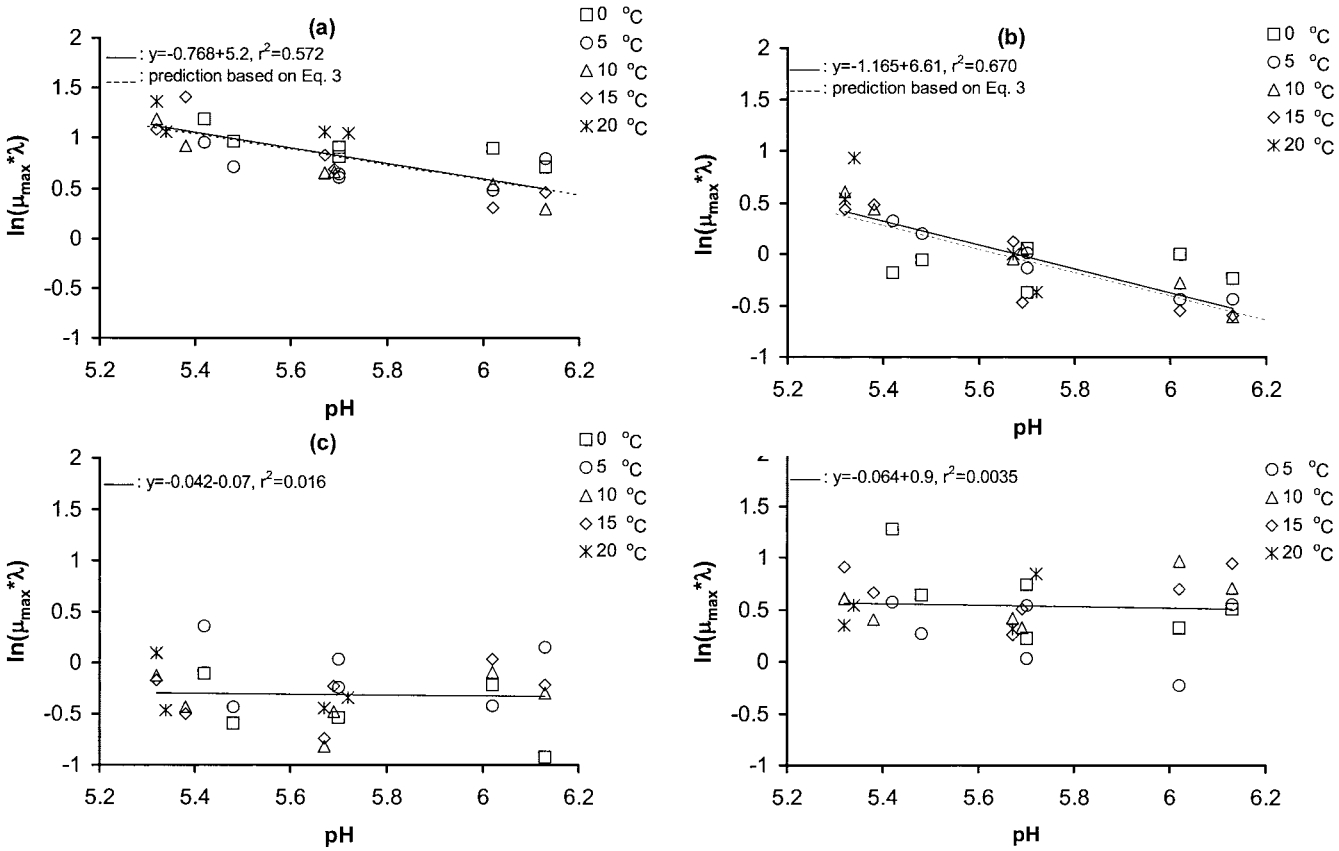

FIG. 5. Effect of initial pH of meat on the natural logarithm of the $\mu_{max} \times \lambda$ product of the different spoilage bacteria (a, pseudomonads; b, *Brochothrix thermosphacta*; c, lactic acid bacteria; d, *Enterobacteriaceae*). Points represent observed values. Solid lines show the linear regression line. In panels a and b, dotted lines show the prediction of equation 3.

below that level could be used as a spoilage level in a microbial spoilage model according to the quality policy and standards of a meat industry.

The kinetic parameters (μ_{max} and λ) of the different spoilage bacteria were modeled as a function of meat pH and storage temperature. In general, initial pH of meat can vary significantly depending on animal feeding and handling or on other factors affecting rigor mortis (11, 26). The pH data of the tested meat samples in combination with information on temperature conditions during meat storage and transportation (13, 15) were used to develop the experimental design. (Fig. 3). By analogy to the minimum convex polyhedron (4), the polygon shown in Fig. 3 encloses the interpolation region of the model.

