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Comparison of individual-based modeling and population approaches for prediction of foodborne pathogens growth

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ABSTRACT

Individual-based modeling (IBM) approach combined with the microenvironment modeling of vacuum-packed cold-smoked salmon was more effective to describe the variability of the growth of a few *Listeria monocytogenes* cells contaminating irradiated salmon slices than the traditional population models. The IBM approach was particularly relevant to predict the absence of growth in 25% (5 among 20) of artificially contaminated cold-smoked salmon samples stored at 8 °C. These results confirmed similar observations obtained with smear soft cheese (Ferrier et al., 2013). These two different food models were used to compare the IBM/microscale and population/macroscale modeling approaches in more global exposure and risk assessment frameworks taking into account the variability and/or the uncertainty of the factors influencing the growth of *L. monocytogenes*. We observed that the traditional population models significantly overestimate exposure and risk estimates in comparison to IBM approach when contamination of foods occurs with a low number of cells (<100 per serving). Moreover, the exposure estimates obtained with the population model were characterized by a great uncertainty. The overestimation was mainly linked to the ability of IBM to predict no growth situations rather than the consideration of microscale environment. On the other hand, when the aim of quantitative risk assessment studies is only to assess the relative impact of changes in control measures affecting the growth of foodborne bacteria, the two modeling approach gave similar results and the simplest population approach was suitable.

1. Introduction

Listeria monocytogenes is a well-known foodborne pathogen bacterium and many quantitative risk assessment studies on listeriosis dealing with food risk ranking or management options were published these last years (Anonymous, 2003, 2004a; Mataragas et al., 2010; Pouillot et al., 2009; Ross et al., 2009a, 2009b). Considering that pathogenic microorganisms generally contaminate foods with a few cells (EFSA, 2013), recent studies have investigated the individual cell behavior to describe more accurately the variability of cell lag times (Baranyi, 1998; Elfwing et al., 2004; Guillier et al., 2005; Métris et al., 2008; Pin and Baranyi, 2006) and cell growth probability (Augustin and Czarnecka-Kwasiborski, 2012; Koutsoumanis, 2008; Koutsoumanis and Lianou, 2013; Koutsoumanis and Sofos, 2005). These results highlighted the significance of the variability of the individual cell behavior in the context of exposure assessment (Anonymous, 2008; Nauta, 2000). As the variability of the microbial behavior is also highly dependent on the variability of food characteristics, we recently published results regarding the characterization of the variability of smear soft cheese surface characteristics (pH and water activity) at the microscale level (Ferrier et al., 2013). We developed an individual-based modeling approach (IBM) to describe the variability of the growth of *L. monocytogenes* cells on the cheese surface taking into account the microenvironment surrounding individual bacterial cells. Published microbial quantitative risk assessment studies usually take into account the variability of food characteristics at a macroscale level on relatively large food portions (Augustin et al., 2011; Koutsoumanis and Angelidis, 2007) and the cell behavior is described at the population level, *i.e.*, no between-cell variability is considered. The macroscale food variability combined with microbial population behavior is then used

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to run stochastic models describing the variability of the growth of foodborne bacteria (Couvert et al., 2010; Koutsoumanis and Angelidis, 2007; Pouillot et al., 2007; Tenenhaus-Aziza et al., 2014). For a given batch, the IBM approach combined with microscale description of the food characteristics was shown more effective to accurately describe the behavior of a few cells contaminating the surface of smear soft cheese in comparison to a population model combined with macroscale food description (Ferrier et al., 2013). This IBM joined to the microscale approach is nevertheless complex, labor intensive and requires the development of individual cell growth models, the development of micromethods to acquire microscopic physicochemical data and the running of complex and tedious computer programs.

The aim of this paper is to assess the usefulness of the IBM approach in comparison to the population one in the context of more global exposure or risk assessment studies. The comparison between the two approaches is performed for *L. monocytogenes* in smear soft cheese and cold-smoked salmon that constitute ready-to-eat foods potentially contaminated by this pathogen and able to support its growth (EFSA, 2013).

