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Use of time–temperature integrators and predictive modelling for shelf life control of chilled fish under dynamic storage conditions

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Abstract

A systematic approach for fish shelf life modelling and Time Temperature Integrator (TTI) selection in order to plan and apply an effective quality monitoring scheme for the fish chill chain was developed. The temperature behaviour of the natural microflora of the Mediterranean fish boque (*Boops boops*) was studied and growth of the specific spoilage bacteria *Pseudomonas* spp. and *Shewanella putrefaciens* was modelled and correlated to organoleptic shelf life. Arrhenius and square root functions were used to model temperature dependence of maximum growth rates. Bacterial growth and shelf life models were validated under dynamic storage conditions with independent variable temperature experiments. The response of several TTIs from similar storage experiments was also modelled. The reliability of the TTI monitoring was cumulatively expressed by the error in the TTI derived effective temperature (T_{eff}) for different variable temperature distributions. T_{eff} was directly translated to shelf life of the fish.

Keywords: Shelf life; Modelling; Arrhenius; Time temperature integrators (TTI); Specific spoilage bacteria; Fish

1. Introduction

The current philosophy for food quality assurance is steadily decreasing the focus on end product testing and verification, traditionally the cornerstones of quality and regulatory control. The efforts of producers and legislation is concentrating on the development and application of structured quality assurance systems based on prevention through monitoring, controlling and recording of critical parameters through the entire product's life cycle extending from production to final use. The fresh fish chill chain, noted for substantial losses due to spoilage is due to benefit significantly from this approach (Huss, 1995). Monitoring and controlling storage temperature, would be of central importance. Temperature largely determines the rate of microbial activity, the main cause of spoilage of fresh and

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minimally processed fish products, and hence constitutes the determining parameter for shelf life under Good Hygiene Practices.

Effective application of this approach requires systematic study and modelling of the temperature dependence of shelf life. Ideally this would mean establishing a time correlation between measured chemical/biochemical changes, microbiological activity and sensory value for the conditions of interest. Since each type of fish product, depending on intrinsic and extrinsic factors, has its own specific spoilage microflora investigation of the spoilage domain provides the fundamentals for understanding the spoilage phenomenon and for reliable shelf life predictive modelling (Dalgaard, 1995; Dalgaard and Huss, 1995). At low temperatures (0–15°C) *Pseudomonas* spp., *Photobacterium phosphoreum, Shewanella putrefaciens, Brochothrix thermosphacta, Aeromonas* spp. have been reported as main spoilage bacteria in different fish stored under different storage conditions (Gram and Huss, 1996; Drosinos and Nychas, 1996; Koutsoumanis and Nychas, 1998).

Having reliably modelled the fish shelf life, what would be potentially useful is the development of practical systems such as Time Temperature Integrators or Indicators (TTI), to monitor, record and translate the effect of temperature, ideally from catch to consumption. TTI are defined as simple, inexpensive devices that indicate with an easily measurable, time–temperature dependent change, the temperature history and quality status of the food they are attached to. The irreversible change TTIs express is usually a mechanical deformation or colour development based on mechanical, chemical or enzymatic systems (Taoukis et al., 1991). The development and application of reliable TTI systems must also be approached based on kinetic principles (Taoukis and Labuza, 1989a,b; Sherlock et al., 1991). Most TTI systems can be designed to have a useful response time matching or correlating to the shelf life at a target constant temperature. On the other hand the temperature dependence of the response (expressed in kinetic terms as activation energy) can only be set at certain limited values. A difference in temperature sensitivity between TTI response and food spoilage can result in an accumulating error in the translation of the response to actual quality loss of the food under the variable temperature conditions of the chill chain (Taoukis and Labuza, 1992).

To establish the application of the afforementioned principles for effectively monitoring the shelf life of fish in the chill chain, models of sensory quality and growth of spoilage microflora were developed and validated in dynamic temperature conditions for the Mediterranean fish boque (*Boops boops*). Kinetic study of alternative TTIs at isothermal and non isothermal conditions was conducted and their use as fish quality monitors was assessed.

2. Materials and methods

2.1. Experimental design

The approach and methodology used aimed in assessing the reliability of the models for the tested fish species and the whole range of conditions of interest. Shelf life experiments were carried out under normal and abusive conditions of storage and distribution.

Boque (*Boops boops*), a Mediterannean fish of high consumption and commercial interest in Greece was studied.

Two replicated storage experiments were carried out with fresh ungutted boque (*Boops boops*). Fish were caught by fish boats who go off-shore fishing at about 2.00 h to 3.00 h and return after about 3 h. The fish were kept in ice in a local fishery-shop until they were bought within 4 to 9 h after catch and transported in ice, within 30 min from their purchase, to the laboratory. The fish were then stored, in individual pouches (not sealed), at controlled isothermal conditions (0, 3, 7 and 10°C) in high precision low temperature incubators (Sanyo MIR). Samples were taken at appropriate time intervals to allow for an efficient kinetic analysis of sensory quality and of microbial growth (Taoukis et al., 1997). Sensory evaluation of fish stored at 13°C was also conducted.