A modified Arrhenius equation was used to model the combined effect of meat pH and storage temperature on microbial growth as follows:

$$\ln(\mu_{max}) = \ln(\mu_{ref}) - d_{\mu} \times (pH_{ref} - pH) - \frac{E_{A\mu}}{R} \times \left(\frac{1}{T} - \frac{1}{T_{ref}}\right) \quad (1)$$

$$\ln(\lambda) = \ln(\lambda_{ref}) + d_{\lambda} \times (pH_{ref} - pH) + \frac{E_{A\lambda}}{R} \times \left(\frac{1}{T} - \frac{1}{T_{ref}}\right) \quad (2)$$

where T is the absolute temperature (in degrees Kelvin), E_A (kJ/mole) is the activation energy, R is the universal gas constant, T_{ref} is the reference temperature ($T_{ref} = 273$°K), pH_{ref} is the reference pH condition (pH = 5.7), μ_{ref} (h^{-1}) and λ_{ref} are the maximum specific growth rate and lag phase at reference storage conditions (T_{ref}, pH_{ref}), respectively, and d_{μ} and d_{λ} are

TABLE 3. Parameters and statistics of the Arrhenius model (equation 2) for the combined effect of temperature and pH on the lag phase of the different spoilage bacteria grown in ground meat

Organism and parameter	Estimated value	Lower 95% CL[b]	Upper 95% CL	r^2
Pseudomonads				
λ_{ref} (h)	40.2	34.9	46.3	0.928
$E_{A\lambda}$ (kJ/mol)	68.8	60.6	77.0	
d_{λ}	1.22	0.91	1.52	
B. thermosphacta				
λ_{ref} (h)	20.7	17.6	24.4	0.915
$E_{A\lambda}$ (kJ/mol)	67.0	57.6	76.4	
d_{λ}	1.73	1.38	2.07	
Lactic acid bacteria				
λ_{ref} (h)	36.2	29.8	43.9	0.926
$E_{A\lambda}$ (kJ/mol)	97.0	85.9	108	
d_{λ}	NS[a]	NS	NS	
Enterobacteriaceae				
λ_{ref} (h)	63.5	49.5	81.4	0.879
E_A (kJ/mol)	93.5	79.1	107	
d_{λ}	0.581	0.056	1.11	

[a] NS, parameter not significant ($P > 0.05$).
[b] CL, confidence limits.

