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ORIGINAL ARTICLE

Comparison of growth limits of *Listeria monocytogenes* in milk, broth and cheese

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Abstract

Aim: To determine growth initiation differences of *Listeria monocytogenes* between a cheesemaking context, milk and tryptic soy broth (TSB).
Methods and Results: A laboratory-scale cheese was made with a mix of two strains of *L. monocytogenes* at four initial pH values, five water activity (a_w) values and two contamination levels at 30°C. Counts of *L. monocytogenes* were determined at time 0 and after 8 h of cheese manufacture. Milk and TSB at the same pH and a_w conditions were inoculated with the *L. monocytogenes* mix in multi-well plates. Growth was determined by plating each well onto Agosti & Ottaviani Listeria Agar after 8 h of incubation at 30°C. Each condition was repeated six times, and growth initiation probability was modelled with logistic regression models. Growth initiation boundaries were obtained for each matrix type. The results showed that the growth limits were matrix dependent. In the three matrix types, a_w was the most important factor affecting the probability of growth initiation. Contamination level affected growth TSB and cheese-making conditions.
Conclusions: The interface wideness and position in cheese, milk and TSB were dissimilar, indicating that the use of models evaluated in TSB or milk could not be used to predict the behaviour of *L. monocytogenes* under cheesemaking conditions.
Significance and Impact of the Study: Predictive models generated in liquid media are not necessarily adaptable to solid food, and the generation of real food models is necessary.

Introduction

Listeria monocytogenes is a widely distributed foodborne pathogen that causes listeriosis in risk groups, such as young, old, pregnant women, neonates and the immuno-compromised, with a mortality rate of 20–30% despite correct antimicrobial treatment (Swaminathan and Gerner-Smidt 2007). It has been responsible for several foodborne outbreaks of listeriosis reported in Europe, USA and Japan. (Dalton *et al.* 1997; Makino *et al.* 2005; Dawson *et al.* 2006; Swaminathan and Gerner-Smidt 2007).

Listeria monocytogenes can be present in all foods, particularly milk products (Harvey and Gilmour 1992; Gaya *et al.* 1996), animal products (Dillon *et al.* 1994; Samelis and Metaxopoulos 1999; Medrala *et al.* 2003; Soultos *et al.* 2003; Miettinen and Wirtanen 2005; Thévenot *et al.* 2005), ready-to-eat foods (Lianou and Sofos 2007) and vegetables (Little *et al.* 2007).

The behaviour of bacterial cells in liquid media may differ from that encountered in certain structured foods. Traditionally, shelf-life and the ability of foods to support the growth of spoilage micro-organisms were assessed by means of challenge testing, where the organism of interest is inoculated onto foods and its growth monitored for a period of time. This approach was later seen as both time-consuming and costly. The development of

predictive microbiology (in laboratory liquid media) complemented the challenge testing by modelling the behaviour of foodborne pathogens as a function of only a few environmental parameters (Wilson *et al.* 2002). For example, Murphy *et al.* (1995) developed a liquid food–based model (milk) and validated the model in a series of dairy products. While liquid-based modelling may yield accurate predictions in liquid-based foods, there can be over estimation of growth in solid food and models become inaccurate for such foods (Pin and Baranyi 1998; Meldrum *et al.* 2003).

When foods support the growth of pathogens it is very important from a practical point of view to be able to predict the growth limits of *L. monoytogenes* under defined environmental conditions. Growth no growth (GNG) models can be used as a method of defining combinations of environmental factors that allow or prevent growth. In this case, the position of the GNG boundaries, rather than the growth rate, are of prime importance. This is because any growth, regardless of the rate, poses a threat to the consumer. Such GNG boundaries are important in process design in order to ensure no growth of a pathogen occurs. The use of laboratory media in the evaluation of GNG boundaries is more common and the extension of these types of work to real food systems is still very poorly developed (Bolton and Frank 1999; Tienungoon *et al.* 2000; Koutsoumanis *et al.* 2004; Boziaris *et al.* 2005; Koutsoumanis and Sofos 2005; Zuliani *et al.* 2007; Hwang 2009). The obvious differences in composition and structure between laboratory media or liquid food, such as milk and certain solid foods (cheese), raises the question about the suitability of these liquid-based models in their application to structured foods.

In this study, we aimed to (i) evaluate and compare the a_w, pH and lactic acid combinations that define the growth limits of *L. monocytogenes* in three different matrices, namely, liquid laboratory media, liquid food (milk) and liquid-to-solid food (during the cheesemaking process) at two contamination levels and to (ii) evaluate and compare the growth initiation probability of *L. monocytogenes* in the three different matrices; the hypothesis being that liquid-based models may not accurately predict the probability of growth initiation of *L. monocytogenes* in a cheesemaking context where liquid media becomes solid.

