

A new approach to predict the fish fillet shelf-life in presence of natural preservative agents

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Abstract

Three data sets concerning the behaviour of spoilage flora of fillets treated with natural preservative substances (NPS) were used to construct a new kind of mathematical predictive model. This model, unlike other ones, allows expressing the antibacterial effect of the NPS separately from the prediction of the growth rate. This approach, based on the introduction of a parameter into the predictive primary model, produced a good fitting of observed data and allowed characterising quantitatively the increase of shelf-life of fillets.

Introduction

Loss of freshness of fish is the consequence of post-mortem biochemical, physicochemical and microbiological processes as well as of several extrinsic factors such as the handling on board and on land, and technological processing (Giuffrida *et al.*, 2013). Several Authors (Dalgaard, 1995; Dalgaard *et al.*, 1997; Koutsoumanis and Nychas, 2000; Neumeyer *et al.*, 1997; Pin and Baranyi, 1998) have proposed a predictive approach to the evaluation of seafood shelf-life. Particularly, two different approaches have been studied taking into account: i) the prediction of the spoilage agents behaviour; ii) the modelling of spoilage compound production.

One of the best strategies to prolong the shelf life of fish and fish products is the use of natural preservative substances (NPS). These are essential oils and different types of dietary fibres (pea, apple, sugar beet, soy and citrus fibres) that are regularly used during foods manufacture for their nutritional and technological properties (Garcia *et al.*, 2007). In this regard the addition of

ingredients rich in bioactive compounds may have a technological purpose as mainly the inhibition of lipid oxidation, effect against spoilage and pathogen bacteria, inactivation of anisakid larvae and influences on tastes and aromas pleasing to the consumer (Busatta *et al.*, 2008; Giarratana *et al.*, 2015a, 2015b; Viuda-Martos *et al.*, 2011).

As well known, the application of predictive microbiology to the modelling of the shelf-life or the safety of foods is generally achieved by coupling a primary and a secondary mathematical model (McMeekin *et al.*, 2002). The former describes the sigmoidal behaviour of bacterial growth and the latter models the growth rate as function of environmental parameters such as temperature, pH, water activity, antibacterial substances, *etc.* Therefore, also the effect of NPS could be modelled according to the above approach providing a specific model for each substance. Anyway, this methodology involves several drawbacks and mostly does not allow to separately model the parameters that influence the growth from those that cause the bacterial decrease. Other different methods have been proposed in order to describe, within a mathematical predictive model, the interference against the growth of a bacterial population. Lotka and Volterra equations, for example, have been applied to several kinds of food systems (Giuffrida *et al.*, 2007). However, the Lotka-Volterra equations model the effect of one or more population against another one therefore the antagonistic effect is related to the concentration of one or more population.

Mejlholm and Dalgaard (2002) introduced a new technique for the modelling the antibacterial effect of some essential oils on *Photobacterium phosphoreum*, based on the expression of the percentage reduction in growth rate (%RGR). This parameter allowed to produce a good fitting of growth curves in presence of essential oils as well as a good prediction of observed data of modified atmosphere-packed cod fillets at 2°C. However, also in this case, the modelling of antibacterial effect is introduced as variable of growth rate, therefore it can predict a reduction of growth but not a decrease of bacterial population. The aim of this work is to study a new approach to modelling the effect of antimicrobial substances, with particular regard to NPS, against main spoilage microorganism of fish flesh. Particularly, the model is based on the introduction of a variable into the primary model rather than the secondary one. This variable takes into account the antibacterial effect of NPS subtracting the related decrease of bacterial load to the population growth.

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Materials and Methods

Data set

Three different data sets (A, B and C) concerning the behaviour of specific spoilage organisms (SSO) in fish fillets added or not with NPS, have been taken into account. According to Dalgaard *et al.* (1997), SSO is a psychrotrophic bacterial population including species mainly belonging to *Pseudomonas* and *Shewanella* genera, with very similar growth and nutritional requirements. Predictive models for this specific microflora have been widely employed for the characterisation of fish shelf-life (Dalgaard *et al.*, 1997; Koutsoumanis and Nychas, 2000).

