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Modelling the effects of modified atmosphere on *Salmonella typhimurium* in packaged meat during storage in the refrigerator and at 12 ºC

Osman ERKMEN*, Aykut BARAZI

Department of Food Engineering University of Gaziantep Faculty of Engineering, 27310 Gaziantep Turkey

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Summary - The effects of storage atmosphere and temperatures on *Salmonella typhimurium* and aerobic plate count (APC) in meat were studied. Experimental results were analysed by non-linear regression of modified-Gompertz and logistic equations to generate "best fit". In the absence of CO_2 in package (with air and 40% O_2 + 60% N_2), in the refrigerator and at 12 ºC, *S. typhimurium* had higher growth rate and reached to higher number, whereas the presence of CO_2 from 40 to 100% reduced the number of *S. typhimurium* by 0.2 to 1.6 logs unit in the refrigerator. CO_2 dissolves in water to form carbonic acid which reduces pH of meat and bacterial cytoplasm. This would be reduced the bacteria or inhibited their growth. The effects of the modified atmospheres on microorganisms in meat seems to be responsible for the data obtained, together with a probable contribution from pH which in turns is likely to be influenced by the gas atmosphere. It is known that CO_2 has a bacteriostatic effect. Parameters of non-linear modified-Gompertz and logistic models of the *S. typhimurium* and APC in meat stored at various atmospheres were matched in a satisfactory way. Both the modified-Gompertz and logistic models showed good fit to all curves as assessed using the root mean square error and the correlation coefficient between the experimental and predicted values.

Key words: modified atmosphere, *Salmonella typhimurium*, modified-Gompertz model, logistic model.

INTRODUCTION

Salmonella typhimurium is one of the most common pathogens associated with foodborne illness. Salmonellosis, the illness caused by this bacterium, is reported to cause illnesses in approximately 40000 people in the United States annually (Juneja *et al*., 2007). Red meat and poultry are good sources of *Salmonella*. Food products from these industries are the most vulnerable foods for the growth of *Salmonella* (Ray, 1996; Juneja *et al*., 2007).

An alternative to time consuming and expensive investigations can be the microbiological prediction. Mathematical models are mainly used for predicting the growth and death of microorganisms. They include the effect of factors such as temperature, a_w, pH, nitrite content, gaseous atmosphere, the content of organic acids or other preserving methods. The process of modelling usually begins with the primary models which are mathematical formulas describing microorganisms growth curves. The microbial growth may be expressed by a total plate count, toxin production, the level of substrates or the level of metabolites (McMeeken *et al*., 1993; Walker and Jones, 1994). Models can allow to predict the changes in time of the number of microorganisms and obtaining information about the number of log cycles of growth (log CFU g^{-1}), the time required to reach the maximum growth rate (h); the specific growth rate (h^{-1}), the maximum specific growth rate (log CFU g^{-1} h^{-1}), the lag phase duration (h), the maximum population density (log CFU g^{-1}) and the others (McMeekin *et al*., 1993; Walker and Jones, 1994; Pin *et al*., 2000; Pernia *et al*., 2005; Gallo *et al*., 2006; Gil *et al*., 2006; Kajak and Kołozyn-Krajewska, 2006; Chowdhury *et al*., 2007). The use of mathematical models to describe microorganisms' behaviour is a helpful tool to improve food safety (Zwietering *et al.*, 1990; Buchanan, 1993; Bozkurt and Erkmen, 1999). Generally, predictive models are built on the basis of data obtained from experiments run to predict the specific growth or inactivation rate, and especially lag phase duration or the phase of disappearance (McMeekin *et al.*, 1992; Palumbo *et al.*, 1992; Buchanan, 1993; Muarmans *et al.*, 1993; Giannuzzi, 1998; Bozkurt and Erkmen, 1999). At present, Gompertz equation has become the most widely used model to describe microbial growth or inactivation (Giannuzzi, 1998; Bozkurt and Erkmen, 1999).

Temperature and oxygen are the major factor on meat deteriorative reactions, especially, for microbial spoilage since specific growth rate and lag phase duration are highly temperature dependent (Giannuzzi, 1998). Hurdle technology, a multifactor procedure, can be employed to accomplish food preservation, where each factor contributes to the stability and safety of the food product (Gallo *et al.*, 2006). Modified atmosphere packaging (MAP) is the substitution of the ambient air in a package with

* Corresponding author. Phone: +90342 3172313,
Fax: +90342 3601105, E-mail: erkmen@gantep.edu.tr

another gas (commonly carbon dioxide, oxygen and nitrogen). MAP and vacuum packaging (this system involves operating pump to extract air from plastic bags before they are sealed) have been used to improve the shelf-life of meat and poultry. MAP offers several unique advantages for retaining the desirable market quality of products (Bodnaruk and Draughon, 1998; Rao and Sachindra, 2002; Skandamis *et al*., 2002). The objective of the present work was to primary modelling and describing (sigmoid) growth or death of *S. typhimurium* and the combined effect of storage temperature (in the refrigerator and at 12 ºC) and various atmospheres on *S. typhimurium* and aerobic plate count (APC) in packaged meat. Different modified atmosphere were chosen to indicate behavior of *S. typhimurium* at these conditions.

MATERIALS AND METHODS

Culture. *Salmonella typhimurium* KUEN 1357 was obtained from Microorganism's Culture Collection Research and Applied Centre, Faculty of Medicine, University of Istanbul, Turkey. The stock cultures were maintained on brain heart infusion agar (BHIA; Difco, Detroit) slants and stored in the refrigerator.

