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Shelf life of modified atmosphere packed cooked meat products: a predictive model

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Abstract

The effect of temperature, concentration of dissolved CO_2 and water activity on the growth of *Lactobacillus sake* was investigated by developing predictive models for the lag phase and the maximum specific growth rate of this specific spoilage organism for gas-packed cooked meat products. Two types of predictive model were compared: an extended Ratkowsky model and a response surface model. In general, response surface models showed a slightly better correlation, but the response surface model for the maximum specific growth rate showed illogical predictions at low water activities. The concentration of dissolved CO_2 proved to be a significant independent variable for the maximum specific growth rate as well as for the lag phase of *L. sake*. Synergistic actions on the shelf life-extending effect were noticed between temperature and dissolved CO_2, as well as between water activity and dissolved CO_2. The developed models were validated by comparison with the existing model of Kant-Muermans et al. (1997) and by means of experiments in gas-packed cooked meat products. Both developed models proved to be useful in the prediction of the microbial shelf life of gas-packed cooked meat products.

Keywords: Modified-atmosphere packaging; Predictive modeling; Carbon dioxide; Cooked meat products; Shelf life; *Lactobacillus sake*

1. Introduction

Cooked meat products are more and more packed under modified atmospheres, containing CO_2 as the antimicrobial component. Contradicting reports are, however, published about the shelf life-extending effect of modified atmospheres for cooked meat products. Kant-Muermans et al. (1997) observed no significant difference in growth behaviour of *Lactobacillus curvatus* between vacuum-packaging, packaging in 35% CO_2/65% N_2 and packaging in 65% CO_2/35% N_2. Papa and Passarelli (1995) noticed a significant prolongation of the shelf life of cooled sliced cooked ham when a 40% CO_2/60% N_2 atmosphere was applied instead of vacuum packaging.

The comparison of the results of many publi-

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cations regarding MAP is, however, very difficult because of the use of different packaging foils with different O_2 and CO_2 permeabilities, and especially because the applied gas/product volume ratio is not mentioned. To avoid the latter problem, the concentration of dissolved CO_2 in the water-phase of the product can be used as a parameter instead of the concentration of CO_2 in the head space (Devlieghere et al., 1998a,b).

Predictive microbiology is nowadays accepted as a useful tool for, among others, product development, HACCP, risk assessment and educational purposes. Most of the research has focused on predictive modelling of growth/inactivation of food pathogens. More recently, predictive microbiology has also been used to predict the growth of spoilage microorganisms in order to determine the shelf life of a food product. Specific spoilage organisms (SSO) are selected for certain food products and used as test organisms. Examples are *Lactobacillus curvatus* in cooked meat products (Kant-Muermans et al., 1997); *Photobacterium phosphorium* in modified atmosphere packed fresh fish from temperate sea water (Dalgaard, 1995); *Pseudomonas* spp. in minimally processed cut endive (Vankerschaver et al., 1996); and *Pseudomonas putida* for chill-stored food products with neutral pH and high water activity, such as milk, cream and fresh meat (Neumeyer et al., 1997a,b).

Predictive models for the growth of microorganisms include temperature, pH and water activity as the main growth-determining factors. However, other factors can significantly influence the growth characteristics of the modelled microorganism, such as nitrite content, organic acid concentration and atmosphere. Recently, more attention has been given to the atmosphere as a fourth important growth-determining factor, but only few models which include the gas atmosphere have been developed until now (Dalgaard et al., 1997; Farber et al., 1996; Fernandez et al., 1997; Sutherland et al., 1996, 1997). All mentioned models include the initial CO_2 concentration (%) in the head space as the independent variable for the gas atmosphere. Devlieghere et al. (1998b) demonstrated that CO_2 exerts its antimicrobial effect in the water-phase of the food product. To exclude the effect of intrinsic, extrinsic and processing parameters on the CO_2 solubility, the concentration of dissolved CO_2 in the water-phase of the food product should be incorporated in the model as independent variable.

The effect of temperature and dissolved CO_2 on the growth of *Lactobacillus sake* was investigated by Devlieghere et al. (1998a). In the current study, the previously developed model is extended by a third parameter, the water activity, in order to predict the shelf life of modified-atmosphere packed cooked meat products. This model is then validated by means of additional experiments with gas-packed cooked meat products.

2. Material and methods

2.1. Development of a predictive model for the effect of temperature, dissolved carbon dioxide and water activity on the growth of Lactobacillus sake

2.1.1. Experimental set-up

The determination of the growth curves necessary for the development of the model, was performed in 600-ml glass jars, specially constructed for this purpose, provided with a Teflon valve and a central opening which is closed with a silicone septum (Devlieghere et al., 1998a). The glass jars were filled with the appropriate amount of broth and autoclaved at 121°C for 15 min. Modified brain heart infusion (Devlieghere et al., 1998a) was used as simulation medium for cooked meat products. Water activity was altered by means of the addition of NaCl (2–6%, w/w). Water activity was measured by means of a hydrometer (Thermoconstanter Humidak − TH2 Novasina Defensor AG − Pläffikon, S.Z.). The pH was adjusted with sterile 2 N HCl after autoclaving to 6.1, corresponding to the average pH of cooked meat products.

Lactobacillus sake (Devlieghere et al., 1998a) was subcultured in APT-broth (BBL®, Becton-Dickinson, 10918) for 24 h at 30°C. A second subculture was incubated in APT broth for 16 h at 30°C and placed for 6 h at the appropriate temperature (4, 8 or 12°C), before inoculation, to allow the test strain to adapt to the chilling temperature. After the adaptation period, the previously cooled simulation medium in the jars was inoculated to a level of 10^4/ml.

