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Mathematical modelling of microbial growth in packaged refrigerated beef stored at different temperatures

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Abstract

Gompertz and logistic models were fitted to experimental counts of microorganisms growing in beef stored at 0, 4, 7, 9 and 10°C. Samples were packaged in polyethylene (high gaseous permeability) and in EVA/SARAN/EVA (low gaseous permeability) films, being EVA ethyl vinyl acetate and SARAN polyvinyl and polyvinylidene chloride copolymer. Lag phase duration (LPD) and specific growth rate (μ) were obtained as derived parameters for lactic acid bacteria, *Enterobacteriaceae*, *Pseudomonas* sp. and psychrotrophic microorganisms. The reciprocal of LPD was fitted to an Arrhenius type equation; LPD of lactic acid bacteria showed a marked dependence on temperature, with activation energy values (E_{LPD}) of 222.2 and 216.9 kJ/mol for polyethylene and ESE respectively. The effect of initial microbial population at different storage temperatures on adaptation period was analyzed. As the initial microbial population increased, adaptation period decreased for all studied microorganisms and for both packaging films. The effect of temperature on specific growth rate was better interpreted by the Arrhenius model than by the linear or the square root equations. Psychrotrophic microorganisms in beef showed the highest activation energy values for specific growth rate (E_μ) in both packaging films, being E_μ 85.50 and 103.10 kJ/mol for polyethylene and ESE film respectively. In both films, *Enterobacteriaceae* showed the lowest E_μ values, being 15.33 and 59.89 kJ/mol in ESE and polyethylene respectively. The final number of microorganisms (maximum population density) did not show significant changes with storage temperature.

Keywords: Mathematical modelling; Microbial growth; Predictive microbiology; Temperature effect; Gas permeability effect

1. Introduction

Vacuum-packaging is increasingly being used as a technique for enhancing shelf-life of persihable foods such as cuts of fresh meat. Low temperature spoilage of fresh meat cuts in contact with air, differs considerably from that of vacuum-packed fresh meat, although in both cases spoilage occurs mainly on the surface of the product (Jay, 1973).

Constantly increasing microbial safety and quality concerns have focused the attention on mathematical

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modelling to quantify and predict microbial behaviour (Zwietering et al., 1990; Buchanan, 1993). Mathematical models can also be used to predict the effect on shelf-life of different time-temperature combinations in production and distribution chains. Generally, predictive models are build up on the basis of data obtained from experiments conducted in liquid media. In laboratory media, different factors can be controlled more easily than in actual food products. Thus, model validation in actual situations becomes a must before a model can be used for predictive purposes (McMeekin et al., 1992; Muermans et al., 1993). At present, Gompertz's equation has become the most widely used model to describe microbial growth (Gibson et al., 1987; Gibson and Roberts, 1989).

Temperature is a major factor on food deteriorative reactions, especially, for microbial spoilage since specific growth rate and lag phase are highly temperature dependent. Besides, although temperature plays a major role on microbial stability, refrigeration temperatures are not always kept constant during food handling. Thus, temperature effects on microbial stability have been widely studied by computer supported models based on heat transfer and microbial growth estimations (McMeekin and Olley, 1986; Buchanan, 1986; Fu et al., 1991; McMeekin et al., 1992; Buchanan, 1992; Almonacid-Merino and Torres, 1993; Li and Torres, 1993).

The square-root model is one of the models that describes the influence of the temperature on specific growth rate (Ratkowsky et al., 1983). Zwietering et al. (1991), modified the extended Ratkowsky model to describe the lag time as a function of temperature. Li and Torres (1993) studied the effect of temperature fluctuations on lag time and specific growth rate in liquid media.

The objectives of the present work were: 1) to analyze the effect of storage temperature (0, 4, 7, 9 and 10°C), initial microbial counts and gaseous permeability of the packaging film on microbial growth parameters obtained by fitting mathematical models to experimental counts of different microorganisms (lactic acid bacteria, *Enterobacteriaceae*, *Pseudomonas* sp. and psychrotrophic microorganisms) growing in packaged beef; 2) to evaluate and compare the fitting of Gompertz and logistic models to experimental microbial growth data; 3) to analyze the effect of temperature on lag phase and specific growth rate of microorganisms using different equations. This information allows the prediction of microbial growth under conditions different from those tested experimentally but within the studied range of temperatures.