Preparation and packaging of the meat. The *S. typhimurium* culture for experiments was subcultured twice from stock culture by inoculating in 10 ml of brain heart infusion broth (BHIB; Difco), and incubated at 35 ºC for 18 h, to obtain cells in the stationary phase. About 200 ml of 24 h *S. typhimurium* culture was added into a sterile bottle containing 1800 ml of 0.1% peptone water (pH 6.5) to obtain a culture suspension used to contaminate *S. typhimurium* with fresh meat. Fresh beef meat was purchased from a local butcher, transported to laboratory within 15 min in bag at 0 ºC. Fresh beef meat with 14% fat was cut to small dishes (diameter of about 0.5 x 0.5 cm) on a clean cutting board under aseptic condition. About 2.5 kg of meat dishes were added into bottle containing 1800 ml culture to contaminate *S. typhimurium*, allowed for 2 min and then the contents of bottle were filtered through a sterile cheese cloth under aseptic conditions. About 30 g of meat dishes was place into sterile polyethylene/polyamide film (PE/PE; Polinas Plastik Sanayii ve Ticareti A.S., Manisa, Turkey) packages (20 x 12 cm) under aseptic condition. According to the manufacturer's data, PE/PA film has permeability at 25 ºC; oxygen: 160 cm^3 m^{-2} day^{-1} and water vapour: 8.5 g m^{-2} day^{-1}. The packages containing the meat dishes were packed with double sealing with heat in seven gas atmospheres in vacuum packing machine (La Minerra, D.V.P. Vacuum Technology, s.r.l., Italy). The CO_2, O_2 and N_2 were mixed in the various combinations using Witt-Gas mixer (GmbH and Co Kg, Deutschland) and the gases were flushed into vacuum machine during packaging. Following seven gas atmosphere were used in packaging of meat: (1) air, (2) vacuum, (3) 100% CO_2, (4) 60% CO_2 + 40% N_2, (5) 60% CO_2 + 40% O_2, (6) 40% CO_2 + 30% O_2 + 40% N_2 and (7) 40% O_2 + 60% N_2. Control meat was also packed from non-contaminated meat dishes (without *S. typhimurium* contamination). Twenty four packages were prepared for each type of gas mixture, vacuum and control packaging.

Twenty four packages from each of gas atmospheres and control were stored in the refrigerator (temperature changed from 4 to 7.5 ºC during storage) and at 12 ± 1 ºC in cold rooms with a relative humidity of 85-90%. *S. typhimurium* and APC were determined at the sampling time.

Microbiological analysis. Two packages for microbiological analysis were taken at the following sampling time: 0, 2, 5, 8, 10 and 12 days of storage. Twenty-five gram portion of meat sample was homogenised in a sterile Warring blender (Torrington, CT, US) containing 225 ml of 0.1% sterile peptone water. Homogenised meat samples were serially diluted using 0.1% sterile peptone water. *S. typhimurium* and APC were counted by spread plating 0.5 ml of diluted (or not diluted) samples on duplicate plates of bismuth sulphide agar (BSA; Merck, Germany) and BHIA respectively. The plates were incubated at 35 ºC for 24 and 48 h, after which all the characteristic visible colonies on BHIA and flat or only slightly raised green colonies on BSA were counted (Erkmen, 2007). The average number of colonies from the duplicate plates was then recorded for each sample. Typical *S. typhimurium* colonies (3 per plate) were streaked onto nutrient agar (Difco) slants for confirmatory tests including gram staining; catalase and oxidase tests; characteristic growth on triple sugar iron agar (Difco), urea agar and citrate agar (Difco) slants; and motility test (Erkmen, 2007). The initial numbers of *S. typhimurium* and APC in contaminated meat were about 4.0 x 10^2 and 7.8 x 10^3 colony forming units (CFU) g^{-1} of meat respectively. Initial APC in fresh meat was about 1.4 x 10^3 CFU g^{-1}.

The whole experimental procedure was performed twice and duplicate samples were taken at each sampling time. The number of *S. typhimurium* was transformed to $\log_{10}$ values and expressed as CFU (N, after treatment) and CFU_o (N_0, initial).

The meat was also tested by selective enrichment method to ensure that the fresh meat was free from *S. typhimurium* (Erkmen, 2007). For this, 25 g of meat was homogenised in 225 ml of sterile lactose broth and incubated at 35 ºC for 24 h and then streak plated onto BSA, which was followed by incubation at 35 ºC for 24 h. The fresh meat showed no *S. typhimurium*, confirming that the samples were not cross-contaminated.

Modelling of microbial growth. One of the recommended models for describing microbial growth is modified-Gompertz equation (Zwietering *et al*., 1990)

$$\text{Log } N = \log N_o + a \exp\{-\exp[-b(t-m)]\}$$

where N is log CFU g^{-1} of cell number at time t, log N_0 is the asymptotic log counts as time decreases indefinitely, approximately equivalent to the log of the initial level of bacteria (log CFU g^{-1}, a is the count increment as time increases indefinitely, that is number of log cycles of growth (log CFU g^{-1}), m is the time required to reach the maximum growth rate (day), and b is the maximum specific growth rate (log CFU g^{-1} day^{-1}).

