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ORIGINAL ARTICLE

Modelling the behaviour of *Listeria monocytogenes* in ground pork as a function of pH, water activity, nature and concentration of organic acid salts

V. Zuliani[1], I. Lebert[2], J.-C. Augustin[3], P. Garry[1], J.-L. Vendeuvre[1] and A. Lebert[2]

1 IFIP-Institut de la filière porcine Antenne de Maisons-Alfort, Maisons-Alfort, France
2 Institut National de la Recherche Agronomique, Saint-Genès Champanelle, France
3 Ecole Nationale Vétérinaire d'Alfort, Maisons-Alfort, France

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Correspondence
Véronique Zuliani, IFIP, 7, avenue du Général de Gaulle, 94704 Maisons-Alfort cedex, France. E-mail: veronique.zuliani@ifip.asso.fr

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Abstract

Aims: to study and model the effect of sodium acetate, sodium lactate, potassium sorbate and combination of acid salts on the behaviour of *Listeria monocytogenes* in ground pork.
Methods and Results: Water activity (a_w), pH and concentration of acid salt of the meat were adjusted. The behaviour of inoculated *L. monocytogenes* was studied and modelled according to physicochemical parameters values. Whatever the acid salt concentration used, we observed an inhibition of the growth of *L. monocytogenes* at pH 5·6 and a_w 0·95. At pH 6·2 and a_w 0·97, addition of 402 mmol l^{-1} of sodium lactate or 60 mmol l^{-1} of potassium sorbate was required to observe a slower growth.
Conclusions: The inhibitory effect of acid salts was a function of pH, a_w, as well as of the nature and concentration of acid salts added. When one acid salt was added, the Augustin's model (Augustin *et al.* 2005) yielded generally correct predictions of either the survival or growth of *L. monocytogenes*.
Significance and Impact of the Study: The suggested model can be used for risk assessment concerning *L. monocytogenes* in pork products.

Introduction

Listeria monocytogenes is an ubiquitous pathogen that can be found in a large number of food products. Pork meat and processed pork products such as delicatessen are parts of major products associated with listeriosis. Indeed, in France in 1992 and 2000, jellied pork tongue was responsible for epidemic listeriosis, as well as potted minced pork in 1993 and 1999 (Jacquet *et al.* 1994; De Valk *et al.* 2000; Thévenot *et al.* 2005).

The widespread occurrence of *L. monocytogenes* in the pork-processing industry from the slaughterhouse to the cutting room (Giovannacci *et al.* 1999) makes it nearly impossible to avoid minor contamination during the meat process (Stekelenburg 2003). As *L. monocytogenes* is a psychotrophic bacterium, it can develop during refrigerated storage. In order to assure the safety of their products, industries can limit the contamination of *L. monocytogenes* using good manufacturing processing. The use of additives to control the growth of pathogens can also limit their development. For example, salts of acids act by (i) lowering water activity (a_w) of the food, it become thus less favourable for the growth of pathogens, (ii) by lowering the cell pH and (iii) by inhibiting enzymes (Houtsma *et al.* 1994).

Sodium lactate is the sodium salt of natural lactic acid (L+) which is a normal component of muscle tissue. It has already been used in the meat industry to improve palatability and shelf life of the product (Jensen *et al.* 2003). Over the past 10 years, the salts of acetic acid such as sodium acetate have been shown to be effective microbial inhibitors (Nerbrink *et al.* 1999). The same results

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have been observed for potassium sorbate (Choi and Chin 2003). Several predictive models integrate the effect of inhibitors on the behaviour of *Listeria*. For example, Le Marc *et al.* (2002) modelled the growth of *Listeria* as a function of undissociated and total amount of lactic acid, acetic acid or propionic acid. This multiplicative model takes into account interactions between temperature, pH and concentration of acids. Coroller *et al.* (2005) extended the model proposed by Le Marc for describing the effects of concentrations of single acid and mixture on the growth of *Listeria* and other pathogens. The model developed by Augustin (1999), modified in order to use the assumptions of Le Marc *et al.* (2002) regarding the effect of the interactions between environmental factors can also predict the growth or the survival of pathogens when inhibitors are added (Augustin *et al.* 2005). In this study, the antilisterial effects of sodium lactate, sodium acetate, potassium sorbate and mixture of acid salts in artificially contaminated raw pork meat were studied. The influence of pH, a_w and their interactions with organic acid salts were also taken into account. The inhibitory effects of the three organic acid salts were modelled using the Augustin's secondary model with interactions (Augustin *et al.* 2005).

Materials and methods

Organism and preparation of the inoculum

Listeria monocytogenes 14, serotype 4b, is a fast growing strain (Bégot *et al.* 1997), isolated from an industrial food environment. It was stored on glass beads at −20°C before use. The stationary phase inoculum was obtained after three subcultures in 10 ml of brain heart infusion (BHI; Oxoid, Dardilly, France), incubated at 37°C in a rotary shaker (Novotron, Infors, France) at 150 rotations per minute. This provided a 3–4 h stationary phase inoculum containing about 10^9 colony forming units (CFU) ml^{-1}.

Ground pork and chemicals added

We used shank-free pork shoulders. The fat was removed from the meat, and the remaining fat percentage was 1·6. The meat was minced and then irradiated with a dose of 15 kGy (Aerial, Illkirch, France) in order to work with a homogeneous and low contaminated meat (Zuliani *et al.* 2006). All chemicals added to the meat are presented in Table 1.

Experimental designs

In order to study the influence of the nature and the concentration of the organic acid salt on the behaviour of *L. monocytogenes* at 20°C according to pH and a_w, three complete factorial designs, one for each organic acid salt, were used. The levels of pH (5·6, 5·9, 6·2) and a_w (0·950, 0·960, 0·970) tested were the same for all experimental designs, and the three concentrations tested varied as a function of the nature of the organic acid salt (Table 2). It represented 81 experiments. In addition, control experiments were carried out without organic acid salt for the nine combinations of pH/a_w. Finally, for 27 pH/a_w conditions where growth was observed when one salt was added, the effect of combinations of two acid salts was also tested. Values of pH and a_w of the experimental designs were chosen in order to frame those frequently found for typical raw meat products (Cole *et al.* 1990; Pidcock *et al.* 2002). Intermediate concentration of each organic acid salt tested was close to concentrations used by French delicatessen manufacturers.

Experimental procedure

Adjusting concentrations of organic acid salt, a_w and pH
We first studied whether the addition of organic acid salt had an influence on a_w of the meat in the range tested. Calibration experiments were then performed to deter-

Table 1 Chemicals, brands and sterilization methods used

Chemicals	Brand	Method of sterilization
HCl, rectapur*‡	Prolabo, Fontenay-sous-bois, France	Autoclaving 15 min at 121°C
NaCl, normapur	Prolabo	Autoclaving 15 min at 121°C in a test tube
NaOH, normapur*‡	Prolabo	Autoclaving 15 min at 121°C
Potassium sorbate, 99%*†	Acros Organics, Noisy-le-grand, France	Filtration with 0·22-μm filter (Steritop, Millipore, Molsheim France)
Sodium lactate, 60% w/w (Purasal S/SP 60)	PURAC, Lyon, France	Filtration with 0·22-μm filter (Steritop)
Trihydrated sodium acetate*†	Sigma, Saint Quentin Fallavier, France	Filtration with 0·22-μm filter (Steritop)

*Product was sterilized in solution with distilled water.
†Concentration of the solution: 0·6 g ml^{-1}.
‡Concentration of the solution: 1 N.

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Table 2 Concentrations of acid salt tested

Organic acid salt	Concentration (mmol l^{-1})
Sodium lactate (NaLac)	134–268–402
Sodium acetate (NaAcet)	22–44–66
Potassium sorbate (KSorb)	20–40–60

mine the percentage of NaCl required to adjust a_w of the meat according to the nature and concentration of the acid(s) salt(s) added.

