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Validation of *Enterococcus faecium* as a surrogate for *Salmonella* under different processing conditions for peanuts and pecans

Pardeepinder K. Brar[1], M.D. Danyluk*

Department of Food Science and Human Nutrition, Citrus Research and Education Center, Institute of Food and Agricultural Sciences, University of Florida, 700 Experiment Station Road, Lake Alfred, FL, 33850, USA

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ABSTRACT

Food Safety and Modernization Act (FSMA) Preventive Control rules require nut processors validate thermal processes to ensure a desirable log reduction of *Salmonella* is achieved. Due to the complex nature of nut and nut products, processes and equipment, it is difficult to use one validation study for all and may requires individual equipment be validated at the plant level. In plant validation studies, pathogens such as *Salmonella* cannot be used due to the risk of contamination, thus the suitability of a non-pathogenic organism, *Enterococcus faecium* as a surrogate for *Salmonella* was evaluated for peanut and pecan thermal processing. Stagnant and forced dry air heating conditions, (120 °C (20, 30, 40 min), 130 °C (10, 20, 30 min), 140 °C (10, 20, 30 min)) were evaluated for unblanched peanut kernels. Oil heating conditions (116 °C, 121 °C, and 127 °C for 0.5, 1.0, 1.5, 2.0, 2.5 min) were evaluated for pecan kernels. Inshell pecans are conditioned in hot or cold water to facilitate the shelling process. Water heating conditions (75 °C (20, 40, 80, 120 s), 80 °C (20, 40, 80, 120 s), 85 °C (20, 40, 80, 120 s), 90 °C (20, 40, 60, 80 s), and 95 °C (20, 40, 60, 80 s)) were evaluated for inshell pecans. Under conditions, except forced air treatment, *E. faecium* reductions (Log N/N_0) were either not significantly different ($P > 0.05$) or significantly lower than *Salmonella* ($P < 0.05$), making it a suitable surrogate for the processes evaluated.

1. Introduction

Peanuts (Killalea et al., 1996; Kirk et al., 2004; Marler, 2006), as well as peanut products (CDC, 2007, 2009, 2012), have been vehicles of *Salmonella*. Six peanut butter related outbreaks, all associated with *Salmonella*, have been reported in the U.S. since 1998 (FOOD Tool, 2016; Harris et al., 2014). These outbreaks have prompted more interest in thermal tolerance of human pathogens on peanuts. Pecans have not been associated with disease outbreaks, but have been recalled due to potential *Salmonella* contamination (FDA, 2010, 2014a, 2015).

The Almond Board of California requires all almonds grown in California for domestic consumption to be processed with a treatment delivering a minimum 4-log reduction of *Salmonella* (Federal register, 2007). However, almonds labeled as "pasteurized" must receive a treatment delivering a minimum 5-log reduction of *Salmonella*. The Preventive Controls for Human Food rule of the U.S. Food Safety Modernization Act (FSMA) of 2011 focuses on the prevention of contamination and requires validation of processes to ensure the desired reduction of foodborne pathogens is achieved (FDA, 2018). Due to the complex nature of nut and nut products, processes and equipment, it is difficult to use a single validation study for all and may require individual equipment be validated at the plant level.

Enterococcus faecium has been validated as a surrogate for *Salmonella* in almond processing (Jeong et al., 2011; Kopit et al., 2014). Two studies published in 2014, evaluated the strain for use as a *Salmonella* surrogate in peanut dry roasting and oil roasting conditions (Poirier et al., 2014; Sander and Calhoun, 2014). Peanuts are exposed to dry heat treatment during blanching to facilitate the removal of red skin (Sanders et al., 1999). The time or temperature profiles of dry heating peanuts changes based on the operational parameters of specific equipment such as bed depth, air flow rate, and air distribution (Shi et al., 2017). Inshell pecans are exposed to water treatments known as "conditioning" to ensure easy cracking of the shells, followed by hot air drying. Pecan kernels can further be exposed to hot oil for roasting purposes. Roasting process helps in developing flavor and crunchiness in nuts. No previous studies have evaluated *E. faecium* in peanut dry heating conditions used prior to blanching or pecan thermal processing conditions.

* Corresponding author. Department of Food Science and Human Nutrition, 700 Citrus Research and Education Center, Institute of Food and Agricultural Sciences, University of Florida, 700 Experiment Station Road, Lake Alfred, FL, 33850, USA.
E-mail address: mddanyluk@ufl.edu (M.D. Danyluk).
[1] Present affiliation: Italian Rose Garlic Products, LLC, Riviera Beach, FL, USA.

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The specific objectives of this experiment were to evaluate the suitability of *E. faecium* to be used as *Salmonella* surrogate for peanut or pecan thermal processing validation studies. Nuts were exposed to four different thermal treatments in the study: (i) Stagnant hot air on peanut kernels (dry heat); (ii) Forced hot air (dry heat) on peanut kernels; (iii) Hot oil on pecan kernels; and (iv) Hot water on inshell pecans.

2. Material and methods

2.1. Peanuts

Raw, unblanched, medium runner peanuts were procured from a local source (Alpharetta, GA) in 2.3 kg bags, and stored at 4 ± 1 °C until use. Runner-type peanuts are most commonly grown in the Southeastern US, including: Alabama, Florida, Georgia, Oklahoma, and Texas. Runner type peanuts account for 80% of the peanut production in the United States (American peanut council, 2014). The moisture content (%) and water activity (a_w) of raw peanuts recorded after a few weeks of storage was 7.7 ± 1.0% and 0.69 ± 0.2, respectively. Peanuts were used for dry heating experiments only.

2.2. Pecans

Mammoth size pecan halves (440–550 per kg) were obtained from local sources (Camilla, GA), in 13.6 kg boxes and stored at 4.4 °C until use in oil heating experiments. The moisture content (%) and a_w of pecan kernels was 4.8 ± 1.2% and 0.66 ± 0.01, respectively. The Southern Improved variety of inshell pecans used for conditioning experiments were donated by pecan shellers from across the southern US states of NM, TX, and GA. The moisture content (%) and a_w of inshell pecans was 6.6 ± 0.6% and 0.64 ± 0.01, respectively. Water activity measurements were conducted at room temperature conditions ca. 20–25 °C. Details on the measurements and replications of moisture content (%) and a_w are provided below.