From these parameters were obtained:
the maximum specific growth rate (or inactivation rate):
$\mu = b \cdot a/e$ (log CFU g^{-1} day^{-1}), where e = 2.7182;
the lag phase duration: LPD = m - (1/b) (day);
and the maximum population density; MPD = log N_0 + a (log CFU g^{-1}).

A logistic model (symmetrical curve) was also applied in order to test its suitability

$$\text{Log } N = \log N_0 + a/[1 + \exp(d\text{-}c\cdot t)]$$

where Log N and log N_0 have the same meaning as above, d is a dimensionless parameter, and c is the specific growth rate at the half-time value of the exponential phase (day^{-1}).

The models were applied to every combination of gas atmosphere and storage temperature where microbial growth occurred.

Statistical analysis. In modelling, the equations were fitted to experimental data by nonlinear regression using SigmaPlot 2002 for windows v.8.0 (SPSS Inc.).

Analysis of variance was applied to the parameters to determine statistical differences between different treatments and was compared using the Student t-test for significant effects identified in the SigmaPlot 2002 ($p < 0.05$).

To compare the performance of different models, the correlation coefficient (R^2) and the root mean square error (RMSE) between experimental data and those predicted using different models were obtained from plot using SigmaPlot 2002 for windows version 8.0 (SPSS Inc.). Additionally, the accuracy of models can be assessed graphically by plotting the predicted values from both models versus the observed values. A simple linear regression was fitted to the points and the intercept, the R^2 was obtained.

RESULTS AND DISCUSSION

All the experimental data obtained from different gas atmosphere conditions of meat were fitted into two growth models (modified-Gompertz and logistic) to detect the effect of temperature and storage atmosphere on *S. typhimurium* and APC in packaged meat. The application of the modified-Gompertz and logistic models for description of bacterial behaviour is widely used and described in literature (Zwietering *et al*., 1990; van Impe *et al*., 1992; Buchanan, 1993; Giannuzzi, 1998; Erkmen, 2001, 2003; Gallo *et al*., 2006; Chowdhury *et al*., 2007).

The modified-Gompertz and logistic models were applied to *S. typhimurium* and APC in which microbial growth was detected and allowed the prediction of the entire curve. Figure 1 shows the fitting of modified-Gompertz model to *S. typhimurium* and APC, respectively, during storage in the refrigerator and at 12 °C in meat packaged with various atmospheric conditions and Fig. 2 shows the fitting of logistic model to *S. typhimurium* and APC respectively. In the experiments, the initial number of *S. typhimurium* and APC were 2.60 and 3.25 log CFU g^{-1}, respectively. The derived parameters from modified-Gompertz model, as the maximum specific growth rate (μ), lag phase duration (LPD) and maximum population density (MPD) in the refrigerator and at 12 °C are given in Tables 1 and 2 respectively. It is quite clear visible that with the