Adjustment of pH, a_w and concentration of organic acid(s) salt(s) were carried out in a laminar air flow cabinet to prevent the contamination of the meat. First, NaCl and solution of organic acid(s) salt(s) were added. The meat was then mixed during 2 min. Second, pH was adjusted: NaOH or HCl 1 N was gradually added and mixed to obtain the desired pH. Third, a_w was measured at 20°C. Uniformity of pH and a_w in the meat using this method was previously demonstrated (Zuliani *et al.* 2006). After adjustment, samples were stored at 4°C during 20–24 h before inoculation of the meat.

Meat inoculation

Meat samples were inoculated at a rate of 10^5 cells per gram of meat. According to the threshold detection of the technique used ($4{\cdot}10^3$ CFU g^{-1} for solid samples prepared by homogenizing a 10-fold dilution of the sample in sterile diluent), this inoculum level made it possible to observe the growth, inhibition and inactivation of *L. monocytogenes*. The subculture was added to the meat in a closed stomacher bag. The stomacher bag was aseptically hand massaged in order to obtain a homogeneous distribution of bacteria in the meat.

Bacterial enumerations

For each experiment, four meat samples were analysed for *L. monocytogenes* enumerations: at the beginning of the experiment (t_0), 1 (t_1), 5 (t_5) and 7 (t_7) days during the storage at 20°C. Moreover, enumeration of the total bacterial flora was performed just before meat inoculation for each experiment in order to confirm that meat had still a low contamination level after adjustment of pH, a_w and concentration of organic acid(s) salt(s).

For each enumeration, 20 g of meat was placed in a stomacher bag with 180 ml of sterile tryptone (0·1 w/v; Biokar, Beauvais, France) salt (0·85 w/v %; Prolabo, Fontenay-sous-bois, France) water. The sample was then stomached in a Model 400 Lab Stomacher (InterScience, Saint-nom-la Bretèche, France) for 1 min and serial dilutions were carried out. All platings were made in duplicate on Palcam agar (Biokar) for enumeration of *L. monocytogenes* and on plate count agar (Biokar) for total bacterial flora using a spiral plater (InterScience). Plates were incubated for 48 h at 37°C for Palcam and 72 h at 30°C for plate count agar. The storage temperature chosen for the meat samples, 20°C, is higher than those found in the commercial cold chain but it made it possible to reduce the time of the experiment. The storage time chosen for the meat samples (7 days) stored at 20°C, corresponded to the length of time between the production and the use-by-date of commercial delicatessen products when stored at refrigeration temperature (for diced bacon mean of 45 days at refrigeration temperature). Indeed, the first signs of microbiological degradation of commercial diced bacon (expansion of the packaging tray, greenness of meat) were observed, in 7 days, when they were stored at 20°C.

Log increase

We calculated G7 that characterized the evolution of the population during the experiment, i.e. 7 days (eqn 1).

$$G7 = \log(N \text{ at } t_7) - \log(N \text{ at } t_0) \quad (1)$$

where N is the concentration (CFU g^{-1}) of *L. monocytogenes*.

G7 values were used to define environmental conditions for which, inhibition or growth of *L. monocytogenes* were observed. We considered that:

– inhibition was assumed when G7 value was below 1·0 log,

– growth corresponded to a G7 value higher than 1·0 log.

Modelling

Models

The aim of our work was to validate models in order to predict the increase of the concentration of *L. monocytogenes* as a function of time in pork products, according to their formulation (pH, a_w, nature and concentration of organic acid salts). Two models were used:

– A primary model (eqn 2) for describing the evolution of the bacterial concentration as a function of time (Rosso *et al.* 1996). Curves were characterized by: (i) the maximum growth rate, (ii) the lag time, (iii) the initial bacterial concentration and (iv) the maximal bacterial concentration. The experimental value was used for N_0 and we considered that N_{max} was equal to $1{\cdot}65\ 10^8$ CFU g^{-1}, the mean of N_{max} measured for experiments carried out in irradiated ground pork and for which the stationary phase was reached.

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$$\begin{cases} \ln(\mathrm{N}) = \ln(N_0) & , \quad t \le \mathrm{lag} \\ \ln(\mathrm{N}) = \ln(N_{\max}) - \ln\left[1 + \left(\frac{N_{\max}}{N_0} - 1\right)\exp(-\mu_{\max}(t - \mathrm{lag}))\right], & t > \mathrm{lag} \end{cases} \quad (2)$$

where N_0 is the initial bacterial concentration (CFU g^{-1}), $N_{\max}$ is the maximal bacterial concentration (CFU g^{-1}), $\mu_{\max}$ is the maximum growth rate (h^{-1}) and lag is the lag time (h).

– A secondary model for describing the influence of a_w, pH, nature and concentration of the organic acid(s) salt(s) on $\mu_{\max}$ and lag. The cardinal model with interactions developed by Augustin and Carlier (2000) and modified (Augustin *et al.* 2005) regarding the effect of interactions between environmental factors was used (eqns 3–10).

$$\begin{cases} \mu_{\max} = \mu_{\mathrm{opt}}.\mathrm{CM}_2(T).\mathrm{CM}_1(\mathrm{pH}).\mathrm{SR}(a_w).\mathrm{SR}(c)\xi(T, \mathrm{pH}, a_w, c) \\ \mathrm{lag} = K/\mu_{\max} \end{cases} \quad (3)$$

where T is the temperature (°C), c is the concentration of the inhibitory substance, μ_{opt} is the optimal value of the maximum growth rate $\mu_{\max}$ when $T = T_{\mathrm{opt}}$ (optimal T for the growth of *Listeria*), $\mathrm{pH} = \mathrm{pH}_{\mathrm{opt}}$ (optimal pH for the growth of *Listeria*), $a_w = a_{w\mathrm{opt}}$ (optimal a_w for the growth of *Listeria*), without inhibitory substance. K is a parameter which is dependent on the preincubation conditions.

$\mathrm{CM}_n(\mathrm{X})$ is defined by eqn 4, $\mathrm{SR}(a_w)$ by eqn 5 and $\mathrm{SR}(c)$ by eqn 6:

$$\mathrm{CM}_n(\mathrm{X}) = \begin{cases} 0 & , \quad \mathrm{X} \le \mathrm{X}_{\min} \\ \frac{(\mathrm{X}-\mathrm{X}_{\max}).(\mathrm{X}-\mathrm{X}_{\min})^n}{(\mathrm{X}_{\mathrm{opt}}-\mathrm{X}_{\min})^{n-1}.[(\mathrm{X}_{\mathrm{opt}}-\mathrm{X}_{\min}).(\mathrm{X}-\mathrm{X}_{\mathrm{opt}})-(\mathrm{X}_{\mathrm{opt}}-\mathrm{X}_{\max}).((n-1).\mathrm{X}_{\mathrm{opt}}+\mathrm{X}_{\min}-n\mathrm{X})]} & , \quad \mathrm{X}_{\min} < \mathrm{X} < \mathrm{X}_{\max} \\ 0 & , \quad \mathrm{X} \ge \mathrm{X}_{\max} \end{cases} \quad (4)$$

where X is temperature or pH, $\mathrm{X}_{\max}$, $\mathrm{X}_{\mathrm{opt}}$ and $\mathrm{X}_{\min}$ are the maximal, optimal and the minimal values of X for the growth of *Listeria*.

$$\mathrm{SR}(a_w) = \begin{cases} 0 & , \quad a_w \le a_{w\,\min} \\ \left(\frac{a_w - a_{w\,\min}}{a_{w\,\mathrm{opt}} - a_{w\,\min}}\right), & a_{w\,\min} < a_w < a_{w\,\mathrm{opt}} \\ 0 & , \quad a_w \ge a_{w\,\mathrm{opt}} \end{cases} \quad (5)$$

where $a_{w\max}$, $a_{w\mathrm{opt}}$ and $a_{w\min}$ are the maximal, optimal and the minimal a_w values for the growth of *Listeria*.

$$\mathrm{SR}(c) = \begin{cases} 1 - \left(\frac{c}{\mathrm{MIC}_u}\right)^a, & c < \mathrm{MIC}_u \\ 0 & , \quad c \ge \mathrm{MIC}_u \end{cases} \quad (6)$$

where MIC_u is the minimal inhibitory undissociated acid concentration for the growth of *Listeria*, α is a shape parameter and c is the undissociated acid concentration.