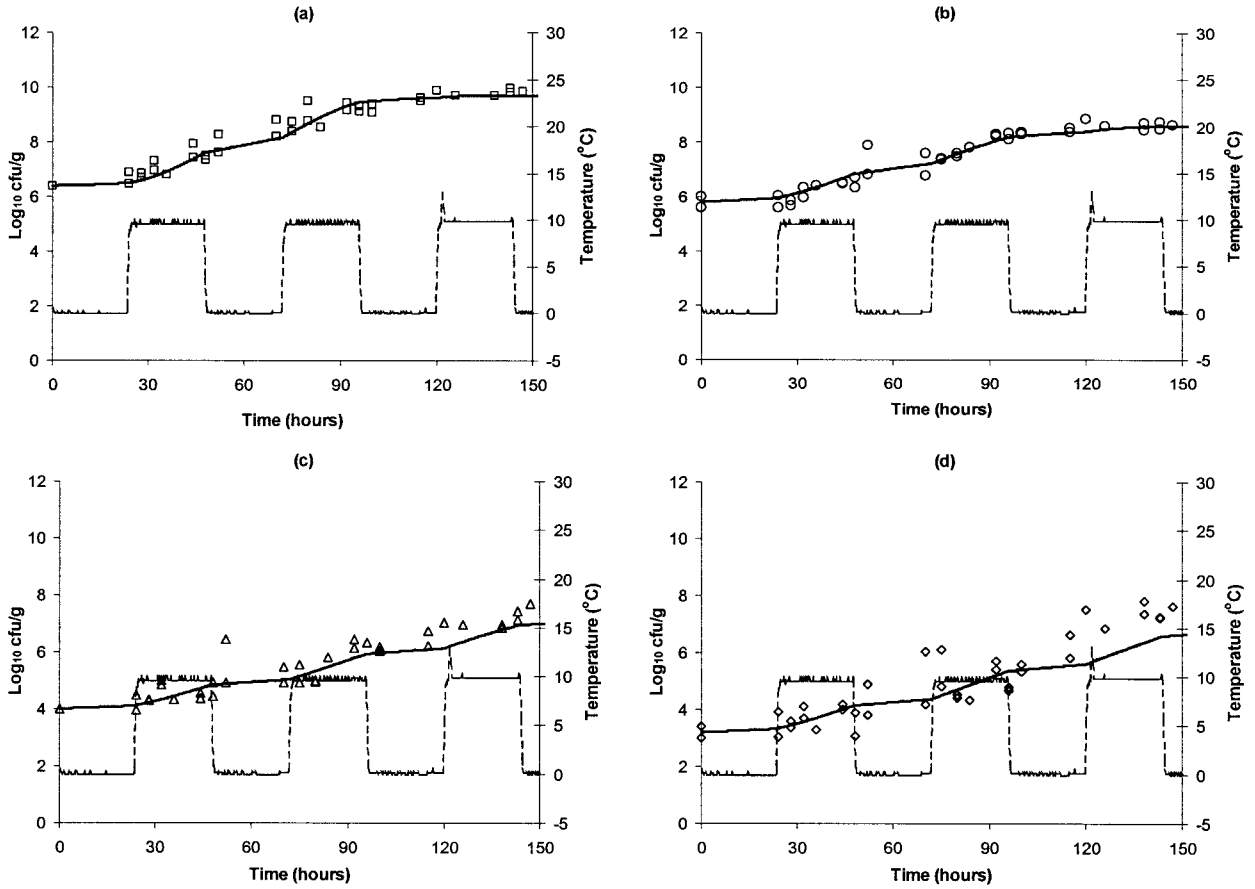

FIG. 6. Comparison between observed (points) and predicted (lines) growth of spoilage bacteria (a, pseudomonads; b, *Brochothrix thermosphacta*; c, lactic acid bacteria; d, *Enterobacteriaceae*) on ground pork (pH 5.65) stored at periodically changing temperature (24 h at 0°C and 24 h at 10°C).

parameters expressing the effect of pH on the maximum specific growth rate and lag phase, respectively.

The modification of the Arrhenius model was based on the observation that pH did not affect the temperature dependence (E_A) of the kinetic parameters. Similar results have been reported for the effect of temperature and CO_2 on growth of spoilage bacteria on fresh fish (19), where the authors used a similar modification of the Arrhenius model to describe the combined effect of these environmental factors. The parameters and statistics of equations 1 and 2 for the tested spoilage bacteria are shown in Tables 2 and 3. In Fig. 4, the predictions of equation 1 are compared to the observed maximum specific growth rates. Activation energies for μ_{max} of pseudomonads and *B. thermosphacta* were 69.3 and 69.5 kJ/mol, respectively. These values are in agreement with the results of other studies on the effect of temperature on the growth of these bacteria on other foods or laboratory media (18, 19, 46). For *Enterobacteriaceae* and lactic acid bacteria, μ_{max} showed much higher temperature dependence, with E_A values of 95.8 and 99.6, respectively. As has been reported previously (18, 19), E_A values for λ were very close to those for μ_{max} for all tested bacteria.

Although the range of meat pH tested in the present study was relatively narrow (5.34 to 6.13), a significant effect of meat pH on the growth kinetics of pseudomonads, *B. thermosphacta*, and *Enterobacteriaceae* was observed. These results are in agreement with the study of Blixt and Borch (5), who reported significant differences in pseudomonads growth on meat at pH 5.35 compared to growth on meat at pH 5.7. However, other studies performed in laboratory media showed pseudomonad growth to be unaffected by pH in the range of 5.3 to 7.8 (30). This discrepancy could be attributed to the fact that in meat, small differences in pH can be translated to significant differences in lactate concentration (5, 26) and thus affect the growth of pseudomonads, which are sensitive to lactic acid (34). Indeed, Blixt and Borch (5) reported lactate concentrations of 599 and 946 mg/100 g for meat samples with pH 5.7 and pH 5.35, respectively. As a consequence, the modified Arrhenius model for the combined effect of pH and temperature described better growth of pseudomonads, *B. thermosphacta*, and *Enterobacteriaceae* than the Arrhenius model for the single effect of temperature. In contrast to the bacterial groups discussed above, meat pH did not affect growth kinetics of lactic acid bacteria. This could be explained by the higher acid tolerance of lactic acid bacteria compared to the rest spoilage bacteria (5, 23).

The majority of mathematical models for spoilage microorganisms have been focused on the effect of the environmental

