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ORIGINAL ARTICLE

Growth of *Listeria monocytogenes* in modified atmosphere packed cooked meat products: a predictive model

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Predictive models for the influence of intrinsic and extrinsic parameters relevant for gas-packed, cooked meat products on the growth parameters (maximum specific growth rate and the lag phase) of Listeria monocytogenes *were developed. Two types of models were compared, i.e. extended Ratkowsky models and response surface models. Temperature, water activity, concentration of sodium lactate (SL) and dissolved CO_2 in the aqueous phase were considered as the relevant parameters influencing the growth of* L. monocytogenes *in gas-packed, cooked meat products and were included in the models. The concentration of CO_2 in the aqueous phase of the food was chosen as the determinative inhibitory factor of modified atmospheres. All four investigated independent variables did influence significantly the growth parameters of* L. monocytogenes*. Interactions between temperature and CO_2 and especially between CO_2 and Na–lactate were observed. The model was validated in real gas-packed, cooked meat products and was then combined with previously developed models for spoilage of gas-packed, cooked meat products to determine the 'Risk Areas' for these type of food products. Low water activity cooked meat products supported outgrowth of* L. monocytogenes *before spoilage had occurred, unless Na–lactate and CO_2 were applied as extra hurdles.*

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Introduction

Modified atmosphere packaging (MAP) is commonly used to prolong the shelf-life of cooked meat products. Despite increasing commercial interest in the use of MAP, the concern about the potential growth of psychrotrophic pathogenic bacteria remains a limiting factor to further expansion of the technology. A quantitative assessment of the safety of products packaged under modified atmospheres is still lacking.

One of the most important psychrotrophic food pathogens related to anaerobically packed cooked meat products is *Listeria monocytogenes*. In a survey of pâté from the Dutch retail trade nine of 83 samples (11%) were found to be contaminated with *L. monocytogenes* (de Boer et al. 1990). The *L. monocytogenes* contamination of 3065 pâté products sampled at the point of retail sale in England and Wales was 2·6% (Nichols et al. 1998). The percentage of samples positive for *L. monocytogenes* shortly after packaging of Danish sliced cooked cured ham and cooked frankfurters was respectively 10%

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and 6% (Qvist and Liberski 1991). A survey of cooked meat products in Germany resulted in 3·7% of samples containing *L. monocytogenes* (Noack and Jöckel 1993). In a recent report (Uyttendaele et al. 1999) a contamination level of 4·9% in 25 g of different cooked meat products (3405 samples) on the Belgian retail market was reported. Moreover, several investigations demonstrated possible proliferation of *L. monocytogenes* in cooked meat products (Barakat and Harris 1999, Blom et al. 1997, Beumer et al. 1996, Farber et al. 1995, Schmidt 1995, Krämer and Baumgart 1993, de Boer and Van Netten 1990, Schmidt and Kaya 1990, Glass and Doyle 1989).

Traditional predictive models for the growth of micro-organisms include temperature, water activity and pH as independent variables. Additional variables, such as nitrite concentration, atmosphere and lactate concentration, are often examined in combination with one or two of the first mentioned variables. For *L. monocytogenes*, specific mathematical models to describe the effect of combinations of temperature, pH, water activity, organic acids, $NaNO_2$, CO_2 concentrations and irradiation on growth and survival have been published (Nerbrink et al. 1999, Razavilar and Genigeorgis 1998, Lebert et al. 1998, Fernández et al. 1997, McClure et al. 1997, Farber et al. 1996, George et al. 1996, Murphy et al. 1996, Farber et al. 1995, Duffy et al. 1994, Duh and Schaffner 1993, Grau and Vanderlinde 1993, Patterson et al. 1993, Buchanan and Philips 1990) and validated (te Giffel and Zwietering 1999). Adequate predictive models are indeed difficult to develop when more than four independent variables have to be included. It is therefore necessary to determine the relevant intrinsic and extrinsic parameters for a specific food product and to develop specific food group related predictive models based on this knowledge.

In this study, temperature, water activity, concentration of sodium lactate (SL) and dissolved CO_2 in the aqueous phase were considered as the relevant parameters influencing the growth of *L. monocytogenes* in gas-packaged cooked meat products and are included in a predictive model. The concentration of CO_2 in the aqueous phase of the food was chosen as the determinative inhibitory factor of modified atmospheres, as described by Devlieghere et al. (1998a). The variation of the pH as well as the residual Na–nitrite content was considered as nonsignificant for the development of *L. monocytogenes* in gas-packaged, cooked meat products and were fixed at respectively 6·2 and $20\,mg\,kg^{-1}$ as these values correspond to the average values in industrial cooked meat products. In a first step, buffered modified brain–heart infusion (BHI) broth was tested for its suitability as a simulation medium for cooked meat products for *L. monocytogenes*. Second, the influence of the natural background flora on the growth of *L. monocytogenes* was evaluated. After model development, the models were validated in real gas-packaged, cooked meat products and were then combined with previously developed models for spoilage of gas-packaged, cooked meat products (Devlieghere et al. 2000a) to determine the 'Risk Areas' of *L. monocytogenes* for these type of food products.