In order to model the growth of *L. monocytogenes* in the presence of two acid salts, we considered the relative effect of the acid salts mixture (Coroller *et al.* 2005) as follows:

$$\mathrm{SR}(c) = \mathrm{SR}(c_1, c_2) = \mathrm{SR}(c_1).\mathrm{SR}(c_2) \quad (7)$$

where c_1 and c_2 are the concentrations of the undissociated forms of acids 1 and 2, respectively.

In order to take into account interactions between environmental factors, eqn 8 was used:

$$\xi(T, \mathrm{pH}, a_w, c) = \begin{cases} 1 & , \quad \psi \le 0{\cdot}5 \\ 2(1-\psi), & 0{\cdot}5 < \psi \le 1 \\ 0 & , \quad \psi > 1 \end{cases} \quad (8)$$

ψ is defined by eqn 9:

$$\psi = \sum_i \frac{\varphi_i}{2\prod_{j=i}[1-\varphi_j]} \quad (9)$$

where φ_i are the contributions to interactions of the environmental factors.

$$\varphi(\mathrm{X}) = \left(\frac{\mathrm{X}_{\mathrm{opt}} - \mathrm{X}}{\mathrm{X}_{\mathrm{opt}} - \mathrm{X}_{\min}}\right)^3 \text{ and } \varphi(c) = (1 - \mathrm{SR}(c))^2 \quad (10)$$

Values of the parameters of the model

The values for optimal growth rate – μ_{opt} – and K (which links lag with $\mu_{\max}$) were respectively equal to 0·85 h^{-1} and 1·93. They were obtained from experiments carried out in irradiated ground meat (data not shown). Maximal and optimal values used for a_w, temperature and pH where those proposed by Augustin and Carlier (2000), minimal values where those proposed by Augustin *et al.* (2005) (Table 3). Coroller *et al.* (2005) estimated that α

Table 3 Cardinal values for *Listeria monocytogenes*

$T_{\min} = -1{\cdot}72$°C	$T_{\mathrm{opt}} = 37$°C	$T_{\max} = 45{\cdot}5$°C
$\mathrm{pH}_{\mathrm{mm}} = 4{\cdot}26$	$\mathrm{pH}_{\mathrm{opt}} = 7{\cdot}10$	$\mathrm{pH}_{\max} = 9{\cdot}61$
$a_{w\,\min} = 0{\cdot}913$	$a_{w\,\mathrm{opt}} = 0{\cdot}997$	$a_{w\,\max} = 1{\cdot}000$

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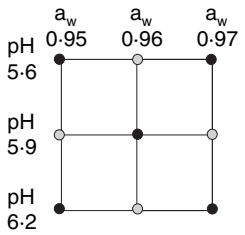

Figure 1 Combination of pH/water activity (a_w) tested for each organic acid salt at each concentration tested: (●) experiments used for the optimization of α and undissociated minimum inhibitory concentration (MIC_u) values; (◎) experiments used to compare predicted curves and experimental points.

(eqn 6) fairly described the general behaviour of species whereas MIC_u (eqn 6) is strain-specific. Therefore, value of α for lactic acid and acetic acid was chosen as proposed for *Listeria* by Le Marc (2001), i.e. 1·0 and 0·5, respectively. For sorbic acid, no value was proposed in the literature; we thus optimized it together with the MIC_u value.

For each organic acid salt, 15 experiments of the experimental design were used to adjust MIC_u (and α for sorbic acid) and 12 experiments were required to compare the predicted curves with experimental data (Fig. 1).

First, the concentration of undissociated organic acid was calculated according to pKa, the total organic acid salt concentration added and the pH using the Henderson-Hasselbach equation. The pKa at 20°C are reported to be 3·86, 4·75 and 4·76, respectively for lactic, acetic and sorbic acids. Second, knowing the pH, a_w, temperature and the concentration of undissociated organic acid, MIC_u and α (for sorbic acid) were estimated. The minimum sum of the squared residuals between predicted and observed bacterial concentrations were computed with the Newton's method (Excel, Microsoft, Courtabœuf, France).

Modelling the boundary between the growth and the no-growth areas

The boundary between the growth and the no-growth areas was defined as the conditions for which concentration of *Listeria* increases were equal to 1·0 log (Le Marc 2001; Legan *et al.* 2004), i.e. in our study, the increase of the concentration of *Listeria* in 7 days of storage at 20°C was 1·0 log. For the prediction of the boundary between the growth and the no-growth areas, the procedure used by Zuliani *et al.* (2006) was followed for each organic acid salt tested and at each concentration. The 90 experiments of the experimental designs were used for validation of predicted boundaries.

Results

Preliminary experiments

When no organic acid salt was added, eqn (11) was used to calculate the percentage of NaCl to be added to the meat for adjusting a_w. The regression coefficient of this calibration curve was 0·991.

$$a_w = -0{\cdot}0097\cdot(\%\ \mathrm{NaCl}) + 0{\cdot}9980 \qquad (11)$$

In the range studied, a_w of meat decreased linearly when concentration of sodium lactate increased. On the other hand, the concentration of sodium acetate and potassium sorbate did not modify a_w of the meat. Calibration experiments were then performed in order to determine the NaCl percentage required to adjust a_w of the meat to 0·970, 0·960 and 0·950 in the presence of acid salt. For sodium acetate and potassium sorbate, one calibration experiment was performed at the intermediate concentration of organic acid salt. For sodium lactate, one calibration experiment was performed for each concentration tested. Calibration curves were also performed in the presence of two acid salts. The percentages of NaCl to adjust a_w were determined from the equation of the calibration curves (Table 4).

Effect of acid salts on the behaviour of *Listeria*

Before inoculation with *Listeria*, the total bacterial flora was estimated for all meat samples. It was always lower than $1{\cdot}10^2$ CFU g^{-1}. This result showed that no contamination occurred during adjustment of physicochemical parameters.

The log increase for *Listeria* in 7 days (G7) was calculated in the 81 conditions depending on the nature and the concentration of organic acid salt, pH and a_w. They were compared with the G7 of the control experiments (Fig. 2). Without addition of an organic acid salt, G7 was always higher than 2·5 log. When organic acid salt was added, a growth (G7 > 1·0 log) was observed for 40 conditions and an inhibition for 41 conditions. Seven, sixteen and eighteen inhibitions were observed, respectively when sodium acetate, potassium sorbate and sodium lactate was added. The inhibitory effect of organic acid salt was influenced not only by pH, a_w, concentration of organic acid salt but also by the nature of the organic acid salt added. When pH and a_w were low, all organic acid salts were efficient to reduce the G7 to a level lower than 0·5 log. On the other hand, at pH 6·2 and a_w 0·97, only addition of 402 mmol l^{-1} of sodium lactate or 60 mmol l^{-1} of potassium sorbate decreased the G7 of *L. monocytogenes* but never below a 1·0 log increase.