Dynamic storage experiments were also conducted by storing the boque fish in individual pouches (not sealed), at controlled non-isothermal conditions, in the programmable high precision low temperature incubators (Sanyo MIR 153, Sanyo Electric Co., Ora-Gun, Gunma, Japan). Two non-isothermal regimes were used. A periodically alternating 10.5 h cycle of 4.5 h at 2°C, 3 h at 8°C and 3 h at 15°C, to simulate a gradually and continuously changing storage environment, and a step wise changing

profile of 24 h at 0°C and 24 h at 10°C, to simulate abrupt changes and sustained abuse periods in the distribution chain. Both sensory and microbiological tests were carried out for fish subjected to the above temperature profiles.

2.2. Evaluation of organoleptic shelf life

Whole fish was evaluated by a trained sensory panel of 8 judges who were asked to evaluate odour of raw and taste and odour of cooked fish. An adaptation of the simple 3 point scoring system (Dalgaard et al., 1993; Koutsoumanis and Nychas, 1998) was used. Taste and odour was judged and recorded in appropriate forms with descriptive terms reflecting the organoleptic evolution of quality deterioration. Rating was assigned on a continuous 0–3 hedonic scale (0 being the highest quality score and 2 the limit of acceptance). Fish were scaled, gutted and gilled before cooking. Fish were cooked whole, individually wrapped steam tightly in aluminum foil, at 180°C for 30 min. They were evaluated whole for odour and filleted for taste and odour.

2.3. Sample preparation and microbiological analysis

Fish (25 g) was transferred to a stomacher bag (Seward Medical, London, UK), 225 ml 0.1% peptone water with salt (NaCl, 0.85%, w/v) were added and homogenized for 60 s with a stomacher (Lab Blender 400, Seward Medical, London, UK). The pH value was recorded by a pH meter (Metrohm 691, Herisan, Switzerland), the glass electrode being applied directly to the flesh.

Samples (0.1 ml) of serial dilutions of fish homogenates were spread on the surface of dried media in Petri dishes for enumeration of: (i) total viable count on a modified Long and Hammer's agar (mLHA) (Van Spreekens, 1974), and incubated at 10°C for 7 d. The medium was composed of (g l^{-1} distilled water): proteose peptone (Sigma P 0431, Sigma Chemical Co., St. Louis, MI, USA), 20; gelatine (Merk4070, Merck, West Point, PA, USA), 40; K_2HPO_4, 1; NaCl, 10; agar (Oxoid L11, Oxoid, Basinstoke, UK), 15; ammonium ferric citrate, 0.25; (ii) Pseudomonads on cetrimide fusidin cephaloridine agar (CFC, Oxoid code CM 559, supplemented with SR 103, Oxoid, Basinstoke, UK) and incubated at 20°C for 2 d (Mead and Adams, 1977), (iii) *Brochothrix thermosphacta* on streptomycin sulphate thallous acetate cycloheximide (actidione) agar, (STAA, Oxoid code CM 881, supplemented with SR 151, Oxoid, Basinstoke, UK) and incubated at 20°C for 3 d (Gardner, 1966).

For enterobacteria and hydrogen sulphide-producing bacteria 1.0 ml was inoculated into 10 ml of molten (45°C) violet red bile glucose agar (VRBGA, Oxoid code CM 485, Oxoid, Basinstoke, UK) and Iron Agar (IA, Oxoid code CM 867, Oxoid, Basinstoke, UK) respectively. After setting, a 10 ml overlay of molten medium was added. For the former, incubation was at 30°C for 24 h. The large colonies with purple haloes were counted (Mossel et al., 1979). Iron agar plates were incubated at 20°C for 4 d (Gram et al., 1987). Black colonies formed by the production of H_2S were enumerated after 2–3 d (Gennari and Campanini, 1991).

Three replicates of at least three appropriate dilutions were enumerated. All plates were examined visually for typical colony types and morphological characteristics associated with each growth medium. In addition, selectivity of each medium was checked routinely by Gram-staining and microscopical examination of smears prepared from randomly selected colonies from all media.

2.4. Data analysis

The growth data were modelled as a function of time using the model of Baranyi and Roberts (1994). For curve fitting the in-house program DMFit of IFR (Institute of Food Research, Reading, UK) was used, kindly provided by Dr J. Baranyi.

