

## Characterization of the temperature fluctuation effect on shelf life of an octopus semi-preserved product

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### Abstract

The aim of this work is to study the effect of temperature fluctuations on spoilage microbial flora behaviour of a semi-preserved seafood product in modified atmosphere packaging (MAP) as well as to find correct interpretation criteria for simulating temperature fluctuations during storage tests. The study concerned 54 packages of “Octopus carpaccio” that were grouped in three batches and stored at 3 different temperature profiles: the first (16 packages - Group 4°C) was stored at  $4\pm 0.5^\circ\text{C}$ ; the second (16 packages - Group 8°C) was stored at  $8\pm 0.5^\circ\text{C}$ ; the third (16 packages - Group F) was stored under a fluctuating temperature regime between  $2^\circ\text{C}$  and  $14^\circ\text{C}$ . Spoilage microflora, pH and AW has been monitored, at regular intervals, along the storage period (44 days). A predictive model was constructed according to the accredited scientific literature and validated against the observed growth curves of the above three groups. Afterwards, the predictive model has been used setting the temperature at the mean value of fluctuations ( $6.72^\circ\text{C}$ ), at the kinetic mean value of fluctuations ( $7.80^\circ\text{C}$ ) and at the 75<sup>th</sup> percentile value of fluctuations ( $11.14^\circ\text{C}$ ). The best fitting to the observed data was obtained with the kinetic mean temperature value and this result shows that this parameter can be proposed to reproduce the temperature fluctuation along the distribution and the domestic storage when a storage test has to be carried out.

### Introduction

Shelf life of food can be defined as the period it will retain acceptable characteristics from a safety, hygienic and organoleptic point of view (Corradini, 2018; Fu and Labuza, 1993). Especially in fresh food with a short shelf life, often labeled with the “use by date” (Reg. 1169/2011 EU), a large part of deterioration is related to the microbial activity since the specific spoilage microflora can produce several kinds of off-

flavors, off-odors and discolorations. Furthermore, the term of consumption is strongly related to the potential growth of some foodborne disease agents, also according to the specific European Regulation 2073/2005 UE. Shelf life can be assessed by the use of storage tests that are carried out through the enumeration of microbial spoilage flora and, at the same time, associating the evaluation of sensorial modifications (Fu and Labuza, 1993). Also, it is widely consolidated the use of challenge tests based on experimental contamination of products and determination of growth rate or growth potential.

Storage tests need to explore the entire storage period at least; in some cases, the use of the Accelerated Shelf Life Tests (ASLT) can help to obtain an earlier response, since the test is carried out at higher storage temperature (Corradini, 2018). Furthermore, ASLT can produce a linear model to predict the shelf-life of product, based on Arrhenius relation and determination of the temperature coefficient (Q10) that represents the factor by which the rate of a reaction increases for every 10-degree rise in the temperature (T).

Concerning the shelf life of seafood products, it is well known it is mainly conditioned by the proliferation of some spoilage psychrotrophic bacteria belonging to *Pseudomonas* genera (Dalgard, 1995). For this product, the application of ASLT and related predictive model based on Arrhenius equation could not produce a good fitting with observed data since the relation between Temperature and bacterial growth is not always linear. Furthermore, the using of Q10 coefficient does not easily allow to predict shelf life under a fluctuating temperature regime (Corradini, 2018).

The fluctuation of food storage temperature under distribution and at domestic level are widely documented (James *et al.*, 2017; Pelletier *et al.*, 2011; Renard *et al.*, 2013; Roccatto *et al.*, 2017; Vergara *et al.*, 2014). Several authors evidence the high level of temperature heterogeneity inside the trucks, refrigerated container, cold room, or warehouse (Pelletier *et al.*, 2011; Raab *et al.*, 2008). Furthermore, significant temperature heterogeneity can also be observed inside a single pallet (Nunes *et al.*, 2014). Recently two contemporary and wide review have been carried out about the domestic refrigerators temperature and they find, beside the temperature mean of several countries, a high heterogeneity of storage condition related to the temperature fluctuation too (James *et al.*, 2017; Roccatto *et al.*, 2017).

There is not a wide knowledge on the relationship between shelf life and fluctuat-

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ing temperature because it is not clear the effect of fluctuations on microorganism behavior. Furthermore, it is not clear which temperature value gives a better representation of the fluctuations in order to simulate the thermal fluctuations during the food distribution and domestic storage when a storage test for assessing a product shelf life is planning.