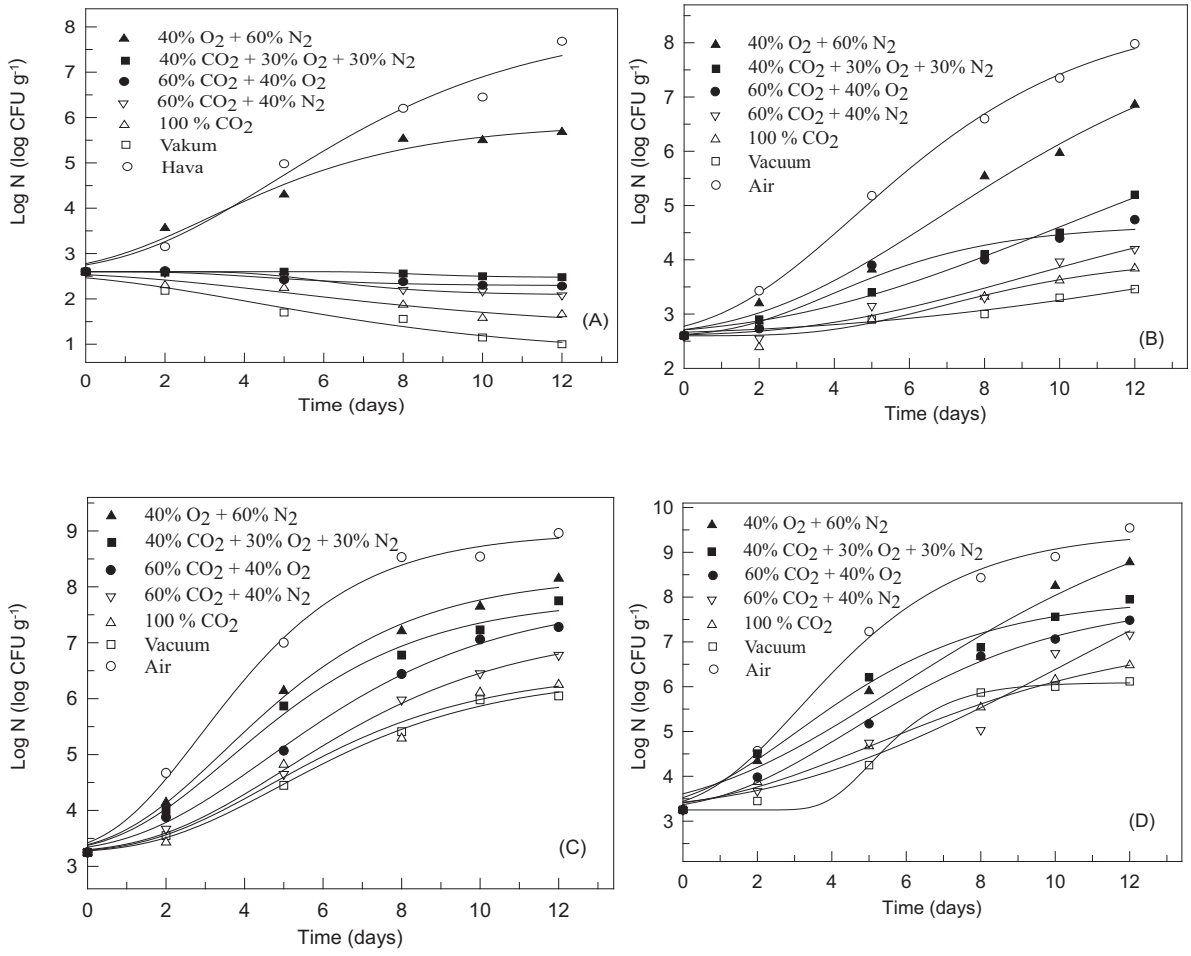

FIG. 1 - Fitting of modified-Gompertz (curves) model to experimental data point of *Salmonella typhimurium* and APC growth in packaged meat at various atmosphere in the refrigerator (A) and 12 °C (B) for *S. typhimurium*, and in the refrigerator (C) and 12 °C (D) for APC.

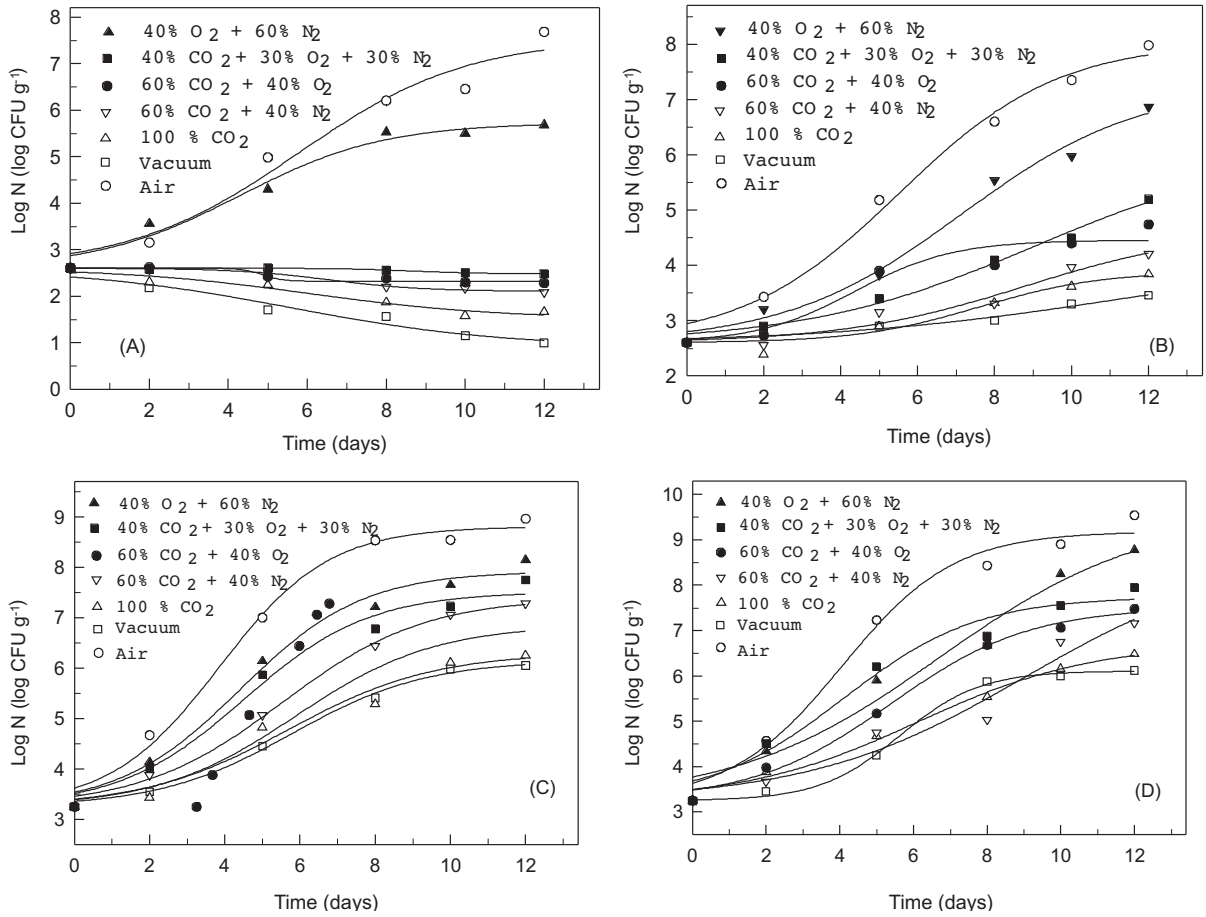

FIG. 2 - Fitting of logistic (curves) model to experimental data point of *Salmonella typhimurium* and APC growth in packaged meat at various atmosphere in the refrigerator (A) and 12 ºC (B) for *S. typhimurium*, and in the refrigerator (C) and 12 ºC (D) for APC.