Materials and Methods

Suitability of the simulation medium

Buffered modified BHI broth OXOID, CM225 + $18\,g\,l^{-1}$ glucose, SIGMA 68270 + $3\,g\,l^{-1}$ yeast extract, OXOID, L21 + $4{\cdot}6\,g\,l^{-1}$ Na_2HPO4, Janssens chimica + $20\,mg\,l^{-1}$ $NaNO_2$, UCB) was tested for its suitablity as a simulation medium for cooked ham. Growth curves of *L. monocytogenes* were determined in duplicate at anaerobic conditions at three temperatures (4, 8 and 12°C) in buffered modified BHI broth and in sterile cooked ham (pH = $6{\cdot}19 \pm 0{\cdot}03$, NaCl content = $2{\cdot}41 \pm 0{\cdot}02\%$ (w/w), dry matter content = $26{\cdot}25 \pm 0{\cdot}60\%$ (w/w), Na–lactate content = $0{\cdot}45 \pm 0{\cdot}08\%$ (w/w)). The pH, NaCl content and Na–lactate content of the broth were adjusted to their respective values in the water phase of the cooked ham.

A cocktail of five *L. monocytogenes* strains (920811, NCTC 11994, Scott A, ATTC 53 and an own isolate from cooked meat products LM6-04) was individually subcultured in Trypton Soy Broth (TSB, OXOID CM129) at 37°C for 24 h. A second subculture (0·1 ml in 5 ml of TSB) was incubated for 16 h at 37°C and left

for 6 h at the experimental temperature (4, 8 or 12°C) before mixing the individual subcultures. After the adaptation period, the broth and the sterile cooked ham were inoculated with the *L. monocytogenes* cocktail at a level of 10^3 cfu ml^{-1} or g^{-1}.

The experimental procedure for gas packaging and sampling described by Devlieghere et al. (1998b) was followed. However, samples were plated on plate count agar (PCA, OXOID CM325) and aerobically incubated at 37°C over 2 days. For every five samples, a sample was also plated on Listeria selective agar base (OXOID CM856) supplemented with Listeria selective supplement (OXOID, SR140E) to detect possible contamination.

Influence of the background flora on the growth of L. monocytogenes

Growth curves of *L. monocytogenes* were determined in buffered modified BHI broth at different initial levels of lactic acid bacteria (10^5, 10^4, 10^3 cfu ml^{-1} and without lactic acid bacteria) to estimate the effect of a typical background flora on the growth characteristics of *L. monocytogenes*. A cocktail of five lactic acid bacteria, isolated from five different gas-packaged cooked meat products at the end of the shelf-life, was used. All strains belonged to the *Lactobacillus sake* species whereas three belonged to *Lb. sake* subsp. *carnosus* (GERT1, GERT5, GERT17) and two to *Lb. sake* subsp. *sake* (GERT2 and GERT3). The five strains were previously tested on possible production of bacteriocins which could be active against *L. monocytogenes* by means of the standard well-diffusion assay, described by Wolf and Gibbons (1996). None of them did produce bacteriocins active against *L. monocytogenes*. The lactic acid bacteria strains were subcultured in APT broth (BBL, 10918, Becton Dickinson) similarly as previously described but incubated at 30°C. The growth of *L. monocytogenes* was followed as described earlier. The growth of the lactic acid bacteria was monitored by plating on MRS agar (OXOID CM361) of which the pH was adjusted to a pH of 5·7 with sorbic acid (SIGMA S-1626). The plates were anaerobically incubated at 30°C over 3 days.

Development of a predictive model for the effect of temperature, dissolved carbon dioxide, water activity and Na–lactate on the growth of L. monocytogenes

Experimental design. Growth curves were determined at all combinations of three temperatures (4, 8 and 12°C), four packaging configurations (100% N_2; 25% CO_2/75% N_2; 50% CO_2/50% N_2 and 80% CO_2/20% N_2 with a constant gas/product volume ratio of 4/1), four water activities (0·9883, 0·9823, 0·9722 and 0·9622) and three Na–lactate concentrations (0·0%, 1·5% and 3·0%). The experiments at a water activity of 0·9823 were performed in duplicate while the other growth curve determinations were single experiments. This resulted in 156 growth curves. As the water activity was altered by adding the investigated level of Na–lactate and additionally the necessary amount of NaCl, the effect of Na–lactate could be estimated independently of its water activity decreasing effect.

The concentration of dissolved CO_2 was used as parameter for the applied modified atmosphere. The concentration of dissolved CO_2 in buffered modified BHI broth at a specific packaging configuration and temperature was determined by means of a previously developed model for the effect of temperature, initial CO_2 concentration in the gas phase and gas/product volume ratio on the amount of dissolved CO_2 in buffered modified BHI broth (Devlieghere et al. 1998a).

Experimental set-up. The procedure for the determination of the growth curves, necessary for the development of the model, was described earlier by Devlieghere et al. (1999). The water activity of the broth was altered by the addition of the investigated level of Na–lactate (0·0, 1·5 or 3·0%) and additionally the necessary amount of NaCl. A 60% DL-Na lactate solution (SIGMA, L-1375) was used. Relationships between the concentration of NaCl and/or Na–lactate and the water activity of buffered modified BHI broth were previously determined (data not shown) and used to calculate the necessary amount of NaCl when a specific water activity and Na–lactate concentration

was required. Water activity was measured with a water activity kryometer, NAGY AWK-20. The pH was adjusted after autoclaving with filter sterilized 2 N HCl or NaOH to 6·2, corresponding to the average pH of cooked meat products.

The same inoculum cocktail of *L. monocytogenes* was used as previously described. After inoculation, the jars were gas packaged at the desired packaging configuration as described by Devlieghere et al. (1999).

Model development. A standard two-stage method was applied to obtain a model for the influence of the temperature (°C), the concentration of dissolved carbon dioxide ($mg\,l^{-1}$), the water activity and the Na–lactate concentration (% w/w) on the growth of *L. monocytogenes*. In the first stage, the maximum specific growth rate (μ_{max}) and the lag phase (λ) were estimated for each combination of the experimental design. The growth parameters were estimated by fitting the data to the modified Gompertz equation (Zwietering et al. 1990) with the Levenberg–Marquardt algorithm of the statistical packet SPSS for Windows, Version 7.5.