The Baranyi model is a non-autonomous, separable, first order ordinary differential equation (Baranyi et al., 1993). The lag phase is formally separated from the exponential and the stationary phase which can be regarded as parts of the potential growth defined by the autonomous model. The model (Baranyi and Roberts, 1994) is based on four parameters. A parameter expressing the lag phase; μ, the exponential growth rate ($\log_{10}$(cfu/g) h^{-1}); y_o representing the low asymptote of the bacterial growth curve ($\log_{10}$(cfu/g)); and y_{end} representing the upper asymptote of the growth curve ($\log_{10}$(cfu/g)). Two more parameters m and n are included in the Baranyi and Roberts equation corresponding to the behaviour of the growth curve at the 'transition' regions (lag to exponential phase and exponential to

stationary phase). In this work the DMFIT suggested default value of 10 was used for *m* and *n*. Use of this model for the determination of the maximum specific growth rate, $\mu_{max} = \ln 10$. $\mu(\ln(\text{cfu/g})\ h^{-1})$, avoids the overestimation often obtained when functions such as the Gompertz (Gibson et al., 1988) are employed for fitting of the growth curves, which can exceed 10%. These parameters can be further expressed as function of temperature. Temperature dependence of μ_{max} was modelled with the Arrhenius and Belehradek (square root) equations (McMeekin et al., 1993):

$$\mu_{max} = \mu_{ref} \exp\left[\frac{-E_A}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right] \quad (1a)$$

$$\sqrt{\mu_{max}} = b(T - T_o) \quad (1b)$$

where T is the absolute temperature, μ_{ref} is the growth rate at a reference temperature T_{ref}, E_A is the activation energy and R the universal gas constant, b the slope of the square root equation and T_o is a nominal minimum temperature.

2.5. Measurement and modelling of TTI response

Two enzymatic TTIs (VITSAB AB, Malmö, Sweden), Type M and Type S were kinetically characterized. They are based on a colour change caused by a pH decrease, due to a controlled enzymatic hydrolysis of a lipid substrate. Before activation, the lipase and the lipid substrate are in two separate compartments. At activation, the barrier that separates them is broken, enzyme and substrate are mixed and the colour change starts. Colour changes from green to yellow can be visually recognized and used as measure of change. A continuous objective instrumental quantitation of the colour change based on measurement of the *L*a*b** coordinates of CIE (CIE, 1978) with a Minolta CR-200 Chroma Meter (Minolta Co., Chuo-Ku, Osaka, Japan) was used. Kinetic characterization was performed by measuring, at appropriate time intervals, the response of multiple TTI samples isothermally stored at temperatures from 0 to15°C. Further, the response function was determined and the temperature dependence of the response rate i.e. the activation energy, E_A, was estimated. Validation of the response under dynamic conditions was also conducted by storing the TTI at controlled non-isothermal conditions, in the programmable high precision low temperature incubators (Sanyo MIR 153, Sanyo Electric Co., Ora-Gun, Gunma, Japan). Also Fresh-Check® indicator (Lifelines Technology, Morris Plains, NJ, USA) Type A6 was similarly tested and modelled. This TTI is based on the solid state polymerization of a thinly coated colourless acetylenic monomer that changes to a highly coloured polymer. The change, X, of the colour of the 'active' center of the TTI is compared to the reference colour of the ring and was also measured with the Minolta CR-200 Chroma Meter.

The basic principles of TTI application for spoilage monitoring are detailed by Taoukis and Labuza (1989a, 1997). In the case of the studied chilled fish, quality indices are the population N_i of the SSB and assuming conservatively that most of the growth occuring during actual distribution is in the exponential phase the quality function is

$$f(N) = \ln(N/N_o) = \mu_{max}(T)\, t \quad (2)$$

The value of the quality function, $f(N)_t$, at time t, after exposure of the fish at a known variable temperature exposure, $T(t)$, can be found based on Eq. (2) by calculating the integral of $\mu_{max}[T(t)]\ dt$, from 0 to time t. We can define the effective temperature, T_{eff}, as the constant temperature, equal exposure to which, results in the same quality value, $f(N)_t$, as the variable temperature distribution, $T(t)$. The same kinetic approach was used to model the measurable change X of the TTI. The response function, $F(X)$, was determined, such that $F(X) = k_I t$, with k an Arrhenius function of T. For an indicator exposed to the same temperature distribution, $T(t)$, as the food product, and corresponding to an effective temperature T_{eff}, the value of the response function will be

$$F(X)_t = k_{I_{T_{eff}}} t = k_{I_{ref}} \exp\left[\frac{-E_{AI}}{R}\left(\frac{1}{T_{eff}} - \frac{1}{T_{ref}}\right)\right] t \quad (3)$$

where k_{Iref} and E_{AI} are the Arrhenius parameters of the indicator.

By solving Eq. (3), the T_{eff} of the exposure is derived. With the T_{eff} and the spoilage kinetic parameters of the fish known, the quality function value is calculated from Eqs. (1a), (1b) and (2), for $\mu_{max} = \mu_{max}(T_{eff})$ and from it the value of N_t. This

gives the extent of the quality deterioration of the fish and allows the calculation of the remaining shelf life.

3. Results and discussion

The experimental data for growth of the different measured constituents of the boque natural microflora are shown in Fig. 1 along with the fitted growth curves from the DMFIT software, for two representative isothermal conditions. The maximum growth rate, μ_{max}, at different storage temperatures from the two replicate studies are listed in Table 1. At all temperatures, growth of pseudomonads and *Shewanella putrefaciens*, which are well established spoilage indexes for air stored chilled fish from cold and temperate water (Gram and Huss, 1996), followed closely the decrease of sensory quality. End of shelf life coincided with an average level of 10^7 for these two bacteria within the studied range of temperatures (see Table 2).

