ELSEVIER International Journal of Food Microbiology 43 (1998) 105–113

International Journal of Food Microbiology

Concentration of carbon dioxide in the water-phase as a parameter to model the effect of a modified atmosphere on microorganisms

F. Devlieghere[a,*], J. Debevere[a], J. Van Impe[b]

[a]Department of Food Technology and Nutrition, Laboratory of Food Microbiology and Preservation, University of Gent, Coupure Links, 653, 9000 Gent, Belgium

[b]Department of Food and Microbial Technology, Katholieke Universiteit Leuven, K. Mercierlaan, 92, B-3001 Leuven, Belgium

Received 27 February 1998; received in revised form 4 June 1998; accepted 15 June 1998

Abstract

The effect of modified atmosphere packaging can mainly be attributed to the bacteriostatic action of CO_2. The dissolved CO_2 in the water-phase of a food product is strongly dependent on several intrinsic and extrinsic parameters and will determine the effectiveness of a modified atmosphere packaging configuration. The effect of pH, gas/product ratio, initial $\%CO_2$ in the gas-phase, lard content and storage temperature on the amount of dissolved CO_2 was screened in a preliminary experiment. The initial CO_2-concentration in the gas-phase and the gas/product ratio turned out to be the two major factors determining the amount of dissolved CO_2. The initial pH also determined significantly the final CO_2-concentration in the broth. Temperature and lard content were shown to have only a minor effect on the amount of dissolved CO_2 compared to the above mentioned parameters. This demonstrates the importance of the packaging configuration in the effectiveness of a modified atmosphere. In a second step, a model was constructed to predict the amount of dissolved carbon dioxide in modified BHI-broth as a function of the gas/product ratio, the initial CO_2-concentration and the temperature by means of Response Surface Methodology (RSM). A second equation was also derived based on Henry's law and was shown to be a powerful tool in the quantification of the effect of intrinsic and extrinsic parameters on the CO_2-solubility in food products. The possibility of the use of the concentration of dissolved CO_2 in the water-phase as a determinative factor for the inhibitory effect of modified atmospheres was examined on *Pseudomonas fluorescens*. Growth curves at 7°C of *P. fluorescens* in different packaging configurations (initial $\%CO_2$ and gas/product ratio) resulting in equal amounts of dissolved CO_2 were compared. *P. fluorescens* was shown to be similarly inhibited by equal amounts of dissolved CO_2-concentrations, independent of the packaging configuration. This demonstrates the potential of the application of the concentration of dissolved CO_2 in the water-phase as a parameter to characterise a modified atmosphere and its inhibition of certain microorganisms.

Keywords: Modified atmosphere packaging; Carbon dioxide; Solubility; Water-phase

1. Introduction

Modified atmosphere packaging (MAP) has been shown to be very effective in extending the shelf-life

---

[*]Corresponding author. Tel: +32 9 264 6177; fax: +32 9 225 5510; e-mail: frank.devlieghere@rug.ac.be

0168-1605/98/$19.00
PII: S0168-1605(98)00101-9

of fresh and processed chilled products. Research regarding MAP has concentrated on the optimisation of gas mixtures for specific products (Zeitoun et al., 1994; Nissen et al., 1996; Jiménez et al., 1997; Boskou and Debevere, 1998) and on the safety of MAP-products (Berrang et al., 1989; Özbas et al., 1996; Beumer et al., 1996; Fernandez et al., 1997; Sutherland et al., 1997). In this type of study, the activity of CO_2 on the microbial growth has always been related to the initial concentration of CO_2 in the gas-phase of the package. Very often, the gas/product volume ratio is not mentioned which makes it very difficult to compare results.

CO_2 is because of its antimicrobial activity the most important component in the applied gas mixtures. When CO_2 is introduced into the package, it is partly dissolved in the water-phase and the fat-phase of the food. This results after equilibrium in a certain concentration of dissolved CO_2 in the water-phase of the product. Little work has been reported regarding the factors affecting the CO_2-solubility in MAP products. Zhao et al. (1994) modeled the CO_2 solubilized in beef during time. Fava and Pergiovanni (1992) correlated the CO_2-solubility in foods packed in modified atmospheres with some chemical-physical characteristics (a_w, pH) and composition of the food (moisture, fat and protein content). Löwenadler and Rönner (1994) demonstrated an important effect of the buffer capacity of the food and the ratio of head space to media volume on the solubility of CO_2. Gill (1988) showed a significant effect of temperature, type of fat tissue and fat content on the solubility of CO_2 in meat. Lower solubility at higher temperatures has been mentioned by many authors (Wolfe, 1980; Wimpfheimer et al., 1990; Zhao et al., 1994) as the reason for the minor effect of MAP at higher temperatures.

