Available online at www.sciencedirect.com
ScienceDirect
Meat Science 79 (2008) 509–520
MEAT SCIENCE
www.elsevier.com/locate/meatsci

Mathematical modelling of microbial growth in ground beef from Argentina. Effect of lactic acid addition, temperature and packaging film

F. Coll Cárdenas, L. Giannuzzi, N.E. Zaritzky *

Centro de Investigación y Desarrollo en Criotecnologia de Alimentos (CIDCA), Universidad Nacional de La Plata – CONICET and Facultad de Ingeniería UNLP, Calle 47 y 116 (1900), La Plata, Argentina

Abstract

The effects of: (i) storage temperature (0, 4 and 10 °C), (ii) gaseous permeability of the packaging film (polyethylene and EVA SARAN EVA for vacuum packaging), and (iii) natural beef pH (5.6, 5.8 and 6.1) on the growth of different bacteria isolated from beef muscle were examined. The bacteria were *Klebsiella, Pseudomonas* sp. and *Escherichia coli*. Microbial growth was modelled using Gompertz and linear equations. The effects of temperature on microbial growth rate (μ) and on lag phase duration were modelled using an Arrhenius type equation. In polyethylene, *E. coli* was the microorganism, that showed the highest μ values and also the greatest effect of pH on μ, especially in samples stored at 4 and 10 °C. In the case of *Klebsiella* sp., neither pH nor temperature had marked effects on μ and on LPD. In ESE film, μ of all the microorganisms were less affected by pH and temperature than in polyethylene. In ESE film *E. coli* showed the highest effect of pH on μ, at 4 and 10 °C. LPD increased significantly with respect to the values in polyethylene, with *Klebsiella* sp., showing the highest values of LPD, followed by *E. coli*. Experiments in ground beef with added lactic acid producing a decrease of the original muscle pH from 6.1 to 5.6 showed that the kinetic parameters of the microbial flora did not differ significantly from those of beef samples in which the original pH was 5.6.

Keywords: Modelling; Microbial growth; Lactic acid; Refrigeration; Packaging film; Ground beef

1. Introduction

The growth of bacteria and contamination are two important issues in the red meat industry. As the industry develops new technologies to produce higher hygienic quality and diverse meat products for increasingly competitive markets, systems must be designed to allow safeguards to be implemented in processing procedures. Traditional approaches to meat safety and quality have relied heavily on regulatory inspection and sampling regimes. However, these systems cannot guarantee total consumer protection (Mc Donald & Da-Wen Sun, 1999).

As with all proteinaceous foods, meat is prone to microbial spoilage, sometimes acting as a vector for pathogens of animal and human origin (Brown, 1982). The presence of pathogens in the food supply in low numbers is undesirable and is considered a major cause of gastrointestinal disease world-wide (Buchanan & Whiting, 1986). All raw meat can have some level of microbial contamination present and cannot be expected to be otherwise without further processing. However, only if spoilage microorganisms such as *Brochothrix thermosphacta*, *Pseudomonas* sp., and lactic acid bacteria are allowed to grow in high numbers does the meat becomes spoiled and unfit for human consumption (Davies, 1992).

The ultimate pH of muscle tissue can vary between 5.5 and 7.0, the value being largely dependent upon the amount of glycogen present in the tissue at slaughter. After

* Corresponding author. Tel./fax: +54 221 4254853.
E-mail address: zaritzky@volta.ing.unlp.edu.ar (N.E. Zaritzky).

0309-1740/$ - see front matter © 2008 Published by Elsevier Ltd.
doi:10.1016/j.meatsci.2007.12.003

death, glycogen is converted to lactic acid via glycolysis, and if glycogen reserves are high, a lactic acid concentration results in a low ultimate pH. A pH below 5.5 is needed to retard microbial growth (Baird-Parker, 1980).

Lactic acid is an acceptable decontaminant because it is a natural, non-toxic, physiological substance produced naturally in meat products, and it offers the possibility of reducing the contamination of carcasses, cuts and beef products. It is used as a terminal decontaminant in combination with good slaughter line hygiene to produce both bactericidal and bacteriostatic effects which result in the extended shelf-life of meat. As reviewed by Doores (1993), lactic acid is able to inhibit the growth of many types of food spoilage bacteria, including gram-negative species of the families Enterobacteriaceae and Pseudomonaceae.

The pH of the beef muscle varies widely between the different types of cuts. There are cuts (such as the extensors and flexors muscle group) which have a natural pH of 6.1 and are considered to be of low hygienic quality. It is therefore interesting from a technological point of view to increase the value of lower hygienic quality cuts by adding a natural metabolic product, such as lactic acid, to obtain a good hygienic quality and low cost product.

Vacuum-packaging and refrigeration are increasingly being used as two techniques for enhancing shelf-life of perishable foods such as cuts of fresh meat, using low-oxygen permeable packing materials (Giannuzzi, Pinotti, & Zaritzky, 1997; Osmanagaoglu, 2002).

The application of mathematical models allows us to quantify and to predict the rate of growth of microorganisms under environmental conditions with the intention of assuring the hygienic quality of food, thus determining its storage life. One of the more frequently used models is that of Gompertz with parameters such as lag phase duration (LPD), specific growth rate (μ) and the maximum population density (MPD) of the microorganisms.

The objectives of the present work were as follows: (1) To analyze the effect of refrigeration temperature, natural beef pH (ranging between 5.6 and 6.1) and gaseous permeability of the packaging film on the growth of three muscle-isolated bacteria (*Pseudomonas* sp., *Klebsiella* sp., and *Escherichia coli*) inoculated in ground beef muscle. (2) To model mathematically the microbial growth curves and to determine the effect of temperature on the corresponding kinetic parameters. (3) To analyze the influence of lactic acid, added to ground beef to decrease pH from 6.1 to 5.6, on microbial growth parameters and to compare results with those of ground beef samples with a natural pH of 5.6.

2. Materials and methods

2.1. Beef muscles of different natural pH

Beef samples were obtained from extensors and flexors muscle group of different natural pH (pH 6.1–5.8) and from *semitendinosus* muscle (pH 5.6), from steers, carcass weighing up to 240 kg, with a post-mortem time of 48 h at 4 °C. Beef samples with different pH values (5.6, 5.8 and 6.1) were used in the experiments. From each muscle the external surface 1 cm depth was cut and discarded. The rest of the tissue was cut in a processor and was comminuted for 1 min.

2.2. Inoculation of ground beef samples with bacteria which had been previously isolated from beef muscle

Ground beef samples were inoculated individually with three bacteria previously isolated from beef tissues. The isolation procedure was detailed in a previous work (Coll Cárdenas, Giannuzzi, & Zaritzky, 2006). The isolated bacteria were classified as *Klebsiella* sp., *Pseudomona* sp., and *E. coli* generic (not O157: H7) from the Centre of Reference of Infectious Illnesses Carlos Malbrán.

The inoculated bacteria were grown in nutritive broth and incubated at 37 °C for 12–18 h.

Suspensions of cells between 10^7 and 10^8 CFU ml^{-1} from each isolated bacteria were inoculated separately in the ground meat samples to reach concentrations of 10^5 CFU g^{-1} and homogenised in a Stomacher.

2.3. Packaging and refrigerated storage of the ground beef samples

The inoculated beef samples were divided into sub-samples of 20 g and packaged in two films with different values of oxygen permeability: (a) low density polyethylene (aerobic condition) of 50 μm thick, water vapour permeability WVP = 12 g m^{-2} day^{-1} atm^{-1} at 30 °C and RH = 78%, oxygen transmission rate OTR = 5000 cm^3 m^{-2} atm^{-1} day^{-1} at 23 °C, and (b) vacuum packaged EVA SARAN EVA (ESE film), being EVA ethyl vinyl acetate and SARAN a polyvinyl and polyvinylidene chloride copolymer (WVP = 7.2 g m^{-2} day^{-1} atm^{-1} at 30 °C and RH = 78%, OTR = 50 cm^3 m^{-2} atm^{-1} day^{-1}). Vacuum packaging was carried out in a Minidual equipment model MW 4980 (Schkolnik SAIC, Bs As, Argentina). Manometric pressure in the vacuum chamber was 70 cm Hg. Storage experiments with packaged refrigerated beef were performed at 0, 4 and 10 °C ± 1. During the storage period microbial counts were determined at 2, 5, 8, 10, 12, 14, 20, 25, 30, 45 days.