2.3. Bacterial cultures

Dry heating of peanuts was conducted both under stagnant and forced air conditions. For stagnant air conditions, three different serotypes of *Salmonella*, Seftenberg 775W, ATCC 43845 typically used in thermal studies; Enteritidis PT 30, ATCC BAA-1045, isolated from raw almonds associated with an 2000–2001 outbreak (Chan et al., 2002); Tennessee, K4643 clinical strain associated with peanut butter outbreak 2006–07 (CDC, 2007) and two species of *Enterococcus*, *E. faecium*, ATCC 8459; *E. faecalis*, ATCC 29212 were studied. Strains used in stagnant dry heating experiments were also used for forced dry heating conditions, with the exception that *E. faecalis* experiments were discontinued after obtaining results from stagnant heat conditions. All the strains in dry heating experiments (stagnant and forced) were inoculated individually onto peanuts.

A cocktail of *Salmonella* serotypes was used for oil heating and conditioning treatments of pecans. Oil heating experiments were conducted using a three-strain cocktail of *Salmonella* (Seftenberg, Enteritidis PT 30, Tennessee) and conditioning treatments were conducted using a five-strain cocktail of *Salmonella* (Seftenberg, Enteritidis PT 30, Enteritidis PT 9c, Oranienburg, Tennessee). *Salmonella* Enteritidis PT 9c, strain RM4635, is a clinical strain isolated from an 2003-04 almond associated outbreak (CDC, 2004), and *Salmonella* Oranienburg, strain 1839, is a pecan isolate. *E. faecium* (ATCC 8459) was used in oil heating and conditioning treatments to determine its validity as a surrogate for *Salmonella*.

All the strains were made resistant to nalidixic acid in order to enumerate the desired bacteria in the presence of the high background microflora found on peanuts and pecans. The levels of background microflora recovered from raw peanuts, pecan kernels, and inshell pecans were 3.3 ± 0.1, 4.8 ± 1.2 and 4.8 ± 0.8 log CFU/g, respectively (n = 3).

2.4. Inoculum preparation

Unless otherwise specified, all media were from BD Difco (BD, Franklin Lakes, NJ), and were supplemented with nalidixic acid (N; Sigma-Aldrich, St. Louis, MO) at 50 μg/ml. Frozen cultures (−80 °C) of *Salmonella* serotypes and *Enterococcus* were streaked onto tryptic soy agar (TSAN; nonselective media) and all plates were incubated at 37 ± 2 °C for 24 h. Each strain was sub-cultured twice in tryptic soy broth (TSBN) and incubated at 37 ± 2 °C for 24 h. After overnight growth, 1 ml of culture was spread over large TSAN (150 by 15 mm) and incubated at 37 ± 2 °C for 24 h to produce a bacterial lawn. Sterile 0.1% peptone water (9 ml) was added to each plate and the bacterial lawn was loosened with a sterile spreader. Cells were collected from three plates for each strain in 50-ml falcon tubes. To prepare *Salmonella* cocktail, the 25-ml preparations for each strain was combined for each pathogen in a 200-ml sterile bottle, and mixed for 1 min on a stir plate (Uesugi et al., 2006). Inoculum populations (log CFU/g) of individual strains (dry heat) and cocktails (oil roasting and hot water conditioning) were determined by serially diluting in 0.1% peptone water and plating onto nonselective media, TSAN (*Salmonella* and *Enterococcus*) and onto selective media: bismuth sulfite agar (BSAN) for *Salmonella*, and bile esculin agar (BHIN) for *Enterococcus*.

2.5. Inoculation procedure and drying

Nuts were inoculated as described by Uesugi et al. (2006) for almonds, with a ratio of 25 ml of inoculum for 400 g of peanut or pecans (kernels and inshell). Inoculation for dry heating experiments was carried out using individual strains, whereas, for oil heating and conditioning treatments, a cocktail of *Salmonella* serotypes was used for inoculation. Nuts (400 g) were weighed into a plastic bag (30.5 by 30.5 cm; Bitran Com-Pac International, Carbondale, IL), 25 ml of inoculum (individual or cocktail) was added, and the bag was sealed. Each bag was shaken and massaged by hand for 1 min to ensure that the nuts were evenly coated with the inoculum. Inoculated nuts were spread onto four layers of filter paper (two sheets folded in half; Qualitative P8 Grade sheets, Fisher) placed on a lid of large plastic container and allowed to dry in biosafety cabinet for the period of 7 h (dry heating and oil heating) or 8 h (conditioning) with the blower on. The temperature and the relative humidity of the biosafety cabinet were 21.3 ± 0.5 °C and 67.5 ± 9.7%, respectively. After drying, the inoculated nuts were transferred into double sterile plastic bags (30.5 by 30.5 cm) and stored at 4 ± 1 °C for up to three weeks.

Inoculated dried nuts (50-g) were placed in 207 ml Whirl-pak bags (Nasco, Fort Atkinson, WI) and 100 ml of cold tryptic soy broth (TSB) was added to it. Nuts were stomached (Smasher, AES Chemunex, Cranbury, NJ) for 2 min, followed by plating onto non-selective (TSAN) and selective media (BSAN, Bismuth sulfite agar supplemented with nalidixic acid (50 μg/ml) for *Salmonella* and BEAN, bile esculin agar supplemented with nalidixic acid (50 μg/ml) for *Enterococcus*), after serially diluting the samples in 0.1% peptone water to determine the initial levels (log CFU/g) present on nuts prior to treatments (n = 6).

Nuts were inoculated with ca. 7 or 8 log CFU/g of *Salmonella* and *Enterococcus* populations; the specific levels obtained from inoculated nuts after drying are listed in Tables 1, 3 and 6. Before treatments, nuts were removed from the 4.4 °C refrigerator and kept at ambient temperature for 3–4 h (Du et al., 2010).

2.6. Measurement of moisture content and a_w

The moisture content was analyzed using an oven dry method and calculations were performed on wet basis (Bradley, 2010). Control nuts (peanut and pecan kernels) (10-g) were finely ground in a food processor (HC306 Type 1, Black and Decker, New Britain, CT) for 30 s and

Table 1
Populations (log CFU/g) of *Salmonella* and *Enterococcus spp.* recovered from unblanched peanut kernels exposed to stagnant and forced dry heat conditions (n = 6).

