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Predictive modelling and selection of Time Temperature Integrators for monitoring the shelf life of modified atmosphere packed gilthead seabream fillets

Theofania Tsironi, Anastasios Stamatiou, Marianna Giannoglou, Eirini Velliou, Petros S. Taoukis*

National Technical University of Athens, School of Chemical Engineering, Laboratory of Food Chemistry and Technology, 5, Iroon Polytechniou, Zografou 15780, Athens, Greece

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ABSTRACT

The objective of the present study was to validate a kinetic model for growth of spoilage bacteria in modified atmosphere packed (MAP) gilthead seabream fillets and to select a Time Temperature Integrator (TTI). The temperature and CO_2 dependence of the growth of lactic acid bacteria in MAP gilthead seabream fillets was expressed by an Arrhenius-type model for the range of 0–15 °C and 20–80% CO_2, which was validated at isothermal, variable and chill chain conditions. A new UV activatable photochemical TTI, was kinetically studied and the influence of the level of activation on the response of the TTI was modelled. Applying the developed models, the required charging levels were estimated so that the TTI response was tailored to monitor the shelf life of fish fillets at selected MAP conditions, during the chill chain storage. A simulation experiment of the product distribution and storage in various chill chain conditions showed the applicability of the TTIs as shelf life monitors.

1. Introduction

The limited and variable shelf life of chilled fish, mainly due to bacterial activity, is a major problem for their quality assurance and commercial viability. Gilthead seabream is one of the most cultured species in the Mediterranean area and its production in Greece was estimated at 44054 tons in 2006, with Greece being the leading world producer with the 47.9% of the total Mediterranean production for gilthead seabream and seabass (FAO, 2006).

Modified atmosphere packaging (MAP) can effectively alter and delay the spoilage process and extend the shelf life of fresh fish (Torrieri, Cavella, Villani, & Masi, 2006). CO_2 inhibits the development of the respiratory organisms like *Pseudomonas* sp. and *Shewanella putrefaciens* and the microflora is dominated by Gram-positive organisms, mainly lactic acid bacteria (Sivertsvik, Jeksrud, & Rosnes, 2002). Lactic acid bacteria have been used as a good spoilage index of modified atmosphere packed fish such as chub mackerel (Stamatis & Arkoudelos, 2007), swordfish (Pantazi, Papavergou, Pournis Kontominas and Savvaidis, 2008) and eel (Arkoudelos, Stamatis, & Samaras, 2007). Drosinos, Lambropoulou, Mitre, and Nychas (1997) reported a co-dominance of lactic acid bacteria and *Brochothrix thermosphacta* in gilthead seabream stored under MA (40% CO_2).

Despite the increasing importance of MAP technology in fish industry and the several studies evaluating the effect of MAP on fish products (Dalgaard, Mejlholm, & Huss, 1997; Lyhs, Lahtinen, & Schelvis-Smit, 2007; Özogul, Polat, & Özogul, 2004; Pantazi, Papavergou, Pournis, Kontominas, & Savvaidis, 2008; Stamatis & Arkoudelos, 2007; Torrieri et al., 2006), a limited number of models including the combined effect of temperature and gas concentration in the packaging environment, that could be vital for shelf life optimization and improvement of the chill chain management,have been proposed for spoilage microorganisms (Dalgaard, 1995; Koutsoumanis, Taoukis, Drosinos, & Nychas, 2000). An Arrhenius-type model was developed by Tsironi, Tsevdou, Velliou and Taoukis (2008) as an effective tool for predicting gilthead seabream (*Sparus aurata*) fillet quality and shelf life under different chilled storage temperatures (0–15 °C) and modified atmospheres (20–80% CO_2).

Effective control of the chilled distribution of fresh fish products is vital to their commercial viability. A substantial portion of chilled products are exposed, throughout the distribution, to effective temperatures that deviate significantly from the recommended range. Application of an optimized quality and safety assurance system for the chilled distribution of products would require continuous monitoring and control of storage conditions, from production to consumption (Tsironi, Gogou, Velliou, & Taoukis, 2008).

Time Temperature Integrators (TTIs) can show an easily measurable, time and temperature dependent change that

* Corresponding author. Tel.: +30 2107723171; fax: +30 2107723163.
E-mail address: taoukis@chemeng.ntua.gr (P.S. Taoukis).

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cumulatively reflects the time–temperature history of the food product (Taoukis & Labuza, 2003). In order to use a TTI based system effectively, mathematical models are needed that describe the effect of temperature on the evolution of spoilage under dynamic storage conditions. Additionally, a full kinetic study of the TTI response is needed. Based on reliable models of the shelf life and the kinetics both of the product and the TTI response, the effect of temperature can be monitored, and quantitatively translated to food quality, from production to the point of consumption (Taoukis & Labuza, 1989a,b; Taoukis, 2001). A TTI based system could lead to realistic control of the chill chain, optimization of stock rotation and reduction of waste and efficient shelf life management.

The objective of this study was to validate the model that predicts the LAB growth and consequently the shelf life of modified atmosphere packed gilthead seabream fillets (*S. aurata*) as a function of packaging and refrigerated storage conditions. The response of UV activatable TTIs was kinetically modelled as a function of activation level and temperature in order to define the appropriate TTIs that can monitor the quality of fish fillets under any selected storage conditions in the range studied. The applicability of the selected TTIs in the real chill chain was also validated by a simulation experiment of the product distribution and storage in various chill chain conditions.