Materials and methods

Listeria monocytogenes cultures preparation

The strains used were 6179 and C5 (Moorepark Food Research Centre culture collection), serotype 4b and 1/2a, respectively, which are natural isolates from cheese and farmhouse cheese environment, respectively. Stock cultures were maintained in tryptic soy broth (TSB; Becton Dickinson Co., Franklin Lakes, NJ, USA) in the presence of 25% v/v glycerol at −80°C. Cultures were activated by growing the strains overnight in TSB at 37°C and streaking a loop full (5 μl) onto tryptic soy agar slopes to use a stock of cells with the same physiological background for the length of the experiment. The inoculum for each experiment was prepared from the slope by growing the strains in TSB overnight at 37°C. Cultures were mixed and diluted in maximum recovery diluent (MRD; Oxoid, Basingstoke, UK) for each particular case to achieve the desired inoculum level of 10^1 CFU ml^{-1} (low population) or *c.* 10^2 CFU ml^{-1} (high population).

Cheesemaking

A factorial design of four pH values, five a_w values and two inoculation levels was undertaken, and six independent replicate cheeses were manufactured under each condition. To evaluate the GNG initiation boundaries, a_w and pH were set to constant values throughout the whole cheesemaking process. Cheeses were made in 800 ml volumes without the addition of starter culture and with pasteurized milk to avoid the possible effect on the behaviour of *L. monocytogenes* of microbial heterogeneity derived from the combination of background flora and starter lactic acid bacteria. Pasteurized milk (with 8% low-heat skim milk powder added; Tipperary Co-operative, Tipperary, Ireland) was adjusted to the required pH (6·5, 6·1, 5·9, 5·6) by adding sterile 10% lactic acid or sterile 10% sodium hydroxide and to the required a_w (0·99, 0·98, 0·97, 0·96 and 0·95) by adding salt at 0, 3, 4·5, 6 or 8% NaCl to the milk. Addition of low-heat skim milk powder was essential to increase the protein content and aid coagulation at low a_w and pH values. Therefore, for consistency, the powder was added to all cheesemaking experiments. In preliminary experiments, it was shown that there was no significant difference (P-value >0·05) between the growth of *L. monocytogenes* with and without powder added. Pasteurized milk was heated to 30°C; following inoculation with *L. monocytogenes* (at 1–10 CFU ml^{-1} or 500 CFU ml^{-1}), rennet was added (approx. 3·18 ml l^{-1} diluted 10-fold in sterile distilled water, depending on protein concentration, CHY-MAX PLUS Fermentation Produced Chymosin; CHR HANSEN, Horsholm, Denmark) and milk was left for coagulation. Once the proper firmness was achieved (tested with a sterile knife), the curd was cut into cubes and left undisturbed for 10 min. The cheesemaking process was followed by cooking to 36°C (at a rate of 1°C every 10 min) and stirring

of the curd for a further 1 h. The whey was then drained and the curd moulded in conical-holed moulds (top diameter 89 mm, bottom diameter 82 mm and height 83 mm; Moorlands Cheesemakers Ltd, Somerset, UK). The cheesemaking process lasted for approximately 8 h from the inoculation of *L. monocytogenes* to milk to the sampling of the cheese.

Milk and laboratory media

A full factorial design of four pH values, five water activity values and two inoculation levels was undertaken in 96-well plate experiments, and six replicates of each condition were tested. Pasteurized and homogenized fresh milk were purchased in a local store or sterile TSB were prepared with the correspondent amount of NaCl (0, 3, 4·5, 6 or 8%) and adjusted to the correspondent pH (6·5, 6·1, 5·9 or 5·6) with 10% lactic acid (v/v) prepared in sterile distilled water. The milk and the broth were inoculated with *L. monocytogenes* to achieve a final concentration of 10^1 CFU ml^{-1} for the low contamination level and 10^2 CFU ml^{-1} for the high contamination level. The inoculated milk/TSB was transferred to the multi-well plates which were placed in an incubator at 30°C for 8 h, the same time as the cheesemaking.