After inoculation, the jars were gas packed at the desired packaging configuration. Gas packaging was

performed by placing the inoculated jars with open valve in an impermeable bag (VPA/PE 15-60, SUDPACK Verpackungen, Ochsenhausen, Germany: O_2 permeability, 2.5 ml/m^2 per 24 h/atm; CO_2 permeability, 8.8 ml/m^2 per 24 h/atm at 20°C, 75% R.H.). The atmosphere in the bag was altered by means of a gas packaging unit (gas mixer, WITT MG18-3MSO, Gasetechnik, Germany; and gas packaging, MULTIVAC A300, 42 Sepp. Haggenmüller KG, Wolfertschwenden, Germany) which inserted the desired gas composition (gasses, Air Products, Vilvoorde, Belgium) in the bags. After sealing, the valve of the jar was closed, the bags opened and removed and the jars stored at the appropriate temperature.

To collect the data necessary for the construction of growth curves, samples (0.2 ml) were taken at regular time intervals with sterile disposable 1-ml syringes, diluted if necessary with peptone physiological salt solution (0.1% peptone, 0.85% NaCl) and plated in duplicate on MRS agar (Oxoid CM361) with a Spiral Plater Model D (Spiral Systems, Cincinatti, USA). The plates were anaerobically incubated during 3 days at 30°C.

2.1.2. Experimental design

Growth curves were determined at all combinations of three temperatures (4, 8 and 12°C), five packaging configurations (100% N_2–20% CO_2; 80% N_2–40% CO_2; 60% N_2–70% CO_2; 30% N_2; and 100% CO_2, with a constant gas/product volume ratio of 4/1) and four water activities (0.986, 0.980, 0.974 and 0.962). Growth curves were determined in duplicate. The data at a water activity of 0.986 were previously obtained by Devlieghere et al. (1998b).

The concentration of dissolved CO_2 was used as parameter for the applied modified atmosphere. The concentration of dissolved CO_2 in modified BHI broth at a specific packaging configuration and temperature was determined by means of a previously developed model for the effect of temperature, initial CO_2 concentration in the gas-phase and gas/product volume ratio on the amount of dissolved CO_2 in modified BHI (Devlieghere et al., 1998b).

2.1.3. Statistical analysis of growth data

A standard two-stage method was applied to obtain a model for the influence of the temperature, the concentration of dissolved carbon dioxide and the water activity on the growth of *Lactobacillus sake*. In the first stage, the maximum specific growth rate (μ_m) and the lag phase (λ) were estimated for each combination of the experimental design. The growth parameters were estimated by fitting the data to the modified Gompertz equation (Zwietering et al., 1990) with the Levenberg–Marquardt algorithm by means of the statistical package SPSS for windows, version 7.0.

The obtained estimates for μ_m (h^{-1}) were primarily fitted towards an extended Ratkowsky model (McMeekin et al., 1987) for the effect of temperature and water activity on the maximum specific growth rate μ_{max} (h^{-1}) of *Lactobacillus sake*, independently of the amount of dissolved carbon dioxide:

$$\mu_{max} = a \cdot (a_w - a_{w\,min}) \cdot (T - T_{min})^2 \quad (1)$$

where a is a constant, T_{min} (°C) and $a_{w\,min}$ are the respective estimated theoretical minimum temperature and water activity for growth of the organism.

Secondly, this model was extended for the amount of dissolved CO_2 to examine the significance of this factor on the maximum specific growth rate:

$$\mu_{max} = b \cdot (a_w - a_{w\,min}) \cdot ([CO_{2\,max}] - [CO_2]) \cdot (T - T_{min})^2 \quad (2)$$

where b is a constant, $[CO_2]$ is the concentration of dissolved CO_2 (ppm) and $[CO_{2\,max}]$ is the estimated theoretical maximum CO_2 concentration (ppm) for growth of the organism.

The extended Ratkowsky models for μ_m were compared with a quadratic response surface model which corresponds to the following equation:

$$\mu_m = I_\mu + m_1 \cdot T + m_2 \cdot [CO_2] + m_3 \cdot a_w + m_4 \cdot T^2 + m_5 \cdot [CO_2]^2 + m_6 \cdot a_w^2 + m_7 \cdot T \cdot [CO_2] + m_8 \cdot T \cdot a_w + m_9 \cdot [CO_2] \cdot a_w \quad (3)$$

where I_μ is the intercept and $m_{1\to9}$ are equation coefficients.

Furthermore, the Ratkowsky model for the growth rate was modified to describe the relation between water activity, temperature and the lag phase λ (h) (Kant-Muermans et al., 1997):

$$\lambda = \frac{1}{c \cdot (a_w - a_{w\,min}) \cdot (T - T_{min})^2} \quad (4)$$

where c is a constant.

The data were also fitted towards an extended Ratkowsky model to include the effect of dissolved CO_2 on the lag phase:

$$\lambda = \frac{1}{d \cdot (a_w - a_{w\,min}) \cdot ([CO_{2\,max}] - [CO_2]) \cdot (T - T_{min})^2} \quad (5)$$

where d is a constant.

Similarly as for μ_m, the Ratkowsky models for λ (Eqs. (4) and (5)) were compared to a quadratic response surface model with the following equation:

$$\lambda = I_\lambda + l_1 \cdot T + l_2 \cdot [CO_2] + l_3 \cdot a_w + l_4 \cdot T^2 + l_5 \cdot [CO_2]^2 + l_6 \cdot a_w^2 + l_7 \cdot T \cdot [CO_2] + l_8 \cdot T \cdot a_w + l_9 \cdot [CO_2] \cdot a_w \quad (6)$$

where I_λ is the intercept and $l_{1\to9}$ are equation coefficients.

All secondary models were obtained by fitting the data to Eqs. (1)–(6) with the Levenberg–Marquardt algorithm by means of the statistical package SPSS for windows, version 7.0. No data transformations of μ_{max} or λ were applied.

2.2. Validation of the model

To validate the model in reel gas-packed cooked meat products, six prime quality boneless hams (quadriceps femoris) with an average weight of 1.2 kg were cut from a pork carcass, brine injected, pasteurised and gas packed. Each ham was injected with approximately 100 ml of brine containing 15 g/l disodium polyposphate, 20 g/l dextrose, 10 g/l sodium ascorbate and either 195 or 286 g/l nitrited curing salt (NaCl + 0.6% $NaNO_2$). The injected hams were vacuum packed and stored during 48 h at 4°C to make diffusion of the brine ingredients possible. The hams were then cooked in the package until a kernel temperature of 68°C was reached. After pasteurisation, the vacuum-packed hams were quickly cooled in the package by means of immersion in an ice/water mixture and stored in a freezer (−24°C). Before use, the hams were defrosted at 4°C and analysed for dry matter content (314±23 g/kg), fat content (13±4 g/kg), residual nitrite content (17.5±7.2 mg/kg), water activity and pH (6.11±0.11).