2. Materials and methods

2.1. Kinetic study of LAB growth on MAP gilthead seabream fillets and validation of the predictive model under isothermal and dynamic conditions

Marine cultured gilthead seabream (*S. aurata*) fillets (weight: 90 ± 10 g, culture zone: Aegean Sea, Greece) came from the same batch and were provided by a leading Greek aquaculture company. Fish was cultivated in net cages and harvested (age 16–20 months). After being ice shocked, fish was put into ice (0 °C), size sorted and transported to the filleting line within 10 h after catch. Fish was scaled, headed, filleted and rinsed with tap water in the industrial filleting line of the company. Fillets were transported directly to the laboratory in polystyrene boxes with appropriate quantity of flaked ice (0 °C, ice/fish ratio 0.5:1 w/w) within 2–4 h. A polyethylene film was placed between fillets, to avoid contact between skin and meat sides of the fillets. Proximate analysis was performed on 5 fillets upon receipt (Grigorakis, 2007).

Fish fillets were packed in HDPE pouches in modified atmosphere (35% CO_2-65% air) (Boss NT42N, Bad Homburg, Germany). Two fillets were packed in each package. Gas headspace was analyzed with the CheckMate 9900 O_2/CO_2 device (PBI Dansensor, Ringsted, Denmark). The gas to product volume ratio was 3:1.

All packages were stored at controlled isothermal conditions of 0, 2.5, 5, 10 and 15 °C in high-precision (±0.2 °C) low-temperature incubators (Sanyo MIR 153, Sanyo Electric, Ora-Gun, Gunma, Japan). Temperature in the incubators was constantly monitored with electronic, programmable miniature dataloggers (COX TRACER ®, Belmont, NC). Samples were taken in appropriate time intervals to allow for efficient kinetic analysis of microbial spoilage. Two independent experiments were also carried out at dynamic conditions. A time–temperature scenario was used, that consisted of three, isothermal steps: 8h at 5 °C, 8h at 9 °C and 8h at 12 °C ($T_{eff} = 9$ °C). A type T thermocouple was inserted into the fish flesh and temperature was constantly monitored during storage, in order to confirm the desired set temperature. Total viable count, *Pseudomonas* sp., lactobacilli and *B. thermosphacta* were enumerated by appropriate plate count methods described in Koutsoumanis, Giannakourou, Taoukis, and Nychas (2002). The sensory attributes of raw and cooked fish were evaluated as described by Tsironi and Taoukis (2010), by a trained sensory panel of 8, selected according to ISO 8586-1 (1993) standard and trained using discriminative tests with practice evaluation methods of determining spoilage characteristics in fish fillets (Botta, 1995).

The microbial growth was modelled using the Baranyi Growth Model (Baranyi & Roberts, 1995). For curve fitting the in-house program DMfit of IFR (Institute of Food Research, Reading, UK) was used, kindly provided by Dr J. Baranyi. Kinetic parameters such as the rate (k_{LAB}) of the microbial growth were estimated. The experimentally measured specific growth rates for LAB and the shelf life of gilthead seabream fillets were compared to the values predicted by the Arrhenius-type model previously developed by Tsironi et al. (2008) based on isothermal experiments at several CO_2 levels, other than the one used in the current experiment (Eq. (1)).

$$k_{LAB} = \left(k_{refLAB}\frac{CO_{2max} - CO_2}{CO_{2max}}\right)\exp\left[\frac{E_a}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right] \quad (1)$$

where CO_2 is the percentage of carbon dioxide, k_{refLAB}, is the specific growth rate at T_{ref} (4 °C, in the absence of carbon dioxide), $CO_{2,max}$ is the nominal maximum CO_2 concentration for LAB growth, T is the temperature in K, E_a is the activation energy of the studied action and R is the universal gas constant. The values of Eq. (1) parameters were determined as $k_{refLAB} = 0.015 \pm 0.003$ h^{-1}, $E_a = 101 \pm 26$ kJ/mol and $CO_{2,max} = 98.3 \pm 23.5\%$.

Based on the limit for LAB growth correlated to the end of the sensory shelf life (Tsironi & Taoukis, 2010; Tsironi et al., 2008) and the dependence of LAB growth on temperature and CO_2 concentration, expressed by the validated combined Arrhenius-type model, an equation for shelf life determination at any temperature and CO_2 level in the package atmosphere was developed (Eq. (2))

$$t_{SL} = \frac{\log N_l - \log N_o}{\left(k_{refLAB}\frac{CO_{2max} - CO_2}{CO_{2max}}\right)\exp\left[\frac{E_a}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right]} \quad (2)$$

where t_{SL} is the shelf life (h) of gilthead seabream fillets, $\log N_l = 6$ is the limit LAB load and $\log N_o$ is the initial LAB load.

2.2. Time Temperature Integrators modelling and application

The OnVu™ TTI (Ciba Specialty Chemicals & Freshpoint, SW), a newly introduced solid state reaction TTI, based on the inherent reproducibility of reactions in crystal phase, was used (Patent EP 1049930 B1). Photosensitive compounds such as benzylpyridines are excited and colored by exposure to low wavelength light. This colored state (dark blue) reverses to the initial colorless at a temperature depended rate. The visual end point can be set by comparison to a light blue printed reference (Fig. 1). By controlling the type of the photochromic compound and the length of UV light exposure during activation the length and the temperature sensitivity of the TTI can be set. The OnVu B1 071031 TTI (Ciba Specialty

Fig. 1. Response scale of solid state photochromic OnVu™ TTI.

