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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),

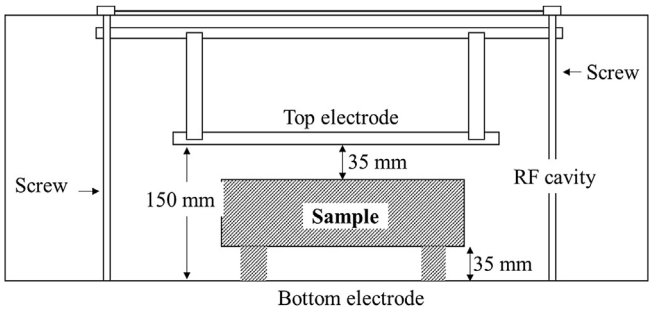


Fig. 1. Schematic diagram of 27.12 MHz, 6 kW radio frequency system heating for wheat flour modified from Tiwari et al.(Tiwari et al., 2011). Note the spacers supporting the sample box in the symmetrical center so equal dielectric heating impacts were expected from top and bottom electrodes.

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}\left[log\left(\frac{N}{N_0}\right)_{data,i} - log\left(\frac{N}{N_0}\right)_{model,i}\right]^2}{n-p}}, \quad (3)$$

where $\log\left(\frac{N}{N_0}\right)_{data,i}$ is the measured log reduction, $\log\left(\frac{N}{N_0}\right)_{model,i}$ is the predicted log reduction from the model, n is the total number of observations, and p is the number of model parameters. RMSE was estimated for three biological independents together. The integrated pathogen modeling program (IPMP) (Huang, 2014) was used for estimating its fitness and providing RSME directly. Differences between D-values among samples were evaluated using ANOVA in Minitab 14 (Minitab Inc., State College, PA).

When the log-linear model fits well to microbial inactivation curves, the log of D-values can be plotted against temperature, this is generally referred to the thermal death time curve. The slope of the log D versus temperature, i.e. $\frac{d(logD)}{d(T)}$, is $-\frac{1}{z}$, where z is the temperature change necessary to alter the thermal-death-time by one log-cycle (Gaillard et al., 1998).

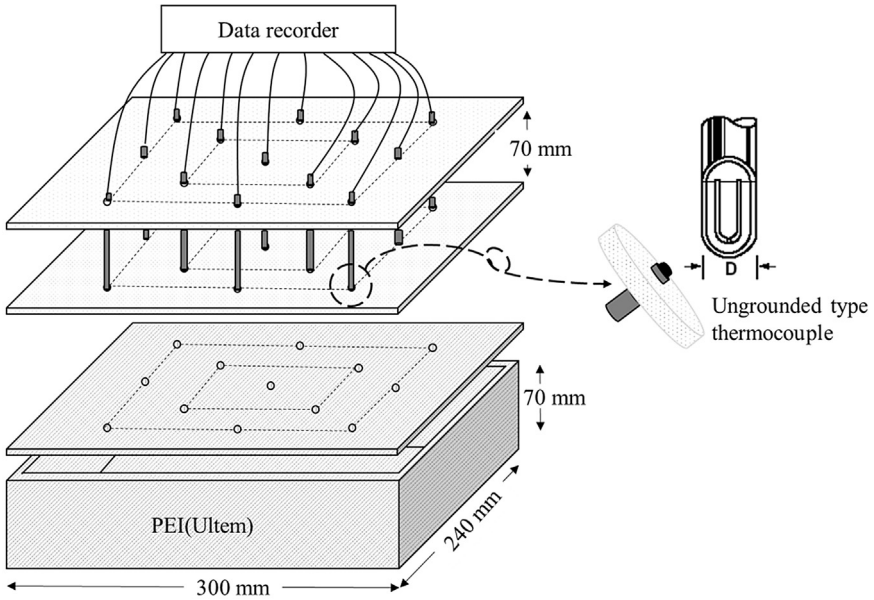
2.3. Microbial validation of RF heating

We did preliminary tests to study the water migration within 3 kg equilibrated wheat flour ($a_{w,25°C} = 0.45$) in a closed container after heating with RF. Minimal variation in water activity (<0.04 measured at 25 °C) was observed among different sites. The moisture distribution of 3 kg wheat flour after pilot-scale RF treatment was relatively uniform (<0.12% water content difference). Thus, the least heated area was considered as the least lethal area for the target microorganisms.

