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ORIGINAL ARTICLE

Growth of *Aeromonas hydrophila* in modified-atmosphere-packed cooked meat products

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Growth of Aeromonas hydrophila *was investigated in modified-atmosphere-packed cooked meat products by developing predictive models. Modified brain–heart infusion (BHI) was shown to be suitable as a simulation medium for cooked meat products. Predictive models were developed for the growth parameters (maximum specific growth rate and lag phase) of* A. hydrophila *in modified BHI as a function of temperature, water activity and concentration of dissolved carbon dioxide. The growth of* A. hydrophila *was compared with the growth of the Specific Spoilage Organism (SSO) for cooked meat products* Lactobacillus sake*, to determine possible risk areas for* A. hydrophila *in modified atmosphere-packed cooked meat products.* Aeromonas hydrophila *was shown to multiply very rapidly at refrigerated temperatures. The developed models clearly demonstrated however that proliferation of* A. hydrophila *could be prevented by the use of carbon dioxide in the package atmosphere in combination with a decreased water activity (<0·985). Gas-packed cured cooked meat products will not sustain the growth of* A. hydrophila *when kept at refrigerated temperatures (<7°C).*

Introduction

Aeromonas spp. has been increasingly recognized as a potential pathogen of commercial and clinical significance. Several studies demonstrated the presence of virulence factors in *Aeromonas* strains (Granum et al. 1998, Handfield et al. 1996, Kirov and Brodribb 1993) and expression of these virulence factors is shown at refrigerated temperatures (Kirov et al. 1993, Krovacek et al. 1991, Majeed and Mac Rae 1991, Knochel 1989). Kirov and Brodribb (1993) showed that exotoxin production by *Aeromonas* spp. is possible in many sterile refrigerated diluted food slurries. Little is known however about the ability of foods to support exotoxin production by *Aeromonas* spp. at these low temperatures.

Aeromonas has a high incidence in food products. Overall, Hudson et al. (1992) reported 23·2% of the samples of ready-to-eat flesh foods purchased from retail outlets positive for the presence of motile Aeromonads. The reported data vary considerably for cooked meat products. Twelve percent of luncheon meat products and 14% of pates of New Zealand origin were positive for *Aeromonas* (Hudson and De Lacy 1991). In a Swiss distribution study of mesophilic *Aeromonas* species in raw and ready-to-eat fish and meat products by Gobat and Jemmi (1993), sliced cooked ham and smoked cooked sausage showed a prevalence of 38·2% and 15·6% respectively for *Aeromonas* spp.

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Aeromonas hydrophila was isolated from 48% of retail cooked tripes (Hunter et al. 1992) with three of the 21 positive samples yielding very high counts of the organism (more than 10^6 g^{-1}).

The psychrotrophic character of *Aeromonas* has been demonstrated in many types of food products such as vegetables (Jacxsens et al. 1999, Callister and Aggar 1987, Berrang et al. 1989) and raw meat and fish products (Özbas et al. 1996, Doherty et al. 1996, Davies and Slade 1995, Palumbo 1988). Gill and Reichel (1989) demonstrated growth of *A. hydrophila* at -2°C on vacuum packed high pH beef. The inhibitory effect of carbon dioxide in the gas atmosphere on the growth of *Aeromonas* has been reported (Özbas et al. 1996, Doherty et al. 1996, Bennik et al. 1995, Davies and Slade 1995, Hudson et al. 1994, Gill and Reichel 1989). Comparison between investigations regarding the effect of CO_2 in the gas atmosphere on the growth of micro-organisms is however very difficult. Devlieghere et al. (1998a) demonstrated that CO_2 exerts its antimicrobial effect in the water-phase of a food product. The concentration of dissolved CO_2 is influenced by the applied gas/product volume ratio next to other intrinsic and extrinsic factors which makes comparison between results of different authors often impossible.

The aim of this study was to estimate the growth of *A. hydrophila* in cooked meat products packed under modified atmospheres. The effect of temperature, water activity and dissolved carbon dioxide on the growth of *A. hydrophila* was quantified by means of predictive models based on growth parameters of the bacterium in a previously tested simulation medium.

Materials and Methods

Testing of the simulation medium

Modified BHI (brain–heart infusion) (Devlieghere et al. 1998b) was tested for its suitability as simulation medium for the growth of *A. hydrophila* in pasteurised and packed meat products. Growth curves of *A. hydrophila* were determined at four different temperatures (4, 8, 10 and 12°C) in modified BHI and in sterile cooked ham (pH $6{\cdot}12 \pm 0{\cdot}02$; NaCl content: $2{\cdot}41 \pm 0{\cdot}08\%$, dry matter; 261 ± 6 g l^{-1}, residual nitrite content: $22{\cdot}1 \pm 5{\cdot}3$). The pH and the NaCl content of the broth was adjusted to the pH and the NaCl content of the aqueous phase of the cooked ham.