Chemicals & Freshpoint, SW) was studied. Kinetic modelling of response was based on measurements, at appropriate time intervals, of the response of a number of TTI tags, isothermally stored at constant temperatures (from 0 to 15 °C).

Biserba GLP80 labelling unit (Biserba GmbH & Co. KG, Balingen, Germany), with 2" thin-layer thermal print head and TTF equipment was used for UV charging of the TTIs for 0.5, 1, 2, 3 and 4 s (charging time of 1 s corresponds to energy of 50 mJ/cm^2) and subsequent laminating with a film (TTR 70QC). This film acts as an optical filter and protects TTI from light exposure and recharging. The charging conditions were selected based on previous studies (unpublished data), where at 6 s charging or higher the TTIs exhibited a supersaturation effect and their response at lower temperatures reached a plateau higher than the expected. Thus the charging range could not exceed 5 s. TTI colour change was measured instrumentally using the Eye-one Pro (X-Rite, Michigan, USA) at D50 illumination and 2° observation angle conditions. Kinetic modelling of TTI response was based on measurements, at appropriate time intervals, of the response of 8 TTI tags, isothermally stored in high-precision low-temperature incubators (Sanyo MIR 153, Sanyo Electric, Ora-Gun, Gunma, Japan) at constant temperatures (2.5, 5, 8, 10 and 15 °C). The overall colour change of the CIELab scale, ΔE, was calculated by Eq. (3)

$$\Delta E = \sqrt{(L - L_{max})^2 + (a - a_{min})^2 + (b - b_{max})^2} \quad (3)$$

The response of the TTI was modelled by defining a mathematical function that better describes the response vs time at all temperature and initial charging conditions. Model coefficients were calculated by non linear regression using SYSTAT 10.2® Software (CLECOM Software Specialists, Birmingham, U.K.).

2.3. Chill chain simulation

In order to test the applicability of the combined Arrhenius-type model to predict the shelf life of gilthead seabream and the effectiveness of the selected TTI as shelf life monitors, the following experiment was designed. 80 samples of MAP (50% CO_2–50% air) gilthead seabream fillets were used. TTIs at appropriate level of activation, selected based on the predictive models for fish product spoilage and the TTI response, were attached on 40 fish packages at packing time. All products were stored in conditions simulating the real chill chain from production to the point of consumption. The simulated chill chain conditions consisted of 5 different time–temperature scenarios (Fig. 2), with effective temperatures, T_{eff}, between 2 and 15 °C, conducted in programmable temperature cabinets (Sanyo MIR 153, Sanyo Electric, Ora-Gun, Gunma, Japan). Products were split at a designated point of the simulated chill chain, 72 h from packing (corresponding to the distribution centre) and followed a simulated path to a "local" and a "distant" market. Products with TTIs were split based on TTI response translated into temperature history based on TTI kinetics. According to the TTI based system, the more temperature burdened products were diverted to the "local" market shortening their shelf life cycle in order to be consumed first (Giannakourou, Koutsoumanis, Nychas, & Taoukis, 2001). The 40 packages without TTI were split randomly. Half of the samples were subsequently stored at 4 °C and half at 8 °C for different times simulating the different final consumption times of products sold at the local and the distant market (Fig. 2).

Microbiological analyses of the respective gilthead seabream fillets were conducted at the 4 different stages. Products that were "sold at local market" were microbiologically analyzed at 72 and 96 h after split. Products "sold at the distant market" were microbiologically analyzed 144 and 168 h after the split. Lactic acid bacteria level was measured at these times, assumed as the end of storage period and time of consumption, and compared to the values predicted by the Arrhenius-type model (Eq. (1)), considering the specific CO_2 concentration in the package (50%) and the respective T_{eff}.

For experimental evaluation of the use of TTIs as shelf life monitors, colour response of the attached TTIs was measured throughout the field test (72 h from packing and at the time of consumption) using the Eye-one Pro (X-Rite, Michigan, USA) at D50 illumination and 2° observation angle conditions. The experimental ΔE value was estimated and it was compared to the ΔE value predicted by the developed model, for the respective charging and

