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Application of an iterative approach for development of a microbial model predicting the shelf-life of packed fish

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Abstract

An iterative approach was used to develop a microbial model for shelf-life prediction of cod fillets packed in modified atmospheres. The effect of temperature (0–15°C) and CO_2 (0–100%) on growth of the specific spoilage organism, *Photobacterium phosphoreum*, was studied in packed cod and in liquid media. *P. phosphoreum* was a dominant part of the spoilage microflora of packed cod stored at the extremes of the range of conditions studied. The organism is therefore likely to be important for spoilage and the development of a microbial model within this domain seems relevant. A liquid medium was developed to provide growth kinetics of *P. phosphoreum* similar to those observed in packed cod. Using this medium, the effect of temperature and CO_2 on the maximum specific growth rate of *Photobacterium phosphoreum* was determined by absorbance measurements and modelled by a square root equation and by a polynomial equation. Product validation studies were carried out during summer and winter using naturally contaminated packed cod fillets which were stored at constant and at changing temperatures. The shelf-life of the packed fillets was predicted on the basis of the initial numbers of *P. phosphoreum*, product temperature profiles and the level of CO_2 in the modified atmosphere. The average deviations between shelf-life determined by sensory evaluation and shelf-life predicted by the square root equation and by the polynomial equation were 17% and 9%, respectively. © 1997 Elsevier Science B.V.

Keywords: Predictive microbiology; Iterative approach; Fish spoilage; *Photobacterium phosphoreum*; Temperature; CO_2

1. Introduction

Fresh fish is perishable and shelf-life may vary from batch to batch. Accurate prediction of shelf-life is, therefore, particularly important to ensure product quality. Different approaches to shelf-life estimation has been used. The effect of the storage temperature was described by relative rate of spoilage models and the responses of numerous sensory, microbial, chemical, biochemical and physical measurements have been correlated with remaining product shelf-life. Temperature models may predict the slope of the regression lines relating sensory or microbial measurements and remaining shelf-life. However, in general the regression models are only applicable to

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products stored at a given condition (Spencer and Baines, 1964; Olley and Ratkowsky, 1973; Gibson, 1985; Bremner et al., 1987). More recently, multivariate statistical methods have been used to relate the sensory attributes of products with spectroscopic or chromatographic measurements (Girard and Nakai, 1994; Jørgensen and Jensen, 1997). Shelf-life predictions or grading of quality may be obtained in this way, but again the predictions are only valid under the storage conditions studied for model development.

Predictive microbiology is an approach to shelf-life prediction that is very different from the product studies mentioned above. A general predictive modelling approach has been suggested and some microbial spoilage models have been developed (Doe and Heruwati, 1988; McMeekin et al., 1993; McClure et al., 1993, 1994). The effect of initial product contamination, product characteristics and storage conditions can be included in predictive models. However, specific spoilage organisms (SSOs) and spoilage reactions in seafood change depending on intrinsic and extrinsic parameters (Huss et al., 1997). Prediction of shelf-life by microbial models is therefore no easy task and an iterative approach taking into account the dynamic nature of microbial seafood spoilage has been suggested for development of spoilage models (Dalgaard, 1995a). The basic idea behind the iterative approach is to validate the components used for model development e.g. the SSO, the spoilage domain, and the growth medium, before large amounts of data are generated and modelled. This philosophy is the same as in quality assurance systems where end-products testing is reduced and more efforts are placed on controlling critical steps in the processing. The iterative approach can be divided into: (i) Initial studies with naturally contaminated products, where SSOs, their spoilage domains, and growth kinetics are determined. The spoilage domain is the range of conditions where a SSO causes spoilage of a product (ii) Generation and mathematical modelling of data, where the effects of extrinsic and intrinsic parameters are quantified and data fitted by mathematical equations and (iii) Product validation, where the final model is compared to data from storage experiments. Thus, the initial studies of naturally contaminated products is the major difference between the general and the iterative approaches for development of predictive models.

P. phosphoreum is the SSO of modified atmosphere-packed (MAP) cod when stored at chill temperatures (Dalgaard, 1995b). The objective of the present study was to test the possibility of developing an accurate spoilage model for MAP cod fillets by using the iterative approach. The combined effect of temperature (0–15°C) and CO_2 (0–100%) on growth of *Photobacterium phosphoreum* and on the shelf-life of MAP cod was studied.