The estimates for μ_{max} were fitted by an extended Ratkowsky model describing the effect of temperature, water activity, dissolved carbon dioxide and Na–lactate concentration on the maximum specific growth rate μ_{max} (h^{-1}) of *L. monocytogenes* (Devlieghere et al. 2000a):

$$\sqrt{\mu_{max}} = a.(T - T_{min}).\sqrt{(A_w - A_{w\,min}).(CO_{2\,max} - CO_2).(NaL_{max} - NaL)} \quad (1)$$

where a is a constant, T_{min} (°C) and $A_{w\,min}$ are the respective estimated theoretical minimum temperature (°C) and water activity for growth of the organism, CO_2 is the concentration of dissolved CO_2 ($mg\,l^{-1}$), NaL is the concentration of Na–lactate (% w/w) and $CO_{2\,max}$ and NaL_{max} are the respective estimated theoretical maximum CO_2 concentration ($mg\,l^{-1}$) and Na–lactate concentration (% w/v) for growth of the organism.

The extended Ratkowsky model for μ_{max} was compared with the following quadratic response surface model:

$$\begin{aligned}\sqrt{\mu_{max}} = {} & I_\mu + m_1.T + m_2.CO_2 + m_3.A_w \\ & + m_4.NaL + m_5.T^2 + m_6.CO_2^2 + m_7.A_w^2 \\ & + m_8.NaL^2 + m_9.T.A_w + m_{10}.T.NaL \\ & + m_{11}.T.CO_2 + m_{12}.A_w.NaL \\ & + m_{13}.A_w.CO_2 + m_{14}.CO_2.NaL\end{aligned} \quad (2)$$

where I_μ is the intercept and $m_{1\,\to 14}$ are equation coefficients.

Furthermore, a Ratkowsky model for the growth rate was used as a basis to describe the dependence of water activity, temperature, dissolved CO_2 and Na–lactate concentration on the lag phase λ (h) of *L. monocytogenes* (Devlieghere et al. 2000a):

$$\ln\lambda = \ln\left[\frac{1}{b.(A_w - A_{w\,min}).(CO_{2\,max} - CO_2).(T - T_{min})^2.(NaL_{max} - NaL)}\right] \quad (3)$$

where b is a constant.

Similarly as for μ_{max} the Ratkowsky model for λ [Eqn (3)] was compared to a quadratic response surface model with the following equation:

$$\begin{aligned}\ln\lambda = {} & I_\lambda + l_1.T + l_2.CO_2 + l_3.A_w \\ & + l_4.NaL + l_5.T^2 + l_6.CO_2^2 \\ & + l_7.A_w^2 + l_8.NaL^2 + + l_9 T.A_w \\ & + l_{10}.T.NaL + l_{11}.T.CO_2 + l_{12}.A_w.NaL \\ & + l_{13}.A_w.CO_2 + l_{14}.NaL.CO_2\end{aligned} \quad (4)$$

where I_λ is the intercept and $l_{1\,\to 14}$ are equation coefficients.

The applied transformations (square root transformation for μ_{max} and natural logarithm for λ) were chosen based on an analysis of the stabilising effect of the variance by specific transformations (Zwietering et al. 1994, McMeekin et al. 1993, Alber and Schaffner 1992).

All secondary models were obtained by fitting the data to eq. 1 to 4 with the Levenberg-Marquardt algorithm of the statistical packet SPSS for Windows, Version 7.5. In general, models were compared based on their adjusted R^2, in order to take into account the different

number of parameters, and Mean Square Error (MSE).

Validation of the model. To assess the performance of the developed models to predict the growth of *L. monocytogenes* in real gas-packaged, cooked meat products, a validation study was performed. This validation consisted of the comparison between the predicted and the actual μ_{max} and λ of *L. monocytogenes* on different types of industrially prepared cooked gas-packaged meat products.

Several cooked meat products were collected from different Belgian meat product producers. After autoclaving (121°C, 15 min), the meat product was aseptically ground and transferred in a sterile, ice chilled Stomacher bag. Every meat product was analysed for dry matter content (% w/w), fat content (% w/w), residual Na–nitrite content (mg kg^{-1}), water activity and Na–lactate concentration (% w/w). The cuttered meat product was inoculated with an inoculum of *L. monocytogenes* of circa 10^4 cfu g^{-1}, similarly prepared as described earlier. After inoculation an appropriate amount of inoculated cooked meat product was transferred in sterile 50-ml glass jars to result in a gas/product water phase volume ratio of 4/1. The glass jars were gas packaged (as described earlier) at two different initial CO_2 concentrations ($\pm 0\%$ and $\pm 50\%$) compensated with N_2, closed and stored at different temperatures.

At regular time intervals the content of a glass jar was sampled, diluted with peptone physiological salt solution, homogenised and plated on PCA (OXOID, CM325) and aerobically incubated at 37°C over 2 days. For every five samples, a sample was also plated on Listeria selective agar base (OXOID, CM856) supplemented with Listeria selective supplement (OXOID, SR140E) to detect a possible contamination. The growth parameters (μ_{max} and λ) of *L. monocytogenes* in the meat product were estimated by fitting the data to the modified Gompertz equation (Zwietering et al. 1990) with the Levenberg–Marquardt algorithm of the statistical packet SPSS for Windows, Version 7.5.