2.4. Microbiological analysis

At different storage times (0–45 days), beef samples were placed in 80 ml sterile 0.1% peptone broth and homogenised for 1 min in Stomacher equipment. Decimal dilutions with the peptone water were then performed. For bacterial enumeration, dilutions were plated on Agar EMB (Merck) for *Klebsiella* sp., and *E. coli* and Masurovsky agar (Masurovsky, Goldblith, & Voss, 1963) for *Pseudomonas* sp., Spread plates were incubated aerobically at 37 °C for 24–48 h.

Determinations were made in duplicate and results were expressed as log N (N: Colony Forming Units/g (CFU g^{-1})).

2.5. Experiments adding lactic acid

Lactic acid was added to decrease natural beef pH from 6.1 to 5.6. A portion of 1000 g of aseptically ground beef with an original pH of 6.1 was used, and enough lactic acid (87.5% W/W) (Merck) was added to reach a pH of 5.6. The quantity of lactic acid added was 5.6 m moles of lactic acid per kg of ground beef.

E. coli and *Pseudomonas* sp., were inoculated separately in 100 g of beef to reach concentrations for 10^5 CFU g^{-1} and homogenised in Stomacher to evaluate the effects of added lactic acid on these bacteria.

The inoculated samples were divided into portions of 20 g, packaged in two types of plastic films as described previously, and stored at 0, 4 and 10 °C.

During the storage period microbial counts of *E. coli* and *Pseudomonas* sp., were determined at 2, 5, 8, 10, 12, 14, 20, 25, 30, 45 days following the procedures already described.

2.6. Mathematical modelling

Mathematical models allow us to describe the effects of the main factors affecting microbial growth parameters. One of the most recommended models (Andrés, Giannuzzi, & Zaritzky, 2001; Gibson, Bratchell, & Roberts, 1988; Zwietering, Jongenburger, Rombouts, & Van't Riet, 1990) is the Gompertz modified equation, whose expression is:

$$\log N = a + c\exp(-\exp(-b(t-m))) \quad (1)$$

where log N is the decimal logarithm of microbial counts [log (CFU g^{-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 g^{-1})]; c is log count increment as time increases indefinitely [log (CFU g^{-1})]; b [log (CFU g^{-1} $days^{-1}$)], 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 $\mu = b\ c/e$ [log (CFU g^{-1}) $days^{-1}$], with $e = 2.7182$; lag phase duration LPD $= m - (1/b)$ (days), maximum population density MPD $= a + c$ [log (CFU g^{-1})].

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

In other cases it is also possible to use the linear regression model, particularly when the microbial counts in food remain constant or decrease during storage. In such a case the equation is expressed as:

$$\log N_t = \log N_0 + at \quad (2)$$

where log N_t is the microbial count expressed in decimal logarithm [log (CFU g^{-1})] at time t [days]; log N_0 is the initial microbial count expressed in decimal logarithm [log (CFU g^{-1})] and a corresponds to the regression slope [(CFU g^{-1})$^{-1}$ $days^{-1}$] (Whiting, 1995), which is negative when there is a bactericidal effect. It was considered that microorganisms are in a lag phase when the slope gets a value lower than 0.01(CFU g^{-1})$^{-1}$ $days^{-1}$, or when the difference between final counts and initial ones are 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 $= 0.5/\mu$).

The effect of the storage temperature on the (μ) derived parameter will be interpreted by means of the Arrhenius equation

$$\mu = A \cdot \exp(-E\mu/RT) \quad (3)$$

where T is the temperature (K), E_μ is the activation energy (KJ/mol), A is a preexponential factor (log (CFU g^{-1})$days^{-1}$) and R is the gas constant 8.31(KJ (Kmol)$^{-1}$).

The activation energy E_μ can be considered as the sensitivity of the microorganisms to temperature change

$$\ln\mu = \ln A - E_\mu/RT \quad (4)$$

Ploting ln μ vs. $1/T$ the values of E_μ for each type of bacteria can be calculated.

Zwietering, de Koos, Hasenack, de Wit, and van't Riet (1991) modified the extended Ratkoswsky model to describe the lag time as a function of temperature. The effect of temperature on LPD reflects how the adaptation period of microorganisms to their new environment changes with temperature. In this regard, the adaptation rate can be considered as the reciprocal of LPD (Li, 1988, and Li & Torres, 1993), and was modelled using an Arrhenius type model

$$1/\mathrm{LPD} = D \cdot \exp(-E_{1/\mathrm{LPD}}/\mathrm{RT}) \quad (5)$$

where T is the temperature in (K), $E_{1/\mathrm{LPD}}$ is the activation energy (KJ/mol), D is a preexponential factor ($days^{-1}$) and R is the gas constant 8.31 (KJ(Kmol)$^{-1}$). The activation energy $E_{1/\mathrm{LPD}}$ can be considered as the sensitivity of the microorganisms to temperature change

$$\ln(1/\mathrm{LPD}) = \ln D \cdot -E_{1/\mathrm{LPD}}/RT) \quad (6)$$

2.7. Experimental design and statistical analysis

In the experiments using ground beef samples without acid lactic addition, a full factorial analysis ($3 \times 3 \times 3 \times 2$) was performed, with three natural pH values in the beef samples (6.1; 5.8 and 5.6), three storage temperatures (0, 4 and 10 °C), three different inoculated microorganisms (*Klebsiella* sp., *Pseudomonas* sp., and *E. coli* generic, not O157:H7) and two different packaging films (polyethylene and ESE). Each set of experiments was run on duplicate samples.

In the experiments where lactic acid was added to decrease beef pH from 6.1 to 5.6, samples inoculated with *Pseudomonas* sp., and *E. coli* were stored at three temperatures (0, 4 and 10 °C), using two different packaging films. Each set of experiments was run on duplicate samples. Analysis variance (ANOVA, 1989) and comparison tests according to the Fisher significant differences table (LSD) were applied with significance levels of 0.05 and 0.01. Statistical computer program SYSTAT (SYSTAT Inc, version 5.0) was used.

3. Results and discussion

3.1. Microbial growth of inoculated microorganisms in ground beef samples under different conditions

Figs. 1a–f, 2a–f and 3a–f show microbial growth of *Klebsiella* sp., *Pseudomonas* sp., and *E. coli*, respectively, on samples of ground beef with different natural pH (5.6, 5.8 and 6.1), packaged in films of different gaseous permeabilities and stored at 0, 4 and 10 ± 1 °C for a maximum storage period of 45 days. The figures also show the application of the mathematical models; full lines represent the

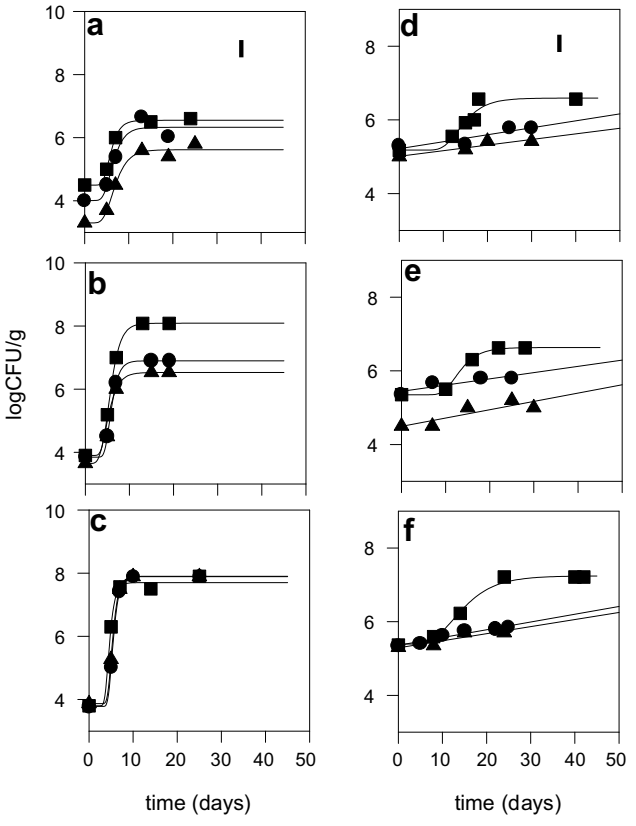

Fig. 1. Effect of temperature, natural pH and gaseous permeability of the packaging film on the growth of *Klebsiella* sp., in ground beef. Bars indicate the least significant difference, LSD ($p < 0.05$). Full lines correspond to Gompertz or linear models at a) 0 °C, (b) 4 °C and (c) 10 °C packaged with polyethylene film (aerobic conditions) and (d) 0 °C, (e) 4 °C and (f) 10 °C vacuum packaged with EVA SARAN EVA. Beef pH values: (■) 6.10, (●) 5.80, (▲) 5.60.

