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A mathematical model to predict early quality attributes in hake during storage at low temperature

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ABSTRACT

In this work we develop a model describing the evolution of the adenosine triphosphate (ATP) degradation products, typically used as early indicators of fish quality loss, during storage and transport conditions. The model is constructed following a modular approach that includes essentially three mechanisms: (1) enzymatic transformation of inosine 5′-monophosphate (IMP), inosine (Ino) and hypoxanthine (Hx) with some reactions catalyzed by bacteria; (2) bacterial growth and (3) nucleotide diffusion through the food matrix. This approach allows us to combine the different underlying mechanisms to account for other fish species and conditions.

We compare alternative mechanisms explaining the catalytic effect of Pseudomonas and Shewanella populations on the reaction linking IMP, Ino and Hx. The selection is carried out in terms of the Akaike Information Criteria.

The predictive capabilities of the selected model are demonstrated with experiments.

1. Introduction

Freshness is one of the most important attributes to define the market value of fish (García et al., 2017). Loss of fish acceptability is generally due to bacterial spoilage (Hong et al., 2017) which occurs several days after fish death at typical storage conditions. However, loss of fish freshness starts early after fish death and before bacterial spoilage can be clearly noticed. Abundance of adenosine triphosphate (ATP) degradation compounds has been widely used as a reliable indicator to assess fish freshness at early storage stages (Saito et al., 1959; Hara and Uda, 1984; Hong et al., 2017). Among the degradation compounds, inosine 5′-monophosphate (IMP) provides a sweet and meaty flavor that contributes to enhance fish quality whereas its conversion in hypoxanthine (Hx) is responsible of unpleasant bitterness (Surette et al., 1988; Haard, 2002; Kawai et al., 2002; Kuda et al., 2008; Li et al., 2015). The K_I-index (Karube et al., 1984), that relates concentration of IMP, inosine (Ino) and Hx, has been proposed as a reliable freshness indicator. The K_I-index is defined as:

$$K_I(\%) = \frac{[\mathrm{Ino}] + [\mathrm{Hx}]}{[\mathrm{IMP}] + [\mathrm{Ino}] + [\mathrm{Hx}]}$$

In this way, studying the degradation pathways of IMP into Ino and Hx will allow us to derive mathematical models that can be used to monitor and predict quality losses of fish at early storage times.

IMP degradation under sterile conditions has been studied by several authors (Davídek et al., 1972; Hara and Uda, 1984; Howgate, 2006). Recently, Vilas et al. (2017a) proposed a biochemical pathway that explained nucleotide degradation kinetics in European hake (*Merluccius merluccius*) under sterile conditions. In that work, the authors started from the complete enzymatic degradation scheme presented in Fig. 2, determined the relevant reactions by fitting the model to experimental data, and reduced the original scheme to the one presented in Fig. 3 by neglecting non relevant reactions.

Under regular storage conditions, and in addition to physical barriers that may limit enzyme accessibility or nucleotide dispersion through the fish muscle, the dynamics of IMP degradation is, at a high extent, influenced by the presence of bacteria (Surette et al., 1988; Howgate, 2006; Zotos, 2010; Li et al., 2017). To illustrate this point, Fig. 1(a) presents the evolution of IMP and Hx for two experiments performed under sterile and non-sterile conditions. Both

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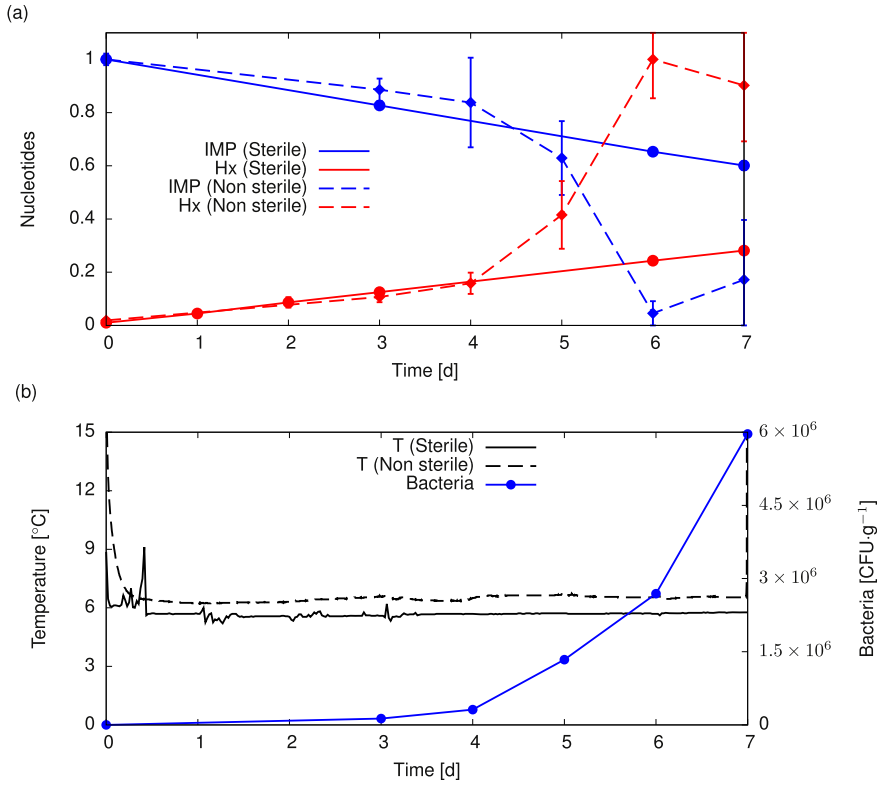