Analyses

Initial numbers of *L. monocytogenes* populations were estimated in milk or TSB by spreading 1 ml onto 20 cm diameter petri dishes prepared with Agosti & Ottaviani Listeria Agar (ALOA, LAB M Lancashire, UK) and incubated at 37°C for 48 h. Final numbers of *L. monocytogenes* were estimated from cheese, milk or TSB by spreading 100 μl from cheese sample dilutions or 30 μl from each well from milk and TSB experiments. Cheeses were sampled according to the IDF Standard (50B, 1985; 122B, 1992) for sampling and microbiological analysis of dairy products. To compare the population of *L. monocytogenes* in milk or TSB (in millilitres) and cheese (in grams), the counts were expressed as gram of dry weight (CFU gdw^{-1}). The CFU gdw^{-1} were calculated by determining first the total solid content of each sample (TSB, milk or cheese). The total solids content in cheese was determined according to IDF Standard (4A, 1982) and in milk and TSB according to IDF Standard (21B, 1987). The number of cells counted in '*x*' g of dry sample was then transformed to counts per 1 g of dry sample by making a 'rule of three' calculation. The a_w was determined placing milk or TSB samples (5 ml) or cheese (as directed in the manufacturers instructions) in a sampling cup and measured with an AquaLab Monitor Series 3T equipment (Labcell, Hampshire, UK). The pH was measured using an Orion pH meter model 420A. Standard BS770:5:1976 was followed for the measurement of pH in cheese. L-lactic acid was measured at the time of addition in milk and TSB, and measured with an L-lactic acid kit (Boehringer Mannheim, Darmstadt, Germany) from cheeses.

Evaluation of growth/no growth initiation of *Listeria monocytogenes*

The difference in populations of *L. monocytogenes* between the 8 h of incubation or 8 h of cheesemaking, expressed in $\log_{10}$ CFU gdw^{-1}, was calculated by subtracting final and initial population size. 'Growth' was considered to have occurred when a statistically significant difference of at least 0·5 $\log_{10}$ CFU gdw^{-1} was observed (tested by the *t*-test, *P*-value <0·05). The remaining cases were considered 'No Growth'. The 0·5 log increase criterion was taken from three studies by Skandamis *et al.* (2007), Koutsoumanis and Sofos (2005) and Bolton and Frank (1999).

Modelling growth/no growth initiation data

Data from each replicate were classified as 1 or 0 for growth or no growth, respectively, based on the aforementioned criteria for the growth of *L. monocytogenes*. Data were treated in two ways, (i) one model was fitted to each set of data (broth, milk and cheese) and (ii) all the data from the three matrices were merged in a single set of data, and each matrix was given a categorical variable A, B and C for cheese, milk and TSB, respectively, for model fitting purposes. The set of data was then analysed with two logistic regression models: the nonlinear logistic regression model (NLRM) and the ordinary logistic regression model (OLRM). The models were fitted to the data by means of the logistic procedure in SAS 9.1 software (SAS Institute Inc., Cary, NC, USA). The data were analysed in two separated sets, namely, low and high contamination level. The factors taken into account in the model were a_w, pH and lactic acid. Temperature was not considered as it was constant for the whole process. The equations used were:

Equation 1: NLRM

$$\mathrm{Logit}(P) = \mathrm{Ln}\left(\frac{P}{1-P}\right) = \beta_0 + \beta_1 \mathrm{Ln}(a_w - a_{w_{min}}) + \beta_2 \mathrm{Ln}(1 - 10^{(pH_{min} - pH)}) \quad (1)$$

Equation 2: OLRM without lactic acid term

$$\text{Logit}(P) = \text{Ln}\left(\frac{P}{1-P}\right) = \beta_0 + \beta_1 \text{pH} + \beta_2 b_w + \beta_3 \text{pH} \cdot b_w + \beta_4 \text{pH}^2 + \beta_5 b_w^2 \quad (2)$$

Equation 3: OLRM with lactic acid term

$$\text{Logit}(P) = \text{Ln}\left(\frac{P}{1-P}\right) = \beta_0 + \beta_1 \text{pH} + \beta_2 b_w + \beta_3 \text{LAC} + \beta_4 \text{pH} \cdot b_w + \beta_5 \text{pH} \cdot \text{LAC} + \beta_6 b_w \cdot \text{LAC} + \beta_7 \text{pH}^2 + \beta_8 b_w^2 + \beta_9 \text{LAC}^2 \quad (3)$$

where P is the probability of growth, receiving a value of 1 or 0 for growth and no growth, respectively; $a_{w_{min}}$, pH_{min} are the minimum (notional) theoretical values of a_w and pH, respectively, below which, no growth of *L. monocytogenes* is likely in milk or broth; LAC is the lactic acid added in mM; b_w is a transformation of a_w, proposed by Gibson *et al.* (1994), to reduce the variance of a_w and enhance the fitting procedure ($b_w = \sqrt{(1 - a_w)}$); and β_{0-9} are the parameters to be estimated by logistic regression.