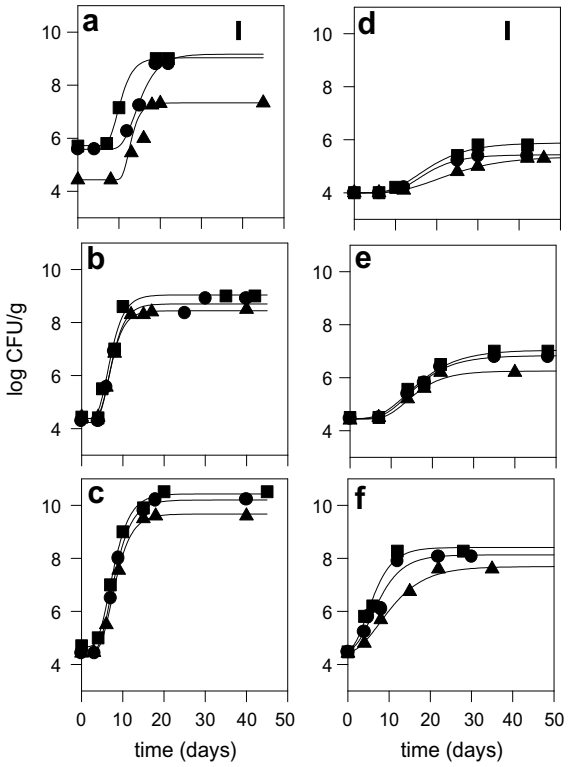

Fig. 2. Effect of temperature, pH and gaseous permeability of the packaging film on the growth of *Pseudomonas* sp., in ground beef. Bars indicate the least significant difference, LSD ($p < 0.05$). Full lines correspond to Gompertz or linear models at: (a) 0 °C, (b) 4 °C and (c) 10 °C with polyethylene film packaging in aerobic conditions and (d) 0 °C, (e) 4 °C and (f) 10 °C in vacuum packaging with EVA SARAN EVA. Beef pH values: (■) 6.10, (●) 5.80, (▲) 5.60.

Gompertz equation or the linear model. A good agreement was observed between the models and the experimental data; the obtained parameters are shown in Tables 1–3 for *Klebsiella* sp., *Pseudomonas* sp., and *E. coli*, respectively. In all cases, the ANOVA analysis shows significant differences ($p < 0.05$) in the microbial counts with the variation in pH, temperature, time and gaseous permeability of the films.

In the case of *Klebsiella* in samples packaged in polyethylene (Fig. 1a–c; Table 1) MPD ranged between 5.62 and 6.55 log CFU g^{-1} at 0 °C and between 6.53 and 8.09 log CFU g^{-1} at 4 °C. At 10 °C MPD reached the highest values ranging between 7.89 and 9.70 according to pH. Decomposition was evident from odours and changes in colour, turning darker due to the formation of metmyoglobin. In all cases, the increase in temperature showed an increase in the specific growth rate (μ). In beef samples with pH values of 6.1 and 5.6, μ increased approximately twice, at 4 °C compared to the value at 0 °C. At 10 °C the value of μ increased up to 3 or 4 times compared to that at 0 °C. The lag phase duration (LPD) showed no significant variation with storage temperature, being lower than 4.2 days for all cases.

In ESE film (Fig. 1d–f; Table 1) it was observed that only the samples with pH 6.1 showed high microbial

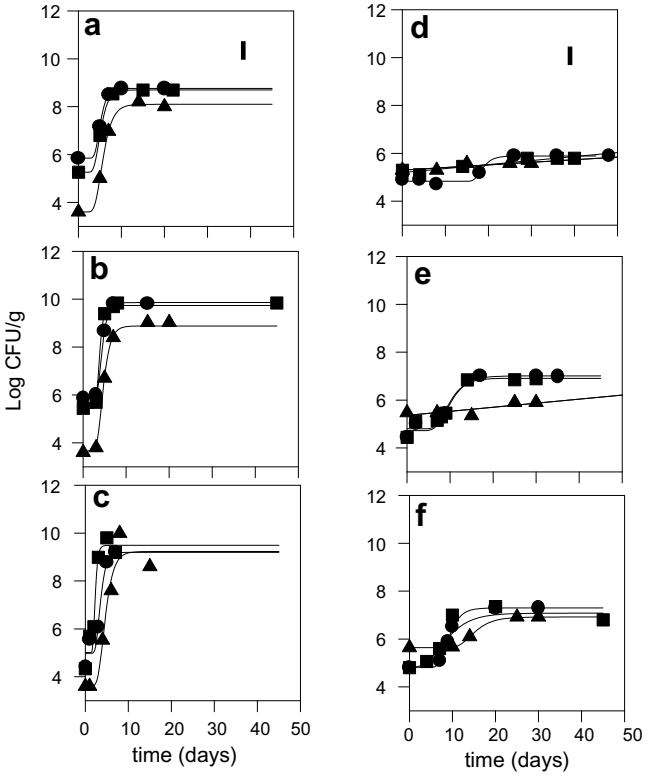

Fig. 3. Effect of temperature, pH and gaseous permeability of the packaging film on the growth of *E. coli* in ground beef. Bars indicate the least significant difference, LSD ($p < 0.05$). Full lines correspond to Gompertz or linear models at: (a) 0 °C, (b) 4 °C and (c) 10 °C packaged in polyethylene film (aerobic conditions) and (d) 0 °C, (e) 4 °C and (f)10 °C vacuum packaging in EVA SARAN EVA. Beef pH values: (■) 6.10, (●) 5.80, (▲) 5.60.

growth of *Klebsiella* sp., at the three temperatures. In all cases the vacuum packaged samples did not show evident changes in sensory properties. The values of μ did not change significantly with temperature for the tested pH values and were lower than in polyethylene. At pH 5.6 and 5.8 and at the three tested temperatures, linear regression were applied and μ values ranged between 0.01 and 0.02 log (CFU g^{-1}) $days^{-1}$. The lag phase duration (LPD) of *Klebsiella* in samples with pH 6.1 packaged in ESE film and stored at 0 and 4 °C increased significantly in comparison to the samples wrapped in polyethylene. A value of LPD = 9.95 days was observed for samples with pH 6.1 at 0 °C; at this pH, LPD = 8.16 days was observed at 10 °C increasing to LPD = 25 days as pH decreased. In ESE film a linear model was applied for pH 5.8 and 5.6 at the three tested temperatures and microbial counts remained below 10^6 CFU g^{-1} at 30 days storage time. The MPD values for samples with pH 6.1 ranged between 6.59 and 7.24 (log CFU g^{-1}) at 0, 4 and 10 °C.

In the case of *Pseudomonas* in polyethylene (Fig. 2a–c; Table 2), ground beef samples began to show evidence of decomposition, changes in colour, unpleasant ammonia smells and the presence of surface slime after 8 days storage at 10 °C. At 4 and 10 °C, μ was not significantly affected by pH; however at 0 °C the effect of pH on μ was more marked. The LPD increased as the pH diminished at the three temperatures, the difference being more marked at 0 °C. The values of MPD ranged between 8.44 and 10.43 CFU g^{-1} observing the highest values at 10 °C.