Experiments in broth 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 (Devlieghere et al. 1998a). The glass jars were filled with 100 ml of broth and autoclaved at 121°C for 15 min. The pH was adjusted after autoclaving with filter sterilized 2N HCl. For each growth curve in cooked ham, portions of 13 g of inoculated cooked ham were aseptically transferred in hermetically sealable 50 ml glass containers (20 times).

A cocktail of five strains of *A. hydrophila* (A1, A2, A3, A4 and A5), isolated from chilled fresh food products (respectively halibut, shark, thornback, haricots and sorrel (Neyts et al. 1998)) was used as inoculum. The strains were individually subcultured in BHI-broth (OXOID C(M2)35) for 24 h at 30°C. A second subculture was incubated in BHI-broth for 16 h at 30°C. The inoculum was then stored for 6 h at the appropriate modelling temperature (4°C, 8°C, 10°C or 12°C) to allow the test strains to adapt to the chilling temperature. After the adaptation period, the previously cooled simulation medium or cooked ham was inoculated to a level of 10^4 cfu ml^{-1}.

After inoculation, the jars and the containers with cooked ham were immediately gas packed in 100% N_2 as described by Devlieghere et al. (1998a) and stored at the appropriate temperature. In case of the broth, samples (0·2 ml) were taken at regular time intervals by means of sterile disposable 1 ml syringes, diluted if necessary with Peptone Physiological Salt solution (0·1% peptone, 0·85% NaCl) and plated in duplicate on PCA agar (OXOID CM325) with a Spiral Plater Model D (Spiral Systems inc. Cincinatti, USA). At regular time intervals one portion of packed cooked ham was removed aseptically, diluted with Peptone Physiological Salt solution, homogenized and plated on PCA. The PCA plates were incubated aerobically for 24 h at 30°C.

The maximum specific growth rate (μ_{max}) and the lag phase (λ) of the growth curves 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 7·5.

Development of predictive models for the growth of A. hydrophila *in the simulation medium*

Experimental design. Growth curves were determined in modified BHI at different combinations of temperature (4°C, 8°C, 10°C, 12°C), packaging configuration (different combinations of CO_2 in the gas-phase compensated with nitrogen at a constant gas/product volume ratio of 4/I) and water activity (0·992, 0·986, 0·980 and 0·974). The water activity was altered by means of the addition of NaCl. Growth curves were determined in duplicate.

The concentration of dissolved CO_2 was used as an independent variable for the applied modified atmosphere to exclude the effect of the several intrinsic, extrinsic and process parameters on the final concentration of dissolved CO_2 in the water-phase of the food (Devlieghere et al. 1998a). 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. 1998a).

The growth parameters of *A. hydrophila* for each combination of independent variables 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 7·5.

Type of secondary models. The different types of models investigated are presented in Table 1 (Devlieghere et al. 1999). For equations (M1), (M3), (L1) and (L3) only the data obtained at experimental conditions in the absence of CO_2 in the head space were included and used to determine the respective model coefficients. For the other equations all data were included. The lag phase values were transformed by the square root of the lag phase to limit the influence of the large measuring error at high numerical values. Maximum specific growth rates values were not transformed.

All secondary models were obtained by fitting the data to the respective equation with

Table 1. Different types of investigated secondary models

μ_{max}		SQRT (λ)
(M1) $a(a_w - a_{w\min})(T - T_{\min})^2$	(L1)	$\frac{1}{c\sqrt{(a_w - a_{w\min})}(T - T_{\min})}$
(M2) $b(a_w - a_{w\min})(CO_{2\max} - CO_2)(T - T_{\min})^2$	(L2)	$\frac{1}{d\sqrt{(a_w - a_{w\min})}\sqrt{(CO_{2\max} - CO_2)}(T - T_{\min})}$
(M3) $I_\mu + m_1 T + m_3 a_w + m_4 T^2 + m_6 a_w^2 + m_8 T a_w$	(L3)	$I_\lambda + l_1 T + l_3 a_w + l_4 T^2 + l_6 a_w^2 + l_8 T a_w$
(M4) $I_\mu + m_1 T + m_2 CO_2 + m_3 a_w + m_4 T^2 + m_5 CO_2^2$ $+ m_6 a_w^2 + m_7 T CO_2 + m_8 T a_w + m_9 CO_2 a_w$	(L4)	$I_\lambda + l_1 T + l_2 CO_2 + l_3 a_w + l_4 T^2 + l_5 CO_2^2$ $+ l_6 a_w^2 + l_7 T CO_2 + l_8 T a_w + l_9 CO_2 a_w$