All parameter values were estimated in the modelling process except the minimum a_w value, which was fixed at 0·913 and the minimum pH value of 4·7 (Augustin *et al.* 2005). The predicted GNG interface was calculated for $P = 0{\cdot}9$, 0·5 and 0·1 using Excel Goal seek (Microsoft® Office 2003 Excel). The statistical indices used to measure the goodness-of-fit of the models were the Log-likelihood, Akaike Information Criterion (AIC) and the concordance rate (PC).

Results

Growth of *Listeria monocytogenes* in three different matrices

Low contamination level

Observed percentages of growth for the three different matrices are shown in Fig. 1. Growth was observed in 100% of the cases at minimum a_w levels of 0·98 in cheese, 0·95 in milk and 0·97 in TSB, respectively. Variable growth was observed during cheesemaking at and below a_w values of 0·97, no growth was observed below a_w 0·96. The lower limit of the interface with a_w and pH combinations leading to no growth observations was not reached either in milk or in TSB. The lower the a_w, less cases of growth were observed in the three matrices, there was no evidence of reduction in growth percentage with changes in pH.

High contamination level

Observed percentages of growth for the three different matrices are shown in Fig. 2. The minimum a_w values at which 100% growth occurred were 0·97 for cheesemaking and 0·95 for milk and TSB. As with low contamination levels, the a_w (but not the pH) affected growth initiation, with less growth initiation observed at low a_w values.

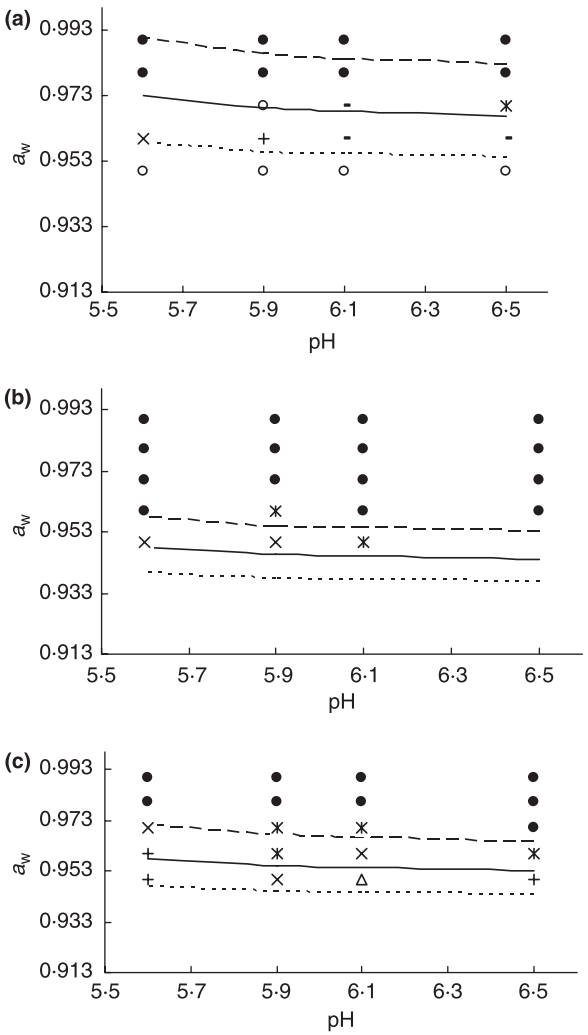

Figure 1 *Listeria monocytogenes* growth boundaries defined by the nonlinear logistic regression model during cheesemaking (a), milk (b) and tryptic soy broth (c) with low contamination levels of *L. monocytogenes* at pH and a_w combinations. Percentage of observed growth: 100% (●), 83% (*), 66% (×), 50% (+), 33% (△), 16% (-) and 0% (○). Boundary for probability 0·9 (– –), 0·5 (—) and 0·1 (- - - -).

Modelling results

The parameter estimates and statistical indices for low and high contamination levels are shown in Table 1 for the NLRM and Table 2 for the OLRM without lactic acid term. The NLRM was not fitted to the data with lactic acid. Tienungoon *et al.* (2000) reported in their study that the term for lactic acid is not necessary in the model

