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Predicting the kinetics of *Listeria monocytogenes* and *Yersinia enterocolitica* under dynamic growth/death-inducing conditions, in Italian style fresh sausage

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ABSTRACT

Traditional Italian pork products can be consumed after variable drying periods, where the temporal decrease of water activity spans from optimal to inactivating values. This makes it necessary to A) consider the bias factor when applying culture-medium-based predictive models to sausage; B) apply the dynamic version (described by differential equations) of those models; C) combine growth and death models in a continuous way, including the highly uncertain growth/no growth range separating the two regions.

This paper tests the applicability of published predictive models on the responses of *Listeria monocytogenes* and *Yersinia enterocolitica* to dynamic conditions in traditional Italian pork sausage, where the environment changes from growth-supporting to inhibitory conditions, so the growth and death models need to be combined. The effect of indigenous lactic acid bacteria was also taken into account in the predictions.

Challenge tests were carried out using such sausages, inoculated separately with *L. monocytogenes* and *Y. enterocolitica*, stored for 480 h at 8, 12, 18 and 20 °C. The pH was fairly constant, while the water activity changed dynamically. The effects of the environment on the specific growth and death rate of the studied organisms were predicted using previously published predictive models and parameters.

Microbial kinetics in many products with a long shelf-life and dynamic internal environment, could result in both growth and inactivation, making it difficult to estimate the bacterial concentration at the time of consumption by means of commonly available predictive software tools. Our prediction of the effect of the storage environment, where the water activity gradually decreases during a drying period, is designed to overcome these difficulties. The methodology can be used generally to predict and visualise bacterial kinetics under temporal variation of environments, which is vital when assessing the safety of many similar products.

1. Introduction

Italian style fresh sausage is a traditional pork product, commonly consumed after cooking. It can also be consumed raw, most frequently in regions of Central Italy, usually after a drying period of varying length. In fact, in some Italian regions, as high as 5% of fresh sausages are eaten raw (Migliorati et al., 2015).

Listeria monocytogenes is a gram positive bacterium causing severe disease in humans. Listeriosis is characterised by gastroenteritis, maternofetal infections and meningoencephalitis (Lecueit, 2005). It is usually related to the consumption of ready-to-eat, uncooked products. *Yersinia enterocolitica* also frequently causes gastroenteric disease, mostly associated with raw or undercooked pork products (CDC, 2005). >2100 cases of listeriosis and 6000 of yersiniosis are reported yearly in the European Union, with a significantly increasing trend for listeriosis since 2009 (EFSA/ECDC, 2014, 2015a, 2015b). Italian style fresh sausage is at risk of contamination by both these hazardous pathogens since it undergoes manual handling during processing before home storage at various temperatures, sometimes for weeks before consumption.

Codex Alimentarius recommends to move food control activities from a hazard-based final testing to a more risk-based management approach (Codex Alimentarius, 1999; Tenenhaus-Aziza and Ellouze, 2015). A consequence of this is that statistical and mathematical tools specifically designed to predict microbial behaviour in foods have become crucial. The European Commission Regulation 2073/2005 (Anonymous, 2005) suggests the use of predictive mathematical modelling. Although predictive models cannot completely replace lab testing or the judgement of an expert food microbiologist, they can aid informed decisions on food safety issues (Whiting and Buchanan, 2001).

Typically, the behaviour of microorganisms in varying environments has been modelled by separate growth or death models. The aim of this

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paper is to describe the development of a predictive model for the kinetic responses of *Y. enterocolitica* and *L. monocytogenes* in Italian style fresh sausage for storage conditions changing dynamically from growth-supporting to inactivating environments.

2. Materials and methods

2.1. Challenge tests

Sausages were produced according to a traditional recipe based on fresh pork (80%), skinned and de-boned, and fat tissue (20%). Salt was added in proportion of 20–22 g/kg; also ground pepper, garlic, rosemary and dried orange peel. These ingredients were ground, mixed and used to fill the casing skin made of pig gut. At the end of the preparation, each piece of sausage was about 10 cm long with a diameter of 4 cm.