Process conditions		*S.* Seftenberg		*S.* Enteritidis PT30		*S.* Tennessee		*E. faecium*		*E. faecalis*	
Temp	Time (min)	TSAN	BSAN	TSAN	BSAN	TSAN	BSAN	TSAN	BEAN	TSAN	BEAN
Inoculum (Log CFU/ml)		11 ± 0.5	11 ± 0.4	11 ± 0.6	11 ± 0.6	11 ± 0.9	11 ± 0.7	10 ± 0.1	10 ± 0.1	12 ± 0.9	12 ± 1.0
Dried nuts (7 h)[b]		8.3 ± 0.1	8.2 ± 0.2	8.3 ± 0.1	8.1 ± 0.2	8.4 ± 0.4	8.3 ± 0.5	8.7 ± 0.2	8.6 ± 0.2	7.7 ± 0.7	7.5 ± 0.8
Stagnant air											
120	20	6.7 ± 0.1	6.6 ± 0.2	7.3 ± 0.2	7.1 ± 0.2	6.4 ± 0.2	6.2 ± 0.2	7.3 ± 0.2	7.1 ± 0.3	4.7 ± 0.5	4.4 ± 0.4
	30	6.0 ± 0.2	5.8 ± 0.2	6.6 ± 0.1	6.6 ± 0.1	6.3 ± 0.2	6.3 ± 0.3	6.7 ± 0.2	6.6 ± 0.2	3.3 ± 0.3	3.1 ± 0.4
	40	5.1 ± 0.2	5.0 ± 0.2	5.6 ± 0.2	5.6 ± 0.2	5.7 ± 0.2	5.6 ± 0.2	5.8 ± 0.2	5.7 ± 0.2	3.4 ± 1.3	3.0 ± 1.5
130	10	7.5 ± 0.1	7.4 ± 0.1	7.3 ± 0.2	7.1 ± 0.2	7.1 ± 0.3	7.0 ± 0.3	7.7 ± 0.2	7.5 ± 0.2	5.9 ± 0.3	5.9 ± 0.2
	20	6.5 ± 0.3	6.4 ± 0.3	6.6 ± 0.2	6.3 ± 0.2	5.4 ± 0.5	5.3 ± 0.4	6.5 ± 0.3	6.3 ± 0.3	3.9 ± 0.4	3.8 ± 0.3
	30	4.9 ± 0.4	4.9 ± 0.4	5.6 ± 0.2	5.4 ± 0.2	4.9 ± 0.2	4.8 ± 0.2	4.4 ± 0.4	4.0 ± 0.6	1.7 ± 0.67	1.33 ± 0.8
140	10	7.1 ± 0.3	7.0 ± 0.3	7.3 ± 0.2	7.1 ± 0.2	6.7 ± 0.2	6.6 ± 0.4	6.7 ± 0.2	6.4 ± 0.1	5.8 ± 0.4	5.5 ± 0.3
	20	5.1 ± 0.5	5.0 ± 0.6	6.0 ± 0.4	5.8 ± 0.4	5.1 ± 0.4	4.9 ± 0.4	5.8 ± 0.3	5.6 ± 0.3	2.5 ± 0.5	1.92 ± 0.8
	30	3.8 ± 1.0	3.8 ± 1.0	4.6 ± 0.7	4.4 ± 0.7	2.6 ± 1.1	2.7 ± 0.9	4.1 ± 0.5	3.6 ± 0.6	1.3 ± 0.8	0.89 ± 0.72
Forced air											
120	20	7.6 ± 0.12	7.6 ± 0.1	7.2 ± 0.2	7.1 ± 0.1	7.2 ± 0.1	7.2 ± 0.1	7.4 ± 0.2	7.2 ± 0.2	ND[a]	ND
	30	6.8 ± 0.1	6.8 ± 0.1	6.8 ± 0.1	6.5 ± 0.1	6.7 ± 0.1	6.5 ± 0.1	6.4 ± 0.1	6.2 ± 0.2	ND	ND
	40	5.7 ± 0.1	5.7 ± 0.1	6.0 ± 0.1	5.8 ± 0.1	5.8 ± 0.1	5.6 ± 0.1	5.6 ± 0.1	5.4 ± 0.1	ND	ND
130	10	7.5 ± 0.2	7.5 ± 0.1	7.6 ± 0.1	7.4 ± 0.1	7.3 ± 0.1	7.3 ± 0.1	7.7 ± 0.1	7.5 ± 0.2	ND	ND
	20	6.7 ± 0.1	6.7 ± 0.1	6.9 ± 0.1	6.7 ± 0.1	6.1 ± 0.5	6.4 ± 0.5	6.8 ± 0.2	6.6 ± 0.2	ND	ND
	30	5.8 ± 0.1	5.7 ± 0.2	6.3 ± 0.0	6.0 ± 0.1	4.8 ± 0.2	4.9 ± 0.2	5.7 ± 0.1	5.5 ± 0.1	ND	ND
140	10	7.5 ± 0.1	7.4 ± 0.1	7.5 ± 0.1	7.4 ± 0.2	7.0 ± 0.1	7.0 ± 0.2	7.5 ± 0.1	7.3 ± 0.2	ND	ND
	20	6.7 ± 0.1	6.6 ± 0.1	6.7 ± 0.2	6.4 ± 0.1	6.4 ± 0.2	6.2 ± 0.2	6.4 ± 0.2	6.2 ± 0.2	ND	ND
	30	5.4 ± 0.2	5.3 ± 0.2	5.7 ± 0.1	5.5 ± 0.1	5.0 ± 0.3	4.8 ± 0.4	5.3 ± 0.1	4.9 ± 0.1	ND	ND

[a] *Enterococcus faecalis* experiments were discontinued due to lack of its validity as a surrogate under stagnant heat experiments.
[b] Dried nuts represents the inoculum level, percent moisture and/or water activity levels obtained from nuts prior to treatments.

placed in an oven at 100 °C for 18–24 h. Inshell pecans were first crushed using a hammer before placing them in the food processor. Moisture content of raw, inoculated and dried nuts was measured before all the treatments. After subjecting the nuts to dry heating (stagnant see section 2.7.1 and forced see section 2.7.2) and oil heating (see section 2.7.3) treatments, moisture content of treated nuts was analyzed at every time point to quantify the loss of water. Since the conditioning treatments (see section 2.7.4) were conducted in water, no attempt was made to calculate moisture content of treated inshell pecans.

Water activity (a_w) of nut samples was measured using water activity meter (Model 3, Aqualab, Decagon Devices, Inc., Pullman, WA) for *E. faecium* inoculated samples only to prevent the contamination of equipment with *Salmonella*. The a_w of a ca. 3-g sample of raw, inoculated, inoculated-dried and treated nuts was measured. The a_w of inshell pecans exposed to conditioning treatments were not measured for the same reasons described previously. Duplicate readings of moisture content and a_w were taken for three separate samples in all the cases. Water activity (a_w) is an important intrinsic property of foods. As per the recent research, a_w of food products can be impacted at constant moisture content with the changes in the temperature (Syamaladevi et al., 2016; Tadapaneni et al., 2017); however, this phenomenon was not studied in the current research and a_w calculations were conducted at the room temperature conditions.