In this paper the effect of intrinsic (pH, fat content) and extrinsic (temperature, gas/product volume ratio and initial CO_2-concentration in the gas-phase) parameters on the amount of CO_2 which dissolves in the water-phase of a food when packed under modified atmospheres is quantified by means of a fractional factorial design screening experiment. Furthermore, a response surface model is developed for the amount of CO_2 which dissolves in broth, as a function of the temperature, the gas/product volume ratio and the initial CO_2-concentration in the gas-phase. In a third part the possibility to use the amount of dissolved CO_2 in the water-phase as a parameter for the effectiveness of a modified atmosphere is illustrated with *Pseudomonas fluorescens* as test organism.

2. Materials and methods

2.1. Screening for the importance of intrinsic and extrinsic factors on the CO_2-solubility

2.1.1. Sample preparation

Experiments were performed in 600-ml jars, specially constructed for this purpose, provided with a Teflon valve and a central opening which was closed with a silicone septum. The glass jars were filled with the appropriate amount of modified BHI (Brain Heart Infusion, Gibco BRL + 18 g/l glucose (Sigma, G8270), 3 g/l yeast extract (Oxoid, L21) and 20 g/l NaCl (Vel, 1723)) and autoclaved at 121°C for 15 min. The pH was adjusted after autoclaving to the appropriate value with sterile 2 N HCl or 2 N NaOH. To investigate the effect of the presence of fat on the CO_2-solubility in the water-phase, a specific quantity of lard was added to the broth. The bottles were placed in an impermeable bag (Sidamil, UCB Transpac, Gent, Belgium; O_2-permeability: 6 $ml/m^2/24$ h, CO_2-permeability: 2 $ml/m^2/24$ h at 25°C, 100% R.H.), the valve opened and gas packed (gas mixer: WITT MG18-3MSO, Gasetechnik, Germany and gas packaging: MULTIVAC A300/42, Sepp. Haggenmüller KG, Wolfertschwenden, Germany) at the desired gas composition (gasses: Air Products, Vilvoorde, Belgium). The bottles were closed 30 s after packaging, removed from the bag and stored at the appropriate temperature ±0.5°C during 24 h to reach an equilibrium.

2.1.2. CO_2-measurement

After equilibration, 25 ml of the broth were taken and if necessary diluted with cooled distilled water. The pH of the mixture was adjusted with a sodium citrate buffer (pH 4) to 5.0. The concentration of CO_2 in the broth was immediately measured with an Orion Carbon Dioxide Electrode, model 95-02, Orion, Boston, USA. A calibration curve was prepared before every measurement with three appropriate $NaHCO_3$ standards. A correction was made for the added buffer solution. The CO_2-concentration in the broth of all bottles was measured in duplicate.

2.1.3. Experimental design

The experiments were designed according to the fractional factorial Box-Behnken Design (Khuri and Cornell, 1987) with five independent variables (gas/product volume ratio, initial CO_2-concentration in the gas-phase, temperature, pH and lard content). A Box-Behnken design is formed by combining two-level factorial designs with balanced incomplete block designs. The different levels of the five investigated parameters are presented in Table 1. The gas/product volume ratio was defined as the ratio of the volume of gas over the volume of broth in the bottles (exclusive of the lard).

The central point was repeated 12 times in order to evaluate the repeatability of the developed method. Each other combination was performed in duplicate, resulting in a total of 92 measurements.

2.1.4. Statistical analysis

From the obtained data a second order response surface equation was derived by stepwise regression with a Householder factorisation algorithm by means of the software package Design Expert Software, version 5.0, Stat-Ease Inc., Minneapolis.

2.2. Development of a model for the amount of dissolved carbon dioxide as a function of the initial CO_2-concentration, gas/product volume ratio and temperature

2.2.1. Experimental design

Sample preparation and CO_2-measurements were performed as described in Section 2.1.1 and Section 2.1.2, respectively.

The amount of dissolved CO_2 was determined by means of a full factorial design of three temperatures (4°C, 8°C and 12°C), five gas/product volume ratios (0.3, 0.8, 1.3, 2.5 and 4.0) and five initial CO_2 gas concentrations (10%, 35%, 60%, 80%, 100% CO_2 compensated with N_2). pH was adjusted to 6.1 and no lard was added. Each combination was performed in duplicate, resulting in a total of 150 measurements.

2.2.2. Statistical modeling

From the obtained data a second order response surface model was developed as described above.

