Modelling of *Listeria Monocytogenes* growth in pre-sterilized ground beef as affected by fat content, temperature, and atmosphere

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Cenkowski, S., Blank, G. and Chung-Lewis, M. 2002. **Modelling of *Listeria Monocytogenes* growth in pre-sterilized ground beef as affected by fat content, temperature and atmosphere**. Canadian Biosystems Engineering/Le génie des biosystèmes au Canada **44**:3.11-3.16. Ground beef samples, previously sterilized using electron beam radiation, containing from 17.8 to 30.2% fat and from 50 to 20% protein, respectively, were inoculated with *Listeria monocytogenes* and stored at either 5 or 11°C in sealed barrier pouches containing either air or modified atmosphere (CO_2:N_2; 15:85% and 30:70%). Following electron beam irradiation (1.75 kGy) the samples were maintained at 11°C and sampled at predetermined time intervals. Controls consisted of samples which were not irradiated following packaging. A logistic equation for the growth of *L. monocytogenes* was derived. The equation closely predicted the growth response of the microorganism under the various storage conditions. The growth of *L. monocytogenes* was predicted in a computer simulated experiment for non-irradiated beef samples and compared to the growth of listeriae on irradiated samples stored at 11°C in air and 30% CO_2 atmosphere. The effect of the fat content in the beef samples on the growth of *L. monocytogenes* was also simulated. Results indicated higher growth rates for the microorganism in ground beef samples containing 18% fat as compared to 30% fat during the initial 14 days of storage in 11°C air.

Des échantillons de boeuf haché, contenant de 17.8 à 30.2 % de gras et de 50 à 20 % de protéines, et qui avaient été irradiés avec un faisceau d'électrons, furent inoculés avec la bactérie *Listeria monocytogenes* et entreposés à 5° ou 11°C dans des sachets scellés contenant de l'air ou une atmosphère modifiée (CO_2:N_2; 15:85% et 30:70%). Après avoir été irradiés (1.75 kGy), les sachets furent maintenus à 11°C, et des échantillons furent prélevés à des intervalles prédéterminés. Les contrôles étaient constitués d'échantillons qui n'avaient pas été irradiés après l'emballage. Une équation logistique simulant la croissance de *L. monocytogenes* fut développée. L'équation prédisait avec justesse la croissance des microorganismes sous les différentes conditions d'entreposage. La croissance de *L. monocytogenes* dans des échantillons de boeuf irradiés fut également prédite lors d'une expérience de simulation par ordinateur, et comparée à celle de listeria dans des échantillons irradiés entreposés à 11°C dans une atmosphère contenant 11% de CO_2. Les effets de la teneur en gras des échantillons de boeuf sur *L. monocytogenes* furent également simulés. Les résultats ont montré que durant la période initiale de 14 jours d'entreposage à 11°C, les taux de croissance des microorganismes étaient plus élevés dans les échantillons de boeuf haché contenant 18% de gras que dans ceux dont la teneur en matières grasses était de 30%.

INTRODUCTION

The ability to derive equations that are capable of predicting microbial growth over a range of temperatures may give a better understanding of the risks involved when subjecting microorganisms to various environmental conditions (Broughall et al. 1983). A set of equations, also named predictive microbial models, can allow prediction of microbial safety or shelf life in products and facilitate development of HACCP (Hazard Analysis and Critical Control Points) programs (Whiting and Buchanan 1994). Predictive microbiology is an alternative to developing technology as it may provide faster microbiological analyses and yet prove to be less costly (McMeekin et al. 1993). Overall, predictive microbiology aims to summarize the probable behaviour of specific spoilage organisms and the progression of spoilage processes in foods (McMeekin and Ross 1996). It relies upon the development of mathematical models that can predict the rate of microbial growth or decline under a given set of environmental conditions. Predictive modelling could be used as a technique for accurate shelf life estimation and for evaluation of food safety. Mathematical models are derived by measuring the responses of microorganisms to various factors like temperature, pH, gaseous atmosphere, presence of chemical preservatives, and water activity. In a true food system, these factors work in combination to inhibit microbial growth/toxin production thereby increasing shelf life and assuring food safety. It is also expected that these models may be used as part of an ongoing quality control program. One such program, HACCP is mandatary in the United States and in Canada for food processors exporting to the United States.