a, b, c and d: constants.
$T_{\min}$ (°C) and $a_{w\,\min}$: respective estimated theoretical minimum temperature and water activity for growth of the organism.
CO_2: concentration of dissolved CO_2 (ppm).
$CO_{2\,max}$: estimated theoretical maximum CO_2 concentration (ppm) for growth of the organism.
I_μ, I_λ: intercepts.
$m_{1\to9}$, $l_{1\to9}$: equation coefficients.

the Levenberg–Marquardt algorithm by means of the statistical package SPSS for windows, version 7·5.

Results and Discussion

Testing of the simulation medium

A first step in model development is the search for a suitable substrate in which data collection (in this study growth curves) will be performed. The necessity of the use of a suitable simulation medium in predictive modelling was already illustrated by Devlieghere et al. (1998b) who demonstrated substantial differences in the growth parameters of *Lactobacillus sake* in a modified MRS medium and in a modified BHI medium. The growth parameters of *A. hydrophila* in a cooked ham and in the simulation medium for pasteurised cooked meat products (modified BHI) at different temperatures are tabulated in Table 2. The growth of *A. hydrophila* was comparable in the simulation medium and in the cooked ham as no significant differences were noticed for λ (h) and μ_{max} (h^{-1}) in both substrates at all investigated temperatures. The growth curves, necessary for the development of a predictive model, were therefore further performed in the modified BHI.

Development of predictive models for the growth of A. hydrophila *in the simulation medium*

Determination of the growth curves. The experimentally determined growth parameters of *A. hydrophila* at different temperatures, concentrations of dissolved CO_2 and water activities are listed in Table 3. All three investigated factors significantly influenced the growth of *A. hydrophila.* At high water activities and in the absence of CO_2 in the gas atmosphere, *A hydrophila* was able to proliferate fast at low temperatures. Many authors reported growth of *A. hydrophila* in refrigerated, high water activity food products. Growth was observed at 3°C in poultry (Özbas et al. 1996), at 0°C in high pH lamb and in trout (Doherty et al. 1996; Davies and Slade, 1995) and even at −1·5°C in vacuum packed sliced roast beef (Hudson et al. 1994) and at −2°C in vacuum packed beef (Gill and Reichel, 1989). In this study the average observed maximum specific growth rate at a water activity of 0·992 and at 4°C was 0·051 h^{-1}. This value is comparable with earlier reported growth rates for *A. hydrophila* in poultry at 3°C (μ_{max} = 0·043 h^{-1}, Özbas et al. 1996) and in nutrient broth (pH = 6·0, concentration NaCl = 1%) at 4°C (μ_{max} = 0·05 h^{-1}, Hudson, 1992).

The growth of *A. hydrophila* was also highly influenced by water activity. The proliferation was limited at the lowest investigated water activity (0·974, corresponding to 4·5% NaCl). Santos et al. (1994) observed a minimum A_w of 0·971 for growth of *A. hydrophila* at 28°C and at pH=7·3 when the water activity of the broth was adjusted with NaCl. Palumbo et al. (1985) however reported a higher minimum A_w (between 0·982 and 0·976) at 5°C and at pH = 7·2. Lowering the water activity will be an important factor in controlling the growth of *A. hydrophila* in vacuum packed cooked meat products as refrigeration temperatures cannot prevent the growth of the organism.

Inclusion of CO_2 in the gas atmosphere highly prolonged λ (h) and decreased μ_{max} (h^{-1}). Several combinations in which CO_2 was included in the gas atmosphere did not sustain the growth of *A. hydrophila* after 60 days, especially at low temperatures. In these conditions, the effect of high concentrations of CO_2 was even shown to be bactericidal as illustrated in Fig. 1. The growth inhibition of *A. hydrophila* by CO_2 at low temperatures has often been reported. Growth did not occur at temperatures $\leq$2°C on beef in an atmosphere of 100% CO_2 (Gill and Reichel, 1989). At 0°C, 20% CO_2 in the gas phase was sufficient to inhibit the growth of *A. hydrophila* on gas packed high pH lamb (Doherty et al. 1996). Bennik et al. (1995) reported a linear decrease in the growth rate of *A. hydrophila* at 8°C, when CO_2 was incorporated in the gas atmosphere. The effect of CO_2 at low and high refrigeration temperatures was also noted by Davies and Slade (1995). Growth of *A. hydrophila* on trout was completely inhibited by an atmosphere containing 60% CO_2 at 0°C while almost no inhibition occurred at 12°C. The effect of dissolved CO_2 in this study was especially pronounced on the