The temperature measurement (session 2.3.2) and inoculated pack study (session 2.3.3) were conducted based on this assumption. If there was significant moisture content transfer, e.g. in open systems like roasting or open air drying, the microbial validation procedures in this study might not apply.

2.3.1. RF pilot-scale system

A pilot-scale 27.12-MHz, 6-kW RF system (COMBI 6-S, Strayfield Fastran, UK) with plate applicators was used as the source of RF energy in our study. A rectangular container (300 × 240 × 70 mm^3,



Distance to bottom (mm)	Temperature (°C)							
	Average	Center	Inner rectangular			Outer rectangular		
			Average	Max	Min	Average	Max	Min
60	59.2±4.3	53.1	61.6±4.4	64.3	57.6	58.8±3.7	64.9	55.1
50	68.3±2.8	57.4	69.7±3.5	73.3	65.2	67.6±2.3	71.8	66.2
40	73.6±3.9	62.5	75.3±2.4	77.9	72.1	74.1±2.0	77.4	71.6
30	77.0±3.5	66.8	77.9±1.4	79.4	76.0	77.8±1.9	80.4	75.2
10	76.3±3.2	68.6	75.4±1.3	76.5	73.9	77.7±2.3	81.1	74.7

Fig. 2. Top: schematic diagram of rectangular polyetherimide (PEI) sample container (▨) and 13 ungrounded type thermocouples fixed in two parallel-boards (□) in two rectangular arrays. The goal was to measure representative sampling points at different horizontal levels. Bottom: typical temperature distribution of 3 kg wheat flour in a PEI box (heated by radio frequency for 12min).

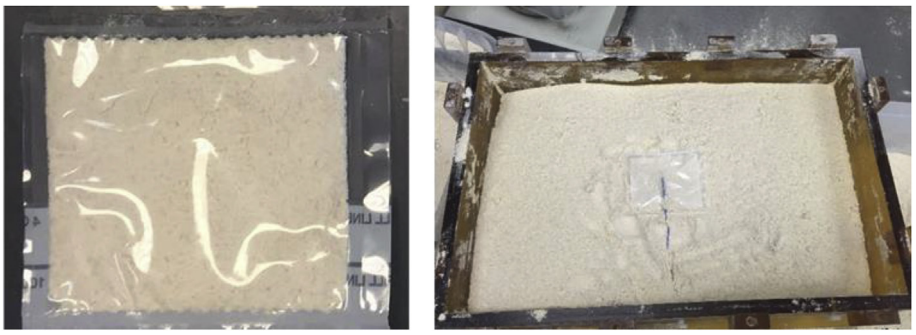


Fig. 3. Left: modified 4 oz whirl-pak bag (76 × 51 × 1 mm^3) bags with 5 g loosely packaged wheat flour; right: sample bag was put in the geometric center of the container (with 3 kg of non-inoculated wheat flour) for temperature profile measurement.

length, width, height) made from a PolyEtherImide (PEI, 4.5 mm thick, HyComp LLC., Cleveland, OH) was used to enclose 3 kg wheat flour for RF heating at a fixed electrode gap of 150 mm. Two 35 mm-height PEI blocks were used as spacers for supporting the sample container in the symmetrical center of the electrodes (Fig. 1). In addition, a lab-made foam case (thickness ~3 mm) was used to cover the surface of the PEI container and act as an insulator to prevent significant heat loss when sample temperature increased.

2.3.2. Temperature measurements

In the present study, two temperature measurements were performed for different purposes. To evaluate the temperature distribution of wheat flour heated by RF, a temperature measurement platform with T-type sensors (OMEGA Engineering In. Stamford, CT) was designed (Fig. 2) (Ozturk et al., 2017). On the lid of the PEI rectangular container (300 × 240 mm^2), five evenly-distributed holes were drilled along each diagonal line of the lid; the two diagonal lines crossed in the center and formed two rectangles (see dashed lines on the lid of Fig. 2). In addition, four more holes were drilled in the geometric centers of the edges of the outer rectangle. Thus, (13) holes on the lid were used for vertically inserting thermocouples from two parallel boards into the wheat flour sample at five horizontal layers (10, 30, 40, 50 and 60 mm from the bottom). All measurements were taken immediately after heating/holding without removing the container lid. Thermocouple temperature readings were recorded by portable thermometers RDXL4SD (OMEGA Engineering Inc. Stamford, CT). The temperature readings of five layers were obtained within 30 s. The average temperature and standard deviation of the outer and inner rectangles at each horizontal layer were calculated. The cold zone was determined based on comparing 13 data points among five layers.