A second equation was also derived based on the physical character of the CO_2-dissolving process. The concentration of CO_2 in solution at equilibrium is normally expressed by Henry's law (Dixon and Kell, 1989):

$$(CO_2)^E_{aq} = K.pCO_2 \tag{1}$$

where K = Henry's constant (g/l atm), pCO_2 = partial pressure of CO_2 in the gas-phase at equilibrium (atm), $(CO_2)^E_{aq}$ = concentration of CO_2 in the water-phase at equilibrium (g/l) .

By application of the ideal gas law, partial mass balances for CO_2 and $W_{CO_2} = \rho_{CO_2}/\mathrm{Vol}_{CO_2}$ where W_{CO_2} is the weight of CO_2 in the gas or water-phase (g), ρ_{CO_2} is the density of CO_2 (g/l) and Vol_{CO_2} is the volume taken by CO_2 (l), the following quadratic equation is obtained:

$$((CO_2)^E_{aq})^2 - \left(\frac{G}{P}.\rho_{CO_2} + K\right).(CO_2)^E_{aq} + K.\%CO^I_{2g}.\rho_{CO_2}/100 = 0 \tag{2}$$

where (G/P) = gas/product volume ratio, $\%CO^I_{2g}$ =

Table 1

Levels of the examined parameters used in a Box-Behnken design to investigate the influence of intrinsic and extrinsic parameters on the CO_2-solubility

Independent variable	Level		
	−1	0	+1
Gas/product volume ratio	0.50	2.25	4.00
Initial CO_2-concentration in the gas-phase (%)	10	55	100
Temperature (°C)	4	7	10
pH	4.5	6.0	7.5
Lard fraction (v/v)	0.0	0.2	0.4

the initial CO_2-concentration in the gas phase (vol.%).

From Eq. (2), the following solution is obtained:

$$(CO_2)_{aq}^{E} = \frac{\Big(\frac{G}{P}.\rho_{CO_2}+K\Big) - \sqrt{\Big(\Big(\Big(\frac{G}{P}.\rho_{O_2}+K\Big)^2 - 4.K.\frac{G}{P}.\%CO_{2g}^{I}.\rho_{CO_2}/100\Big)}}{2} \quad (3)$$

2.3. Influence of different packaging configurations on the growth of Pseudomonas fluorescens

2.3.1. Test organism

Pseudomonas fluorescens (LMG 1794) was chosen as test organism.

2.3.2. Experimental set-up

To illustrate the possible use of the concentration of CO_2 in the water-phase of a food as a parameter for the effectiveness of a modified atmosphere, growth curves of *P. fluorescens* were measured at 7°C. For experiment 3A growth curves were determined for five different CO_2-concentrations (after equilibrium) in the broth (Table 2). In experiment 3B growth curves of *P. fluorescens* were determined at three different combinations of initial CO_2-concentration and G/P volume ratio (Table 2). All the combinations of experiment 3B resulted in a final CO_2-concentration of 800 ppm in the broth. A jar filled with modified BHI (gas/product volume ratio = 1.5) and gas packed under 80% N_2/20% O_2 was functioning as a blank. All growth curves were determined in duplicate.

P. fluorescens was subcultured in APT-broth (BBL, 10918) for 24 h at 30°C. A second subculture was incubated in APT-broth for 16 h at 30°C and was placed for 6 h at 7°C before inoculation, to allow the test strain to adapt to the chilling temperature. After the adaptation period, the modified BHI-broth in the jars (see Section 2.1.1) was inoculated to a level of $\pm 10^4$/ml.

After inoculation, the jars were immediately gas packed as described in Section 2.1.1 and stored at 7°C. At regular time intervals, samples (0.2 ml) were taken with a sterile disposable 1-ml syringe, diluted if necessary with Peptone Physiological Salt solution (0.1% peptone, 0.85% NaCl) and plated in duplicate on Plate Count Agar (Oxoid, CM325) with a Spiral Plater Model D (Spiral Systems Inc., Cincinatti, USA). The plates were aerobically incubated during 3 days at 30°C before counting.

3. Results and discussion

3.1. Screening for the importance of intrinsic and extrinsic factors on the CO_2-solubility

Fig. 1 represents the perturbation plot for the effect of the investigated parameters on the amount of CO_2 which is dissolved in modified BHI. In a perturbation plot, the response (here the amount of dissolved CO_2 (ppm)) is plotted by changing only one factor over its range while holding all other factors constant at the central level.

