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Short communication

Predictive model for the growth of spoilage bacteria on modified atmosphere packaged Atlantic salmon produced in Australia

S.M. Powell [a, *], D.A. Ratkowsky [b], M.L. Tamplin [a]

[a] Australian Seafood Cooperative Research Centre and School of Land and Food, University of Tasmania, Private Bag 54, Hobart, Tasmania, Australia
[b] School of Land and Food, University of Tasmania, Private Bag 54, Hobart, Tasmania, Australia

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ABSTRACT

Most existing models for the spoilage of modified atmosphere packed Atlantic salmon are based on the growth of the spoilage organism *Photobacterium phosphoreum*. However, there is evidence that this organism is not the specific spoilage organism on salmon produced and packaged in Australia. We developed a predictive model for the growth of bacteria in Australian-produced Atlantic salmon stored under modified atmosphere conditions (30–98% carbon dioxide in nitrogen) at refrigeration temperatures (0–10 °C). As expected, both higher levels of carbon dioxide and lower temperatures decreased the observed growth rates of the total population. A Bělehrádek-type model for growth rate fitted the data best with an acceptably low root mean square error. At low temperatures (~0 °C) the growth rates in this study were similar to those predicted by other models but at higher temperatures (~10 °C) the growth rates were significantly lower in the current study.

1. Introduction

Atlantic salmon, *Salmo salar* L., is subject to the same autolytic and microbial spoilage processes as other finfish harvested from temperate waters. Usually, bacterial growth and metabolism results in the production of off-odours and flavours before autolytic degradation processes impact on fish quality (Gram and Huss, 1996). Storage at low temperature and packaging in modified atmospheres of carbon dioxide (CO_2) and nitrogen are two methods that are used to limit the growth of bacteria and increase the shelf-life of seafoods. The use of carbon dioxide in modified atmosphere packaging (MAP) has been the subject of many studies in seafood (for a review see Sivertsvik et al., 2002) as, in some cases, it does not produce the same extension of shelf-life as it does for products such as red meat. The ability of some bacteria found on fish to tolerate high levels of carbon dioxide is responsible for this. *Photobacterium phosphoreum* and lactic acid bacteria (LAB), such as *Carnobacterium maltaromaticum* in particular, are capable of growing in the presence of CO_2 and have been implicated in the spoilage of MAP seafood (Emborg et al., 2002; Macé et al., 2012).

Understanding the growth of the spoilage community under different CO_2 and temperature regimes is important for understanding spoilage rates. Models that predict the numbers of bacteria present in foods based on the conditions in which the food is stored have become useful tools assisting the food industry in making decisions about the safety and quality of their products (Membré and Lambert, 2008). These models are based on knowledge of which microorganisms are of concern in a particular product either because they are known to produce off-odours or off-flavours or because they can cause disease in humans.

Several predictive models exist that relate the concentration of carbon dioxide in the atmosphere and the storage temperature to the growth rate of specific bacterial species in a variety of different seafoods. For example the Seafood Spoilage and Safety Predictor (SSSP), version 2.0, contains several models for the spoilage of different types of seafood in air, MAP and vacuum packs as well as models for the growth of *P. phosphoreum* (MAP), *Shewanella putrefasciens* (air) and *Listeria monocytogenes* (Dalgaard et al., 2002). FishMap (Alfaro et al., 2013) is based on the growth of *C. maltaromaticum*, *Serratia proteamaculans*, *Yersinia intermedia* and *Shewanella baltica*, either individually or as a mixture, in Atlantic horse mackerel. The growth of *Lactobacillus* was modelled by Mejlholm and Dalgaard (2013) and validated against both meat and seafood products. However the authors of this paper acknowledge

* Corresponding author. Tel./fax: +61 3 62266286.
E-mail address: shane.powell@utas.edu.au (S.M. Powell).

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that it is unsuitable for describing the growth of other LAB including *Carnobacterium*. Because *Carnobacterium* is one of the genera that we have previously observed in our MAP Atlantic salmon, this model is not suitable for use with our product. Other models exist for organisms that may be of interest to seafood producers (for example Mejlholm and Dalgaard, 2007 or Pin and Baranyi, 1998) but were developed in matrices such as artificial broth or red meat that may not be realistic proxies for fish as a growth medium.

The only predictive model specifically for MAP Atlantic salmon is the model in the SSSP based on the growth of *P. phosphoreum*. Our previous work has shown that this organism is not commonly found in fresh chilled (not frozen) MAP Atlantic salmon produced in Australia (Powell and Tamplin, 2012; Milne and Powell, 2014) and therefore is unlikely to be a valid model for the prediction of shelf-life of Australian product. DNA-based analysis of commercially produced MAP Atlantic salmon portions found that LAB, mainly the genera *Carnobacterium*, *Lactococcus* and *Vagococcus*, were the most common organisms in spoilt product (Powell and Tamplin, 2012). Other recent work on the spoilage of MAP seafood found that *C. maltaromaticum* produces off odours in Atlantic salmon (Macé et al., 2013) and shrimp (Macé et al., 2014) and other bacteria, including Enterobacteriaceae such as *Hafnia alvei*, can also be responsible for off odours and flavours in MAP Atlantic salmon (Macé et al., 2013).

