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Inactivation of *Salmonella*, *Listeria monocytogenes* and *Enterococcus faecium* NRRL B-2354 in a selection of low moisture foods

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ABSTRACT

The aims of this study were to obtain data on survival and heat resistance of cocktails of *Salmonella*, *Listeria monocytogenes* and the surrogate *Enterococcus faecium* (NRRL B-2354) in four low moisture foods (confectionery formulation, chicken meat powder, pet food and savoury seasoning) during storage before processing. Inoculated samples were stored at 16 °C and cell viability examined at day 0, 3, 7 and 21. At each time point, the heat resistance at 80 °C was determined. The purpose was to determine a suitable storage time of inoculated foods that can be applied in heat resistance studies or process validations with similar cell viability and heat resistance characteristics. The main inactivation study was carried out within 7 days after inoculation, the heat resistance of each bacterial cocktail was evaluated in each low moisture food heated in thermal cells exposed to temperatures between 70 and 140 °C. The Weibull model and the first order kinetics (D-value) were used to express inactivation data and calculate the heating time to achieve 5 log reduction at each temperature.
Results showed that the pathogens *Salmonella* and *L. monocytogenes* and the surrogate *E. faecium* NRRL B-2354, can survive well (maximum reduction <0.8 log) in low moisture foods maintained at 16 °C, as simulation of warehouse raw material storage in winter and before processing. The D_{80} value of the pathogens and surrogate did not significantly change during the 21 day storage ($p > 0.05$). The inactivation kinetics of the pathogens and surrogate at temperatures between 70 and 140 °C, were different between each organism and product. *E. faecium* NRRL B-2354 was a suitable *Salmonella* surrogate for three of the low moisture foods studied, but not for the sugar-containing confectionery formulation. Heating low moisture food in moisture-tight environments (thermal cells) to 111.2, 105.3 or 111.8 °C can inactivate 5 log of *Salmonella*, *L. monocytogenes* or *E. faecium* NRRL B-2354 respectively.

1. Introduction

Although low moisture foods cannot support microbial growth, and were historically considered as 'low risk' in terms of pathogen contamination and no growth potential compared to high water activity animal- or vegetal-derived products, they have significantly contributed to the total number of food-borne infections and outbreaks (Chen et al., 2009; Podolak et al., 2010; Beuchat et al., 2013). For example, it has been estimated that 1000 people were infected by contaminated paprika present on potato chips in the 1993 outbreak in Germany (Lehmacher et al., 1995); over 400 cases (126 in 1981 and 283 in 2009) were associated with black pepper outbreaks (Gustavsen and Breen, 1984; Gieraltowski et al., 2013); and >200 cases were attributed to toasted oats cereal in the USA between April and June 1998 (Centers for Disease Control, 2001) Contaminated peanut butter was responsible for >400 cases in the USA between August 2006 and May 2007 (Centers for Disease Control, 2007), and again between 2008 and 2009 in 46 states resulting in a further 700 cases. It is generally recognised that many cases of food poisoning e.g. of salmonellosis, are unreported or not investigated, for all types of products; this in turn suggests that association of food-borne infections from dry ingredients, is much higher. According to RASFF (the Rapid Alert System for Food and Feed) a total of 477 notifications related to *Salmonella* in all types of food was recorded in 2014, of which 101 were related to low moisture foods – 21.2%. In 2015, 517 notifications were recorded of which 116 were related to low moisture food – 22.4%. Notifications related to *Listeria* are very much lower; 91 in 2014 with only two related to low moisture foods (butter and halva with pistachio nuts) and 99 in 2015 with three recorded notifications related to low moisture food (dry ham, sesame pasta and dried pork sausage).

The high percentage of *Salmonella* notifications in low moisture foods indicates that current methods of harvesting e.g. of seeds, drying and primary processing for control or elimination of *Salmonella*, are not efficacious or are not correctly implemented. Attention should therefore be focused on improving harvesting methods, and evaluating the ability of pathogens to survive in low moisture foods both during

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storage and throughout processes. Appropriate and validated, processes and processing conditions should be developed and applied industrially, to control or eliminate food-borne pathogens in dry foods and ingredients for ready to eat products that are not heat treated before packaging and distribution. A number of studies relating to survival of pathogens in low moisture foods have been published, (e.g. Danyluk et al., 2005; Uesugi et al., 2006; Komitopoulou and Peñaloza, 2009; Blessington et al., 2012), but the product range, product composition, storage conditions and heating methods differ; therefore obtaining data for specific products, organisms and conditions is necessary.

Although the number of cases of listeriosis is low, and those causally related to dry foods, very low, the infection can be life-threatening (20–30% mortality). For this reason, the survival and heat resistance of *Listeria monocytogenes* in a selection of four dried foods was investigated. This data is necessary for evaluating potential hazards and taking data-based decisions in HACCP studies. The use of clinical strains is a prudent option as there seems to be some evidence that strains isolated from foods and food-processing environments tend to exhibit reduced virulence (Liu et al., 2007).

While most publications show no limitations in using *Enterococcus faecium* NRRL B-2354 as a surrogate for *Salmonella* (Almond Board of California, 2007, 2014; Jeong et al., 2011; Enache et al., 2015) other studies have shown some limitations of using this surrogate (Rachon and Gibbs, 2015).

Survival curves obtained during heat inactivation studies are not always linear. Non-linear curves are very common in both laboratory experiments and in pilot plant scale trials, (Humpheson et al., 1998; Drosinos et al., 2006; Leguérinel et al., 2007). While for linear curves a first order kinetic model has been used and D- and z-values calculated, for non-linear curves, several different models have been proposed (Smith, 1991; Xiong et al., 1999; Juneja et al., 2001; Pasquali et al., 2016).