The amount of CO_2 dissolved in the broth was shown to be mainly affected by two factors: the

Table 2
Different applied packaging configurations resulting in a specific target concentration of dissolved CO_2

Experiment no.	Target dissolved CO_2 (ppm)	Packaging configuration			
		O_2 (%)	N_2 (%)	CO_2 (%)	Gas/product (ml/ml)
3A	0	20.0	80.0	0.0	1.50
	500	20.0	56.0	24.0	1.50
	1000	20.0	20.0	60.0	1.50
	1500	20.0	16.0	64.0	3.40
	2000	14.0	10.0	86.0	4.00
3B	0	20.0	80.0	0.0	1.50
	800	20.0	20.0	60.0	0.97
	800	20.0	39.8	40.2	1.74
	800	20.0	48.6	31.4	4.00

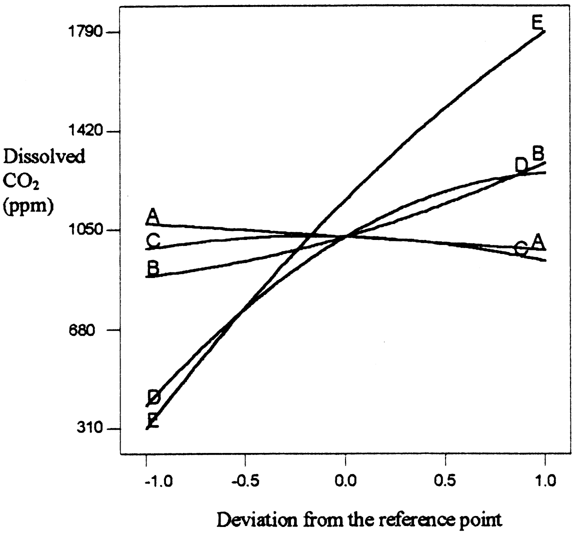

Fig. 1. Perturbation plot for the effect of the fat/product volume fraction (A), pH (B), temperature (°C) (C), gas/product volume ratio (D) and the initial CO_2-concentration in the head space (%) (E) on the amount of CO_2 dissolved in modified BHI (ppm).

initial CO_2-concentration in the gas-phase and the gas/product volume ratio. Temperature was shown to influence the solubility of CO_2 only slightly in the applied temperature range (4–12°C) and in comparison with the effect of the other investigated parameters. The effect of temperature, initial CO_2-concentration and gas/product volume ratio will be further discussed in Section 3.2.

Fig. 1 also illustrates the significant effect of pH on the CO_2-solubility. An increase in pH resulted in an increase of the CO_2-solubility and this effect was strongest at high pH values. Similar results were demonstrated by Löwenadler and Rönner (1994). This can be explained by the equilibrium reactions which occur when CO_2 dissolves in the food. At the moment CO_2 is dissolved, it will be hydrated and carbonic acid will be formed.

This will be dissociated to form a bicarbonate ion and a hydrogen ion. At low pH (read high H^+-concentrations) the reaction will be pushed towards the direction of CO_2 and consequently lower amounts of CO_2 will be able to dissolve in the water-phase.

When CO_2 dissolves in the fat-phase of a food, part of the CO_2 in the gas-phase will be consumed and thus less will be left to dissolve in the water-phase of the food which results in a lower CO_2-concentration in the water-phase in fatty foods. It is generally known that products with a high fat content (salads, fatty fish,···) very often show collapsing because of the CO_2 dissolving in the fat-phase. The fat-phase of the above mentioned products mainly consists of unsaturated oils. Fig. 1 shows that the lard content did not strongly influence the amount of CO_2 dissolved in the water-phase. The low solubility of CO_2 in the lard can possibly be explained by the high solid fat index of lard at the applied low temperatures.

3.2. Development of a model for the amount of dissolved carbon dioxide as function of the initial CO_2-concentration, gas/product volume ratio and temperature

The coefficients, the square root of the mean square error and the adjusted R^2 for the quadratic response equation for the amount of dissolved CO_2 as function of the gas/product ratio (G/P), the initial CO_2-concentration in the gas phase (%) and the temperature are given in Table 3. In Fig. 2 the observed values for the dissolved CO_2 are plotted against the values predicted by the developed response surface model without modification of the response. The observed values were close to the predicted values which demonstrates, in combination with the high adjusted R^2, the usefulness of the model to predict the amount of dissolved CO_2 in

Table 3
Unscaled coefficients of the quadratic response surface equation for the amount of carbon dioxide dissolved as a function of the gas/product volume ratio, the initial CO_2 gas concentration and the temperature

Parameters	Coefficient	Prob>|t|
Intercept	−249.37	
Gas/product (G/P)	342.42	<0.0001
Initial %CO_2	14.11	<0.0001
Temperature (T)	7.1	<0.0001
$(G/P)^2$	−63.44	<0.0001
(Initial %$CO_2)^2$	−0.045	<0.0001
$G/P \times$ initial %CO_2	3.85	0.0137
$G/P \times T$	−5.49	<0.0001
Initial %$CO_2 \times T$	−0.35	<0.0001
RMSE	87.1	
Adjusted R^2	0.9801	

RMSE, square root of the mean square error.