The present work aims to study the effect of temperature fluctuations on spoilage microbial flora behavior of a semi-preserved seafood product in modified atmosphere packaging (MAP) as well as to find a correct interpretation of criteria for simulating temperature fluctuations during storage tests.

### Materials and Methods

For the present study, 54 packages of

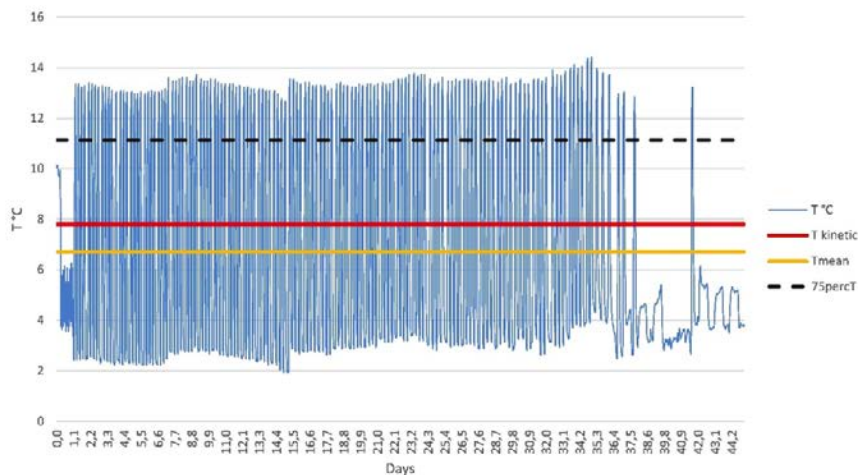
“Octopus carpaccio” of 100g in modified atmosphere packaging (MAP; 70% Nitrogen – 30% Carbone dioxide) were sampled, from a seafood industry located in the western Sicily, at the end of production and transferred at laboratory within 3 hours, in refrigerated conditions. Briefly, “Octopus carpaccio” is a semi-preserved product obtained by boiled octopus, inserted into plastic casing, opportunely pressed and, after 12 hours of refrigeration, sliced and packaged.

Samples were grouped in three batches and stored at 3 different temperature profiles: the first (16 packages - Group 4°C) was stored at  $4\pm 0.5^\circ\text{C}$ ; the second (16 packages - Group 8°C) was stored at  $8\pm 0.5^\circ\text{C}$ ; the third (16 packages - Group F) was stored under a fluctuating temperature regime between  $2^\circ\text{C}$  and  $14^\circ\text{C}$ . For this group, temperature monitoring was carried out by a data logger Thermo fisher mod. 3475.

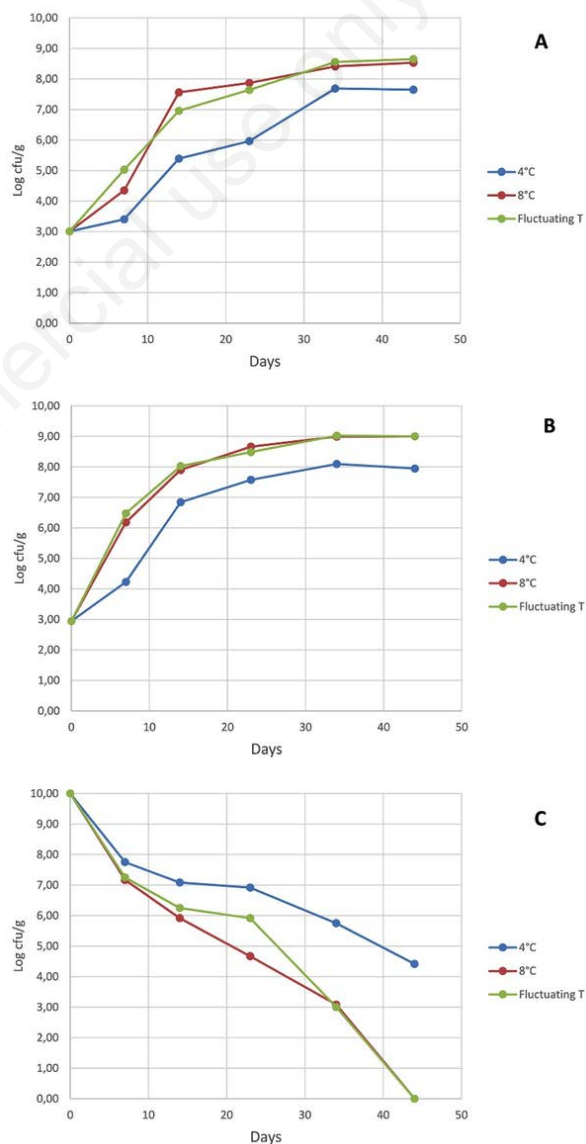
Three samples for each group were sampled at 0, 7, 14, 23, 34 and 44 days from the end of production, for the microbial analysis, for the determination of pH and AW as well as for the sensorial evaluation.

