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Modeling the heat inactivation of foodborne pathogens in milk powder: High relevance of the substrate water activity

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ABSTRACT

Due to the ability of foodborne pathogens to survive in low moisture foods, the decontamination of these products is an important issue in food hygiene. Up to now, such decontamination has mostly been achieved through empirical methods. The intention of this work is to establish a more rational use of heat treatment cycles. The effects of thermal treatment cycles on the inactivation of dried *Salmonella* Typhimurium, *Salmonella* Senftenberg, *Cronobacter sakazakii* and *Escherichia coli* were assessed. Bacteria were mixed with whole milk powder and dried down to different water activity levels (0.11, 0.25, 0.44 and 0.58). The rate of inactivated bacteria was determined after thermal treatment at 85 °C, 90 °C, 95 °C and 100 °C, from 0 s to 180 s in closed vessels, in order to maintain a_w during treatment. In a first step, logarithmic bacterial inactivation was fitted by means of a classical loglinear model in which temperature and a_w have a significant effect ($p < 0.05$). $D_{T,aw}$ values were estimated for each T, a_w condition and the results clearly showed that a_w is a major parameter in the thermal decontamination of dried foods, a lower a_w involving greater thermal resistance. In a second step, Bigelow's law was used to determine z_T, a classical parameter relative to temperature, and y_{aw} values, a new parameter relative to a_w resistance. The values obtained for z_T and y_{aw} showed that the bacterium most resistant to temperature variations is *Salmonella* Typhimurium, while the one most resistant to a_w variations is *Escherichia coli*. These data will help design decontamination protocols or processes in closed batches for low moisture foods.

1. Introduction

Low water activity (a_w) foods are defined by a water activity value below 0.60 which prevents any further microbial development. Such foods represent 80% of food products in the food industry (Cuq, Rondet, & Abecassis, 2011). Water activity, which varies between 0 (no water) and 1 (pure water), represents the water available for biochemical, biological and physiological reactions in a food product and this parameter is of primary importance in establishing the inactivation rate for low moisture foods. Indeed, under a threshold of 0.60, no microorganisms can grow and for pathogenic bacteria, a first growth is observed in media with an a_w of > 0.87. This is the reason why a_w is widely used as a reference to limit pathogen growth in food products (Sofia M Santillana Farakos & Frank, 2014) and to reduce pathogen contamination. Indeed, recent works propose to manage a_w in order to inactivate foodborne pathogens on food surface or products (Lang et al., 2016, 2017; Zoz et al., 2016).

Among low a_w food products, whole milk powder (a_w generally comprised between 0.20 and 0.45) production represents a very large volume (2.5 million tons per year in the European Union) which is directly consumed or included in the formulation of various food products such as dairy foods, cakes, cereal products, chocolate and infant formula (Forsythe, 2014). The milk used to make the powder was pasteurized but the further drying and conditioning processes could result in cross-contamination of the obtained powder (Carrasco, Morales-Rueda, & García-Gimeno, 2012; Podolak, Enache, Stone, Black, & Elliott, 2010; Sofia M Santillana Farakos & Frank, 2014).

This risk has been documented by outbreaks involving milk powder and products containing milk powder, for which several pathogens were involved, including both Gram positive and Gram negative bacteria. The vegetative pathogens associated with dried milk products are principally *Salmonella enterica* and *Cronobacter sakazakii* (Beuchat et al., 2013; CDC, 2009, 2012; Finn, Condell, McClure, Amézquita, & Fanning, 2013; Forsythe, 2014; Institut de veille sanitaire, 2006), for which

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outbreaks have been recorded annually by national and international public health institutes. Besides the outbreaks, foodborne pathogens contamination can lead to product recalls, which have an impact on both the reputation and the finances of the industry (FDA, n.d.; Dai, Tseng, & Zipkin, 2015).

Many decontamination processes in liquid or high water activity food products involve a heating step and more precisely a specific "time-temperature" couple in order to provide the optimal conditions for bacterial inactivation (Podolak et al., 2010; van Asselt & Zwietering, 2006). The loglinear model is widely used in the food industry and a great number of D values, relative to different temperatures, is available in the literature for high water activity media and foods. In these high water activity media, a_w can be considered as constant and very close to 1 and the thermal properties of water make heat treatment simple and homogeneous and enable precise temperature control during the thermal process, which is not the case for solid foods, especially for dried foods (Fine, Ferret, & Gervais, 2005). Furthermore, other models could be used, such as the Weibull model which allows to represent a two-phase bacterial inactivation cycle (Santillana Farakos, Frank, & Schaffner, 2013; Silva & Gibbs, 2012).

The thermal decontamination process of dried food products such as milk powder is difficult to predict due to the heterogeneous thermal properties of dried foods (Fine et al., 2005; Laroche, Fine, & Gervais, 2005). Indeed, several investigations report an increased resistance in low moisture foods of *S. enterica, Escherichia coli* or *C. sakazakii* unlike what is found in high water activity foods. For example, in the case of *S.* Weltevreden in flour, a decrease in a_w from 0.40 to 0.10 involved an increase in the D_T value by a factor of 30 (Grasso, Stam, Anderson, & Krishnamurthy, 2014). At low a_w, an increased thermal resistance is generally observed in several microorganisms, including *Saccharomyces cerevisiae*, *Lactobacillus plantarum* or *Salmonella enterica* (Juneja & Eblen, 2000; Laroche & Gervais, 2003; Laroche et al., 2005).