2. Materials and methods

Beef samples were obtained from semitendinosus muscles removed from steers classified as U2 grade A according to the Argentine National Meat Classification (carcass weight up to 240 kg) with a post-mortem time of 48 h at 4°C; pH values ranged between 5.5–5.6 and were determined using a meat electrode Ingold lot 405-M4.

Different initial microbiological loads were obtained storing the muscles packaged in polyethylene at 10°C for up to 36 h previously to the cutting and final packaging procedure. The muscles were then removed from the polyethylene bags, and cut in slices, transversely to the fibres (1.5 cm thick and 10 cm in diameter, approx. 117 g weight). Samples were packaged using two films with different values of oxygen permeability: a) low density polyethylene of 60 μm thick, water vapour permeability WVP = 12 $g\,m^{-2}\,day^{-1}\,atm^{-1}$ at 30°C and RH = 78%, oxygen transmission rate OTR = 6500 $cm^3\,m^{-2}\,atm^{-1}\,day^{-1}$ at 23°C, and b) 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 = 37 $cm^3\,m^{-2}\,atm^{-1}\,day^{-1}$). ESE film was used for vacuum packaging obtaining partial gaseous evacuation in a Minidual equipment model MW 4980 (Schocolnik SAIC, Buenos Aires, Argentina)). Manometric pressure in the vacuum chamber was 4.5 mm Hg. Storage experiments with packaged refrigerated beef were performed at 0, 4, 7, 9 and 10°C. Temperature of the storage cabinets was recorded using thermocouples, inserted within the beef slices and connected to a Data Logger Fluke, 2240-C. During the storage period microbial counts were carried out periodically.

2.1. Microbiological analysis

Microbial evaluation of beef slices was performed by cutting a ribbon (20 cm^2 of the surface and approximately 0.3 cm thick) along the lateral area of

the muscle. Each sample was placed in 180 ml sterile 0.1% peptone broth and the maceration stage was carried out in an Omni mixer 17106 Homogenizer at 6000 rpm for 2 min. Dilutions with 0.1% peptone water were then performed to prepare the culture media for the following microbial determinations:

1. Psychrotrophic microorganisms: 1 ml of the necessary dilutions were inoculated in Plate Count Agar (Merck) following the pour plate procedure and was incubated at 4°C for 7 days.
2. *Enterobacteriaceae* counts: 0.1 ml of the necessary dilutions were inoculated in Bilis red violet glucose agar (Merck). Spread plates were incubated aerobically at 37°C for 12 to 24 h.
3. *Pseudomonas* sp. counts: 0.1 ml of the necessary dilutions were inoculated in Masurovsky agar (Masurovsky et al., 1963). Spread plates were incubated aerobically at 30°C for 2 days.
4. Lactic acid bacteria: 0.1 ml of the necessary dilutions were inoculated in MRS agar (Merck). Spread plates were incubated aerobically at 30°C for 3 days.

Determinations were made in duplicate and results were expressed as log N (N: Colony Forming Units ($\log(\mathrm{CFU}/\mathrm{cm}^2)$)).

2.2. Modelling of microbial growth

Mathematical models allow to analyze the effect of temperature and gaseous permeability of the packaging film on microbial growth parameters. One of the recommended models is the modified-Gompertz equation (Gibson et al., 1987) 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 ($\mathrm{CFU}/\mathrm{cm}^2$)] at time t, A the asymptotic log count as time decreases indefinitely (approximately equivalent to the log of the initial bacteria counts) [log ($\mathrm{CFU}/\mathrm{cm}^2$)], C is the log count increment as time increases indefinitely, that is the number of growth cycles [log ($\mathrm{CFU}/\mathrm{cm}^2$)], B is the relative maximum growth rate at time M [days]$^{-1}$, M is the time required to reach the maximum growth rate [days].

Using these parameters, the specific growth rate ($\mu = B.C/e$ [log ($\mathrm{CFU}/\mathrm{cm}^2$)day^{-1}], with $e =$ 2.7182), lag phase duration (LPD $= M - (1/B)$ [days]) and maximum population density (MPD $=$ A + C[log($\mathrm{CFU}/\mathrm{cm}^2$)]) were derived.

The logistic model was also applied according to:

$$\log N = A + C/(1 + \exp(D - F.t)) \quad (2)$$

where log N and A have the same meaning as was previously defined, D is a dimensionless parameter, F is the relative growth rate at half time of the exponential phase [days]$^{-1}$.

From these parameters, the specific growth rate ($\mu = C.F/4$ [log($\mathrm{CFU}/\mathrm{cm}^2$) days^{-1}]), and the lag phase duration (LPD $= D - 2/F$ [days]), were derived.