The amount of dissolved CO_2 in the water phase of each meat product and at each temperature was separately determined by measuring the under pressure into a closed volumetric flask after equilibrium (Devlieghere et al. 1999). There was assumed that all CO_2 dissolved in the water phase of the meat product. This assumption is justified as Devlieghere et al. (1998a) demonstrated that at 7°C almost no CO_2 dissolved in pork fat.

The validation was quantified by determining the Bias factor (B_f) and Accuracy factor (A_f) as described by Ross (1996).

Results and Discussion

Suitability of the simulation medium

The growth parameters of *L. monocytogenes* in cooked ham and in buffered modified BHI broth which was adapted to the intrinsic parameters of the cooked ham, were compared (Table 1). A good agreement was achieved for λ

Table 1. Estimated growth parameters for *L. monocytogenes* at three temperatures in cooked ham and buffered modified BHI

Temperature (°C)	Estimated growth parameters			
	Sterile cooked ham		Buffered modified BHI	
	λ (h)	μ_{max} (h^{-1})	λ (h)	μ_{max} (h^{-1})
4	252 (203–301)[a]	0·0332 (0·0271–0·093)	230 (204–256)	0·0310 (0·0255–0·0364)
8	85 (54–114)	0·0643 (0·0542–0·0744)	70 (49–90)	0·0662 (0·0577–0·0746)
12	39 (29–49)	0·109 (0·095–0·122)	35 (26–43)	0·120 (0·104–0·136)

[a](95% confidence interval).

as well as for μ_{max}. Previously, the suitability of the applied simulation medium was also demonstrated for *L. sake* (Devlieghere et al. 1998b) and *Aeromonas hydrophila* (Devlieghere et al. 2000b). The choice of a suitable medium is of great importance in the development of predictive models. As in this study, predictive models are developed for a specific type of food products (i.e. gas-packaged, cooked meat products), it was possible to determine a priori a suitable simulation medium rather than developing models by means of experiments in broth without the assurance that the simulation medium and the experimental conditions correspond to the reality.

Influence of the background flora on the growth of L. monocytogenes

The background flora can influence the growth characteristics of a food pathogen, mainly due to the production of antimicrobial metabolites and nutrient competition. Therefore, the influence of a background flora, originating from gas packaged cooked meat products, on the growth of *L. monocytogenes* was estimated (Fig. 1). The growth of *L. monocytogenes* was not influenced when the level of lactic acid bacteria was $<10^7$ cfu ml^{-1}. However, from the moment that the lactic acid bacteria number reached a level $>10^7$ cfu ml^{-1}, a significant increase of the lactic acid concentration and a significant decrease in the pH was measured (data not shown). This was also reported in vacuum packaged cooked ring sausages (Korkeala et al. 1990), where a sharp decrease in the pH as well as a sharp increase of the total lactic acid concentration was noticed after lactobacilli count reaching 10^7–10^8 cfu g^{-1}. The aroma as well as the taste score of cooked ring sausages also sharply decreased at the moment 10^7cfu g^{-1} lactobacilli were determined (Korkeala et al. 1987). Moreover, the CNERNA–CNRS (Jouve 1996) postulated the following criterium regarding lactic acid bacteria for the shelf-life of post-contaminated cooked meat products: m = 10^7 cfu g; M = $5{\cdot}10^7$ cfu g; c/n = 2/5. Debevere (1996) also suggested a maximum of 10^7cfu g^{-1} lactic acid bacteria at the end of the shelf-life of pasteurized products which are subjected to post-contamination. It can therefore be concluded that the natural background flora in cooked meat products will normally not influence the growth of *L. monocytogenes* during its shelf-life unless bacteriocin producing strains are developing.

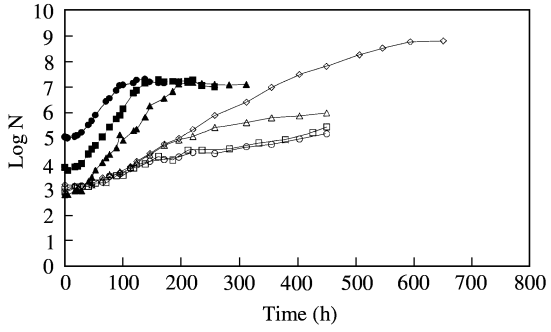

Figure 1. Influence of cooked ham background flora (closed symbols) on the growth of *L. monocytogenes* (open symbols). (Initial lactic acid bacteria contamination = 0 cfu ml^{-1} (diamonds), 10^3 cfu ml^{-1} (triangles), 10^4 cfu ml^{-1} (squares) and 10^5 cfu ml^{-1} (circles).

Development of a predictive model for the effect of temperature, dissolved carbon dioxide, water activity and Na–lactate on the growth of L. monocytogenes

Listeria monocytogenes did not show growth over 60 days at the 37 experimental conditions listed in Table 2. Only the 119 data where growth was observed were used to develop the predictive models. Tables 3 and 4 show the coefficients of the different types of equations estimated for respectively μ_{max} and λ of *L. monocytogenes* while Table 5 lists the statistical parameters of the different proposed models.

