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Microbial validation of radio frequency pasteurization of wheat flour by inoculated pack studies

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ABSTRACT

This study developed a microbial validation method for radio frequency (RF) pasteurization of low-moisture food powders. Wheat flour with water activity of 0.45 ± 0.02 was used as a model. In this study, heat resistance parameters (D- and z-values) of *Salmonella* Enteritidis PT 30 (*S.* Enteritidis) and its potential surrogate *Enterococcus faecium* NRRL B-2354 (*E. faecium*) in wheat flour were determined. The results showed that, while both microorganisms yielded the similar z-values, *E. faecium* was more heat-resistant than *S.* Enteritidis. For process validation, a 5-g pack of wheat flour inoculated with either microorganism was placed in the geometric center of 3 kg wheat flour and subjected to various processing times of up to 39 min in a 27 MHz RF unit. The inactivation kinetics matched but yielded slightly greater reduction than pasteurization modeled from measured temperature profiles and microbial thermal resistance parameters. This investigation concluded that *E. faecium* is a valid surrogate for *Salmonella* in wheat flour. A conservative validation can be obtained by inoculated pack protocol. RF heating technology has potential for pasteurizing wheat flour.

1. Introduction

Industrial thermal processes for food safety are generally established based on two key sets of information: 1) the heat resistance of microorganisms related to specific product formulation, and 2) the heating rate and duration of the specific product in the least heated zone (Awuah et al., 2007). Novel food safety processes may require microbial validation, but pathogens are rarely allowed in the food processing environment. Thus, non-pathogenic surrogate microorganisms, with thermal resistance similar to or slightly higher than the target pathogens (FDA, 2015a), are often used to study the fate of the pathogens in these processes.

Salmonella in low-moisture foods has been identified as a potential hazard and requires further preventive controls to minimize or prevent its outbreaks (FDA, 2015b). Its potential surrogate *Enterococcus faecium* NRRL B-2354 (*E. faecium*), identified by the Almond Board of California (Almond Board of California, 2007) in almond processing, has been utilized in validation studies of extrusion (Bianchini et al., 2012), moist-air convection heating (Jeong et al., 2011) and infrared pasteurization (Bingol et al., 2011) of low-moisture foods.

Radio frequency (RF) heating is a novel, chemical-free pasteurization method for dry food materials. It has been previously studied for inactivation of bacteria in meat lasagna (Wang et al., 2012), almonds (Gao et al., 2012, 2011, 2010), flour (Villa Rojas, 2015; Tiwari et al., 2011), peanut butter (Villa Rojas, 2015) and pepper spices (Kim et al., 2012). Similar to microwave heating, a main challenge for commercial application of RF heating technology is in heating uniformity. Computer simulations have demonstrated a relatively uniform heat distribution can be achieved in some dry food materials (Tiwari et al., 2011; Wang et al., 2007). However, no protocols are available for RF decontamination of *Salmonella* in low-moisture foods. No systematic studies have been reported on microbial validation of RF pasteurization for low-moisture foods.

This study aimed to develop procedures to validate RF treatments of *Salmonella* in low-moisture foods. We selected wheat flour as a model food and used *E. faecium* as a surrogate. Specific

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objectives were to: 1) evaluate the suitability of *E. faecium* as a potential surrogate for *Salmonella* in wheat flour in commercial applications, 2) determine the heating pattern and obtain temperature distribution of wheat flour in a RF system and develop a procedure for inoculated pack studies, and 3) validate RF treatment of wheat flour by comparing survivor kinetics with the calculated F-value obtained from temperature-time histories.

2. Materials and methods

2.1. Materials

Organic soft winter wheat flour was purchased from Eden Foods (Clinton Township, MI). Water activity was measured at 25 °C ($a_{w,25°C}$) using a water activity meter (AQUA PRE, Decagon Devices, Pullman, WA). The geometric mean particle size of the flour was 144 ± 60 μm, which was measured with an ATM sonic sifter L3P (ATM Corporation, Milwaukee, WI). The nutritional composition of the wheat flour was provided by Northern California Laboratory of Silliker Inc. (Salida, CA) (Table 1). Enumeration of background microflora was obtained from five random 1 g samples diluted in 9 mL of 0.1% peptone water, plated on trypticase soy agar (BD Diagnostics, Sparks, MD) and incubated for 48 h at 37 °C.

S. Enteritidis and *E. faecium* (ATCC 8459) were obtained from Dr. Linda Harris (University of California, Davis) and kept at −80 °C in tryptic soy broth (TSB; BD Diagnostics, Sparks, MD) supplemented with 20% (vol/vol) glycerol. Working cultures of each microorganism were prepared by streaking for isolation onto TSA plates supplemented with 0.6% (wt/vol) yeast extract (TSAYE). Plates were incubated 24 h at 37 °C.