The use of functions is largely empirical and the type selected to represent growth is sometimes chosen by simply looking at the data and making an informed guess. The PC software available on the market (e.g. SigmaPlot, FitCurve, etc.) can even increase the success of an informed guess by enhancing the data fitting procedure with statistical analysis at the click of a button. However, selecting an empirical formula or a modified version of known models (e.g. Gompertz) and finding their parameters may lead to routine determination of meaningless parameters. Therefore, it is preferable to develop mathematical relationships whose parameters may characterize underlying physiological or biochemical mechanisms or constraints.

The Gompertz model can be used to simulate the sigmoidal response of a microbial growth curve. For example, researchers have used the Gompertz model to statistically compare parameters of the model to simulate the effects of substrate composition (e.g. salt concentration, pH, and temperature) on the growth of *L. monocytogenes* (Buchanan et al. 1989) and to study the effects of pH, sodium chloride, and sodium nitrite on the growth kinetics of *Aeromonas hydrophila* (Palumbo et al. 1991). The accuracy in predicting growth depends on a number of parameters used in the sigmoidal model. Modified versions of the Gompertz equation can include three or more parameters to describe the behaviour of the bacterial growth curve. The higher the number of the parameters, the better the fit of the equation to the data. The primary Gompertz model for growth curves is of the form (Buchanan and Phillips 1990; Whiting and Buchanan 1994):

$$Y_t = Y_0 + C\exp\left\{-\exp\left[-B\left(t - t_M\right)\right]\right\} \quad (1)$$

where:

Y_t = $\log_{10}$ of colony forming units (cfu) at time t,
Y_o = $\log_{10}$ of inoculum at time 0,
C = change in cell numbers between inoculum and stationary phase,
B = relative growth rate (1/time),
t_M = time when the maximum specific growth rate is achieved, and
t = time.

However, the Gompertz equation has several drawbacks including: (i) the lag period is not always horizontal, (ii) the mathematical asymptote or inoculum value is off-scale in negative time as its magnitude is in decimal values before the logarithm is taken, (iii) the equation is continually curving and the resultant curve does not contain a period of linear increase which is observed with most growth curves, and (iv) the exponential growth rate is determined by the curve's inflection point.

A major factor in determining the specific growth rate of a microorganism in chilled foods is temperature. To accommodate the temperature effect, a modified version of the Gompertz model was used by Zwietering et al. (1991) in which three parameters (A, μ_m, λ) of the equation were correlated to the storage temperature:

$$Y_t = A\exp\left\{-\exp\left[\frac{\mu_m \exp(1)}{A}\left(\lambda - t\right) + 1\right]\right\} \quad (2)$$

where:

A = asymptote, the maximum value of the growth reached,
μ_m = maximum specific growth rate, tangent to the growth curve at the inflection point, and
λ = lag time, t-axis intercept of the tangent in the inflection point on the growth curve.

Although the description of the coefficient μ_m in Eq. 2 is different from the parameter B in Eq. 1, they are both determined in the same manner. However, the remaining parameters in the both equations are not interchangeable.

The objective of this research was to develop and verify a mathematical model for the growth of *L. monocytogenes* in ground beef. Although all components in our food system (ground beef) are known to affect the growth of microorganisms, only the influence of the fat was examined in the model. The main reason for choosing this food component is that during and following radiation treatment it, probably more than any other, impacts on the development of off-flavours and odours (Diehl 1990). In many instances, the maximum or threshold radiation dose that can be applied to a specific food without incurring sensory alterations is predicated on its fat or lipid level (Sudarmadji and Urbain 1972). To more easily assess the growth of listeriae in the samples, the meat was previously sterilized by radiation. According to the protocol, the sterile meat samples were inoculated and packaged in barrier pouches containing either air or a modified atmosphere consisting of $C0_2$:N_2 at two temperatures and subsequently irradiated (radurized). The choice for *L. monocytogenes* as the target microorganism in the study was based on its wide distribution in nature and its ability to grow and survive under common refrigeration temperatures (Chawla et al. 1996). In addition, this bacterium is a known human and animal pathogen (Chakraborty and Goebel 1988). In humans, ingestion of food containing listeriae can result in gastroenteritis or listeriosis.

