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Predictive modelling of *Escherichia coli* O157:H7: Inclusion of carbon dioxide as a fourth factor in a pre-existing model

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Abstract

Two models for *Escherichia coli* O157:H7 are compared, one with growth-controlling factors pH (4.5–7.0), temperature (10–30°C) and NaCl concentration (0.5–6.5%w/v) and the other with the same factors and ranges, but with the addition of carbon dioxide (CO_2; 10–80% v/v). Validation of the four-factor model, to include food packed in modified atmospheres containing CO_2, was not possible due to lack of published data. However, where CO_2 concentration was entered as 0%, only minor differences occurred between the predictions from the two models for the same conditions of pH, NaCl and temperature; consequently reliable, safe predictions using the four-factor model, with CO_2 concentration recorded as 0%, can be made for foods packed in air. At temperatures from 10 to 30°C, it was found that lower (10 and 20%) concentrations of CO_2 had little effect on lag times and growth rates, and higher concentrations still permitted growth of *E. coli* O157:H7 under a wide range of conditions of NaCl concentration, pH value and temperature, suggesting that the organism is relatively CO_2-tolerant. © 1997 Elsevier Science B.V.

Keywords: *Escherichia coli* O157:H7; Growth; Predictive model; Carbon dioxide; Food safety

1. Introduction

Carbon dioxide (CO_2) inhibits the growth of Gram negative aerobic spoilage organisms such as *Pseudomonas* and related species (Sutherland et al., 1977; Silliker and Wolfe, 1980; Stier et al., 1981). Exploitation of this has led to increasing use of modified atmosphere packaging (MAP) to extend the shelf life of perishable foods such as raw meat (Silliker et al., 1977; Newton et al., 1977; Enfors et al., 1979; Shay and Egan, 1987), poultry (Gardner et al., 1977; Bailey et al., 1979; Baker et al., 1985) and fish (Wang and Brown, 1983; Barnett et al., 1987). There is concern that MAP might suppress growth of spoilage microorganisms, but permit growth of food poisoning organisms, particularly those that grow at low temperatures, in a relatively non-competitive environment (Hintlian and Hotchkiss, 1986). Although the effect of CO_2 on growth of pathogens has been reported, including *Salmonella typhimurium* (Eklund and Jarmund, 1983; Gill and DeLacy,

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1991), *Listeria monocytogenes* (Marshall et al., 1992) *Bacillus cereus* (Molin, 1983), *Yersinia enterocolitica* (Eklund and Jarmund, 1983; Zee et al., 1984), *Staphylococcus aureus* (Molin, 1983) and *Escherichia coli* (Molin, 1983; Gill and DeLacy, 1991; Hao and Brackett, 1993), data are limited.

Predictive modelling of microbial growth responses is becoming accepted as a means of reducing the amount of challenge testing required to determine product safety, and three- and four-factor models exist for a range of pathogens including *S. aureus* (Sutherland et al., 1994), *E. coli* O157:H7 (Buchanan and Klawitter, 1992; Sutherland et al., 1995), *Y. enterocolitica* (Adams et al., 1991; Hudson, 1993; Sutherland and Bayliss, 1994), salmonellae (Gibson et al., 1988), *B. cereus* (Baker and Griffiths, 1993) and *L. monocytogenes* (Buchanan et al., 1989; Buchanan and Phillips, 1990; Cole et al., 1990).

The aim of this investigation is to extend the existing 3-factor (pH, temperature and sodium chloride) model for *E. coli* O157:H7 (Sutherland et al., 1995) to 4 factors by the inclusion of data systematically generated in varying concentrations of CO_2, NaCl, pH value and temperature and to compare predicted growth responses from the new model with those from the original 3-factor model for *E. coli* using data from the literature.

2. Materials and methods

2.1. Strains

Four strains of *E. coli* O:157 were used as a mixed strain inoculum: *E. coli* NCTC 12079, *E. coli* 204P (isolated from pork), *E. coli* W2-2 (isolated from poultry), and *E. coli* 505B (isolated from beef). The last three cultures were supplied by Dr M.P. Doyle, University of Georgia, Griffin, Georgia, USA.

