Original article

Modelling microbial growth in meat broth with added lactic acid under refrigerated storage

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Summary Lactic acid is a classical preservative in meat industry and it is used with high efficiency on sanitization of meat surfaces. This work analyses the effect of temperature and added lactic acid on the growth of different beef muscle-isolated bacteria: *Pseudomonas* sp. and two enterobacteria identified as *Klebsiella* sp. and *Escherichia coli*, in a liquid model system. Inoculation levels between 10^4 and 10^6 CFU mL^{-1} in a culture medium of meat extract broth at 2% were studied, with addition of lactic acid in concentrations of 0.29, 0.39 and 0.58 M, which allowed to reach pH values of 6.1, 5.8 and 5.6 respectively. Culture media inoculated with each micro-organism were incubated at three temperatures: 0, 4 and 10 °C in aerobiosis; bacterial counts as a function of time were mathematically modelled by applying Gompertz equation. Derived parameters: lag phase (LPD), specific growth rate (μ) and maximum population density were calculated. When the observed effect was bacteriostactic a linear regression was used. Results were compared with a meat extract broth control at pH = 7. At 10 °C added lactic acid produced a higher variation in μ values for *Klebsiella* sp. and *E. coli*, whereas LPD values were modified between six and three times for each micro-organism. The highest LPD values were observed for *E. coli*, followed by *Pseudomonas* and finally by *Klebsiella*. LPD values for *E. coli* at 0 °C ranged between 40 and 50 days, whereas at 10 °C they varied between 3 and 6.5 days. *Escherichia coli* did not grow at 0 °C, except when the control system was at pH 7. The effect of temperature on μ values was modelled through an Arrhenius type equation and the corresponding activation energies were determined; μ and LPD values were correlated with the undissociated acid concentration for the three tested temperatures.

Keywords Lactic acid, meat, microbial growth, modelling, refrigeration storage.

Introduction

Meat is one of the most nutritive food-stuffs; however, because of its chemical composition and organoleptic features it becomes not only highly sensible to microbial action but also it is frequently implicated on food-borne diseases. In natural conditions its pH could range from about 6.0 (being close to the optimum level for most pathogenic and alteration-causing bacteria) to values close to 5.5, at witch microbial growth rate decreases significantly. Combining low pH with other factors such as low temperatures they could even prevent microbial growth to occur almost completely (Baird-Parker, 1980). Muscle pH variation is highly dependent on the tissue glycogen level at the slaughter. After animal death, glycogen becomes lactic acid through the glycolitic via; when glycogen level is high, lactic acid concentration is close to 0.9% g/100 g/of meat, with the corresponding decrease in pH. Instead, if glycogen concentration was low before animal death, final lactic acid concentration could be lower than half the normal value, and meat is described as dark, dried and hard with a pH higher than 6.0 (Gill & Newton, 1982). Usual meat microflora is mainly composed of *Acinetobacter*, *Moraxella*, *Brochothrix termosphacta*, *Lactobacillus*, *Pseudomonas* and Enterobacteriaceae. There are other factors that could act together on bacterial growth, such as water activity, redox potential or food features as well as the initial microbial population of both saprophyte and pathogenic flora. The growth of micro-organisms occurs at the expense of its soluble components, mainly carbohydrates, amino acids and lactic acid (Ingram &

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Simonsen, 1980). The latter could have a bacteriostatic effect when added at certain concentrations.

Weak organic acids tend to be more effective as antimicrobials than strong acids because they acidify the interior of the cell (Anderson *et al*., 1992). Antimicrobial activities exerted by organic acids is given by (i) pH reduction, (ii) minimizing dissociation of the acid and (iii) maximizing toxicity of the acid molecule (Anderson *et al*., 1987; Hsiao & Siebert, 1999). Lactic acid produces inhibitory effect because of the decrease in pH (Greer & Dilts, 1995); this acid could act both on the meat muscle flora itself and on that of the grease, although such antimicrobial effect varies not only according to the type of acid used but also according to the microbial variety to be treated. Sometimes it could be bacteriostatic and sometimes it could have a bactericidal action. High efficiency in meat surface sanitization due to the lactic acid addition has been widely reported (Ockerman *et al*., 1974; Gill & Newton, 1982; Nassos *et al*., 1985; Woolthuis & Smulders, 1985; Visser *et al*., 1988; Greer & Dilts, 1995; Ellebracht *et al*., 1999; Nakai & Siebert, 2004).

The use of organic acids as decontaminants is an emerging preventive procedure, despite the fact that this application is not new. However, for reasons of solubility, taste and low toxicity, the short chain organic acids are more commonly used as preservatives or acidulating systems (Baird-Parker, 1980). Nevertheless, despite its effectiveness as decontaminant, acetic acid has been considered unacceptable because of its pungent odour and its provoking discoloration of the meat surface (Mountney & O'Malley, 1965; Belmuller *et al*., 1973; Egan, 1984). Gill & Badoni (2004) have studied the effect of a 0.02% peroxyacetic acid solution, 0.16% sodium chloride with 2% and 4% lactic acid on the natural flora of the distal surfaces from chilled beef carcasses. Peroxyacetic acid and the acidified sodium chloride solution had little effect on the number of aerobes, coliforms or *Escherichia coli* on meat, and were less effective than 4% lactic acid for reducing the number of bacteria on meat. Lactic acid is an acceptable decontaminant because it is a natural, non-toxic, physiological product produced naturally in meat products, and it offers the possibility of reducing the contamination of carcasses meat, cuts and products. According to Snijders *et al*. (1984), the use of lactic acid as a terminal decontaminant in combination with the perfect slaughter line hygiene produces both bactericidal and bacteriostatic effects which result in extended shelf-lives of meat.