DERIVATION OF A LOGISTIC EQUATION FOR THE GROWTH OF MICROORGANISMS

The mathematical model derived hereafter stems from two fundamental assumptions that also are used in the Gompertz model (Ozilgen 1998):

1. The quantitative growth of bacteria (dS/dt) is proportional to their quantity and depends on the proportionality constant, μ:

$$\frac{dS}{dt} = \mu S \quad (3)$$

where:
S = bacterial count at any time (cfu/g), and
μ = specific growth rate (d^{-1}).

2. The proportionality constant μ or the specific growth rate can change with time and is governed by:

$$\frac{d\mu}{dt} = -D\,\mu \quad (4)$$

where D describes the decay in the specific growth rate (d^{-1}).

Separation and integration of variables in Eq. 4 allows to express the specific growth rate μ of microorganisms at any time. Thus, substituting the solution for the specific growth rate into Eq. 3 and introducing the integration limits (S and S_0 for corresponding times t and t_0) leads to:

$$\ln S - \ln S_0 = -\frac{\mu_0}{D}\left[\exp(-Dt) - \exp\left(-Dt_0\right)\right] \quad (5)$$

where:

S_0 = initial bacterial count at time 0 (cfu/g), and
μ_0 = specific growth rate at time zero (d^{-1}).

Table 1. Proximate analysis (percent wet basis) of ground beef samples.

Ground beef	moisture	fat	protein
regular (beef 30)	41.4 ±2.0[a]	30.2 ±3.6	20.0 ±1.8
lean (beef 20)	29.2 ±0.4	19.8 ±0.1	47.0 ±0.1
extra lean (beef 18)	28.1 ±0.5	17.8 ±1.4	49.7 ±3.0

[a]values represent means ±standard deviation; n=3

For t_0=0, the exponential expression in Eq. 5 becomes 1 and after rearrangement the growth ratio can be expressed as:

$$\frac{S}{S_0} = \exp\left\{\frac{\mu_0}{D}\left[1-\exp(-Dt)\right]\right\} \quad (6)$$

MATERIALS and METHODS

Ground beef and listeriae preparation

Retail display packages (styrofoam base, polyethylene overwrap) of regular, lean, and extra lean ground beef were purchased locally. In the present study these products are referred to as beef 30, beef 20, and beef 18, respectively, and the number refers to the proximate level of fat in the samples (Table 1). For all products, protein and fat were determined by micro-Kjeldahl and Soxhlet extraction (16 h) using hexanol, respectively. Moisture was determined using an oven drying procedure (100-102°C, 18 h) (AOAC 1995). All proximate analyses were performed according to AOAC (1995) in triplicate.

Listeria monocytogenes (No. 10-112) was obtained from the Cadham Provincial Laboratory (Winnipeg, MB). The organism was maintained on trypticase soy agar (TSA; BBL, Becton Dickinson, Cockeysville, MD) slants at 4°C following growth at 35°C for 48 h. On a monthly basis, the listeriae were transferred to fresh TSA slants to maintain viability. Inocula were prepared by transferring a loopful of *L. monocytogenes* (maintained on TSA) into 25 mL of trypticase soy broth (TSB; BBL, Becton Dickinson, Cockeysville, MD). Following incubation (35°C, 18 h) a portion of the resultant growth ($OD_{420}\approx 0.10$; 10 mL) was diluted with sterile, fresh TSB (75 mL). The resulting listeriae concentration was approximately 10^7 cfu/mL (confirmed using direct plating with TSA following incubation at 37°C for 24 h). The TSB was further diluted by transferring 10 mL into 90 mL of 0.85% sterile NaCl and served as a stock inoculum (10^6 cfu/mL).