2.2. Media

Tryptone soya broth (TSB; Unipath CM 129) was used for the growth of the *E. coli* strains with NaCl added to give final concentrations up to 6.5% (w/v). The pH values of broths were adjusted to between 4.0 and 7.0 inclusive using 1 M HCl. The broths were dispensed in 100 ml amounts and autoclaved at 121° for 15 min. Tryptone soya agar (TSA: Unipath CM 132) incubated at 37°C was used for determination of changes in viable numbers with time.

2.3. Inoculum

The strains were grown separately in TSB (pH 7.2, no added NaCl) and subcultured on three successive days. The third subculture was grown for 24 h at 37° to the previously determined stationary phase. Ten ml aliquots of broth containing similar numbers of each strain were mixed and diluted in TSB to approximately 10^5 cfu/ml.

2.4. Experimental procedure

Ten ml aliquots of TSB were aseptically withdrawn and the pH value measured but not adjusted. The remaining 90 ml of broth were inoculated with 1 ml of mixed strain inoculum prediluted to 10^5 cfu/ml.

Sterile membrane pads (Sartorius Ltd., Epsom, UK) of 47 cm diameter were aseptically placed in 60 cm diameter sterile plastic vented petri dishes (Bibby Sterilin Ltd, Stone, UK). To each pad was added 2 ml of the inoculated broth containing approximately 10^3–10^4/ml *E. coli*. Each petri dish containing an inoculated filter pad was individually packaged in a Dyno HPDE 150 ml tray (Dynopack Ltd., Reading, UK) with a top web of polyamide laminate (Suprovac 90; Kempner, Witham, UK) of water vapour permeability ca. 1.1 $g/m^2/d$ at 23°C, 85% RH and gas permeability: oxygen ca. 25, CO_2 ca. 90 and nitrogen ca. 6 $cm^3/m^2/d$ at 20°C and 50% RH. Packs were flushed with an atmosphere of 10, 20, 40, 60 or 80% CO_2, with a balance of nitrogen, using a Mecapac M500 modified atmosphere packaging machine (ECM Mecapac, Bagonet, France). The effectiveness of MAP can depend on the relative volumes of gas and food. In these experiments, the volume of gas was relatively large compared with that of the filter pad supporting the organisms, so gaseous conditions should be conducive to maximum inhibition of growth.

Immediately after gas packaging the atmosphere was checked using a Gowmac Gas Chromatograph Model 5292/202 (Gowmac, Gillingham, UK). The gas flushed filter pads were stored at a temperature of 4°C until transport to the laboratory in insulated boxes, a journey time of just over 1 h. At the

laboratory, one pack from each set of conditions was sampled initially and the remainder were incubated at the appropriate temperatures. At intervals during incubation, inoculated pads were sampled by opening the gas flushed pack, aseptically removing and macerating the entire pad in 90 ml 1/4 strength Ringer's solution, preparing decimal dilutions and plating 20 μl volumes on duplicate TSA plates. Plates were incubated for 24 h at 37°C, the mean count on duplicate plate counts was determined and the number of colony-forming units per ml (of suspension saturating the pad) was calculated.

2.5. Experimental design

Representative conditions from the following matrix (intended to cover most conditions encountered in foods) were selected:

Incubation temperature (°C)	10, 15, 20, 25, 30
pH	4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0
NaCl (%w/v)	0.5, 2.5, 4.5, 6.5
CO_2 (% v/v; balance nitrogen)	10, 20, 40, 60, 80

Fig. 1 shows the distribution of experimental data generated at different concentrations of CO_2. A total of 79 growth curves from this matrix were combined with the 53 used for the original *E. coli* O157:H7 model (Sutherland et al., 1995) and the resulting 132 curves used to construct the model.

2.6. Construction of the model

The growth responses at each set of conditions were expressed as $\log_{10}$ numbers of *E. coli* as a function of time. Growth curves were fitted to the data using the technique of Baranyi et al., (Baranyi et al., 1993; Program 2, McClure et al., 1993).