Cudjoe (1988), studied the effect of lactic acid sprays on meat during storage. The spraying of the meat surface of a skinned cow head with 1% (v/v) lactic acid resulted in a significant reduction in total viable counts of bacteria during storage at 4, 15 and 20 °C. Shelf-lives of all sprayed heads were observed to have been extended to about 3 days at 4 °C and to 1 day at both 15 and 20 °C.

A variety of antimicrobial solutions have been tested for their effects on warm carcasses or pieces of meat inoculated with cultures or faecal preparations, and most have been reported to reduce bacterial numbers under such experimental conditions (Belk, 2001). However, fewer antimicrobials have been tested against the natural meat flora, and findings from such studies have been far from uniform (Dorsa, 1997; Gill & Badoni, 2004).

Predictive microbiology is a powerful tool for quantify and predict micro-organism growth rate under ambient conditions, with the aim of guarantee food quality, thereby determining its effective life. One of the most frequently used mathematical models is that of Gompertz (Gibson *et al*., 1988; Zwietering *et al*., 1990; Giannuzzi *et al*., 1998), which describes the micro-organism response under different factor combinations (Buchanan, 1992, Andrés *et al*., 2001). It permits to estimate parameters such as lag phase duration (LPD), specific growth rate (μ) and maximum population density (MPD) of micro-organism under such conditions.

The objectives of this work were to analyse and mathematically model the effect of storage temperatures (0, 4 and 10 °C) on the growth of three micro-organisms isolated from beef: *Klebsiella* sp., *E. coli* and *Pseudomonas* sp., inoculated in culture broth with different concentrations of lactic acid leading to pH values ranging between 5.6 and 6.1.

Materials and methods

Isolation and identification of the micro-organisms

Isolations were carried out from beef samples (about 100 g each), obtained in local markets, aseptically treated and collected in sterile bags. The samples were immediately carried to the laboratory, where 20 g subsamples were aseptically and randomly taken. Subsamples were placed in sterile bags with 180 mL peptone water in 0.1% (w/v) solution (Merck KGaA, Darmstadt, Germany) and were homogenized for 1 min in Stomacher equipment (Model 400) (Seward Medical, London, UK). The isolations were performed using (i) bile agar red violet glucose culture medium (37 °C for 24 h), and characterized through Gram coloration, catalase and oxidase plus biochemical tests Sensident EM-Iden Enterobacteriaceae (Merck KGaA) (Mac Faddin, 1979), (ii) agar Masurovsky (Masurovsky *et al*., 1963); the micro-organisms was characterized through Gram coloration, oxidase test and oxide fermentation assay, (iii) according to AOAC (1984) Method 46016, isolating in lauril sulphate triptose (Merck KGaA) (37 °C, 48 h); positive results were replicate in

MacConkey broth (Merck KGaA) as well as in EC (*E. coli*) broth (Merck KGaA), with incubation at 37 and 44.5 °C respectively for 48 h. Positive tubes were spread in EMB (Eosyn methylene blue) (Merck KGaA) (37 °C, 48 h); typical metallic bright colonies were isolated in nutritive agar (37 °C, 48 h) being identified through the corresponding biochemical tests (IMViC): indole, methyl red, Voges Proskauer, citrate (Mac Faddin, 1979).

Meat model broth system

Model system was prepared from a 2% meat extract solution (Merck), to which different lactic acid volumes were added, allowing to reach lactic acid concentrations of 0.28, 0.39 and 0.58 M. After sterilization, the pH values were 6.10, 5.80 and 5.60 respectively. The control medium at pH = 7 (without acid addition) was also inoculated. Cell suspensions between 10^7 and 10^8 CFU mL^{-1} from each of the isolated bacteria were inoculated separately in 50 mL of the model system up to reach concentrations ranging between 10^4 and 10^6 CFU mL^{-1}. Inoculated culture media were incubated at three storage temperatures (0, 4 and 10 °C) without agitation; at different storage times aliquots were taken and microbial counts were determined in agar plate count (37 °C, 48 h).

Mathematical modelling

Mathematical models allow to describe the effect of the main factors affecting microbial growth parameters. One of the most recommended models (Gibson *et al.*, 1988; Zwietering *et al.*, 1990; Andrés *et al.*, 2001) is the Gompertz modified equation, whose expression is:

$$\log N = a + c\exp\{-\exp[-\mathrm{b}(t-m)]\}, \qquad (1)$$

where $\log N$ is the decimal logarithm of microbial counts [log (CFU mL^{-1})], at time t; a is asymptotic log count as time decrease indefinitely (approximately equivalent to log of the initial level of bacteria) [log(CFU mL^{-1})]; c is log count increment as time increases indefinitely [log(CFU mL^{-1})]; b [log (CFU mL^{-1} day^{-1})] is the maximum growth rate at time m; m is time required to reach the maximum growth rate (days).

From these parameters, the following derived parameters were obtained: specific growth rate $\mu = bc/e$ [log(CFU mL^{-1}) day^{-1}], with $e = 2.7182$; LPD = $m - (1/b)$ (days), MPD = $a + c$[log(CFU mL^{-1})].

Data fits obtained from Gompertz model were analysed by means of statistical software (Systat Software Inc., Richmond, CA, USA, 1990). The Systat software calculates the set of parameters with the lowest residual sum of squares and their 95% confidence interval. Besides, it provides for each data fit, the sum of squares, the degree of freedom (d.f.) and the mean square due to the regression and the residual variation.