2. Materials and methods

2.1. Initial studies with naturally contaminated products

Cod (*Gadus morhua*) from Denmark was kept in ice for less than one day after catch until they are filleted and packed. Skin-off fillets of 100–200 g were packed in two volumes of 100% N_2 or 100% CO_2 in Riloten bags with low gas permeability as previously described (Dalgaard et al., 1993). The fish was stored at 0°C and 15°C. Two different batches of cod were studied at each of the four storage conditions. Growth curves with approximately fifteen replicated measurements of *P. phosphoreum* (see Section 2.4) were obtained for each batch. Estimates of the lag phases, maximum specific growth rates (μ_{max}) and maximum cell numbers (N_{max}) were obtained by the log-transformed form of the 4-parameter Logistic model (Dalgaard, 1995a). Estimates of variation for significance testing were obtained from the replicated experiments. The 3-parameter Logistic model (Eq. (1)) was fitted to growth curves without lag phases.

$$\log(N_t) = \log\left\{ N_{max} / \left[1 + \left(\frac{N_{max}}{N_0} - 1 \right) \times \exp(-\mu_{max} \times t) \right] \right\} \quad (1)$$

In Eq. (1), t is the time (h), N_t the number of micro-organisms at time t (cfu/g), N_{max} the maximum asymptotic number of microorganisms (cfu/g), N_0 the initial number of microorganisms (cfu/g) at time zero and μ_{max} the maximum specific growth rate (h^{-1}).

The effect of temperature and CO_2 on μ_{max} values was fitted with a simple interpolation model (Eq. (2))

where T is the temperature (°C) and C the level of carbon dioxide (% CO_2).

$$\mu_{max} = p_{2a} + p_{2b} \times T + p_{2c} \times C + p_{2d} \times T \times C \quad (2)$$

The shelf-life, in the eight storage experiments, was determined by assessment of off-odours and at the time of sensory rejection the microflora in the fillets was evaluated by direct microscopy (see Section 2.4).

2.2. Generation and mathematical modelling of data

2.2.1. Inoculum and growth media

A mixture of eight strains of *Photobacterium phosphoreum* (MIX-Pp) was studied. The isolates has been deposited in the National Collections of Industrial and Marine Microorganisms (NCIMB). MIX-Pp was prepared as previously described with strains from spoiled MAP cod stored at 0°C (NCIMB 13476-81) or at 15°C (NCIMB 13482-83) (Dalgaard, 1995a).

Brain Heart Infusion (BHI) (Oxoid CM 225), Tryptone Soya Broth (TSB) (Oxoid CM 129) and a growth medium (GM) with different levels of NaCl were tested for generation of growth data. GM contained: 1% Lab-Lemco powder (Oxoid), 1% Bacto-peptone (Difco), 0.75% NaCl, 0.4% trimethylamine-oxide dihydrate (Fluka), 0.3% yeast extract (Oxoid), 0.3% H_2KPO_4, 0.3% HK_2PO_4, 0.075% $MgSO_4 \cdot_7 H_2O$, 0.075% KCl, and 0.0014% $FeSO_4 \cdot 7 H_2O$.

2.2.2. Selection of a liquid medium

The objective was to select or develop a medium that providing the same growth kinetics of MIX-Pp as observed for *P. phosphoreum* in naturally contaminated MAP cod fillets stored at different temperatures and CO_2 levels. Growth in BHI, TSB and GM containing 0.50% and 0.75% NaCl was studied in Hungate tubes at 0.0°C and 15.0°C with 100% N_2 and 100% CO_2 (see Section 2.2.3). At each storage condition, two growth curves with 12 to 16 measurements of MIX-Pp was obtained by spread plating on Long and Hammers medium (see Section 2.4). Each Hungate tube was only sampled once. The log-transformed 4-parameter Logistic model (Eq. (3)) was used to estimate μ_{max}-values.

2.2.3. Growth experiments in Hungate tubes

The Hungate technique was used to quantify the effect of temperature and CO_2 of growth of MIX-Pp as well as for the evaluation of growth media. Media were autoclaved (121°C, 15 min) in flasks with cotton stopper that allowed diffusion of gas into the flask. Flasks with chilled sterile media were incubated for 24 h at 0–2°C in anaerobic jars with different modified atmospheres as previously described (Dalgaard, 1995a). After this pre-incubation, the pH of media was adjusted to 6.6. Media were added pre-cultures of MIX-Pp to a final cell concentration of 10^3 cfu/ml. Seven ml of inoculated media were then transferred to sterile Hungate tubes (Bellco Hungate tubes, A/S E. Pedersen and Søn, Oslo, Norway) under a flow of a sterile modified atmosphere. The tubes were sealed with a sterile butyl-rubber stopper.