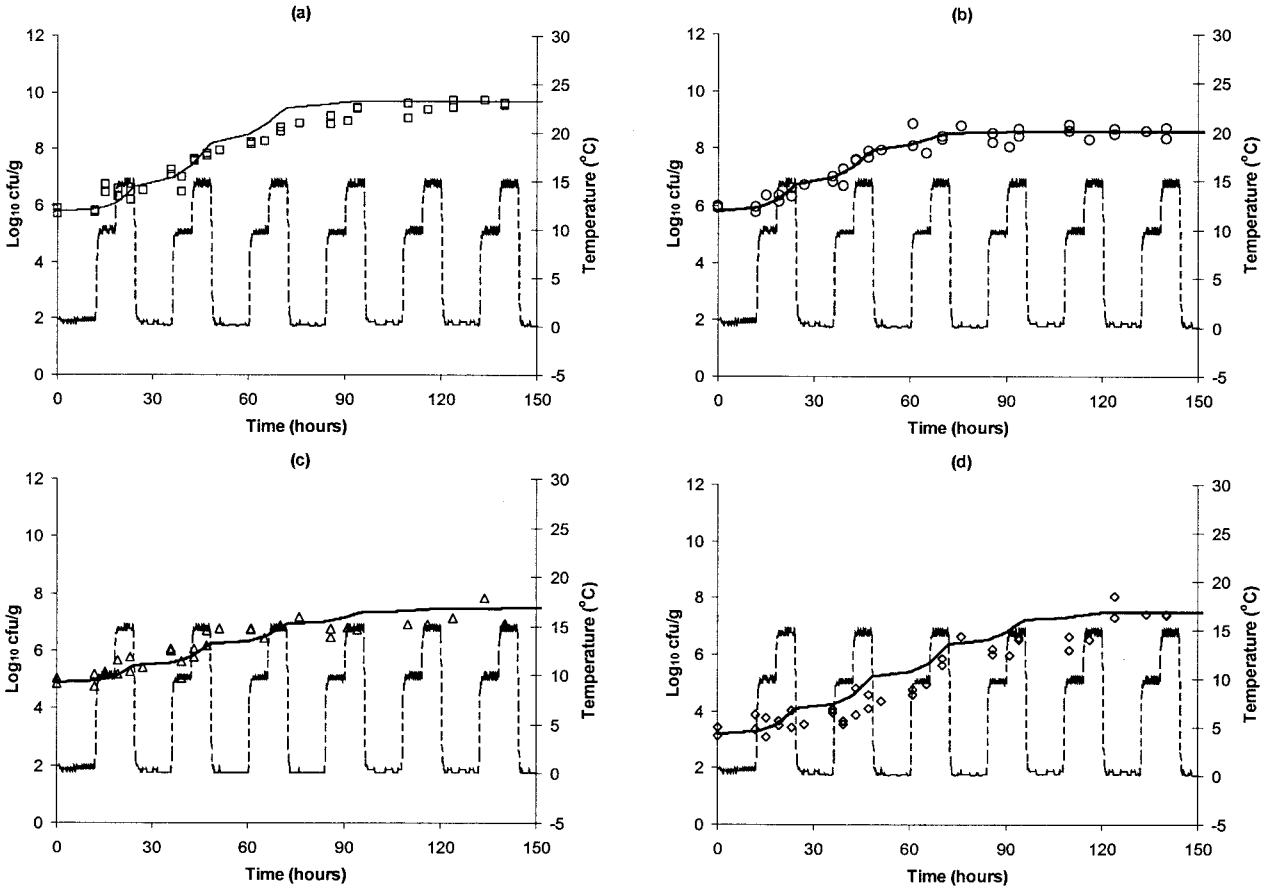

FIG. 7. Comparison between observed (points) and predicted (lines) growth of spoilage bacteria (a, pseudomonads; b, *Brochothrix thermosphacta*; c, lactic acid bacteria; d, *Enterobacteriaceae*) on ground pork (pH 5.95) stored at periodically changing temperature (12 h at 0°C, 6 h at 10°C, and 6 h at 15°C).

factors on maximum specific growth rate without taking into account the lag phase. It has been shown, however, that the lag-phase duration of the SSO can be a significant part of the total shelf life of foods (18, 23); thus, ignoring lag phase may lead to underestimated shelf life predictions with significant economic losses for the food industry.

In biological terms, lag can be determined as the ratio between the amount of "work" that a cell has to perform in order to adapt to its new environment and the rate at which it is able to perform that work which may be identified with μ_{max} (7, 8, 39, 41). In that case the "adaptation work" is given by the product of μ_{max} and λ ($\mu_{max} \times \lambda$). The study of this product can be more useful than the study of λ, which can be considered as the consequence of the "adaptation work" and μ_{max}. The product of μ_{max} and λ has been integrated into primary growth models as a parameter related to the physiological state of the cells (h_0 and p_0 parameters in the models of Baranyi and Roberts, [2] and McKellar et al. [29], respectively). Several studies have shown that the physiological state of microbial populations depends on both preincubation and growth conditions (1, 7, 8, 10, 38, 41), while in some of them these effects were described quantitatively (1, 38). All of the studies described above were performed in laboratory media with defined and well-controlled preincubation conditions. In practice, however, the history of microbial cells in foods is unknown. Thus, the study of physiological state of naturally contaminated bacteria in food products would provide useful information.