Sample sterilization, inoculation, and modified atmosphere packaging

Samples (10 g) of ground beef contained in Surevak Paxe 2050 pouches (Winpak, Winnipeg; MB; 185×215 mm) were folded over, taped shut, and frozen at -20°C. Sample sterilization was achieved via irradiation using a I-10/1, 10 MeV electron accelerator (Atomic Energy of Canada, Limited, Pinawa, MB). The time of irradiation under the beam was approximately 20-25 s at 25 kGy (sterilizing energy dose in kiloGrays; 1 kGy = kJ/kg of the product) with a dose rate at approximately 1 kGy/s. Product sterility was assessed using a direct plating technique (TSA, 37°C, 48 h). The oxygen and vapour transmission rates of the pouches were 8-10 cm^3/m^2 per 24h and 4.96 g/m^2 per 24h, respectively, at 37.8°C and 90% relative humidity (Winpak information data).

Following sterilization, the pouches were aseptically opened and the contents inoculated with *L. monocytogenes* (0.1 mL stock inoculum; 10^6 cfu/mL). The pouches were then taped closed and the contents were gently massaged by hand for approximately 30 s to distribute the inoculum. A Bizerba (model GM 2002, Bizerba Canada Inc., Mississauga, ON) packaging machine was used for the modified atmosphere packaging (MAP) studies. In this regard, the pouches were evacuated to -980 mbar and back-flushed with either 15:85% CO_2:N_2 or 30:70% CO_2:N_2 (Union Carbide Gas, Winnipeg, MB) via a pressurized regulating valve. Samples in pouches containing air were also inoculated. All pouches were subsequently heat sealed and stored at either 5 or 11°C.

Sample irradiation at 1.75 kGy following MAP

Irradiation of all samples was performed using a linear accelerator (Impela I-10/1, 10 MeV, AECL, Pinawa, MB). The irradiation time under the beam was approximately 10 s. In all cases, this dose was 1.75 kGy ± 10%. Irradiated samples were subsequently stored at 11°C in thermostatically controlled refrigeration units. Actual doses received by the irradiated samples were determined using radiochromic GAF dosimiters (Miller and McLaughlin 1981).

Survivor growth

Testing of samples at day 0 was carried out within 6 h of packaging. At specified time periods (7, 14, 21 d, etc.) samples were removed from their storage environment and microbiologically examined. In this regard, the pouches were aseptically opened and the samples were homogenized with 90 mL of 0.1% peptone for approximately 60 s using a stomacher (model 400 A.J. Seeward, London, UK). Samples were serially diluted (0.1% peptone) and evaluated using a pour plate technique with TSA (BBL). Colony forming units were determined following incubation at 35°C for 48 h. Results are expressed as means of triplicate trials, each performed in triplicate giving a total of nine tests pear mean.

RESULTS and DISCUSSION

Growth profiles of listeriae in the ground beef samples exposed to either air or CO_2:N_2 are presented as average cell count S and were plotted versus storage time. A function of the form of Eq. 6 was generated and coefficients μ_0 and D were determined based on the best fit criterion (SigmaPlot 1997) from the individual trials.

Two examples of cell count profiles of listeriae in the ground beef samples stored at 5 and 11°C, in the package atmosphere containing air only or either 15:85 and 30:70% CO_2 are shown in Figs. 1 and 2. The ratio, CO_2:N_2 used for MAP, will hitherto be referred to as the CO_2 level. The data points are the averages of nine trials and the vertical bars associated with them are the 95% confidence limits. The three lines (solid, dashed dot, and dashed) show the generated relationships based on Eq. 6 and developed for storage in three package atmospheres at one temperature. The values of the μ_0 and D coefficients for cell count of *L. monocytogenes* in ground beef of 17.8, 19.8, and 30.2% fat content exposed to three package atmospheres and stored at two temperatures (5 and 11°C), are summarized in Table 2.