increase of the storage temperature of meat the m increases for both *S. typhimurium* and APC. The packaging with vacuum and 40% O_2 + 60% N_2 and storing in the refrigerator, *S. typhimurium* grew. The MPD for *S. typhimurium* with air and 40%O_2 + 60% N_2 packaging were 8.12 and 5.67 log CFU g^{-1}, respectively, in temperature and 8.61 and 8.62 log CFU g^{-1}, respectively, at 12 ºC. Higher values for air and 40%O_2 + 60% N_2 (without CO_2 and vacuum packaging) were also obtained for APC. On the other hand higher MPD values were also obtained with the modified atmosphere packaging containing oxygen for *S. typhimurium* and APC (Tables 1 and 2) than the packaging with vacuum and containing CO_2. Storage temperature and atmosphere are responsible in a significant way for the kinetics of bacterial growth in packaging meat (Skandamis *et al.*, 2002; Zardetto, 2005). All values of the calculated parameters from modified-Gompertz values (Tables 1 and 2) are statistically significant ($p < 0.05$).

There was a decline trend of *S. typhimurium* in meat stored in the refrigerator (Fig. 1A) while it was grown during the storage at 12 ºC (Fig. 1B) in VP and packages containing CO_2. On the other hand *S. typhimurium* was grown in meat packaged with air and 40% O_2 (without CO_2) during storage in the refrigerated and at 12 ºC. Growth of aerobic bacteria were observed in meat packaged with and without CO_2 during storage at both refrigerator temperature and 12 ºC but the level of growth was lower in CO_2 packaged meat than without CO_2 packaging (Fig. 1C and 1D). The inactivation rate (μ) of *S. typhimurium* in CO_2 packaged meat was ranged from 0.02 to 0.16 (log CFU g^{-1} day^{-1}) while the growth rate was 0.55 and 0.41 (log CFU g^{-1} day^{-1}) in meat packaged with air and 40% O_2 respectively.

The correlation between observed and estimated data for the behavior of *S. typhimurium* and APC in packaged meat at various atmospheres calculated with modified-Gompertz and logistic models are given in figures 3 and 4 respectively. Both the modified-Gompertz and logistic models showed good fit to all the curves as assessed using the root mean square error (RMSE) and the correlation coefficient (R^2) between the experimental and predicted values but the modified-Gompertz model best fit ($R^2 > 0.98$) than the logistic model ($R^2 > 0.96$). Additionally, good agreement between experimental data and predicted values of *S. typhimurium* and APC were obtained with $R^2 \geq 0.98$ for the modified-Gompertz model (Fig. 3A-3D) in the refrigerator and at 12 ºC. Thus we concluded that these equations allowed a good prediction of the effects of various atmospheric conditions on *S. typhimurium* and APC in packaged meat because of the closer fit.

High R^2 values result from a small number of degree of freedom and in such a situation a very good matching of a model to the data is not surprising (McMeekin and Ross, 2002). It seems that the growth parameters of microorganisms occurring in packaged meats with various atmosphere conditions were estimated relatively well (Tables 1

TABLE 1 - Gompertz and logistic parameters for *Salmonella typhimurium* growth the refrigerator and at 12 °C in modified atmosphere packaged meat[a]

Parameters	Refrigerator						
Gompertz	Air	Vacuum	100% CO_2	60% CO_2 + 40% N_2	60% CO_2 + 40% O_2	40% CO_2 + 30 O_2 + 40% N_2	40% O_2 + 60% N_2
log N_0	2.60						
a	5.52 ± 0.98	−1.85 ± 0.44	−1.27 ± 0.51	0.30 ± 0.04	−0.12 ± 0.03	0.13 ± 0.02	4.27 ± 0.33
b	0.27 ± 0.93	0.23 ± 0.10	0.21 ± 0.13	0.56 ± 0.14	0.50 ± 0.31	0.80 ± 0.49	0.34 ± 0.10
m	4.81 ± 0.98	4.23 ± 49	5.11 ± 0.51	5.83 ± 0.33	4.26 ± 0.71	8.17 ± 0.37	3.13 ± 0.62
R^2	0.98	0.96	0.95	0.99	0.96	0.97	0.98
RMSE	0.36	0.15	0.12	0.03	0.04	0.01	0.25
m	0.55	0.16	0.10	0.06	0.02	0.04	0.41
LPD	1.11	−0.12	0.35	4.04	2.26	6.92	0.19
MPD	8.12	0.75	1.33	2.90	2.43	2.73	5.67
Logistic							
log N_0	2.60						
a	4.94 ± 0.70	−1.70 ± 0.34	−1.09 ± 0.22	−0.50 ± 0.03	−0.28 ± 0.03	−0.12 ± 0.02	3.12 ± 0.24
b	2.69 ± 0.73	2.08 ± 0.56	2.52 ± 0.77	5.44 ± 0.14	19.3 ± 0.74	9.60 ± 0.05	2.36 ± 0.60
m	0.47 ± 0.16	0.37 ± 0.15	0.42 ± 0.18	0.83 ± 0.18	4.00 ± 0.38	1.11 ± 0.50	0.55 ± 0.16
R^2	0.97	0.95	0.94	0.99	0.94	0.97	0.97
RMSE	0.46	0.17	0.12	0.03	0.04	0.01	0.26
Parameters	12 °C						
Gompertz	Air	Vacuum	100% CO_2	60% CO_2 + 40% N_2	60% CO_2 + 40% O_2	40% CO_2 + 30 O_2 + 40% N_2	40% O_2 + 60% N_2
log N_0	2.60						
a	6.01 ± 0.35	4.39 ± 0.51	1.47 ± 0.46	2.90 ± 0.39	2.06 ± 0.32	5.47 ± 0.21	6.02 ± 0.15
b	0.28 ± 0.33	0.12 ± 0.02	0.38 ± 0.20	0.18 ± 0.14	0.38 ± 0.19	0.14 ± 0.04	0.20 ± 0.06
m	4.95 ± 0.32	18.10 ± 0.52	6.80 ± 0.72	8.98 ± 0.60	4.38 ± 0.89	10.03 ± 0.30	6.89 ± 0.15
R^2	0.99	0.97	0.97	0.96	0.96	0.99	0.99
RMSE	0.14	0.07	0.13	0.17	0.26	0.10	0.23
m	0.62	0.19	0.21	0.19	0.14	0.28	0.44
LPD	1.38	9.67	4.17	3.42	1.75	2.89	1.89
MPD	8.61	6.99	4.07	5.50	4.66	8.07	8.62
Logistic							
log N_0	2.60						
a	5.43 ± 0.38	1.58 ± 0.21	1.28 ± 0.25	2.04 ± 0.79	1.85 ± 0.20	3.48 ± 0.80	4.66 ± 0.56
b	2.70 ± 0.41	2.99 ± 0.55	4.79 ± 0.08	3.71 ± 0.08	3.40 ± 0.90	3.03 ± 0.31	3.81 ± 0.47
m	0.49 ± 0.09	0.27 ± 0.11	0.63 ± 0.31	0.42 ± 0.20	0.78 ± 0.43	0.34 ± 0.07	0.44 ± 0.09
R^2	0.99	0.97	0.96	0.96	0.94	0.99	0.99
RMSE	0.28	0.07	0.15	0.19	0.29	0.17	0.24