Particularly, data set concern shelf life of gilthead seabream in presence of Allyl-isothiocyanate (Giarratana *et al.*, 2015c; Muscolino *et al.*, 2016), shelf life of gilthead seabream in presence of (R) Limonene (Giarratana *et al.*, 2016d) and shelf-life of Tilapia fillets treated with Thyme essential oil (Khalafalla *et al.*, 2015). Figure 1 summarises growth curve of spoilage bacteria and concentration of NPS for each trial.

Predictive model

Predictive model is based on the set of differential equations of Baranyi and Roberts model (Baranyi and Roberts, 1994), where the bacterial concentration N at time t is generically expressed as follows:

$$\frac{dN}{dt} = \mu_{max} N \frac{Q}{1+Q} \left(1 - \frac{N}{N_{max}}\right) \quad \text{Eq. 1}$$

Here μ_{max} is the maximum specific growth rate and N_{max} the theoretically maximum population densities of the bacterial population; Q represents the physiological state of the species and, as expressed in Eqs. (2a-b), allows to calculate the Lag-time (λ) duration (hours).

$$\lambda(t) = \frac{-\ln \alpha(t)}{\mu_{max}(t)} \quad \text{Eq. 2a}$$

$$\alpha(t) = \frac{Q(t)}{1 + Q(t)} \quad \text{Eq. 2b}$$

In order to take into account the antibacterial activity of NPS, the term ζ is introduced into equation 1 obtaining the equation 3:

$$\frac{dN}{dt} = \mu_{max} N \frac{Q}{1 + Q} \left(1 - \frac{N}{N_{max}}\right) \zeta \quad \text{Eq. 3}$$

with $1 > \zeta > 0$ and $N_{(t0)} < N$

In this way, the term ζ reduce the concentration of N without interfering with the calculation of μ_{max} . The expression of antibacterial effect is inversely proportional to the ζ values.

Model was numerically solved by Eulero method and the *Solver* function of Microsoft Excel was used, for each growth curve of each data set, in order to calculate appropriate ζ values related to each data set. Furthermore, for the numerical solving of