Vacuum packaged samples in low gaseous permeability film (ESE) stored at 0 °C, showed final microbial counts of

Table 1
Application of Gompertz equation to the microbial growth of *Klebsiella* sp. in ground beef samples with different natural pH values, packaged in polyethylene (aerobic condition) and ESE film (vacuum packaging) and stored under refrigeration at 0, 4 and 10 °C

T (°C)	pH	Gompertz parameters				Derived parameters		
		a	*c*	*b*	*m*	μ	LPD	MPD
Polyethylene (aerobic condition)								
0	6.1	4.50 ± 0.06	2.05 ± 0.08	0.75 ± 0.09	5.45 ± 0.14	0.56	4.11	6.55
	5.8	4.01 ± 0.47	2.32 ± 0.59	0.60 ± 0.50	5.82 ± 1.06	0.51	4.15	6.33
	5.6	3.30 ± 0.20	2.32 ± 0.24	0.50 ± 0.19	6.15 ± 0.52	0.42	4.15	5.62
4	6.1	3.90 ± 0.01	4.19 ± 0.01	0.68 ± 0.009	5.24 ± 0.016	1.05	3.78	8.09
	5.8	3.85 ± 0.001	3.05 ± 0.001	0.88 ± 0.001	5.49 ± 0.001	0.99	4.15	6.90
	5.6	3.65 ± 0.001	2.88 ± 0.001	0.89 ± 0.001	5.22 ± 0.001	0.94	4.15	6.53
10	6.1	3.80 ± 0.28	5.90 ± 0.34	1.24 ± 1.10	4.35 ± 0.64	1.79	3.54	9.70
	5.8	3.78 ± 0.001	4.12 ± 0.005	1.15 ± 0.005	5.17 ± 0.002	1.74	4.13	7.90
	5.6	3.87 ± 0.009	4.02 ± 0.01	1.15 ± 0.01	5.04 ± 0.006	1.68	4.13	7.89
ESE film (vacuum packaging)								
0	6.1	5.18 ± 0.24	1.41 ± 0.34	0.30 ± 0.17	13.29 ± 1.70	0.15	9.95	6.59
	5.8	–	–	–	–	0.02	25	–
	5.6	–	–	–	–	0.01	50	–
4	6.1	5.35 ± 0.02	1.29 ± 0.04	0.32 ± 0.03	12.38 ± 0.33	0.15	9.34	6.64
	5.8	–	–	–	–	0.02	25	–
	5.6	–	–	–	–	0.02	25	–
10	6.1	5.40 ± 0.10	1.84 ± 0.13	0.22 ± 0.06	12.71 ± 0.94	0.15	8.16	7.24
	5.8	–	–	–	–	0.02	25	–
	5.6	–	–	–	–	0.02	25	–

a: log (CFU g^{-1}), *c*: log (CFU g^{-1}), *b*: $days^{-1}$, *m*: days, μ: log (CFU g^{-1})$days^{-1}$, MPD: (log (CFU g^{-1}), LPD: (days).

Table 2
Application of Gompertz equation to the microbial growth of Pseudomonas sp. in ground beef samples with different natural pH values, packaged in polyethylene (aerobic condition) and ESE film (vacuum packaging) and stored under refrigeration at 0, 4 and 10 °C

T (°C)	pH	Gompertz parameters				Derived parameters		
		a	*c*	*b*	*m*	μ	LPD	MPD
Polyethylene (aerobic condition)								
0	6.1	5.72 ± 0.01	3.31 ± ± 0.02	0.48 ± 0.02	9.61 ± 0.04	0.59	7.55	9.03
	5.8	5.58 ± 0.16	3.59 ± 0.46	0.32 ± 0.11	13.85 ± 0.66	0.43	10.79	9.17
	5.6	4.45 ± 0.24	3.02 ± 0.41	0.36 ± 0.14	13.64 ± 0.90	0.40	11.00	7.47
4	6.1	4.37 ± 0.35	4.67 ± 0.44	0.45 ± 0.10	6.23 ± 0.58	0.78	4.02	9.04
	5.8	4.23 ± 0.25	4.47 ± 0.30	0.46 ± 0.12	6.51 ± 0.39	0.76	4.36	8.70
	5.6	4.38 ± 0.08	4.06 ± 0.10	0.49 ± 0.04	6.52 ± ± 0.14	0.74	4.51	8.44
10	6.1	4.66 ± 0.16	5.77 ± 0.22	0.37 ± 0.04	6.73 ± 0.27	0.78	1.46	10.43
	5.8	4.39 ± 0.07	5.81 ± 0.10	0.36 ± 0.02	7.03 ± 0.12	0.78	1.74	10.20
	5.6	4.43 ± 0.05	5.24 ± 0.07	0.38 ± 0.02	7.23 ± 0.09	0.75	1.87	9.67
ESE film (vacuum packaging)								
0	6.1	3.99 ± 0.09	1.89 ± 0.16	0.15 ± 0.03	15.58 ± 0.99	0.10	8.83	6.03
	5.8	4.00 ± 0.03	1.43 ± 0.05	0.19 ± 0.02	15.50 ± 0.69	0.09	10.38	5.43
	5.6	3.99 ± 0.01	1.38 ± 0.02	0.11 ± 0.06	19.98 ± 0.38	0.05	10.85	5.33
4	6.1	4.45 ± 0.14	2.59 ± 0.21	0.15 ± 0.03	13.96 ± 0.99	0.30	7.70	7.04
	5.8	4.45 ± 0.07	2.38 ± 0.11	0.17 ± 0.02	13.97 ± 0.67	0.30	7.87	6.83
	5.6	4.44 ± 0.11	1.81 ± 0.20	0.20 ± 0.10	13.71 ± 0.99	0.13	8.71	6.25
10	6.1	4.45 ± 0.60	3.96 ± 0.82	0.30 ± 0.18	4.69 ± 1.27	0.45	4.02	8.41
	5.8	4.46 ± 0.48	3.67 ± 0.60	0.25 ± 0.09	5.74 ± 1.26	0.35	4.30	8.13
	5.6	4.34 ± 0.23	3.35 ± 0.33	0.17 ± 0.03	7.78 ± 1.05	0.22	4.50	7.69

a: log (CFU g^{-1}), *c*: log (CFU g^{-1}), *b*: $days^{-1}$, *m*: days, μ: log (CFU g^{-1})$days^{-1}$, MPD: (log (CFU g^{-1}), LPD: (days).

Table 3
Application of Gompertz equation to the microbial growth of Escherichia coli in ground beef samples with different natural pH values, packaged in polyethylene (aerobic condition) and ESE film (vacuum packaging) and stored under refrigeration at 0, 4 and 10 °C

T (°C)	pH	Gompertz parameters				Derived parameters		
		a	*c*	*b*	*m*	μ	LPD	MPD
Polyethylene (aerobic condition)								
0	6.1	5.85 ± 0.008	2.91 ± 0.01	1.04 ± 0.02	4.70 ± 0.01	1.12	3.70	8.76
	5.8	5.26 ± 0.01	3.43 ± 0.01	0.93 ± 0.01	4.76 ± 0.01	1.12	3.70	8.69
	5.6	3.60 ± 0.14	4.50 ± 0.18	0.69 ± 0.09	5.22 ± 0.15	0.99	3.78	8.10
4	6.1	5.44 ± 0.08	4.30 ± 0.09	1.74 ± 0.16	3.59 ± 0.08	2.76	3.01	9.74
	5.8	5.87 ± 0.09	3.99 ± 0.13	1.17 ± 0.17	4.10 ± 0.15	1.72	3.19	9.86
	5.6	3.57 ± 0.06	5.44 ± 0.08	0.83 ± 0.04	4.31 ± 0.06	1.58	3.22	9.01
10	6.1	5.01 ± 0.52	4.48 ± 0.75	2.49 ± 1.92	2.14 ± 0.23	4.11	1.74	9.49
	5.8	4.98 ± 0.41	4.22 ± 0.60	1.20 ± 0.93	3.22 ± 0.36	1.85	2.36	9.20
	5.6	3.61 ± 0.64	5.62 ± 1.03	0.78 ± 0.59	4.16 ± 0.60	1.61	2.89	9.23
ESE film (vacuum packaged)								
0	6.1	4.83 ± 0.05	1.06 ± 0.08	0.67 ± 2.59	18.08 ± 0.53	0.26	16.58	5.89
	5.8	–	–	–	–	0.01	31.25	–
	5.6	–	–	–	–	0.01	50	–
4	6.1	4.72 ± 0.17	2.29 ± 0.24	0.39 ± 0.27	9.12 ± 0.80	0.33	6.55	7.02
	5.8	4.80 ± 0.18	2.11 ± 0.27	0.43 ± 0.20	8.94 ± 0.76	0.30	6.64	6.91
	5.6	–	–	–	–	0.01	29.41	–
10	6.1	4.93 ± 0.22	2.14 ± 0.30	1.16 ± 1.65	7.13 ± 0.42	0.90	6.26	7.07
	5.8	4.82 ± 0.07	2.48 ± 0.09	0.57 ± 0.06	8.54 ± 0.17	0.52	6.54	7.30
	5.6	5.64 ± 0.01	1.28 ± 0.02	0.36 ± 0.07	14.11 ± 0.12	0.17	11.37	6.92

a: log (CFU g^{-1}), *c*: log (CFU g^{-1}), *b*: $days^{-1}$, *m*: days, μ: log (CFU g^{-1})$days^{-1}$, MPD: (log (CFU g^{-1}), LPD: (days).