In other cases it was also possible to use the linear regression model, particularly when the micro-organisms number in food remained constant or decreased during storage. In such a case the equation was expressed as:

$$\log N_t = \log N_0 + \mu t, \qquad (2)$$

where $\log N_t$ is the final microbial counts decimal logarithm [log (CFU mL^{-1})] at the end of the time t,

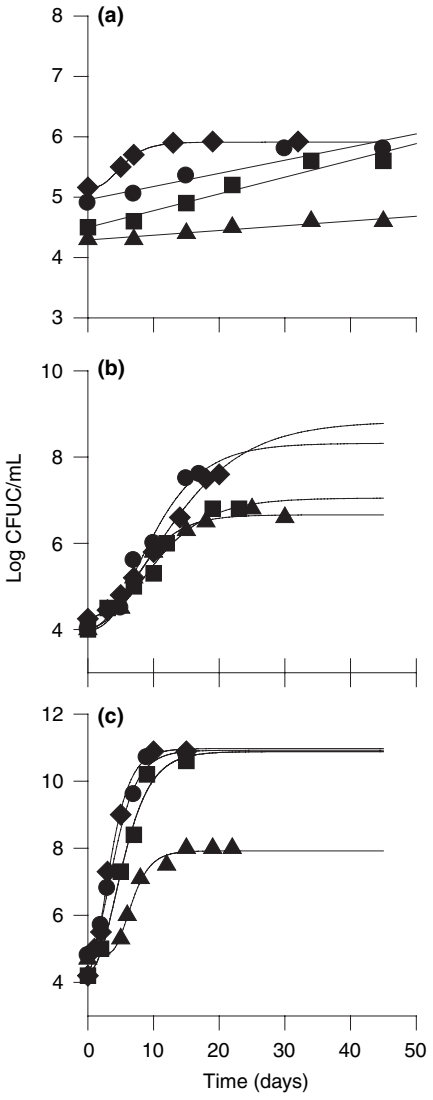

Figure 1 Effect of temperature and pH (reached by addiction of lactic acid) on the growth of *Klebsiella* sp. in a meat model system. Bars indicate the least significant difference ($P < 0.05$). Full lines correspond to Gompertz or linear model at (a) 0 °C, (b) 4 °C and (c) 10 °C, and at different pH values: ◆ 7.0, ■ 6.10, ● 5.80, ▲ 5.6.

given in days; $\log N_0$ is the initial microbial counts decimal logarithm [log (CFU mL^{-1})] and μ corresponds to the regression slope [(CFU $mL^{-1})^{-1}$ day^{-1}] (Whiting, 1995), which was negative when a bactericidal effect was observed. It was considered that micro-organisms were in a lag phase when the slope gets a value lower than 0.01 (CFU $mL^{-1})^{-1}$ day^{-1}, or when the difference in microbial counts with reference to the initial ones was lower than 0.5 logarithm cycle. Lag phase was calculated as the time necessary to increase initial microbial counts in 0.5 log cycle (LPD = $0.5/\mu$).

Experimental design and statistical analysis

A full factorial analysis ($4 \times 3 \times 3$) was performed using four pH values in the meat broth (7.0, 6.1, 5.8 and 5.6), three storage temperatures (0, 4 and 10 °C) and three different inoculated micro-organisms. Each set of experiments was run on duplicate samples. Analysis of variance (ANOVA) and comparison tests according to the Fisher significant differences table (least significant

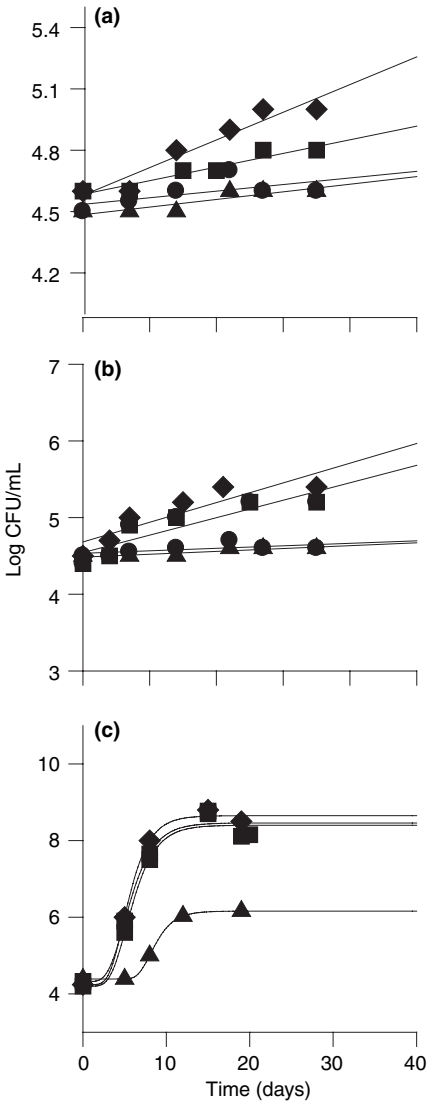

Figure 2 Effect of temperature and pH (reached by addiction of lactic acid) on the growth of *Escherichia coli* in a meat model system. Bars indicate the least significant difference ($P < 0.05$). Full lines correspond to Gompertz or linear model at (a) 0 °C, (b) 4 °C and (c) 10 °C, and at different pH values: ◆ 7.0, ■ 6.10, ● 5.80, ▲ 5.6.