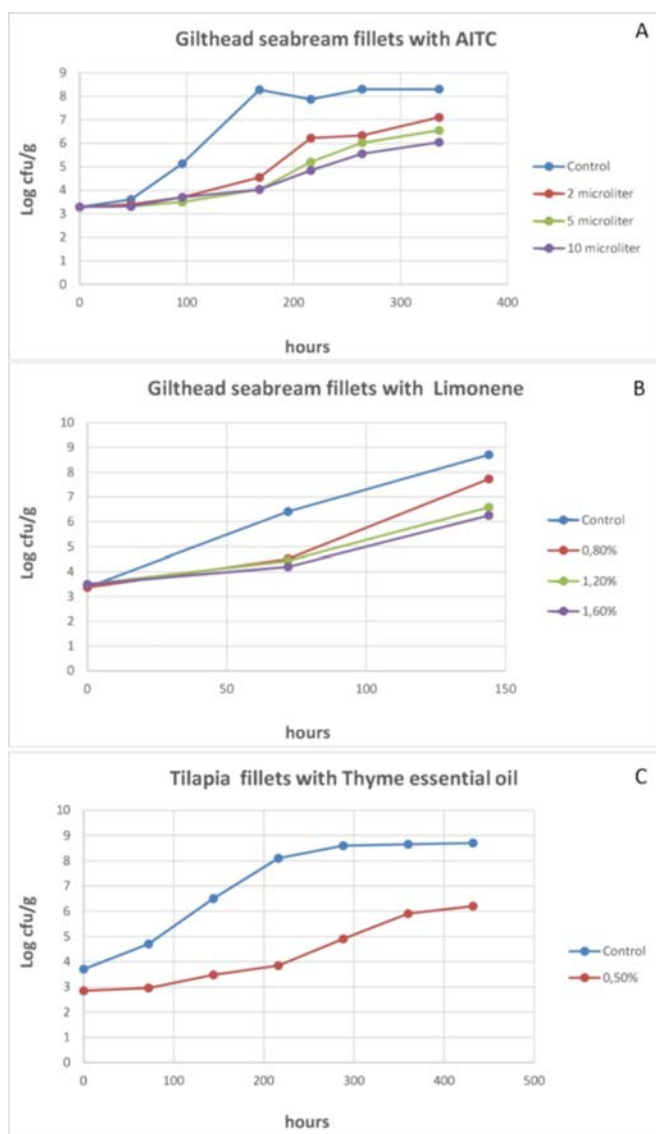


Figure 1. Trend of spoilage bacteria for each considered data set. A) (data set A) shows bacterial growth of Gilthead seabream fillets exposed to vapours of allyl isothiocyanate at concentrations of 2, 5 and 10 μL ; B) (data set B) shows bacterial growth of Gilthead seabream fillets treated with 500 μL of a solution with 0.8, 1.2 and 1.6% of limonene; C) (data set C) shows bacterial growth of Tilapia fillets with 0.5% of time essential oil. In all panels, *control* series indicates the trial without natural preservative substances.

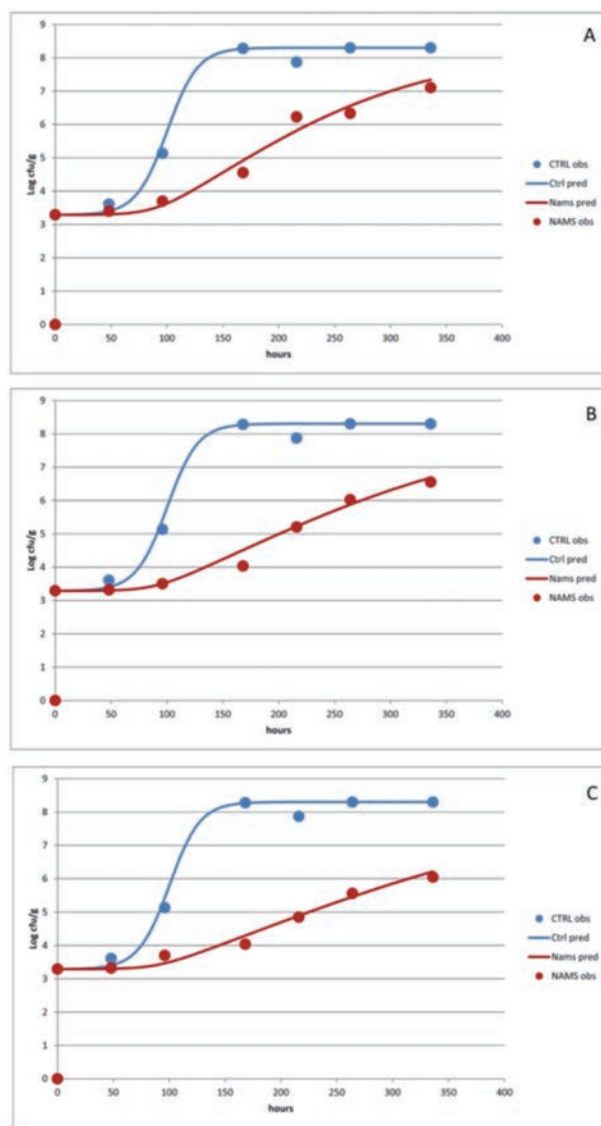


Figure 2. Observed and predicted spoilage bacteria growth curves for data set A, obtained from Gilthead seabream fillets exposed to vapour of allyl-isothiocyanate from 2 μL (A), 5 μL (B) and 10 μL (C).

equation 3 the secondary model of Ratowsky *et al.* (1983) as modified by Kotsoumanis and Nychas (2000) for fish spoilage flora, was introduced into the equation 3 to calculate the μ_{max} values as function of Temperature, according to the below equation 4:

$$\sqrt{\mu_{max}} = b (T - T_{min}) \quad \text{Eq. 5}$$

For the resolution of secondary model (equation 4) the procedure suggested by Kotsoumanis and Nychas (2000), and Neumeyer *et al.* (1997).

Results and discussion

Values of ζ obtained by the solving procedures (observed data against predicted) are showed in Table 1, whereas Figures 2-4 show observed and predicted data for each data set. Predicted data are obtained resolving equations 3 and 4 with the specific ζ values (Table 1).