[a] $p < 0.05$.

N_0 = the asymptotic log counts (log CFU g^{-1}); a = the count increment as time increases indefinitely (log CFU g^{-1}); b = the maximum specific growth rate (log CFU g^{-1} day^{-1}); m = the time required to reach the maximum growth rate (day); m = maximum specific growth rate (log CFU g^{-1} day^{-1}), e = 2.7182; LPD = the lag phase duration (h); MPD = the maximum population density (log CFU g^{-1}).

TABLE 2 - Gompertz and logistic parameters for aerobic plate count the refrigerator and at 12 ºC in modified atmosphere packaged meat[a]

Parameters	Refrigerator						
Gompertz	Air	Vacuum	100% CO_2	60% CO_2 + 40% N_2	60% CO_2 + 40% O_2	40% CO_2 + 30 O_2 + 40% N_2	40% O_2 + 60% N_2
log N_0	3.25						
a	5.73 ± 0.90	−1.85 ± 0.18	−1.27 ± 0.47	−4.02 ± 0.19	4.57 ± 0.23	4.55 ± 0.26	4.67 ± 0.22
b	0.43 ± 0.05	0.23 ± 0.04	0.21 ± 0.11	0.30 ± 0.03	0.30 ± 0.03	0.36 ± 0.06	0.58 ± 0.05
m	2.89 ± 0.20	4.23 ± 0.29	5.11 ± 0.75	5.01 ± 0.24	3.27 ± 0.27	3.20 ± 0.33	3.13 ± 0.26
R^2	0.99	0.99	0.99	0.99	0.99	0.99	0.99
RMSE	0.19	0.08	0.24	0.08	0.10	0.19	0.17
m	0.91	0.16	0.10	0.44	0.44	0.60	0.99
LPD	0.56	−0.12	0.35	1.68	0.94	0.42	1.41
MPD	8.98	1.40	1.98	−0.77	7.82	7.80	7.90
Logistic							
log N_0	3.25						
a	6.22 ± 0.21	2.84 ± 0.12	4.00 ± 0.39	8.89 ± 0.12	4.70 ± 0.17	4.74 ± 0.26	7.43 ± 0.25
b	0.40 ± 0.43	0.83 ± 0.35	0.23 ± 0.87	0.13 ± 0.26	0.29 ± 1.30	0.34 ± 0.60	0.19 ± 0.54
m	3.14 ± 0.11	5.05 ± 0.07	5.16 ± 0.19	10.31 ± 0.05	4.42 ± 0.06	3.06 ± 0.15	5.74 ± 0.13
R^2	0.99	0.99	0.98	0.96	0.99	0.98	0.98
RMSE	0.25	0.12	0.12	0.42	0.14	0.28	0.41
Parameters	12 ºC						
Gompertz	Air	Vacuum	100% CO_2	60% CO_2 + 40% N_2	60% CO_2 + 40% O_2	40% CO_2 + 30 O_2 + 40% N_2	40% O_2 + 60% N_2
log N_0	3.25						
a	6.22 ± 0.28	2.84 ± 0.11	4.00 ± 0.45	6.19 ± 0.90	4.70 ± 0.29	4.74 ± 0.37	7.43 ± 0.22
b	0.40 ± 0.06	0.83 ± 0.25	0.23 ± 0.04	0.13 ± 0.11	0.29 ± 0.04	0.36 ± 0.08	0.19 ± 0.08
m	3.14 ± 0.27	5.05 ± 0.15	5.16 ± 0.67	10.31 ± 0.86	4.42 ± 0.34	3.36 ± 0.47	5.74 ± 0.92
R^2	0.99	0.99	0.99	0.99	0.99	0.98	0.98
RMSE	0.25	0.12	0.12	0.42	0.14	0.28	0.41
m	0.91	0.87	0.34	0.30	0.50	0.59	0.52
LPD	0.64	3.85	0.81	2.62	0.97	0.58	0.48
MPD	9.47	6.09	7.25	9.44	7.95	7.99	10.68
Logistic							
log N_0	3.25						
a	5.93 ± 0.30	2.86 ± 0.06	3.47 ± 0.32	5.47 ± 0.60	4.27 ± 0.21	4.50 ± 0.35	6.33 ± 1.30
b	2.67 ± 0.56	5.15 ± 0.60	2.57 ± 0.35	3.07 ± 0.65	2.85 ± 0.35	2.24 ± 0.59	2.41 ± 0.48
m	0.66 ± 0.14	0.92 ± 0.12	0.42 ± 0.08	0.34 ± 0.15	0.53 ± 0.08	0.55 ± 0.16	0.36 ± 0.12
R^2	0.99	0.99	0.99	0.96	0.99	0.97	0.97
RMSE	0.39	0.08	0.17	0.43	0.17	0.39	0.49