Growth of MIX-Pp in Hungate tubes was measured by absorbance at 540 nm (Novaspec II, Pharmacia Biotech, Allerød, Denmark) without opening the tubes. Growth curves with approximately thirty measurements were produced and μ_{max} was estimated by fitting of the non-transformed 4-parameter Logistic model (Eq. (3)).

$$ABS_t = ABS_{min} + \frac{ABS_{max} - ABS_{min}}{1 + \exp(-\mu_{max}(t - t_i))} \quad (3)$$

In Eq. (3), t is the time (h), ABS_t is the absorbance at time t (absorbance units), ABS_{min} and ABS_{max} the minimum and maximum asymptotic absorbance values, μ_{max} the maximum specific growth rate (h^{-1}) and t_i the inflection point, i.e. the time when $ABS_t = ABS_{max}/2$.

2.2.4. Effect of temperature and CO_2 on μ_{max}

The effect of temperature (0, 3, 6, 9, 12 and 15°C) and CO_2 (0%, 25%, 50%, 75% and 100%) on μ_{max} of MIX-Pp was quantified in a full factorial experiment. Duplicate experiments were carried out for all storage conditions. In addition, experiments were repeated at 0°C and 15°C with 0% and 100% CO_2 and at 6°C with 0%, 50% and 100% CO_2. In this way 74 μ_{max} values were estimated from the absorbance growth curves by Eq. (3).

2.2.5. Modelling of the μ_{max} data

Eq. (4) (Ratkowsky et al., 1982) and Eq. (5) (Dalgaard, 1995a) were fitted to μ_{max} values ob-

tained at constant CO_2 levels and constant temperatures, respectively. Eqs. (6) and (7) were fitted to the entire μ_{max} data set.

$$\sqrt{\mu_{max}} = b_4 \times (T - T_{min}) \quad (4)$$

$$\sqrt{\mu_{max}} = b_5 \times (\%CO_{2max} - \%CO_2) \quad (5)$$

$$f(\mu_{max}) = f(\{b_6 \times (T - T_{min}) \times [(\%CO_{2max} - \%CO_2)/\%CO_{2max}]\}^2) \quad (6)$$

$$f(\mu_{max}) = p_{7a} + p_{7b} \times T + p_{7c} \times C + p_{7d} \times T \times C + p_{7e} \times T^2 + p_{7f} \times C^2 \quad (7)$$

In Eqs. (4) and (5) T_{min} and $\%CO_{2\ max}$ are the temperature (°C) and the percentage of CO_2 where μ_{max} theoretically becomes zero. In Eqs. (6) and (7), f represents three different transformations of the μ_{max} data i.e. no transformation, a square root-transformation and a logarithmic transformation. As in most systems studied in predictive microbiology only the response variable of the polynomial model (Eq. (7)) was transformed.

Standard software was used to fit the primary (Eqs. (1) and (3)) and secondary (Eqs. (2), (4)–(7)) growth models and for the analyses of variance (Anon., 1993a,b). The statistically significant parameters in Eq. (7) were determined by stepwise regression (F-value equal to 4). F-tests for lack-of-fit (F_{lof}) indicated the goodness-of-fit of Eqs. (6) and (7). The sum of squares of pure error (SS_{pe}) was calculated from the replicated μ_{max} values. The sum of squares of lack-of-fit (SS_{lof}) was then determined as the difference between the residual sum of squares (RSS) obtained by model fitting and SS_{pe} (Weisberg, 1985, pp. 89–99).

2.3. Product validation

Eqs. (6)–(8) were tested by comparison of predicted shelf-life and shelf-life observed in independent storage experiments. Series of product validation experiments were carried out in summer (June) and winter (January). Each series included four storage experiments with skin-off cod fillets (*Gadus morhua*) from the Baltic sea. The fillets were packed (see Section 2.1) with two volumes of a modified atmosphere containing 60% CO_2 and 40% N_2. The packed fillets were randomly divided into four batches. Batch I was stored at 0°C, batch II at 0°C initially and then transferred to 5°C, batch III at 0°C and then transferred to 10°C, batch IV at 5°C initially and then transferred to 0°C. In the summer and winter experiments batches II–IV were initially kept for 69 h and 92 h, respectively before transferring to new storage temperatures. From each batch, temperature profiles in two packs were recorded by loggers (EPI 125, ebro Electronics GmbH, Ingolstadt, Germany) and in two other packs the level of CO_2 was measured after four days of storage by gas chromatography (Dalgaard et al., 1993). Numbers of *P. phosphoreum*, total viable counts (LH plates), and the development of off-odours (see Section 2.4) were analysed at regular intervals during storage in two packs from each batch of fillets. In the summer experiments the spoilage microflora was also evaluated by direct phase contrast microscopy and ten colonies from each batch were representatively isolated from the L and H plates. The isolates were characterized as previously described (Dalgaard et al., 1997) by tests for shape, size, motility, Gram reaction, oxidase, catalase, Hugh and Leifson, o/129 sensitivity, production of NH3 from arginine, utilization of D-mannitol, reduction of TMAO and NO_3^- and growth at 0°C and 35°C. The isolates were tentatively identified (Dainty et al., 1979; Dalgaard et al., 1997).