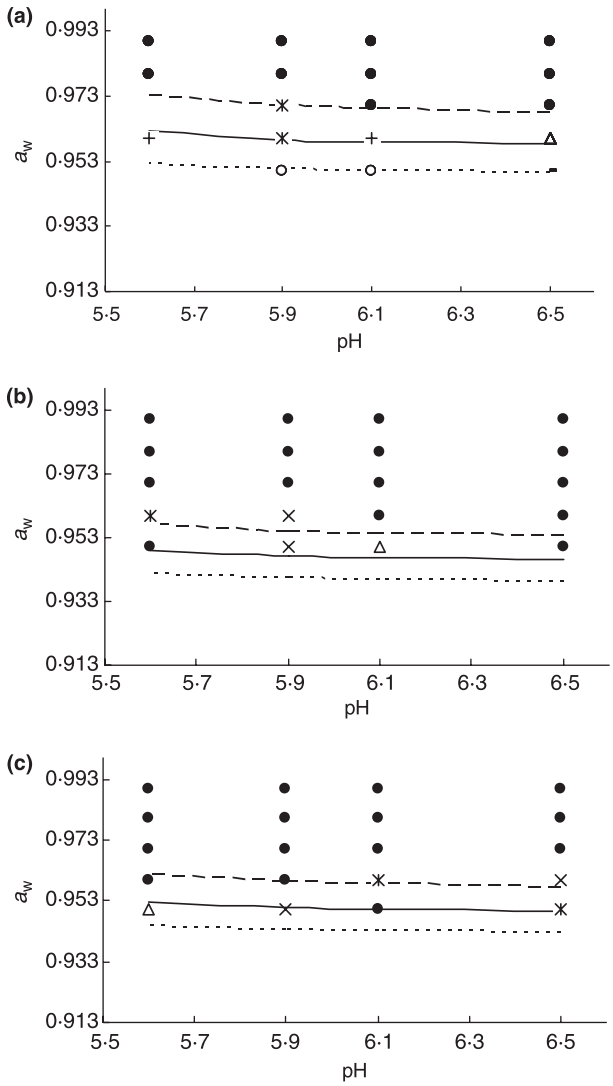

Figure 2 *Listeria monocytogenes* growth boundaries defined by the nonlinear logistic regression model during cheesemaking (a), milk (b) and tryptic soy broth (c) with high contamination levels of *L. monocytogenes* at pH and a_w combinations. Percentage of observed growth: 100% (●), 83% (*), 66% (×), 50% (+), 33% (△), 16% (-) and 0% (○). Boundary for probability 0·9 (– –), 0·5 (—) and 0·1 (- - - -).

if the total lactic acid concentration is below 500 mmol l^{-1}. Maximum lactic acid concentrations of lactic acid measured in milk, broth or cheese were 275 mmol l^{-1} (data not shown). Lactic acid term was included in the OLRM [Eqn (3)], but the lactic acid parameter was not significant for both low and high contamination data (*P*-value = 0·157 and 0·3891 for low and high contamination data, respectively). The AIC for the OLRM with lactic acid data was 395 and 348 for low and high contamination data, respectively, higher than the AIC for the OLRM without lactic acid data (see Table 2). The data were therefore fitted to the OLRM without lactic acid [Eqn (2)] and compared to the fit of the NLRM. The statistical indices indicated that the NLRM fitted the data better than the OLRM with low contamination data and vice versa with high contamination data, but the intercepts for TSB yielded by the OLRM were not significant (*P*-value = 0·4947 and 0·2204 for low and high contamination data, respectively) and it was not possible to obtain boundaries with this model. In consequence, the NLRM was chosen for data fitting and comparison.

Nonlinear logistic regression model

The NLRM yielded significant intercepts for the three types of matrices for both low and high contamination levels. The parameter for pH [β_2 in Eqn (2)] was statistically not significant either with low or high contamination levels in a backward analysis. The statistical indices indicated a better fit of the data with low contamination levels. The growth boundaries for probabilities 0·9, 0·5 and 0·1 are plotted in Fig. 1 (low contamination levels) and Fig. 2 (high contamination levels). The interface resulting from the 0·1–0·9 probability boundaries was wider in cheesemaking conditions than in milk or broth. The range of a_w values for the interface 0·1–0·9 probability was 0·95-0·99, 0·94-0·95 and 0·94–0·97 for cheesemaking, milk and TSB, respectively for low contamination data and 0·94–0·97, 0·94–0·95 and 0·94–0·96 for cheesemaking, milk and TSB, respectively and high contamination levels. Percentage of observed cases of growth for each a_w/pH combination and 0·9, 0·5 and 0·1 probability of growth boundaries for each matrix type are plotted in Fig. 1 (low contamination level) and Fig. 2 (high contamination level).

Simulations of the predicted probability of growth are plotted with the observed percentage of growth in Fig. 3 (low contamination level) and Fig. 4 (high contamination level). From a graphical point of view, the NLRM predicted well the data except for low contamination levels at pH 5·6 in cheesemaking where there was underestimation of the probability of growth.