[a] $p < 0.05$. The legends of abbreviations was indicated on Table 1.

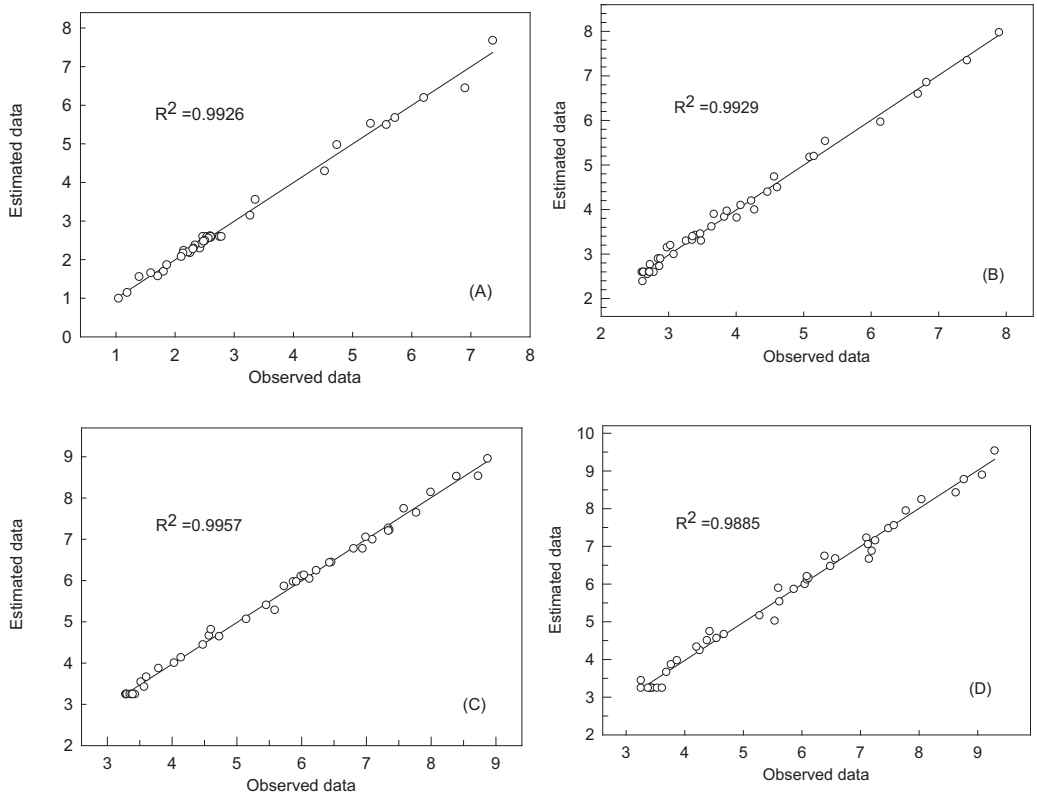

FIG. 3 - Correlation between observed and estimated data for the behavior of *Salmonella typhimurium* and APC in packaged meat at various atmospheres calculated with Gompertz model in the refrigerator (A) and 12 ºC (B) for *S. typhimurium*, and in the refrigerator (C) and 12 ºC (D) for APC.