2.7. Thermal treatments

2.7.1. Stagnant hot air

Peanuts are exposed to hot air treatment during blanching to ensure easy removal of red skin (Woodroof, 1983). Peanuts (50 g) were weighed into an aluminum foil tray (5 by 5 cm) and spread into a single layer. Uninoculated (control) peanuts, and peanuts inoculated with individual bacterial strains were exposed to 120 ± 1 °C (20, 30, 40 min), 130 ± 1 °C (10, 20, 30 min) and 140 ± 1 °C (10, 20, 30 min) in a stagnant air oven (Lindburg/Blue M, Ashville, NC). Aluminum foil trays were placed on to the top shelf of the oven and exposed to the treatment temperature; two samples trays were placed on the top shelf at the same time. Within less than 30 s of placing the tray in the oven and closing the door, the oven temperature reached the set treatment temperature. The digital display of oven was monitored before and during the heating process and upon fluctuation greater than 1 °C, nuts were discarded.

2.7.2. Forced hot air

Experiments were conducted using same procedure described stagnant air. Inoculated and un-inoculated peanuts were exposed to the given time and temperature conditions in a forced air oven (Isotemp, model 6921, Fisher scientific, Pittsburg, PA).

2.7.3. Oil heating

Inoculated and uninoculated pecan kernels were exposed to pre-heated peanut oil (all natural LouAna peanut oil, Brea, CA), obtained from a local grocery store (Winter Haven, FL) in a Hi-temp water bath (160A, Fisher Scientific, Pittsburg, PA). Pecan kernels (50-g) were placed in a wire mesh basket and submerged in peanut oil (2.8 L) heated to 116 ± 1, 121 ± 1 and 127 ± 1 °C for 0.5, 1, 1.5, 2 and 2.5 min at each temperature. A digital thermometer (Trace 10 memory thermometer, Fisher scientific, Pittsburg, PA) was placed in the water bath and temperature of peanut oil was monitored throughout the process.

2.7.4. Hot water conditioning

Inshell pecans (50-g; inoculated and uninoculated) were submerged in pre-heated water in a Hi-temp water bath (160A, Fisher Scientific, Pittsburg, PA) at 75 ± 1 °C (20, 40, 80, 120 s), 80 ± 1 °C (20, 40, 80, 120 s), 85 ± 1 °C (20, 40, 80, 120 s), 90 ± 1 °C (20, 40, 60, 80 s), 95 ± 1 °C (20, 40, 60, 80 s) using a wire-mesh basket. A digital thermometer (Trace 10 memory thermometer, Fisher scientific, Pittsburg, PA) was used to monitor the temperature throughout the process. Inshell pecans were submerged beneath the water to ensure uniform

heating of nuts.

After exposure to a treatment, nuts were placed in 207 ml Whirl-pak bags (Nasco, Fort Atkinson, WI) and 100 ml of cold tryptic soy broth (TSB) was added to the bags immediately to stop thermal lethality in nuts removed from high temperature conditions (Du et al., 2010). Residual oil was drained from hot oil treated kernels for 5 s, followed by placing them into Whirl-pak bags. Inshell pecans were placed into double 207 ml Whirl-pak bags and further into a double layer of zip top bags (17.78 by 30.48 cm; Fisherbrand, Pittsburg, PA) before crushing them with a hammer and stomaching, to prevent potential leakage while processing. Nuts were stomached for 2 min (dry heating and oil heating) and 1 min (conditioning treatment), followed by plating onto non-selective and selective media, after serially diluting the samples in 0.1% peptone water as explained above. Each treatment was replicated six times with duplicate samples plated in each replication (n = 6).

2.8. Statistics

Reductions of pathogen populations (Log N/N_0) were analyzed using ANOVA in JMP 10 software (SAS institute, Cary, NC), and if F ratio was significant ($P < 0.05$), the differences between means were determined using Tukey-Kramer's test. Linear or Weibull models were used to fit the collected data and determined on the basis of R^2 values.

3. Results

3.1. Hot air treatment

The concentration of inoculum used to inoculate peanut kernels varied between 10 and 12 log CFU/ml for all the strains (Table 1). The levels recovered from inoculated and dried peanut kernels ranges from 7.5 to 8.7 log CFU/g (Table 1). Significant reductions of pathogen populations were observed during drying period; *E. faecalis* reductions of up to 4.3 log CFU/g and *E. faecium* reductions of up to 1.3 log CFU/g were observed. *Salmonella* serotypes decreased by up to 2.6 (*S.* Tennessee) and 2.7 (*S.* Seftenberg and Enteritidis PT 30) log CFU/g during drying (Table 1). Counts obtained from selective media were lower and occasionally significantly lower ($P < 0.05$) than non-selective media for all strains and under all process parameters of the study (Table 1). Comparisons between different strains were conducted using non-selective media (TSAN) values only.

The moisture content and a_w of raw peanut kernels increased upon inoculation (Table 2). The moisture content (and water activity) of peanut kernels inoculated with *S.* Seftenberg, *S.* Enteritidis PT 30, *S.* Tennessee, *E. faecium* and *E. faecalis* were 12 ± 2.0%, 10 ± 0.5%, 12 ± 0.4%, 13 ± 0.4% (0.90 ± 0.01), 12 ± 0.4% (0.89 ± 0.04) after inoculation. Drying of inoculated peanut kernels in biosafety cabinet for 7 h reduced the moisture content and water activity levels significantly to 9.7 ± 0.8%, 8.7 ± 0.5%, 8.7 ± 2%, 9.1 ± 2% (0.7 ± 0.1), 8.7 ± 2% (0.6 ± 0.2), respectively ($P < 0.05$; Table 2). Moisture content of peanuts reduced to the pre-inoculation levels in most of the cases, except for nuts inoculated with *S.* Seftenberg ($P < 0.05$). Water activity (a_w) of *E. faecium* inoculated nuts reduced to the original un-inoculated levels ($P > 0.05$) after drying, whereas *E. faecalis* inoculated peanuts reduced to significantly lower water activity levels than the raw nuts ($P < 0.05$). Reduction in moisture content and a_w were observed further as the process temperatures were increased from 120 ± 1 to 140 ± 1 °C for all the strains under both stagnant and forced air study (Table 2).

3.2. Stagnant air

E. faecalis decreased significantly more than other strains at all stagnant dry air process parameters ($P < 0.05$), except at 140 ± 1 °C, 10 min exposure, where no significant difference between *S.* Tennessee, *E. faecium*, and *E. faecalis* reductions were observed ($P > 0.05$; Fig. 1). Population reductions (log CFU/g) of *E. faecium* were either not significantly different ($P > 0.05$) or significantly lower ($P < 0.05$) than all the *Salmonella* serotypes under all the treatment conditions, except, 130 ± 1 °C, 30 min and 140 ± 1 °C, 10 min exposure, where *S.* Enteritidis PT 30 reduced significantly lower than all other strains ($P < 0.05$; Fig. 1). Among the three *Salmonella* serotypes, Enteritidis PT 30 population reductions were lower and occasionally significantly lower than other *Salmonella* serotypes. The maximum log reductions were observed after 140 ± 1 °C, 30 min exposure and *S.* Seftenberg, Enteritidis PT 30, Tennessee, *E. faecium* and *E. faecalis* populations decreased by 4.5 ± 1.1, 3.8 ± 0.7, 5.7 ± 1.3, 4.6 ± 0.5, 6.5 ± 1.3 log CFU/g, respectively (Fig. 1).