Discussion

The percentages of growth observed in the three different matrices for low and high contamination levels (shown in Figs 1 and 2) presented considerable differences and this was reflected by the NLRM. Growth was considered to have been initiated at the tested experimental conditions when a significant statistical difference of 0·5 logs was observed between the two samplings. During cheesemaking, the difference in logs between the two samplings was on some occasions negative,

Table 1 Parameter estimates and statistical performance indices for the Nonlinear Logistic Regression Model fitted for low and high contamination level growth/no growth data of *Listeria monocytogenes* in TSB, milk and during cheesemaking

	Low contamination level		High contamination level	
Coefficient	Estimate	*P*-value	Estimate	*P*-value
Int TSB (β_0*)	24·6864	<0·0001	32·8726	<0·0001
Int milk (β_0)	29·2418	<0·0001	36·093	<0·0001
Int cheesemaking (β_0)	27·1176	<0·0001	35·0751	0·029
β_1	8·4081	<0·0001	14·6666	<0·0001
β_2	7·8409	0·05	15·9338	0·135
Loglikelihood	204		280	
AIC	214		282	
PC	92		91·3	

Int, intercept; AIC, Akaike Information Criterion; PC, concordance rate; TSB, tryptic soy broth.
*Coefficient of the Ordinary Logistic Regression Model described by Eqn (2).

Table 2 Parameter estimates and statistical performance indices for the Ordinary Logistic Regression Model (OLRM) fitted for low and high contamination level on growth/no growth data of *Listeria monocytogenes* in TSB, milk and during cheesemaking

	Low contamination level		High contamination level	
Coefficient	Estimate	*P*-value	Estimate	*P*-value
Int TSB (β_0*)	−1·1652	0·4947	−7·0991	0·0129
Int milk (β_0)	3·3463	<0·0001	−4·0583	<0·0001
Int cheesemaking (β_0)	1·3265	<0·0001	−5·0185	<0·0001
β_1	4·698	<0·0001	4·1028	<0·0001
β_2	ns	0·8348	ns	0·2392
β_3	−65·1372	0·0013	−14·7460	<0·0001
β_4	1·1847	0·0006	ns	0·6036
β_5	853·7	0·0058	ns	0·8582
Log-likelihood	390		141	
AIC	392		151	
PC	94·0		93·5	

Int, intercept; AIC, Akaike Information Criterion; PC, concordance rate; TSB, tryptic soy broth.
*Coefficient of the OLRM described by Eqn (2).

indicating that the populations of *L. monocytogenes* were inactivated at those tested conditions (data not shown). In milk and TSB, the a_w and pH combinations tested did not result in any no growth cases. This difference is supported by the theory that liquid media presents a better substrate for the growth of bacteria, the reason being that solid foods may limit the diffusion rate of organisms throughout space in food. Antwi *et al.* (2007) suggested that the inhibitory effect of undissociated lactic acid and pH on micro-organisms in structured foods may be modified by the food matrix structure and/or buffering capacity and that the food matrix structure may reduce the rate of microbial multiplication. Dens and Van Impe (2000) found that microbial growth on a medium that is not perfectly mixed resulted in fundamentally different behaviour than in homogeneous media. These results are also in agreement with those published by Koutsoumanis *et al.* (2004) who observed that *L. monocytogenes* growth was less supported by solid agar medium than by TSB and Hwang (2009) who observed in his study that TSB would support better the growth of *L. monocytogenes* than solid food (cooked salmon). The position of the growth boundaries (Figs 1 and 2) with respect to a_w was higher in cheesemaking and lower in milk. These results are in accordance with the observed data. On the other hand, the interface was wider in cheesemaking and narrower in milk. The lower and narrower characteristics of the milk interface reflect the possible buffering capacity of the milk and the homogeneity of the liquid state.

During cheesemaking, 'no growth' cases were observed at the lowest a_w values tested during cheesemaking (0·95), and the minimum a_w value at which growth was observed in 100% of the replicates was lower at high contamination levels than at low. In milk, the percentage of replicates showing growth was very similar in both low and high contamination levels. In TSB, the behaviour of *L. monocytogenes* at low and high contamination levels was similar to cheesemaking where growth was initiated in more cases at low a_w values and high contamination levels than at low a_w values and low contamination levels; although this effect was more marked in cheesemaking,