Table 2. Comparison of the growth parameters of *A. hydrophila* in cooked ham and in modified BHI

	Estimated growth parameters			
	Lag phase λ (h)		Max. specific growth rate μ_{max} (h^{-1})	
T (°C)	Cooked ham	Modified BHI	Cooked ham	Modified BHI
4	352[a]	343	0·018	0·019
	(317–387)	(312–374)	(0·014–0·023)	(0·014–0·024)
8	184	165	0·070	0·062
	(163–205)	(135–195)	(0·058–0·082)	(0·057–0·067)
10	104	124	0·090	0·085
	(86–122)	(104–144)	(0·073–0·108)	(0·070–0·100)
12	64	64	0·108	0·097
	(61–67)	(50–72)	(0·084–0·132)	(0·082–0·112)

[a]Estimated growth parameter.
(95% confidence interval)

lag phase of *A. hydrophila*. At 12°C and at a water activity of 0·980, the lag phase was extended tenfold by the addition of 874 ppm CO_2 (42 h to an average of 439 h).

Development of the models. Predictive secondary models were developed to quantify the effect of the three investigated factors. The estimated values of the coefficients of the proposed equations listed in Table 1 for the maximum specific growth rate and the lag phase are given in Tables 4 and 5 respectively. The adjusted determination coefficient was also calculated to make comparison between the models possible.

High adjusted determination coefficients were obtained for equations (M1), (M3), (L1) and (L3). For these equations, only the data obtained at experimental conditions in the absence of CO_2 in the head space were included. The modified Ratkowsky equation performed very well in spite of the limited number of parameters (=3) in comparison with the response surface equation (=6). When models were developed for all data, i.e. including the data obtained under experimental conditions in which CO_2 was included in the head space, lower determination coefficients were obtained. The determination coefficients were especially low for the models for the lag phase (eq. (L2) and eq. (L4)). The measured maximum specific growth rates and lag phases were compared in Fig. 2 with the values predicted by the developed models (M4) and (L4). The sensitivity of *A. hydrophila* to CO_2, especially at low temperatures or low water activities, resulted in high variations of the estimated growth parameters, resulting in low determination coefficients. This was especially the case for the lag phase.

The developed models (M1) and (L1) for the influence of temperature and water activity on the growth parameters of *A. hydrophila* were compared with three existing models (Hudson, 1992, Food Micro Model and Pathogen Modelling Program) by means of previously described (Devlieghere et al. 1999) M-factors and A-factors (Fig. 3). The M-factor is a measure of the mean difference between the predictions of compared models while the A-factor is a measure of the mean absolute difference between the predictions of compared models. An M-factor <1 means that the proposed model generally predicts larger values for a specific growth parameter than the reference model.

The M-factor of the lag phase for all three reference models is >1. The currently developed model for the lag phase, model (L1), thus predicts shorter lag phases in comparison with the three existing models. High differences in the predictions of the lag phase can be due to many reasons with the preparation of the inoculum being the most important. In this study, the inoculum was placed at the modelling temperature for 6 h to allow the test strains to adapt to the chilling temperature before inoculation.

Table 3. Experimentally determined growth parameters of *A. hydrophila* at different temperatures, concentrations of dissolved CO_2 and water activities