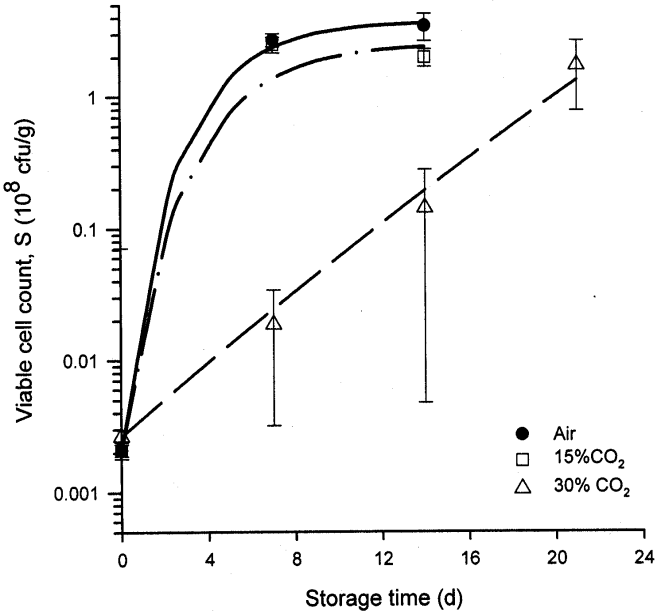

Fig. 1. Determination of coefficients μ_0 and D of Eq. 6 of growth of *L. monocytogenes* for beef 20 stored at 11°C in air and two levels of CO_2. Data points represent averages of nine determinations. Vertical bars indicate 95% confidence limits. The lines represent the best fit for the corresponding data.

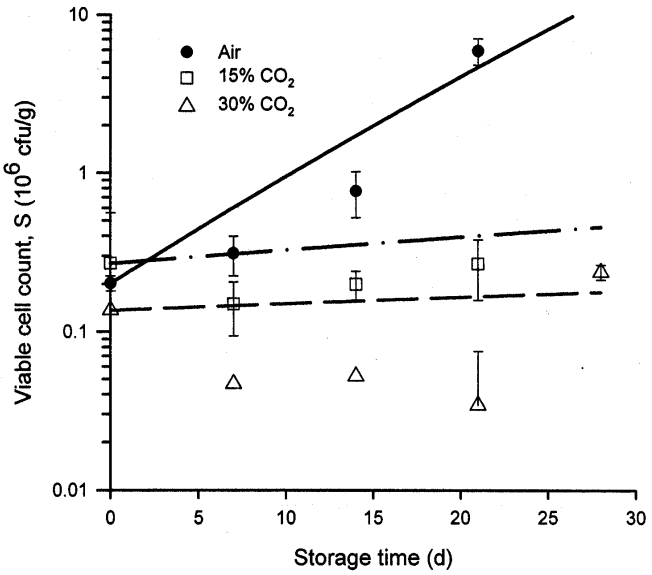

Fig. 2. Determination of coefficients μ_0 and D of Eq. 6 of growth of *L. monocytogenes* for beef 30 stored at 5°C in air and two levels of CO_2. Data points represent averages of nine determinations. Vertical bars indicate 95% confidence limits. The lines represent the best fit for the corresponding data.

The specific growth rate μ_0 of listeriae was affected by the package atmosphere, fat content of beef, and the storage temperature of the package. As expected, an increase in the CO_2 content in the package was reflected in a decrease of the specific growth rate μ_0 and a decrease in the decay in the specific growth rate D. For example, for the regular ground beef (30.2% fat) stored at 11°C, the specific growth rate decreased from 2.25 to 0.30 when the CO_2 content in a pouch was increased to 30%. The decay in the specific growth rate D also decreased with the increase in the CO_2 content in a pouch. Again, for the regular beef of 30.2% fat content and stored at 11°C, the D values are 0.30 and 0.01 for air and 30% CO_2. This regularity was observed in all the experiments. Lowering the storage temperature from 11 to 5°C also caused the lowering of the growth rate of listeriae which was reflected in a smaller magnitude of both coefficients.

The effect of fat content in ground beef is also visible in Table 2. The increase in fat content from 17.8 to 30.2% resulted in a decrease in both the specific growth rate and the decay coefficient when ground beef was stored at 11°C. This effect diminished at the 5°C. The prediction results for the growth of *L. monocytogenes* correlated to fat content for non-irradiated beef stored in the air temperature of 11°C are shown in Fig. 3. The symbols indicate data points determined from the experiments. In the simulation, it was assumed that beef was initially inoculated with 4500 cfu of *L. monocytogenes* per gram. It is noticeable that in the first 7 days of storage the slowest growth was obtained in beef of high fat content (30.2%). After 14 days, differences in growth related to the fat content were rather small.