The current study was undertaken to obtain data and information on the viability of two important pathogens, *Salmonella* and *L. monocytogenes*, in four dried food materials of different compositions during storage for 21 days (currently raw materials are generally ordered according to food processing schedules and kept temporarily in warehouses for short periods of time and therefore processed within 3 weeks), and to evaluate the utility of a non-pathogenic organism – *E. faecium* NRRL B-2354 – as a surrogate for these pathogens for potential use in food processing environments in case that biological validations/verifications are necessary to demonstrate that specific processing steps are appropriate to ensure food safety. Additionally, the heat resistance of the pathogens and surrogate in the four low moisture foods, was determined to evaluate the kinetics of inactivation to achieve a 5 log or greater inactivation levels.

2. Materials and methods

2.1. Low moisture foods

Four low moisture food formulations in powder form, were supplied by Nestec Ltd in sealed, flexible aluminised plastic pouches and stored at 16 °C. The samples had been decontaminated at 25–50 kGy for this study at an external company.

Table 1
Composition and a_w of low moisture foods.

Composition	Confectionery	Seasoning	Chicken meat powder	Pet food
Moisture (%)	8.36	8.95	3.63	10.94
a_w	0.434	0.648	0.235	0.576
Protein (N_2% × 6.25)	3	24.2	69.5	30
Fat (%)	1	1.2	25	6
Carbohydrate (%)	87.5	26	3	53.8

The composition of the products and water activity (a_w) before inoculation are shown in Table 1. In addition to the proximate composition, the confectionery formulation contained starch (35%), sucrose (20%), maltodextrin (20%), wheat flour (20%), and natural flavouring ingredients (5%). The savoury seasoning contained salt (30%), glutamate (30%), sucrose (20%), rice flour, chicken meat, egg, spices. The chicken meat powder is an industrial raw material mix of chicken meat meal (85%) and salt (15%). The pet food formulation contained corn, rice, wheat flours (40%), and protein-rich materials like corn gluten, soybean meal, fish meal (35%), chicken by-product meal (20%), mineral/vitamin premixes and natural flavouring (5%).

2.2. Bacterial strains

A cocktail of six *Salmonella* strains was used in this study: *S.* Enteritidis PT 30; ATCC BAA-1045 (a strain associated with the first recorded food-borne outbreak linked to consumption of raw almonds, USA/Canada, 2001), *S.* Senftenberg 775W; ATCC 43845 (heat resistant in moist foods), *S.* Typhimurium; ATCC 14028 (chicken isolate), *S.* Anatum; ATCC BAA-1592 (a strain isolated from a tomatoes linked outbreak in the USA, 2004), *S.* Montevideo; ATCC BAA-710 (tomato isolate), *S.* Tennessee; K4643 (a human isolate from the 2006 peanut butter outbreak in the United States). These strains were selected for their survival above average among >30 strains in selected low moisture foods (data not shown). Selections was focused on the most frequently used strains with heat resistance above average, and strains from outbreaks linked to low moisture foods.

All strains were obtained from American Type Culture Collection (ATCC) except for *S.* Tennessee K4643 which was supplied by Nestec Ltd. All strains were recovered on Tryptone Soya Agar (TSA, Oxoid CM0131) incubated aerobically for 18 h at 37 ± 0.5 °C and a number of colonies (<20) were dispersed in Cryo-preservation beads (TS/80-BL; TSC Ltd, Heywood, UK) containing Cryopreservative fluid: beef extract, peptone, sodium chloride, glycerol (20%), de-ionised Water. Three vials of each strain were prepared and stored at −70 °C and used for preparing three independent replicates.

Preliminary screening of seventeen *L. monocytogenes* strains for ability to survive in low moisture foods identified five suitable strains with survival above average (data not shown). A cocktail of the five *L. monocytogenes* strains was used in this study: *L. monocytogenes* ATCC 15313 – 53 XXIII, DSMZ 20600 (serovar 1a, mammal isolate), *L. monocytogenes* ATCC 49594 – Petite Scott A (serovar 4b human isolate, the strain widely used as a reference strain for efficacy testing of food processing and preservation techniques, establishment of detection methods in foods, growth and heat resistance studies, and virulence studies, (Briers et al., 2011), *L. monocytogenes* ATCC 35152 – NCTC 7973 (serovar 1a, isolated from mammal), *L. monocytogenes* ATCC 13932 – LMG 21264 (isolated from child with meningitis, Germany; serotype 4b), DSMZ 27575 (serovar 4b, human isolate) and L. *monocytogenes* - FRRB 2542 (Barotolerant salami isolate). Strains were obtained from ATCC and Leibniz-Institut DSMZ - Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH and *L. monocytogenes* FRRB 2542 was supplied by Nestec Ltd. All strains were grown and stored at −70 °C as described above.

A single strain of *Enterococcus* was used in this study: *E. faecium* NRRL B-2354 (ATCC 8459) - (strain most frequently used in heat inactivation studies as a surrogate for *Salmonella*). This strain was obtained from ATCC and grown and stored at −70 °C as described above.

2.3. Inocula preparation

This study was conducted using a cocktail of *Salmonella* strains, a cocktail of *L. monocytogenes* strains and an *E. faecium* NRRL B-2354 inoculum. The *Salmonella* cocktail combined all 6 strains (grown as individual cultures); *L. monocytogenes* cocktail combined all 5 strains (grown separately), and *E. faecium* NRRL B-2354 was used as a single