The product $\mu_{max} \times \lambda$ for the tested spoilage bacteria in ground meat is shown in Fig. 5. The results showed that the "adaptation work" was not affected by storage temperature. This can also be derived from the estimated values of E_A for μ_{max} and λ which were found to be very close (see Tables 2 and 3). Indeed, by adding equations 1 and 2 and assuming the same E_A for μ_{max} and λ, we have:

$$\ln(\mu_{max} \times \lambda) = \ln(\mu_{ref} \times \lambda_{ref}) + (d_\lambda - d_\mu) \times (pH_{ref} - pH) \quad (3)$$

The temperature independence of the physiological state has been also reported by other researchers who found that the product $\mu_{max} \times \lambda$ remains constant under different storage temperature conditions (20, 38, 41). In contrast to storage temperature, a negative linear correlation between meat pH and $\ln(\mu_{max} \times \lambda)$ for pseudomonads ($r^2 = 54\%$) and *B. thermosphacta* ($r^2 = 67\%$) was observed (Fig. 5). As shown in Fig. 5a and b, the above regression lines were almost identical with the predictions of equation 3. A similar correlation has been also reported by Delignette-Muller (7) for other spoilage and

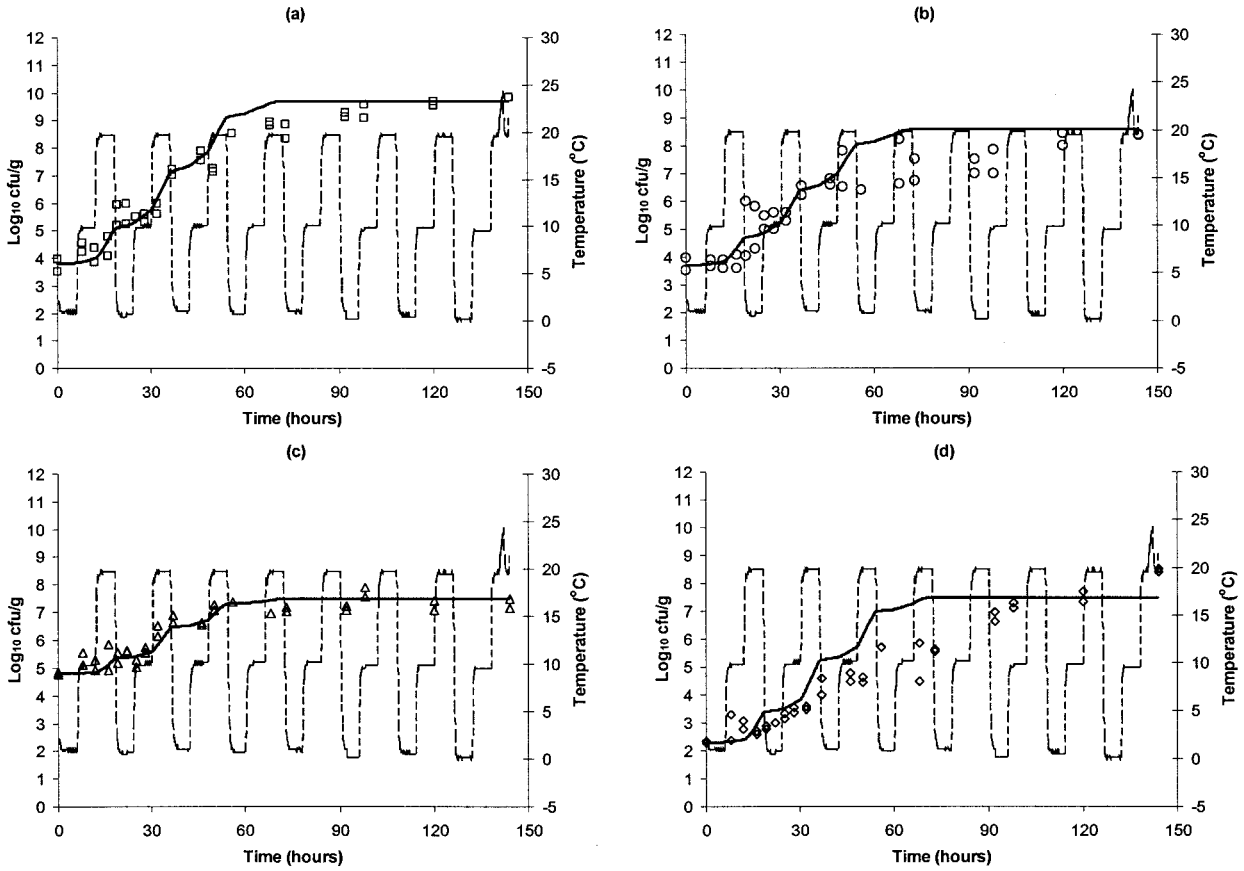

FIG. 8. Comparison between observed (points) and predicted (lines) growth of spoilage bacteria (a, pseudomonads; b, *Brochothrix thermosphacta*; c, lactic acid bacteria; d, *Enterobacteriaceae*) on ground pork (pH 6.10) stored at periodically changing temperature (6 h at 2°C, 6 h at 10°C, and 6 h at 20°C).

pathogenic bacteria. The relation between the physiological state and meat pH could be attributed to the physiological stress of the cells induced by their introduction to a more acidic environment. Indeed, the increased lactic acid concentration in meat with low pH may contribute to an additional "adaptation work" (i.e., proton pumping by membrane-bound H^+-ATPase) (25) needed by the cells in order to raise the internal pH above a threshold value required to enter the exponential phase (17). The dependence of physiological state on environmental factors other than temperature has been reported by Pin et al. (38), who also found an exponential correlation between the "adaptation work" of *Yersinia enterocolitica* and CO_2 concentration in packaging atmosphere.