2. Materials and methods

2.1. Development and validation of IBM combined with microscale variability of food characteristics

2.1.1. Overview of the modeling approach

The spatial and temporal variability of physicochemical characteristics (pH and water activity – a_w) was characterized with different batches of smear soft cheese (Munster) and cold-smoked salmon during ripening and cold storage, respectively. Models were developed to describe the variability at the micro- and macroscale levels. The sources of variability were the batches, the cheeses surfaces and salmon slices and the location on cheese and salmon surfaces in the specific case of microscale level. The microscale pH of food surfaces was determined with a miniaturized 50 μm diameter pH electrode (Unisense, Aarhus, Denmark). The microelectrode was manipulated with a micromanipulator (Unisense, Aarhus, Denmark) and the position of the electrode on the food surface was checked with binocular glasses. The macroscale pH was determined with a pH-meter (HI pH 213, Hanna Instruments, Tanneries, France) according to the FD V04-035 standard (Anonymous, 2009). The pH-meter was equipped with a combination pH electrode (HI 1131B, Hanna Instruments, Tanneries, France) calibrated with pH 4.01 and pH 9.18 buffer solutions (Schott Glas, Mainz, Germany). Measurements were performed on analytical portions consisting in 5 g of food homogenized in 5 ml of deionized water. The microscale a_w of food surfaces was estimated using a cryoscopic micro-osmometer (Osmomat 030, Gonotec, Berlin, Germany). Food extracts were prepared by mixing 100 mg of food with 300 μl of deionized water. The mixture was blended, centrifuged (2000 rpm for 5 min for smear soft cheese and 5000 rpm for 10 min for cold-smoked salmon) and then allowed to stand at room temperature for 1 h. The osmolality of the food extract solutions was measured on the intermediate phase (Cabezas et al., 1988). A calibration curve linking the osmolality of cheese and salmon extracts with the a_w measured with the dew-point analyzer (FA-st/1, GBX Scientific Instruments, Romans sur Isère, France) was established by preparing 10 artificial samples of cheese and salmon exhibiting a_w ranging from 0.85 to 0.99 and from 0.70 to 0.90, respectively, by adding and mixing NaCl or water to natural food matrices. The macrosacle a_w of food surfaces was determined with a dew-point analyzer according to the ISO 21807 standard (Anonymous, 2004b) after calibration with a saturated solution of K_2SO_4. The growth of *L. monocytogenes* in homogeneously blended and irradiated cheese and salmon was studied to adjust predictive microbiology models to smear soft cheese and cold-smoked salmon (μ_{opt} and K).

The environmental and microbiological models were then used to predict the behavior of small or high inoculums of *L. monocytogenes* on the surface of smear soft cheese and cold-smoked salmon. Twenty samples of the two food matrices contaminated with approximately 10 and 1000 *L. monocytogenes* cells on surface were used to validate model predictions. Food surfaces were previously irradiated to eliminate indigenous microflora and avoid microbiological interactions not taken into account in current models. Inoculated food surfaces were enumerated after storage and observed distributions of *L. monocytogenes* counts were compared to simulated ones with the IBM/microenvironmental and population/macroenvironmental approaches.

2.1.2. Smear soft cheese model

The development and the validation of the IBM approach combined with the description of the microenvironment surrounding *L. monocytogenes* on smear soft cheese during ripening were previously published (Ferrier et al., 2013). Briefly, the increase in pH with time during ripening was described by a logistic-type model and the parameters of this model at the microscale level were dependent on the location on the cheese surface (hollows or crests and radius). A random between-microlocation variability was assumed and normal distributions were used to describe this variability. The variability of a_w at the microscale level was dependent on the cheese surface and on the location. Normal distributions were also used to describe the between- and within-surface variability. The IBM approach was shown more effective than the traditional population/macroenvironmental one to describe the actual bacterial behavior variability when cheese surfaces are contaminated with a few cells.

2.1.3. Cold-smoked salmon model

Vacuum-packed cold-smoked salmon sales units coming from three independent batches were obtained from an industrial manufacturer. Only the slices on the top of the sales units were used in the study since we chose to study the behavior of *L. monocytogenes* cells contaminating salmon surface and growing between the slice surface and the packaging film.

pH and a_w of cold-smoked salmon surface were measured during storage at 4 °C under vaccum at three different times from seven days after the packaging to approximately 28 days of storage (end of product shelf life). The macro- and micro-scale pH and a_w were determined for the three batches and on three salmon slices at each measurement time. For microscale measurements, different locations of the salmon surface were examined to reveal potential spatial effects (between 15 and 20 micro-measurements were obtained to characterize the within-slice variability).

The variability of pH and a_w was related to the following random factors: batch and slice, and location for micro-scale measurements. The factor “batch” described the between-batch variability, the factor “slice” described the between-slice variability and the factor “location” described the within-surface variability. The random effects of these factors followed normal distributions centered on 0 with standard deviations σ_{batch}, σ_{slice} and $\sigma_{location}$, respectively, and the variance of pH and a_w can be expressed with the following formula:

$$\sigma^2 = \sigma^2_{batch} + \sigma^2_{slice} + \sigma^2_{location} + \sigma^2_{residual} \quad (1)$$

The factor “slice” was not used for the microscale pH and a_w because the addition of this random factor did not increase the

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