The maximum specific growth rate of *L. monocytogenes* was very well described by the extended Ratkowsky equation (Eqn 1) as well as by the response surface equation (Eqn 2). However, model (Eqn 1) has to be preferred above (Eqn 2) as only five instead of nine parameters were necessary to describe the effect of the four investigated independent variables. No structural deviations could be noticed when the predicted values were plotted against

Table 2. Experimental conditions at which no growth of *L. monocytogenes* occurred within 60 days

Temperature (°C)	Water activity	[Na–lactate] (%)	[dissolved CO_2] ($mg\,l^{-1}$)
12	0·9723	3	1608
12	0·9623	3	1061 ; 1625
8	0·9823	3	1136 ; 1818
8	0·9723	1·5	1786
8	0·9723	3	1136 ; 1818
8	0·9623	1·5	1818
8	0·9623	3	698 ; 1136; 1818
4	0·9883	1·5	1295 ; 1940
4	0·9883	3	722 ; 1327 ; 1940
4	0·9823	1·5	1240 ; 1984
4	0·9823	3	712 ; 1240 ; 1984
4	0·9723	1·5	620 ; 1240 ; 1984
4	0·9723	3	662 ; 1240 ; 1984
4	0·9623	0	1932
4	0·9623	1·5 ; 3·0	0 ; 620 ; 1240 ; 1984

Table 3. Estimated values of the coefficients of two equations for the maximum specific growth rate μ_{max} of *L. monocytogenes*

Equation type	Parameters	Estimated value	95% confidence interval
Eqn (1)	a	$7{\cdot}1277 \cdot 10^{-04}$	$6{\cdot}2866 \cdot 10^{-04}$–$7{\cdot}9689 \cdot 10^{-04}$
	$A_{w\,min}$	0·9295	0·9233–0·9358
	T_{min}	−3·5419	−4·2222–−2·8616
	$CO_{2\,max}$	3140	2917–3365
	NaL_{max}	5·9547	5·4944–6·4150
Eqn (2)	I_μ	−1·0636	−2·0706–−0·0565
	m_1	−0·1469	−0·2485–−0·04533
	m_2	$5{\cdot}8168 \cdot 10^{-04}$	$0{\cdot}8006 \cdot 10^{-04}$–$10{\cdot}8329 \cdot 10^{-04}$
	m_3	1·1532	0·1225–2·1838
	m_4	$-2{\cdot}8161 \cdot 10^{-04}$	$-85{\cdot}584 \cdot 10^{-04}$–$79{\cdot}952 \cdot 10^{-04}$
	m_5	not significant[a]	–
	m_6	not significant[a]	–
	m_7	not significant[a]	–
	m_8	not significant[a]	–
	m_9	0·1733	0·0692–0·2773
	m_{10}	$-2{\cdot}5119 \cdot 10^{-03}$	$-3{\cdot}3603 \cdot 10^{-03}$–$-1{\cdot}6635 \cdot 10^{-03}$
	m_{11}	$-4{\cdot}4792 \cdot 10^{-06}$	$-5{\cdot}9749 \cdot 10^{-06}$–$-2{\cdot}9835 \cdot 10^{-06}$
	m_{12}	not significant[a]	–
	m_{13}	$-6{\cdot}0063 \cdot 10^{-04}$	$-11{\cdot}1128 \cdot 10^{-04}$–$-0{\cdot}8999 \cdot 10^{-04}$
	m_{14}	not significant[a]	–

[a]Not significant because $P < t < 0{\cdot}05$.

the experimental values (not shown). All quadratic terms as well as the interaction terms between Na–lactate and water activity and Na–lactate and CO_2 of the response surface equation for μ_{max} showed to be not significant (Eqn (2)). A quadratic relation between temperature and μ_{max} is however generally accepted in predictive microbiology. The non-significance of the quadratic term of temperature could possibly be explained by the limited investigated temperature range at refrigerated temperatures (4–12°C).

The lag phase of *L. monocytogenes* was much better described by the response surface equation (Eqn 4) compared to the extended Ratkowsky equation (Eqn 3), as can be derived from the adjusted R^2 and the MSE of both models (Table 5). In both cases no structural

Table 4. Estimated values of the coefficients of two equations for the lag phase λ (h) of *L. monocytogenes*

Equation type	Parameters	Estimated value	95% confidence interval
Eqn (3)	b	$4{\cdot}5231 \cdot 10^{-07}$	$2{\cdot}5324 \cdot 10^{-07}$–$6{\cdot}5137 \cdot 10^{-07}$
	$A_{w\ min}$	0·9485	0·9432–0·9538
	T_{min}	−1·5390	−2·633–−4·4485
	$CO_{2\ max}$	2476	2235–2718
	NaL_{max}	3·7494	3·5304–3·9683
Eqn (4)	I_λ	51·1874	45·5459–56·8289
	l_1	−0·4967	−0·6111–−0·3822
	l_2	$6{\cdot}0668 \cdot 10^{-04}$	$4{\cdot}9999 \cdot 10^{-04}$–$7{\cdot}1343 \cdot 10^{-04}$
	l_3	−44·8051	−50·5327–−39·0774
	l_4	0·5566	0·3906–0·7226
	l_5	$1{\cdot}4918 \cdot 10^{-02}$	$0{\cdot}8071 \cdot 10^{-02}$–$2{\cdot}1764 \cdot 10^{-02}$
	l_6	not significant[a]	–
	l_7	not significant[a]	–
	l_8	$-4{\cdot}8613 \cdot 10^{-02}$	$-1{\cdot}0095 \cdot 10^{-01}$–$0{\cdot}3723 \cdot 10^{-02}$
	l_9	not significant[a]	–
	l_{10}	not significant[a]	–
	l_{11}	not significant[a]	–
	l_{12}	not significant[a]	–
	l_{13}	not significant[a]	–
	l_{14}	$3{\cdot}1252 \cdot 10^{-04}$	$2{\cdot}3954 \cdot 10^{-04}$–$3{\cdot}8551 \cdot 10^{-04}$

[a]Not significant because $\text{Prob} > |t| > 0{\cdot}05$.