Exp. n°	Temperature (°C)	Dissolved CO_2 (ppm)	A_w	μ_{max} (h^{-1})	λ (h)
1	12	0	0·992	0·187	26·9
2	12	0	0·992	0·199	27·2
3	12	415	0·992	0·110	26·3
4	12	415	0·992	0·120	24·7
5	12	1080	0·992	0·082	8·0
6	12	1095	0·992	0·084	7·1
7	12	1441	0·992	0·056	38·2
8	12	1978	0·992	0·058	246·7
9	12	1980	0·992	no growth[a]	
10	12	0	0·986	0·148	2·5
11	12	0	0·986	0·107	21·6
12	12	434	0·986	0·130	64·7
13	12	442	0·986	0·109	68·7
14	12	646	0·986	0·099	142·4
15	12	855	0·986	0·029	208·2
16	12	1479	0·986	no growth	
17	12	1480	0·986	no growth	
18	12	1979	0·986	no growth	
19	12	1982	0·986	no growth	
20	12	0	0·980	0·067	42·2
21	12	439	0·980	0·032	174·9
22	12	425	0·980	0·028	235·9
23	12	874	0·980	0·011	513·9
24	12	874	0·980	0·020	364·4
25	12	0	0·974	0·028	57·6
26	12	439	0·974	0·028	188·6
27	12	462	0·974	0·040	147·6
28	10	217	0·986	0·074	71·2
29	10	215	0·986	0·091	59·8
30	10	697	0·986	0·065	146·8
31	10	697	0·986	0·076	186·7
32	10	1340	0·986	0·044	144·5
33	10	1342	0·986	0·045	159·9
34	8	0	0·992	0·100	17·2
35	8	0	0·992	0·136	39·5
36	8	407	0·992	0·082	164·9
37	8	407	0·992	0·074	105·7
38	8	1114	0·992	0·035	591·7
39	8	1778	0·992	no growth	
40	8	0	0·986	0·086	88·3
41	8	528	0·986	0·067	130·9
42	8	902	0·986	0·069	234·3
43	8	902	0·986	0·062	300·6
44	8	981	0·986	0·022	124·6
45	8	988	0·986	0·024	294·5
46	8	1617	0·986	no growth	
47	8	1620	0·986	no growth	
48	8	2222	0·986	no growth	
49	8	2188	0·986	no growth	
50	8	0	0·980	0·038	165·6
51	8	0	0·974	0·015	343·0
52	8	0	0·974	0·014	289·1
53	4	0	0·992	0·052	80·7
54	4	0	0·992	0·049	68·8
55	4	559	0·992	0·035	115·6
56	4	574	0·992	0·028	124·5

Table 3. Continued

Exp. n°	Temperature (°C)	Dissolved CO_2 (ppm)	A_w	μ_{max} (h^{-1})	λ (h)
57	4	1030	0·992	0·023	199·9
58	4	1041	0·992	no growth	
59	4	1771	0·992	no growth	
60	4	1777	0·992	no growth	
61	4	0	0·986	0·045	265·1
62	4	0	0·986	0·046	271·5
63	4	317	0·986	0·028	411·1
64	4	317	0·986	0·037	473·0
65	4	567	0·986	0·013	1230·0
66	4	1109	0·986	no growth	
67	4	1120	0·986	no growth	
68	4	1778	0·986	no growth	
69	4	1769	0·986	no growth	
70	4	2411	0·986	no growth	
71	4	2403	0·986	no growth	
72	4	0	0·980	0·007	428·3
73	4	0	0·980	0·006	408·8
74	4	0	0·974	0·011	414·7
75	4	0	0·974	0·007	259·3

[a]no growth observed in 60 days.

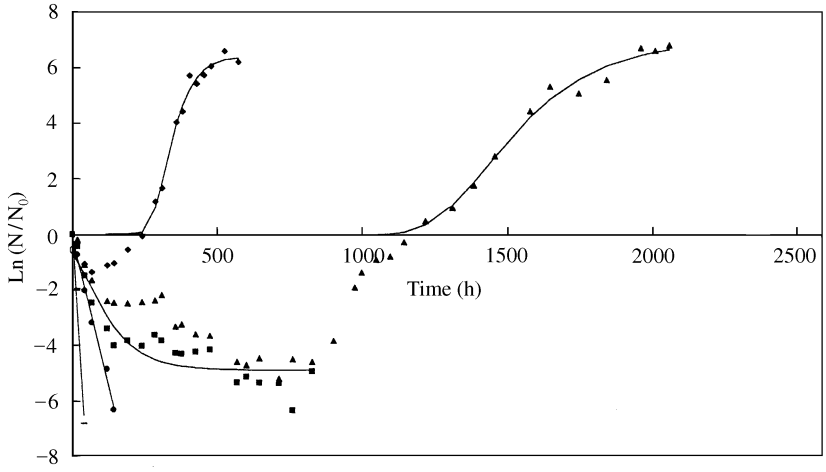

Figure 1. Influence of CO_2 on the growth of *A. hydrophila* at 4°C, at a water activity of 0·9885 and at different concentrations of dissolved CO_2 (◆ = 0 ppm, ▲ = 567 ppm; ■ = 1109 ppm, ● = 1778 ppm, – = 2411 ppm).

The Food Micro Model is predicting comparable maximum specific growth rates (M-factor and A-factor are close to one) while the predictions of μ_{max} by the other two existing models are much lower than the predictions of model (M1). The maximum specific growth rate is not very dependent on the inoculum preparation but can also differ because of the different types of basic broths applied to prepare the simulation medium. It is therefore essential to test the applied simulation medium for its simulation ability by comparing growth parameters in the broth and in the specific food products before using the broth for modelling.