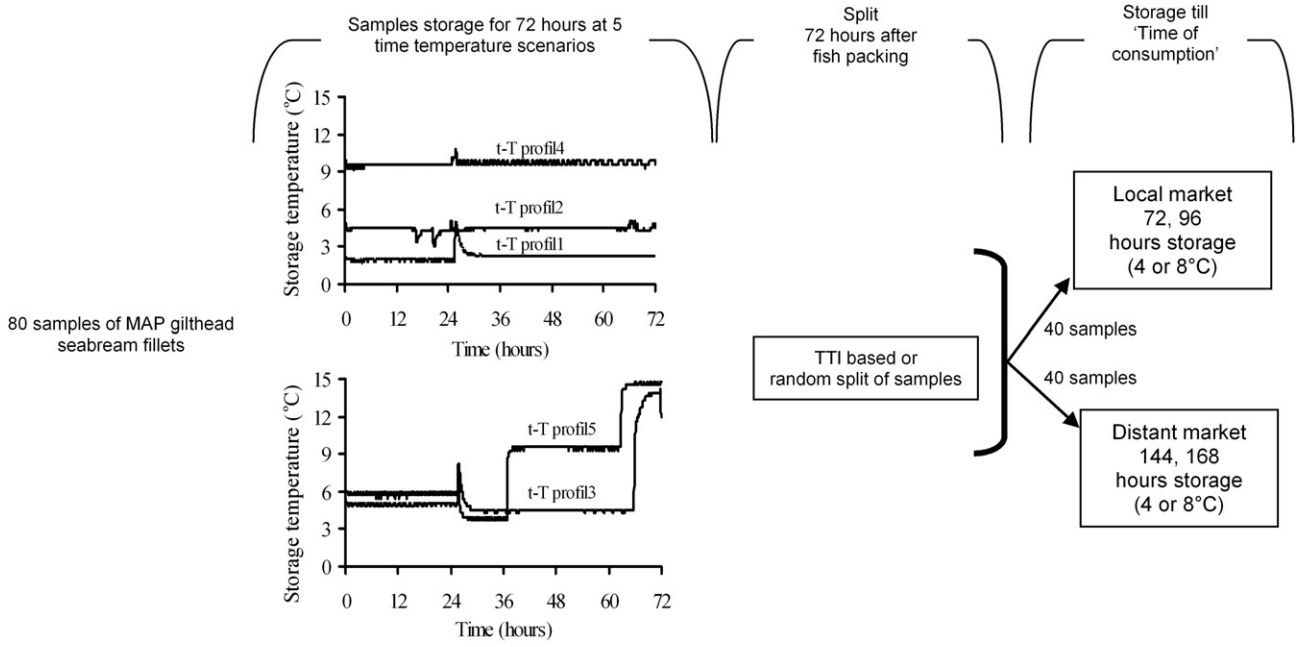

Fig. 2. Steps of simulated chill chain shelf life experiment of MAP fish fillets.

storage conditions of the attached TTIs. At the end of storage period the experimental ΔE value from which the T_{eff} of the exposure was derived, allowed the calculation of the remaining shelf life (RSL) of fish fillets at a reference temperature. These RSL values were compared to the RSL based on the enumerated LAB counts at the time of consumption and Eq. (2).

3. Results and discussion

3.1. Prediction of the LAB growth on MAP gilthead seabream fillets

Proximate analysis showed a total protein content of 19.7 ± 1.3%, total fat amounts 9.7 ± 0.8%, moisture 75.3 ± 0.8% and ash content 1.4 ± 0.3% (wet basis). Initial total viable count, *Pseudomonas* sp., lactic acid bacteria and *B. thermosphacta* were 4.1 ± 0.2, 2.4 ± 0.3, 2.1 ± 0.3 and 2.0 ± 0.1 log cfu/g, respectively. These values were in agreement with the initial microbial load reported by Tsironi, Salapa, and Taoukis (2009) for gilthead seabream fillets and lower than those reported by Paleologos, Savvaidis, and Kontominas (2004) for Mediterranean sea bass fillets. The experimental data for LAB of the MAP (35% CO_2) gilthead seabream fillets stored at 0, 2.5, 5, 10 and 15 °C are presented in Fig. 3 with the DMFIT growth curves. The level of lactic acid bacteria in the initial population was low but dominated the final population, which supports the hypothesis that LAB defined spoilage in MAP gilthead seabream. Growth curves for all populations were constructed (data not shown). The average of microbial counts at the end of storage periods at all temperatures, was 8.0 ± 0.3, 4.2 ± 0.2, 7.4 ± 0.4 and 4.7 ± 0.2 log cfu/g for TVC, *Pseudomonas* sp., LAB and *B. thermosphacta*, respectively. The end of shelf life, i.e. the limit of sensory acceptability, was correlated to a 6 log LAB level, as also reported in previous studies for MAP gilthead seabream (Tsironi & Taoukis, 2010; Tsironi, Tsevdou et al., 2008). The temperature dependence of the rates of LAB growth was described by the Arrhenius equation ($R^2 > 0.99$). The estimated E_a value ($E_a = 100.2 \pm 2.6$ kJ/mol) was in agreement with the fitted parameter value of 101.0 ± 25.9 kJ/mol, reported by Tsironi, Tsevdou et al. (2008) (Eq. (1)), indicating a strong dependence of LAB growth on storage temperature.

The LAB dominance occurs for modified atmosphere conditions (CO_2>20% in the packages) whereas *Pseudomonas* sp. defined spoilage in aerobically stored seabream (Gram & Huss, 1996; Koutsoumanis & Nychas, 2000; Kyrana, Lougovois, & Valsamis, 1997; Tsironi et al., 2009; Tsironi, Tsevdou et al., 2008). In previous study on MAP (50% CO_2) gilthead seabream fillets, TVC and LAB counts reached 8.2 ± 0.2 and 7.8 ± 0.3 log cfu/g, respectively, after one month of refrigerated storage, while *B. thermosphacta* did not exceed 5.3 ± 0.2 log cfu/g (Tsironi & Taoukis, 2010). Dalgaard, Mejlholm, Christiansesn, and Huss (1997) reported contribution of *Photobacterium phosphoreum* in the spoilage of chilled MAP Mediterranean fish, that was associated with high concentrations of trimethylamine. The low levels of TMA-N (<6 mg N/100 g) reported by Tsironi and Taoukis (2010), did not indicate *P. phosphoreum* as a factor of spoilage.