Table 2. Values of the specific growth rate (μ_0) and the decay coefficient (D) in Eq. 6 for growth of *L. monocytogenes* in ground beef of various fat content stored at 5 and 11°C in air and modified atmosphere.

Fat content (%)	Atmosphere	Storage temperature 5°C		Storage temperature 11°C	
		μ_0	D	μ_0	D
17.8	Air	0.22	0.008	3.75	0.50
	15% CO_2	0.02	0.002	3.48	0.49
	30% CO_2	0.01	0.001	1.26	0.18
19.8	Air	0.22	0.008	3.00	0.40
	15% CO_2	0.04	0.003	2.49	0.35
	30% CO_2	0.02	0.002	0.33	0.01
30.2	Air	0.16	0.006	2.25	0.30
	15% CO_2	0.02	0.003	1.26	0.18
	30% CO_2	0.01	0.002	0.30	0.01

Figure 4 shows the experimental results marked by data points (solid circles, squares, and triangles) of the viable cell count of *L. monocytogenes* on ground beef (17.8, 19.8, and 30.2% fat content), packaged in three different atmospheres (air, 15 and 30% CO_2) and then exposed to 1.75 kGy electron beam radiation. The samples were stored at 11°C. Irradiation of the beef samples decreased the initial population of listeriae by approximately a

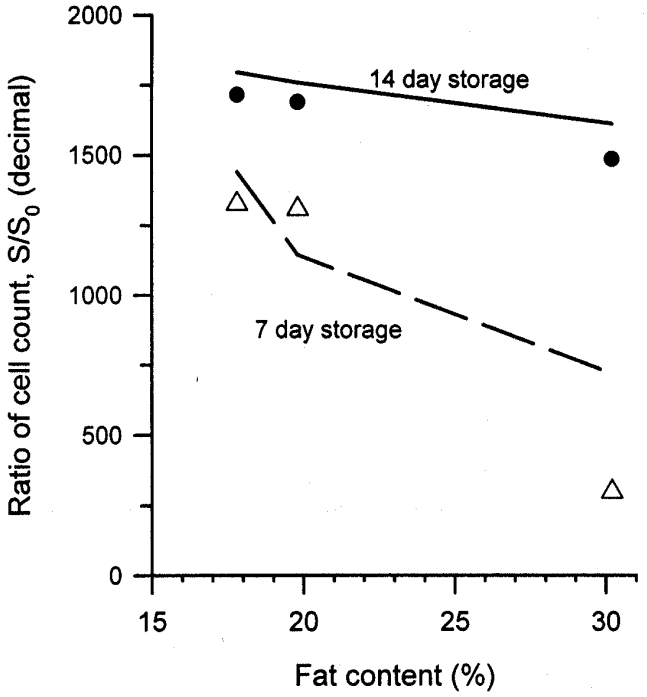

Fig. 3. Simulation and verification of the prediction results for the growth of *L monocytogenes* in ground beef samples with various fat contents after 7 and 14 days of storage in air at 11°C.