As Figures show, model allows to obtaining a good fitting with the observed values. Particularly, for each data set, the combination of primary and secondary model reproduced, with a good fitting, the

growth curve of specific spoilage organism in fish products not treated with NPS. In this way, it is validated the goodness of predictive approach derived from equations 1, 2 and 4.

The introduction of ζ parameter is specific for each NPS and reproduces the antibacterial effect, independently to the prediction of growth rate, therefore it is not introduced into the secondary model. The numerical parametrisation of ζ is easily obtained with a simple fitting procedure and allows to find a set of ζ values concentration dependent.

Furthermore, the proposed mathemati-

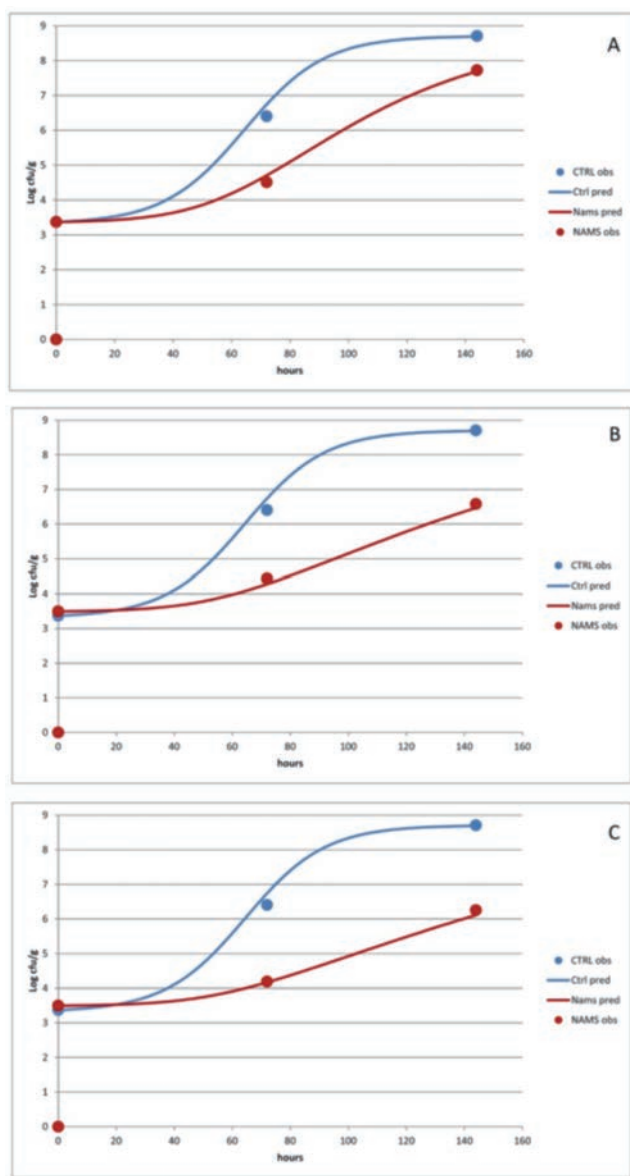


Figure 3. Observed and predicted spoilage bacteria growth curves for data set B, obtained from Gilthead seabream fillets treated with 500 μ L of a solution with 0.8% (A), 1.2% (B) and 1.6% (C) of limonene.

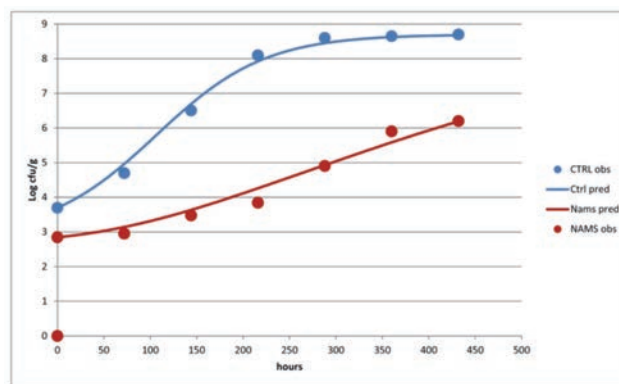


Figure 4. Observed and predicted spoilage bacteria growth curves for data set C, obtained from Tilapia fillets treated with a solution with 0.5% of thyme essential oil.