Tryptic soy agar, TSB, yeast extract and peptone were purchased from BD Diagnostics (Sparks, MD); ammonium iron (III) citrate was purchased from Sigma-Aldrich Corporation (St. Louis, MO); sodium thiosulfate, 5-Hydrate, was made by J. T. Baker (Avantor Performance Materials, Center Valley, PA).

2.2. Determination of thermal resistance of microorganisms in wheat flour

2.2.1. Bacterial strains and inoculation

Frozen *S.* Enteritidis or *E. faecium* was subjected to two consecutive transfers (24 h each at 37 °C) in 9 ml of TSB supplemented with 0.6% (wt/vol) yeast extract (TSBYE), and then 1 ml was evenly spread onto a plate (150 × 15 mm) of TSAYE. The bacterial lawn on TSAYE was harvested with 20 ml of sterile 0.1% peptone water and centrifuged for 15 min at 6,000*g*, 4 °C. Then, the supernatant was discarded and the pellet was re-suspended in 3 ml 0.1% peptone water. One ml of concentrated pellet was hand-mixed into 10 g flour in a sterile stomacher bag until the pellet was visibly mixed. After mixing, this seed flour sample was used to further inoculate 90 g flour, which was mixed and stomached (Seward Stomacher, 400 Lab System, Norfolk, United Kingdom) at 260 rpm for 5 min. Then, ten of 1 g samples were randomly selected and enumerated on TSA plates as described subsequently to confirm the uniformity of inoculum distribution.

Table 1
General composition (wet basis) of the wheat flour sample used in the study.

Component	Content % (w/w)
Carbohydrate	78.92 ± 0.16
Total dietary fiber	12.92 ± 0.09
Moisture	8.34 ± 0.12
Protein	5.70 ± 0.00
Fat	3.28 ± 0.09
Ash	1.55 ± 0.03

2.2.2. Equilibration

To avoid the impact of water activity on thermal resistance of microorganisms, the inoculated samples were placed in sterile trays and then put into a Hotpack 435315 humidity chamber (SP Industries, Inc., Warminster, PA) (Villa Rojas, 2015) for a minimum of four days to ensure equilibrium with the target water activity ($a_{w,25°C} = 0.45 \pm 0.02$) (Hildebrandt et al., 2016).

2.2.3. Isothermal heating

A full factorial experiment was performed at three inactivation temperatures (75, 80, 85 °C) and at $a_{w,25°C}$ 0.45 ± 0.02. All tests were conducted in triplicate. To obtain thermal death curves for *S.* Enteritidis and *E. faecium*, inoculated wheat flour samples were subjected to isothermal heating in aluminum test cells (Chung et al., 2008; Wang et al., 2013). Briefly, 0.7 g of samples were loaded in aluminum test cells (18 mm inner diameter, 4 mm height) and immersed in an oil bath (Neslab GP-400, Newington, NH) maintained at 75, 80 and 85 °C. The come-up time (CUT) was verified using a T-type thermocouple located at the center of the test cell loaded with non-inoculated sample. Thermal treatment at the same time intervals was performed at each temperature, starting from the end of CUT. Once removed from the oil bath, the test cells were immediately placed in an ice-water bath for at least 30 s to stop isothermal inactivation.

2.2.4. Enumeration

To enumerate *S.* Enteritidis and *E. faecium* survivors, samples were transferred from the test cells into sterile stomacher bags, diluted 1:10 with 0.1% peptone water, and homogenized for 3 min at 260 rpm with a Seward Stomacher (Seward, London, UK) (Harris et al., 2012). Appropriate tenfold serial dilutions were spread-plated in duplicate onto modified TSAYE (TSAYE plus 0.05% ammonium iron (III) citrate and 0.03% sodium thiosulfate pentahydrate ($5H_2O$)) for *S.* Enteritidis and TSA for *E. faecium*, respectively. The plates were incubated aerobically at 37 °C for 48 h, then the colonies were enumerated and the populations were converted to log CFU per gram. Log reductions were calculated by subtracting the survivor counts from the initial population.

2.2.5. Modeling of inactivation kinetics

Two primary models were used for describing the thermal resistance: the first order kinetic model (Eq. (1)) and the Weibull-type model (Eq. (2)) (Peleg, 2006)

$$\log\left(N/N_0\right) = -t/D, \tag{1}$$

$$\log\left(N/N_0\right) = -\left(t/\delta\right)^{\alpha}, \tag{2}$$

where N and N_0 are the populations (CFU/g) at times t and 0, respectively; t is the time of the isothermal treatment (min) after CUT; and D is the time (min) required to reduce the microbial population by 10-fold at a specified temperature (°C); δ refers to the overall steepness of the survival curve; α describes the general shape of the curve and whether it is linear ($\alpha = 1$) or nonlinear ($\alpha \neq 1$) with a decreasing ($\alpha < 1$) or increasing ($\alpha > 1$) inactivation rate with time.

Data were fitted to the models, and the goodness of fit for each candidate model was quantified by the root mean square error (RMSE) (log CFU/g) (Motulsky and Christopoulos, 2004),