The second stage of modelling described the variation of the growth parameters μ_{max}, (maximum specific growth rate) λ, (lag time) and γ (maximum population density) as a function of growth conditions in different combinations of pH, NaCl, temperature and CO_2. To stabilise the variance of the response parameter, the natural logarithm was taken and expressed as a quadratic function of the controlling factors as shown below:

$$g = p_1 + p_2x_1 + p_3x_2 + p_4x_3 + p_5x_4 + p_6x_1x_2 + p_7x_1x_3 + p_8x_1x_4 + p_9x_2x_3 + p_{10}x_2x_4 + p_{11}x_3x_4 + p_{12}x_1^2 + p_{13}x_2^2 + p_{14}x_3^2 + p_{15}x_4^2 + \epsilon$$

where g is the natural logarithm of the modelled growth parameter

p_i (i = 1, 2...15) are the coefficients to be estimated
x_1 = pH value
x_2 = NaCl concentration
x_3 = temperature
x_4 = CO_2
ϵ is a random error.

Thus, a four-factor response surface model was generated for curves fitted by the function of Baranyi et al. (1993), the coefficients entered into a spreadsheet and predictions of growth response (doubling time) obtained for conditions across the ranges of the matrix.

3. Results and discussion

The volumes of CO_2 and N_2 measured immediately after packaging were within 3% of the target volumes. No attempt was made to maintain these concentrations throughout storage, since the conditions were intended to be representative of commercial modified atmosphere packaging procedures. Consequently, the volume of CO_2 declined during storage, while that of N_2 increased and this was dependent on temperature, the 10°C-stored packs showing less atmospheric change than the 30°C packs (Fig. 2).

A few hours after packing, the pH values of pads in all atmospheres had increased slightly and during storage there were further changes. At low temperatures e.g. 8°C, after seven days, initial pH values of 4.5–5.5 increased by 0.5–0.6 units and a pH of 6.5 increased by about 0.3 units, irrespective of CO_2 concentration. At 25°C, pH values of 4.5 increased by about 0.4 units, but pH values of 5.5 decreased by less than 0.1 unit and values of 6.5 decreased by 0.2–0.3 units. The lesser effect on pH at the higher temperature may be a result of CO_2 gas being driven off into the atmosphere surrounding the pads, while