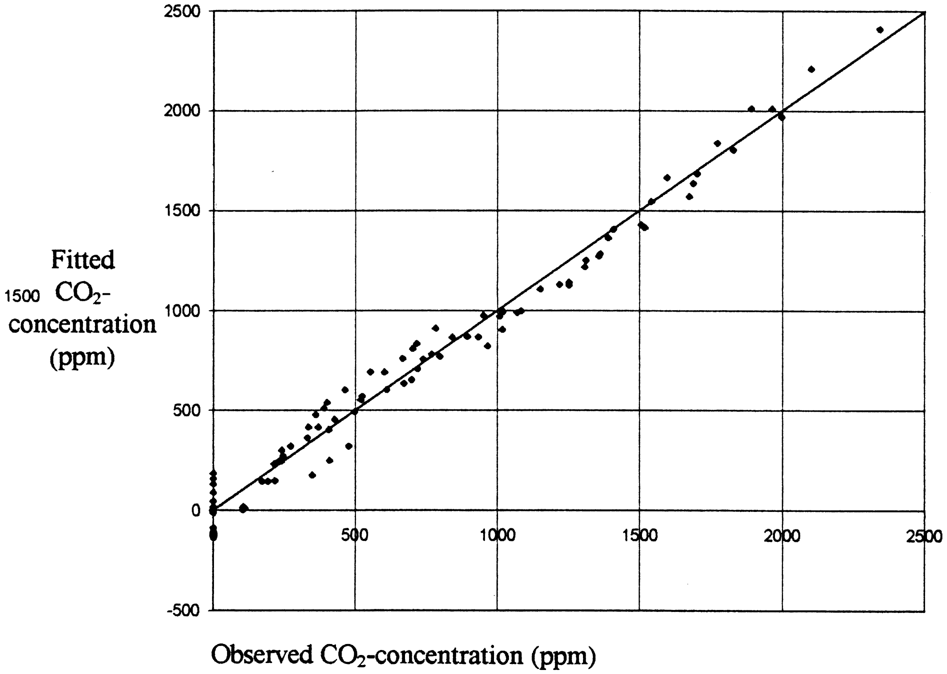

Fig. 2. Observed versus fitted values of the developed model for the dissolved CO_2 as a function of temperature, gas/product volume ratio and initial CO_2-concentration.

modified BHI-broth as a function of the investigated parameters.

When the data at the different experimental temperatures were separately fitted towards Eq. (3), good correlation was obtained. The derived Henry constants K and the corresponding correlation coefficients for the different temperatures are presented in Table 4. Eq. (3) shows how to describe the amount of dissolved carbon dioxide as a function of the initial CO_2 gas concentration and the gas/product volume ratio. When Eq. (3) will be used in practice, Henry constants of the packed product will have to be determined. These Henry constants are dependent on temperature and the type of food (a_w, pH, water content, buffer capacity,···). Eq. (3) can be a powerful tool in the quantification of the effect of different intrinsic and extrinsic parameters on the solubility of CO_2 in food products. For further discussion however, the response surface equation will be used because of the inclusion of temperature as an independent variable.

Fig. 3 represents the contour plots of the response surface equation for the amount of CO_2 (ppm) dissolved in modified BHI-broth as a function of the initial CO_2-concentration (%) in the gas-phase, the gas/product volume ratio (A) and the temperature (B). The initial CO_2-concentration in the gas-phase was shown to have a strong influence on the amount of dissolved CO_2 in the water-phase, as also reported by Löwenadler and Rönner (1994). More remarkable however was the significant effect of the gas/product volume ratio (G/P) on the amount of CO_2 which

Table 4
Henry's constants K and correlation coefficients R^2 for Eq. (3) at the different experimental temperatures

Temperature (°C)	K	95% lower confidence interval value	95% upper confidence interval value	R^2
4	2.841	2.583	3.098	0.950
8	2.541	2.331	2.751	0.956
12	2.223	2.030	2.417	0.948