Table 5. Statistical parameters of the different proposed models for the growth parameters μ_{max} and λ of *L. monocytogenes*

	Equation type	Number of parameters	Adjusted R^2	MSE[a]
SQRT (μ_{max})	(1)	5	0·9356	$2{\cdot}55 \cdot 10^{-04}$
	(2)	9	0·9397	$2{\cdot}38 \cdot 10^{-04}$
Ln (λ)	(3)	5	0·7931	0·233
	(4)	8	0·9288	0·080

[a]Mean square of error.

deviations were present when the predicted values were plotted against the experimental values, but the deviations in the case of model (Eqn 3) were much higher (not shown). Eight of the 15 parameters of the response surface model showed significance of which only one interaction term, i.e. between Na–lactate and CO_2.

To our knowledge, no models were developed in the past for the prediction of the effect of temperature, water activity, dissolved CO_2 and Na–lactate concentration on μ_{max} and λ of *L. monocytogenes*. Moreover, very few models include the effect of various parameters on the lag phase. A validation in industrially prepared cooked meat products will have to show the performance of the developed models in real food.

Validation of the model

The developed models were validated in inoculated industrially prepared cooked meat products. The type and the characteristics of the meat products used for validation are listed in Table 6. When CO_2 was included in the headspace the amount of CO_2 dissolved in the water phase of the cooked meat product varied from $590\,mg\,kg^{-1}$ for cooked ham at 12°C to $903\,mg\,kg^{-1}$ for pâté at 47°C. The bias factor B_f and the accuracy factor A_f for the different

Table 6. Industrially prepared cooked meat products used for validation and their characteristics

Type of meat product	Water activity	[Na–lactate] (%w/w)	[$NaNO_2$] (mg kg^{-1})	pH	Dry matter (%)	Fat content (%w/w)
Cooked ham1	0·977 ± 0·002	0·455 ± 0·15	< 10	6·16 ± 0·02	27·6 ± 0·2	3·4 ± 0·4
Cooked ham2	0·970 ± 0·002	1·954 ± 0·11	< 10	6·36 ± 0·01	28·7 ± 1·1	5·2 ± 0·8
White sausages	0·974 ± 0·002	2·066 ± 0·13	< 10	6·34 ± 0·03	38·6 ± 0·3	22·4 ± 0·2
Luncheon meat	0·970 ± 0·001	0·846 ± 0·05	58 ± 9	6·19 ± 0·01	37·1 ± 1·0	18·3 ± 0·3
Chicken white	0·974 ± 0·002	2·676 ± 0·16	33 ± 7	6·40 ± 0·03	29·2 ± 0·7	1·8 ± 0·6
Pâté	0·960 ± 0·001	1·537 ± 0·13	< 10	6·42 ± 0·02	50·0 ± 0·9	31·4 ± 0·6

Table 7. Validation factors for the developed models on industrially prepared cooked meat products inoculated with *L. monocytogenes*

	μ_{max}		λ	
Validation factor	Extended Ratkowsky	Response surface	Extended Ratkowsky	Response surface
B_f	0·968	0·977	1·330	1·030
A_f	1·211	1·158	1·508	1·172

developed models for μ_{max} and λ, calculated as described by Ross (1996), are tabulated in Table 7.

A good agreement between the experimentally determined and the predicted values of μ_{max} was obtained for both types of models. The response surface model for μ_{max} showed a slightly better performance than the extended Ratkowsky model, which is expressed by its lower A_f-factor.

A much bigger difference between the two proposed models was noticed in the case of λ. The extended Ratkowsky model clearly overestimated the lag phase which was not the case for the response surface model. An overestimation of the lag phase results in fail-dangerous predictions which has to be avoided. The response surface model will therefore be preferred for the prediction of the lag phase of *L. monocytogenes*. It is important to notice that this validation was performed with inoculated studies. The inoculation procedure was identical to the one used for model data collection, which could explain the good correspondence between the predicted and the experimentally determined data. The lag phase is inherently more difficult to predict than the growth rate because it depends on the physiological state of the inoculum as well as growth conditions (Robinson et al. 1998). In reality, the physiological state of *L. monocytogenes* at the moment of contamination of a cooked meat product is not known and will probably show a high degree of variation. The lag phase predicted by models should therefore always be carefully interpreted. However, a knowledge of the lag time is important when predicting the risk for bacterial growth during storage of meat products. In the estimation of product safety the lag time should therefore be included (Nerbrink et al. 1999).

Application of the developed model

To assess the individual effect of the studied independent variables on the growth of *L. monocytogenes*, perturbation plots for μ_{max} and λ can be used (Fig. 2). A perturbation plot represents the effect of each individual independent variable on the growth parameters while the other independent variables remain constant at the central level of the design. The perturbation plots in Fig. 2 are calculated with the obtained response surface equations (Eqns (2) and (4)). All investigated independent variables did influence the growth parameters of *L. monocytogenes*. μ_{max} and λ were most influenced by temperature while the water activity did not