Growth of *P. phosphoreum* and shelf-life of MAP cod fillets were predicted by Eq. (1) and by the spoilage criterion developed from the initial product studies (see Section 2.1). Values of μmax were obtained by Eqs. (6)–(8) at the storage conditions measured. A simple spreadsheet was used for stepwise calculation of growth from the recorded temperature profiles.

2.4. Analyses

Fifty grams of fillets were diluted fivefold in chilled (5°C) physiological saline (0.85% NaCl and 0.1% bacto-peptone) and homogenized for 30 s in a stomacher. When needed for viable counting the homogenate was further diluted in physiological saline. In naturally contaminated cod (see Section 2.2 Section 2.4), the numbers of *P. phosphoreum* were specifically detected by a Malthus conductance method, total viable counts (TVC) were determined by spread plating on Long and Hammers medium with

1% NaCl (LH), incubated for 7 days at 15°C, the microflora in the spoiled cod fillets was evaluated by direct microscopy as previously described (Dalgaard et al., 1996). The development of off-odours in the MAP cod fillets was evaluated by two trained panellists. Two packs were analysed at each sampling time and a batch of fillets was rejected when both panellist detected unacceptable off-odours in at least one of the two packs.

3. Results

3.1. Initial studies with naturally contaminated products

Specific enumeration and direct microscopy determined *P. phosphoreum* as a dominant part of the spoilage microflora in MAP cod fillets stored with 100% N_2 and 100% CO_2 at both 0°C and at 15°C. *P. phosphoreum* is known to have a high spoilage activity (Dalgaard, 1995b). The organism is therefore likely to be important for spoilage and development of a microbial spoilage model within the range of conditions studied seems relevant. The importance of *P. phosphoreum* was confirmed by the product validation studies where strains were isolated and identified (see Section 3.3).

No statistically significant lag phases of *P. phosphoreum* were observed except at 15°C with 0% CO_2 where a short lag phase of 3.3±0.1 h was found. Eq. (1) was therefore an acceptable growth model (Fig. 1). Temperature and CO_2 had no significant effect of Log(N_{max}) being 7.9±0.4 (SD). With a constant N_{max}-value and Eq. (1) as the primary growth model, estimates of N_0 and μ_{max} were sufficient to predict growth of *P. phosphoreum*. The interpolation model developed from the initial studies with naturally contaminated products and including the effect of temperature and CO_2 (Eqs. (2) and (8)) described 99.3% of the total variability of the μ_{max} data set. In Eq. (8), T is temperature (0–15°C) and C the level of carbon dioxide (0–100% CO_2).

$$\mu_{max} = 0.082 + 0.032 \times T - 3.6 \times 10^{-4} \times C - 1.1 \times 10^{-4} \times T \times C \quad (8)$$

The MAP cod fillets did not spoil until the numbers of *P. phosphoreum* had reached and remained at their maximum level for some time (Fig. 1). On average the fillets spoiled four generation times ($t_g = \mathrm{Ln}\ 2/\mu_{max}$) after *P. phosphoreum* reached the inflection point of the Logistic growth curve. A spoilage criterion for prediction of shelf-life was derived from different parametrizations of Eq. (1). The inflection point ($t = t_i$) was expressed as a function of N_0 and N_{max} and the spoilage criterion (Eq. (9)) was obtained as the storage time equal to $t_i + 4 \times t_g$. With this spoilage criterion shelf-life could be predicted on the basis of N_0 and μ_{max}-values only.

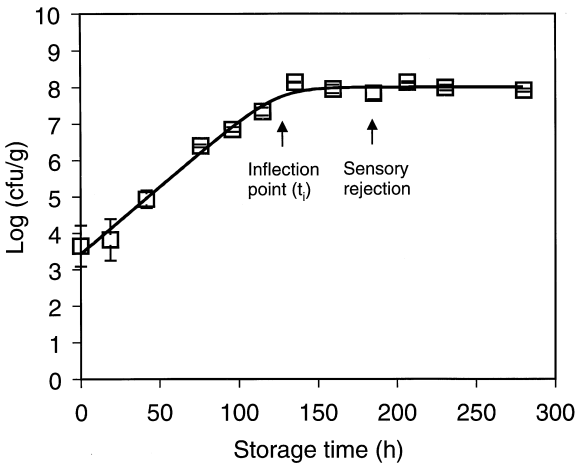

Fig. 1. Growth of *P. phosphoreum* in naturally contaminated cod fillets stored at 0°C in an atmosphere with 100% N_2. Error bars indicate standard deviations ($n = 2$).