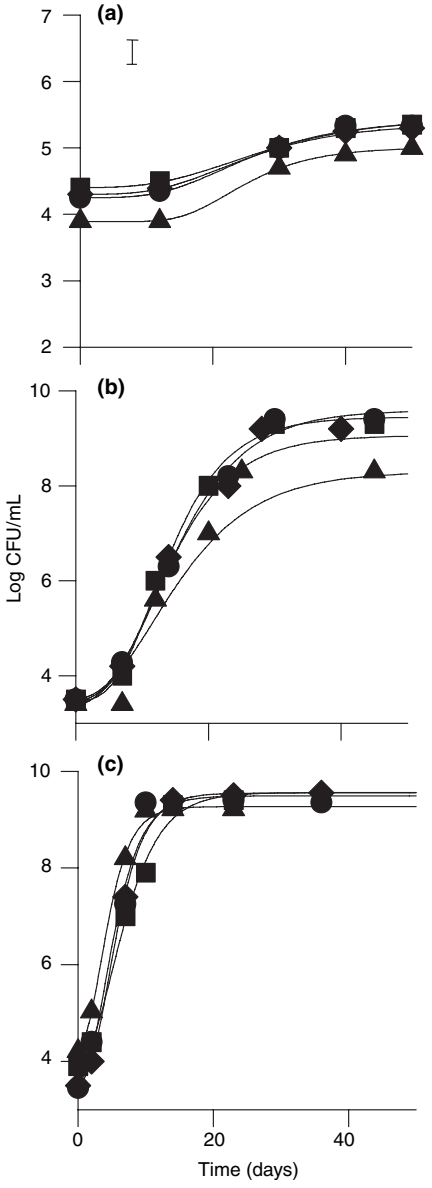

Figure 3 Effect of temperature and pH (reached by addition of lactic acid) on the growth of *Pseudomonas* sp. in a meat model system. Bars indicate the least significant difference ($P < 0.05$). Full lines correspond to Gompertz or linear model at (a) 0 °C, (b) 4 °C and (c) 10 °C, and at different pH values: ◆ 7.0, ■ 6.10, ● 5.80, ▲ 5.6.

difference) were applied with significance levels of 0.05 and 0.01. Statistical computer program Systat (Systat Inc., version 5.0) was used.

Results and discussion

Identification of the isolated bacteria

Isolated bacteria were identified as: (i) *Klebsiella* sp., growing in bile agar red violet glucose culture medium, Gram negative, positive catalase, negative oxidase and subsequent identification through biochemical tests, (ii) *Pseudomonas* sp., growing in Masurovsky agar, Gram negative, positive oxidase, not fermentative and with an oxidative metabolism, (iii) *E. coli* growing in EMB agar, Gram-negative, lactose and glucose fermentative and characterized as on the basis of biochemical tests IMViC.

Table 1 Application of Gompertz equation and linear model to *Klebsiella* sp., *Pseudomonas* sp. and *Escherichia coli* microbial growth in a model system at different pH and temperatures

		Gompertz parameters				Derived parameters			
Temperature (°C)	pH	*a*	*c*	*b*	*m*	μ	LPD	MPD	Time 10^6 (days)
Klebsiella sp.									
0	7.0	5.15 ± 0.01	0.76 ± 0.01	0.42 ± 0.01	4.46 ± 0.01	0.12	2.1	6.77	28
	6.1	—	—	—	—	0.03	16.6	–	>50
	5.8	—	—	—	—	0.02	5.0	–	>50
	5.6	—	—	—	—	0.015	33.3	–	>50
4	7.0	4.12 ± 0.15	4.71 ± 0.63	0.13 ± 0.02	10.21 ± 0.82	0.22	2.51	8.82	10.9
	6.1	3.93 ± 0.41	4.39 ± 1.40	0.19 ± 0.11	7.85 ± 1.64	0.20	2.54	8.32	9.35
	5.8	3.97 ± 0.22	3.08 ± 0.43	0.18 ± 0.05	8.05 ± 0.94	0.19	2.55	7.05	12.9
	5.6	3.96 ± 0.13	2.70 ± 0.16	0.26 ± 0.04	6.46 ± 0.48	0.19	2.62	6.66	11.4
10	7.0	4.08 ± 0.43	6.88 ± 0.53	0.49 ± 0.08	2.65 ± 0.33	1.24	0.62	10.97	2.2
	6.1	4.69 ± 0.34	6.23 ± 0.48	0.43 ± 0.08	3.28 ± 0.34	0.98	0.96	10.92	2.2
	5.8	4.18 ± 0.55	6.70 ± 0.87	0.35 ± 0.10	4.28 ± 0.69	0.86	1.43	10.88	3.6
	5.6	4.83 ± 0.13	3.09 ± 0.17	0.53 ± 0.18	6.03 ± 0.27	0.60	4.14	7.92	6.1
Escherichia coli									
0	7.0	—	—	—	—	0.013	38	—	>50
	6.1	—	—	—	—	0.006	50	—	>50
	5.8	—	—	—	—	0.003	50	—	>50
	5.6	—	—	—	—	0.003	50	—	>50
4	7.0	—	—	—	—	0.05	10.0	—	>50
	6.1	—	—	—	—	0.04	12.5	—	>50
	5.8	—	—	—	—	0.03	16.6	—	>50
	5.6	—	—	—	—	0.02	25.0	—	>50
10	7.0	4.24 ± 0.21	4.41 ± 0.27	0.58 ± 0.14	4.85 ± 0.28	0.95	3.00	8.65	5.0
	6.1	4.32 ± 0.45	4.14 ± 0.57	0.51 ± 0.23	4.94 ± 0.70	0.80	3.00	8.46	5.2
	5.8	4.20 ± 0.43	4.20 ± 0.54	0.51 ± 0.27	5.20 ± 0.64	0.80	3.27	8.40	5.5
	5.6	4.39 ± 0.001	1.77 ± 0.001	0.6 ± 0.001	8.09 ± 0.001	0.43	6.57	6.16	12.4
Pseudomonas sp.									
0	7.0	4.33 ± 0.03	1.06 ± 0.06	0.10 ± 0.01	21.21 ± 1.30	0.08	11.2	5.40	>50
	6.1	4.25 ± 0.08	1.16 ± 0.16	0.10 ± 0.03	21.50 ± 3.12	0.04	11.5	5.41	>50
	5.8	4.40 ± 0.06	1.00 ± 0.15	0.08 ± 0.02	22.86 ± 2.80	0.03	11.7	5.40	>50
	5.6	3.89 ± 0.02	1.11 ± 0.05	0.14 ± 0.03	22.19 ± 1.60	0.05	15.0	5.01	>50
4	7.0	3.40 ± 0.40	6.05 ± 0.62	0.16 ± 0.03	11.65 ± 1.44	0.36	4.99	9.45	12.8
	6.1	3.48 ± 0.28	6.11 ± 0.43	0.14 ± 0.02	12.23 ± 1.03	0.32	5.04	9.59	13.1
	5.8	3.44 ± 0.22	5.62 ± 0.31	0.15 ± 0.02	11.43 ± 0.69	0.30	5.53	9.06	13.1
	5.6	3.25 ± 0.48	5.05 ± 0.71	0.12 ± 0.07	11.53 ± 1.64	0.22	6.45	8.30	15.7
10	7.0	3.51 ± 0.85	5.89 ± 1.02	0.26 ± 0.07	3.89 ± 1.240	0.56	0.04	9.40	4.5
	6.1	3.26 ± 0.80	6.12 ± 0.90	0.25 ± 0.07	3.90 ± 1.120	0.56	0.06	9.38	4.8
	5.8	2.73 ± 0.39	6.13 ± 0.49	0.24 ± 0.03	4.54 ± 0.590	0.55	0.45	8.86	6.6
	5.6	2.74 ± 0.65	5.69 ± 1.19	0.25 ± 0.12	8.09 ± 1.470	0.54	4.15	8.43	10.5