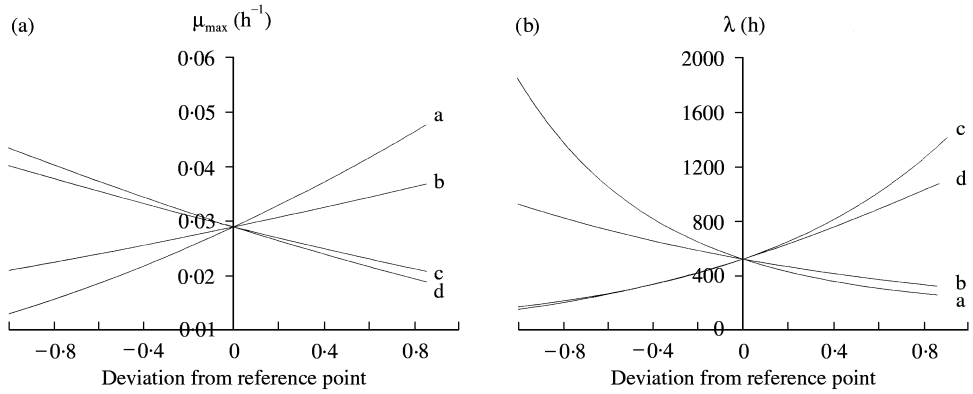

Figure 2. Perturbation plots of the effect of temperature (a), water activity (b), Na–lactate concentration (c) and dissolved CO_2 (d) on the growth parameters of *L. monocytogenes* calculated by response surface Eqns (2)(a) and (4)(b).

influence the growth to a great extent at the investigated levels. The relatively low sensitivity of *L. monocytogenes* for low water activities was previously reported (Marth 1993, Chen and Shelef 1992, Nolan et al. 1992).

Perturbation plots do not express possible interactive effects between the independent variables. Therefore, the effect of temperature and dissolved CO_2 on one side and the effect of Na–lactate and dissolved CO_2 on the other side on the time needed to increase the number of *L. monocytogenes* with 2 logarithmic units (cfu g^{-1}) was represented in Figs 3 and 4, which were calculated based on the Eqns (2) and (4).

Synergistic effects between temperature and CO_2 and especially between CO_2 and Na–lactate were noticed. The synergistic effect between temperature and CO_2 is independent of the influence of temperature on the solubility of CO_2 as differentiation of this effect was made by including the concentration of dissolved CO_2 in the model. This synergistic effect was already noticed for *Lactobacillus sake* (Devlieghere et al. 1999), but the effect is more pronounced for *L. monocytogenes* due to the higher sensitivity of this bacterium for CO_2. Jones (1989) suggested a decrease of membrane fluidity occurring in the presence of carbon dioxide which could explain the enhanced growth inhibition observed at lower temperatures.

The observed synergistic effect between dissolved CO_2 and Na–lactate can, at least partly, be explained by the pH decreasing effect of CO_2

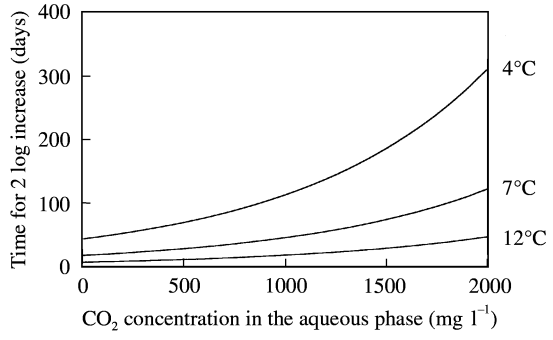

Figure 3. Influence of dissolved CO_2- concentration on the time for a 2 log cfu g^{-1} increase of *L. monocytogenes* at different temperatures. (Na–lactate concentration = 1·50% (w/w), A_w = 0·9723) calculated by Eqns (2) and (4).

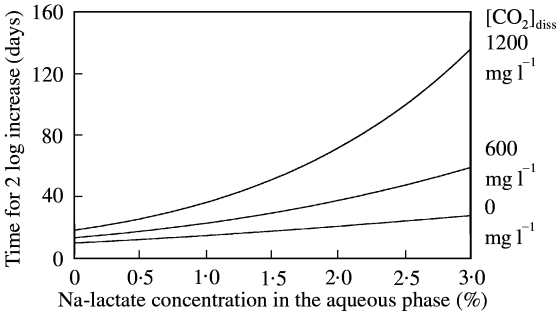

Figure 4. Influence of Na–lactate concentration on the time for a 2 log cfu g^{-1} increase of *L. monocytogenes* at different dissolved CO_2-concentrations calculated by Eqns (2) and (4). (Temperature = 7°C, A_w = 0·9723).

in the medium which was already suggested by Devlieghere et al. (2000b). When 1800 mg l^{-1} of CO_2 was dissolved in modified buffered BHI broth, a decrease of 0·3 pH units (from 6·2 to 5·9) was noticed. This pH drop was similar for cooked ham (data not shown). Such pH drop doubles the amount of undissociated lactic acid molecules in the aqueous phase of the food and therefore possibly results in an increased growth inhibitory effect of Na–lactate. The changes in pH due to gas packaging were not included as such in the model. However, as the pH drop in the medium was similar to the pH drop in cooked ham, the influence of this factor is included in the influence of CO_2 on the micro-organism. The synergistic effect of CO_2 and Na–lactate could possibly also be explained by the cooperative effect of both inhibiting factors on lowering the intracellular pH. Further research is necessary, however, to confirm this assumption.