After defrosting, the ham was aseptically cut and transferred in a sterile stomacher bag. The cut ham was inoculated with an inoculum of *L. sake* of circa 10^4/g, similarly prepared as in Section 2.1.1. After inoculation an appropriate amount of inoculated ham was transferred in sterile 50-ml conical flasks to result in a gas/product water-phase volume ratio of 4/1. The conical flasks were gas packed (see Section 2.1.1) at different initial CO_2 concentrations (±17% and ±75%) compensated with N_2, closed and stored at different temperatures (4, 7 and 10°C).

At regular time intervals the content of a conical flask was sampled, diluted with peptone physiological salt solution, stomached and plated on MRS agar (Oxoid CM 361). The plates were anaerobically incubated during 3 days at 30°C. The growth parameters (μ_m and λ) of *L. sake* in the ham were estimated by fitting the data to the modified Gompertz equation (Zwietering et al., 1990) with the Levenberg–Marquardt algorithm by means of the statistical package SPSS for windows, version 7.0.

The amount of dissolved CO_2 in the water-phase of the ham was separately determined by measuring the underpressure in the closed flask after equilibrium (24 h after packaging). The underpressure was measured with a U-tube filled with mercury. The concentration of dissolved CO_2 in the ham was calculated by the formula of Zhao and Wells (1995):

$$A_{CO_2} = \frac{44 V_H}{R \cdot T \cdot M_m} \cdot [P_i - P_f] \quad (7)$$

where V_H is volume head space (m^3); R is universal gas constant (J/mol·K); T is temperature (K); P_i is initial pressure in the flask at the moment of packaging (Pa); P_f is final pressure in the flask after equilibrium (Pa); M_m is the mass of packed product (kg).

By taking the water content of the ham into account, the concentration of CO_2 in the water-phase of the ham could be calculated.

3. Results and discussion

3.1. Development of a predictive model for the effect of temperature, dissolved carbon dioxide and water activity on the growth of Lactobacillus sake

Table 1 shows the estimated growth parameters μ_{max} (h^{-1}) and λ (h) of *L. sake* at the different tested

Table 1
Experimentally determined growth parameters of *L. sake* at different temperatures, concentrations of dissolved CO_2 and water activities

Expt. no.	Temperature (°C)	Dissolved CO_2 (ppm)	a_w	μ_{max} (h^{-1})	λ (h)
1[a]	12	0	0.986	0.212	13.1
2[a]	12	0	0.986	0.229	13.4
3[a]	12	400	0.986	0.181	11.6
4[a]	12	408	0.986	0.199	12.3
5[a]	12	865	0.986	0.191	13.4
6[a]	12	870	0.986	0.193	13.6
7[a]	12	1470	0.986	0.184	13.1
8[a]	12	1470	0.986	0.186	13.1
9[a]	12	1931	0.986	0.179	12.3
10[a]	12	1950	0.986	0.175	13.7
11	12	0	0.980	0.151	18.5
12	12	0	0.980	0.149	19.7
13	12	450	0.980	0.137	17.8
14	12	415	0.980	0.132	16.0
15	12	867	0.980	0.123	15.4
16	12	896	0.980	0.118	16.1
17	12	1452	0.980	0.110	13.5
18	12	1458	0.980	0.094	13.7
19	12	1994	0.980	0.089	18.3
20	12	1966	0.980	0.081	15.6
21	12	0	0.974	0.125	11.5
22	12	0	0.974	0.125	12.4
23	12	427	0.974	0.093	9.1
24	12	429	0.974	0.092	10.9
25	12	861	0.974	0.080	8.0
26	12	867	0.974	0.078	10.1
27	12	1485	0.974	0.070	9.4
28	12	1477	0.974	0.070	6.0
29	12	1946	0.974	0.062	5.78
30	12	1974	0.974	0.064	6.5
31	12	0	0.962	0.049	28.6
32	12	0	0.962	0.052	28.6
33	12	396	0.962	0.018	8.4
34	12	404	0.962	0.021	13.1
35	12	857	0.962	0.018	7.9
36	12	863	0.962	0.015	10.0
37	12	1490	0.962	0.015	5.2
38	12	1490	0.962	0.017	21.8
39	12	1950	0.962	0.012	0.6
40[a]	8	0	0.986	0.118	24.9
41[a]	8	0	0.986	0.123	22.9
42[a]	8	514	0.986	0.103	17.4
43[a]	8	514	0.986	0.099	17.1
44[a]	8	1000	0.986	0.094	18.5
45[a]	8	1000	0.986	0.087	16.1
46[a]	8	1652	0.986	0.087	17.3
47[a]	8	1652	0.986	0.082	13.9
48[a]	8	2236	0.986	0.073	18.0
49[a]	8	2236	0.986	0.072	19.7
50	8	0	0.980	0.124	27.2
51	8	0	0.980	0.122	25.5
52	8	513	0.980	0.110	24.5
53	8	515	0.980	0.112	28.1