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Table 4 Percentage of NaCl to adjust the a_w of the meat according to the nature and the concentration of the organic acid(s) salt(s)

Organic acid(s) salt(s)	Concentration (mmol l^{-1})	Equation of the calibration curve, a_w =	Desired a_w	Percentage (w/w) of NaCl added
Sodium lactate	134	−0·0079 × (% NaCl) + 0·9842	0·97	1·8
		R^2 = 0·983*	0·96	3·1
			0·95	4·3
	268	−0·0078 × (%NaCl) + 0·9794	0·97	1·2
		R^2 = 0·990	0·96	2·5
			0·95	3·8
	402	−0·0080 × (%NaCl) + 0·9700	0·97	0·0
		R^2 = 0·969	0·96	1·3
			0·95	2·5
Sodium acetate	22 to 66	−0·0074 × (%NaCl) + 0·9926	0·97	3·1
		R^2 = 0·999	0·96	4·4
			0·95	5·8
Potassium sorbate	20 to 60	−0·0084 × (%NaCl) + 0·9963	0·97	3·1
		R^2 = 0·995	0·96	4·3
			0·95	5·5
Potassium sorbate + sodium acetate	20	−0·0085 × (%NaCl) + 0·9865	0·97	1·9
	22 to 44	R^2 = 0·968	0·96	3·1
			0·95	4·3
Potassium sorbate + sodium lactate	20 to 40	−0·0095 × (%NaCl) + 0·9838	0·97	1·5
	134	R^2 = 0·964	0·96	2·5
			0·95	3·6
Sodium acetate + sodium lactate	22 to 44	−0·0074 × (%NaCl) + 0·9798	0·97	1·3
	134	R^2 = 0·985	0·96	2·7
			0·95	4·0

R^2 is the R^2 error (linear regression coefficient).

When two acid salts were added, inhibitory effect was also highly linked to pH and a_w values. For a_w 0·97 and pH 6·2, no interactions between organic acid salts were observed whatever the combination tested: effect of the two acid salts was equivalent to the effect of the acid salt added at the highest undissociated concentration. When pH and/or a_w were lower we rarely (3 on a total of 27 conditions tested) observed synergic or additive effect.

Modelling the *Listeria* behaviour

Estimation of model parameters

α and MIC_u values for the three organic acids associated with the organic acid salts tested are presented in Table 5.

Performance evaluation of the model

One acid salt added. For each organic acid salt, the 12 kinetics which were not used for the estimation of the parameters were compared with the predicted curves. Good predictions were obtained whatever the organic acid salt added (Fig. 3). Mean of log difference (absolute value) between G7 observed (log increase in 7 days) and G7 predicted was 0·6 (Table 6). The two higher measures of the difference between observed and predicted G7 where when sodium acetate was added. These two conditions (pH 5·6, a_w 0·96, 22 mmol l^{-1} sodium acetate and pH 5·9, a_w 0·97, 66 mmol l^{-1} sodium acetate) were near the boundary between the growth and the no-growth areas.

In the experimental design studied, CM(T) was equal to 0·44, CM(pH) were respectively equal to 0·70, 0·81 and 0·89 for pH 5·6, 5·9 and 6·2. SR(a_w) were 0·44, 0·56 and 0·68 for a_w equal to 0·95, 0·96 and 0·97, respectively. SR(c) and ξ values are reported in Fig. 4. In the presence of sodium lactate, concentration of inhibitor was often too high [SR(c) = 0] and the model did not predict growth. For the combinations where growth was predicted for sodium lactate, interactions contributed to significantly slowing down the predicted growth rate for the two combinations (Fig. 4). When sodium acetate was added, interactions between environmental factors contributed to slowing down the predicted growth ($\xi < 1·0$) or prevent it for the five conditions tested ($\xi = 0$). For eight conditions, in the presence of potassium sorbate, the predicted no-growth was attributed to interactions.

The boundary between the growth and the no-growth areas was then predicted for the three organic acid salts (Figs 5–7). The aim was to verify if the

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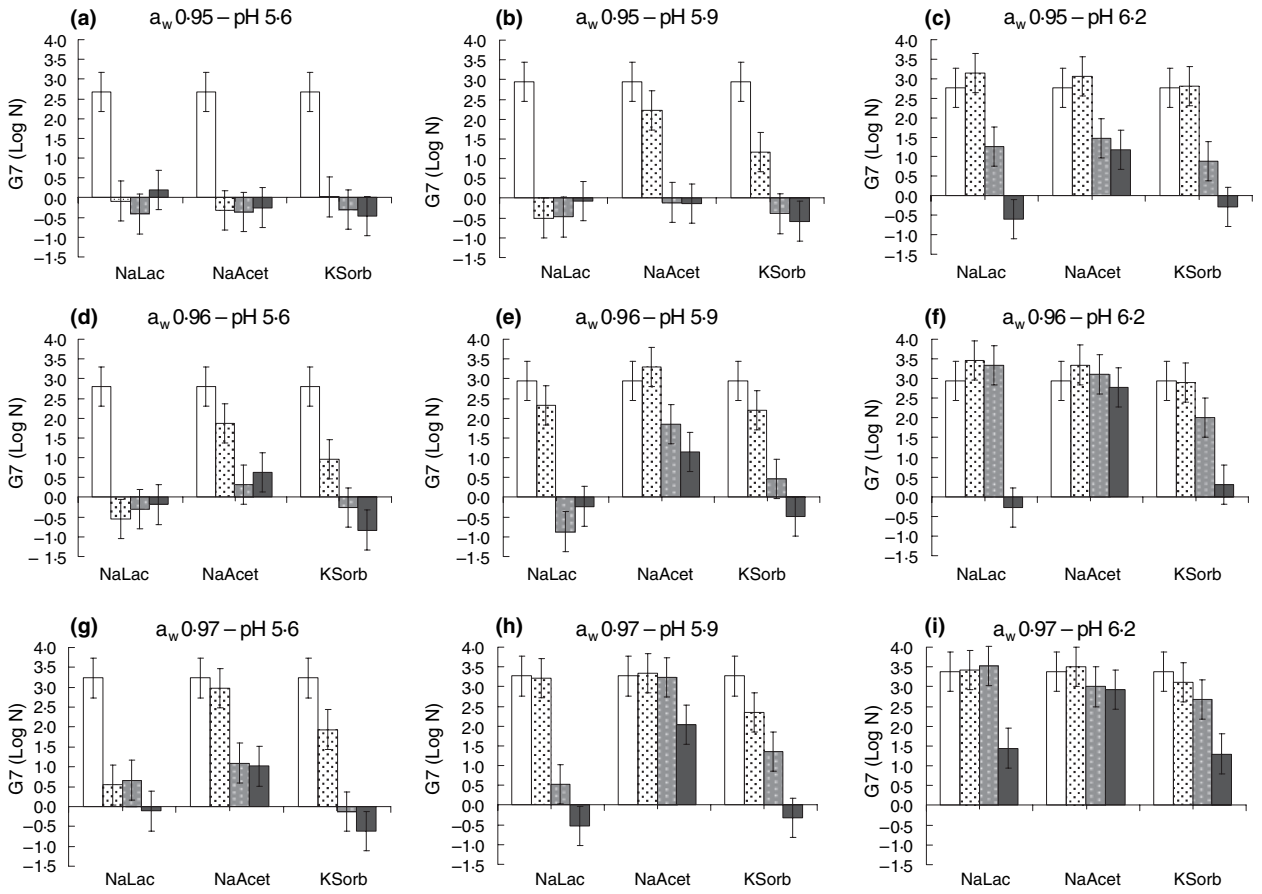

Figure 2 G7, log increase in 7 days for *Listeria monocytogenes* in irradiated ground pork stored at 20°C as a function of water activity (a_w), pH, nature and concentration of organic acid salt added: (□) control; (▨) level 1 tested (134 mmol l^{-1} for sodium lactate, 22 mmol l^{-1} for sodium acetate and 20 mmol l^{-1} for potassium sorbate); (▩) level 2 tested (268 mmol l^{-1} for sodium lactate, 44 mmol l^{-1} for sodium acetate and 40 mmol l^{-1} for potassium sorbate); (■) level 3 tested (402 mmol l^{-1} for sodium lactate, 66 mmol l^{-1} for sodium acetate and 60 mmol^{-1} for potassium sorbate); (|—|) experimental error.