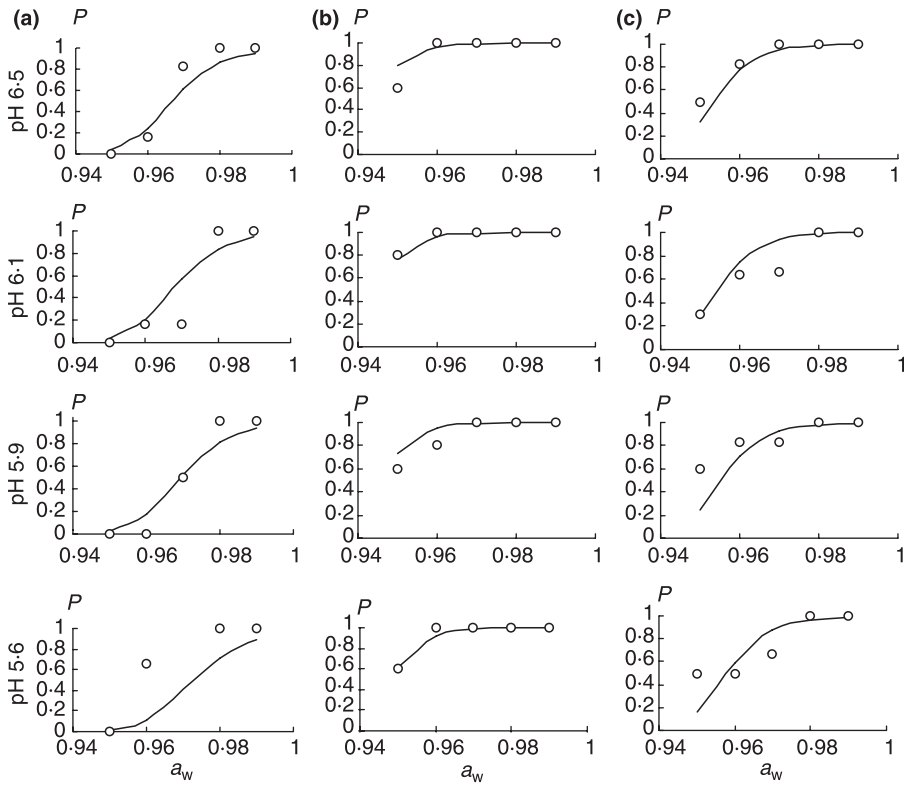

Figure 3 Probability of growth for low contamination levels at pH 6·5, 6·1, 5·9 and 5·6, obtained from the nonlinear logistic regression model predictions (line), against the percentage of observed cases of growth (dots) for each matrix type [Cheesemaking (a), milk (b) and tryptic soy broth (c)].

probably because of the matrix structure effect. As milk has a higher buffering capacity, the contamination level had little effect in this case. In TSB and during cheesemaking, the environmental factors influenced the effect of the inoculum size on the growth limits. Skandamis *et al.* (2007) studied the effect of inoculum size on GNG interface with *Escherichia coli* and found that the lower the contamination level, the higher were the minimum pH and a_w values permitting growth. In the present study, the pH did not show to have this effect probably because of the narrow range of pHs tested, which in turn was a consequence of the cheesemaking limitations (rennet does not coagulate the milk below pH 5·6 and milk has a maximum pH of 6·8) and may subsequently explain the lack of significance of the lactic acid term. In accordance with these observations, the NLRM yielded a statistically nonsignificant parameter for the pH term. In other words, the range of pH values tested in this experiment was within the pH limits for the growth of *L. monocytogenes* (pHmin = 4·7, Augustin *et al.* 2005) and therefore within the interface for growth. Despite the lack of significance of the pH term, this was used to calculate the 0·9, 0·5 and 0·1 probability boundaries.

The pH and a_w combinations tested allowed the definition of the limits of growth of *L. monocytogenes* during cheesemaking, while the lower limits that define the no growth boundary were not observed in milk or TSB. Nonetheless, these limits in milk and broth could be defined by the modelling process. The OLRM estimated a nonsignificant value for the intercepts. The use of OLRM or NLRM differs in the meaning of their parameters. The OLRM includes interaction between terms ($b_w \times$ pH) and the quadratic form of the terms (pH^2 and b_w^2). In contrast, the NLRM includes more biologically realistic terms, namely, $a_{w_{min}}$ and pH_{min} (cardinal values) which are specific for *L. monocytogenes*. Other studies carried out in laboratory media and salmon used the OLRM for data fitting (Boziaris *et al.* 2005; Skandamis *et al.* 2007; Hwang 2009), where the range of combinations studied was wider and there was probably higher significance in the interaction between environmental factors. In other studies (Presser *et al.* 1998; Tienungoon *et al.* 2000), the NLRM was used to model the growth limits of *L. monocytogenes* and *E. coli* as a function of multiple environmental factors. Neither of these studies makes clear the reason for the choice of model. In this study,