Pseudomonas of 10^6 CFU g^{-1} independent of the pH values (Fig. 2d; Table 2). At 4 °C MPD values were higher than 10^7 CFU g^{-1} and at 10 °C, MPD reached 10^8 CFU g^{-1} (Fig. 2e and f; Table 2). The effect of temperature on MPD values was higher than that of muscle pH. The values of μ in ESE film were lower than in polyethyl-

ene and increased progressively as the temperature increased; μ values ranged between 0.22 to 0.45 log (CFU g^{-1})$days^{-1}$ and between 0.05 to 0.10 log (CFU g^{-1})$days^{-1}$ at 10 and 0 °C respectively for the different muscle pH. The lag phase duration in ESE film, ranged between 4.02 to 4.50 and 8.83 to 10.85 days at 10 °C and 0 °C respectively.

In the case of *E. coli* in polyethylene (Fig. 3a–c; Table 3), a notable growth at the different pH values was observed, reaching 10^8 and 10^9 CFU g^{-1} at 4 and 10 °C respectively. Ground beef samples with pH 5.6 stored at 10 °C in polyethylene showed specific growth rates 2.5 times lower than those obtained at pH 6.1. At 10 °C the LPD values ranged between 1.74 and 2.89 days for ground beef samples with pH 6.1 and 5.6 respectively; at 0 and 4 °C, LPD ranged between 3.01 and 3.78 days for the three tested pH. MPD ranged between 8.10 and 9.89 log CFU g^{-1} at the three temperatures and the tested pH.

In ESE film (Fig. 3d–f; Table 3) MPD of *E. coli* at 0 °C, reached 5.89 log (CFU g^{-1}), only in samples with pH 6.1. For lower pH values this parameter could not be calculated since a linear regression model was applied. At 4 °C, ground beef samples with pH values of 5.8 and 6.1 reached MPD close to 7 log (CFU g^{-1}). Gompertz model could not be applied in samples of pH 5.6 stored at 4 °C and linear regressions were calculated. At 10 °C MPD values ranged between 6.92 and 7.30 log CFU g^{-1}. In all cases it was observed that the values of μ, were lower in ESE than those obtained under the same conditions in polyethylene. The highest values of μ occurred in the samples stored at 10 °C with pH 6.1 reaching a value of 0.90 log (CFUg^{-1})$days^{-1}$. The value of LPD at 0 °C in samples of pH 5.6 was 45 days considering that this was the maximum experimental time. In samples stored at 4 °C and 10 °C, LPD values ranged between 6.26 and 6.64 days at pH 5.8 and 6.1 respectively. For samples with pH 5.6, LPD increased to 29.41 and 11.37 days at 4 °C and 10 °C respectively.

Based on the obtained results, we can conclude that in the case of samples packed with polyethylene, *E. coli* was the microorganism, that showed the highest μ values at the different pH and also the greatest effect of pH on μ, especially in samples stored at 4 and 10 °C.

Gill and Badoni (2004) working with beef carcasses, sprayed with lactic acid solutions ranging between 2% and 4% and stored at 7 °C determined that lactic acid decreased two log units *E. coli* counts. In the case of *Klebsiella* sp., neither pH nor temperature had marked effects on specific microbial growth rate and on LPD in meat samples packaged in polyethylene.

Pseudomonas sp., was the microorganism that showed the lowest effect of pH on the specific growth rate, at the three assayed temperatures in polyethylene. Confirming these results, studies by Hsiao and Siebert (1999) showed that some strains of *Pseudomonas*, can present some acid-resistance. In meat samples packaged in ESE film, microbial growth rates of all the analyzed microorganisms were less affected by pH and temperature than in polyethylene.

The effect of pH was more noticeable as the temperature increased. In ESE film *E. coli* showed the highest effect of pH on μ, at 4 and 10 °C. Grau (1981) reported that at pH 6.1 lactate did not prevent aerobic or anaerobic growth of Enterobacteriacea, however at low pH (5.5), this acid inhibited its anaerobic growth. In vacuum packaging lag phase duration increased significantly with respect to the values in polyethylene, being *Klebsiella* sp., the microorganism that showed the highest values of LPD, followed by *E. coli*. Smulders and Woolthuis (1984) confirmed these findings, reporting that vacuum packaging alone did not present inhibitory action on the growth of *E. coli*, which was observed in conjunction with the addition of lactic acid.

3.2. Effect of temperature on specific growth rate (μ) and lag phase duration (LPD) of the inoculated bacteria

The values of E_μ for each type of bacteria were obtained by plotting $\ln_\mu$ vs. $1/T$ (Fig. 4). The values obtained and the coefficients of regression for the four types of microorganisms studied are shown in Table 4. In all the cases an

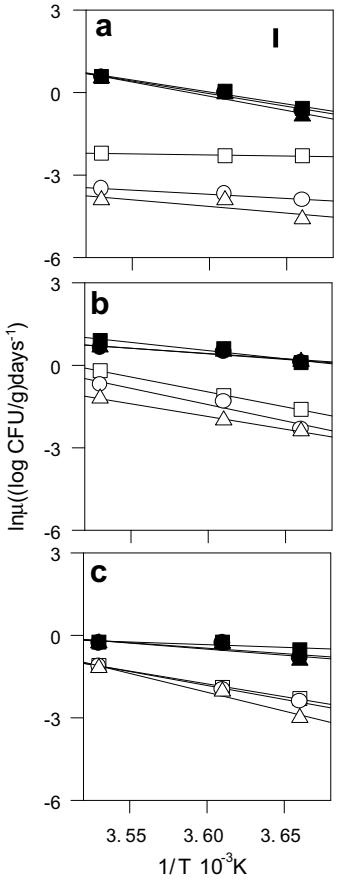

Fig. 4. Application of the Arrhenius equation to evaluate the temperature effect on the specific microbial growth rate (μ) for: (a) *Klebsiella* sp., (b) *E. coli* and (c) *Pseudomonas* sp., in ground beef. Filled symbols correspond to experiments using ground beef packaged in polyethylene film (aerobic conditions) at different pH values: (■) 6.10, (●) 5.80, (▲) 5.60. Empty symbols correspond to experiments using vacuum packaging with EVA SARAN EVA at different pH values: (△) 6.10, (O) 5.80, (Δ) 5.60.