Fig. 1. (a) Evolution of IMP and Hx in sterile conditions (continuous lines) and in the presence of bacteria (dashed lines). Nucleotide concentrations are normalized with respect to their maximum concentration. (b) Bacteria concentration (*Pseudomonas* plus *Shewanella*) in the non-sterile experiment and temperature in the experiments carried out under sterile (continuous line) and non-sterile conditions (dashed line).

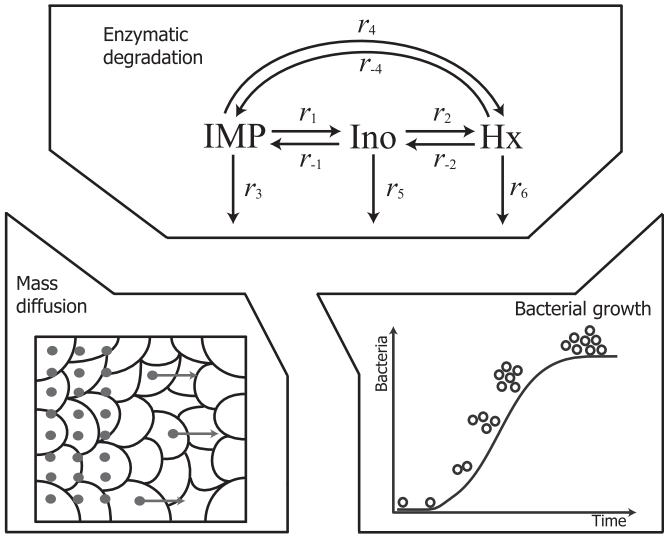

Fig. 2. Combination, in a modular way, of the different mechanisms relevant in nucleotide degradation to obtain the complete model.

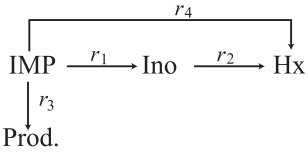

Fig. 3. Postmortem degradation scheme of IMP in European hake as reported by Vilas et al. (2017a).

experiments were carried out under similar storage temperatures which lied in the order of 6 °C (Fig. 1(b)).

As shown in the figure the rates of IMP degradation and Hx formation increase considerably as bacteria concentration becomes significant (from approximately day 4) in the non sterilized experiment. This suggests a bacterial catalytic action.

In this regard, alkaline phosphatase and 5′−nucleotidase, the major enzymes involved in IMP degradation, are widespread in bacteria (Zimmermann, 1992); including *Shewanella* (Ishida et al., 1998; Tsuruta et al., 2010; Kuribayashi et al., 2017) and *Pseudomonas* (Lazdunski et al., 1990; Beveridge and Kadurugamuwa, 1996). Ino can turn to Hx by purine nucleoside phosphorylase and inosine nucleosidase, which are also widely distributed in bacteria, including spoilage organisms of fresh fish such as *Shewanella* and *Pseudomonas*, as found in protein databases (e.g. UniProt, STRING). Previously, Surette et al. (1988) suggested that *Pseudomonas* was responsible for the production of intracellular inosine nucleosidase in fresh fish. We assume that *Pseudomonas* and *Shewanella* are the predominant bacterial species involved in the spoilage of fresh fish (Gram and Dalgaard, 2002; Gram and Huss, 1996) and make use of the model developed by García et al. (2015) to describe their growth as a function of temperature.

Competition among bacteria may constitute an important issue to be considered. Modeling such competition has been addressed in a number of works (Giuffrida et al., 2007, 2009a, 2009b), typically using Lotka–Volterra models. We assume that, for the bacteria considered in the present study, competition is not relevant for the following reasons: (i) the growth of *Pseudomonas* in fish stored at low temperature is unaffected by the presence of *Shewanella* (Gram and Melchiorsen, 1996); (ii) the growth of *Shewanella* is only