The models, including dissolved CO_2 as an independent variable, could not be compared

Table 4. Estimated values of the coefficients of four equations and adjusted correlation coefficient for the maximum specific growth rate μ_{max} of *Aeromonas hydrophila*

Equation type	Parameters	Estimated value	95% confidence interval
Eq (1)	a	0·032	0·021–0·043
	$A_{w\ min}$	0·9718	0·9698–0·9737
	T_{min}	−5·25	−7·75–−2·75
	Adjusted R^2	0·9574	
Eq (2)	b	1·17E-05	5·89E-06–1·74E-05
	$A_{w\ min}$	0·9715	0·9691–0·9739
	T_{min}	−5·68	−8·84–−2·53
	$CO_{2\ max}$	2·30E03	1·90E3–2·69E3
	Adjusted R^2	0·8074	
Eq (3)	I_μ	89·6	−75·5–254·8
	m_1	−0·80	−1·07–−0·52
	m_3	−181	−518–154
	m_4	1·30E-04	−7·27E-04–9·88E-04
	m_6	92·1	−78·9–263·2
	m_8	0·82	0·54–1·10
	Adjusted R^2	0·9551	
Eq (4)	I_μ	74·6	−72–221
	m_1	−0·74	−1·02–−0·45
	m_2	5·7E-3	2·7E-03–8·6E-03
	m_3	−151	−449–146
	m_4	1·24E-04	−5·65E-04–8·12E-04
	m_5	4·62E-08	2·28E-08–6·97E-08
	m_6	77	−74–228
	m_7	−10·0E-06	−14·9E-06–−5·1E-06
	m_8	0·76	0·47–1·05
	m_9	−5·7E-03	−8·8E-03–−2·7E-03
	Adjusted R^2	0·8477	

with reported literature data as no models are available regarding the effect of dissolved CO_2 on the growth of *A. hydrophila*.

Application of the developed models. Predictive models are useful tools in the assessment of the microbial safety of specific food products. By means of models, global risk areas can be detected for a food pathogen in a specific food product which have to be fine tuned by additional experiments when required. Previously, a predictive model for spoilage of modified atmosphere packed cooked meat products was developed and validated (Devlieghere et al. 1999). This model was based on the growth of *Lactobacillus sake* as a Specific Spoilage Organism (SSO) for these type of products. The dashed lines of Fig. 4 represent the time to reach 10^7 cfu g^{-1} of *L. sake* for different water activities when the initial lactic acid load of the meat product was $5{\cdot}10^2$ cfu g^{-1}. The time to reach a 2 log increase of *A. hydrophila* is represented by the full lines and was calculated with the actual developed models (equations (M1) and (L1)). No data concerning the dose/response values for pathogenic *Aeromonas* species are available until now. The 2 log increase was chosen as it represents significant growth of the opportunistic pathogen without claiming that this increase is a condition for illness by the organism when ingested.

When stress conditions are present (low water activity and low temperature), spoilage occurs much faster than outgrowth of *A. hydrophila*. For high water activity cooked meat products however, temperature abuse (e.g.

Table 5. Estimated values of the coefficients of four proposed equations for the square root of the lag phase λ (h) of *A. hydrophila*

Equation type	Parameters	Estimated value	95% confidence interval
Eq (5)	c	1·43E-01	0·85E-01–2·01E-01
	$A_{w\,min}$	0·9740	0·9706–0·9774
	T_{min}	−4·85E-01	−2·27–1·31
	Adjusted R^2	0·8826	
Eq (6)	d	1·13E-03	0·27E–03-1·99E-03
	$A_{w\,min}$	0·9638	0·9546–0·9730
	T_{min}	−0·43E01	−0·86E01–1·0E-03
	$CO_{2\,max}$	2·1E03	1·1E3–3·2E3
	Adjusted R^2	0·4283	
Eq (7)	I_λ	−1·5E04	−4·9E04–1·8E04
	l_1	−44·7	−101·1–11·7
	l_3	3·2E04	−3·7E04–10·1E04
	l_4	−4·8E-02	−2·2E-01–1·3E-01
	l_6	−1·7E04	−5·2E04–1·8E04
	l_8	44·9	−12·6–102·4
	Adjusted R^2	0·8264	
Eq (8)	I_λ	−1·8E04	−5·3E04–1·E04
	l_1	−35·6	−105·1–33·8
	l_2	4·7E-01	−2·5E-01–1·2
	l_3	3·7E04	−3·4E04–1·1E-05
	l_4	5·3E-02	−1·1E-01–2·2E-01
	l_5	7·6E-08	−5·6E-06–5·7E-06
	l_6	−1·9E04	−5·6E04–1·7E04
	l_7	−3·5E-04	−1·5E-03–8·4E-04
	l_8	3·4E01	−3·6E01–1·1E02
	l_9	−4·6E-01	−1·2–2·7E-01
	Adjusted R^2	0·5745	

12°C) could allow proliferation of the pathogen before spoilage has occurred.