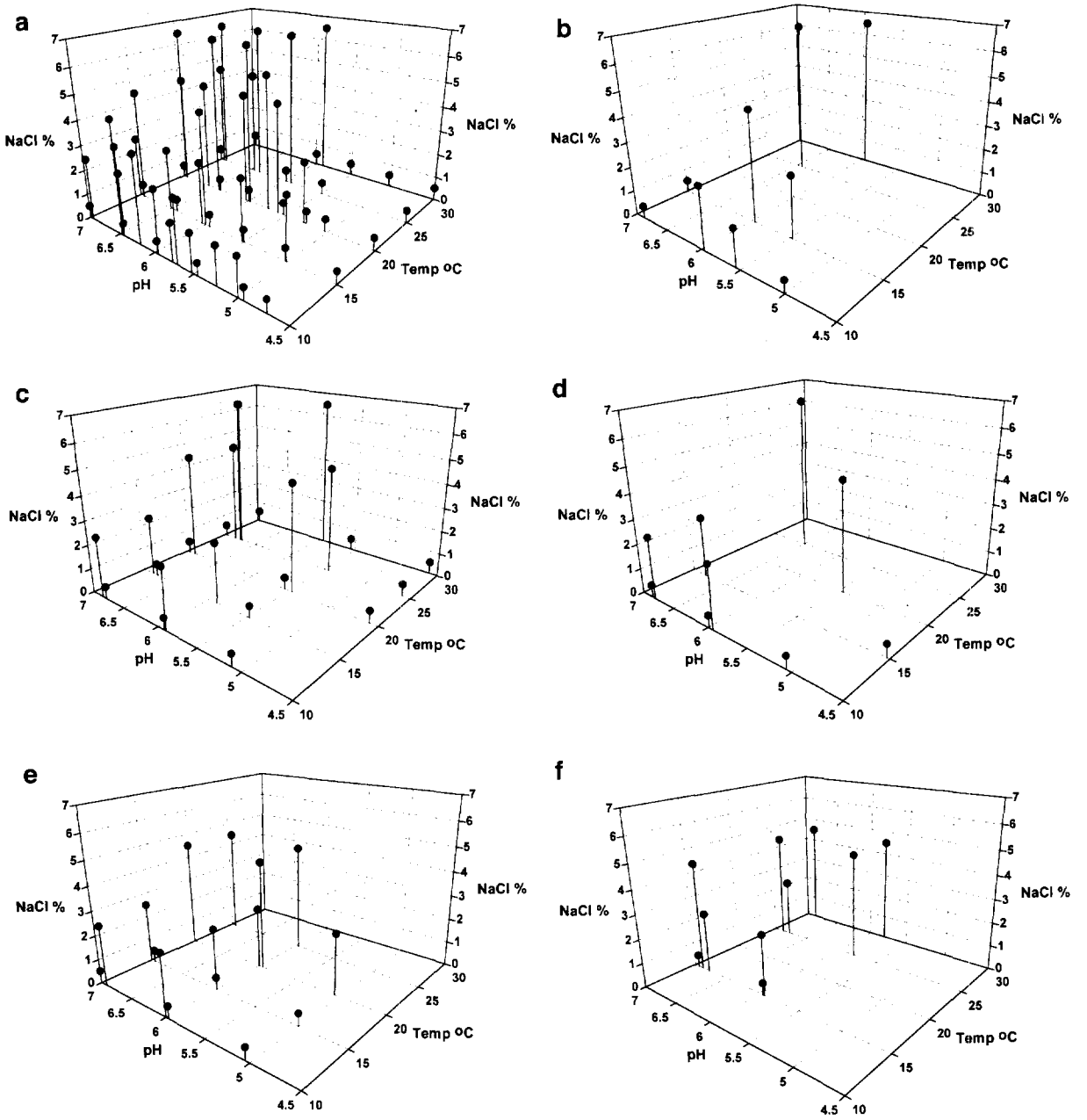

Fig. 1. Distribution of growth curves of *E. coli* O157:H7 generated in various concentrations of carbon dioxide at different temperatures, pH values and sodium chloride concentrations. (a) 0% CO_2, (b) 10% CO_2, (c) 20% CO_2, (d) 40% CO_2, (e) 60% CO_2, (f) 80% CO_2.

the greater effect at low temperature may be a consequence of increased solubility. However, the chemical and microbiological mechanisms of action of CO_2 are complex and not fully researched (Daniels et al., 1985; Dixon and Kell, 1989).

Fig. 3 demonstrates examples of fitting by the method of Baranyi et al. (1993) of growth curves generated in a range of experimental conditions.

Table 1 compares a limited number of predictions of doubling time for a representative range of foods,

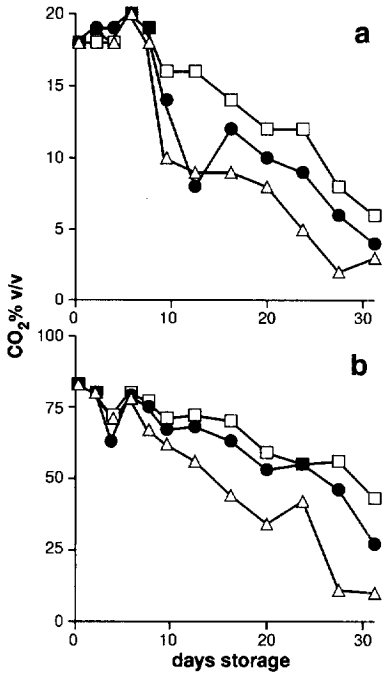

Fig. 2. Decrease in CO_2 concentration in empty packs during storage at 10, 20 and 30°C. (a) Initial CO_2 concentration 20% (80% N_2). (b) Initial CO_2 concentration 80% (20% N_2). □ = 10°C; ● = 20°C; △ = 30°C.