Particularly, microbiological analysis regarded the count of *Enterobacteriaceae* (ISO 21528-2:2017), the determination of Total Viable Count on plate of Iron Agar (Lingby), incubated at  $25^\circ\text{C}$  for 72 hours (Gram *et al.*, 1987) and the count of presumptive *Pseudomonas* spp. on plates of Pseudomonas Agar (Biolife, Italia) supplemented with CFC Pseudomonas Supplement incubated at  $25^\circ\text{C}$  for 40-48 hours (ISO 13720:2010). Each sample was analysed for pH value too (pHmeter HI90023CW - Hanna Instruments – with electrode Mettler Toledo InLab 427) as well as for the activity water (AW - Decagon Devices, AquaLab Serie 3TE). For the sensorial analysis four panelists, regularly trained in fish quality assessment, applied a demerit point system considering three parameters (odour, colour and taste) and assigning a demerit score from 10 to 0; the average of each score has provided the total sensorial score of the product.

In order to characterize the bacterial behaviour, a microbial predictive model has been implemented through the association of a primary and a secondary model. The former is the well-known Baranyi and Roberts model (1994) [Eq. 1] and the latter is the equation of Martinez-Rios *et al.* (2016) [Eq. 2] for calculating the growth rate as function of environmental parameters (Temperature, pH and AW) for Pseudomonads.



**Figure 1.** Representation of temperature fluctuation applied for “Group F” and related mean value ( $6,72^\circ\text{C}$ ), kinetic mean value ( $7,80^\circ\text{C}$ ) and 75<sup>th</sup> percentile value ( $11,14^\circ\text{C}$ ) of temperature fluctuations.



**Figure 2.** Observed growth curves for *Pseudomonas* spp. (A), Total Viable Count in Iron Agar (B) and sensorial scores (C) along the storage at  $4^\circ\text{C}$ ,  $8^\circ\text{C}$  and under fluctuating regime.

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

$$\mu_{max} = \mu_{ref25^{\circ}C} \left(\frac{T - T_{min}}{T_{ref} - T_{min}}\right)^2 (1 - 10^{(pH_{min} - pH)}) \left(\frac{aw - aw_{min}}{1 - aw_{min}}\right)^2 \quad [\text{Eq. 2}]$$

First of all, predictive model has been validated by reproducing the mean growth curves of *Pseudomonas* at 4°C and 8°C. In this way the initial value of “*Q*” (Eq. 1) has been calculated by fitting predicted to observed data. For “*N<sub>0</sub>*” (initial value) and “*N<sub>max</sub>*” in Eq. 1, the observed values were used. After this, the validated model has been applied to the prediction of *Pseudomonas* mean growth curve under temperature fluctuating regime. Further resolution has carried out applying the mean value of temperature fluctuations, the 75<sup>th</sup> percentile of temperature fluctuations and the mean kinetic value of temperature fluctuations, obtained according [Eq. 3]

$$T_K = \frac{\frac{\sum_{i=1}^n \Delta H_i}{R}}{-\ln\left(\frac{\sum_{i=1}^n e^{-\frac{\Delta H_i}{RT_i}}}{n}\right)} \quad [\text{Eq. 3}]$$

Where *T<sub>k</sub>* is the Mean Kinetic

Temperature in Kelvin degree;  $\sum_{i=1}^n \Delta H_i$  is the activation energy (generally between 60 and 100 kJ.mol<sup>-1</sup>); *R* is the gas constant; *T<sub>i</sub>* is the temperature of *i*<sup>th</sup> sample in Kelvin degree; *n* is the number of temperature samples.

In all cases, the predictive performance of model has been evaluated by the calculation of Root Mean Square Error (RMSE). All numerical resolutions of model have been carried out by the application of Eulero techniques.