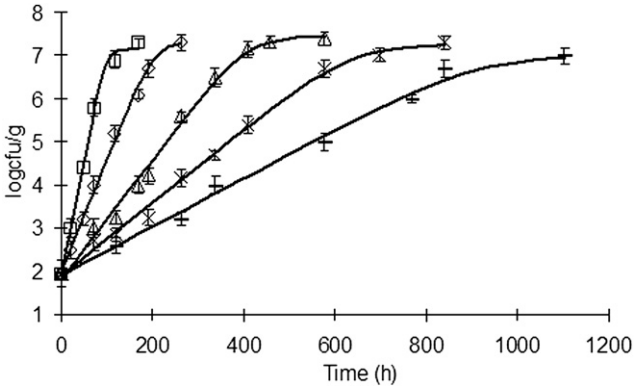

Fig. 3. Growth of lactic acid bacteria on gilthead seabream fillets packed under modified atmosphere (35% CO_2) at ▬ 0, ✳ 2.5, △ 5, ◇ 10 and □ 15 °C (Mean values ± standard deviation and statistical fit to the Baranyi equation).

The experimental values for the exponential growth rates of LAB in gilthead seabream fillets stored in MAP (35% CO_2) at 0, 2.5, 5, 10 and 15 °C compared with the growth rates predicted by the Arrhenius-type model, are shown in Table 1. Relative errors (RE) were calculated as

$$\%RE = \left[\left(k_{observed} - k_{predicted}\right)\Big/ k_{observed}\right] \times 100 \quad (4)$$

The model provided satisfactory predictions of the growth rates of LAB. The highest value for RE was 18.1%, below the limit of 20% that is used in the literature as criterion of applicability (Dalgaard, Mejlholm, & Huss et al., 1997; Gougouli, Angelidis, & Koutsoumanis, 2008), indicating that the combined Arrhenius-type model can describe satisfactorily the growth of LAB in MAP gilthead seabream fillets during isothermal refrigerated storage at levels of CO_2 packing within the 20–80% range.

The applicability of the Arrhenius-type model was also validated at dynamic chilling temperature conditions by using a periodically changing temperature profile. The time–temperature scenario used to validate the model and the temperature of fish flesh during storage under non-isothermal conditions together with the growth data for LAB in MAP (35% CO_2) gilthead seabream fillets are illustrated in Fig. 4.

The observed growth rates compared with the values predicted by the Arrhenius-type model, are also shown in Table 1. The % error of the prediction was 16.9 and 15.5 for the two experiments. The validation experiments support the applicability of the developed model to predict the growth of LAB on MAP gilthead seabream fillets under non-isothermal conditions and hence in the dynamic temperature conditions of the real chill chain. The shelf life of MAP gilthead seabream fillets can be thus estimated by Eq. (2), for any selected CO_2 level and storage temperature in the range studied (20–80% CO_2, 0–15 °C), as shown in Fig. 5. Such models are prerequisite for developing a monitoring system based on TTIs (Giannakourou, Koutsoumanis, Nychas, & Taoukis, 2005).

3.2. Time Temperature Integrators modelling and application

The visual colour change of the TTIs was adequately described by Δ*E* value (Eq. (3)). Δ*E* was modelled by an exponential decay function with time Eq. (5), $R^2 = 0.97$), as shown in Fig. 6a and b

Table 1
Specific growth rates (h^{-1}) of lactic acid bacteria of gilthead seabream fillets stored at 35% CO_2, calculated by the Arrhenius-type model and determined experimentally at isothermal and non-isothermal conditions.

Temperature	$k_{predicted}$ (h^{-1})	$k_{observed}$ (h^{-1})
0 °C	0.0051 ± 0.0002[a]	0.0050 ± 0.0003[b]
2.5 °C	0.0077 ± 0.0002	0.0085 ± 0.0004
5 °C	0.0115 ± 0.0003	0.0138 ± 0.0007
10 °C	0.0245 ± 0.0005	0.0259 ± 0.0009
15 °C	0.0522 ± 0.0015	0.0531 ± 0.0029
Variable 1 (T_{eff} = 9 °C)	0.0213 ± 0.0006	0.0256 ± 0.0013
Variable 2 (T_{eff} = 9 °C)		0.0252 ± 0.0007

[a] Mean values ± 95% Confidence Intervals based on the statistical variation of the parameters - regression analysis.
[b] Fitted parameter values ± standard error based on the statistical variation of the kinetic parameters of the Baranyi growth model - regression analysis.

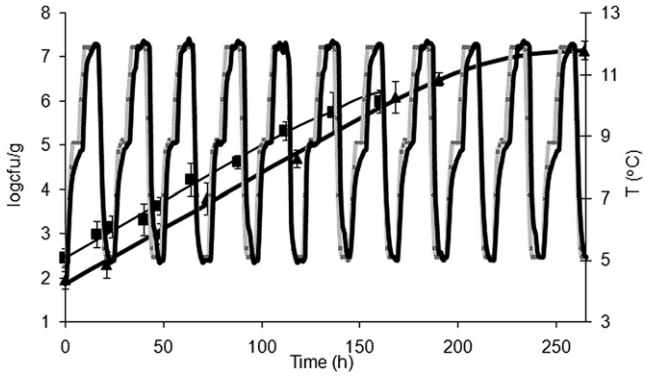

Fig. 4. Growth of lactic acid bacteria on gilthead seabream fillets packed under modified atmosphere (35% CO_2) at non-isothermal conditions (■ Experiment 1 and ▲ Experiment 2, storage temperature, fish temperature) (T_{eff} = 9 °C).