a, log(CFU mL^{-1}); *c*, log(CFU mL^{-1}); *b*, day^{-1}; *m*, days; μ, log(CFU mL^{-1}) day^{-1}; maximum population density (MPD), log(CFU mL^{-1}); lag phase duration (LPD), (days); —, indicates that a linear regression was applied in this case.

Modelling of *Klebsiella* sp., *E. coli* and *Pseudomonas* sp. growth in meat broth systems

Figures 1, 2 and 3a–c show microbial counts of *Klebsiella* sp., *E. coli* and *Pseudomonas* sp. inoculated in the meat broth with different added lactic acid concentrations and stored at 0, 4 and 10 °C. The ANOVA test indicated that the three bacteria show significant differences ($P > 0.05$) for each studied condition. In the same Figs 1–3 the linear and the non-linear (Gompertz) regressions applied to bacterial counts are shown.

The values of parameters *a*, *c*, *b* and *m* as well as the derived parameters μ, LPD and MPD from the application of Gompertz equation or from the linear model and time 10^6, the time (days) necessary to reach 10^6 CFU mL^{-1} for *Klebsiella* sp., *E. coli* and *Pseudomonas* sp. respectively are shown in Table 1. The decrease in temperature and the added lactic acid led to a decrease in growth rate (μ) as well as a decrease in MPD and a progressive increase of the lag phase (LPD) for the three studied bacteria.

Growth rate values for *Klebsiella* notably decreased with the decrease in temperature; thus, for instance, at pH 5.6 and at 10 °C a μ value of 0.60 log(CFU mL^{-1}) - day^{-1} was obtained; at 4 °C such value decreased three times, and at 0 °C a maximum reduction in growth rate, of up to ten times was observed for this bacterium. Lag phase value was 33.3 days at 0 °C; at 4 °C lag phase ranged between 2.51 and 2.62 days, and at 10 °C it ranged from 0.62 to 4.14 days for the different pH assayed.

Escherichia coli did not grow at 0 and 4 °C; at 10 °C lag phase varied between 3.00 and 6.57 days at pH 7.0 and 5.6 respectively. MPD values for the two Enterobacteriaceae (*Klebsiella* sp. and *E. coli*) diminished with the decrease of both temperature and pH; thus for *Klebsiella* sp. at pH 5.6 and at 10 °C LPD was 7.92 log(CFU mL^{-1}) decreasing to 6.66 log(CFU mL^{-1}) at 4 °C. *Pseudomonas* also showed low μ values at 0 °C ranging from 0.08 and 0.03 log(CFU mL^{-1}) day^{-1} which increased between four and ten times at 4 °C and between seven and eighteen times at 10 °C; for the lag phase, values between 11.2 and 15.0 days at 0 °C were observed, decreasing between 5.00 and 6.45 days at 4 °C and reaching the lowest values of 0.04 and 4.15 days at 10 °C. Besides, maximum population densities both at 10 °C and at 4 °C did not show important variations between them, ranging between 8.30 and 9.59 log(CFU mL^{-1}). None of the tested conditions showed bactericidal effect.

Effect of temperature on specific growth rate (μ)

The effect of temperature on specific growth rate (μ) was modelled through an Arrhenius type equation:

$$\mu = A \exp\left(\frac{-E_\mu}{RT}\right), \tag{3}$$

where T is the temperature (°K), E_μ is the activation energy (kJ mol^{-1}), A is a pre-exponential factor and R is the gas constant (8.31 kJ/°K mol). Activation energy (E_μ) can be considered as the sensitivity of the specific growth rate to thermal changes.