Mixed cultures of *L. monocytogenes* (ATCC 7644 and two wild type strains, serotypes 1/2a and 1/2c, isolated from sausage) and *Y. enterocolitica* (NCTC 10463 and two wild type strains isolated from sausage) were prepared and inoculated according to Version 2 of the technical guidance document for shelf-life studies on *L. monocytogenes* (EURL Lm, 2008). Once late exponential growth phase was reached, successive dilutions of the mixed culture in physiological water resulted in an initial concentration of 10^2–10^4 CFU/ml, as checked on Tryptone Soy Agar (TSA). Sausages were inoculated in depth with 0.6 ml of the obtained cocktail (<1% of the volume of the food) injected at three different points. Sausages inoculated with *L. monocytogenes*, sausages inoculated with *Y. enterocolitica*, and control samples inoculated with 0.6 ml of physiological water were stored at four different temperatures (8, 12, 18 and 20 °C), in order to simulate plausible household storage conditions. The observation time was 480 h, as domestic storage before consumption of raw sausages may last up to 20 days after purchase, resulting in a gradual drying of the sausage. Initial concentration in the product, checked at time 0, was between 10^3 and 10^4 CFU/g at 8 and 20 °C; and between 10^2 and 10^3 CFU/g at 12 and 18 °C. The temporal variation of their concentration was followed by regular sampling and enumeration. Lactic acid bacteria, enterobacteriaceae and micrococci were only counted in the controls at 8 and 20 °C. Water activity (a_w) and pH were measured at all temperatures.

2.2. Laboratory analysis

L. monocytogenes was detected and enumerated according to ISO 11290-1:1996/Amendment 1:2004 and 11290-2:1998/Amendment 1:2004. *Y. enterocolitica* was detected according to ISO 10273:2003. Lactic acid bacteria, enterobacteriaceae and micrococci were counted only in the controls at 8 and 20 °C, according to ISO 15214:1998, 21528-4:2004 and 6888-1:1999, respectively. Water activity (a_w) and pH were measured according to ISO 21807:2004 and using a potentiometric method with a Beckman 360 pH meter (Beckman Instruments, Fullerton, CA, USA). *Y. enterocolitica* was enumerated following the FDA Bacteriological Analytical Manual (FDA, 2007).

2.3. Model development

Our aim was to predict the combined growth/death kinetics of *L. monocytogenes* and *Y. enterocolitica* under dynamic water activity conditions during the storage of the fresh sausages, using publicly available data, predictive models and new laboratory measurements.

2.3.1. Primary models of growth and death

For growth-supporting environments, the $y(t)$ natural logarithm of the concentration (cell/ml) of the studied two pathogens were predicted by the model of Baranyi and Roberts (1994):

$$\begin{cases} \dfrac{dy}{dt} = \dfrac{1}{1+q}\mu(1-e^{y_{\max}-y}) & (1a) \\ \dfrac{dq}{dt} = \mu q & (1b) \end{cases}$$

Here, $q(t)$ is a certain quantity typical of the actual physiological state of the cells that continuously improves according to linear kinetics, immediately after the inoculation. The specific rate is assumed to depend on it according to a Michaelis-Menten inhibition function. μ is the maximum specific growth rate (the maximum of the dy/dt rate), characteristic of the given environment. As usual, the specific "improvement-rate" of q is taken the same as the μ maximum specific rate of the cells. We assume that the instantaneous specific growth rate of the population instantaneously adjusts to the variation of the environmental factors (temperature, pH, water activity). The initial value for the first equation is a free parameter ($y(0) = y_0$); i.e. it is determined by the inoculation procedure, controlled by the experimenter.

The lag time does not directly appear in this model. The lag is in fact a derived parameter determined partly by the actual environment, partly by the initial physiological state of the cells that, on the other hand, depends on the history of the cells. The history-effect can be quantified by α_0, the fraction of the inoculum that could have produced the same exponential phase, had that fraction started to grow immediately after inoculation. Here we used its reparameterisation:

$$q_0 = \frac{1-\alpha_0}{\alpha_0}. \quad (2)$$

The lag in a static environment would be

$$\lambda = \frac{h_0}{\mu} = \frac{-\ln\alpha_0}{\mu}. \quad (3)$$