## Results and Discussion

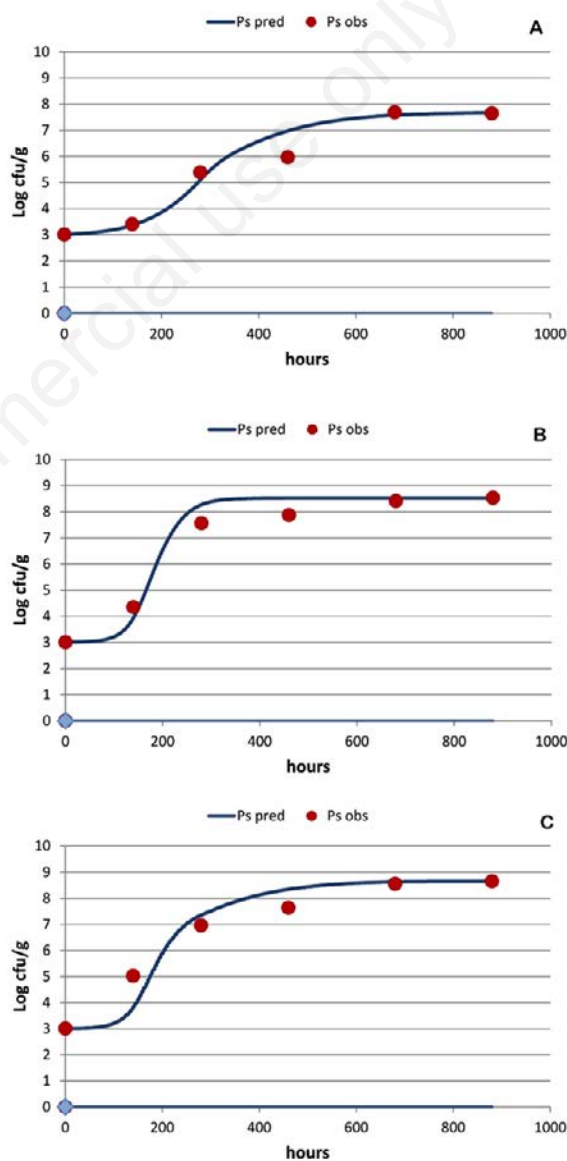
Figure 1 shows the temperature fluctuation applied for “Group F” and the related mean value (6.72°C), kinetic mean value (7.80°C) and 75<sup>th</sup> percentile value (11.14°C) of temperature.

Microbiological and sensorial result are summarised in Figure 2 that shows a shelf life of almost 33 days for product of Group 4°C while samples stored at 8°C and under fluctuating temperatures have reached a shelf life of 15 days and 22 days respectively. On this regard, note that, under the fluctuating temperature regime (“Group F”),

samples have a slightly longer shelf life than samples of “Group 8°C” despite the storage temperature of both groups are meanly very close, especially if it is taken into account the kinetic mean temperature value.

The application of predictive model has allowed to obtain a good fitting with the observed curves of Group 4°C (RMSE = 0.4312), Group 8°C (RMSE = 0.4322) and Group F (RMSE = 0.5906) (Figure 3). Particularly, the prediction for samples stored under a fluctuating temperature profile has been obtained by the dynamical resolution of the equations 1 and 2 (numerical resolution step by step with the Eulero techniques), allowing to take into account the instantaneous modification of environmental parameters.

Concerning the other predictions obtained with mean value of temperature fluctuations, kinetic mean value of temperature fluctuations and 75<sup>th</sup> percentile of temperature fluctuations (Figure 4), the best reproduction has been obtained with the kinetic mean temperature (RMSE = 0.5612) in respect of mean fluctuating temperature (RMSE = 0.8623) and the 75<sup>th</sup> percentile of fluctuating temperature (RMSE = 1.0855). This result show that the kinetic mean temperature could be considered a useful method to represent the fluctuating temperature regime of the retail and domestic storage or when it is necessary to summary several studies about the storage temperature during distribution in order to individuate a representative value.



**Figure 3.** Predicted growth curves against observed ones for *Pseudomonas* spp. at 4°C (A), 8°C (B) and under fluctuating regime (C).