Table 1. Continued

Expt. no.	Temperature (°C)	Dissolved CO_2 (ppm)	a_w	μ_{max} (h^{-1})	λ (h)
54	8	986	0.980	0.109	26.4
55	8	997	0.980	0.096	25.2
56	8	1643	0.980	0.087	25.3
57	8	1637	0.980	0.083	28.5
58	8	2200	0.980	0.077	27.1
59	8	2209	0.980	0.071	23.9
60	8	0	0.974	0.090	32.0
61	8	0	0.974	0.092	30.3
62	8	515	0.974	0.102	32.5
63	8	510	0.974	0.089	32.1
64	8	997	0.974	0.078	30.9
65	8	981	0.974	0.080	32.7
66	8	1638	0.974	0.057	28.8
67	8	1647	0.974	0.071	36.2
68	8	2210	0.974	0.054	30.9
69	8	2207	0.974	0.062	38.2
70	8	0	0.962	0.039	67.9
71	8	0	0.962	0.038	70.5
72	8	508	0.962	0.038	59.5
73	8	508	0.962	0.04	62.3
74	8	979	0.962	0.028	46.6
75	8	995	0.962	0.036	65.7
76	8	1494	0.962	0.035	66.4
77	8	1513	0.962	0.038	63.7
78	8	2211	0.962	0.024	50.7
79	8	2211	0.962	0.034	66.5
80	4	52	0.986	0.067	43.5
81	4	49	0.986	0.073	47.6
82	4	567	0.986	0.066	45.8
83	4	572	0.986	0.062	43.5
84	4	1089	0.986	0.060	46.0
85	4	1097	0.986	0.056	40.6
86	4	1767	0.986	0.051	42.1
87	4	1767	0.986	0.051	43.2
88	4	2411	0.986	0.042	42.8
89	4	2411	0.986	0.045	42.5
90	4	52	0.980	0.053	60.8
91	4	49	0.980	0.054	67.0
92	4	606	0.980	0.048	63.8
93	4	606	0.980	0.050	71.4
94	4	1114	0.980	0.050	77.4
95	4	1119	0.980	0.047	69.4
96	4	1791	0.980	0.041	71.6
97	4	1786	0.980	0.040	74.4
98	4	2411	0.980	0.036	75.6
99	4	2411	0.980	0.036	82.5
100	4	0	0.974	0.051	77.4
101	4	0	0.974	0.050	74.9
102	4	609	0.974	0.047	79.4
103	4	600	0.974	0.047	76.1
104	4	1119	0.974	0.040	72.8
105	4	1097	0.974	0.041	75.6
106	4	1793	0.974	0.039	81.1
107	4	1791	0.974	0.039	82.0
108	4	2411	0.974	0.035	88.8
109	4	2411	0.974	0.038	95.0

Table 1. Continued

Expt. no.	Temperature (°C)	Dissolved CO_2 (ppm)	a_w	μ_{max} (h^{-1})	λ (h)
110	4	0	0.962	0.017	132.8
111	4	0	0.962	0.016	128.7
112	4	606	0.962	0.017	126.8
113	4	590	0.962	0.020	131.5
114	4	1114	0.962	0.021	144.4
115	4	1119	0.962	0.020	127.9
116	4	1793	0.962	0.020	138.1
117	4	1795	0.962	0.022	145.7
118	4	2411	0.962	0.020	168.6
119	4	2411	0.962	0.021	176.5

[a]Adapted from Devlieghere et al. (1998b)

combinations of temperature, dissolved CO_2 and water activity. By means of this dataset, the coefficients of the different types of models were estimated for μ_{max} (Table 2) and λ (Table 3). The statistical parameters of the different proposed models for the growth parameters μ_{max} and λ of *L. sake* are presented in Table 4.

To make comparison between the models possible, the adjusted correlation coefficient was calculated instead of the multiple correlation coefficient, since the addition of an independent variable into a model always increases the multiple correlation coefficient, even when the added variable is not significant.

The Ratkowsky model for μ_{max} in which only temperature and water activity is included (model (1)) showed a lower adjusted R^2 compared to model (2) in which CO_2 is included. Whether the three-parameter model is sufficient to describe the data could be validated by means of an F-test (Zwietering et al., 1990). In this test, the difference between the residual sum of squares values (RSS) f for model (2) and model (1) was compared to the RSS of model (2). The difference in RSS of model (1) and model (2) is the profit obtained including the CO_2 factor: f is then calculated as follows:

$$f = \frac{(RSS_1 - RSS_2)/(Df_1 - Df_2)}{RSS_2/Df_2}$$

where RSS_1 and RSS_2 are the RSS of model (1) and model (2), respectively, Df_1 and Df_2 are the number of degrees of freedom of model (1) and model (2),

Table 2
Estimated values of the coefficients of three equations for the maximum specific growth rate μ_{max} of *L. sake*

Equationtype	Parameters	Estimated value	95% confidence interval
Eq. (1)	a	0.0141	0.0105–0.0177
	$a_{w\,min}$	0.9561	0.9540–0.9582
	T_{min}	−8.1	−10.2– −6.0
Eq. (2)	b	2.5E-06	1.8E-6–3.2E-6
	$a_{w\,min}$	0.9560	0.9544–0.9576
	T_{min}	−9.0	−10.7– −7.2
	$[CO_{2\,max}]$	6.1E03	5.0E3–7.3E3
Eq. (3)	I_μ	0.90	0.03–1.77
	m_1	−0.61	−0.69– −0.53
	m_2	4.9E-4	1.5E-4–8.3E-4
	m_3	−0.91	−1.77– −0.03
	m_4	$P>0.05$	–
	m_5	$P>0.05$	–
	m_6	$P>0.05$	–
	m_7	−2.3E-6	−3.3E-6– −1.3E-6
	m_8	0.63	0.55–0.72
	m_9	−5.0E-4	−8.6E-4– −1.5E-4

Table 3
Estimated values of the coefficients of three equations for the lag phase λ (h) of *L. sake*

Equation type	Parameters	Estimated value	95% confidence interval
Eq. (4)	c	0.012	0.009–0.014
	$a_{w\ min}$	0.9469	0.9452–0.9486
	T_{min}	−2.31	−2.92– −1.71
Eq. (5)	d	9.3E-07	5.3E-07–1.3E-06
	$a_{w\ min}$	0.9470	0.9455–0.9485
	T_{min}	−2.38	−2.95– −1.81
	$[CO_{2\ max}]$	1.4E4	8.8E3–19E4
Eq. (6)	I_λ	6.04E8	−3.05E16–3.06E16
	l_1	−504	−547– −461
	l_2	0.16	0.09–0.22
	l_3	−5.70E3	−6.11E3–5.29E3
	l_4	0.83	0.63–1.02
	l_5	3.28E-6	1.72E-6–4.82E-6
	l_6	−6.04E8	−3.06E16–3.06E16
	l_7	−1.07E-3	−1.59E-3– −5.55E-3
	l_8	495	451– −540
	l_9	−0.16	−0.23– −0.09

respectively, and equals the number of data points minus the number of parameters of the respective model.