$$\text{Spoilage criterion} = \frac{4 \times Ln2 + Ln[(N_{max}/N_0) - 1]}{\mu_{max}} \quad (9)$$

3.2. Generation and mathematical modelling of data

3.2.1. Selection of a liquid medium

Growth rates of *P. phosphoreum* were considerably lower in BHI and TSB than in MAP cod fillets (Table 1). Consequently, these substrates cannot be used for the development of an accurate spoilage model. However, similar μ_{max}-values were obtained in MAP cod and in GM with 0.75% NaCl. In this liquid medium, growth rates determined from viable counts and absorbance measurements were also

Table 1
Maximum specific growth rates (μ_{max}, h^{-1}) of *Photobacterium phosphoreum* determined in naturally contaminated MAP cod fillets and in liquid media

	0°C		15°C	
	0% CO_2	100% CO_2	0% CO_2	100% CO_2
MAP cod: Specific counting of *P. phosphoreum*				
	0.082±0.004[a]	0.045±0.006	0.57±0.024	0.37±0.052
Liquid media: Viable counts				
BHI	0.044±0.001	0.029±0.002	0.48±0.034	0.33±0.004
TSB	0.042±0.004	0.035±0.013	0.41±0.006	0.31±0.011
GM with 0.50% NaCl	0.067±0.011	0.038±0.004	0.64±0.004	0.45±0.024
GM with 0.75% NaCl	0.076±0.006	0.036±0.002	0.58±0.024	0.41±0.016
Liquid medium: Absorbance measurements				
GM with 0.75% NaCl	0.076±0.006	0.036±0.0024	0.58±0.006	0.41±0.010

[a] Maximum specific growth rate (h^{-1}). Average±standard deviation, $n=2$.

almost identical (Table 1) and this medium was selected for the generation of growth data.

3.2.2. Modelling of μ_{max} data

A regression method as well as a graphical method were used to determine a model-independent variance-stabilizing transformation of the μ_{max} data. Both methods indicated that a square root transformation would stabilizing the variance. However, the results were not unambiguous (results not shown) and, therefore, different transformations of the μ_{max} data were studied.

The average T_{min} and $\%CO_{2\ max}$-values in Eqs. (4) and (5) were -9.0 ± 0.8(SD) and 376 ± 39 (SD), respectively. The $\%CO_{2\ max}$-value of 376%CO_2 correspond to a partial pressure of 3.76 atm. These parameter values showed no systematic changes depending on CO_2 levels or temperatures and Eq. (6) was therefore fitted to the entire μ_{max} data set (Fig. 2). This figure include temperatures as low as T_{min} but Eq. (6) should only be used within the range of conditions studied (0–15°C and 0–100% CO_2). Data transformations had a pronounced effect on the lack-of-fit tests for Eqs. (6) and (7) (Table 2). The transformations had little effect on the fitted parameters in Eq. (6) but they influenced the confidence intervals of T_{min} and $\%CO_{2max}$ (Table 2). As shown by the residual sum of squares the polynomial model (Eq. (7)) explained a slightly higher fraction of the total variability in the μ_{max} data than Eq. (6) (Table 2). The parameters estimated from the square root transformed μ_{max} data were used for prediction of growth and shelf-life.

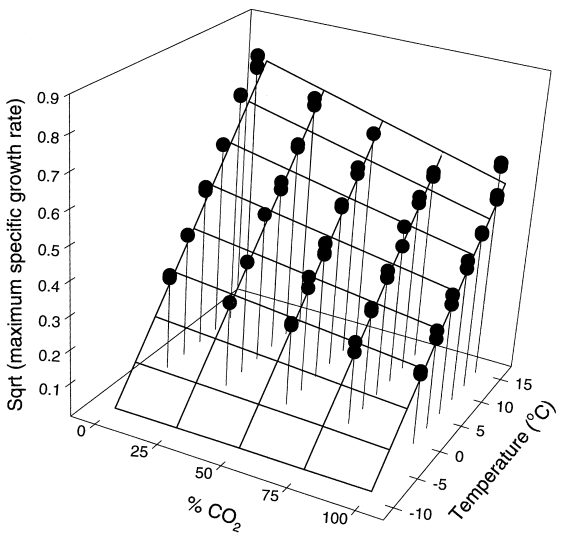

Fig. 2. Response surface plot of the effect of temperature and CO_2 on the μ_{max}-values of *P. phosphoreum* fitted by the square root model (Eq. (6)).