No correlation between the physiological state and meat pH was observed for lactic acid bacteria and *Enterobacteriaceae* (Fig. 5). For lactic acid bacteria, as mentioned in the case of μ_{max} and λ, this could be attributed to their higher acid tolerance compared to the rest of the bacterial groups. Similar results have been observed for *Listeria monocytogenes* by McKellar et al. (29), who reported that growth pH does not affect the physiological state of the pathogen. These findings indicate that the effect of the environmental factors on microbial physiological state depends not only on the nature of the factor but also on the type of the microorganism and its physiology.

The developed models were further validated under dynamic temperature conditions. Ground meat was stored under four different fluctuating temperature scenarios with temperature shifts from 0 to 20°C. For growth predictions the numerical solution of the model of Baranyi and Roberts (2) was used based on the procedure used by Baranyi et al. (3). As in the case of the latter study, it was assumed that during exponential growth in a dynamic temperature environment, the specific growth rate defined by temperature is adopted instantaneously. In addition, it was assumed (based on the results of the present study) that the parameter h_0 ($= \mu_{max} \times \lambda$) is temperature independent. The maximum population density (y_{max}) was assumed to be constant, therefore being taken as the average of the values estimated for each bacterial group from primary fitting at isothermal conditions. For the initial population parameter (y_0) the initial bacteria level of meat estimated with plate count was used. The parameter μ_{max} was taken from the developed secondary model (equation 1) based on the initial pH of the meat and the "momentary" temperature conditions (temperature within a very short time interval "dt" was assumed to be constant). For pseudomonads and *B. thermosphacta* the parameter h_0 was calculated from the relation between meat pH and $\ln(\mu_{max} \times \lambda)$ shown in Fig. 5a and b, based on the initial value of meat pH. In the cases of lactic acid bacteria and *Enterobacteriaceae* where the initial pH of meat did not affect the parameter h_0, the latter was set equal to