$$\Delta E = \Delta E_o \exp(-k \cdot t) \quad (5)$$

where k is a function of initial charging time, t_c, and storage temperature T (K). The L_{max}, a_{min} and b_{max} values are the values of the "white" coloured, uncharged TTI ($L_{max} = 80$, $a_{min} = -3.5$ and $b_{max} = 1.2$). The end point of the OnVu TTI was determined at $\Delta E = 11.9$ ($L_f = 69$, $a_f = -5$ and $b_f = -6$), corresponding to the visual endpoint. The response rate constants were plotted as a function of temperature in Arrhenius plots (Fig. 6c). The total response time (time from activation to endpoint) of the TTIs are shown in Fig. 7.

Based on the results of the testing of the TTI, a composite model that allows the calculation of the response rate, k_{TTI}, at any selected charging time was developed. The model was based on the observation that the response rate k_{TTI} at any temperature is a power function of the charging time, t_c. It was also observed that the effect of charging time on the E_a values of the TTIs is within the statistical variation ($P > 0.05$) (note in Fig. 6c the slope of the Arrhenius lines of the response rates of the TTI charged at different times which does not differ significantly). Thus the E_a value can be assumed not to change with charging time. The form of the composite model is expressed by Eq. (6)

$$k = k_{\mathrm{ref}_{\mathrm{Tref,1s}}} \cdot t_c^{-A} \cdot \exp\left(\frac{-E_a}{R} \cdot \left(\frac{1}{T} - \frac{1}{T_{\mathrm{ref}}}\right)\right) \quad (6)$$

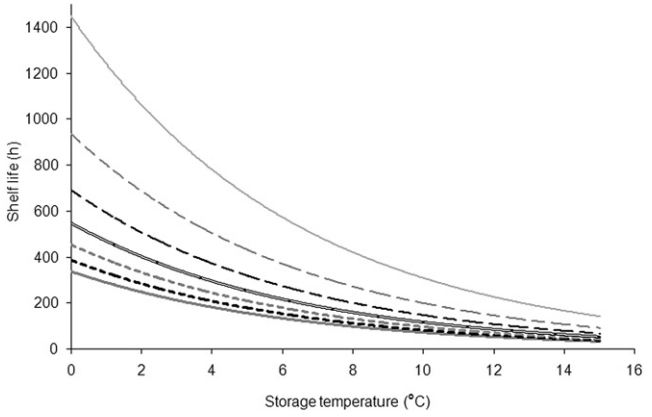

Fig. 5. Shelf life (d) of gilthead seabream fillets stored in MAP (20% CO_2, 30% CO_2, 40% CO_2, 50% CO_2, 60% CO_2, 70% CO_2 and 80% CO_2) at different storage temperatures, calculated using the combined Arrhenius-type model.

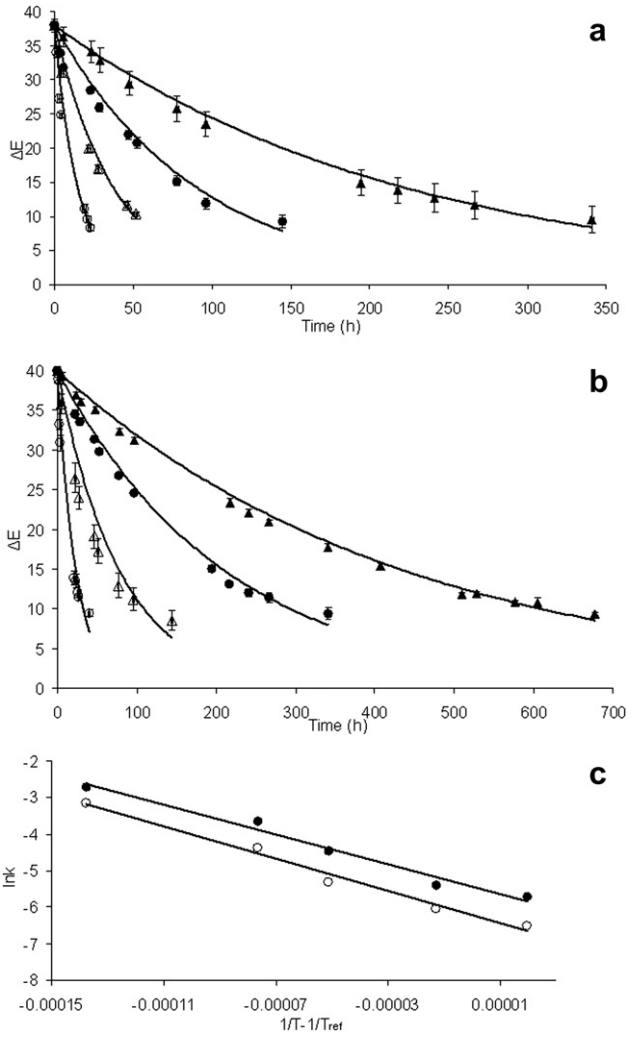

Fig. 6. Response of TTIs at different isothermal storage conditions (▲ 5, ● 8, △ 10 and ○ 15 °C, experimental points with error bars and potential fit) for (a) $t_c = 1.2$ s and (b) $t_c = 2$ s, (c) Arrhenius plots of the response rate of the TTIs (● $t_c = 1.2$ s and ○ $t_c = 2$ s).