To measure the real-time temperature at a specific spot, fiber-optic temperature sensors (30-mm-long polyimide tubing, Fiso Technologies Inc., Quebec City, Québec, Canada) were inserted through holes on the side-wall of the sample container (or attached to the central surface of an inoculated pack). The fiber-optic sensors were connected to a UMI4 multichannel instrument (Fiso Technologies Inc., Quebec City, Québec, Canada) for recording the temperature-time history. Differences between temperatures at different locations were evaluated using ANOVA in Minitab 14.7.

2.3.3. Temperature measurement for inoculation pack in RF heating

The purpose of these tests was to assess if inserting a small inoculation pack inside the sample container would alter the temperature rise in the sample. Sterile plastic bags with a dimension of 76 × 51 × 1 mm^3 (length × width × thickness) (VWR International, Radnor, PA) were used to hold 5 g inoculated samples (Fig. 3, left), which were placed in the center of the sample container (Fig. 3, right). A pair of fiber-optic sensors was used; one inserted into a small (uninoculated) pack and the other attached onto the surface, to obtain the temperature history of wheat flour in the small pack. The temperature history of the same position without inserting the small pack was measured as a control.

2.3.4. Inoculation method

The initial population levels of *S.* Enteritidis and *E. faecium* were 8.6 ± 0.1 CFU/g and 7.8 ± 0.1 CFU/g, respectively. The RF system was shut down immediately when the central temperature reached 85 °C and then held for up to 39 min. The survival of the two microorganisms during RF treatment was studied by utilizing different time/temperature conditions (Table 2). To obtain inactivation kinetics of microorganisms during RF pasteurization process, six data points were designated at different temperatures (before CUT) or times (during holding session) until the time that 5-log reduction of *E. faecium* was attained. When the selected temperature/time was reached, the inoculated pack was immediately removed and placed in an ice-bath for cooling (10 s to reach <20 °C). One gram of treated sample was collected, tenfold serially diluted and plated as described previously to enumerate the survivors.

2.3.5. Comparison of inactivation kinetics and F-value

The D- and z-values determined in 2.2.3 with fixed sample moisture content were used in calculation of the lethal rate of heat on microorganisms at a given temperature (Gaillard et al., 1998):

$$F = \int_0^t 10^{\frac{T-T_{ref}}{z}} dt, \tag{5}$$

where F is the total equivalent heating time at the reference

Table 2
Inoculated pack treated time/temperature points in radio frequency pasteurization[a].

Strains	Time(min)/Temperature(°C)							
S. PT 30	5.0/50.2	8.7/65.1	13.1/80.1	14.9/85.0	20.9/83.4	26.5/81.6	32.5/80.0	NA
E. faecium	5.0/50.2	8.7/65.1	13.1/80.1	14.9/85.0	20.9/83.4	27.0/81.4	33.5/79.8	39.0/78.7

[a] Each point was duplicated in independent runs. The inoculated packs were sampled at 50.2, 65.1, and 80.1 °C, and every 6–8 min after CUT depends on the treatment temperature and total treatment time.

temperature; T is the real-time temperature at time t, and D_{ref} is the D-value at reference temperature T_{ref}. Thus, the log reduction in a process can be calculated as

$$\text{Log reduction in a process} = \frac{F_{ref}}{D_{ref}}, \quad (6)$$

All graphs were drawn with Excel or PowerPoint 2016, and all tables were made with Excel 2016.