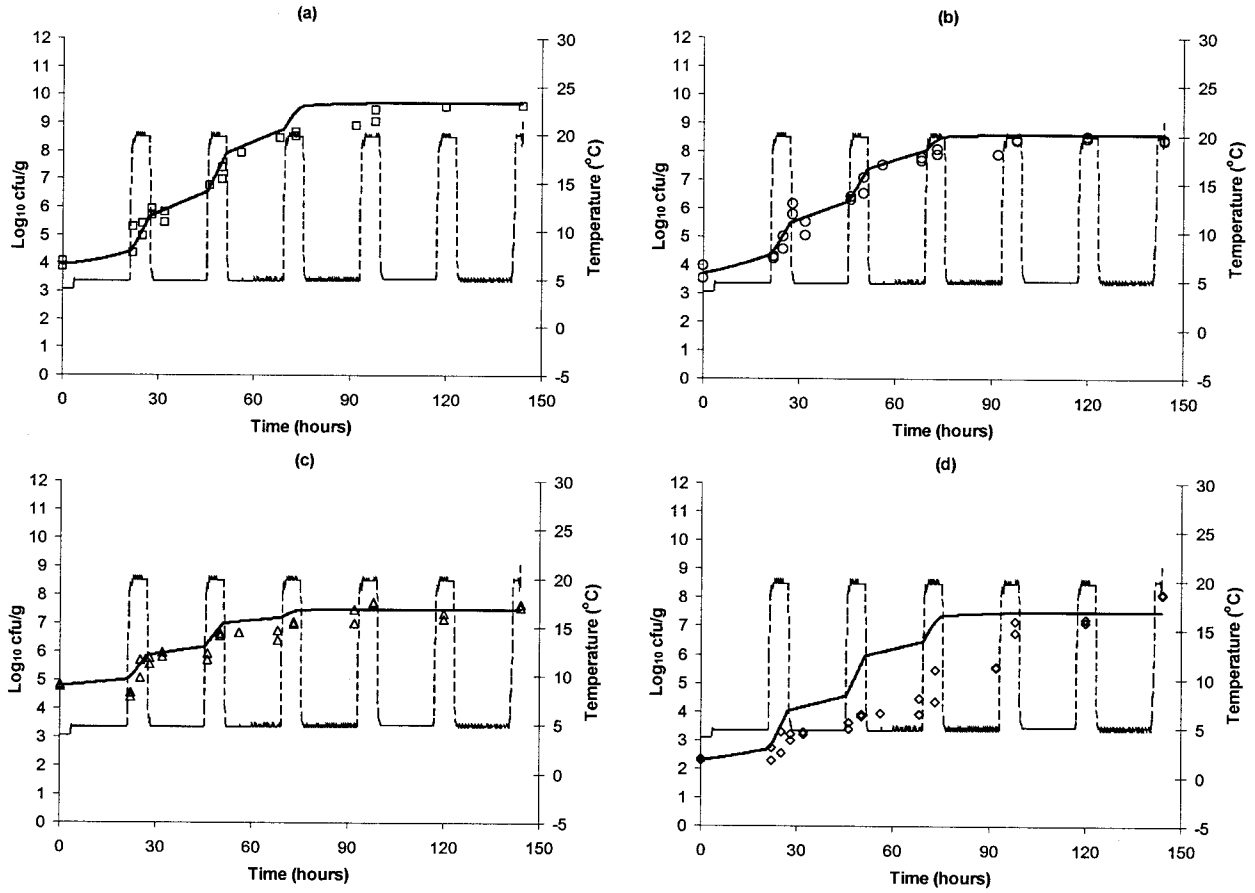

FIG. 9. Comparison between observed (points) and predicted (lines) growth of spoilage bacteria (a, pseudomonads; b, *Brochothrix thermosphacta*; c, lactic acid bacteria; d, *Enterobacteriaceae*) on ground pork (pH 6.10) stored at periodically changing temperature (18 h at 5°C and 6 h at 20°C).

the average value of the product $\mu_{max} \times \lambda$ estimated from the tested meat samples.

The results from the comparison between observed and predicted growth at dynamic temperature conditions are shown in Fig. 6 to 9. In general, at all temperature scenarios tested, the model predicted the growth of meat spoilage bacteria well. Better predictions were obtained with milder temperature shifts (Fig. 6 and 7). For temperature shifts from 20 to 2°C (Fig. 8) a slight overprediction of the model was observed especially during the late phase of growth. This overprediction was more pronounced in the case *Enterobacteriaceae*. Similar results have been reported in other studies on model validation at changing temperatures. Baranyi et al. (3) tested a model for the growth of *B. thermosphacta* and reported that predictions were good when temperature profile contained step changes from an upper temperature of 17 to 25°C down to 5°C, but with step changes down to 3°C a significant overprediction was observed. These authors attributed this observation to an additional lag phase induced by the sudden cold shock, which altered the physiological state of the organism. It needs to be noted, however, that the extent of overprediction observed in the present study was much lower than in the study of Baranyi et al. (3).

The performance of the developed models in dynamic temperature conditions was also evaluated by using the percent relative errors (%RE) (8):

$$\%\ \text{relative error (RE)} = \frac{(N_{\text{observed}} - N_{\text{predicted}})}{N_{\text{observed}}} \times 100 \qquad (4)$$

The %RE of prediction at the four temperature scenarios tested is shown in Fig. 10. For pseudomonads, 93.3% of predictions were within the −10 to 10% RE zone, while none was outside the −20 to 20% RE zone. For *B. thermosphacta* and lactic acid bacteria, 90.1 and 88.1% of predictions, respectively, were within the −10 to 10% RE zone. For *Enterobacteriaceae* 77.8% of predictions were within the −20 to 20% RE zone, and the rest were within the −50 to 50% RE zone.

The ability of pseudomonads growth model to predict shelf life of ground meat under dynamic temperature conditions was also evaluated. Predicted shelf life was estimated as the time required by pseudomonads to multiply from the initial to the spoilage level (10^9 CFU/g). In Table 4 a comparison between model predictions and shelf life estimated by sensory analysis is shown for the different tested scenarios. Overall, the model predicted satisfactorily shelf life with a mean percent difference between predicted and observed values of 13.1%.