These models were numerically fitted to experimental data of microbial counts of lactic acid bacteria, *Enterobacteriaceae*, *Pseudomonas* sp. and psychrotrophic microorganisms; data were collected at various temperatures from beef samples packaged in two plastic films of different gaseous permeabilities (polyethylene and ESE films).

2.3. Statistical analysis

Data fits obtained from Gompertz and logistic models were performed by means of a statistical software (Systat, Inc 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 due to the residual variation. Lack of fit test ($F_{\text{lack of fit}}$) was performed in each case for both models (Draper and Smith, 1981).

Fitness of Gompertz and logistic models were statistically compared by the F test (Zwietering et al., 1990). Considering that both models have the same number of parameters the residual sum of squares (RSS) allows their direct comparison. F values were calculated as the ratio between RSS values of both models (F_{comp}), being the numerator the larger RSS. Degrees of freedom (number of data points – number of model parameters) were equal for the two models; these numbers were also used to obtain F_{table}; F_{comp} was tested against F_{table} to compare both models.

3. Results and discussion

3.1. Application of the mathematical models to fit microbial growth curves.

Fig. 1a, b, c, d and Fig. 2a, b, c, d show the fitting of Gompertz model to experimental data of microbial growth in beef, packaged in ESE and polyethylene films respectively. In all cases, a good agreement between experimental data and predicted values was obtained. The lack of fit of each model was compared with the measuring error by the $F_{\text{lack of fit}}$ The ratio between mean square due to lack of fit and the mean square due to measuring error was in all cases less than F_{table} (Draper and Smith, 1981). Since $F_{\text{lack of fit}}$ of each model was not significant, both models were found to be adequate.

When F test was used to compare both models, non significant differences were observed ($P<0.05$), because F_{comp} value was smaller than the F_{table}; thus only Gompertz equation fitting is shown in Figs. 1 and 2.

Obtained results of microbial growth curves were similar to data reported by Fournaud et al. (1973). These authors showed that *Pseudomonas* sp. and *Lactobacillus* reached counts of 10^4 and 10^3 CFU/cm^2 after 4 weeks of storage at 0°C in vacuum packaging.

Sheridan (1982) reported that the time necessary to reach the stationary phase for *Pseudomonas* sp. and *Lactobacillus* sp. growing in vacuum packaged beef (pH 5.6) stored at 4°C, was 15 days for both microorganisms. These values are similar to the results shown in Fig. 1.

The derived parameters: specific growth rate (μ), lag phase duration (LPD) and maximum population density (MPD) for the different microorganisms growing in beef samples packaged in both films are shown in Table 1. In beef samples packaged in polyethylene, *Pseudomonas* sp. grew at the highest rates with μ values ranging from 0.447 to 1.672 log(CFU/cm^2) $days^{-1}$; LPD diminished from 5.579 to 0.662 days for 0 and 10°C respectively. In the case of psychrotrophic microorganisms μ values changed