3.3. Forced air

At 120 ± 1 °C, 20 min exposure, *E. faecium* reductions were not significantly different from *S.* Enteritidis PT 30 and Tennessee ($P > 0.05$) and significantly higher than *S.* Seftenberg ($P < 0.05$; Fig. 2). At 120 ± 1 °C, 30 and 40 min exposure, log reductions recovered from *E. faecium* were significantly higher than all other strains. At 130 ± 1 °C, 10 and 20 min exposure, higher or equal thermal resistance of *E. faecium* was observed when compared to *Salmonella* serotypes, however after 30 min exposure, log reductions from *E. faecium* were significantly higher than *S.* Seftenberg and Enteritidis PT 30 ($P < 0.05$). At 140 ± 1 °C, *E. faecium* reductions were significantly higher than *S.* Seftenberg after 10 min exposure and than both *S.* Seftenberg and Enteritidis PT 30 after 20 and 30 min exposure. The highest reductions were observed at 140 ± 1 °C, 30 min exposure, where *S.* Seftenberg, Enteritidis PT 30, Tennessee and *E. faecium* populations decreased by 2.9 ± 0.2, 2.6 ± 0.1, 3.4 ± 0.6 and 3.4 ± 0.2 log CFU/g, respectively.

3.4. Oil heating

Bacterial populations recovered from non-selective media were higher and occasionally significantly higher than selective media. Log reductions of *E. faecium* were not significantly different from *Salmonella* except when *E. faecium* reductions were significantly less than *Salmonella* ($P < 0.05$; TSAN values only; Table 3). Greater than 5 log reductions of *Salmonella* were observed after 121 ± 1 °C, 2.5 min (5.6 ± 0.4 log CFU/g), 127 ± 1, 2.0 (5.7 ± 0.9 log CFU/g) and 127 ± 1, 2.5 min (6.3 ± 0.7 log CFU/g) treatment conditions (Table 3).

After drying inoculated pecan kernels in the biosafety hood, the moisture content decreased to the original un-inoculated levels for *Salmonella* and *E. faecium* inoculated pecans ($P > 0.05$) (Table 4). Processing conditions did not decrease the moisture content values significantly ($P > 0.05$; Table 4), indicating insignificant loss of water during oil roasting treatments.

Data obtained from oil heating experiments fit into a linear model determined on the basis of R^2 values (Table 5). The D-values of *Salmonella* and *E. faecium* calculated at 116 ± 1, 121 ± 1 and 127 ± 1 °C were 0.63, 0.43, 0.36 min and 0.68, 0.52, 0.39 min, respectively (Table 5). A z-value of 44.8 and 45.2 °C was calculated for *Salmonella* and *E. faecium*, respectively under the oil heating treatment of pecan kernels.

3.5. Hot water conditioning

Under all the treatment conditions, log reductions (Log N/N_0) for *E. faecium* were either not significantly different from ($P > 0.05$), or significantly lower than ($P < 0.05$), *Salmonella* (Table 6). A maximum 4.9 log reduction of *Salmonella* was observed following exposure to 95 ± 1 °C water for 80 s. Data obtained from conditioning treatments did not fit into a linear or Weibull model, due to low R^2 values. The highest and lowest R^2 calculated using linear model were 0.72 and

Table 2
Moisture content (%) and water activity (a_w) changes in peanut kernels upon exposure to dry heat conditions (n = 3).

Process conditions		*S.* Seftenberg	*S.* Enteritidis PT30	*S.* Tennessee	*E. faecium*		*E. faecalis*	
Temp	Time (min)	% Moisture	% Moisture	% Moisture	% Moisture	a_w	% Moisture	a_w
Raw Nuts		7.7 ± 1.0	7.7 ± 1.0	7.7 ± 1.0	7.7 ± 1.0	0.69 ± 0.02	7.7 ± 1.0	0.69 ± 0.02
Inoculated Nuts		12 ± 2.0	10 ± 0.5	12 ± 0.4	13 ± 0.4	0.90 ± 0.01	12 ± 0.4	0.89 ± 0.04
Dried Nuts (7 h)[c]		9.7 ± 0.8	8.7 ± 0.5	8.7 ± 2	9.1 ± 2	0.70 ± 0.1	8.7 ± 2	0.61 ± 0.02
Stagnant Air								
120	20	6.7 ± 0.8A[b]	7.0 ± 0.0AB	4.7 ± 0.8BC[c]	7.5 ± 0.5A	0.6 ± 0.01	6.7 ± 0.7AB	0.6 ± 0.01
	30	5.6 ± 0.9A	6.2 ± 0.8BC	5.7 ± 0.8AB	5.8 ± 0.9BCD	0.5 ± 0.03	6.6 ± 0.7AB	0.4 ± 0.03
	40	5.1 ± 1A	4.8 ± 0.4D	3.6 ± 1BC	4.4 ± 0.5E	0.5 ± 0.05	5.1 ± 0.9BC	0.4 ± 0.04
130	10	6.7 ± 0.5A	7.3 ± 0.8A	7.3 ± 2A	6.8 ± 0.4AB	0.6 ± 0.02	7.3 ± 1.2A	0.5 ± 0.0
	20	5.7 ± 0.7A	5.7 ± 0.5CD	4.5 ± 1.8BC	6.7 ± 0.5ABC	0.6 ± 0.04	6.7 ± 0.7AB	0.5 ± 0.0
	30	5.0 ± 0.6A	5.0 ± 0.0D	2.5 ± 1C	5.4 ± 0.6CDE	0.5 ± 0.04	4.3 ± 0.9C	0.4 ± 0.02
140	10	7.3 ± 0.9A	7.2 ± 0.4A	5.5 ± 1AB	7.1 ± 0.2A	0.6 ± 0.01	6.8 ± 0.8AB	0.5 ± 0.02
	20	5.5 ± 0.6A	5.7 ± 0.5CD	3.7 ± 1BC	4.8 ± 1DE	0.4 ± 0.03	5.4 ± 2BC	0.4 ± 0.05
	30	2.8 ± 0.4B	3.1 ± 0.5E	3.3 ± 0.7C	4.6 ± 0.9DE	0.30 ± 0.02	3.7 ± 0.6C	0.3 ± 0.03
Forced Air								
120	20	6.8 ± 0.8A	7.3 ± 0.5A	5.5 ± 0.8A	7.5 ± 1A	0.5 ± 0.02	NA[a]	NA
	30	4.3 ± 0.8CD	6.5 ± 0.6AB	5.2 ± 0.8A	5.8 ± 0.7 BCD	0.5 ± 0.01	NA	NA
	40	4.8 ± 0.8BC	6.5 ± 0.8AB	4.5 ± 1AB	5.0 ± 0.06CD	0.40 ± 0.01	NA	NA
130	10	6.0 ± 0.6AB	6.3 ± 0.5AB	6.2 ± 1A	7.4 ± 1AB	0.5 ± 0.01	NA	NA
	20	5.8 ± 1ABC	5.5 ± 0.8BC	5.2 ± 1A	6.2 ± 1ABC	0.4 ± 0.03	NA	NA
	30	4.5 ± 0.6BC	4.7 ± 1C	5.2 ± 1A	5.4 ± 0.5CD	0.4 ± 0.04	NA	NA
140	10	5.5 ± 0.8ABC	7.0 ± 0.9A	4.7 ± 0.8A	7.6 ± 0.9A	0.6 ± 0.03	NA	NA
	20	4.9 ± 0.8BC	5.3 ± 0.5BC	4.5 ± 1AB	4.8 ± 0.41CD	0.5 ± 0.03	NA	NA
	30	2.8 ± 1.3D	4.7 ± 1C	2.8 ± 0.8B	4.2 ± 0.8D	0.5 ± 0.04	NA	NA