3. Results and discussion

3.1. D- and z-values of S. Enteritidis and E. faecium in wheat flour

The background microflora count in wheat flour samples was 2.20 ± 0.45 log CFU/g, low enough that it wouldn't interfere with the inactivation treatment counts (Villa Rojas, 2015). The CUT for the sample core to reach target temperature ±0.5 °C was ~150 s. Inactivation kinetics of *S.* Enteritidis and *E. faecium* at $a_{w,25°C} = 0.45$ is presented in Fig. 4. Survival data of both microorganisms fit well to the primary models and showed similar RMSE values (Table 3). In this study, the log-linear model was used for describing thermal resistances and plotting z-values (Fig. 5). The D-values at 75, 80 and 85 °C for *S.* Enteritidis were 17.65 ± 1.58, 7.17 ± 0.35 and 2.92 ± 0.35 min, respectively. These D-values were comparable to values reported in the literature. For example, $D_{80°C}$ of the same strain in all-purpose wheat flour was reported as 6.9 ± 0.7 min at $a_{w,20°C} = 0.45$ (Syamaladevi et al., 2016a); $D_{80°C}$ for the same strain in the same wheat flour was reported as 5.51 ± 0.22 min at the same water activity level (Smith, 2014); $D_{70°C}$ of ~50 min was reported for *Salmonella* Weltevreden in wheat flour at $a_{w,25°C} = 0.4$ (Archer et al., 1998). Different D-values of the same microorganisms in similar food samples may be due to different moisture adsorption/desorption isotherms, protocols of bacterial inoculation, and isothermal treatments. The z-values of *S.* Enteritidis and *E. faecium* in wheat flour samples were 12.8 ± 0.3 and 11.7 ± 0.3 °C, respectively. These data were slightly lower than the calculated z value of *S.* Enteritidis (14.8 °C) based on the reported D-values of that in wheat flour at $a_{w,25°C} = 0.43$ (Smith, 2014). Lacking the original data of this published work, we are unable to compare and interpret.

In our study, *E. faecium* had significantly higher ($P < 0.05$) D-values at all temperatures and had an equivalent z-value in the 75–85 °C treatment range compared to *S.* Enteritidis. For example, $D_{75°C}$ of *E. faecium* and *S.* Enteritidis were 29.35 ± 1.10 and 17.65 ± 1.58 min, respectively (Table 3). Consistently, *E. faecium* showed higher thermal resistance in different low-moisture foods than *Salmonella* (Bianchini et al., 2014; Enache et al., 2015; Jeong et al., 2011; Rachon et al., 2016). These comparisons indicate that *E. faecium* is a valid surrogate for *S.* Enteritidis in wheat flour at $a_{w,25°C}$ of 0.45.

3.2. Temperature distribution of wheat flour heated by RF

Fig. 2 shows a typical temperature distribution of 3 kg wheat flour in a PEI box heated by RF for 12 min. Small temperature deviations (≤4.4 °C) in all layers indicate that good temperature uniformity within industrial-size batches of wheat flour was achieved with RF heating. The middle layer experienced 1.4–1.9 °C deviation. Although the average-temperature differed among layers, the central temperature in each layer was always coldest. This heating pattern indicated that the cold zone is located in the geometric center of the package and matched with that of a previous study (Tiwari et al., 2011). Among horizontal layers, the average and central temperatures increased from the top to bottom layer. This was mainly because heat loss from the top layer was more severe than from the bottom: air circulation above the lid reduced temperature. It may also be due to density-dependent dielectric properties of wheat flour impacted by gravity: higher density at the bottom increases dielectric loss for transferring dielectric energy into heat (Nelson, 1984). Several strategies have been utilized to improve the heating uniformity of low-moisture foods in RF systems. Polyetherimide material was used around peanut butter and wheat flour samples to improve heating uniformity in a 27.12 MHz, 6 kW RF system (Jiao et al., 2014). A combination of RF and forced hot air (Wang et al., 2010) or infrared heating (Fasina et al., 2001) was used to balance volumetric and surface heating uniformity. Intermittent mixing or stirring the product after each pass of the load through the RF system was also reported to benefit treatment uniformity of in-shell walnuts (Wang et al., 2005, 2007). Since the focus of this study was to validate the RF pasteurization effect, the cold spot was used as the worst case for embedding inoculated packs and monitoring real-time temperatures.

3.3. Temperature profile of inoculated pack

In Fig. 6, temperature profiles labeled center of inoculation pack and center on pack surface, respectively, were compared with the temperature profile of the same position without inserting the pack (line labeled center-no pack). No significant difference ($P > 0.05$) was observed among the three temperature profiles of the central spot: measured without the pack, in the center of the pack (filled with uninoculated sample), and on the center of the pack surface. Hence, a small plastic sample pack embedded in wheat flour did not