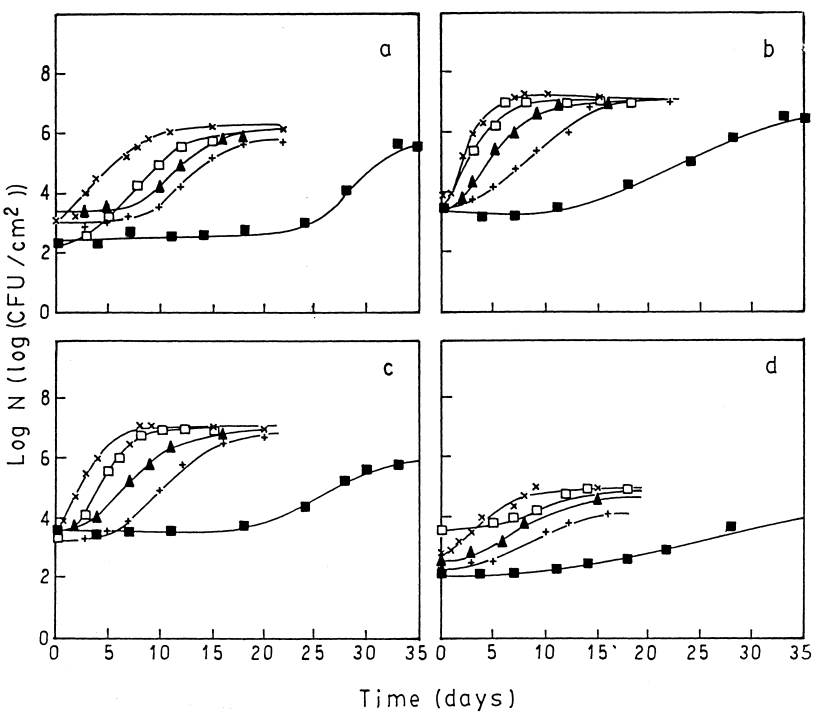

Fig. 1. Fitting of the Gompertz model to microbial counts of different microorganisms growing in beef samples packaged in EVA/SARAN/EVA film at 0 (■), 4 (+), 7 (▲), 9 (□) and 10°C (×). a) *Enterobacteriaceae*, b) Psychrotrophic microorganisms, c) Lactic acid bacteria, d) *Pseudomonas* sp.

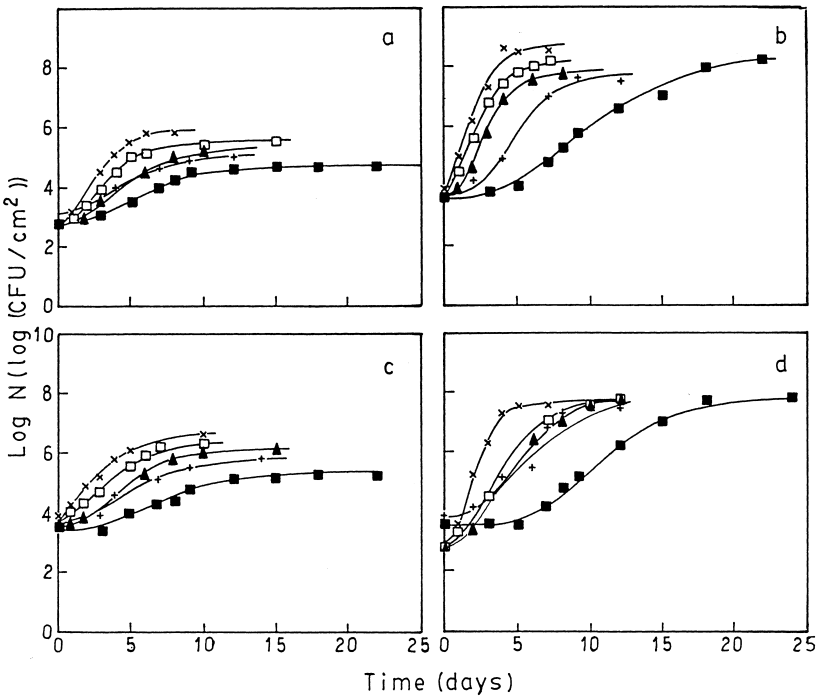

Fig. 2. Fitting of the Gompertz model to microbial counts of different microorganisms growing in beef samples packaged in polyethylene film at 0 (■), 4 (+), 7 (▲), 9 (□) and 10°C (×). a) *Enterobacteriaceae*, b) Psychrotrophic microorganisms, c) Lactic acid bacteria, d) *Pseudomonas* sp.

from 0.374 to 1.548 log(CFU/cm^2) days^{-1} and LPD diminished from 3.724 to 0.264 days respectively, when temperature increased from 0 to 10°C. *Enterobacteriaceae* showed μ values ranging from 0.284 to 0.758 log(CFU/cm^2) days^{-1} and LPD values between 2.477 to 0.537 days, while lactic acid bacteria grew at the lowest rates with μ values from 0.242 to 0.556 log(CFU/cm^2) days^{-1} and LPD values of 3.660 and 0.035 days at 0 and 10°C respectively.

In meat samples packaged in low gaseous permeability film, lactic acid bacteria and psychrotrophic microorganisms grew at the highest rates. For psychrotrophic microorganisms when temperature increased from 0 to 10°C, μ values changed from 0.173 to 0.904 log(CFU/cm^2) days^{-1} and LPD decreased from 13.165 to 0.566 days. *Pseudomonas* sp. grew at the lowest rates (0.090 log(CFU/cm^2) days^{-1} at 0°C and 0.345 log(CFU/cm^2) days^{-1} at 10°C); LPD decreased from 11.961 to 0.906 days when temperature increased from 0 to 10°C.

The final number of microorganisms (maximum population density) did not show significant changes with storage temperature (Table 1).