Figure 4 shows Arrhenius regressions for the μ values of *Klebsiella* sp., *E. coli* and *Pseudomonas* sp. at the different tested pH. E_μ values and regression coefficients are shown in Table 2. It can be observed that activation energy values (E_μ) for each micro-organism were affec-

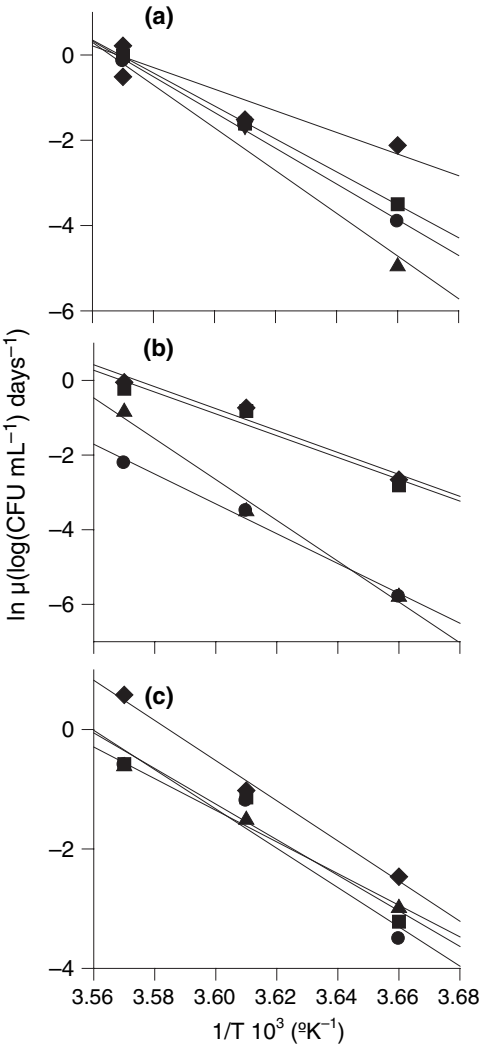

Figure 4 Application of the Arrhenius equation to evaluated the temperature effect on the specific microbial gowth rate (μ) for (a) *Klebsiella* sp., (b) *Escherichia coli*, (c) *Pseudomonas* sp. at different pH values: ◆ 7.0, ■ 6.10, ● 5.80, ▲ 5.6.

Table 2 Application of the Arrhenius type equation for evaluating the effect of temperature on the specific growth rate of *Klebsiella* sp., *Pseudomonas* sp. and *Escherichia coli* at different pH levels

pH	ln *A* [ln(CFU mL^{-1}) day^{-1}]	E_μ (kJ mol^{-1})	R^2*
Klebsiella sp.			
7.0	90.55	210.8 ± 8.8	0.89
6.1	137.9	320.2 ± 1.5	0.99
5.8	149.1	347.4 ± 4.2	0.99
5.6	178.1	416.5 ± 1.1	0.96
Escherichia coli			
7.0	105.1	244.4 ± 6.1	0.96
6.1	104.3	242.4 ± 7.3	0.95
5.8	140.1	332.4 ± 9.0	0.99
5.6	194.2	451.4 ± 5.9	0.99
Pseudomonas sp.			
7.0	120.54	279.3 ± 8.2	0.99
6.1	106.11	257.8 ± 10.5	0.94
5.8	117.23	273.4 ± 8.8	0.93
5.6	94.07	277.2 ± 12.1	0.99

* Coefficient of determination.

ted by the different pH, thus obtaining the lower E_μ values at pH 7 for *E. coli* and *Klebsiella* sp. E_μ values for *Pseudomonas* sp. did not show significant variations at the different pH values, ranging between 257.8 and 279.3 kJ mol^{-1}. The effect of pH on decreasing E_μ was more marked for Enterobacteriaceae than for *Pseudomonas* sp.

Effect of undissociated acid concentration on the derived parameter μ

Antimicrobial effect of organic acids is mainly due to its undissociated fraction rather than to hydrogen ions. Undissociated acid concentration (UAC) from a monoprotic acid such as lactic acid can be calculated through the following expression:

$$[\mathrm{UAC}] = \frac{C_a[\mathrm{H}^+]}{[\mathrm{H}^+] + K}, \quad (4)$$

where C_a is the added acid total concentration (0.29, 0.39 and 0.58 M), $[H^+]$ is the proton concentration and K the lactic dissociation constant ($10^{3.86}$); UAC values, calculated by applying eqn 4 for the different pH (6.10, 5.80 and 5.60) are 1.66×10^{-3}, 4.42×10^{-3} and 1.01×10^{-2} M respectively; at pH = 7, without added lactic acid ($C_a = 0$) and UAC = 0.

Figure 5a–c shows the effect of UAC on μ values at the three studied temperatures for *Klebsiella* sp., *E. coli* and *Pseudomonas* sp. respectively. In all cases the parameter μ decreased when UAC increased. It can be observed that for the three micro-organism *Klebsiella* sp., *E. coli* and *Pseudomonas* sp. at 0 and 4 °C there were