In Australia, Atlantic salmon is grown only in Tasmania, the southern-most part of the country, and the transport chains to both domestic and international markets can be lengthy. Knowledge of the spoilage organisms and their growth under realistic storage and transport conditions is required for producers to be able to guarantee the quality of the product throughout this transport chain. This knowledge can be used by managers to make decisions on the benefits of changing their processes – for example the costs and benefit of increasing the CO_2 content of MAP packs or the temperature during transport. The goal of the current study was to produce a model for the growth of spoilage bacteria on Australian produced Atlantic salmon stored under modified atmospheres of CO_2 and nitrogen, by measuring the growth of naturally occurring bacteria.

2. Material and methods

2.1. Experimental design

The growth rates of total viable bacteria (as measured by standard plate count methods) were determined on commercially produced Atlantic salmon stored in varying modified atmospheres of nitrogen and carbon dioxide at temperatures between 0 and 10 °C. Packs of commercially harvested and MAP packaged fresh Atlantic salmon were obtained from processing facilities in Tasmania on the day they were MAP packed. The packs were transported to the University of Tasmania on ice where they were aseptically opened and individual pieces removed. Pieces were subsampled into similarly sized portions of approximately 30 g and repacked into bags (Orved 95 GR smooth bags 2332025) in modified atmospheres (initial CO_2 content from 30 to 100%, remainder nitrogen) at a gas: product ratio of approximately 3:1 with a Technovac vacuum packer. Samples were stored at a range of temperatures between 0 and 10 °C. Data loggers (Thermochron ibuttons®) were used to monitor the product storage temperature.

2.2. Microbial enumeration

At each sampling time, two random bags were chosen. Each piece was stomached in saline peptone diluent (0.85% NaCl and 0.1% bacteriological peptone) and serially diluted. Total viable counts were obtained by pour-plating with Standard Plate Count (APHA) agar (Oxoid CM0463). Plates were incubated at 25 °C for 48 h prior to enumeration.

2.3. Model development

At each combination of temperature and percentage CO_2, DMFit (web version available from ComBase: http://www.combase.cc) was used to fit the data on the log of the number of colony forming units per g (CFU g^{-1}) as a function of time to a growth model (Baranyi and Roberts, 1994) and to obtain the maximum specific growth rates (hereafter referred to simply as growth rate). The growth rates were then used to derive a secondary growth model (e.g. see Ratkowsky et al., 1982). Following the work of Koutsoumanis et al. (2000), a Bělehrádek-type model was fitted to the growth data:

$$\sqrt{\mu max} = a\left(T - T_{min}\right)\sqrt{\left(CO_{2max} - CO_2\right)} \quad (1)$$

where a is a constant, T is temperature (K), CO_2 is the initial percentage CO_2 in the pack, T_{min} is the theoretical (i.e. notional) minimum temperature (K) and CO_{2max} (%) is the theoretical (i.e. notional and not necessarily realistic) maximum CO_2 level at which the bacteria will grow. The square root transformation serves to stabilise the variance.

3. Results and discussion

3.1. Growth curves

Growth curves were obtained for total viable bacteria on Atlantic salmon stored in modified atmospheres at temperatures between 0 and 10 °C. Examples of these curves at different temperatures in 98% CO_2 are presented in Fig. 1, where the base 10 logarithm of the number of CFU g^{-1} is plotted against time (h). The curves generated fitted well, giving low root mean square errors. The maximum growth rates obtained from 22 different combinations of CO_2 and temperature were used to generate the secondary model.

Lag phases were observed in some of the growth curves (for example 3.5 °C in Fig. 1) but not all. The appearance of a lag phase was not consistently observed at any particular temperature or in a particular atmosphere. The lag phase was not included in the model because of this inconsistency: the assumption was that growth began immediately. These observations are consistent with other studies (for example Bovill et al., 2000). The assumption that there was no lag phase is a conservative approach which will overestimate bacterial numbers by assuming that growth began immediately rather than hours (or days) later.

The growth rates determined in this study were compared to those predicted by the Seafood Spoilage and Safety Predictor (version 2.0, Dalgaard et al., 2002) for *P. phosphoreum* on MAP salmon (Table 1) after converting the initial CO_2 concentration (used in our model) to the equilibrium CO_2 concentration (used in the SSSP) based on the conversion tool present in SSSP. Although the growth rates are similar at low temperatures (around 0 °C), at higher storage temperatures (10 °C) the rates obtained in the current study are significantly lower. In practical terms, starting with the same number of bacteria (1×10^3 CFU g^{-1}) for salmon stored at 10 °C in 55% CO_2, the SSSP predicts that it takes 1.7 d for bacterial numbers to reach 1×10^6 CFU g^{-1}, whereas our growth rates indicate 3 d. We also compared the growth rates we obtained to those used by Alfaro et al. (2013) in the development of the