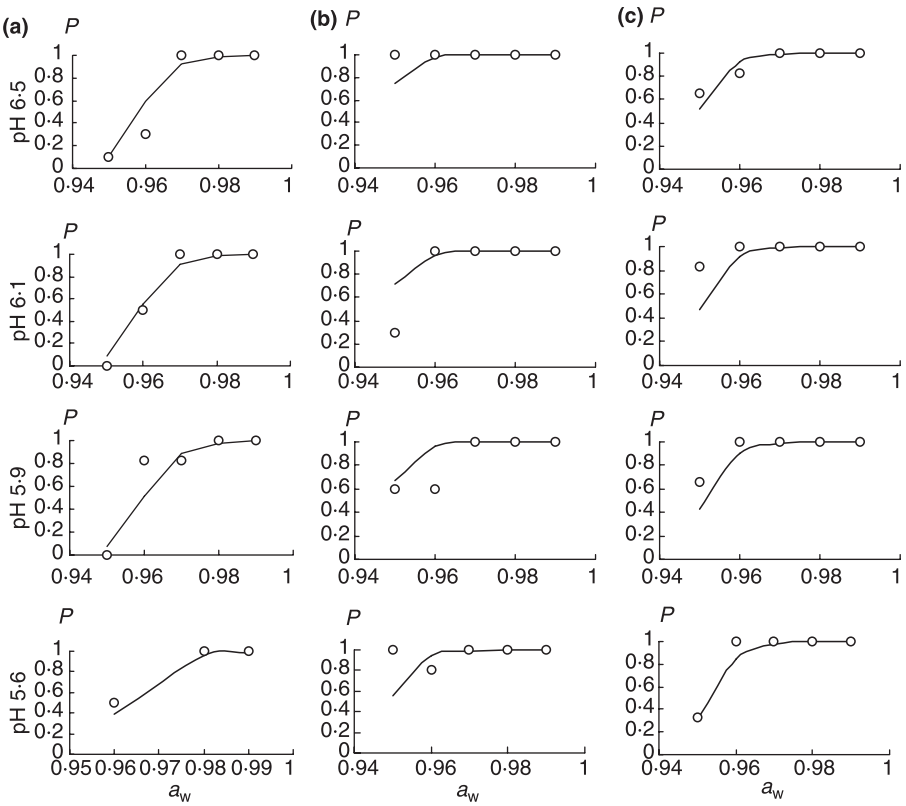

Figure 4 Probability of growth for high contamination levels at pH 6·5, 6·1, 5·9 and 5·6, obtained from the nonlinear logistic regression model predictions (line), against the percentage of observed cases of growth (dots) for each matrix type [Cheesemaking (a), milk (b) and tryptic soy broth (c)].

the NLRM performed better with the three different types of data (liquid media, liquid food, liquid-to-solid food) and was therefore chosen for the comparison of matrix influence on the growth initiation of *L. monocytogenes*.

The agreement between the model predictions and the observed responses were 92 and 91·3% for low and high contamination levels, respectively (PC, Table 1). These values seem acceptable as Hwang (2009) obtained values of 91 and 90% for salmon and TSB, respectively. The AIC and Log-Like indices indicate a better fit with low contamination data.

The predicted probability of growth was calculated for each pH in a range of a_w values and plotted together with observed responses (Figs 3 and 4). The outcome of the model predicted the general trend encountered in the different matrices. These results suggest that there are limitations on the use of models based on data gathered from either liquid food or laboratory media as the models developed in this study would not accurately predict the behaviour observed in a cheesemaking scenario. Overall, the probability of growth initiation would be overestimated. Koutsoumanis *et al.* (2004) pointed out the importance of providing more accurate predictive models to improve the safety of liquid and solid foods.

Conclusions

In this study, we wanted to evaluate the suitability of liquid-based models to predict the behaviour of *L. monocytogenes* encountered in foods, e.g. milk and cheese substrate. In conclusion, cheesemaking conditions facilitate the less growth of *L. monocytogenes* than TSB and milk (in order of importance). Cheesemaking conditions allowed for less growth than liquid media most likely because of diffusion rate limitations. The NLRM predicted accurately the observed responses in the three matrix types and could therefore be used to evaluate the suitability of liquid-based models for solid foods. The results showed that liquid-based models overestimated the growth encountered in cheesemaking conditions. The effect of the food matrix and composition is not taken into account in a liquid-based model, and the subsequent variability is therefore not accounted for by

the model. Further work should be focused on the development of specific growth models for cheese.

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