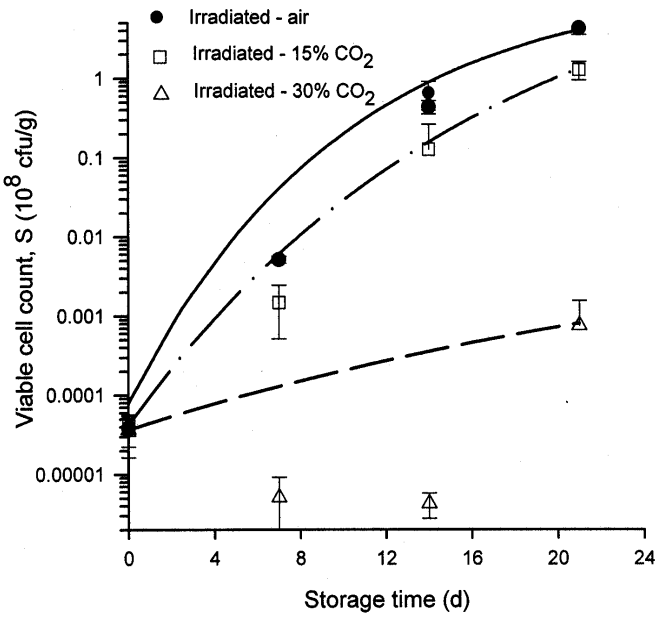

Fig. 4. Determination of coefficients μ_0 and D of Eq. 6 of growth of *L. monocytogenes* after irradiation (1.75 kGy) and storage at 11°C of beef 20 maintained in air, 15% and 30% CO_2. Data points represent averages of nine determinations. Vertical bars indicate 95% confidence limits. The lines represent the best fit for the corresponding data.

Table 3. Values of the specific growth rate (μ_0) and the decay coefficient (D) in Eq. 6 for growth of *L. monocytogenes* in ground beef of various fat content after irradiation at 1.75kGy and storage at 11°C in air and modified atmosphere.

Fat content (%)	Atmosphere	Storage temperature 11°C	
		μ_0	D
17.8	Air	1.25	0.10
	15% CO_2	0.97	0.07
	30% CO_2	0.50	0.02
19.8	Air	1.24	0.10
	15% CO_2	0.87	0.06
	30% CO_2	0.20	0.03
30.2	Air	0.63	0.07
	15% CO_2	0.39	0.04
	30% CO_2	0.03	0.01

factor of 100. The symbols on the graphs represent the average of nine measurements and the vertical bars show the 95% confidence limits. The three lines correspond to the three different package atmospheres and they are plotted as the best fit lines using Eq. 6. The plotted lines show a reasonable agreement with the measured growth values. A discrepancy between the experimental and prediction results has been observed for samples stored at 30% CO_2. This was a typical situation in a number of simulations for 30% CO_2 atmosphere. The logistic equation (Eq. 6) did not predict well the lag phase of the bacterial growth curve, however, both the exponential and stationary phases were predicted reasonably well. The coefficients μ_0 and D for this set of experiments and for the samples of three fat content are given in Table 3.

Figure 5 shows the simulation results for the growth of listeriae for irradiated and non-irradiated beef of 30.2% fat content stored at 11°C in air and in 30% CO_2 atmosphere. As a result of irradiation, the initial cell count of the irradiated samples with respect to non-irradiated product was decreased by a factor of 100. The effect of irradiation is visible in both storage conditions (air and modified atmosphere). Initially, the slope of the growth curve for the non-irradiated product stored in the air remains more steep than the slope of the curve for the irradiated samples, but after 3 to 4 days of storage it begins to level off reaching the stationary phase while the growth of listeriae on irradiated samples is still in the exponential phase. When non-irradiated and irradiated samples are stored in 30% CO_2, the slope of the simulated growth for non-irradiated product is more steep in comparison to irradiated ground beef.

CONCLUSIONS

The developed logistic equation predicted reasonably well the growth of *L. monocytogenes* for two temperatures, with and without MAP, and with and without application of irradiation.

Simulated growth patterns of *L. monocytogenes* in ground beef of various fat contents exposed to similar storage conditions (air and 11°C) indicated a higher growth rate in

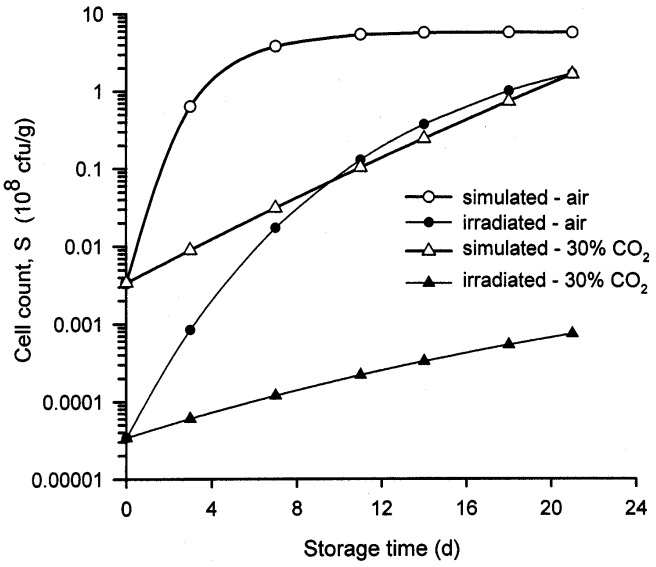

Fig. 5. Simulation experiments of the growth of listeriae for non-irradiated and irradiated ground beef stored at 11°C in air and in 30% CO_2 modified atmosphere.

samples containing 18% fat as compared to 30% fat during the first 7 to 14 days of storage.