Table 4
Effect of temperature on the specific growth rate (μ) and on lag phase duration (LPD) of *Klebsiella* sp., *Pseudomonas* sp., and *E. coli* in ground beef samples with different natural pH values, packaged in polyethylene (aerobic conditions) and ESE film (vacuum packaged)

pH	$E_{1/LPD}$ [KJ/mol]	R^2 [coef. of determination]	E_μ [KJ/mol]	R^2 [coef. of determination]
Polyethylene (aerobic condition)				
Klebsiella sp.,				
6.1	9.27 ± 0.28	0.95	72.46 ± 1.58	0.97
5.8	0.67 ± 0.03	0.85	76.59 ± 1.67	0.97
5.6	0.67 ± 0.03	0.85	85.95 ± 2.39	0.95
Pseudomonas sp.,				
6.1	120.91 ± 4.86	0.98	16.18 ± 1.49	0.98
5.8	118.83 ± 4.11	0.98	33.01 ± 3.05	0.98
5.6	116.83 ± 3.98	0.98	36.57 ± 3.25	0.98
Escherichia coli				
6.1	48.79 ± 0.77	0.98	36.97 ± 1.50	0.90
5.8	29.08 ± 0.28	0.99	37.06 ± 1.50	0.90
5.6	16.04 ± 0.52	0.93	37.22 ± 1.53	0.90
ESE film (vacuum packaged)				
Klebsiella sp.,				
6.1	12.56 ± 0.20	0.98	6.39 ± 0.31	0.85
5.8	11.59 ± 1.07	0.98	25.27 ± 0.58	0.96
5.6	11.59 ± 1.07	0.98	40.18 ± 3.72	0.85
Pseudomonas sp.,				
6.1	55.75 ± 5.06	0.90	77.41 ± 0.49	0.99
5.8	53.53 ± 4.76	0.92	84.18 ± .0.53	0.99
5.6	51.59 ± 4.78	0.93	113.51 ± 2.24	0.97
Escherichia coli				
6.1	86.96 ± 8.05	0.85	76.90 ± 0.31	0.99
5.8	86.95 ± 8.05	0.85	85.84 ± 4.00	0.87
5.6	85.92 ± 0.26	0.99	90.82 ± 1.20	0.99

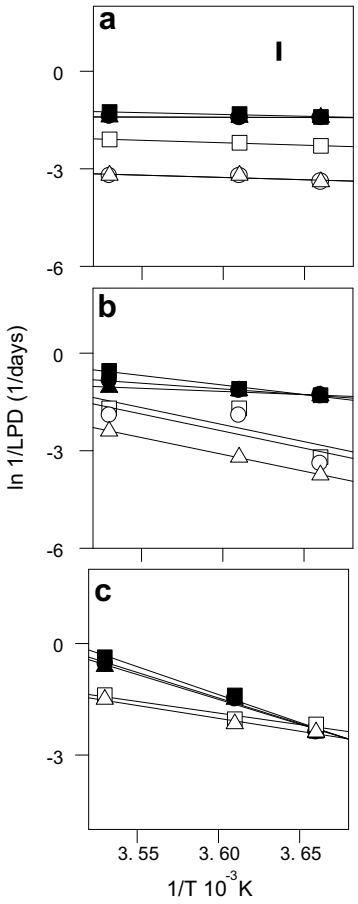

Fig. 5. Application of the Arrhenius equation to evaluate the temperature effect on adaptation period (1/LPD) for (a) *Klebsiella* sp., (b) *E. coli* and (c) *Pseudomonas* sp., in ground beef. Filled symbols correspond to experiments using ground beef packaged in polyethylene film (aerobic conditions) at different pH values: (■) 6.10, (●) 5.80, (▲) 5.60. Empty symbols correspond to experiments using vacuum packaging with EVA SARAN EVA at different pH values: (△) 6.10, (O) 5.80, (Δ) 5.60.

increase in the value of activation energy was observed as the pH diminished. *Klebsiella* sp., showed the highest value of activation energy in polyethylene and *Pseudomonas* sp., in ESE for all the tested pH. In all cases a good coefficient of linear determination was observed. Similar results were obtained by Coll Cárdenas et al. (2006) who reported that the highest activation energy values were obtained for *Klebsiella* sp., at 6.1 and 5.6 in model systems of a meat product.

The values of $E_{1/LPD}$ for each type of bacteria were obtained by plotting $\ln_{1/LPD}$ vs. $1/T$ (Fig. 5).

The values of $E_{1/LPD}$ obtained and the regression coefficients for the analyzed microorganisms are shown in Table 4. The highest values of $E_{1/LPD}$ were for *Pseudomonas* sp., in polyethylene and *Klebsiella* sp., in ESE film.

3.3. Effect of lactic acid addition on pH stability

The effect of temperature and gaseous permeability of the packaging plastic film on the growth of inoculated bacteria into ground beef samples with natural pH values of 5.6, 5.8 and 6.1 was described in the previous sections. In this section, the effect of acid lactic addition on ground beef samples is analysed.

Lactic acid was added on ground beef samples with a natural pH of 6.1 reaching a final pH of 5.6. The lactic acid was mixed in the ground beef sample and the growth of bacteria as affected by storage temperatures and packaging films was determined. Besides, during storage, the pH stability after addition of lactic acid was analysed.

Ground beef samples whose final pH was 5.6 due to the addition of lactic acid, maintained this pH during storage at 0, 4, and 10 °C while *E. coli* and *Pseudomonas* sp., were in the exponential phase. However, when microbial counts were higher than 7 log CFU g^{-1}, the pH of the samples increased at the end of the storage period indicating that in these conditions the buffer capacity of the beef was overtaken by the metabolic products of the bacteria.

Naveena, Muthukumar, Sen, Babji, and Murthy (2006), reported that during storage, the pH of meat treated with lactic acid slightly decreased initially and then significantly ($p < 0.05$) increased with few exceptions at the end of storage. This initial decrease might be attributed to the acid treatment, whereas the final increase in pH may be attributed to the microbial metabolites (Goddard, Mikel,

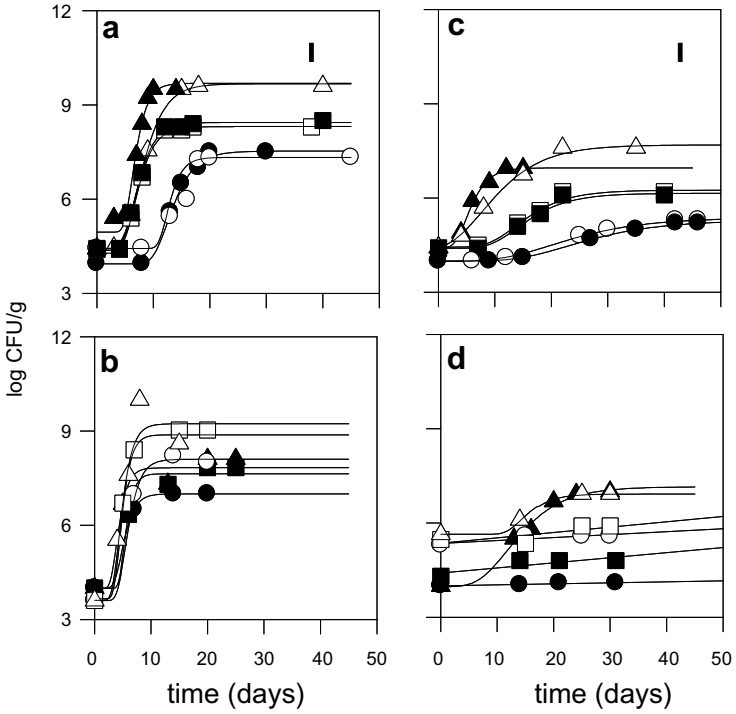

Fig. 6. Effect of lactic acid addition, storage temperature and gaseous permeability of the packaging film on the growth of *Pseudomonas* sp., (a and c) and *E. coli* (b and d) in ground beef. Bars indicate the least significant difference, LSD ($p < 0.05$). Full lines correspond to Gompertz or linear model. Filled symbols correspond to experiments in which lactic acid was added to ground beef of pH 6.10 to reach a pH 5.60. (●) 0 °C, (■) 4 °C and (▲) 10 °C. Empty symbols correspond to experiments in ground beef with a natural pH 5.60 (○) 0 °C, (△) 4 °C and (Δ) 10 °C. Packaging with polyethylene film in aerobic condition (a and b); packaging in vacuum with EVA SARAN EVA (c and d).

Conner, & Jones, 1996). As stated by Gill (1983), bacteria on exhaustion of stored glucose, utilize amino acids released during protein breakdown and ammonia accumulates as a product of amino acid degradation leading to a higher pH. Jose, Iyer, and Prabhakaran (1984) also observed that pH values decreased at the onset of spoilage but then increased as the spoilage developed.