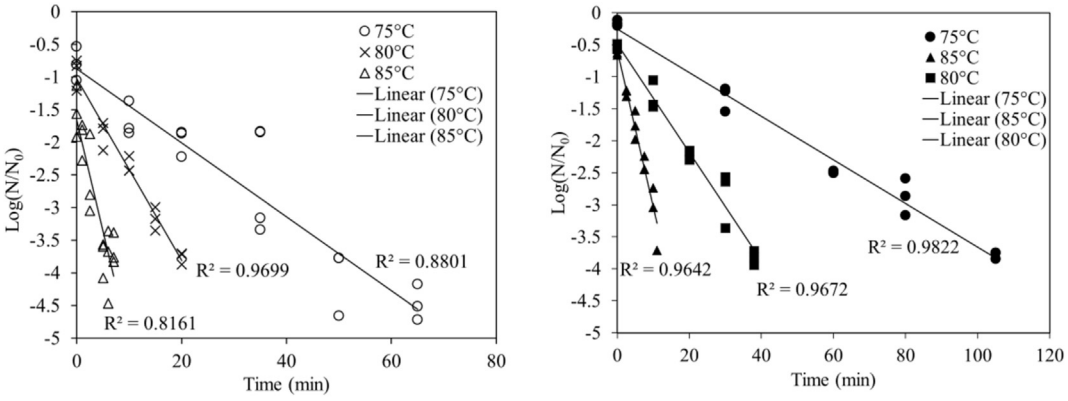


Fig. 4. Inactivation kinetics of *S.* Enteritidis (open circles) and *E. faecium* (solid circles) in wheat flour at 75, 80, and 85 °C ($a_w = 0.45 \pm 0.02$ at 25 °C).

Table 3
Parameter estimates for the primary models, as well as the root mean square error (RMSE)[a]. Parameters were estimated from triplicate experimental data using IPMP software.

Bacteria	Linear model			Weibull Model		
	T (°C)	D-value (min)	RMSE (log CFU/g)	δ(min)	α	RMSE (log CFU/g)
S. Enteritidis	75	17.65 ± 1.58	0.49	17.99 ± 5.50	1.01 ± 0.22	0.50
	80	7.17 ± 0.35	0.19	6.12 ± 0.89	0.87 ± 0.01	0.18
	85	2.92 ± 0.35	0.45	1.99 ± 0.87	0.73 ± 0.21	0.44
E. faecium	75	29.35 ± 1.10	0.18	24.23 ± 3.08	0.87 ± 0.07	0.17
	80	11.80 ± 0.60	0.23	12.92 ± 1.91	1.08 ± 0.13	0.23
	85	4.08 ± 0.22	0.18	4.51 ± 0.53	1.11 ± 0.14	0.19

[a] Values are means ± standard errors. Parameters were estimated separately for each data set. Smaller RMSE values indicate a better fitness of the model. Comparison can be made only horizontally within each row (Villa Rojas, 2015).

impact RF heating or temperature distribution. For process development, the temperature reading of the center of the pack surface was used to track heating and holding temperature.

A heating rate of 3.85°C/min was achieved with the RF system. The central temperature of the 3 kg wheat flour load reached 85 °C within 16 min. During the holding time, the center temperature decreased slowly to ~77 °C because of heat loss from wheat flour to the system (Fig. 6). However, the temperature during holding time was within 75–85 °C, and hence, the D-values and z-value obtained from isothermal inactivation can be applied to calculate the F-value.

3.4. Comparison of survivor curves and F-value models

Fig. 6 shows the F-values at T_{ref} = 85 °C. Both predictive lines were stable (<1 log reduction) up through 12 min RF treatment and then declined during the rest of the treatment. The predicted survivor curve for *E. faecium* declined less than that of *S.* Enteritidis. This is because *E. faecium* has higher D-values, therefore higher thermal resistance. By following the predictive lines, time needed for 5-log reduction of each strain was CUT of 3 kg wheat flour (~15 min) to reach 85 °C plus holding for 25 min (*E. faecium*) or 18 min (*S.* Enteritidis).

The experimental survivor data had a similar trend to that of the predicted curves with respect to RF treatment (Fig. 6). At any given treatment time, *E. faecium* had more survivors than *S.* Enteritidis because of the higher thermal resistance of *E. faecium*. Predictions based both on D- and z-values and on the temperature profile of experimental data support the use of *E. faecium* as a surrogate to validate RF treatments for control of *S.* Enteritidis.