3.3. Product validation

The ability of *P. phosphoreum* to outgrow the other microflora in MAP cod fillets was confirmed although the initial numbers of the SSO were much

Table 2
Lack-of-fit testing and parameters values of Eqs. 6 and 7 fitted to the μ_{max}-data.

		No transformation	Square Root transformation	Logarithmic transformation
Lack-of-fit testing[a]				
SS_{pe}		0.020	0.016	0.055
RSS	Eq. 6	0.048	0.036	0.104
	Eq. 7	0.056	0.029	0.092
F_{lof}	Eq. 6	2.25[b]	1.98[c]	1.46[d]
	Eq. 7	3.02[b]	1.39[d]	1.19[d]
Parameter values Eq. 6				
b($°C \times h^{-0.5}$)		0.033 ± 0.0016[e]	0.032 ± 0.0012	0.032 ± 0.0012
T_{min} (°C)		-8.6 ± 0.9	-8.8 ± 0.6	-9.0 ± 0.4
$\%CO_{2\ max}$ ($\%CO_2$)		358 ± 30	368 ± 30	383 ± 29
Parameter values Eq. 7				
p_{7a}		0.063 ± 0.014	0.29 ± 0.014	-1.071 ± 0.022
p_{7b}		0.021 ± 0.004	0.032 ± 0.001	0.082 ± 0.006
p_{7c}		ns[f]	$-1.6 \times 10^{-3} \pm 5 \times 10^{-4}$	$-0.0037 \pm 8.2 \times 10^{-4}$
p_{7d}		$-1.8 \times 10^{-4} \pm 2 \times 10^{-5}$	$9 \times 10^{-5} \pm 2 \times 10^{-5}$	ns
p_{7e}		$9.1 \times 10^{-4} \pm 3 \times 10^{-4}$	ns	$1.7 \times 10^{-3} \pm 2 \times 10^{-4}$
p_{7f}		ns	$-9 \times 10^{-6} \pm 4 \times 10^{-6}$	$-1 \times 10^{-5} \pm 8 \times 10^{-6}$

[a] Abbreviations. SS_{pe}, sum of squares of pure error. RSS, residual sum of squares.F_{lof}, calculated F-value for lack-of-fit. The μ_{max}-data set included 74 data points. The degrees of freedom for lack-of-fit testing differed for Eq. 6 with 3 parameters and Eq. 7 with 4 or 5 parameters dependent on the transformations.
[b] F_{lof} > F-table ($\alpha = 0.01$) indicating a significant lack-of-fit.
[c] F-table ($\alpha = 0.05$) < F_{lof} < F-table ($\alpha = 0.01$).
[d] No indication of a lack-of-fit, F_{lof} < F-table ($\alpha = 0.05$).
[e] Fitted parameter value ±95% confidence interval.
[f] Non-significant parameter value.

lower than the TVC (Fig. 3 and Table 3). As expected some variability was observed for growth of *P. phosphoreum* in MAP cod fillets. However, the models developed by the iterative approach (Eqs. (6) and (7)) predicted growth of *P. phosphoreum* with a reasonable accuracy (Fig. 3 and Fig. 4). The predicted shelf-lifes, at constant as well as at varying temperatures, were only slightly shorter than observed in product studies (Table 4). The average deviation between the predicted shelf-life and the shelf-life determined by sensory assessments in the eight storage experiments was −17% and −9% for Eq. (6) and Eq. (7), respectively. The simple interpolation model (Eq. (8)) developed directly from the initial studies with naturally contaminated product was less accurate, the average deviation was −30% (Table 4). The concentration of CO_2 in the modified atmosphere-packs was $50 \pm 3\%CO_2$ and this value was used for prediction of the growth and of the shelf-life.

4. Discussion

4.1. Initial studies with naturally contaminated products

It is well known that the activity of specific groups of microorganisms in an ecosystem is related to a certain domain (Boddy and Wimpenny, 1992). The relatively extended spoilage domain of *P. phosphoreum* justified the development of a microbial spoilage model for MAP cod. If other micro-organisms had dominated the spoilage microflora at 0°C or 15°C with 100% N_2 or 100% CO_2, a model for *P. phosphoreum* would most likely be of no use for shelf-life prediction. Although, the spoilage domains of microorganisms in foods are usually unknown microbial spoilage models have been developed for wide ranges of environmental factors by the general predictive modelling approach (Doe and Heruwati, 1988; McClure et al., 1993; McMeekin and Ross,