During refrigerated storage at 0, 4, and 10 °C in ESE film, the pH values of the ground beef samples with added lactic acid showed stability. This is due to the fact that the predominant microbes in stored beef samples in ESE film are lactic flora (LAB) which produce lactic acid as a metabolic process and hence the pH values did not increase. It should also be noted that the growth of the inoculated bacteria is limited under vacuum, which helps to maintain the stability of pH.

Reducing the redox potential by vacuum-packaging and storage at refrigerated temperatures are two of the factors that enhance growth of LAB (Davies & Roberts, 1999; Samelis, Kakouri, & Rementzis, 2000). Buffering capacity is the ability of meat to resist the change in pH when acid or alkali is added. Usually the buffering capacity of beef muscle balances the pH changes and prevents the rapid pH decrease followed by rapid glycolysis. Buffering capacity is an important property of both living muscles and post-mortem beef. The same chemical compounds that regulate pH in a living muscle also regulate the post-mortem pH (Kivikari, 1996).

3.4. Effect of lactic acid addition on microbial growth in beef

Fig. 6 shows the growth of *Pseudomonas* sp., and *E. coli* in beef having an original pH 6.1, as was affected by the addition of lactic acid, decreasing pH values of ground beef to 5.6; samples were packaged with two films of different gaseous permeability and were stored at refrigerating temperatures (0, 4 and 10 °C). In the same Fig. 6 we can compare the growth rates of *Pseudomonas* sp., and *E. coli* in ground beef samples of natural pH 5.6 without addition of lactic acid.

It may be observed that in the case of the samples inoculated with *Pseudomonas* sp., in both films, there are no differences in the counts with respect to the samples with original pH 5.6 (open symbols). In contrast, in the case of meat inoculated with *E. coli*, packed with ESE and stored at 0 and 4 °C (filled symbols), there are variations in the initial counts. This difference indicates that the technology of adding lactic acid in conjunction with the packaging films with low gas permeability and refrigeration temperatures can be effective in keeping the meat with a minimum growth of *E. coli* for 30 days.

Gompertz and linear models are also shown in Fig. 6 and it can be observed that adding lactic acid to a beef sample of pH 6.1 to obtain a final pH 5.6 allowed the microorganisms to grow in a similar way that in beef with a natural pH 5.6.

Table 5
Kinetic parameters μ, LPD and MPD of the growth *E. coli* and *Pseudomonas* sp., in ground beef samples with original pH 6.1 and lactic acid added (LA) to reach pH 5.6 and packaged in polyethylene (aerobic condition) and ESE film (vacuum packaged) and stored at refrigeration temperature

Polyethylene (aerobic condition)					ESE film (vacuum packaged)		
T (°C)	pH initial	μ	LPD	MPD	μ	LPD	MPD
E. coli							
0	5.6	0.99 ± 0.51	3.78 ± 0.74	8.01 ± 0.57	0.01	50	–
	6.1 + LA	0.83 ± 0.05	3.45 ± 0.09	7.00 ± 0.05	0.03	50	–
4	5.6	1.58 ± 0.41	3.22 ± 0.38	9.01 ± 0.37	0.001	50	–
	6.1 + LA	1.41 ± 0.54	4.26 ± 0.99	7.64 ± 0.81	0.001	50	–
10	5.6	1.61 ± 1.20	2.89 ± 1.48	9.23 ± 1.29	0.17 ± 0.13	11.37 ± 2.07	6.92 ± 0.17
	6.1 + LA	1.30 ± 0.38	2.79 ± 1.04	7.83 ± 0.98	0.22 ± 0.05	6.68 ± 2.13	7.16 ± 0.63
Pseudomonas sp.							
0	5.6	0.40 ± 0.17	11.00 ± 3.04	7.47 ± 0.81	0.05 ± 0.02	10.85 ± 0.03	5.33 ± 0.44
	6.1 + LA	0.39 ± 0.11	10.00 ± 1.92	7.50 ± 0.49	0.05 ± 0.01	12.90 ± 1.69	5.29 ± 0.05
4	5.6	0.74 ± 0.09	4.51 ± 0.83	8.44 ± 0.18	0.13 ± 0.64	8.71 ± 1.57	6.25 ± 0.65
	6.1 + LA	0.75 ± 0.20	4.71 ± 0.84	8.31 ± 0.16	0.12 ± 0.27	9.96 ± 1.65	6.15 ± 0.29
10	5.6	0.75 ± 0.28	4.50 ± 1.02	9.67 ± 0.35	0.22 ± 0.04	1.87 ± 1.03	7.69 ± 0.75
	6.1 + LA	0.75 ± 0.18	2.30 ± 1.24	9.80 ± 1.11	0.26 ± 0.03	2.73 ± 1.83	6.98 ± 0.52

μ: log (CFU g^{-1})$days^{-1}$, MPD: (log (CFU g^{-1}), LPD: (days).

Serdengecti, Yildirim, and Gokoglu (2005) reported that 2.5% of sodium lactate significantly ($p < 0.95$) affected the aerobic plate counts. Although growth of lactic acid bacteria, psychrotrophs and coliforms were delayed in treated samples, they were not completely inhibited.

Sanitizing meat surfaces with lactic acid have been reported to be highly efficient. (Anderson, Marshall, & Dickson, 1992; Gill & Newton, 1982; Nassos, King, & Sttaford, 1985; Visser, Koolmees, & Bijker, 1988). However, the mechanistic action on the microorgnamisms of the lactic acid is not clear. Lactic acid inhibits by lowering pH (Baird-Parker, 1980; Davidson, 2001).

Lactic acid, in addition to its antimicrobial property due to the lowering of the pH, also acts as a permeabilizer of gram-negative bacterial membrane and may act potentiating the effects of other antimicrobial substances (Alakomi et al., 2000).

The kinetic parameters of *E. coli* and *Pseudomonas* sp., growing in beef with an initial natural pH of 6.1 in which lactic acid was added to give a pH of 5.6, are shown in Table 5. Obtained results correspond to ground beef samples stored at 0, 4 and 10 °C, and packaged in polyethylene and ESE.

The kinetic parameters obtained show that the samples with added lactic acid do not show significant differences from those whose natural pH was 5.6.

The above results indicate that it is possible to increase the quality value of ground beef with a high natural pH (6.1), by adding lactic acid to retard the growth of contaminant microflora.

4. Conclusions

In this work the growth of three bacteria isolated from beef muscle (*Klebsiella* sp., *E. coli* and *Pseudomonas* sp.) inoculated on ground meat samples of different pH values (6.1, 5.8 and 5.6) at different storage temperatures (0, 4 and 10 °C) and packaged in two different gaseous permeability films (polyethylene and ESE) was analysed. Microbial growth was modelled using the Gompertz and linear equations.

In polyethylene, *E. coli* was the microorganism, that showed the highest μ values at the different pH and also the greatest effect of pH on μ, especially in samples stored at 4 and 10 °C.

In the case of *Klebsiella* sp., neither pH nor temperature had marked effects on μ and on LPD. *Pseudomonas* sp., showed the lowest effect of pH on μ at the assayed temperatures in polyethylene. In ESE film, microbial growth rates of all the analyzed microorganisms, were less affected by pH and temperature than in polyethylene. The effect of pH was more noticeable as the temperature increased. In ESE film *E. coli* showed the highest effect of pH on μ, at 4 and 10 °C. LPD increased significantly with respect to the values in polyethylene, being *Klebsiella* sp., the microorganism that showed the highest values of LPD, followed by *E. coli*.

The effect of temperature on specific microbial growth and lag phase duration values were modelled through an Arrhenius type equation, determining the corresponding activation energies.

Klebsiella sp., showed the highest value of activation energy for the specific growth rate in ground beef samples packaged in polyethylene while in ESE films the highest activation energy values corresponded to *Pseudomonas* sp., for the tested pH values.

The highest values of activation energy for the adaptation period ($E_{1/LPD}$) were for *Pseudomonas* sp., in polyethylene and *Klebsiella* sp., in ESE film.

According to the results of the present study, lactic acid can be used as a decontaminant in ground fresh meat to

improve and increase the hygiene quality of beef samples of high pH.