For the comparison of model (2) with model (1) $f=82.8$. This value has to be compared with the F-table value (Df_1-Df_2, Df_2) which equals 3.92. The high level of f compared to F demonstrates the significance of the factor dissolved CO_2, and thus the necessity of incorporating this factor as an independent variable in the model for the maximum specific growth rate of *L. sake*. It has to be remarked that the F-test should be considered as an informal process rather than a rigorous statistical analysis because of the non-linearity of the proposed models (Schnute, 1981).

When the extended Ratkowsky model (2) is compared with the response surface model (3) for μ_{max}, a slightly better correlation of the latter model is noticed. The response surface model did not contain any quadratic terms which were significant ($Prob>|t|>0.05$). At low water activities (<0.970), the response surface model predicts, however, illogical growth rates at high CO_2 concentrations (Fig. 1). At these conditions, higher growth rates are predicted at low temperatures compared to high temperatures. From these results, the importance of thorough evaluation of response surface models becomes evident. In environmental regions at which the target microorganism is highly stressed (e.g., near the minimum a_w), growth experiments result very often in determinations with high experimental error. In these regions response surface models often predict illogical behaviour of the target microorganism. Because of the mentioned anomaly on one hand, the small difference in correlation between models (2) and (3) on the other hand, and because of the higher number of parameters of the response surface model compared to the extended Ratkowsky model (7 and 4, respectively), the latter model should be preferred to predict the maximum specific growth rate of *L. sake*.

The measured maximum specific growth rates were compared with the values predicted by the extended Ratkowsky model in Fig. 2A. The predic-

Table 4
Statistical parameters of the different proposed models for the growth parameters μ_{max} and λ of *L. sake*

	Equation type	Adjusted R^2	F value	$P>F$
μ_{max}	Eq. (1)	0.8412	207	<0.0001
	Eq. (2)	0.9070	285	<0.0001
	Eq. (3)	0.9264	211	<0.0001
λ	Eq. (4)	0.9408	620	<0.0001
	Eq. (5)	0.9501	558	<0.0001
	Eq. (6)	0.9656	421	<0.0001

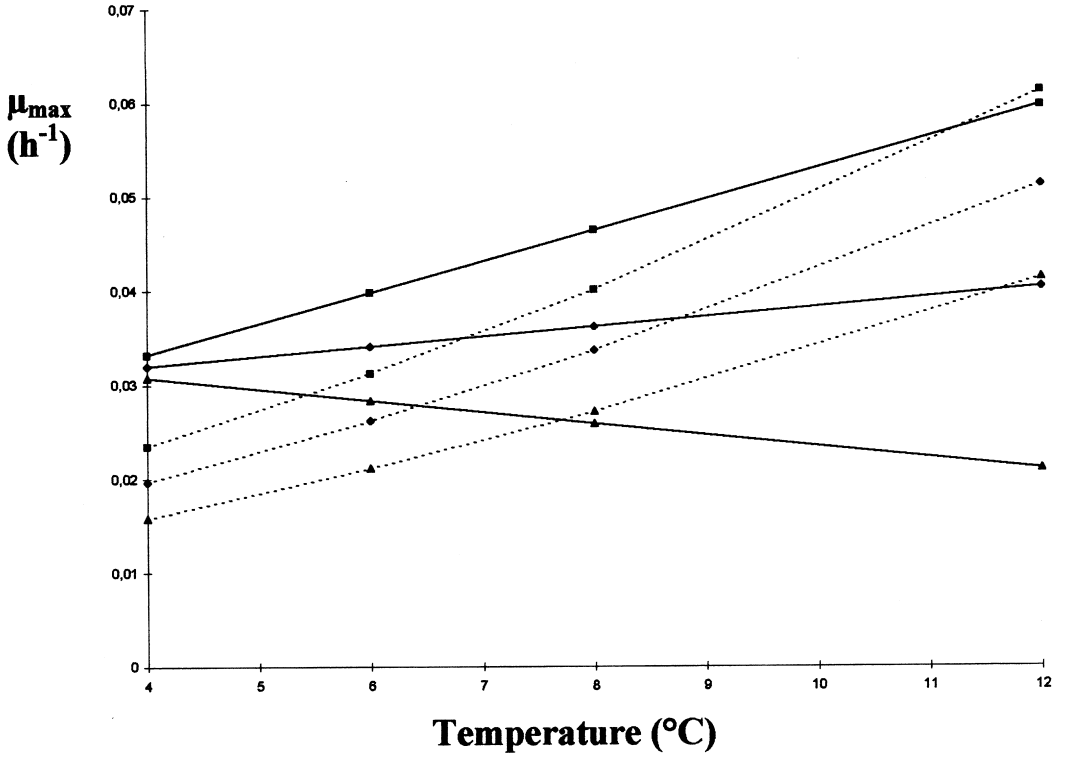

Fig. 1. Illustration of the illogical predictions of μ_{max} (h^{-1}) at low water activities (here $a_w = 0.965$) by the response surface model (Eq. (3)) (full lines) in comparison with the extended Ratkowsky model (Eq. (2)) (dashed lines). (□) 0 ppm CO_2, (△) 1000 ppm CO_2, (◇) 2000 ppm CO_2).

tions were close to the measured values. At high μ_{max} values the model shows a minor tendency of underestimation of μ_{max}.