[a] *Enterococcus faecalis* experiments were discontinued due to lack of its validity as a surrogate under stagnant heat experiments.
[b] Same letters represent no significant difference within columns of each treatment (stagnant and forced) separately.
[c] Dried nuts represents the inoculum level, percent moisture and water activity levels obtained from nuts prior to treatments.

0.24, respectively. To determine the applicability of Weibull model on the experimental data, a classic test is generally employed in the studies involving analyzing the straight line relationship between double logarithmic plot of ln (-ln N/N_0) vs ln t (Du et al., 2010a; Ma et al., 2009). The highest and lowest R^2 values obtained were 0.43 and 0.10, indicating the unsuitability of the Weibull model to describe the thermal inactivation of *Salmonella* and *E. faecium* on inshell pecans exposed to hot water conditioning.

Upon inoculation, the moisture content of the inoculated in-shell pecans increased from initial un-inoculated levels of 6.6 ± 0.6%. Drying the in-shell pecans in the biosafety cabinet for 8 h reduced the moisture content to 8.1 ± 0.9% (*Salmonella*) and 6.9 ± 1.5% (*E. faecium*). Moisture levels of inoculated inshell pecans reduced to the original levels after drying for *E. faecium* ($P > 0.05$), but not for

Table 3
Populations (Log CFU/g) of *Salmonella* cocktail and *Enterococcus faecium* on pecan kernels treated with hot oil (n = 6).

Treatment		*Salmonella* cocktail				*Enterococcus faecium*			
		TSAN (log CFU/g)		BSAN (log CFU/g)		TSAN (log CFU/g)		BEAN (log CFU/g)	
Temp(°C)	Time (min)	Populations	Reductions	Populations	Reductions	Populations	Reductions	Populations	Reductions
Inoculated nuts		12 ± 0.1		12 ± 0.1		10 ± 0.4		10 ± 0.2	
Dried Nuts (7 h)[b]		8.0 ± 0.1		7.5 ± 0.3		7.9 ± 0.3		7.7 ± 0.3	
116	0.5	7.2 ± 0.1	0.82 ± 0.2A[a]	6.9 ± 0.2	0.61 ± 0.4A	7.3 ± 0.2	0.61 ± 0.3A	7.2 ± 0.2	0.51 ± 0.3A
116	1.0	6.5 ± 0.1	1.5 ± 0.2A	6.3 ± 0.1	1.2 ± 0.3A	6.5 ± 0.1	1.4 ± 0.4A	6.3 ± 0.2	1.4 ± 0.4A
116	1.5	5.9 ± 0.3	2.1 ± 0.4A	5.4 ± 0.3	2.1 ± 0.6A	6.0 ± 0.3	1.9 ± 0.4A	5.6 ± 0.3	2.1 ± 0.5A
116	2.0	4.7 ± 0.5	3.3 ± 0.4A	4.3 ± 0.3	3.3 ± 0.4A	5.0 ± 0.2	2.9 ± 0.4A	4.4 ± 0.3	3.3 ± 0.5A
116	2.5	4.1 ± 0.2	3.9 ± 0.2A	3.9 ± 0.5	3.7 ± 0.6A	4.2 ± 0.2	3.5 ± 0.2A	4.1 ± 0.2	3.6 ± 0.3A
121	0.5	7.2 ± 0.1	0.76 ± 0.2A	7.0 ± 0.2	0.64 ± 0.2A	7.3 ± 0.2	0.54 ± 0.3A	7.2 ± 0.1	0.52 ± 0.3A
121	1.0	6.1 ± 0.1	1.9 ± 0.2A	6.0 ± 0.2	1.6 ± 0.4A	6.3 ± 0.1	1.6 ± 0.4A	6.2 ± 0.2	1.5 ± 0.4A
121	1.5	4.6 ± 0.4	3.4 ± 0.5A	4.3 ± 0.3	3.2 ± 0.3A	4.9 ± 0.6	3.0 ± 0.8A	4.5 ± 0.6	3.2 ± 0.8A
121	2.0	3.5 ± 0.4	4.5 ± 0.3A	3.4 ± 0.4	4.1 ± 0.4AB	4.3 ± 0.3	3.5 ± 0.6C	3.8 ± 0.4	3.9 ± 0.6BC
121	2.5	2.3 ± 0.4	5.6 ± 0.4A	2.1 ± 0.5	5.4 ± 0.6AB	3.2 ± 0.4	4.7 ± 0.7B	2.3 ± 0.6	5.4 ± 0.8AB
127	0.5	7.2 ± 0.1	0.77 ± 0.1A	7.2 ± 0.1	0.44 ± 0.2B	7.3 ± 0.1	0.60 ± 0.3AB	7.0 ± 0.1	0.70 ± 0.4AB
127	1.0	6.0 ± 0.2	2.0 ± 0.2A	5.8 ± 0.3	1.8 ± 0.5AB	6.2 ± 0.1	1.7 ± 0.3AB	6.2 ± 0.3	1.5 ± 0.4B
127	1.5	3.3 ± 0.5	4.7 ± 0.5A	3.3 ± 0.3	4.3 ± 0.5AB	4.1 ± 0.1	3.8 ± 0.3C	3.8 ± 0.4	3.9 ± 0.2BC
127	2.0	2.3 ± 0.9	5.7 ± 0.9A	2.2 ± 0.8	5.3 ± 0.9A	2.7 ± 0.4	5.1 ± 0.6A	1.8 ± 0.5	5.9 ± 0.6A
127	2.5	1.7 ± 0.7	6.3 ± 0.7A	1.9 ± 0.6	5.7 ± 0.7A	2.0 ± 0.7	5.9 ± 0.5A	1.7 ± 0.5	6.1 ± 0.6A

[a] Letters represent significant differences within rows using Tukey Kramer test in JMP 10 software ($p < 0.5$).
[b] Dried nuts represents the inoculum levels obtained from nuts prior to treatments.