Experiments in ground beef samples with added lactic acid that produced a decrease of the original muscle pH from 6.1 to 5.6 showed that the kinetic parameters of the microbial flora did not significantly differ from those corresponding to beef samples in which the original pH was 5.6. Thus it is possible to increase the quality value of beef cuts with a natural high pH by adding lactic acid to retard the deterioration caused by microorganisms which grow on beef, packaged with different plastic films at refrigeration temperatures.

Acknowledgments

The authors gratefully acknowledge the financial support given by the Universidad Nacional de La Plata, CONICET and the Agencia Nacional de Promoción Científica y Tecnológica.

References

Alakomi, H. L., Skytta, E., Saarela, M., Mattila-Sandholm, T., Latva-Kala, K., & Helander, M. (2000). Lactic acid permeabilizes gram-negative bacteria by disrupting the outer membrane. *Applied and Environmental Microbiology, 66*(5), 2001–2005.

Anderson, M., Marshall, R., & Dickson, J. (1992). Efficacies of acetic, lactic and two mixed acids in reducing numbers of bacteria on surfaces of lean meat. *Journal of Food Safety, 12*, 139–147.

Andrés, S., Giannuzzi, L., & Zaritzky, N. E. (2001). Mathematical modelling of microbial growth in packaged refrigerated orange juice treated with chemical preservatives. *Journal of Food Science, 66*, 724–728.

ANOVA (1989). *Super ANOVA: Assessable General Linear Modelling*. Berkley, California: Abacus Concepts Inc.

Baird-Parker, G. (1980). Microbial Ecology of Foods. In *Factors affecting life and death of microorganisms. International Commissions on Microbiological Specifications for foods (Chapter 7. Organic Acids)* (Vol. 1). New York: Academic Press.

Brown, M. H. (1982). Introduction. In M. H. Brown (Ed.), *Meat Microbiology* (pp. 1–12). London and New York: Applied Science Publishers Ltd.

Buchanan, R. L., & Whiting, R. C. (1986). Processed meats as a Arrhenius model and the square root model for predicting microbial environment. *Food Technology, 40*, 134–138.

Coll Cárdenas, F., Giannuzzi, L., & Zaritzky, N. (2006). Modelling microbial growth in meat broth with added lactic acid under refrigerated storage. *International Journal of Food Science & Technology, 42*(2), 175–184.

Davies, A. (1992). Meat microbiology. In *Introduction to meat product manufacture* (pp. 231–235). UK: Leatherhead Food Research Association.

Davies, R., & Roberts, T. A. (1999). Antimicrobial susceptibility of enterococci recovered from commercial swine carcasses: effect of feed additives. *Letters in Appied Microbiology, 29*(5), 327–333.

Davidson, P. M. (2001). Chemical preservatives and natural antimicrobial compounds. In M. P. Doyle, L. R. Beuchat, & T. J. Montville (Eds.), *Food Microbiology – Fundamentals and Frontiers* (pp. 593–627). Washington, D.C.: American Society for Microbiology.

Doores, S. (1993). Organic acids. In P. M. Davidson & D. L. Branen (Eds.), *Antimicrobials in foods* (pp. 95–136). New York: Marcel Dekker, Inc.

Giannuzzi, L., Pinotti, A., & Zaritzky, N. (1997). Mathematical modelling of microbial growth in packaged refrigerated beef stored at different temperatures. *International Journal of Food Microbiology, 39*(1–2), 101–110.

Gibson, A. M., Bratchell, N., & Roberts, T. A. (1988). The effect of sodium chloride and temperature on rate and extent of growth of *Clostridium botulinum* type A in pasteurized pork slurry. *Journal of Applied Bacteriology, 62*, 479–490.

Gill, C. O. (1983). Microbial interaction with meats. In M. H. Brown (Ed.), *Meat Microbiology* (pp. 225–264). London and New York: Applied Science Publishers.

Gill, C. O., & Badoni, M. (2004). Effects of peroxyacetic acid, acidified sodium chlorite or lactic acid solutions on the microflora of chilled beef carcasses. *International Journal of Food Microbiology, 91*, 43–50.

Gill, C., & Newton, K. (1982). Effect of lactic acid concentration on growth on meat of gram-negative psychrotrophs from a meat works. *Applied and Environmental Microbiology, 43*(2), 284–288.

Goddard, B. L., Mikel, W. B., Conner, D. E., & Jones, W. R. (1996). Use of organic acids to improve the chemical, physical, and microbial attributes of beef strip loins stored at −1 °C for 112 days. *Journal of Food Protection, 59*(8), 849–853.

Grau, F. (1981). Role of pH, lactate, and anaerobiosis in controlling the growth of some fermentative gram-negative bacteria on beef. *Applied and Environmental Microbiology, 42*, 1043–1050.

Hsiao, C., & Siebert, K. (1999). Modelling the inhibitory effects of organic acids on bacteria. *International Journal of Food Microbiology, 47*(3), 189–201.

Jose, M. T., Iyer, P., & Prabhakaran, P. (1984). Influence of pH on keeping quality of beef. *Kerala Journal of Veterinary Science, 15*(2), 135–137.

Kivikari, R. (1996). Buffering capacity of meat. EKT-Series 1203. Academic Dissertation. University of Helsinki, Helsinki. Department of Food Technology, University Press.

Li, K.Y. (1988). Microbial stability of intermediate moisture foods. Ph.D. Thesis. Corvallis, OR: Oregon State Univ.

Li, K. Y., & Torres, J. A. (1993). Microbial growth estimation in liquid media exposed to temperature fluctuations. *Journal of Food Science, 58*, 644–648.

Masurovsky, E. B., Goldblith, S. A., & Voss, J. (1963). Differential medium for selection and enumeration of members of the *Pseudomonas*. *Journal Bacterial, 85*, 722–723.

Mc Donald, K., & Da-Wen Sun, S. (1999). Predictive food microbiology for the meat industry: A review. *International Journal of Food Microbiology, 52*, 1–27.

Naveena, B. N., Muthukumar, M., Sen, A. R., Babji, Y., & Murthy, T. R. K. (2006). Improvement of shelf-life of buffalo meat using lactic acid, clove oil and vitamin C during retail display. *Meat Science, 74*, 409–415.

Nassos, P., King, A., & Sttaford, A. (1985). Lactic acid concentration and microbial spoilage in anaerobically and aerobically stored ground beef. *Journal of Food Science, 50*, 710–712.

Osmanagaoglu, O. (2002). Behaviour and biological control of bacteriocin-producing Leuconostocs associated with spoilage of vacuum-packaged sucuk. *Turkish Journal of Veterinary and Animal Sciences, 27*, 471–480.

Samelis, J., Kakouri, A., & Rementzis, J. (2000). Selective effect of the product type and the packaging conditions on the species of lactic acid bacteria dominating spoilage microbial association of cooked meats at 4 °C. *Food Microbiology, 17*, 329–340.

Serdengecti, N., Yildirim, I., & Gokoglu, N. (2005). Effects of sodium lactate, sodium acetate and sodium diacetate on microbiological quality of vacuum-packed beef during refrigerated storage. *Journal of Food Safety, 26*(1), 62–71.

Smulders, F., & Woolthuis, C. (1984). Immediate and Delayed Microbiological Effects of Lactic Acid Decontamination of Calf Carcasses-Influence on Conventionally Boned Versus Hot-Boned and Vacuum-Packaged Cuts. *Journal of Food Protection, 48*(10), 838–847.

Visser, I., Koolmees, P., & Bijker, P. (1988). Microbiological conditions and keeping quality of veal tongues as affected by lactic acid

decontamination and vacuum packaging. *Journal of Food Protection, 51*, 208–213.

Whiting, R. C. (1995). Microbial modelling in foods. *Critical Reviews in Food Science and Nutrition, 35*, 467–494.

Wilkinson, L. (1990). *SYSTAT, The System for Statistics*; SYSTAT. Evanston, IL.

Zwietering, M. H., Jongenburger, I., Rombouts, F. M., & Van't Riet, K. (1990). Modelling of the bacterial growth curve. *Applied and Environmental Microbiology, 56*, 1875–1881.

Zwietering, M. H., de Koos, J. T., Hasenack, B. E., de Wit, J. C., & van't Riet, K. (1991). Modeling of bacterial growth as a function of temperature. *Applied and Environmental Microbiology, 57*, 1094–1101.