3.2. Effect of temperature on lag phase duration

Adaptation rate of microorganisms, defined as the reciprocal of lag phase (1/LPD) (Li, 1988; Li and Torres, 1993) was fitted to an Arrhenius type equation:

$$1/\mathrm{LPD} = Z \exp(-E_{\mathrm{LPD}}/RT) \quad (3)$$

where Z is the preexponential factor [days^{-1}], E_{LPD} is the activation energy [kJ/mol] and R is the gas constant (8.31 J/K/mol). Fig. 3 shows the plot for psychrotrophic microorganisms in beef packaged in polyethylene and ESE film. The other microorganisms also showed an Arrhenius dependence on temperature. Activation energy values (E_{LPD}) for both films are shown in Table 2. Lactic acid bacteria were the most sensitive to temperature, with E_{LPD} values of 222.2 and 216.9 kJ/mol in polyethylene and ESE respectively. *Enterobacteriaceae* showed

Table 1
Derived parameters μ (specific microbial growth rate), LPD (lag phase duration) and MPD (maximum population density) obtained from Gompertz parameters.

		EVA/SARAN/EVA					Polyethylene				
	Temp.	μ	A.S.E	LPD	A.S.E	MPD	μ	A.S.E	LPD	A.S.E.	MPD
Enterobacteriaceae	0	0.335	0.120	22.865	1.552	5.6	0.284	0.021	2.477	0.412	4.7
	4	0.390	0.059	8.780	0.560	5.7	0.310	0.310	1.348	0.466	5.1
	7	0.398	0.105	7.944	0.829	5.8	0.398	0.042	1.080	0.538	5.2
	9	0.419	0.028	3.526	0.393	5.7	0.611	0.033	1.018	0.205	5.5
	10	0.434	0.055	0.845	0.771	6.1	0.759	0.054	0.537	0.321	5.7
Lactic acid bacteria	0	0.192	0.048	18.861	1.992	5.9	0.242	0.036	3.660	0.717	5.2
	4	0.380	0.063	5.388	0.646	6.7	0.298	0.098	1.927	1.218	5.8
	7	0.410	0.051	2.941	0.423	6.7	0.431	0.034	1.572	0.448	6.0
	9	0.776	0.046	1.932	0.189	7.0	0.483	0.044	0.582	0.597	6.3
	10	0.756	0.098	0.319	0.621	7.0	0.556	0.044	0.035	0.564	6.5
Pseudomonas sp.	0	0.090	0.043	11.961	5.146	3.9	0.447	0.043	5.579	0.497	7.8
	4	0.184	0.057	5.232	1.028	4.9	0.597	0.169	2.299	1.476	7.4
	7	0.256	0.041	4.452	1.136	4.4	0.906	0.062	1.850	0.243	7.6
	9	0.235	0.095	2.915	1.476	4.7	0.989	0.191	1.276	0.696	7.7
	10	0.345	0.061	0.906	0.440	5.0	1.672	0.182	0.662	0.229	7.5
Psychrotrophic microorganisms	0	0.173	0.026	13.165	1.848	6.5	0.374	0.044	3.724	0.900	8.2
	4	0.334	0.042	3.554	0.976	7.0	0.756	0.457	2.250	2.287	7.5
	7	0.526	0.029	1.191	0.313	7.0	1.234	0.106	1.225	0.199	7.9
	9	0.714	0.179	1.083	0.772	7.1	1.221	0.044	0.310	0.141	8.2
	10	0.904	0.149	0.596	0.298	7.2	1.548	0.257	0.264	0.567	8.5

Temp. [°C], μ[log (CFU/cm^2) days^{-1}], LPD [days], A.S.E.: average standard error.

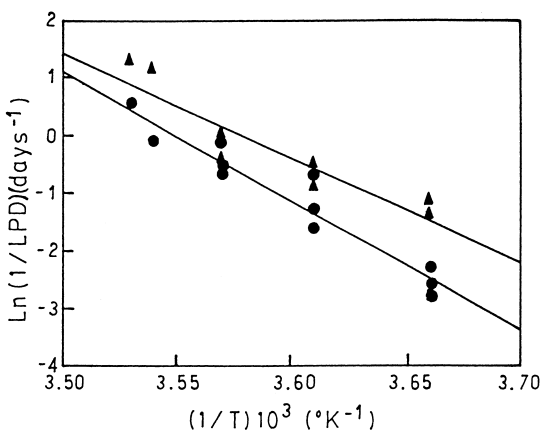

Fig. 3. Arrhenius plot of the adaptation rate (1/LPD) for psychrotrophic microorganisms in beef packaged in polyethylene (▲) and ESE films (●).