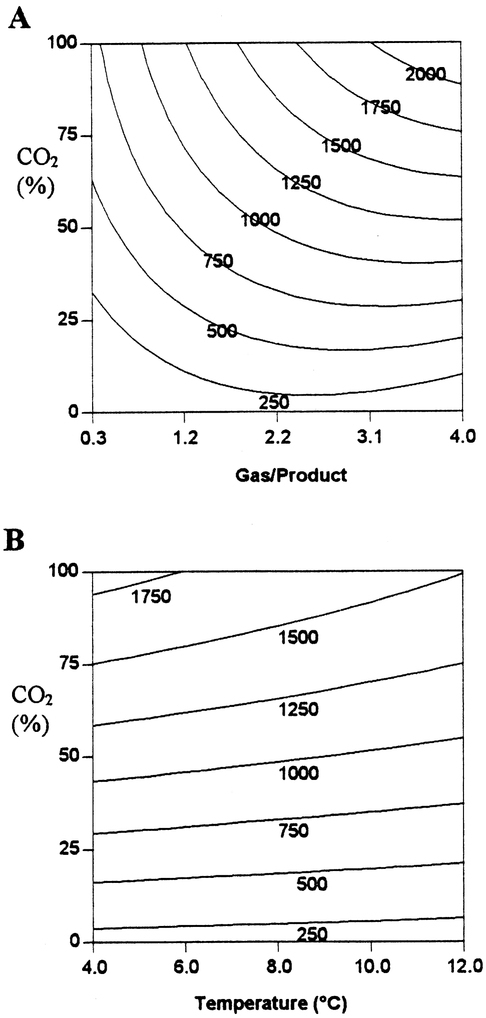

Fig. 3. Contour plots of the amount of carbon dioxide (ppm) dissolved in modified BHI-broth as a function of the initial CO_2-concentration in the gas phase (%), gas/product volume ratio (A) and temperature (°C) (B).

was dissolved in the broth. An increase of the *G/P* from 0.3 to 2.0 (at 8°C and initial CO_2-concentration in the gas-phase = 50%), resulted in an increase of the amount of CO_2 dissolved in the modified BHI-broth of 145% (402 ppm to 989 ppm). For all CO_2-concentrations, the influence of the *G/P* was highest at low G/P ratios. This can be explained by the larger drop of the CO_2 concentration with the gas-phase in case of low *G/P* ratios when CO_2 starts dissolving.

At equilibrium, this will result in a low residual CO_2-concentration in the gas-phase and thus a low amount of CO_2 dissolved in the water phase. This illustrates clearly the importance of the optimisation of a packaging configuration of a modified atmosphere package. Moreover, the importance of the gas/product volume ratio on the amount of CO_2 which will dissolve in the water-phase of a food demonstrates the incompatibility of the diverse published data concerning MAP. Data without mentioning the applied gas/product volume ratio and the moisture content of the product will be difficult to compare.

Temperature was shown to influence only slightly the amount of dissolved CO_2 compared to the other investigated parameters (Fig. 2B). When the temperature was increased from 4 to 12°C ($G/P = 2$, initial CO_2-concentration in the gas-phase = 50%), a decrease in the solubility of CO_2 of 16% (1073 ppm to 901 ppm) was noticed. This is comparable with the results of Löwenadler and Rönner (1994) and Lange (1956), who reported a decrease of, respectively, 17.1% and 22.8% (in nutrient broth and water, respectively) when temperature was increased from 4 to 12°C. Although the minor effect of modified atmospheres at higher temperatures has been attributed by many authors to the lower solubility of carbon dioxide (Wolfe, 1980; Wimpfheimer et al., 1990; Zhao et al., 1994), the present results demonstrate only a minor effect of the temperature on the CO_2-solubility at a realistic chilling temperature range (4–12°C). This demonstrates that the high effect of CO_2 at low temperatures will not only be attributed to the higher solubility of CO_2 but also to a higher sensitivity of the microbial cell for CO_2 at low temperatures. Further investigations are however necessary to prove this statement.

3.3. Influence of different packaging configurations on the growth of Pseudomonas fluorescens

The growth of *P. fluorescens* was highly influenced by CO_2 as shown in Fig. 4. A significant increase in the lag phase and decrease in the growth rate of *P. fluorescens* was, as could be expected, noticed with increasing CO_2-concentrations. A more remarkable result is however shown in Fig. 5. The growth characteristics of *P. fluorescens* in the three packaging configurations resulting in 800 ppm of CO_2 in the medium, showed no significant differ-