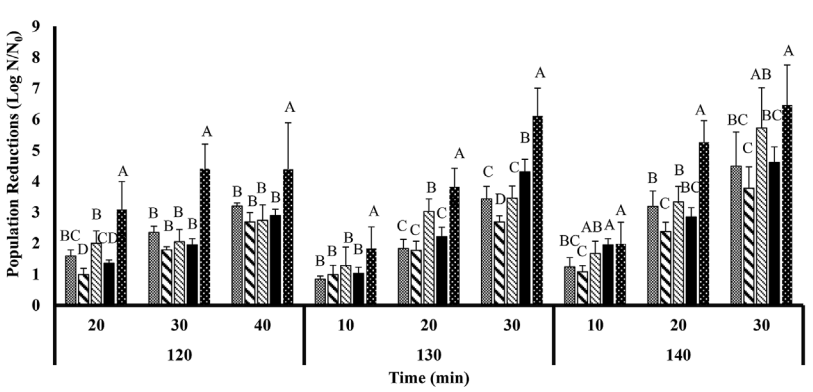

Fig. 1. Populations reductions (Log N/N_0) obtained from non-selective, TSAN media for *Salmonella* and *Enterococcus* spp. after exposure of peanut kernels. The figure depicts population reductions of *S.* Seftenberg, *S.* Enteritidis PT30, *S.* Tennessee, *E. faecalis* and *E. faecium* on peanut kernels exposed to 120 ± 1 (20, 30, 40 min), 130 ± 1 (10, 20, 30 min), and 140 ± 1 °C (10, 20, 30 min) in a stagnant air oven (n = 6).

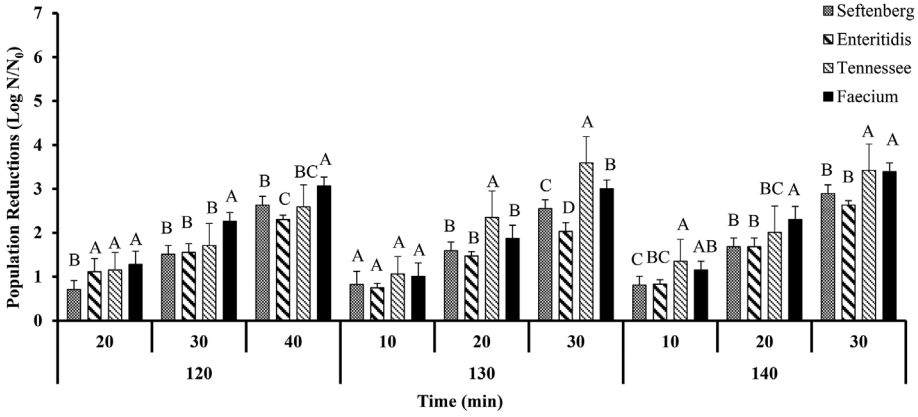

Fig. 2. Population reductions (Log N/N_0) obtained from non-selective, TSAN media for *Salmonella* and *Enterococcus faecium* after exposure of peanut kernels. The figure depicts population reductions of *S.* Seftenberg, *S.* Enteritidis PT30, *S.* Tennessee, and *E. faecium* on peanut kernels exposed to 120 ± 1 (20, 30, 40 min), 130 ± 1 (10, 20, 30 min), and 140 ± 1 °C (10, 20, 30 min) in a forced air oven (n = 6).

Table 4
Moisture content (%) and water activity (a_w) of inoculated pecan kernels exposed to hot oil treatments (n = 3).

		Salmonella	*Enterococcus faecium*	
Temperature (°C)	Time (min)	Moisture (%)	Moisture (%)	a_w
Raw nuts		4.8 ± 1.2	4.8 ± 1.2	0.66 ± 0.01
Inoculated nuts		8.8 ± 0.8	7.7 ± 1.3	0.94 ± 0.00
Dried Nuts (7 h)[c]		3.9 ± 0.6	4.8 ± 1.3	0.69 ± 0.01
116	0.5	2.4 ± 0.5AB[b]	3.5 ± 1.4A	0.49 ± 0.01
116	1.0	1.5 ± 0.3[a]B	3.3 ± 1.2A	0.45 ± 0.02
116	1.5	2.0 ± 0.4AB	4.0 ± 1.9[a]AB	0.40 ± 0.04
116	2.0	2.2 ± 0.4AB	3.0 ± 0.9AB	0.40 ± 0.04
116	2.5	2.3 ± 0.6AB	2.2 ± 0.4AB	0.41 ± 0.03
121	0.5	2.7 ± 0.4AB	2.2 ± 1.2AB	0.46 ± 0.02
121	1.0	2.1 ± 0.4AB	2.0 ± 0.6AB	0.40 ± 0.02
121	1.5	2.5 ± 0.04AB	1.8 ± 1.0AB	0.41 ± 0.04
121	2.0	2.3 ± 0.7AB	2.7 ± 1.0AB	0.39 ± 0.03
121	2.5	2.2 ± 0.4AB	1.3 ± 0.8B	0.40 ± 0.02
127	0.5	2.8 ± 0.7A	2.3 ± 0.3AB	0.51 ± 0.01
127	1.0	2.3 ± 0.4AB	2.0 ± 0.5AB	0.43 ± 0.03
127	1.5	2.0 ± 0.4AB	1.8 ± 0.4AB	0.39 ± 0.04
127	2.0	1.7 ± 0.8B	2.2 ± 0.7AB	0.41 ± 0.02
127	2.5	2.2 ± 0.7AB	1.9 ± 0.4AB	0.33 ± 0.03

[a] An outlier was omitted before statistical analysis.
[b] Same letters indicate no significant different ($p > 0.05$) within columns using Tukey Kramer in JMP 10.
[c] Dried nuts represents the percent moisture and water activity levels obtained from nuts prior to treatments.

Salmonella ($P < 0.05$) inoculated pecans. The a_w of *E. faecium* inoculated nuts reduced (0.56 ± 0.1) to the original un-inoculated levels (i.e. 0.64 ± 0.01) after drying period of 8 h ($P > 0.05$).