Table 5 Values of α and undissociated minimum inhibitory concentration (MIC_u) for the three organic acid salts tested

Organic acid (salt added)	MIC_u (mmol l^{-1}) [confidence interval (5%)]	α [confidence interval (5%)]
Lactic acid (sodium lactate)	1·76 [1·67–1·84]	1·0*
Acetic acid (sodium acetate)	5·83 [5·40–6·25]	0·5*
Sorbic acid (potassium sorbate)	4·31 [3·11–5·51]	0·3 [0·23–0·42]

*Value from the literature (Le Marc *et al.* 2002). Other values were adjusted using our data.

boundary predicted with the Augustin's model and the logistic model with delay correctly delimited the experimental G7, lower or higher than 1·0 log. Comparison between prediction and experimental results was only made on the bacterial concentration after 7 days of storage at 20°C. For each organic acid salt, the area which permitted the *Listeria* growth according to pH and a_w was smaller when the concentration of the inhibitor was increased.

These boundaries were correctly predicted when 134 or 268 mmol l^{-1} of total sodium lactate was added. The growth area was larger than predicted in the presence of 402 mmol l^{-1} of total lactate. Whatever the total concentration of sodium acetate added, the growth areas were larger than predicted: one environmental condition where a growth was observed was located in the predicted no-growth area when 22 or 44 mmol l^{-1} of sodium acetate was added and three environmental conditions in the presence of 66 mmol l^{-1}.

When 20 or 40 mmol l^{-1} of potassium sorbate was added, predicted growth area was slightly smaller than the one observed: for each concentration, one environmental condition where a growth was observed was located in the predicted no-growth area. On the other hand, in the

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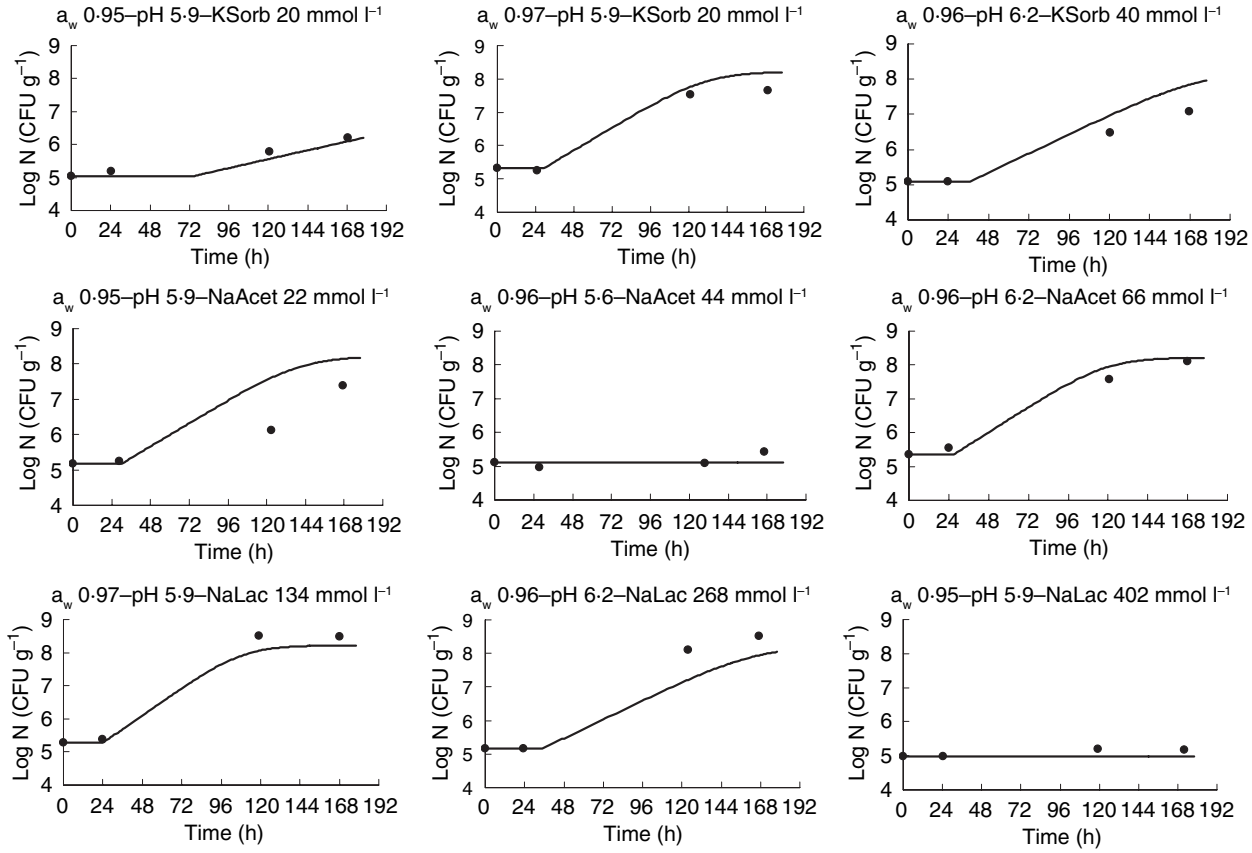

Figure 3 Comparison between bacterial enumerations and predicted curves in the presence of one organic acid salt: (●) experimental points; (¯¯) predicted curve.

Table 6 Log increase in seven days, G7 observed minus G7 predicted, for experiments used for the validation of the predictions

		Sodium lactate			Sodium acetate			Potassium sorbate		
a_w	pH	134 mmol l^{-1}	268 mmol l^{-1}	402 mmol l^{-1}	22 mmol l^{-1}	44 mmol 1^{-1}	66 mmol l^{-1}	20 mmol l^{-1}	40 mmol l^{-1}	60 mmol l^{-1}
0·97	5·9	+0·3	+0·5	−0·5	+0·3	+0·2	+0·2	−0·5	+1·0	−0·3
0·96	6·2	+0·6	+0·6	−0·3	+0·4	+0·1	−0·1	−0·3	−0·8	−2·0
0·96	5·6	−0·5	−0·3	−0·2	+1·4	+0·3	+0·6	+1·0	−0·3	−0·8
0·95	5·9	−0·5	−0·5	0·0	−0·7	−0·7	−0·1	+0·1	−0·4	−0·6

presence of 66 mmol l^{-1}, predicted growth area was slightly larger than predicted.

Two acid salts added. Good predictions were obtained for the growth or survival (G7 value between 0 and 1·0 log) of *Listeria* when combination of sodium acetate and sodium lactate was added. When potassium sorbate was used in combination with sodium lactate or sodium acetate, predictions were less accurate. For the first acid salts combination (potassium sorbate and sodium lactate), the great majority of incorrect predictions underestimated the growth and for the second combination (potassium sorbate and sodium acetate), incorrect predictions under- or overestimated the growth. Figure 8 shows examples of comparison between bacterial enumerations and predicted curves in the presence of two organic acid salts. According to our results showing that the behaviour of *Listeria*, when a mixture of acid salts was added, was in the majority equivalent to its behaviour in the presence of the major undissociated acid, we proposed a new model to substitute for eqn 7:

$$SR(c) = SR(c_1, c_2) = SR(c_1) \qquad (12)$$

where c_1 and c_2 are the concentrations of the undissociated forms of acids 1 and 2, respectively and $c_1 > c_2$.