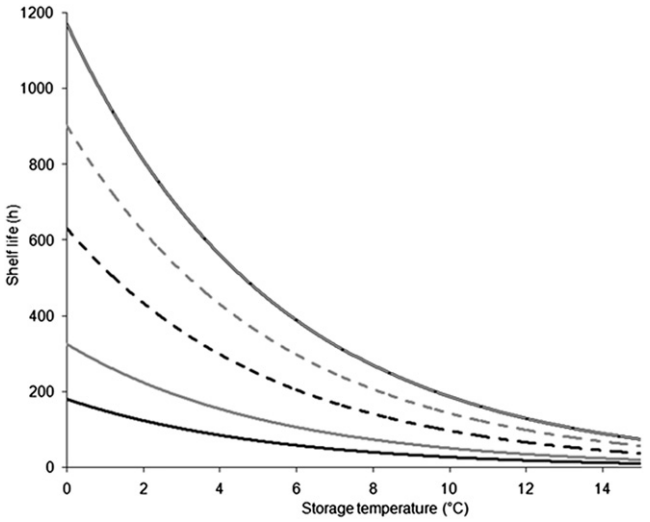

Fig. 7. Total response time of OnVu TTIs as a function of temperature at different charging times, t_c (1 s, 2 s, 4 s, 6 s and 8 s) calculated by the composite model.

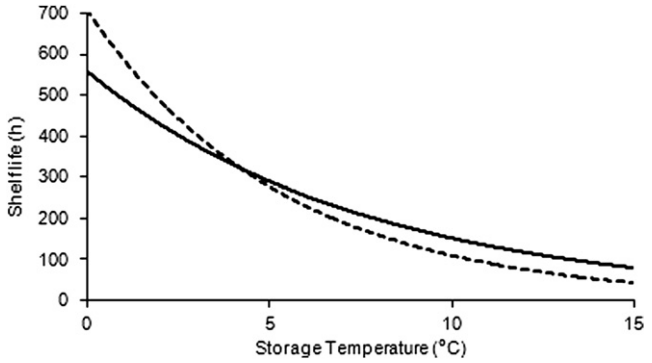

Fig. 8. Shelf life curve of MAP gilthead seabream fillets (solid lines) together with the matching TTI response curves (dotted lines), as estimated by the shelf life model (Eq. (2)) for 50% CO_2 and the TTI response rate model (Eq. (6)) for $t_c = 2.3$ s.

where T is the absolute temperature (K), E_a is the activation energy (kJ/mol), R is the universal gas constant, T_{ref} is a reference temperature (4 °C), $k_{ref_{Tref,1s}}$ is the TTI response rate constant at T_{ref} (with charging time $t_c = 1$ s). The composite model allows the calculation of the charging time needed in order to achieve the required colour change rate and response time for the TTI in order to better match the respective kinetics of the product.

The activation energy value, E_a, for all different charging levels of the TTI was defined at 122 ± 16.6 kJ/mol, $A = 0.7103 \pm 0.062$ and $k_{refTref,1s} = 0.00667 \pm 0.002$. The TTIs showed a slightly higher temperature dependence than LAB growth in MAP gilthead seabream fillets ($E_a = 101.0 \pm 25.9$ kJ/mol).

To select the appropriate charging time, based on the kinetic studies on MAP gilthead seabream fillets and the response profiles of the TTIs, the composite model can be solved to obtain an exact match between fish shelf life at any specific MAP condition and response time of the TTI at a reference temperature in the chilled range (e.g. 4 °C). By combining Eqs. (2) and (6), the optimal charging level for the OnVu TTI can be estimated for different packaging conditions of gilthead seabream fillets. It can be calculated that charging time of 1.3, 2.3 and 3.0 s could lead to suitable TTIs for monitoring the quality of gilthead seabream fillets stored under 20, 50 and 80% CO_2, respectively, during refrigerated storage. In Fig. 8 the shelf life of fish fillets at 50% CO_2 is shown together with the matching TTI total response time curve. If the fish fillets are stored at very low temperatures of 0–2 °C the end of shelf life will be determined and limited by the expiration date on the food package. On the other hand, if abuse temperatures of 6–10 °C prevail, then the TTI will conservatively signal poor quality products slightly before the end of shelf life.

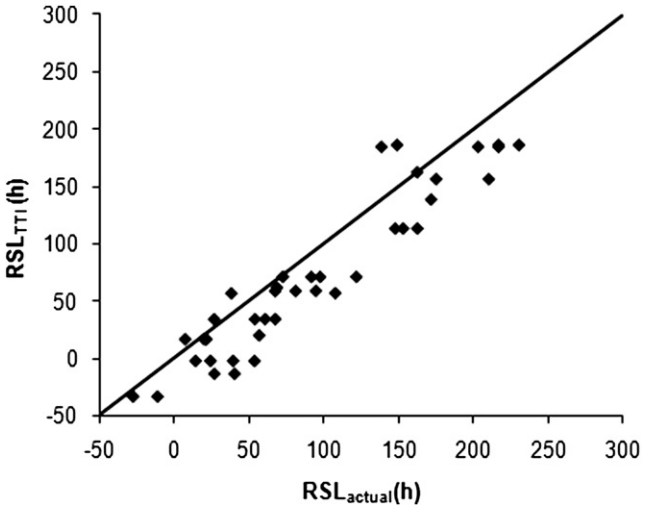

Fig. 10. Correlation of remaining shelf life of MAP (50% CO_2) gilthead seabream fillets based on TTI response (RSL_{TTI}) with RSL value based on LAB counts (RSL_{actual}) at the time of consumption.