Table 2
Application of Arrhenius model to evaluate the effect of temperature on lag phase duration for different microorganisms growing in beef samples packaged in polyethylene and ESE film.

Microorganism	Packaging	ln Z	E_{LPD}	R^2
Pseudomonas sp.	Polyethylene	49.61	116.5	0.921
	ESE	55.53	131.8	0.811
Lactic acid bacteria	Polyethylene	97.37	222.2	0.634
	ESE	92.47	216.9	0.848
Enterobacteriaceae	Polyethylene	34.96	81.3	0.894
	ESE	71.84	170.6	0.796
Psychrotrophic microorganisms	Polyethylene	75.33	174.7	0.911
	ESE	80.69	188.8	0.967

Z [days^{-1}], E_{LPD} [kJ/mol].

the lowest E_{LPD} (81.3 kJ/mol) in polyethylene; *Pseudomonas* sp. showed the lowest E_{LPD} (131.8 kJ/mol) in ESE film. Correlation coefficients (R^2) are also shown in Table 2.

LPD values for *Pseudomonas* sp. in aerobically packaged fresh meat stored at 0.7, 3.3, 7.1 and 9.4°C were reported by Muermans et al. (1993); their fitted values were 6, 3.44, 2.5 and 1.94 days respectively, similar to those obtained in the present work.

Christopher et al. (1979) reported microbial counts of *Lactobacillus sp.* and psychrotrophic microorganisms growing in vacuum packaged beef stored between 1 and 3°C. From these data, LPD values were calculated, obtaining 14.05 days for *Lactobacil-*

lus and 12.8 days for psychrotrophic microorganisms, that are comparable to our results shown in Table 1.

3.3. Effect of initial microbial counts on adaptation period

Adaptation period of microorganisms causing deteriorative changes in beef is highly dependent on initial microbial population as shown in Fig. 4 for beef samples packaged in polyethylene and ESE film with different initial microbial counts stored at different temperatures. Adaptation period was short for samples with high initial counts ranging from 10^4 to 10^5 CFU/cm^2; within this range the effect of temperature was not significant ($P<0.05$). In all cases, adaptation period of samples packaged in ESE film were higher than those in polyethylene. At 0°C, *Pseudomonas* sp. and lactic acid bacteria showed values of 5 days, while in ESE film it increased to 24 days. As temperature increased, adaptation period decreased for both films; at 4 and 7°C, corresponding values in ESE film were 100% higher than in polyethylene. At temperatures higher than 7°C, adaptation periods were close to zero.

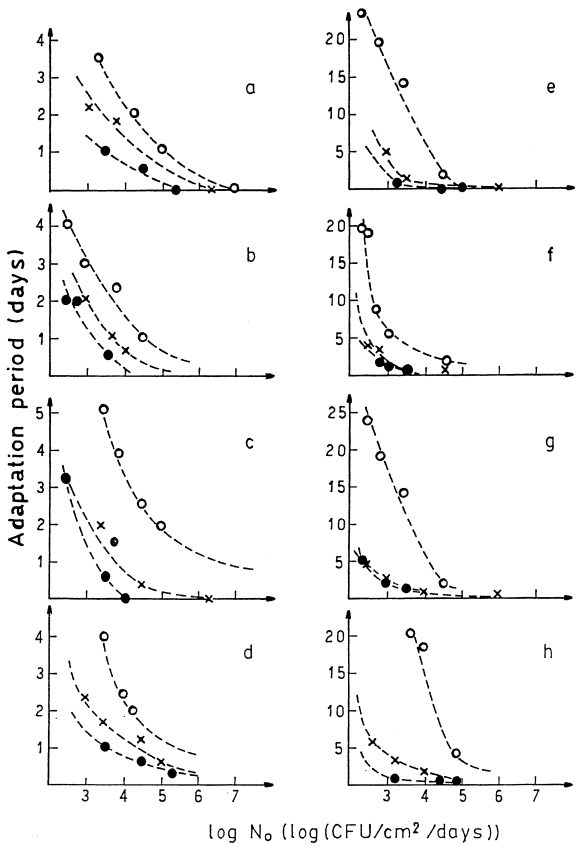

Fig. 4. Effect of temperature and initial microbial population on adaptation period for psychrotrophic microorganisms (a, e), *Enterobacteriaceae* (b, f), *Pseudomonas* sp. (c, g) and lactic acid bacteria (d, h), growing in polyethylene (a, b, c, d) and ESE (e, f, g, h) films, at 0 (○), 4 (×) and 7°C (●).