The average sensory score evolution of the cooked fish is shown in Fig. 2(a) for boque stored at different temperatures. The results of the shelf life estimation based on the scoring of the sensory panelists are shown in Table 1. Shelf life at 0°C was determined as 174 h. In Fig. 2(b) the Arrhenius plot of shelf life (logarithm of shelf life versus inverse absolute temperature) is shown (correlation coefficient, $R^2 = 0.986$). The temperature dependence was expressed as a value of activation energy obtained from the slope of the Arrhenius plot as 86.6 kJ/mol±8.3 kJ/mol (95% confidence limits). The Arrhenius empirical shelf life model can be used for prediction of shelf life (SL) at any constant or effective

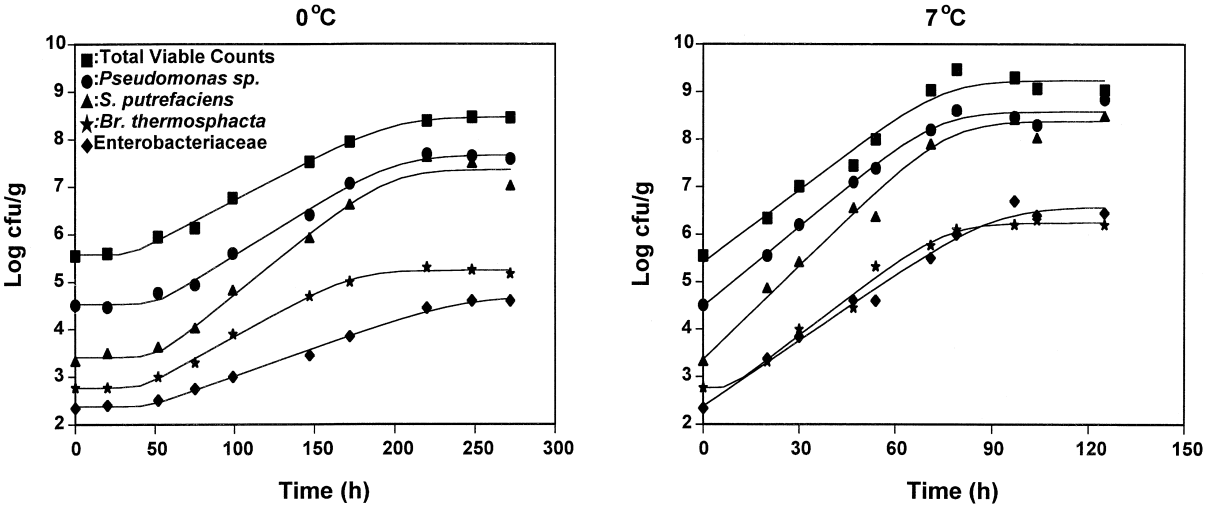

Fig. 1. Development of the natural microflora of Mediteranian fish (*Boops boops*) stored aerobically at 0°C and 7°C. The growth data were fitted with DMFit programme according to the Baranyi model.

Table 1
Maximum growth rate, μ_{max} (h^{-1}), estimated by the DMFIT software based on the Baranyi model, and end of sensorial shelf life at 0°C, 3°C, 7°C and 10°C: results from two replicate studies, A and B (results for B at 0°C were not obtained)

T (°C)	Shelf life (h)		TVC		*Pseudomonas*		*Brochothrix thermosphacta*		*Shewanella putrefaciens*		Enterobacteria	
	A	B	A	B	A	B	A	B	A	B	A	B
0	174	174	0.044	–	0.042	–	0.041	–	0.055	–	0.028	–
3	103	108	0.071	0.071	0.065	0.074	0.053	0.071	0.083	0.097	0.046	0.055
7	60	55	0.147	0.147	0.117	0.104	0.117	0.120	0.150	0.150	0.106	0.127
10	44	40	0.161	0.161	0.143	0.161	0.131	0.161	0.189	0.209	0.143	0.175

Table 2
Growth of the spoilage indices (*Pseudomonas* sp. and *Shewanella putrefaciens*) at the end of sensorial shelf life and time for the spoilage indices to reach $N=10^7$ as estimated by the DMFIT software based on the Baranyi model (average of the replicate studies)

T (°C)	Sensorial Shelf life: SL (h)	Log N for *Pseudomonas* at SL	Log N for *Shewanella p.* at SL	Time for *Pseudomonas* Log $N=7$	Time for *Shewanella p.* Log $N=7$
0	174	7.07	6.70	170	190
3	106	6.70	6.40	117	126
7	57	7.32	6.95	50	58
10	42	7.07	7.11	42	41