3.3. Application of the Arrhenius-type model to predict the microbial spoilage of MAP gilthead seabream fillets in the real chill chain and the use of TTI as shelf life monitors

The experimentally measured log LAB values at the end of storage period as illustrated in Fig. 2 were compared with the LAB counts determined by the Arrhenius-type model, in order to evaluate its applicability in the real chill chain. The predictions obtained using the Arrhenius-type model for the spoilage of gilthead seabream fillets stored under MAP in the real chill chain were satisfactory, with 96.3% of the predictions lying within the ±20% RE zone. The positive RE values averaged 3.5% and the negative values averaged −6.8%, well below the 20% limit of applicability. The observed slight overpredition of the LAB growth rate (73.8% of the RE values are negative, indicating slightly higher predicted than observed log LAB values) is on the conservative side.

Initial LAB load was 3.8 ± 0.4 cfu/g, higher than the values determined for the batches used for the kinetic modelling of LAB growth (Tsironi et al., 2008), which is attributed to sample and batch variability of fillets. This initial load is taken into account in the calculations with the developed shelf life model (Eq. (2)). If the initial value of LAB counts cannot be measured or reliably estimated, the error due to this variability could be up to 30%.

The effectiveness of the TTI based system was evaluated by the simulated chill chain experiment based on the level of LAB at the end of storage. Respectively, measured TTI response was close to the expected based on the TTI response model (Eqs. (5), and (6)

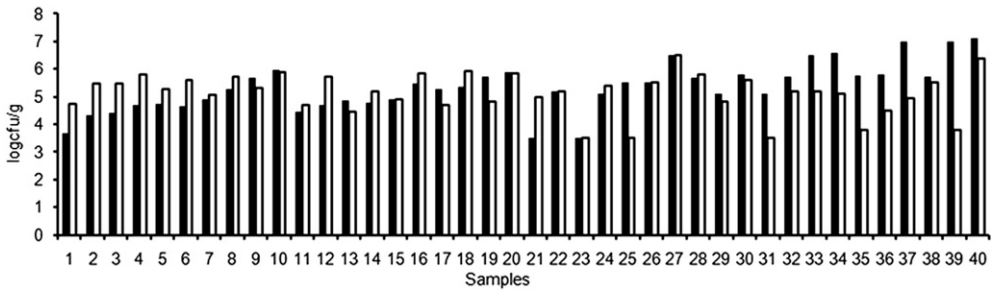

Fig. 9. Measured LAB counts (logcfu/g) in MAP (50% CO_2) gilthead seabream fillets at the end of the simulated chill chain, "local" and "distant" market (■ : products distributed randomly and □ : distributed based on TTI response).

with the 92.5% of the predictions 72 h after packaging lying within the ±20% relative error (RE) zone, indicating that the selected TTIs can be used for monitoring the spoilage of gilthead seabream fillets stored under MAP in the real chill chain.

In Fig. 9 the distribution of observed LAB counts of MAP gilthead seabream fillets at the "time of consumption" (end of storage) is depicted. With the random approach 6 out of 40 samples have exceeded the spoilage threshold (logN = 6). When the TTI based sorting was applied, only 2 of the 40 samples reached the spoilage level, significantly reducing the number of rejected products before the "time of consumption".

The predicted remaining shelf life based on the TTI response (RSL_{TTI}) compared to the "actual" remaining shelf life (RSL_{actual}), as it is estimated based on the actual measured LAB counts at the "end of storage" and Eq. (2), with logLAB = 6 as the limit of sensory acceptability, is illustrated in Fig. 10.

Overall (for both "local" and "distant" market) the TTI based system resulted in reducing the number of spoiled products. The use of TTIs at appropriate points of the chill chain (e.g. at a central distribution centre) would help making decisions for the further management of products based on their temperature history and hence microbial status.

4. Conclusions

The objective of the present study was to test a model that predicts reliably the shelf life of modified atmosphere packed gilthead seabream fillets during refrigerated storage and to define the appropriate TTIs that can monitor the quality of fish fillets under any selected storage conditions in the range studied. An Arrhenius-type model developed for MAP gilthead seabream fillets was validated at different packaging and temperature conditions and at various chill chain conditions. The agreement of the experimental measurements of microbial spoilage with the predictions from the developed model supports the assumption that it can be applied reliably in the dynamic temperature conditions of the real chill chain.

Seeking a suitable label for monitoring the quality of gilthead seabream fillets, a new UV activatable photochemical TTI was studied and its response was kinetically modelled. A composite model to predict the TTI response at any charging time was developed and the appropriate charging times for monitoring the quality of MAP gilthead seabream fillets at any selected packaging and storage conditions were estimated. The selection and use of the optimum TTIs for a particular product, with regard to its visual response characteristics and temperature sensitivity, could lead to realistic control of the chill chain, reduction of waste and efficient shelf life management. The models developed combined with the use of TTIs could be an effective tool for monitoring of the quality of chilled fish fillets during distribution and storage. If the temperature conditions of the products could be continuously monitored by TTIs, reliable estimation of the quality status and the remaining shelf life could be performed, allowing better management and optimization of the chill chain from production to the point of consumption.

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