When reliable predictive models are available for spoilage and for development of food pathogens, both models can be combined to define 'Risk Areas' for a specific food product. 'Risk Areas' are areas or combinations of

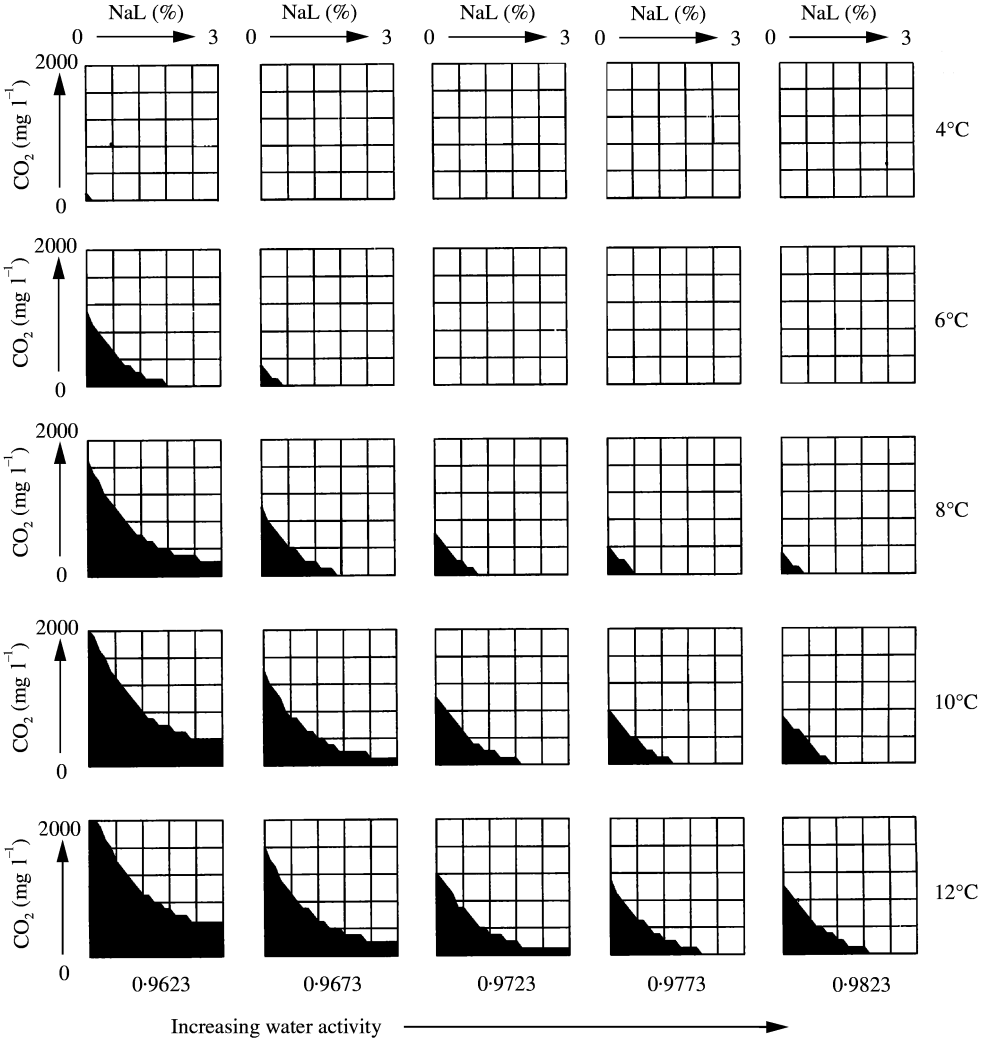

Figure 5. *Listeria monocytogenes* Risk Areas in gas-packed, cooked meat products (initial contamination lactic acid bacteria g^{-1} = 10 g^{-1}, end of the shelf-life = 10^7 *L. sake* g^{-1}, maximum initial contamination with *L. monocytogenes* = 1 g^{-1}, at day of consumption <100 g^{-1} *L. monocytogenes* g^{-1}).

intrinsic and extrinsic factors of the specific food product at which food pathogens can develop before spoilage has occurred or before the end of the indicated shelf-life. These 'Risk Areas' were calculated here by means of the developed models for spoilage (Devlieghere et al. 2000a) and growth of *L. monocytogenes* (Eqns (2) and (4)) in gas packaged cooked meat products and are represented in the grey zones of Fig. 5. For the calculations, the following assumptions were made:

(1) the meat products were contaminated with 10 lactic acid bacteria g^{-1};
(2) the end of the shelf-life was reached when 10^7 cfu g^{-1} *L. sake* on the products were present;
(3) a maximum initial contamination of 1 cfu g^{-1} with *L. monocytogenes* at the day of production was tolerated;
(4) an increase of 2 log cfu g^{-1} of *L. monocytogenes* or more during the shelf life leads to unacceptable results.

The last two assumptions are based on the criteria proposed by CNERNA-CNRS (Jouve 1996) which postulates absence of *L. monocytogenes* in 0·01 g of cooked meat products at the day of consumption. Similar criteria have been proposed by Debevere (1996).

The importance of respecting the cold chain can clearly be deduced from Fig. 5. At 4°C, no Risk Areas are present but when temperature rises the surface of the Risk Areas increases rapidly. This illustrates that for guaranteeing microbial safety of cooked meat products, a maximum storage temperature of 4°C should be preferred above 7°C. As *L. monocytogenes* is less sensitive for low water activities than the specific spoilage organism *L. sake*, low water activity cooked meat products ($A_w < 0{\cdot}970$) seems to represent a greater risk for *L. monocytogenes* outgrowth before spoilage has occurred. When one wants to avoid proliferation of *L. monocytogenes* in low water activity cooked meat products, a combination of Na–lactate and a CO_2 enriched atmosphere has to be applied, as *L. monocytogenes* is much more sensitive for both factors in comparison with lactic acid spoilage bacteria. It is therefore essential that the shelf-life determination of cooked meat products is not only based on the assessment of spoilage phenomena but also takes the possible proliferation of *L. monocytogenes* into account. Calculation of 'Risk Areas' allows the producer of cooked meat products to understand and quantify the consequences of the addition of specific quantities of salt and Na–lactate, differences in storage temperature and applied packaging configurations on the shelf life and the safety of their product.

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