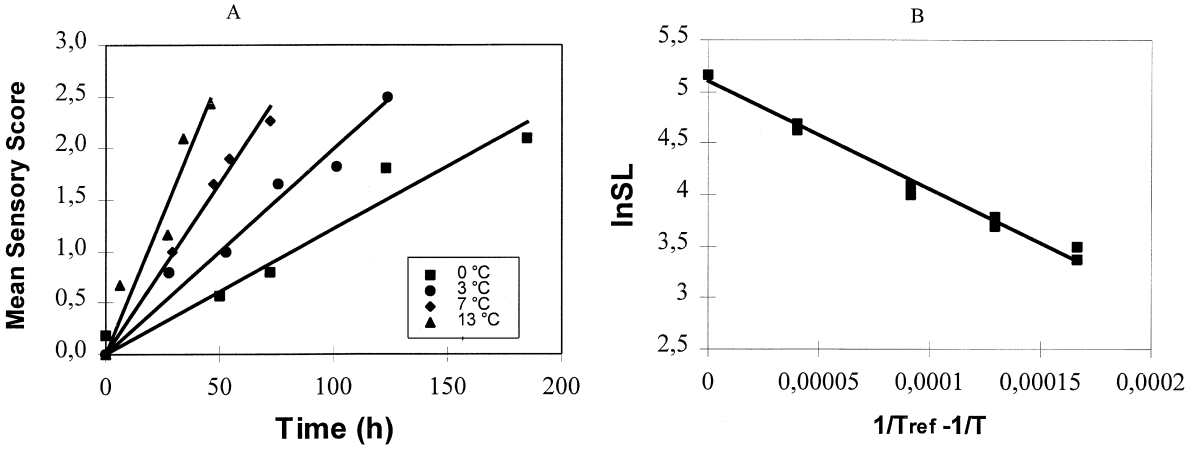

Fig. 2. (a) Sensory scoring of cooked boque (*Boops boops*) fish at four storage temperatures (0, 3, 7, 13°C). (b) Arrhenius plot of sensory shelflife of boque. (Reference temperature=0°C).

temperature T_{eff} (corresponding to a variable temperature profile) based on the Eq. (4):

$$SL = SL_{ref} \exp\left[\frac{E_A}{R}\left(\frac{1}{T_{eff}} - \frac{1}{T_{ref}}\right)\right] \quad (4)$$

Further the temperature dependence of the growth parameters of Pseudomonads and *Shewanella putrefaciens* was modelled. In Fig. 3 the effect of temperature on μ_{max} is shown in Arrhenius and Belehradek plots. Both models describe sufficiently the temperature dependence of μ_{max}.

In Table 3 the kinetic parameters for the Arrhenius and Belehradek equations and the relevant statistics for the two spoilage indices of boque are shown.

Based on the $L^*a^*b^*$ measurements the index that quantified better the change of TTI colour with time for the enzymatic TTIs M and S was the chroma value, $C=(a^{*2}+b^{*2})^{1/2}$. The normalized chroma $X_c=(C-C_{min})/(C_{max}-C_{min})$, where C_{min} is the minimum measured chroma value at the time of TTI activation and C_{max} the chroma value that corresponds to the colour reached long after what is considered as the end point colour of the TTI, was used as the response X of the TTI. The response function of the TTI, such that $F(X)=k_1t$, based on the Gaussian sigmoidal shape of X_c vs. time has the form shown in Eq. (5).

$$F(X_C) = \sqrt{\ln\left(\frac{1}{1-X_C}\right)} = kt \quad (5)$$

From the $F(X_c)$ vs. time plots, the value of the rate of the TTI response k was determined at each temperature by linear regression analysis (Fig. 4a). The correlation coefficients of the fit were 0.9 or larger. The temperature dependence of the response rates, k, was modelled by the Arrhenius equation. In Fig. 4b the Arrhenius plot of TTI Type M is shown (values of parameters in Table 4).

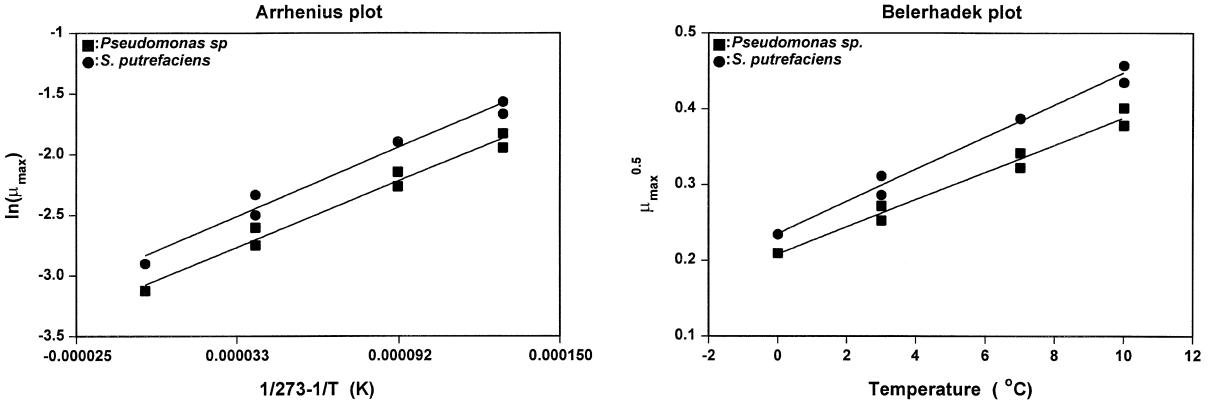

Fig. 3. Arrhenius and Belehradek plots, showing the temperature dependence of μ_{max} of pseudomonads and *Shewanella putrefaciens* growth on boque fish. (Values and statistics of fit in Table 3).