3.4. Effect of temperature on specific growth rate

Effect of storage temperature (0, 4, 7, 9 and 10°C) on specific growth rate (μ) was described by the following equations:

a) Arrhenius model:

$$\mu = A'.\exp(-E_\mu/RT) \quad (4)$$

where μ is the specific growth rate [log(CFU/cm^2) days^{-1}], T the absolute temperature, E_μ the activation energy [kJ/mol], A' the preexponential factor [log(CFU/cm^2) days^{-1}], and R the gas constant.

b) Linear model:

$$\mu = \mu_o + rT \quad (5)$$

where μ_o is the specific growth rate at 0°C [log(CFU/cm^2) days^{-1}], T temperature in (°C), r is slope of the linear regression [log(CFU/cm^2) days^{-1}°C^{-1}] (Spencer and Baines, 1964; Li and Torres, 1993).

c) Square root equation:

The square root equation is probably the most studied and widely used model to analyze the effect of temperature on specific microbial growth rate. Ratkowsky et al. (1983) proposed the following relationship:

$$\sqrt{\mu} = g.(T' - T'_0) \quad (6)$$

where g is a regression coefficient [(log(CFU/cm^2) days^{-1})$^{1/2}$°C^{-1}], T' is the incubation absolute temperature [K], T'_o is a conceptual temperature with no metabolic significance for psychrophiles, psychrotrophs and mesophiles (Ratkowsky et al., 1983).

Eq. (6) was modified as follows:

$$\sqrt{\mu} = p + q.T \quad (7)$$

where T is the incubation temperature [°C], q is the slope of the regression line [(log(CFU/cm$^{2)}$ days^{-1})$^{1/2}$°C^{-1}] and p[(log(CFU/cm^2) days^{-1})$^{1/2}$] is the intercept at 0°C.

Fig. 5 shows the effect of temperature on μ values of lactic acid bacteria. Table 3 summarizes the regression coefficients (R^2) obtained with the three models applied to the microorganisms growing in packaged beef. The highest correlation coefficients were obtained with Arrhenius model. In both films, psychrotrophic microorganisms showed the highest E_μ values (85.50 and 103.10 kJ/mol in polyethylene and ESE film respectively), while *Enterobacteriaceae* showed the lowest E_μ values (15.33 and 59.89 kJ/mol in ESE and polyethylene respectively).

Similar μ values were reported by Zamora and Zaritzky (1985) for *Pseudomonas* sp. and lactic acid bacteria growing in beef packaged in EVA–SARAN–EVA and polyethylene during storage at 0 and 4°C.

From data reported by Christopher et al. (1979) of *Lactobacillus* sp. and psychrotrophic microorganisms growing in vacuum packaged beef stored between 1 and 3°C, μ values were calculated, obtaining results that agree with those shown in Table 1.

An early report by Cooper (1963) noted that in some cases the ratio of growth rate to generation time was nearly constant. This suggested a linear relationship between lag time duration and the reciprocal of specific growth rate, that was confirmed in the present work for different microorganisms growing in beef. Fig. 6 shows the linear regression obtained for *Pseudomonas* sp. in both packaging films. Similar regressions were obtained for the other microorganisms with correlation coefficients R^2 ranging between 0.869 and 0.998. Li and Torres (1993), reported that a linear relationship was also observed for *P. fluorescens* growing in media with NaCl or glycerol as the controlling solute.

The present work, allowed to predict microbial growth and storage life of beef at different temperatures, by means of activation energy values for LPD and μ, derived from Arrhenius type models. Besides,