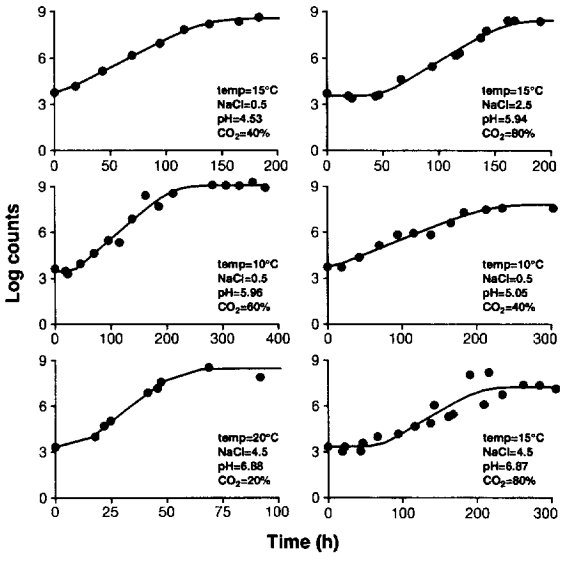

Fig. 3. Growth curves fitted by the method of Baranyi et al. (1993) to data generated in different conditions of temperature, NaCl, pH and CO_2 concentration.

using the four-factor model for *E. coli* O157:H7, with predictions using the original 3-factor model described by Sutherland et al. (1995). That paper also discusses aspects of the observed doubling times shown in Table 1. For making predictions, a CO_2 concentration of 0% was assumed in the atmosphere surrounding the foods. Table 1 shows very little difference between the two models in predictions of doubling times for varying conditions of temperature, pH value and NaCl concentration. The scale of difference between the two models can be determined by the statistical comparison:

$$\Sigma(\ln dt_{publ} - \ln dt_{pred})$$

If all the predicted values and observed values were identical, the sum would be zero. For the 3-factor model, the sum derived from all the predicted and observed values that we compared (not only those shown in Table 1) is 88.72, and for the 4-factor model it is 90.16. Thus the 3-factor model gives predictions which are very slightly closer to observed values of doubling time taken from the literature, while the 4-factor model predictions are marginally safer, but the difference between the two models is so small as to be negligible for the practical purpose of making predictions. It can therefore be concluded that the 4-factor model which includes CO_2 is also satisfactory for predicting growth responses for *E. coli* in foods in the absence of CO_2. There is limited published data that is suitable for validation of the CO_2 aspect of the model. Gill and DeLacy (1991) investigated the effect of inoculating high pH (>6.0) beef with *E. coli* (not O157:H7) and storing in 100% CO_2 at temperatures between 8 and 30°C. Comparison of the predicted and observed doubling times in these conditions (assuming a pH value for the meat of 6.2) are shown in Table 1 (ref. 4), but the predictions were made using 80% CO_2 rather than 100%, since this is the maximum concentration used in the preparation of our model. The predicted values of doubling times were less than those observed; thus the model gives safe predictions and the difference in inhibitory effect between 80% and 100% CO_2 is likely to be small under these environmental conditions, as demonstrated by Fig. 4a which shows the effect of varying CO_2 concentrations (from 0 to 80%) on predictions of growth of *E. coli* O157:H7 in conditions of 1.5% (w/v) NaCl, pH value of 5.0 and

Table 1
Comparison of observed doubling times and predictions from two models for *E. coli* O107:H7

Substrate	Temp (°C)	pH	NaCl (%w/w)	CO_2 (%v/v)	DT publ (h)	DT pred* (h)	DT pred** (h)	Ref
Skim milk	21	6.5	0.5	0	1.1–1.82	1.0	1.0	1
soft ripened cheese	21	6.8	3.36	0	3.14–4.82	1.6	1.6	2
Non-fat dried milk	10	6.5	0.5	0	8.6–10.7	8.4	6.8	3
High pH beef	15	6.2	0.5	0	3.70	2.9	2.6	4
High pH beef	10	6.2	0.5	80	20.60	10.6	NA	4
High pH beef	12	6.2	0.5	80	14.50	6.7	NA	4
High pH beef	15	6.2	0.5	80	8.40	3.6	NA	4
High pH beef	20	6.2	0.5	80	2.30	1.4	NA	4
High pH beef	30	6.2	0.5	80	0.90	0.4	NA	4
Raw meat (aerobic)	20	5.5	0.5	0	1.8	1.3	1.3	5
Barbecued chicken	30	6.5	1	0	0.66	0.4	0.4	6
Barbecued duck	30	5.5	1	0	0.6	0.4	0.4	6
Lean (beef)	25	5.5	0.5	0	1.24	0.6	0.7	7