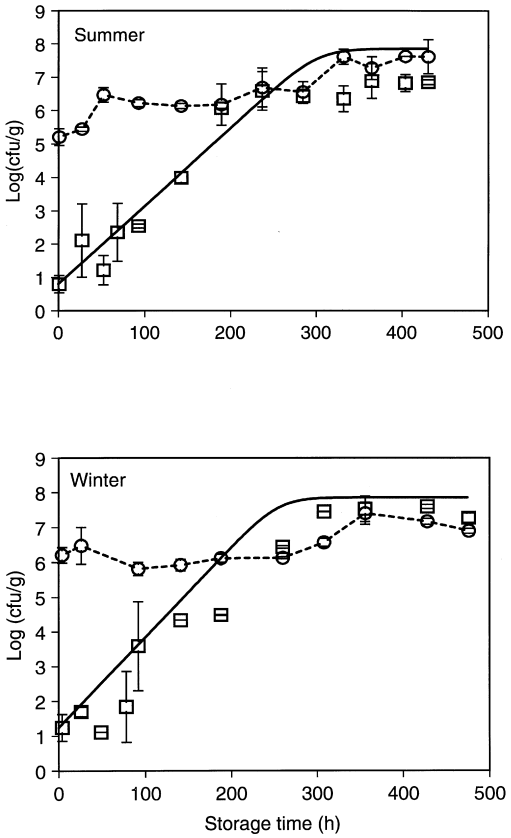

Fig. 3. Observed and predicted growth of *P. phosphoreum* in MAP cod fillets (60% CO_2 and 40% N_2) stored at 0°C. Total viable counts (circles) and counts of *P. phosphoreum* (squares). Error bars indicate standard deviations ($n=2$). The solid lines show growth predicted by Eq. (7).

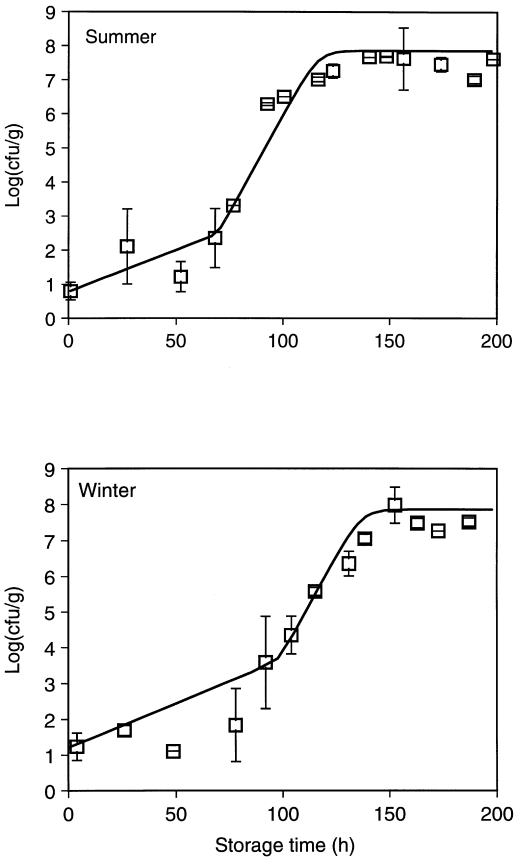

Fig. 4. Observed and predicted growth of *P. phosphoreum* in packed cod fillets (60% CO_2 and 40% N_2) stored at varying temperatures. In summer and winter experiment at 0°C for 69 h and 92 h, respectively, followed by storage at 10°C. Error bars indicate standard deviations ($n=2$). The solid lines show growth predicted by Eq. (7).

Table 3
Numbers and types microorganisms in MAP cod fillets at the time of sensory rejection

Batch of fish	I	II	III	IV
Initial storage temp.	0°C	0°C	0°C	5°C
Final storage temp.	0°C	5°C	10°C	0°C
TVC, log(cfu/g)	7.6±0.5[a]	7.2±0.3	7.9±0.1	7.5±0.3
Direct microscopy, log(cfu/g)	7.8±0.1	8.0±0.1	7.3±0.2	7.9±0.1
P. phosphoreum, log(cfu/g)	6.9±0.1	7.5±0.3	7.7±0.0	7.5±0.9
Microflora in spoiled cod fillets. Number of strains[b]				
P. phosphoreum	9	5	7	7
Enterobacteriaceae	0	2	0	1
Micrococcus spp.	0	2	1	0
Others	1	1	2	2

[a] Average±standard deviation, $n=2$.[b] Ten isolates from each batch were randomly selected and identified according to simple taxonomic keys (Dainty et al., 1979; Dalgaard et al., 1997).