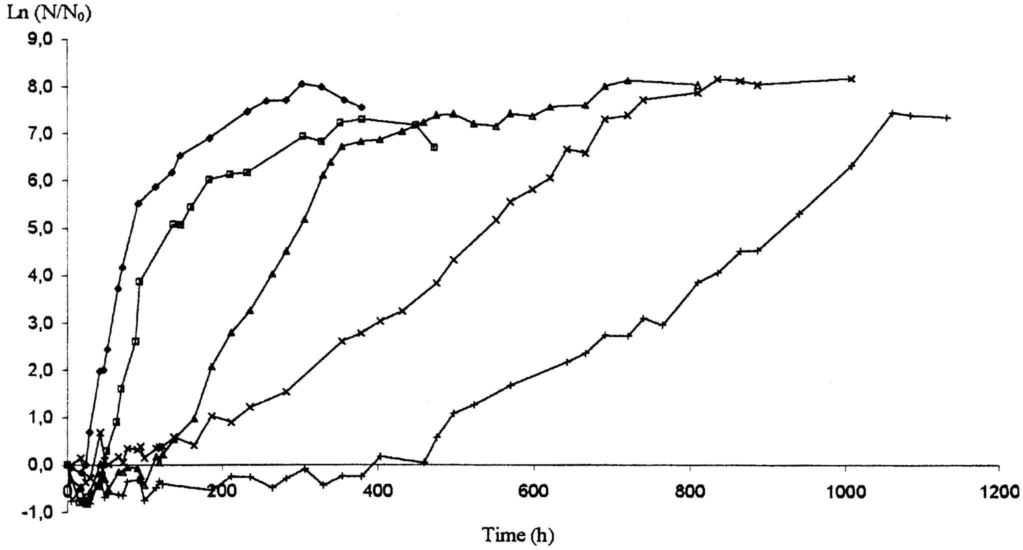

Fig. 4. Influence of CO_2 on the growth of *P. fluorescens* at 7°C for different CO_2-concentrations (◇ 0 ppm, □ 500 ppm, △ 1000 ppm, × 1500 ppm and + 2000 ppm) in modified BHI-broth (experiment 3A).

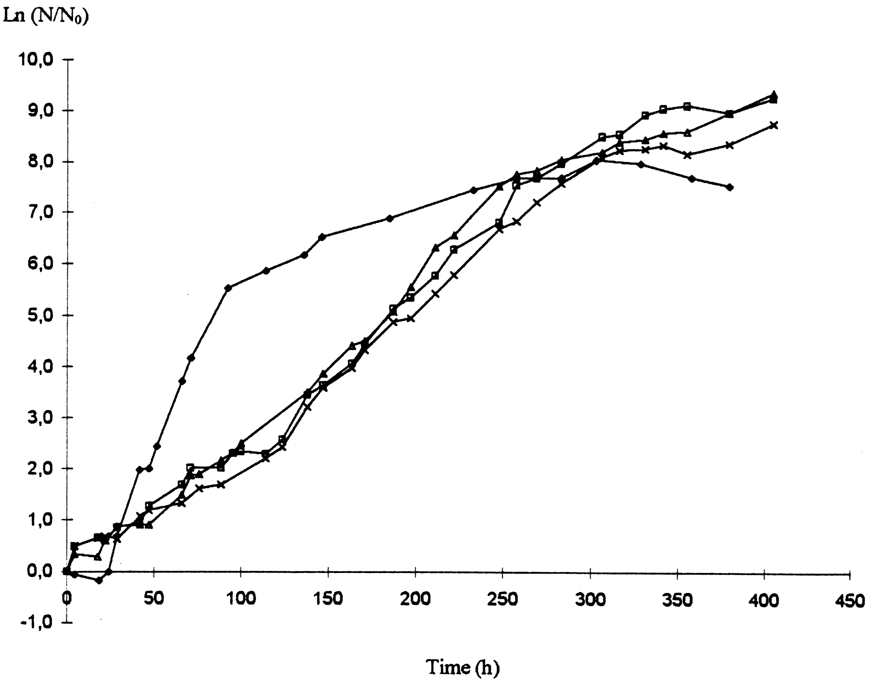

Fig. 5. Influence of CO_2 on the growth of *P. fluorescens* at 7°C for different packaging configurations (◇ 0% CO_2 — 1.5 *G/P*, □ 31.4% CO_2 — 4.0 *G/P*, △ 40.2% CO_2 — 1.74 *G/P*, × 60% CO_2 — 0.97 *G/P*), resulting in 800 ppm of CO_2 in modified BHI-broth (experiment 3B).

ences at 7°C. The three configurations resulted in the same inhibition rate of *P. fluorescens* compared to the growth in the packaging configuration without CO_2. The three packaging configurations differed strongly in initial gas composition. This proves the statement that CO_2 exerts its antimicrobial effect in the water-phase of a food product. The combination of the results of Figs. 4 and 5 illustrates the usefulness of the dissolved CO_2 in the water-phase of a food product to define a modified atmosphere packaging system regarding its antimicrobial effect. Optimisation of modified atmosphere packaging should be based on a maximisation of the amount of CO_2 dissolved in the water-phase of the specific food product. During this process, the antimicrobial effects will however always be confronted with sensorial and marketing aspects which will at the end result in an optimal product. The initial CO_2-concentration is however mostly used as the only parameter to characterise a modified atmosphere packaging configuration. When the effect of modified atmospheres on the microbial spoilage or safety of food products is modeled, the CO_2 dissolved in the water-phase of the food should be incorporated in the model as an independent variable (Devlieghere et al., 1998).