The singular resistance to heat in the dried state can be attributed to two major factors. The main one is the low molecular mobility resulting from the low water content characteristic of dried food. The breaking of disulfide and hydrogen bonds and the active site injuries due to the thermal treatment are reduced, preventing the heat denaturation of biomolecules essential to vital bioprocesses or membrane alterations (Alvarez-Ordóñez, Fernández, López, & Bernardo, 2009). The second factor is the active phenomenon of cross-protection by the activation of bacterial genetic and metabolic pathways during the transition to the dried state (Shen & Fang, 2012; Spector & Kenyon, 2012), involving, for example, a large synthesis of Heat Shock Proteins which protect protein conformations by preventing thermal damage (Gruzdev et al., 2012). This high heat resistance in dried state gives a great importance to a_w parameter, which has to be taken into account in dried food decontamination processes.

The objective of the present study was to model the simultaneous effects of time, temperature and a_w on the thermal inactivation of *Escherichia coli*, *Salmonella enterica* ssp. *enterica* serovar Typhimurium, *Salmonella enterica* ssp. *enterica* serovar Senftenberg and *Cronobacter sakazakii* in milk powder, and then to propose a decimal reduction time as well as suitable parameters to represent the effect of temperature and water activity on bacterial inactivation.

2. Materials and methods

2.1. Bacterial strains and cultivation conditions

Escherichia coli K12TG1, *Salmonella enterica* subspecies *enterica* serovar Typhimurium DT104 DSM 10506 (DSMZ, Braunschweig, Germany), *Salmonella enterica* subspecies *enterica* serovar Senftenberg 775 W DSM 10062 (DSMZ) and *Cronobacter sakazakii* CIP 103183T (Institut Pasteur, Paris, France) strains were used in this study. *E. coli* K12TG1 was chosen as a reference strain. *S.* Typhimurium DSM 10506 was chosen for its high thermal resistance in dried state and its relevance in outbreaks linked to low-moisture foods (Beuchat et al., 2013). *S.* Senftenberg DSM 10062 was chosen for its high thermal resistance (Ng, Bayne, & Garibaldi, 1969). Finally, *C. sakazakii* 103183T was chosen for its resistance to stress and its relevance in outbreaks (Dancer, Mah, Rhee, Hwang, & Kang, 2009). All cultures were stored in Tryptic Soy Broth (TSB, Sigma-Aldrich, Saint-Quentin-Fallavier, France) with 20% glycerol (Sigma-Aldrich) at − 80 °C. For recovery, the bacteria were inoculated on Tryptic Soya Agar (TSA, Sigma-Aldrich) at 37 °C for 24 h, five colonies of each bacterium were subsequently picked up in 50 mL of Tryptic Soya Broth (TSB, Sigma-Aldrich) and incubated for 8 h at 37 °C. Suspensions were then diluted in 50 mL of fresh TSB in order to adjust the Optical Density at 600 nm (OD_{600}) of 0.01 (corresponding approximately to 10^6 CFU/mL) before incubation for 14 h at 37 °C to reach stationary growth phase cultures.

2.2. Inoculation of the powder

For each strain, the cell concentration of previous stationary phase cultures was checked and adjusted to 1×10^8 CFU/mL through the measurement of the OD_{600}, according to calibration curves (established upstream of the experiment by three independent measures of the OD_{600} and bacterial concentration in CFU/mL in bacterial culture over time in the same growth conditions) showing the link between OD_{600} and cell concentration in CFU/mL. 50 mL cultures were centrifuged (3400g, 10 min at 25 °C) and washed twice with 25 mL of Phosphate Buffered Saline (PBS, Sigma-Aldrich). In a final step, the supernatant was removed and cell pellets were weighed. Milk powder (fat: 26% of which saturated fat: 17%, sugar: 41%, proteins: 25%, salt: 1%, initial microbiological quality: < 100 CFU/g, Regilait, Saint–Martin–Belle–Roche, France) was added to the pellets with a 1:20 ratio (w_{pellet}:w_{powder}) and homogenized using a mortar and pestle. An inoculated milk powder was obtained with an a_w of about 0.80. The achievement of a homogeneous contamination was checked by enumeration of samples collected from the inoculated milk powder (around 5×10^8 CFU/g; SD < 0.3 log CFU/g).

2.3. Drying process

To dry the inoculated milk powder, we used hermetic boxes with saturated salt solutions at the bottom to regulate the a_w and consequently the atmosphere RH. We used lithium chloride (LiCl, Sigma–Aldrich) to obtain an a_w of 0.11 (corresponding to an RH of 11%), potassium acetate to reach an a_w of 0.25 (corresponding to an RH of 25%), potassium carbonate to obtain an a_w of 0.44 (corresponding to an RH of 44%) and finally sodium bromide (all procured from Sigma–Aldrich) to obtain an a_w of 0.58 (corresponding to an RH of 58%). The atmospheres were maintained under convection using a fan (Sunon, Radiospare, France) as described in a previous work (Lemetais, Dupont, Beney, & Gervais, 2012). All drying processes were performed at room temperature. For each bacterium, 2 min after inoculation, about 5 g of the inoculated milk powder were spread on four Petri dishes (which corresponds to a thin layer), and placed inside hermetic boxes without a lid for 16 h in order to reach the final a_w level which we checked using an a_w-meter (Aqualab, Dardilly, France).

2.4. Heat treatment

Each 0.2 mL capped tube (propylene tube with flat waterproof cover and self-locking hinge) was completely filled with 0.1 g of dried inoculated milk powder and treated at different holding temperatures (85 °C, 90 °C, 95 °C, and 100 °C) over a given holding time (0 s, 30 s, 60 s, 90s, 120 s, and 180 s) using a thermocycler (Bioer, France). It took 7 s for the tubes to reach the holding temperature. The samples were then cooled down to 4 °C (15 s of ramp-down time) to stop the impact of the thermal treatment. The tubes were checked for water-tightness through weighting before and after thermal treatment. These specific treatment cycles were chosen because they did not involve any visual