The experimental data (scatter plots) showed fewer survivors for both microorganisms than those of the predicted curves. This was because the temperature profile of the inoculated pack in Fig. 6 was tracked at the least heated spot. The remaining portion of the pack experienced greater lethality. That is, we observed fewer survivors, and thus more inactivation, during RF treatment. To eliminate differences between observed and predicted survivors, a smaller inoculated pack could be used in the future. In this study, the use of a 5-g inoculated pack in the geometric center of a wheat flour sample provided conservative validation for RF treatment technology. For example, since *E. faecium* was shown to be more heat resistant, only 3.7-log reduction needs to be achieved to ensure microbial validation of any thermal processing line targeting 5-log reduction (5D) of *S.* Enteritidis (Fig. 6). In the plate counting method, the lower limit of enumeration can be based on the limit of qualification (LOQ) (25 CFU, from a countable range of 25–250) or the limit of detection (LOD) (BAM, 1998). Therefore, the use of *E. faecium* as a surrogate microorganism can reliably simulate a 5D pathogen reduction, while simultaneously providing more lethality of processing (~6.6-log reduction of *S.* Enteritidis by targeting 5D of *E. faecium*).

Our results (Fig. 6) indicate that the F-value model would provide a reliable prediction for RF thermal processes in closed systems. But open systems, such as roasting or baking, moisture content of the heated food changes with treatment time due to external water evaporation and internal migration. Because thermal resistance of microorganisms increased sharply with reduced

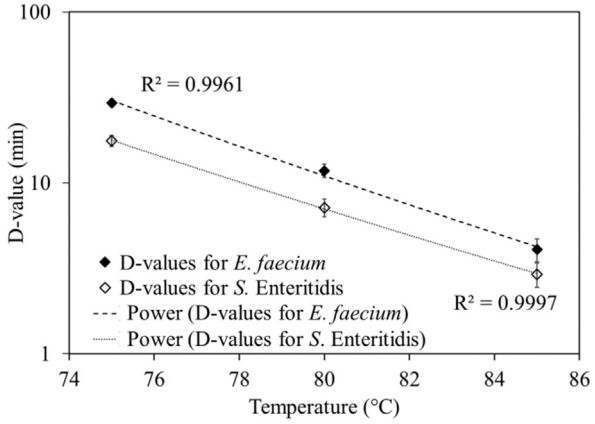


Fig. 5. D-values of *S.* Enteritidis and *E. faecium* at $a_{w,25°C}$ = 0.45 and their power trend lines (round dot line for *S.* Enteritidis, dashed line for *E. faecium*): experiments were done in triplicate.

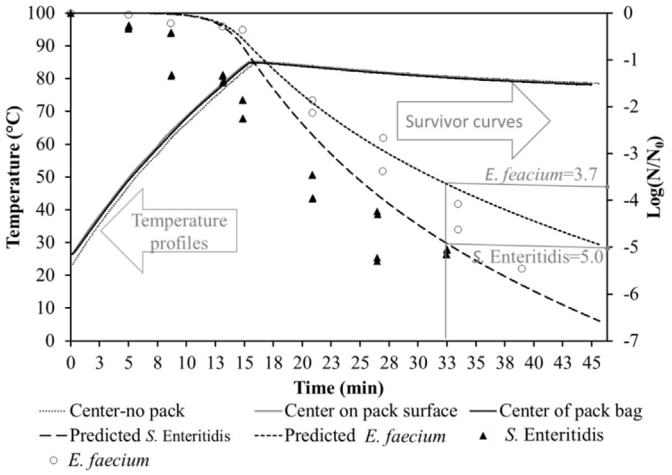


Fig. 6. Thermal inactivation curves of *S.* Enteritidis and *E. faecium* in inoculated-pack with predicted inactivation curves modeled on temperature profile (RF heating for 15min, holding for 25min).

water content (Syamaladevi et al., 2016a, 2016b; Wang et al., 2013), the least heated zones might not be the locations for least lethality. Thus, lethality calculations for process determination will be more challenging.

4. Conclusion

In this study, comparison of thermal resistance of *S.* Enteritidis and *E. faecium* inoculated in wheat flour at 75, 80 and 85 °C supports the use of *E. faecium* as a surrogate for *Salmonella* in wheat flour. RF pasteurization of wheat flour evaluated by inoculated pack studies suggest that: 1) the temperature pattern of 3 kg wheat flour treated by a 27.12 MHz, 6 kW RF oven was not impacted by inserting an inoculated pack; 2) *E. faecium* can be used as a surrogate in an inoculated pack to provide conservative validation of RF pasteurization technology; 3) microbial destruction by pilot-scale RF pasteurization satisfied a designed degree of pasteurization (F-value). This study confirms that RF heating technology has potential for pasteurizing low-moisture foods, and it is practical to use *E. faecium* or the F-value to design RF pasteurization processes.

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