References

Berrang, M.E., Brackett, R.E., Beuchat, L.R., 1989. Growth of *Aeromonas hydrophila* on fresh vegetables stored under a controlled atmosphere. Appl. Environ. Microbiol. 9, 2167–2171.

Beumer, R.R., te Giffel, M.C., de Boer, E., Rombouts, F.M., 1996. Growth of *Listeria monocytogenes* on sliced cooked meat products. Food Microbiol. 13, 333–340.

Boskou, G., Debevere, J., 1998. Optimum modified atmospheres for the packaging of fresh cod fillets. Int. J. Food Sci. Technol., submitted.

Devlieghere, F., Debevere, J., Van Impe, J., 1998. Effect of dissolved carbon dioxide and temperature on the growth of *Lactobacillus sake* in modified atmospheres. Int. J. Food Microbiol., in press.

Dixon, N.M., Kell, D.B., 1989. The inhibition by CO_2 of the growth and metabolism of microorganisms. J. Appl. Bacteriol. 67, 109–136.

Fava, P., Pergiovanni, L., 1992. Carbon dioxide solubility in foods packed with modified atmosphere. II. Correlation with some chemical-physical characteristics and composition. Ind. Aliment. 21 (5), 424–430.

Fernandez, P.S., George, S.M., Sills, C.C., Peck, M.W., 1997. Predictive model of the effect of CO_2, pH, temperature and NaCl on the growth of *Listeria monocytogenes*. Int. J. Food Microbiol. 37, 37–45.

Gill, C.O., 1988. The solubility of carbon dioxide in meat. Meat Sci. 22, 65–71.

Jiménez, S.M., Salsi, M.S., Tiburzi, M.C., Rafaghelli, R.C., Tessi, M.A., Coutaz, V.R., 1997. Spoilage microflora in fresh chicken breast stored at 4°C: influence of packaging methods. J. Appl. Microbiol. 83, 613–618.

Khuri, A.I., Cornell, J.A., 1987. Response Surfaces Designs and Analysis. D.B. Owen, Dallas, pp. 105–148.

Lange, N.A., 1956. Solubility of gases in water. Handbook of Chemistry. McGraw-Hill, pp. 1091–1093.

Löwenadler, J., Rönner, U., 1994. Determination of dissolved carbon dioxide by coulometric titration in modified atmosphere systems. Lett. Appl. Microbiol. 18, 285–288.

Nissen, H., Sorheim, O., Dainty, R., 1996. Effects of vacuum, modified atmospheres and storage temperature on the microbial flora of packaged beef. Food Microbiol. 13, 183–191.

Özbas, Z.Y., Vural, H., Aytac, S.A., 1996. Effects of modified atmosphere and vacuum packaging on the growth of spoilage and inoculated pathogenic bacteria on fresh poultry. Z. Lebensm. Unters. Forsch. 203, 326–332.

Sutherland, J.P., Bayliss, A.J., Braxton, D.S., Beaumont, A.L., 1997. Predictive modelling of *Escherichia coli* O157:H7. Inclusion of carbon dioxide as a fourth factor in a pre-existing model. Int. J. Food Microbiol. 37, 113–120.

Wimpfheimer, L., Altman, N.S., Hotchkiss, J.H., 1990. Growth of *Listeria monocytogenes* Scott A, serotype 4 and competitive spoilage organisms in raw chicken packaged under modified atmospheres and in air. Int. J. Food Microbiol. 11, 205–214.

Wolfe, S.K., 1980. Use of CO and CO_2 enriched atmospheres for meats, fish and produce. Food Technol. 34, 55–58.

Zeitoun, A.A.M., Debevere, J.M., Mossel, D.A.A., 1994. Significance of Enterobacteriaceae as indicator organisms for hygiene in fresh untreated poultry, poultry treated with lactic acid and poultry stored in a modified atmosphere. Food Microbiol. 11, 169–176.

Zhao, Y., Wells, J.H., McMillin, K.W., 1994. Applications of dynamic modified atmosphere packaging systems for fresh red meats: a review. J. Muscle Foods 5, 299–328.