Table 3
Kinetic parameters of the spoilage indices (*Pseudomonas* sp. and *Shewanella putrefaciens*) of *Boop boops* (reference temperature = 0°C; 95% confidence intervals are reported for all parameters)

	Pseudomonas spp.	*Shewanella putrefaciens*
f(*N*)	$\ln(N/N_o)$	$\ln(N/N_o)$
E_A(kJ/mol)	81.6±11.6	82.7±11.1
μ_{ref} (h^{-1})	0.044±0.005	0.058±0.006
R^2	0.981	0.982
T_o (°C)	−11.36±0.7	−11.14±0.5
b ($h^{-1/2}$/°C)	0.0180±0.0030	0.0212±0.0029
R^2	0.977	0.985

Based on the $L^*a^*b^*$ measurements the index that quantified better the change of TTI colour with time for the polymer reaction based TTI A6 was the b^* value i.e. $X = b^*$. The change of b^* followed a first order behaviour, expressed as $F(X) = \ln(X_o/X)$, where X_o is the b^* value at 0 time.

In Table 4 the kinetic parameters and the temperature dependence of the TTI response based on the experimentally determined response rates at all temperatures is shown. The parameters of the Arrhenius and Belehradek equations and the relevant statistics for the response of the three tested TTI are shown.

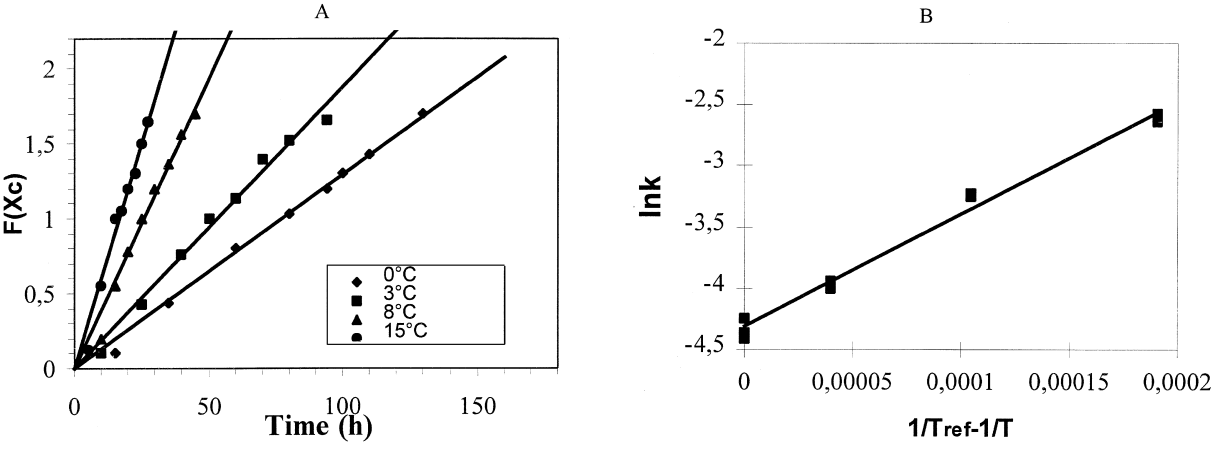

Fig. 4. (a) Response of TTI Type M at different isothermal storage conditions. $F(X_c)$ is the response function of the colour changing 'window' of the indicator given by Eq. (5). Points are average of measured response of twelve TTI units at each temperature. Lines are the regression fit lines for $F(X_c)$ vs. time. (b) Arrhenius plot of the response rate of TTI Type M.

Table 4
Kinetic parameters of the response of the tested TTIs

	TTI M	TTI S	TTI A6
F(X)	$[\ln(1/1-X)]^{1/2}$	$[\ln(1/1-X)]^{1/2}$	$\ln(X_o/X)$
E_A (kJ/mol)	68.70±9.03	102.1±6.2	83.60±10.71
R^2	0.966	0.982	0.960
T_o (°C)	−12.6±0.3	−14.9±0.4	−6.23±0.2
b ($h^{-1/2}$/°C)	0.0091±0.0009	0.0066±0.0011	0.0042±0.0008
R^2	0.983	0.940	0.983

The activation energies of the studied TTIs cover a range of about ±20 kJ/mol of the ones for the spoilage bacterial growth and thus are good candidates for monitoring the fish shelf life (Taoukis and Labuza, 1989a).