Simulated growth patterns of listeriae in ground beef stored in 30% CO_2 atmosphere indicated a higher growth rate over a period of 21 days in non-irradiated samples as compared to irradiated samples. Listeriae growth in samples stored in air at the same temperature exhibited the same trend only in the first several (3 to 4) days of storage. After this period the growth of listeriae on non-irradiated beef reached a stationary phase while the growth of listeriae on the irradiated product was still in the exponential phase.

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REFERENCES

AOAC. 1995. Meat and meat products. In *Official Methods of Analysis of the Association of Food Chemists*, ed. K. Herlich. Arlington, VA: Association of Official Analytical Chemists.

Broughall, P.A., D.C. Anslow and D.C. Kilsby. 1983. Hazard analysis applied to microbial growth in foods: Development of mathematical models describing the effect of water activity. *Journal of Applied Microbiology* 5:101-110.

Buchanan, R.L. and J.G. Phillips. 1990. Response surface model for predicting the effects of temperature, pH, sodium chloride content, sodium nitrite concentration and atmosphere on the growth of *Listeria monocytogenes*. *Journal of Food Protection* 53:370-376.

Buchanan, R.L., H.G. Stahl and R.C. Whiting. 1989. Effects and interactions of temperature, pH, atmosphere, sodium chloride, and sodium nitrite of growth of *Listeria monocytogenes* in bovine milk phagocytes. *Applied and Environmental Microbiology* 54:364-370.

Chakraborty, T. and W. Goebel. 1988. Recent developments in the study of virulence in *Listeria monocytogenes*. *Microbiology and Immunology* 138:41-58.

Chawla, C.S., H. Chen and C.W. Donnelly. 1996. Mathematically modelling the repair of heat-injured *Listeria monocytogenes* as affected by temperature, pH, and salt concentration. *International Journal of Food Microbiology* 30:231-242.

Diehl. J.F. 1990. *Safety of Irradiated Foods*. New York, NY: Marcel Dekker Inc.

McMeekin, T.A. and T. Ross. 1996. Shelf-life prediction: Status and future possibilities. *International Journal of Food Microbiology* 33:65-83.

McMeekin, T.A., J. Olley, T. Ross and D.A. Ratkowsky. 1993. *Predictive Microbiology: Theory and Application*. New York, NY: John Wiley.

Miller, A. and W.L. McLaughlin. 1981. Evaluation of radiochromic dye films and other plastic dose meters under radiation processing conditions. In *High-Dose Measurements in Industrial Radiation Processing*. Technical Report 205:119-138. Vienna, Austria: International Atomic Energy Agency.

Ozilgen, M. 1998. *Food Process Modeling and Control: Chemical Engineering Applications*, vol. 12. Amsterdam, The Netherlands: Gordon and Breach Science Publishers.

Palumbo, S.A., A.C. Williams, R.L. Buchanan and J.G. Phillips. 1991. Model for the aerobic growth of *Aeromonas hydrophila K144*. *Journal of Food Protection* 54:429-435.

SigmaPlot. 1997. *User's Manual. SigmaPlot 4.0 for Windows*. San Rafael, CA: Jandel Scientific Software.

Whiting, R.C. and R.L. Buchanan. 1994. Microbial modelling. *Food Technology* 48:113-119.

Sudarmadji, S. and W.M. Urbain. 1972. Flavor sensitivity of selected animal protein foods to gamma radiation. *Journal of Food Science* 37:671-672.

Zwietering, M.H., J.T. De Koos, B.E. Hasenack, J.C. De Wit and K. van't Reit. 1991. Modelling of bacterial growth as a function of temperature. *Applied and Environmental Microbiology* 57:1875-1881.