## Conclusions

The characterization of seafood shelf-life is a complex matter that have to take into account several variables such as food characteristics, specific spoilage agents and environmental parameters with particular regard to the storage temperature, as well as the using natural or synthetic additives (Khalafalla *et al.*, 2015; Giarratana *et al.*, 2015; Giarratana *et al.*, 2016; Muscolino *et al.*, 2016; Aponte *et al.*, 2018). Predictive microbiology could represent an interesting method for the characterization of these variables and their influence on product shelf life. Concerning the seafood, several mathematical methods have been proposed for the shelf life predicting (Dalgaard, 1995; Dalgaard *et al.*, 1997; Giuffrida *et al.*, 2007;

Giuffrida *et al.*, 2013; Koutsoumanis and Nychas, 2000; McClure *et al.*, 1994; Pin and Baranyi, 1998; Ratkowsky *et al.*, 1983; Ratkowsky *et al.*, 1996). Some studies have explored the possibility to simulate, with an appropriate dynamic model, the combined influence of environmental parameter fluctuations and presence of natural preservative agents (Giuffrida *et al.*, 2017; Mejlholm and Dalgaard, 2002).

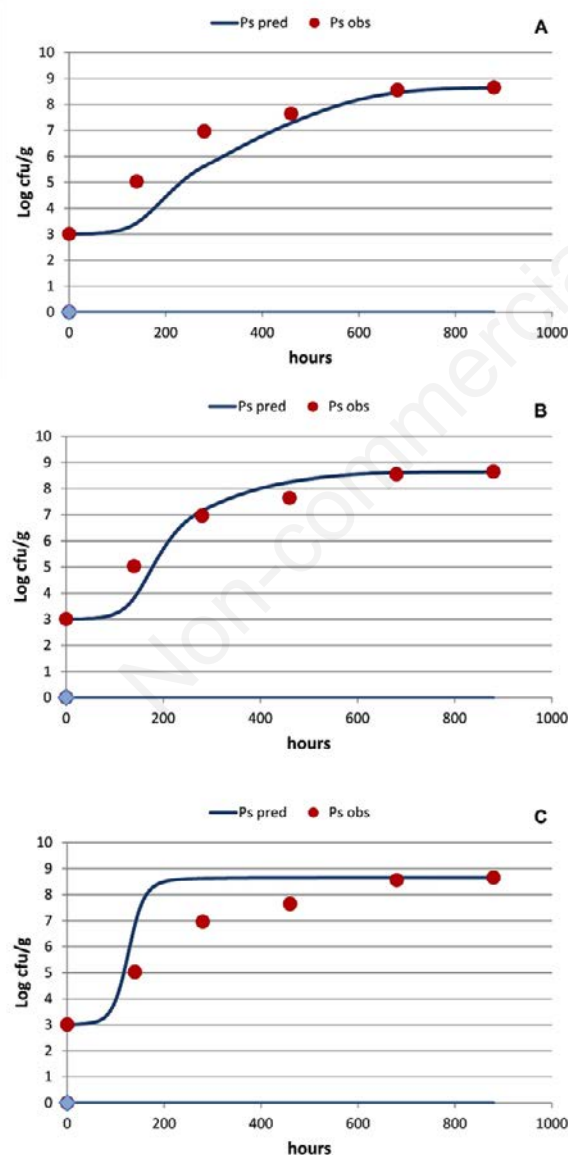
In the present study, the using of a dynamic predictive model has allowed to show that the kinetic mean temperature is the best method to represent a fluctuating temperature regime of storage while the using of the mean temperature underestimates the spoilage bacteria growth. On the other hand, the application of 75<sup>th</sup> percentile of temperature fluctuations has provided an

overestimation of the bacterial growth and a significative reduction of the predicted shelf life. In this regard, note that the use of the 75<sup>th</sup> percentile of temperature is suggested in the EURL *Lm* Guidance Document for implementing challenge tests and durability studies related to *Listeria monocytogenes* in ready-to-eat foods (v2, 07/05/2018), where, probably, a more precautionary approach is preferred.

Generally, we found the using of kinetic mean temperature more useful for the planning of shelf life studies, but this finding needs to a more robust validation in order to explore the effect of frequency and amplitude of fluctuations on the bacterial behaviour. At the same time, the development of probabilistic predictive models (Giuffrida *et al.*, 2009) that allow to take into account the temperature fluctuation as a stochastic variable, could represent an interesting method to consider the fluctuating temperature during the storage at retail and domestic level.

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**Figure 4.** Observed growth curves of *Pseudomonas* spp. under fluctuating regime against mean temperature (A), kinetic mean temperature (B) and 75<sup>th</sup> percentile of temperature (C).

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