The applicability of the shelf life models, developed based on isothermal experiments, has to be validated in dynamic conditions (Fu et al., 1991). This will allow the effective use in the real variable handling, storage and distribution conditions. The independent non isothermal experiments showed that the models can give an accurate prediction in the variable conditions of the experiments. The change of the average sensory score at these conditions is shown in Fig. 5. The sensory evaluation at the first variable temperature regime i.e. a periodically alternating 10.5 h cycle of 4.5 h at 2°C, 3 h at 8°C and 3 h at 15°C, to simulate a gradually and continuously changing storage environment is shown in Fig. 5(a). The scoring of the panel is plotted with time. End of shelf life (score of 2) is experimentally determined as 41 h. The experimentally determined time of 41 h is compared to the empirical model prediction which in this case is based on the T_{eff} of the temperature profile which was calculated to be 9.2°C. The predicted time was 47 h. The difference between predicted and actual shelf life is less than 13% which is well within the acceptable variability of the model and practically shows applicability of the model in dynamic conditions.

The sensory evaluation at the second variable temperature regime i.e. a step wise changing profile of 24 h at 0°C and 24 h at 10°C, to simulate abrupt changes and sustained abuse periods in the distribution chain is shown in Fig. 5(b). The scoring of the panel is plotted with time. End of shelf life (score of 2) is experimentally determined as 52 h. The experimentally determined time is compared to the empirical model prediction which in this case is based on the T_{eff} of the temperature profile which

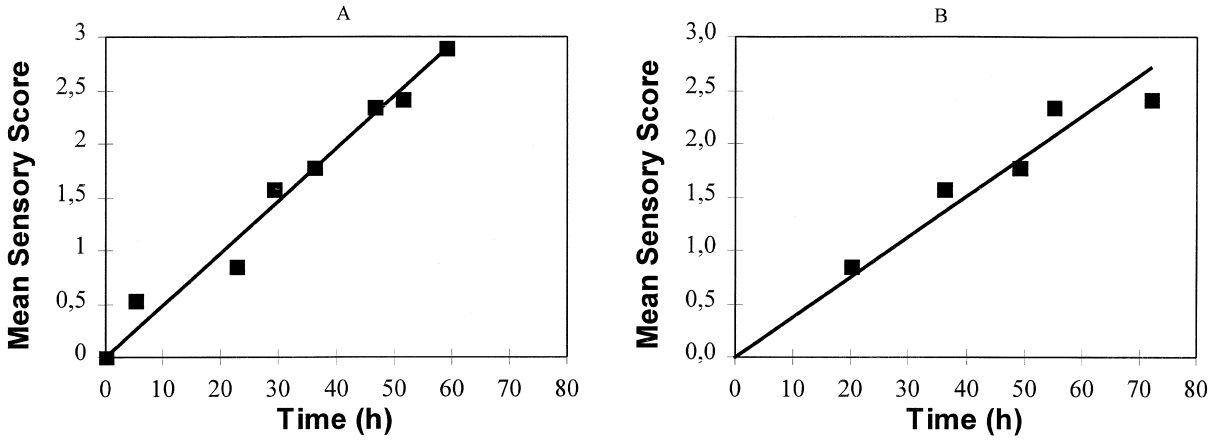

Fig. 5. Sensory scoring of cooked boque (*Boops boops*) fish at variable temperature storage. (Linear regression fit lines are also shown.) (a) Cycle of 4.5 h at 2°C, 3 h at 8°C and 3 h at 15°C. (b) Cycle of 24 h at 10°C and 24 h at 0°C.

was calculated 7.3°C. The predicted time was 58 h. The difference between predicted and actual shelf life is less than 15% which is within the acceptable variability of the model and the sensory methodology and practically shows the predicting ability of the model in abusive dynamic conditions.

The comparison of actual growth of the microbial spoilage indexes measured in independent non-isothermal experiments and the prediction of the growth models for the particular temperature profiles are shown in Fig. 6. The continuous growth lines are constructed piecewise from the μ_{max} predicted from the Arrhenius model at each temperature step. It is noted that here, as well as in all calculated predictions, there is no lag phase considered. Additionally, the exponential phase growth prediction is extended to the point where the experimental stationary phase begins. Straight interrupted lines is exponential growth based on the μ_{max} at the effective temperature, T_{eff}, of each total temperature profile. A very good agreement is obtained between measured values and the predicted curve and with the predicted value of time to reach a level of 10^7 for the respective effective temperatures.

Having established the validity of the shelf life predictive models in dynamic conditions in the temperature range of interest the effectiveness of the tested TTIs can be evaluated based on the ability to translate their response to the effective temperature of the food undergoing the same temperature exposure. The error in the T_{eff} estimation increases as the difference in the activation energies of TTI response and spoilage rate of the monitored fish increases.