The results of the present study showed that growth of

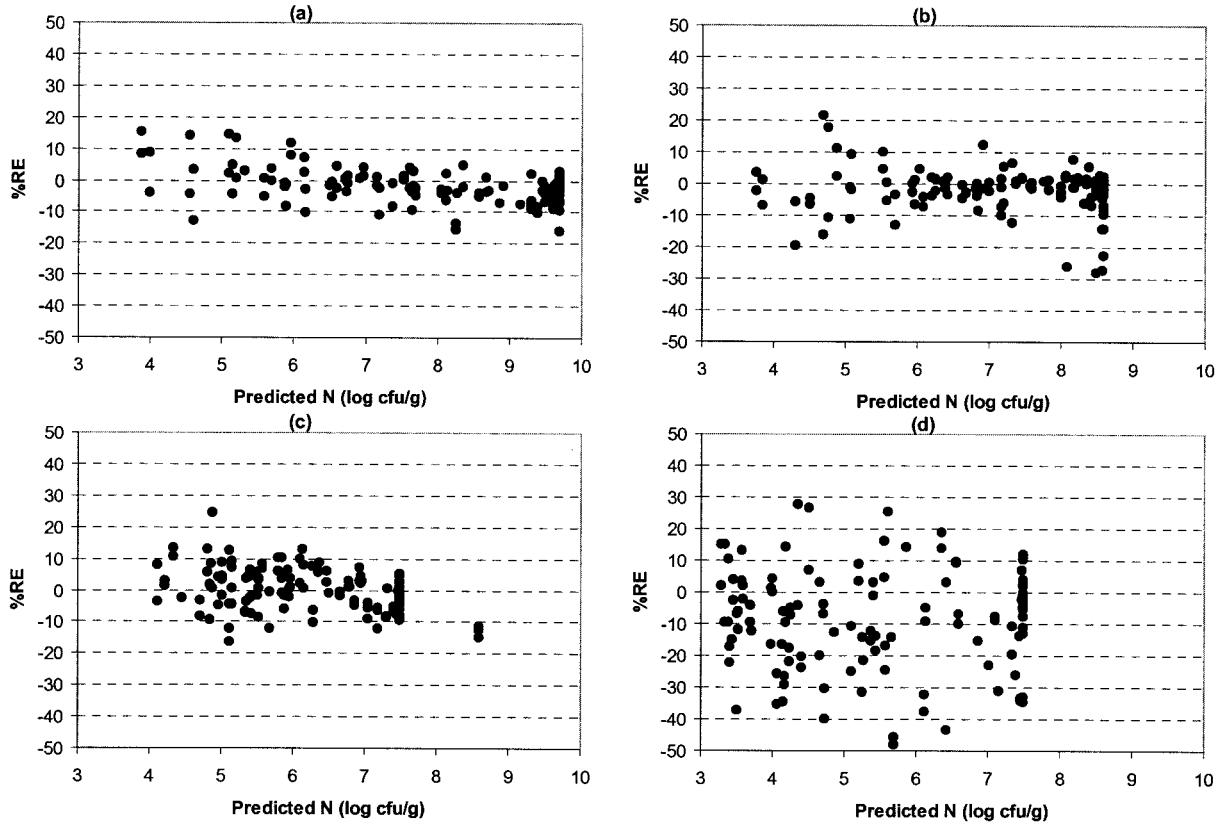

FIG. 10. %RE values for the comparison between observed and predicted growth of spoilage bacteria (a, pseudomonads; b, *Brochothrix thermosphacta*; c, lactic acid bacteria; d, *Enterobacteriaceae*) on ground pork (pH 6.10) stored at changing temperature.

pseudomonads followed closely sensory changes during storage and thus a growth model for this group can be used for predicting spoilage of aerobic stored ground meat. However, further research is needed in order to evaluate the possible effect of meat composition (e.g., glucose, lactate, etc.) on model applicability and especially on the spoilage level of pseudomonads. This is particular evident for glucose, a carbon source that has been found to be an important intrinsic factor, among others, for describing or predicting the degree of spoilage (35, 43). Indeed, this compound plays the key role for the rate as well as the type of spoilage in meat and meat products (9, 35, 42, 43). The good microbiological quality (low bacterial numbers) of retail beef, lamb, and even wild boar stored under different conditions has been correlated with glucose concentration (35). It was observed that when the glucose concentration had become very low the first signs of spoilage were evident. This was due to the fact that glucose limitation promotes a switch from a saccharolytic to an amino acid-degrading metabolism in pseudomonads. On the other hand, it has been demonstrated that by increasing the availability of glucose in meat, spoilage defined as proteolysis, slime, or off-odor production is postponed. This is due to the fact that the physiological behavior (i.e., expressed as metabolic products that are produced or assimilated) from pseudomonads is drastically (negatively or positively) affected. The interaction with the other members of microbial association for glucose should also be taken into consideration (6, 16, 45). In addition, other metabolic end products, e.g., gluconate may also be considered for the meat ecosystem and its shelf life prediction (35).

TABLE 4. Comparison between predicted and observed shelf life of ground meat stored at dynamic temperature conditions

Temp profile	Shelf life observed (h)	Shelf life predicted (h)
T1 (Fig. 6)	85.3	85.5
T2 (Fig. 7)	98.0	66.8
T3 (Fig. 8)	68.8	53.6
T4 (Fig. 9)	71.5	70.5

In conclusion, the microbial growth models, data, and information presented here provide a "ready-to-use" model for predicting spoilage of aerobic stored ground meat. In addition, the fact that the model is developed based on data from commercially available products in combination with the extensive validation under dynamic temperature conditions increases our confidence in the model's accuracy. The application of this model by the meat industry can lead to effective management systems (13, 21, 23), which will optimize the quality of meat products

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