A good correlation was obtained for all proposed models for λ (Eqs. (4)–(6)). The addition of the concentration of dissolved CO_2 as an independent variable did not result in a much higher adjusted R^2 of the extended Ratkowsky model (Eq. (5)) for λ (Table 4). An F-test, however, proved the significance of including a CO_2 term in the equation ($f = 22.58$ and $F = 3.92$). CO_2 terms also proved to be significant in the response surface equation when an F-test was performed by comparing Eq. (6) with the same equation in which no CO_2 terms were included ($f = 147$ and $F = 2.46$). The significant effect of CO_2 on the lag-phase of *L. sake* is in contradiction with the results which were obtained at constant and high water activities ($a_w = 0.985$) (Devlieghere et al., 1998b). Enfors and Molin (1980) also noticed no significant influence of the presence or absence of CO_2 on the lag phase of *P. fragi*. Farber et al. (1996), however, observed an influence of CO_2 on the growth rate as well as on the lag phase of several microorganisms. The non-significance of CO_2 on the lag phase of *L. sake* can be explained by the low sensitivity in general of lactic acid bacteria for CO_2 (Borch et al., 1996; Stanbridge and Davies, 1998) in combination with the relatively large measuring errors when the lag phase was determined. When the microbial cells become more stressed by low water activities, the effect of CO_2 on the lag-phase of *L. sake* becomes more evident and could be demonstrated by means of the F-test. The estimated values of T_{min}, $a_{w\,min}$ and $[CO_{2\,max}]$ changed with μ_{max} and λ Ratkowsky equations (Table 2 versus Table 3). This illustrates the empirical character of square root models.

Fig. 2B represents the plot of the measured lag

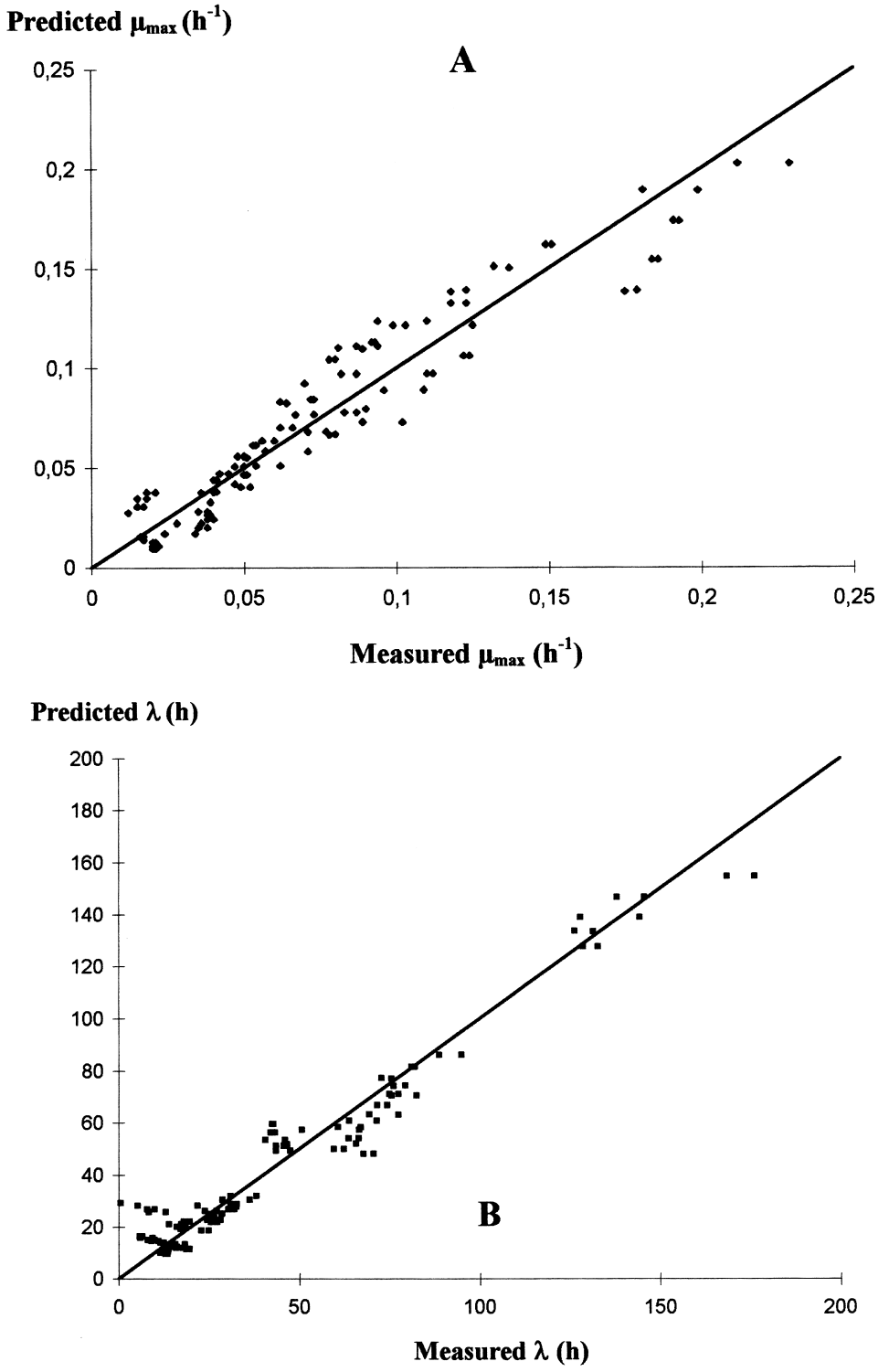

Fig. 2. Comparison of predicted and measured maximum specific growth rate μ_{max} (h^{-1}) (A) and lag phase λ (h) (B) of the extended Ratkowsky models for the growth of *Lactobacillus sake* in modified BHI.

Table 5
Comparison of the extended Ratkowsky models with the model of Kant-Muermans et al. (1997) by means of *M*- and *A*-factors for μ_{max} and λ for observations without CO_2 in the head space and when all observations were included

	μ_{max}		λ	
	0% CO_2	All observations	0% CO_2	All observations
M-factor	1.053	1.278	0.81	0.88
A-factor	1.29	1.39	1.22	1.17
n	12	119	12	119

values versus the values predicted with the extended Ratkowsky model (Eq. (5)). A good correspondence between measured and predicted values was obtained and no systematic deviations could be noticed.