The procedure to translate the TTI response to the T_{eff} of the variable temperature exposure is described in the data analysis section. Shelf life calculations were based on Eq. (3) using as index the growth of *Shewanella putrefaciens* with an average initial count of 10^3 and 10^7 corresponding to the end of shelf life. For the temperature profiles 1 and 2 used in the validation testing (see above and in Fig. 6) the T_{eff} for the fish for a 36 h exposure is 8.55 and 7.85°C respectively. The corresponding T_{eff} calculated based on TTI M, TTI S and TTI A6 were 8.23, 8.99, 8.57°C and 7.68, 8.06, 7.86°C for the temperature profiles 1 and 2 respectively. It can be seen that all TTI give less than 0.5°C difference, with the ones closer in activation energies giving the smaller error. To demonstrate the effect of the error in T_{eff} estimation, in Table 5, the remaining shelf life, t_r, at 0°C as it is predicted by the TTI response at the different temperature profiles, is compared to the actual (Real t_r) which is based on the model for *S. putrefaciens* growth and the true temperature exposure. The predictions are based on Arrhenius models for temperature dependence of fish spoilage and TTI. Similar results are obtained if the square root model is applied. The error in T_{eff} estimation depends not only on the activation energy difference between the TTI and the spoilage index of the fish but on the

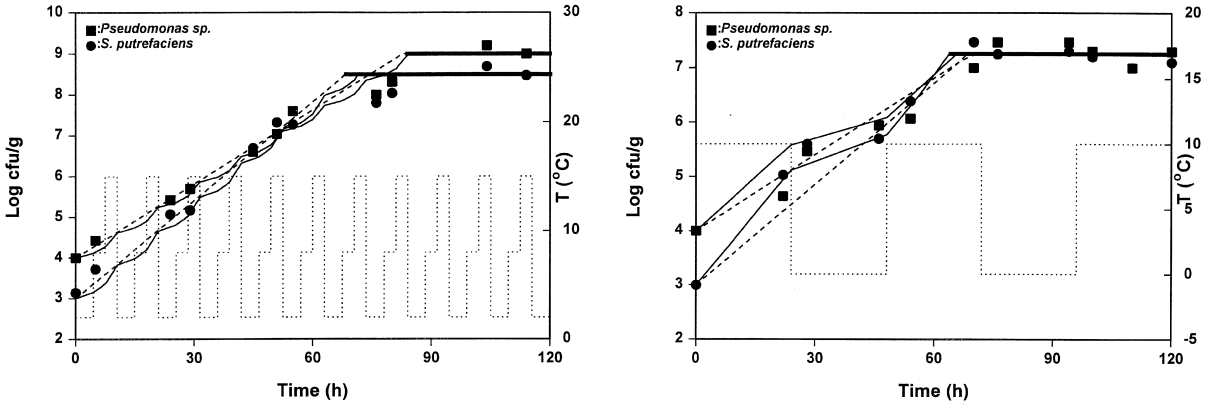

Fig. 6. Comparison of measured growth of the microbial spoilage indices (*Pseudomonas* sp. and *Shewanella putrefaciens*) at two dynamic conditions with the prediction of the growth models. The cycling temperature profile is shown in each graph (values at right axis). Continuous growth lines are based on μ_{max} at each step. Straight interrupted lines is exponential growth for μ_{max} at the effective temperature, T_{eff}, of each temperature profile. Thick asymptotes show level of stationary phase based on experimental data.

Table 5
Estimation of remaining shelf life (t_r) of boque at 0°C storage, after 36 h exposure at different variable Temperature Profiles (TP)

	Real t_r (h)	TTI M t_r (% error)	TTI S t_r (% error)	TTI A6 t_r (% error)
TP 1	50	54 (+8%)	44 (−12%)	50 (−0,4%)
TP 2	59	61 (+3%)	57 (+4%)	59 (−0,0%)
TP 3	57	66 (+15%)	45 (−21%)	56 (−2%)
TP 4	58	61 (+5%)	53 (−9%)	57 (−2%)

specific temperature profile. Profiles that show gradual changes generate less error than profiles with shorter but abrupt changes. This is demonstrated by Temperature Profile 3 that includes an abrupt breakdown of the chilled storage, exposing the fish for 9 h at 17°C, after 20 h of ice storage with the fish returning to 0°C for the rest of the 36 h period. In that case for TP3 the real T_{eff} is 8°C whereas the estimated by TTI M, TTI S and TTI A6 is 7.3, 8.9, and 8.1°C respectively. On the other hand for TP4, representing a more gradually changing temperature profile with the same T_{eff} of 8°C, the TTI estimations range from 7.7 to 8.4°C. In Table 5 these differences are reflected in the predictions of remaining shelf life. Even in that case however the least accurate prediction is within the practically acceptable range of ±20% and has to be put into perspective by comparing with the 5.5 days shelf life that would be assumed to be left without the changing response of the attached TTI.

Based on the above analysis a TTI with an activation energy (E_A) within ±20 kJ/mol (±5 kcal/mol) of the activation energy of the spoilage index of the fish could be used to satisfactorily monitor its shelf life. Other factors being equal, the TTI with the closest E_A will give the most accurate estimation. These statements are applicable for monitoring of the chill chain based on a continuous scale response, translatable to the effective temperature history as described above. This methodology can be applied to other chilled fish products if appropriate models are available (Taoukis and Nychas, 1996).

The present study shows the systematic approach to be followed for chilled fish shelf life modelling and TTI selection in order to achieve an effective monitoring of the quality during distribution. The applied methodology allows development of an application scheme specific to the studied fish and selection of the most appropriate TTI without the need of extensive side by side testing of the product and the indicator.

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