Due to the sensitivity of *A. hydrophila* to CO_2, inclusion of CO_2 in the head space which results in a significant amount of dissolved CO_2 in the water-phase of the product, will retard the development of the organism. At 12°C and at a water activity of 0·990, inclusion of 800 ppm dissolved CO_2 resulted in a 2 log increase of *A. hydrophila* after 272 h compared to 107 h when no CO_2 was added. The effect of CO_2 at 7°C on the growth of *A. hydrophila* (full lines) and of the SSO *Lactobacillus sake* (broken lines) is illustrated in Fig. 5. CO_2 inhibits the growth of *A. hydrophila* to the extent that spoilage will occur much faster than development of *A. hydrophila*.

Conclusions

A. hydrophila was shown to multiply very rapidly at refrigerated temperatures. At high water activity (0·992), a generation time of 13·6 h was noticed at 4°C. The developed models clearly demonstrated, however, that proliferation of *A. hydrophila* could be prevented by the use of CO_2 in the packaging atmosphere in combination with a decreased water activity (<0·985). The water activity of cured cooked meat products is generally about 0·980–0·960 (Roberts et al. 1998). Gas packed cured cooked meat products will not sustain the growth of *A. hydrophila* when kept at refrigerated temperatures (<7°C).

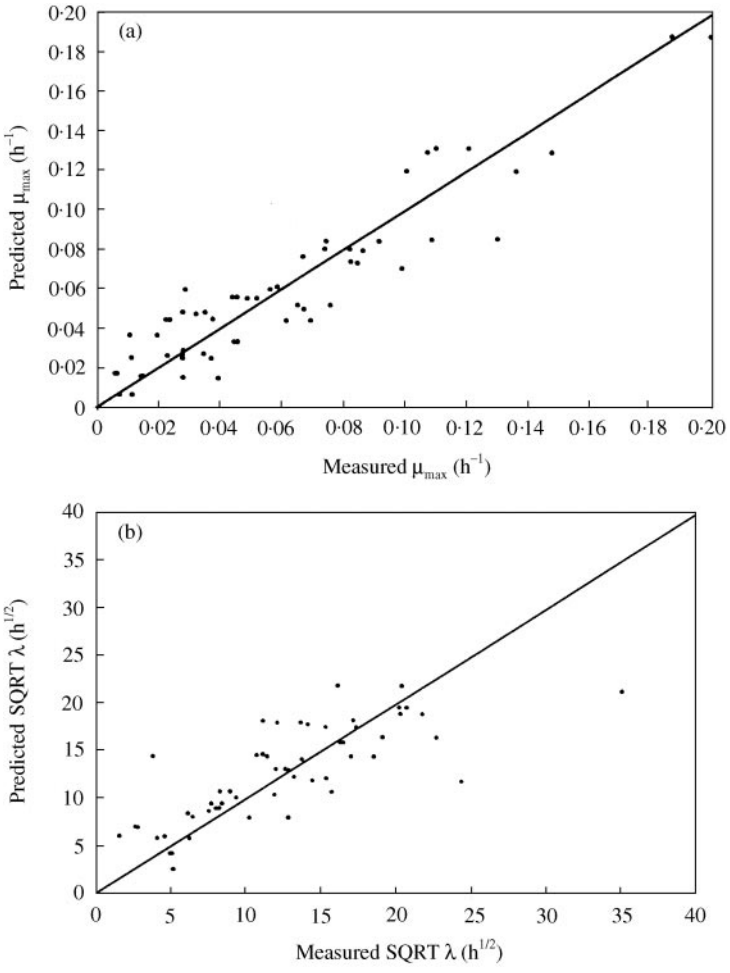

Figure 2. Measured maximum specific growth rate μ_{max} (h^{-1}) (a) and square root of the lag phase SQRT(λ) ($h^{1/2}$) (b) vs predicted values models (4) and (8) respectively.