Table 4
Observed and predicted shelf-life of MAP cod fillets stored at constant and at varying temperatures

Batch of fish	I	II	III	IV
Initial storage temp.	0°C	0°C	0°C	5°C
Final storage temp.	0°C	5°C	10°C	0°C
Winter experiments				
Observed shelf-life, days	15.8	8.2	6.4	9.3
Predicted shelf-life, days				
Interpolation model (Eq. 8)	11.7 (−26%)[a]	6.5 (−21%)	5.5 (−14%)	4.1 (−56%)
Square root model (Eq. 6)	12.5 (−21%)	7.5 (− 9%)	5.8 (− 8%)	7.2 (−22%)
Polynomial model (Eq. 7)	13.9 (−12%)	8.1 (− 2%)	6..1 (− 4%)	8.5 (− 9%)
Summer experiments				
Observed shelf-life, days	16.8	9.2	5.9	11.8
Predicted shelf-life, days				
Interpolation model (Eq. 8)	12.4 (−26%)	6.4 (−30%)	4.7 (−20%)	6.2 (−47%)
Square root model (Eq. 6)	13.2 (−21%)	7.4 (−19%)	5.1 (−13%)	8.9 (−25%)
Polynomial model (Eq. 7)	14.7 (−13%)	8.0 (−13%)	5.4 (−8%)	10.3 (−13%)

[a] % deviation between observed and predicted shelf-life.

1996). These models must therefore be used together with systems that identify the microorganisms of importance in different foods. Such systems have been suggested (Zwietering et al., 1992; Schellekens et al., 1994) but their ability to predict shelf-life of food accurately needs to be confirmed.

The pronounced differences between growth of *P. phosphoreum* in MAP cod and in BHI and TSB (Table 1) stressed the importance of the initial product studies in order to obviate the generation of large amounts of unrealistic growth data in inappropriate media. Of course modelling of product data eliminates the risk of using inappropriate media. However, product studies are laborious and the modest accuracy of the simple interpolation model (Eq. (8)) shows that a larger number of product experiments would be required for the development of an accurate model (Table 4). Absorbance measurements have been little used within predictive microbiology but in combination with the iterative approach this simple and inexpensive technique for generation of growth data allowed an accurate model to be developed (Table 1, Table 4). The initial product studies also enabled the selection of a simple primary growth model that did not included a lag phase and therefore facilitated shelf-life prediction (Eq. (1)). The initial product studies finally enabled the establishment of a spoilage criterion. A minimum spoilage level (MSL) e.g. 10^7 cfu/g has been used for shelf-life determination of food (Mossel et al., 1995). Clearly, an MSL value was inappropriate for MAP cod fillets (Fig. 1), but the empirical spoilage criterion equal to $t_i + 4 \times t_g$ (Eq. (9)) enabled the accurate prediction of shelf-life (Table 4).

4.2. Generation and mathematical modelling of data

The complex polynomial equations sometimes used in predictive microbiology have been criticized (Baranyi et al., 1996). However, microbial spoilage models only need to cover the spoilage domain of a particular SSO and simple polynomials can then be used reliably as shown in the present study (Table 4). Compared to Eq. (7), the square root model, Eq. (6) has the advantage of being simple and to contain parameters (T_{min} and $\%CO_{2\,max}$) that are easy to compare with values from other studies. Kalina (1993) suggested another simple CO_2 model. However, Eq. (7) explained a higher fraction of the variability in the entire μ_{max} data set for MIX-Pp than Eq. (6) (Table 2) and the Kalina-model combined with Eq. (5) (results not shown).

4.3. Product validation

Graphical methods or indices of performance e.g. the bias and accuracy factors have been used for product validation of predictive models (McClure et al., 1993; Avery et al., 1996; Ross, 1996). However,

growth of spoilage micro-organisms may be predicted accurately outside their spoilage domain and comparisons of growth kinetics may not be optimal for validation of microbial spoilage models (Dalgaard, 1995a). It has been mentioned that MAP cod stored with very high concentrations of CO_2 (>90%) spoil partly due to texture changes e.i. non-microbial spoilage reactions (Dalgaard et al., 1993). Clearly, in these situations, shelf-life cannot be predicted accurately by a microbial model. In commercial practice, levels of CO_2 of 30 to 60% are often used with MAP seafood. The product validation studies with 60% CO_2 and 40% N_2, together with the initial product studies and unpublished result with lower levels of CO_2 indicate that the developed model (Eq. (7)) is useful for prediction of shelf-life of MAP cod fillets under the conditions used in commercial practice.

5. Conclusion

Modified atmospheres are used increasingly for retail packs and for bulk transport of seafood. The present study has shown that microbial models developed with the iterative approach can predict accurately the shelf-life of packed cod. The extended spoilage domain of *P. phosphoreum* in MAP cod fillets allowed the microbial model to be used under practical storage conditions. The iterative approach is product-related and for development of microbial spoilage models this is important to make sure that microbial growth data are generated only for a range of conditions where a given micro-organism is actually relevant for product spoilage.

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