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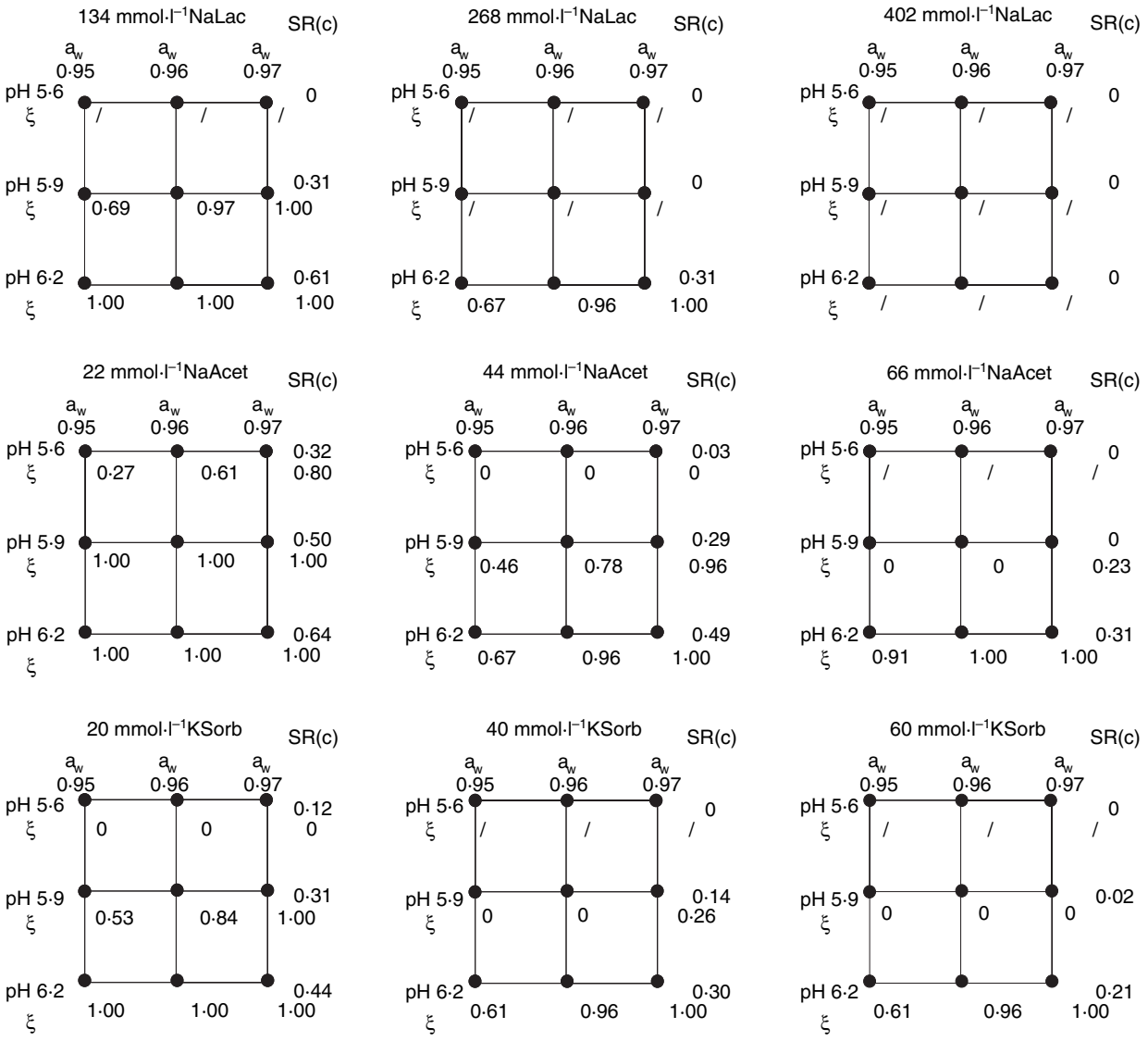

Figure 4 SR(c) and ξ values according to pH, water activity (a_w), nature and concentration of organic acid salt: /: predicted $\mu_{max} = 0$ because of acid already added without interaction; 0: predicted $\mu_{max} = 0$ because of interaction.

The model using eqn 12 provided accurate, if not better, description of kinetics of *Listeria* as use of eqn 7 (Fig. 9). Moreover, using eqn 12, the great majority of incorrect predictions were fail safe. However, regarding food safety, it is preferable to slightly overestimate the concentration of *Listeria* than to underestimate it.

Discussion

Use of organic acid salt as an additive to control the bacterial growth in food is now well established (Durand 1999; Jensen *et al.* 2003; Nakai and Siebert 2003). The inhibitory effect of organic acid salts has been in part associated with their capacity to decrease a_w (Houtsma *et al.* 1994; Mbandi and Shelef 2002; Deumier and Collignan 2003; Stekelenburg 2003). In pork meat, we showed that addition of 268 mmol l^{-1} of sodium lactate reduced a_w of 0·010. For other organic acid salts, the concentrations tested were probably too low to modify a_w. The inhibitory effect has also been attributed to their capacity to decrease the pH (Eifert *et al.* 1997; Dubal *et al.* 2004) following their dissociation in acid. Moreover, it has been shown that inhibitory effect of organic acids was mainly seen when it is undissociated (Blom *et al.* 1997). Finally, their specific inhibitory effect has been associated with their nature (Ahamad and Marth 1989; Houtsma *et al.* 1993). Indeed, Houtsma *et al.* (1993) and Le Marc (2001) proposed a possible role of the dissociated molecule, the

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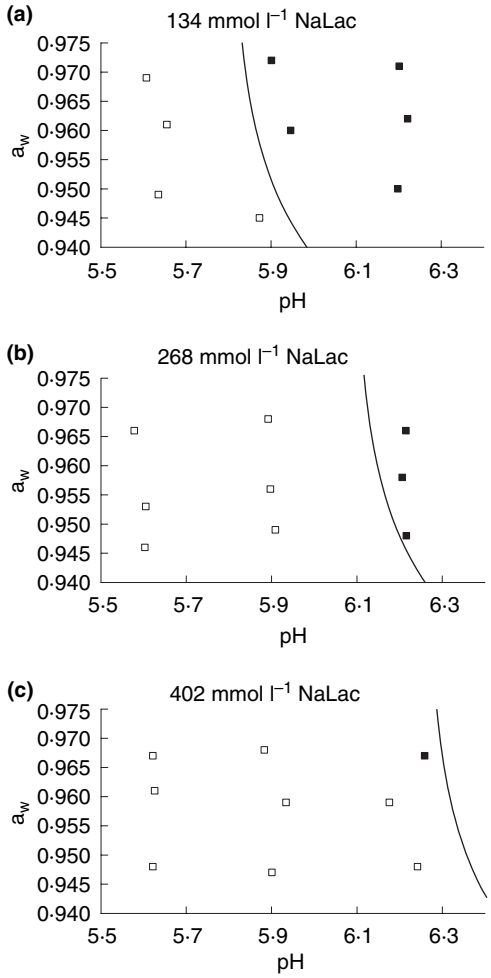

Figure 5 Comparison of the predicted boundary of the growth/no-growth areas for *Listeria* as a function of concentration of sodium lactate added, pH and water activity (a_w) with experimental G7 (log increase in 7 days) lower or higher than 1·0 log: (■) growth (G7 > 1·0 log); (□) no growth (G7 < 1·0 log); (————) predicted boundary.

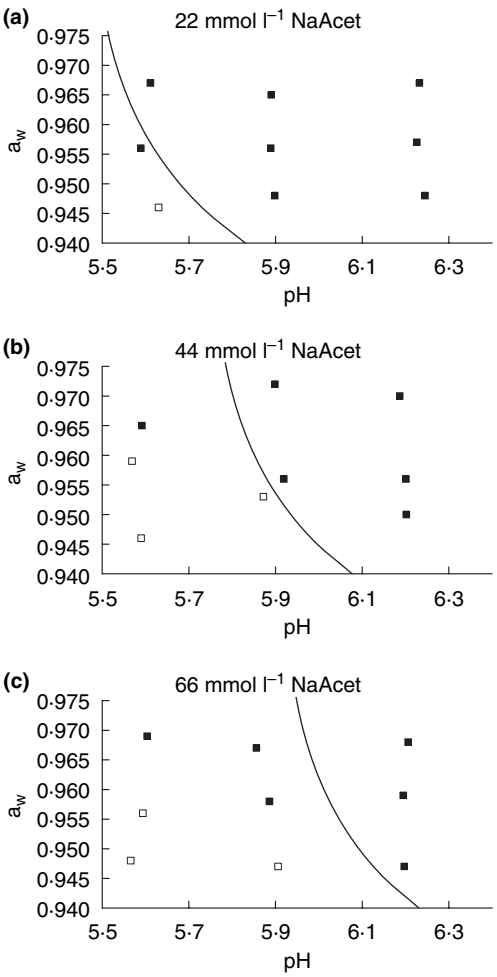

Figure 6 Comparison of the predicted boundary of the growth/no-growth areas for *Listeria* as a function of concentration of sodium acetate added, pH and water activity (a_w) with experimental G7, log increase in 7 days, lower or higher than 1·0 log: (■) growth (G7 > 1·0 log); (□) no growth (G7 < 1·0 log); (————) predicted boundary.

lactate ion, in the inhibition of microbial growth: the lactate anion inhibits enzymes involved in the pyruvate-to-lactate conversion (Houtsma *et al.* 1994). Additional toxic effect of acetic acid on cells by modification of physiological and metabolic activities was also demonstrated (Eifert *et al.* 1997; Jensen *et al.* 2003). Even if the mechanism of action of potassium sorbate has remained uncertain, it is partly based on its ability to inhibit the amino acid uptake in *Penicillium chrysogenum* and in vesicles of some bacteria (Ronning and Hilmer 1987).