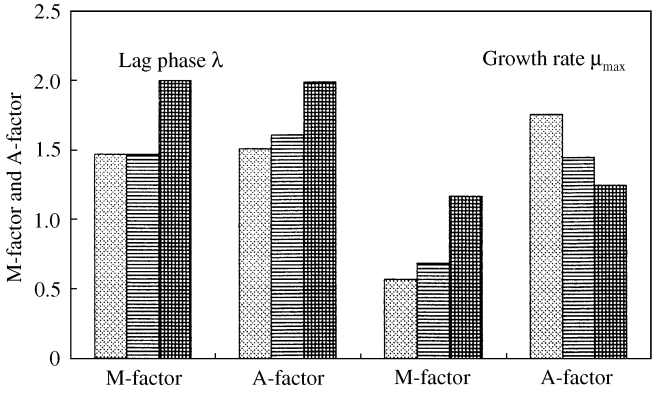

Figure 3. M-factors and A-factors of the lag phase λ and the maximum specific growth rate μ_{max} of the models (5) and (1) respectively in comparison with the models of Hudson (1992) (▨), Pathogen Modeling Program (▤) and Food Micro model (▦).

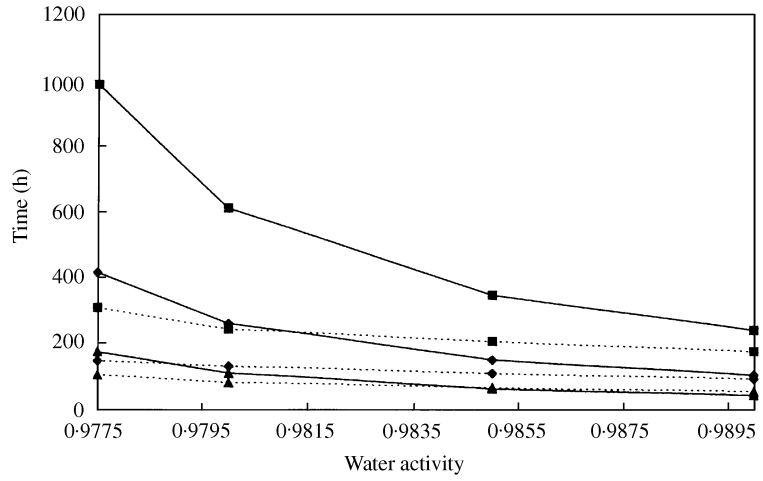

Figure 4. Time (h) to reach a 2 log increase for *A. hydrophila* calculated with models M1 and L1 (full lines) and time (h) to reach 10^7/g for *L. sake* (initial contamination with lactic acid bacteria is $5{\cdot}10^2$/g) calculated with the model of Devlieghere et al., 1999 (broken lines) at different temperatures (■ = 4°C, ◆ = 7°C, ▲ = 12°C) and water activities in the absence of carbon dioxide.

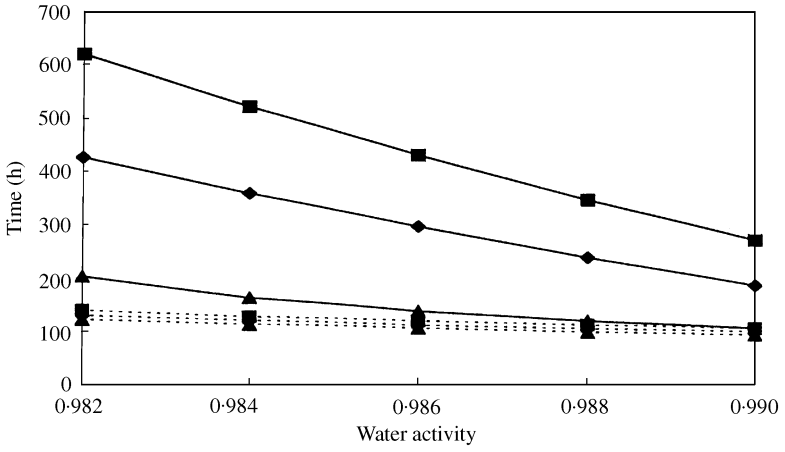

Figure 5. Time (h) to reach a 2 log increase for *Aeromonas hydrophila* calculated with models M4 and L4 (full lines) and time (h) to reach 10^7 g^{-1} for *L. sake* (initial contamination with lactic acid bacteria is $5{\cdot}10^2$ g^{-1}) calculated with the model of Devlieghere et al., 1999 (broken lines) at different concentrations of dissolved carbon dioxide (■ = 800 ppm, ◆ = 400 ppm, ▲ = 0 ppm) and water activities at 7°C.

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