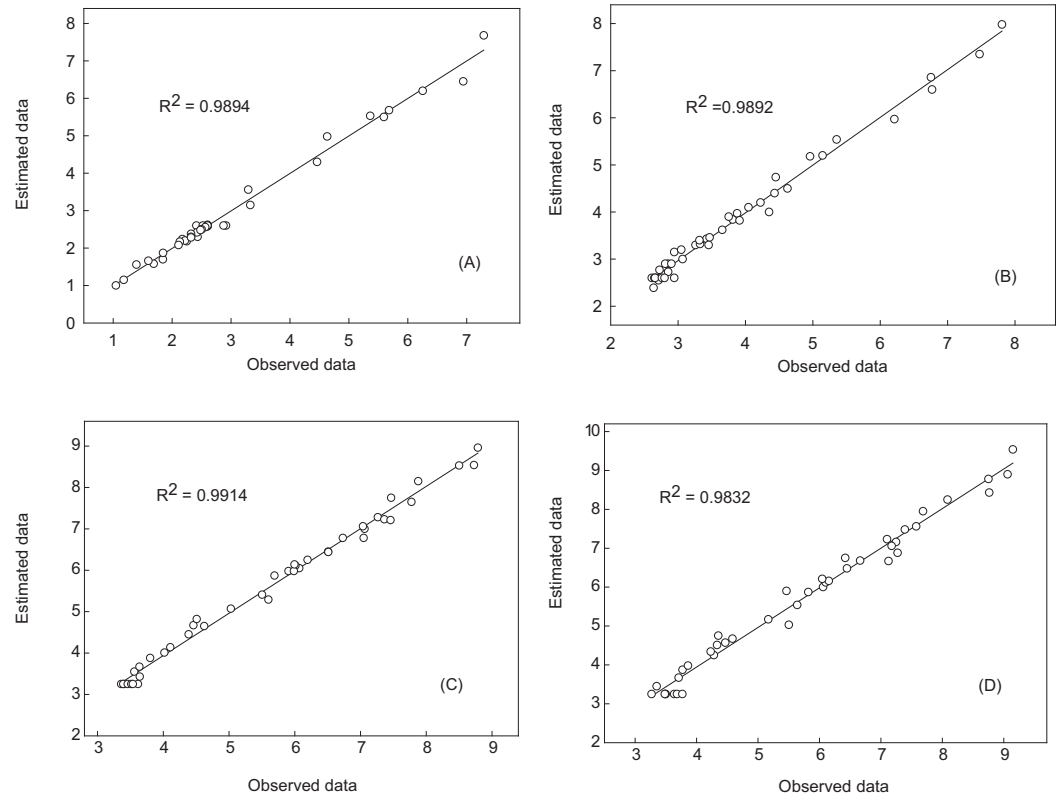

FIG. 4 - Correlation between observed and estimated data for the behavior of *Salmonella typhimurium* and APC in packaged meat at various atmospheres calculated with logical model in the refrigerator (A) and 12 ºC (B) for *S. typhimurium*, and in the refrigerator (C) and 12 ºC (D) for APC.

and 2). Most of them are statistically significant at the level of p value = 0.05. It seems that quite clear differences occur among parameters of growth characteristics of *S. typhimurium* and APC in at various atmospheric conditions ($p < 0.05$). With the level of CO_2 within the range fro 40 to 100% inhibitory effect on bacterial growth was observed. It was also reported in the literature that the presence of CO_2 in storage atmosphere inhibits the growth of bacteria (Daniels *et al*., 1985; Rao and Sachindra, 2002). In the absence of CO_2 in package (with air and 40% O_2 + 60% N_2), in the refrigerator and at 12 ^oC, *S. typhimurium* grew immediately (from 4 to 5 log units were increased with air and 40% O_2 + 60% N_2), whereas the presence of CO_2 at 100 and 60% reduced the number of *S. typhimurium* by 0.2 to 1.6 log units from initial number (2.60 log CFU g^{-1} in the refrigerator. The bacteriostatic activity of CO_2 was highly dependent on the initial concentration of CO_2 in packages. A lower population density of 1.0 log CFU g^{-1} was reached in the presence of 100% CO_2 in packaged meat in the refrigerator after 12 days. Depression of MPD's in response to an increase of CO_2 amount or a reduction of storage temperature was observed. An increment of CO_2 level from 40 to 100% in package, in the refrigerator, reduced the MPD ($p < 0.05$), whereas a change in temperature from 4 to 12 ^oC were increased the MPD ($p < 0.05$). CO_2 was required to cause a significant reduction ($p < 0.05$) of *S. typhimurium* and APC in packaged meat. CO_2 is the most important gas in the gas mixture and bacteriostatic (Daniels *et al*., 1985). Hintlian and Hotchkiss (1987) reported that the three pathogens (*Staphylococcus aureus*, *Salmonella typhimurium* and *Clostridium perfringens*) were unable to grow in the MA packaging of cooked roast meat at 4.4 ^oC, probably due to the effect of temperature. Growth of *Listeria monocytogenes*, *Escherichia coli* O:157:H7, *Yersinia enterocolitica* and *Salmonella* spp. was not inhibited in ground meat packed in high CO_2/low CO_2 mixture and stored at 10 ^oC (Nissen *et al*., 2000). The use of MAP for pork meat at 4 ^oC showed a bacteriostatic effect on *Listeria monocytogenes* (López-Mendoza *et al.,* 2007).

Consequently, the combination of a reduced storage temperature and increased CO_2 level in package resulted a final number of *S. typhimurium* and APC lower than packaging with air after 12 days of storage in the refrigerator and at 12 ^oC. A reduction in the storage temperature from 4 to 12 ^oC produced an important change in the behavior of *S. typhimurium* and APC. In consequence, m and MPD values achieved were dependent on the immediate antimicrobial effect of CO_2 level, highly affected by storage temperature. These data shows the importance of temperature and gases at storage as a controlling factor for *S. typhimurium* and APC in packaged meat. It is possible to obtain an important reduction of *S. typhimurium* and APC in packaged meat with a combination of gases (CO_2, N_2 and O_2) and vacuum storage in the refrigerator. A drop in storage temperature showed a dramatic reduction of *S. typhimurium* population and allowed a level of cells after 12 days (1.0 log CFU g^{-1}) without re-growth of the bacterium. On the basis of data obtained in the experiment parameters of non-linear modified-Gompertz and logistic models of the *S. typhimurium* and APC in meat at various different atmospheres were matched in a satisfactory way. The presence of CO_2 at different level effected the inhibition of the number of microorganisms.

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