Our results confirmed that organic acid salts did not have the same inhibitory effect according to their nature. We also showed that pH and a_w had an influence on the inhibitory effect of the organic acid salt: when pH and a_w increased, the concentration of organic acid salt required to observe an inhibitory effect increased, showing interactions between environmental factors (Augustin and Carlier 2000; Le Marc 2001; Coroller *et al.* 2005). Moreover, contrary to several studies (Mbandi and Shelef 2002; Zheng *et al.* 2005) we did not show additive effect of organic acid salts added in combination: the usefulness of mixing different organic acid salts was not shown.

In the literature, most of the experiments that study the behaviour of *Listeria* were performed in broth rather than in a food model matrix but it is well established that the structure of the medium has a great influence on the bacterial behaviour (Robins and Wilson 1994); therefore, we carried out our experiments in meat. Wang (2000) demonstrated that addition of 3% sodium lactate (the equivalent of 268 mmol l^{-1}) to Chinese-style sausage stored at 20°C (a_w 0·91, pH 6·6) maintained low microbial numbers. Stekelenburg and Kant-Muermans (2001)

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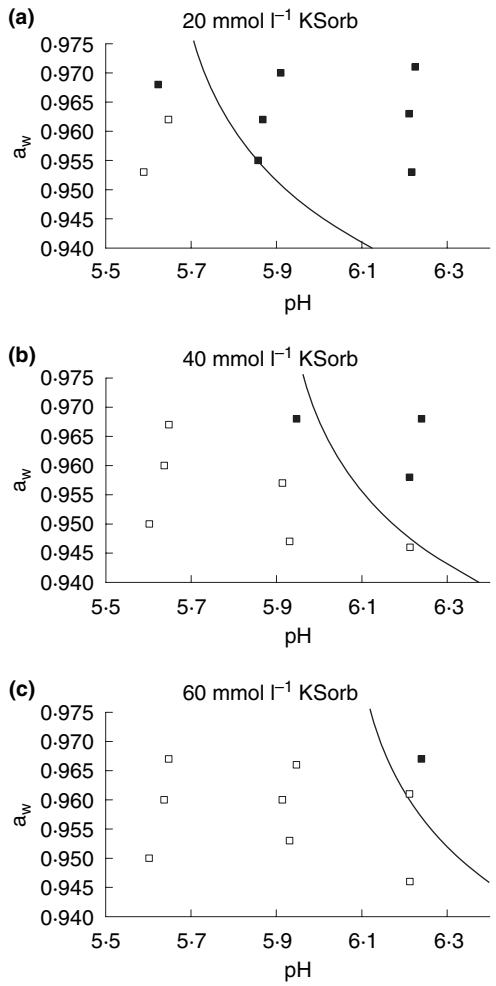

Figure 7 Comparison of the predicted boundary of the growth/no-growth areas for *Listeria* as a function of concentration of potassium sorbate added, pH and water activity (a_w) with experimental G7, log increase in 7 days, lower or higher than 1·0 log: (■) growth (G7 > 1·0 log); (□) no growth (G7 < 1·0 log); (————) predicted boundary.

showed that the addition of sodium lactate to cooked ham stored at 4°C (pH between 6·1 and 6·2; a_w between 0·96 and 0·97), in percentages ranging from 1·9% to 2·8% (170–250 mmol l^{-1}) inhibited the development of *L. monocytogenes*. Choi *et al.* (2003) demonstrated that sodium lactate had an anti-*Listeria* effect (after 8 weeks at 4°C) in regular-fat sausages (pH 6·1, a_w 0·93) when added to a concentration of 295 mmol l^{-1}. In our study, inhibitory effect of sodium lactate was significant at 134 mmol l^{-1} when a_w and pH were respectively 0·95 and 5·6. For less drastic values (pH 6·2 and a_w 0·97), addition of 402 mmol l^{-1} of sodium lactate was required to observe a significant reduction of the final increase of the *L. monocytogenes* population compared with the control condition. Potassium sorbate has principally been used to inhibit the growth of yeasts and moulds (Lund *et al.* 1987; El-Shenawy and Marth 1988; Durand 1999; Marin *et al.* 2002, 2003). However, Choi and Chin (2003) found that changes of microbial counts for inoculated *L. monocytogenes* of regular-fat sausages (pH 6·1, a_w 0·94) during refrigerated storage was affected by addition of 0·05% (3 mmol l^{-1}) of potassium sorbate (reduction of 1·5 log after 8 weeks). In our study, at pH 6·2 and a_w 0·95, 40 mmol l^{-1} of potassium sorbate was needed to observe an inhibitory effect. The inhibitory effect of such a low concentration of potassium sorbate reported by Choi and Chin (2003) was probably because ofthe lower storage temperature they used.

Addition of 5 g kg^{-1} (61 mmol l^{-1}) of sodium acetate in turkey bologna (pH 6·58, a_w 0·945) stored at 4°C provided a significant inhibition of *L. monocytogenes* (Wederquist *et al.* 1995). In our study, such a concentration was also effective except when a_w was 0·97 and pH equal or higher than 5·9 and for a_w 0·96 and pH 6·2.

In this work, we first optimized undissociated MIC values for lactic, acetic and sorbic acids. We also optimized α (eqn 6) associated with sorbic acid. For lactic acid, we obtained an undissociated MIC value of 1·76 mmol l^{-1}. This value is lower than the one found by Augustin (1999) – 5·4 mmol l^{-1} – or by Coroller *et al.* (2005) – between 3·6 and 5·7 mmol l^{-1}.

For acetic acid, Coroller *et al.* (2005) obtained a range of estimated undissociated MIC values between 6·2 and 18·6 mmol l^{-1}; a value of 20·1 mmol l^{-1} was obtained by Augustin (1999). In this study, we found a MIC_u value equal to 5·83 mmol l^{-1}. For undissociated potassium sorbate, El-Shenawy and Marth (1988) proposed a MIC value equal to 5·1 mmol l^{-1}; this value is slightly higher than the one we found – 4·31 mmol l^{-1}. The difference between undissociated MIC values proposed in this study and other data obtained from the literature came from either: (i) the strain, (ii) the matrix, (iii) the way of calculating the acid concentration in the meat, (iv) the model and/or (v) the experimental procedure used. Indeed, *L. monocytogenes* 14 used in this work was probably more sensitive to acid than other *L. monocytogenes* strains used in previous studies. Nevertheless, Coroller *et al.* (2005) proposed to adjust the MIC_u for all strains of one species and to establish the variability of sensitivity of the species by adjusting α (eqn 6) according to each strain. In the work by Coroller *et al.* (2005), MIC values were estimated using experiments carried out in BHI. In the work by Augustin (1999), data were collected in several studies performed on broth or food. We suppose that other inhibitors naturally found in the meat were not taken into account in this work. For example, concentration of lactic acid naturally present in pork muscle was not taken into account for the μ_{max} prediction. We thus