DT pred* = predictions from 4-factor model with CO_2 in Food MicroModel Version 1.
DT pred** = predictions from 3-factor model (without CO_2; Sutherland et al., 1995).
DT publ = published generation time.
NA = not applicable.
Ref = reference.
References
1. Frank et al., 1977.
2. Fantasia et al., 1975.
3. Skelton and Harmon, 1964.
4. Gill and DeLacy, 1991.
5. Gill and Newton, 1980.
6. Stiles and Ng, 1977.
7. Grau, 1983.

temperature 10°C. Even at this temperature, there is relatively little difference in effect on lag times and growth rates between the different concentrations of CO_2. At a higher temperature (20°C) and more inimical conditions of NaCl (5.5% w/v) and pH (4.5) there is more inhibition by increasing CO_2 concentration (Fig. 4b), but the possibility of growth in 80% CO_2 still exists. Hao and Brackett (1993) evaluated the influence on *E. coli* O157:H7, in broth, of modified atmospheres, including CO_2 concentrations of 5 and 10%, but found that these concentrations were not inhibitory at 10 and 20°C, consistent with the results of our experiments. Molin (1983) measured the maximum specific growth rate of *E. coli* in the presence of 5% and 100% (v/v) CO_2 using absorbance changes. Optical techniques such as absorbance measurements have considerable limitations, including a minimum detection level of ca. 10^6 cells/ml and an upper limit of sensitivity of $10^{7.5}$ cells/ml, resulting in linearity over only about one order of magnitude (McMeekin et al., 1993). Thus, growth responses determined using these techniques are not comparable to those achieved by plate counting methods and have not been used for validation of the model, but nevertheless, the results of Molin (1983) support the results from this work that *E. coli* is relatively resistant to high (80–100%) concentrations of CO_2.

The predictions and Fig. 4 are derived from the 4-factor growth model for *E. coli* O157:H7, available on a disc for personal computers (Food MicroModel Version 2.53), obtainable through Food MicroModel Ltd., Randalls Road, Leatherhead, Surrey KT22 7RY, UK.

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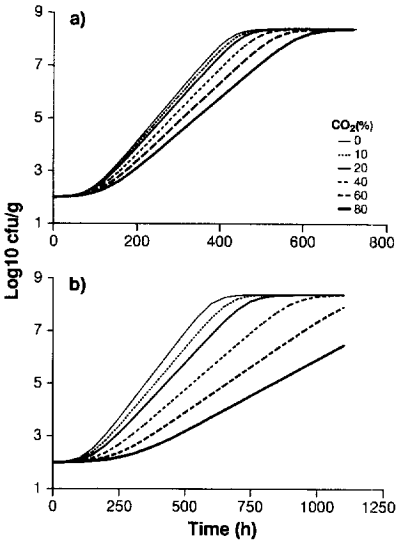

Fig. 4. Comparison of the effect of different concentrations of CO_2 on growth of *E. coli* O157:H7 in different conditions of temperature, pH value and NaCl concentration. (a) Temperature, 10°C; pH value 5.0; NaCl concentration 1.5% (w/v). (b) Temperature, 20°C; pH value 4.5; NaCl concentration 5.5% (w/v).

ing information on change in pH value of the CO_2-packed filter pads and for providing the information for Fig. 2. They also wish to thank Dr. J. Baranyi for assistance with the modelling, Miss S. Sumner for technical assistance, Dr. T.A. Roberts for constructive discussion and the Ministry of Agriculture, Fisheries and Food for funding the work.

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