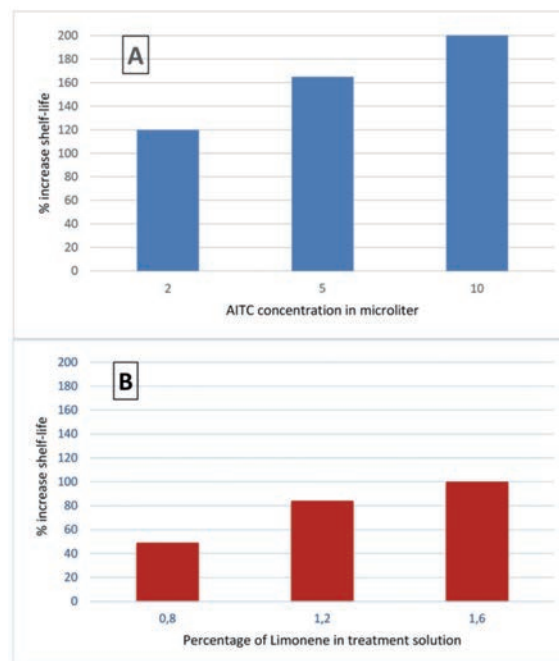


Figure 5. Percent increase in shelf life of fillets, according to the predicted behaviour of spoilage bacteria for data set A (A) and data set B (B).

Table 1. ξ values for each growth curve of each considered data set.

Conditions for each data set		NAMs	ξ
Data set A	AITC - Gilthead seabream (vapours from μL)	0	1.000
	AITC - Gilthead seabream (vapours from μL)	2	0.136
	AITC - Gilthead seabream (vapours from μL)	5	0.102
	AITC - Gilthead seabream (vapours from μL)	10	0.084
Data set B	LMN - Gilthead seabream (%/500 μL)	0	1.000
	LMN - Gilthead seabream (%/500 μL)	0.8	0.368
	LMN - Gilthead seabream (%/500 μL)	1.2	0.216
	LMN - Gilthead seabream (%/500 μL)	1.6	0.186
Data set C	TEO - Tilapia (%)	0	1.000
	TEO - Tilapia (%)	0.5	0.180

NAMs, natural actomyosins; AITC, allyl isothiocyanate; LMN, limonene; TEO, thyme essential oil.

cal predictive model with the ξ parameter allows to calculating the prolongation of product shelf-life when an antimicrobial substance is added to the food. In this study where we are considering two different data set obtained with two kind of NPS at different concentration, we predict a shelf-life prolongation proportional to the concentration of the substance, as showed in Figure 5. Moreover, model shows quantitatively the higher antimicrobial effect of a substance (AITC) compared to the other one (Limonene) and the related impact on the shelf life.

Conclusions

Foods are complex systems where biotic hazards such as bacteria and parasite (EFSA, 2015; Shifko *et al.*, 2000; Bucca *et al.*, 2011) and spoilage organism are subjected, during processing and storage, to several factors able to increase or reduce their concentration. For these reasons, the mathematical prediction of bacterial concentration could be interpreted as the results of growth and reduction of a microbial population that should be modelled separately. According to this point, the present study represents the starting point for a new class of predictive model for food hygiene and safety, able to separately model the growth and the decrease of a bacterial population. Obtained results show that the predictive approach can express the microbial concentration in presence or not of an antimicrobial substance without any modification of secondary model.

Beyond the satisfactory results of this study concerning the shelf-life prediction of fish fillets in presence of some NPS, the proposed approach could allow to describe the microbial behaviour of a population in a food system as the result of growth and inactivation phenomena that are separately modelled. This approach can appear very useful when a predictive model has to be

applied to a food processing based on the application of several mild technological *hurdles*. Therefore, the proposed model allows applying the predictive microbiology to a more complex context where the bacterial growth is alternated to the inactivation. In this context, the stochastic expression of ξ parameter, which reproduces the variability of *antimicrobial activity* (NPS, additives or other antimicrobials), could allow to express a probability distribution on the final concentration/presence of a microorganism according to a risk assessment based approach.

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