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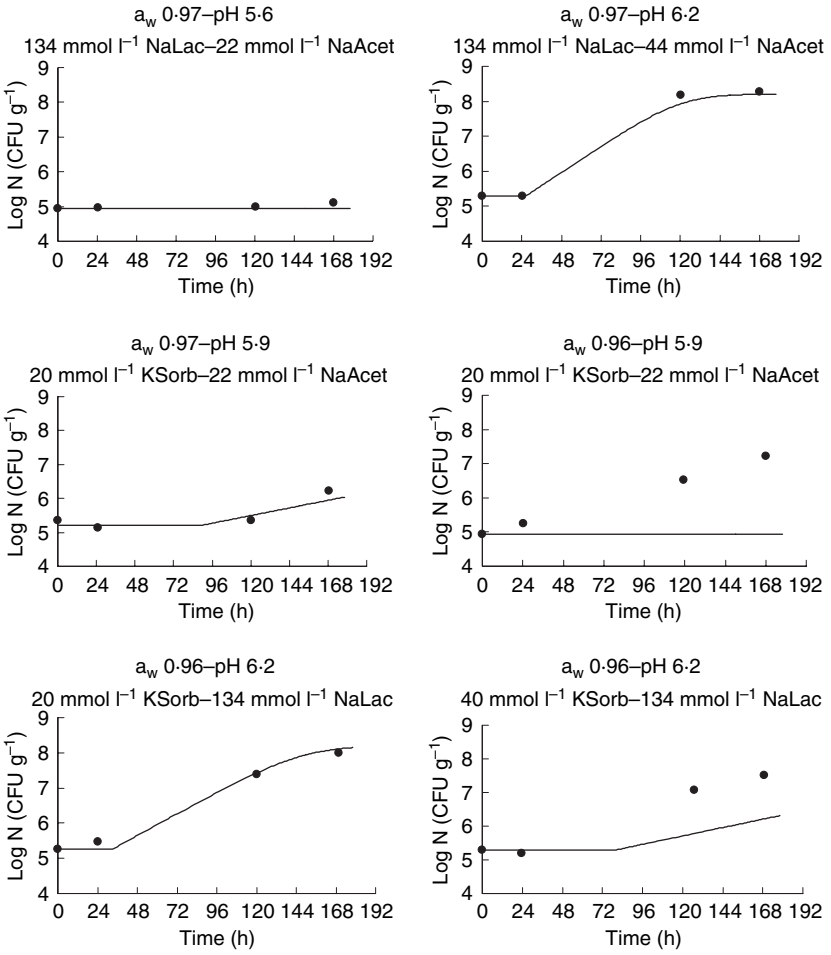

Figure 8 Comparison between bacterial enumerations and predicted curves in the presence of two organic acid salts: (●) experimental points; (——) predicted curve.

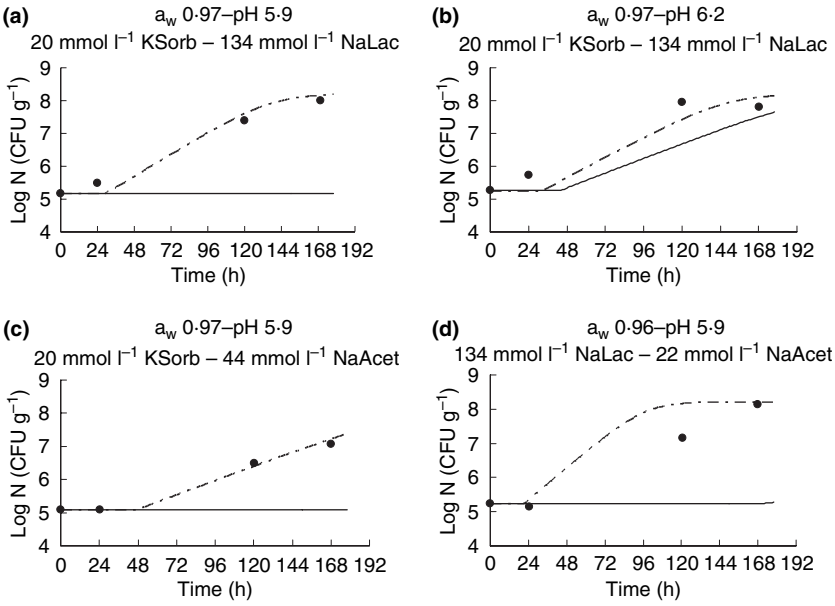

Figure 9 Comparison between bacterial enumerations and predicted curves using the model proposed by Coroller *et al.* (2005) or the equation proposed in this study, in the presence of two organic acid salts: (●) experimental points; (——) predicted curve using eqn 7 (Coroller *et al.* 2005); (- - -) predicted curve using eqn 12.

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neglected endogenous inhibitor(s) and its (their) interaction(s) with added organic acid salt(s). The MIC value obtained in this work could be a global MIC value for all inhibitors present in the meat: the organic acid salt(s) added and endogenous inhibitors (e.g. lactic acid). Calculation of the concentration of added acid was based on the total volume of meat. Nevertheless, acid was dissociated in the extracellular water of unknown volume. Acid concentration in this compartment was higher than the concentration based on the total meat volume, which we used for calculation. This can partly explain the lower MIC value obtained in this study. Moreover, models used in the different studies were also different (for the function used to take into account interactions or for the value of α); it thus influenced the optimized MIC values. Finally, the experimental procedure used also influenced the optimized MIC values. Indeed, in our study, organic acid salts were added and then pH was adjusted using HCl or NaOH. For other studies, pH was only controlled by addition of organic acid (salts).

We compared (data not shown) the sum of the squared residuals between experimental data (data which were not used for the optimization) and predicted curves for the three models when one organic acid salt was added (Augustin 1999; Augustin *et al.* 2005; Coroller *et al.* 2005). Equivalent correct results were obtained with the three models. We therefore investigated the ability to predict the boundary between the growth and the no-growth areas with the model proposed by Augustin *et al.* (2005). For sodium lactate, the majority of the predictions were correct. For sodium acetate and potassium sorbate, predicted growth areas were sometimes underestimated, particularly when pH was low (5·6), i.e. for the conditions where proportion of undissociated acid *vs* dissociated acid increased. For sodium lactate, pKa was lower (3·86) than for the other acids tested (4·75 and 4·76): the range of pH tested was more distant from its pKa.

Moreover, figures were proposed to facilitate the comparison between experimental data and predicted growth/no-growth boundaries. On these figures, boundaries were always predicted after 7 days of storage, i.e. 168 h. Nevertheless, experimental enumerations have been made at 168 ± 5 h. Such time difference was sometimes enough to shift a condition from the no-growth (or the growth) area to the growth (no-growth) area.

Concerning the modelling of mixture of acids, we obtained good predictions when sodium lactate and sodium acetate were added. In the presence of potassium sorbate with another salt, predictions were less accurate but incorrect ones in a great majority failed safely using the equation proposed in this study. Other models exist to predict the effect of mixture of acids (Coroller *et al.* 2005) but the equation we proposed has the advantage of being simple. We thus have proposed a model which is useful to predict the behaviour of *L. monocytogenes* in pork products containing one or a mixture of organic acid salts and especially when their pH is equal to or higher than 5·9.

Conclusions

The inclusion of additives is amongst strategies employed to minimize or inhibit undesirable bacterial growth in fresh meat. Efficiency of organic acid salts such as sodium lactate, sodium acetate and potassium sorbate against *L. monocytogenes* was demonstrated in ground pork.

In order to predict the evolution of *L. monocytogenes* concentration in pork meat when organic acid salt was added, or when mixture of acid salts was used, the Augustin's model with interactions (Augustin *et al.* 2005) was well adapted, particularly in the pH area of delicatessen such as diced bacon (pH near 5·9–6·0).

As consumer demand is currently driven towards food products that are 'natural' but still safe, combination of low pH and a_w permit to minimize the concentration of organic acid salt required to inhibit or limit the bacterial growth. To conclude, the Augustin's model is an additional tool for the optimization of the formulation and to guarantee the microbiological quality of the product in minimizing the organic acid salt added.

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