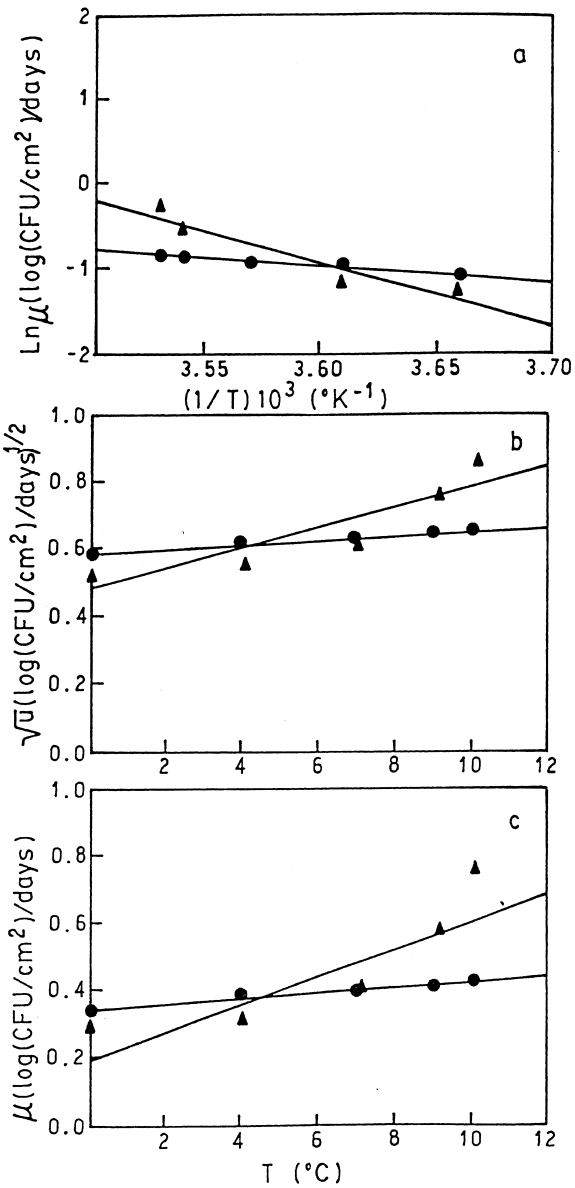

Fig. 5. Effect of temperature on specific growth rate of lactic acid bacteria in ESE (●) and polyethylene (▲) films. a) Arrhenius model, b) linear model, c) square root model.

the obtained results will help to develop a software to predict storage life of packaged beef submitted to thermal variations within 0–10°C range, characteristic of transport and storage stages. On the basis that microbial testing in foods is expensive and time consuming, mathematical models become a useful

Table 3
Application of Arrhenius, linear and square root models to evaluate temperature effect on specific growth rate for different microorganisms growing in beef packaged in polyethylene and EVA/SARAN/EVA films.

		Arrhenius model			Linear model			Square root model		
Microorganism	Packaging	ln A'	E_μ	R^2	μ_0	r	R^2	p	q	R^2
Pseudomonas sp.	Polyethylene	31.838	74.29	0.995	0.313	0.101	0.755	0.612	0.054	0.834
	ESE	29.582	72.43	0.904	0.086	0.021	0.856	0.306	0.024	0.910
Lactic acid bacteria	Polyethylene	21.847	52.90	0.979	0.216	0.031	0.952	0.475	0.025	0.%8
	ESE	35.764	84.66	0.939	0.159	0.056	0.861	0.429	0.043	0.905
Enterobaaeriaceae	Polyethylene	24.983	59.89	0.854	0.207	0.043	0.764	0.481	0.032	0.806
	ESE	5.667	15.33	0.949	0.340	0.009	0.965	0.583	0.007	0.962
Psychrotrophic	Polyethylene	36.772	85.50	0.946	0.360	0.111	0.957	0.625	0.061	0.966
microorganisms	ESE	42.916	103.10	0.996	0.114	0.069	0.934	0.393	0.052	0.979

A'[log(CFU/cm^2) days^{-1}], E_μ[kJ/mol], μ_0[log(CFU/cm^2) days^{-1}], r [log(CFU/cm^2) days^{-1}°C^{-1}], p[(log(CFU/cm^2) days^{-1})$^{1/2}$], q [(log(CFU/cm^2) days^{-1})$^{1/2}$°C^{-1}].

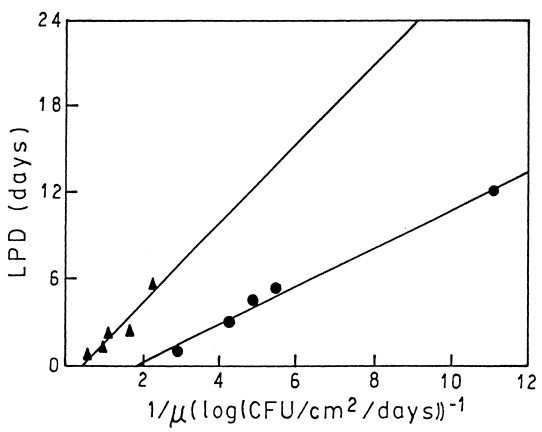

Fig. 6. Correlation of lag phase duration (LPD) and reciprocal of specific growth rate ($1/\mu$) for *Pseudomonas* sp. in beef samples packaged in polyethylene (▲) and ESE (●) films.

tool to provide a matrix of microbial growth responses to a broad range of storage conditions.

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