3.2. Validation of the developed model

The proposed extended Ratkowsky models were primarily validated by comparison with the model developed by Kant-Muermans et al. (1997). Comparison of the models was performed by calculating bias and accuracy factors (Ross, 1996) which were modified to make the factors useful for model comparison:

$$M\text{-factor} = 10^{(\sum \log(g_{KM}/g_{PM})/n)} \quad (8)$$

$$A\text{-factor} = 10^{(\sum |\log(g_{KM}/g_{PM})|/n)} \quad (9)$$

where g_{KM} is growth parameter predicted by the model of Kant-Muermans et al. (1997); g_{PM} is growth parameter predicted by the proposed model; *n* is number of predictions used in the calculations.

The *M*-factor is a measure for the mean difference between the predictions of compared models while the *A*-factor is a measure for the mean absolute difference between the predictions of compared models. The *M*-factors and the *A*-factors for μ_{max} and λ are presented in Table 5. Difference is made between the factors for observations without CO_2 in the head space and the factors when all observations were included. When only the observations without CO_2 in the head space are considered, both models correspond well for the maximum specific growth rate. However, when the observations with CO_2 in the head space are included, the extended Ratkowsky model clearly predicts lower growth rates in comparison with the model of Kant-Muermans et al. (1997). This can be explained by the significant negative effect of CO_2 on the growth rate which is not included in the model of Kant-Muermans et al. (1997). In general, the prediction of λ by the extended Ratkowsky model is 12% higher compared to the model of Kant-Muermans et al. (1997).

The developed models were also validated towards experiments performed in gas-packed cooked ham. The combinations of temperature, concentration of dissolved CO_2 and water activity which were tested together with the determined growth parameters of *L. sake* in cooked ham at the corresponding conditions

Table 6
Experimentally determined growth parameters of *L. sake* in gas-packed cooked ham

T (°C)	Dissolved CO_2 (ppm)	a_w	μ_{max} (h^{-1})[a]	λ (h)[a]
10	304	0.980	0.147 (0.120–0.174)	15.6 (9.7–21.5)
10	1062	0.980	0.139 (0.120–0.158)	14.6 (10.4–18.9)
7	302	0.982	0.068 (0.057–0.080)	20.5 (11.3–29.7)
7	1293	0.982	0.063 (0.052–0.075)	24.3 (12.9–35.7)
4	346	0.985	0.046 (0.038–0.054)	43.9 (27.2–60.7)
4	1381	0.985	0.042 (0.036–0.048)	41.9 (26.5–57.2)
10	259	0.979	0.135 (0.118–0.150)	9.8 (5.7–13.9)
10	838	0.979	0.122 (0.099–0.145)	9.6 (2.2–17.1)
7	284	0.980	0.072 (0.063–0.081)	22.9 (14.5–31.4)
7	1111	0.980	0.069 (0.057–0.080)	26.1 (15.9–36.3)

[a]Estimated value (95% lower confidence interval value − 95% upper confidence interval value).

Table 7
Bias and accuracy factors for the extended Ratkowsky models (Eqs. (2) and (5)) and the response surface models (Eqs. (3) and (6)) in comparison to the observed values μ_{max} and λ of *L. sake* in gas-packed cooked ham

	λ		μ_{max}	
	Extended Ratkowsky	Response surface	Extended Ratkowsky	Response surface
Bias factor	1.26	1.27	0.90	0.90
Accuracy factor	1.26	1.27	1.26	1.23
n	10	10	10	10

are listed in Table 6. These data were then used to calculate bias factors and accuracy factors (Ross, 1996) of μ_{max} and λ for the corresponding extended Ratkowsky model and the response surface model (Table 7). Few differences between the factors of both developed models could be noticed. An average overestimation of 10% for μ_{max} and 26% for λ was noted. Almost all predictions of λ were systematically overestimated, as the accuracy factor almost equals the bias factor. From the values of the mentioned factors, one can conclude that both developed models can be applied to predict the microbial shelf life of gas-packed cooked meat products.

3.3. Application of the developed model

The developed models can be very useful in practice to assess in a quick way the influence of one or a combination of the incorporated factors on the microbial shelf life of cooked meat products. The