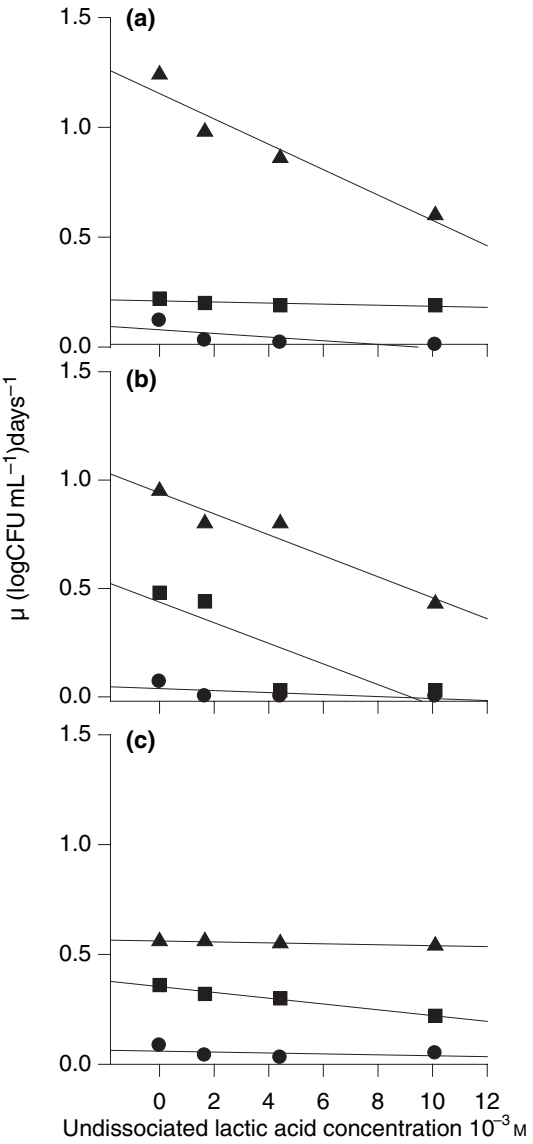

Figure 5 Effect of the undissociated acid concentration on μ values at different temperatures: ● 0 °C, ■ 4 °C, ▲ 10 °C: (a) *Klebsiella* sp., (b) *Escherichia coli* and (c) *Pseudomonas* sp.

no variations in μ values with an increase of the UAC, whereas at 10 °C a variation in the μ values was evidenced by an increase in UAC. A linear regression was applied to μ values as a function of the UAC according to the following equation:

$$\mu = p + q(\mathrm{UAC}). \quad (5)$$

A linear correlation between μ values and UAC was obtained for the three bacteria. The coefficients p and q of eqn 5 and the determination coefficients (R^2) for each linear regression are shown in Table 3. It can be

Table 3 Coefficients of the linear regressions obtained for μ (specific growth rate, p, q) and LPD (lag phase duration, p', q') as functions of the undissociated lactic acid concentration (UAC) at 0, 4 and 10 °C for *Klebsiella* sp., *Escherichia coli* and *Pseudomonas* sp

Temperature (°C)	p	q	R^2*	p'	q'	R^2
Klebsiella sp.						
0	0.09	−0.008	0.86	8.21	2.64	0.86
4	0.20	−0.008	0.89	2.51	0.01	0.97
10	1.15	−0.057	0.93	0.36	0.35	0.95
Escherichia coli						
0	0.94	−0.048	0.94	44.72	0.69	0.67
4	0.43	−0.048	0.77	10.02	1.48	0.99
10	0.038	−0.004	0.79	2.47	0.37	0.87
Pseudomonas sp.						
0	0.56	−0.002	0.96	10.80	0.38	0.91
4	0.35	−0.001	0.98	4.89	0.15	0.98
10	0.059	−0.002	0.73	0.35	0.35	0.89

p and q are the coefficients of the linear regression $\mu = p + q(\text{UAC})$.
p' and q' are the coefficients of the linear regression $\text{LPD} = p' + q'(\text{UAC})$.
* Coefficient of determination.

observed that the specific growth rate values of the analysed enterobacteria (*Klebsiella* sp. and *E. coli*) were more dependent on pH than in the case of *Pseudomonas* sp. in agreement with the results reported by Gill & Newton (1982), who used lactic acid for adjusting pH to 5.5 and 6.0 with an incubation temperature of 2 °C. Such authors also modelled Enterobacteriaceae growth at 2 °C, obtaining specific growth rate values of 0.04 and 0.05 log(CFU mL^{-1}) day^{-1}; the values measured in the present work were 0.01 log(CFU mL^{-1}) day^{-1} at pH 5.6 and 0.03 log(CFU mL^{-1}) day^{-1} at pH 6.1 for the particular case of *Klebsiella* sp., while μ values ranged between 0.003 and 0.006 log(CFU mL^{-1}) day^{-1} at pH 5.6 and 6.1 respectively for *E. coli*.

The simultaneous effects of both, storage temperature and UAC on μ values of each micro-organism are shown in Fig. 6a–c. Fig. 7a–c shows the effect of UAC on LPD values at the three studied temperatures for *Klebsiella* sp., *Pseudomonas* sp. and *E. coli* respectively. It can be observed that the parameter LPD increases along with the increase of UAC for the three bacteria. As was previously commented, the highest LPD values were obtained for *E. coli*, followed by *Pseudomonas* and *Klebsiella*. At 0 °C *Klebsiella* sp. was the most affected by pH variation; at pH 7, LPD value was 2.1 days, whereas with 10.1 mM undissociated lactic acid (pH = 5.6) LPD value was 33.3 days. For this micro-organisms LPD was the most affected by lactic acidity. A linear regression between LPD values and the UACs was applied according to the following equation:

$$\text{LPD} = p' + q'(\text{UAC}). \quad (6)$$

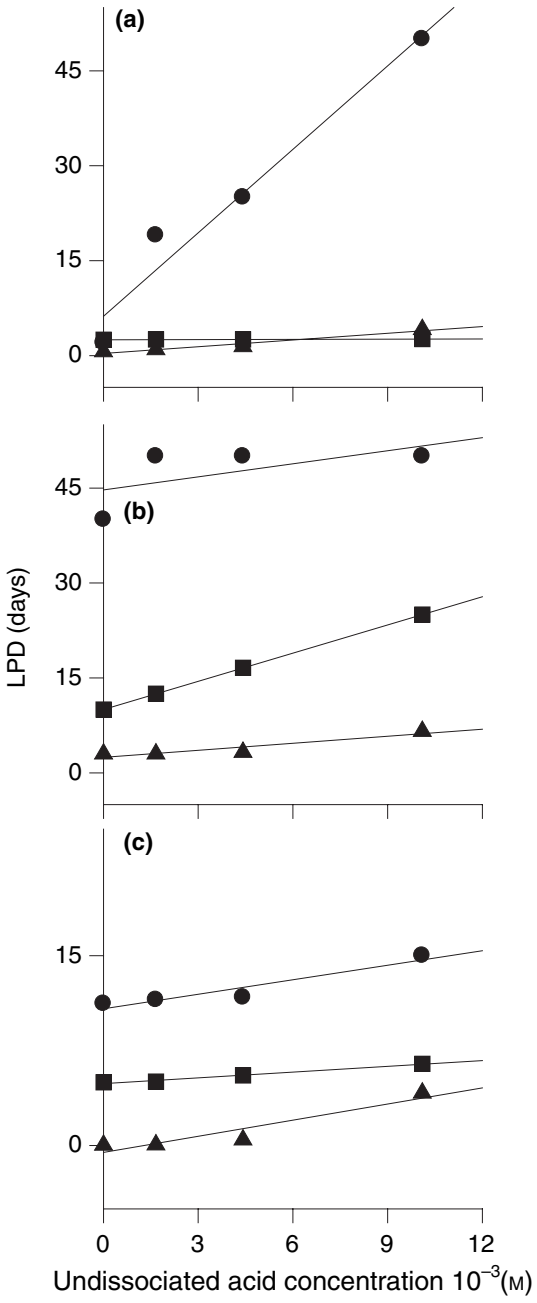

Figure 6 Effect of the undissociated acid concentration on lag phase duration values at different temperatures: ● 0 °C, ■ 4 °C, ▲ 10 °C; bars indicate the least significant difference ($P < 0.05$) (a) *Klebsiella* sp., (b) *Escherichia coli* and (c) *Pseudomonas* sp.

Satisfactory linear regressions between LPD and UAC values were obtained for the three bacteria. The coefficients of the linear regressions (p', q') for each micro-organism are shown in Table 3. As can be observed LPD values of *Klebsiella* sp. and *E. coli* were the most sensitive to the pH changes given by added lactic acid.

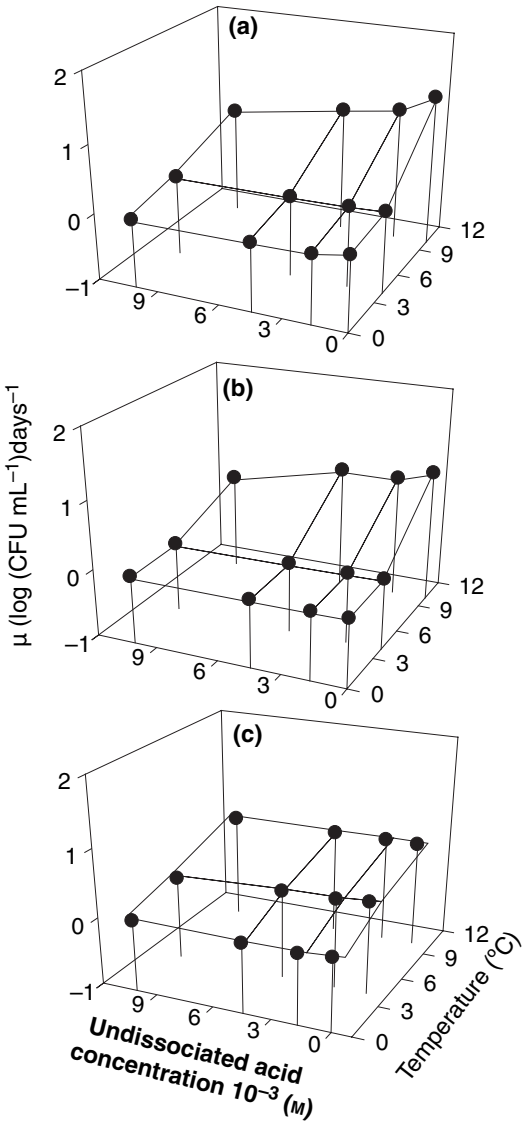

Figure 7 Simultaneous effect of temperature and undissociated acid concentration on μ values for (a) *Klebsiella* sp., (b) *Escherichia coli* and (c) *Pseudomonas* sp.

From the obtained Gompertz parameters it was possible to estimate the time necessary to reach microbial counts of 10^6 CFU mL^{-1} under different tested conditions (Table 1), noting that temperature of 0 °C and a pH of 5.6 reached by addition of lactic acid, extended this time to more than 50 days.

Conclusions

In this work the growth of three isolated bacteria from beef (*Klebsiella* sp., *E. coli* and *Pseudomonas* sp.) were analysed in a meat broth system and modelled at different temperatures (0, 4 and 10 °C) and pH levels (7.0, 6.1, 5.8 and 5.6) reached by adding different concentrations of lactic acid. The highest values of μ and LPD were observed for *Klebsiella* sp. and *Pseudomonas* sp. respectively. LPD of *Klebsiella* sp. was the most affected by lactic acidity.

The effect of temperature on μ values was modelled through an Arrhenius type equation, determining the corresponding activation energies. The highest activation energy was obtained for *Klebsiella* sp. at pH 6.1 and 5.6. Satisfactory correlations between μ and LPD with the undissociated lactic acid concentration were obtained for the three micro-organisms at the tested temperatures. Lactic acid is a natural non-toxic decontaminant that can be applied in the food industry for the sanitization of meat surfaces.

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