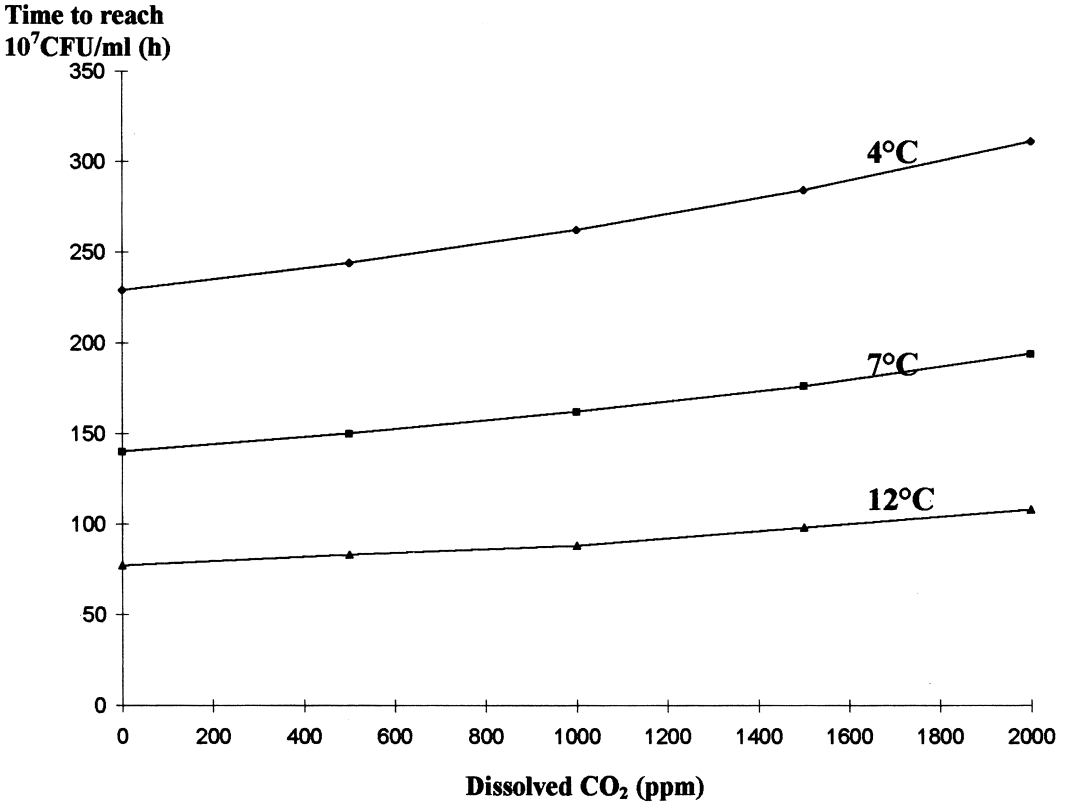

Fig. 3. Temperature dependence of the effect of carbon dioxide on the time (h) to reach 10^7/g *Lactobacillus sake* in modified BHI (inoculation level is 5.10^2/g, $a_w = 0.980$).

influence of temperature and CO_2 on one side and of water activity and CO_2 on the other hand on the time to reach 10^7/g of *L. sake*, when the contamination level is 5.0×10^2/g (a realistic contamination level for cooked meat products), is illustrated in Figs. 3 and 4, respectively.

Temperature as well as water activity proved to have a synergistic action on the shelf life-extending effect of carbon dioxide. This effect of temperature is independent of the influence of temperature on the solubility of CO_2 because differentiation is made of this effect by including the concentration of dissolved CO_2 in the model. The synergistic effect of CO_2, independently of solubility, was already noticed when the effect of dissolved CO_2 and temperature was studied on *L. sake* (Devlieghere et al., 1998a). Enfors and Molin (1980) on the contrary demonstrated the CO_2 inhibitory effect on *Pseudomonas fragi* independent of temperature when taking the higher solubility of CO_2 into account at lower temperatures. However, a similar analysis of data from *Bacillus cereus* showed that the increased solubility of CO_2 at lower temperatures accounted for some but not all of the synergistic effect of reduced temperatures and CO_2. Fernandez et al. (1997) also observed a synergistic inhibitory effect of NaCl and CO_2 on the growth of *Listeria monocytogenes*. *Escherichia coli* O157:H7 was also more inhibited by CO_2 under more inimical conditions of NaCl (Sutherland et al., 1997). However, to study quantitatively the synergistic effect of CO_2 and other inhibitory factors on microorganisms, further investigations on CO_2-sensitive microorganisms will be necessary.

This paper demonstrated that models can be developed to predict the shelf life of gas-packed cooked meat products based on a specific spoilage organism (here *L. sake*). The determination of the

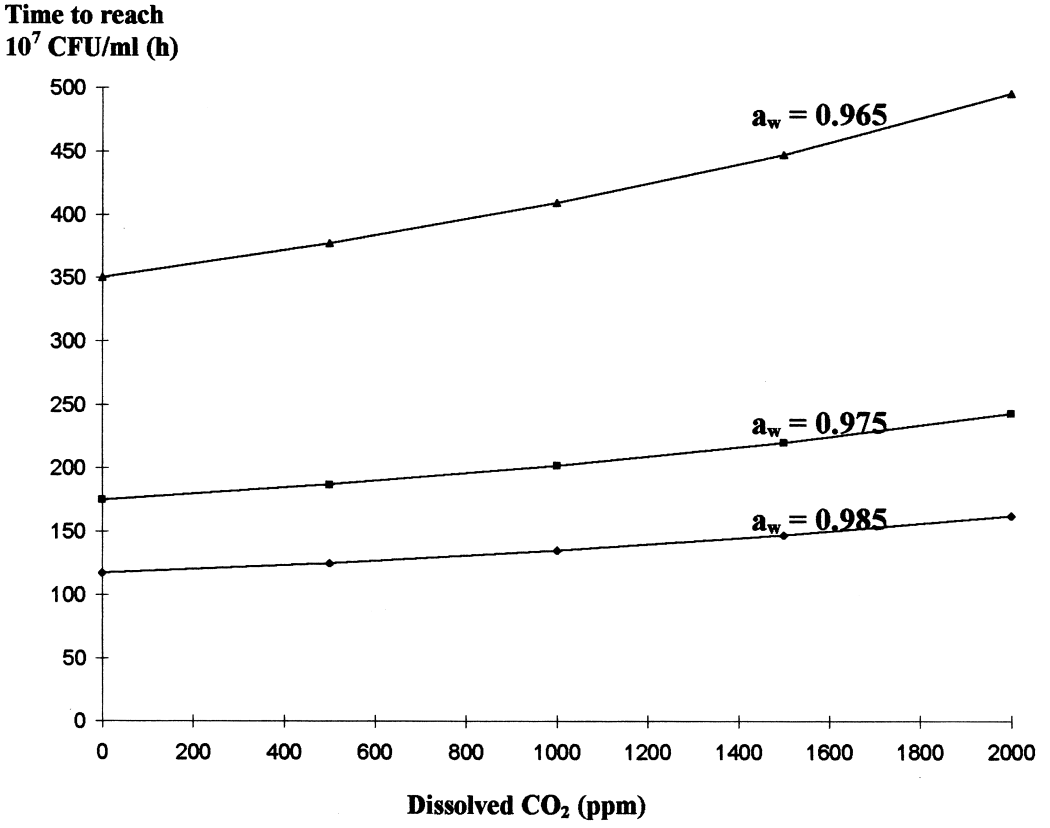

Fig. 4. Water activity dependence of the effect of carbon dioxide on the time (h) to reach 10^7/g *Lactobacillus sake* in modified BHI (inoculation level is 5.10^2/g, $T=7$°C).

shelf life of a food product should, however, not be solely based on the development of the responsible spoilage microorganisms. Safety aspects, i.e. the outgrowth of psychrotrophic food-borne pathogens, will also have to be considered when the shelf life of a product is determined. Future research will have to focus on the development of predictive models for the inhibitory effect of modified atmospheres on the growth of these food-borne pathogens, taking possible interactions with other inhibitory factors into account.

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