Table 5
D-values (min) obtained from oil roasting experiments using *Salmonella* and *Enterococcus faecium* inoculated pecan kernels. Data fit in linear model and linear equations were calculated, where Y represents log CFU/g and X represents treatment time in min.

Oil temp (°C)	Line equation	R^2	D-value (min)
Salmonella			
116	Y = −1.58X + 8.04	0.99	0.63
121	Y = −2.35X + 8.22	0.99	0.43
127	Y = −2.79X + 8.24	0.96	0.36
Enterococcus faecium			
116	Y = −1.48X + 8.00	0.99	0.68
121	Y = −1.94X + 8.07	0.99	0.52
127	Y = −2.59X + 8.28	0.97	0.39

4. Discussion

An ideal surrogate for *Salmonella* is a non-pathogenic microorganism with similar or higher resistance when exposed to same conditions. *Enterococcus faecium* (ATCC 8459) originally known as *Micrococcus freudenreichii* ATCC 8459 and then, *Pediococcus* sp. NRRL B2354, was originally isolated from milk and is a heat resistant and a non-pathogenic strain (Kornacki, 2012). This strain has been evaluated as a potential surrogate for *Salmonella* during almond and peanut thermal treatments (Jeong et al., 2011; Poirier et al., 2014; Sanders and Calhoun, 2014). Upon extensive research, Almond Board of California (ABC) recommended this particular strain for almond thermal validation studies (ABC, 2014).

Two different methods of inoculation have been used in the literature for low water food related studies, surface inoculation (Uesugi et al., 2006) and immersion inoculation (Beuchat and Mann, 2010). Surface inoculation represents the contamination present on the surface of nuts, whereas immersion inoculation represents contamination

Table 6
Populations (log CFU/g) of *Salmonella* and *Enterococcus faecium* on in-shell pecan during conditioning treatment with hot water (n = 6).

Temp (°C)	Time (s)	*Salmonella*				*Enterococcus faecium*			
		TSAN		BSAN		TSAN		BSAN	
		Population	Reduction	Population	Reduction	Population	Reduction	Population	Reduction
Inoculum (Log CFU/ml)		11 ± 0.7		11 ± 0.8		9.7 ± 0.5		9.7 ± 0.4	
Dried Nuts (8 h)[b]		8.0 ± 0.2		7.9 ± 0.2		6.5 ± 0.9		6.5 ± 0.9	
75	20	6.0 ± 0.8	1.9 ± 0.7A[a]	5.8 ± 0.7	2.1 ± 0.6A	5.2 ± 0.5	1.5 ± 1.0A	4.4 ± 0.8	1.8 ± 1.3A
	40	4.5 ± 1.1	3.4 ± 1.1A	4.1 ± 1.1	3.8 ± 1.2A	4.6 ± 0.9	1.9 ± 1.1B	4.4 ± 0.8	1.8 ± 1.1B
	80	4.5 ± 0.7	3.5 ± 0.7A	4.3 ± 0.7	3.7 ± 0.6A	4.8 ± 0.3	1.8 ± 1.0B	4.4 ± 0.4	1.8 ± 1.0B
	120	4.2 ± 0.8	3.8 ± 0.8A	4.3 ± 0.7	3.7 ± 0.8A	4.4 ± 0.8	2.1 ± 0.9B	4.3 ± 1.1	2.0 ± 1.6B
80	20	4.9 ± 0.5	3.0 ± 0.4A	4.5 ± 0.8	3.4 ± 0.7A	5.0 ± 1.0	1.4 ± 0.8B	5.0 ± 0.7	1.3 ± 0.7B
	40	5.1 ± 0.7	2.8 ± 0.7A	5.1 ± 0.6	2.8 ± 0.7A	4.4 ± 0.8	2.2 ± 1.4A	3.5 ± 1.3	2.7 ± 1.6A
	80	4.7 ± 0.6	3.3 ± 0.6AB	4.6 ± 0.5	3.4 ± 0.6A	4.5 ± 1.0	2.0 ± 1.7AB	4.1 ± 1.2	1.6 ± 1.7B
	120	4.2 ± 0.6	3.7 ± 0.6A	4.1 ± 0.7	3.8 ± 0.8A	4.4 ± 1.0	2.2 ± 1.2B	3.8 ± 1.6	2.8 ± 1.6AB
85	20	5.2 ± 0.8	2.7 ± 0.8A	5.1 ± 0.6	2.9 ± 0.8A	4.8 ± 1.2	1.7 ± 0.6B	4.8 ± 1.0	1.7 ± 0.7B
	40	5.0 ± 0.7	2.9 ± 0.8A	4.8 ± 0.8	3.1 ± 0.9A	5.0 ± 0.9	1.8 ± 0.9B	4.7 ± 1.2	2.1 ± 0.9AB
	80	4.0 ± 0.8	4.0 ± 0.8A	3.8 ± 0.7	4.1 ± 0.7A	3.8 ± 1.2	2.9 ± 1.3B	3.7 ± 1.2	2.6 ± 1.0B
	120	3.9 ± 0.7	4.0 ± 0.8A	3.8 ± 0.7	4.1 ± 0.9A	3.7 ± 1.0	2.9 ± 0.9B	3.1 ± 1.5	3.4 ± 1.0AB
90	20	4.0 ± 1.1	4.0 ± 1.0A	4.0 ± 1.0	4.0 ± 0.8A	4.8 ± 1.1	1.7 ± 1.4B	4.4 ± 1.1	1.9 ± 1.4B
	40	3.7 ± 0.9	4.3 ± 1.0A	3.6 ± 0.9	4.3 ± 1.0A	4.0 ± 0.8	2.5 ± 1.4B	3.3 ± 1.4	2.9 ± 2.0AB
	60	3.6 ± 0.9	4.3 ± 0.9A	3.6 ± 0.9	4.3 ± 0.9A	4.0 ± 0.6	2.5 ± 0.8B	3.1 ± 1.3	3.3 ± 1.3AB
	80	3.1 ± 0.8	4.8 ± 0.8A	3.1 ± 0.8	4.9 ± 0.9A	3.8 ± 0.4	2.7 ± 1.2B	2.9 ± 1.2	3.0 ± 2.2B
95	20	4.8 ± 1.1	3.2 ± 1.1A	4.6 ± 1.1	3.4 ± 1.1A	4.7 ± 0.7	2.2 ± 1.0A	4.3 ± 0.8	2.2 ± 1.5A
	40	3.8 ± 0.8	4.2 ± 0.8A	3.7 ± 0.8	4.2 ± 0.9A	4.5 ± 0.2	2.1 ± 1.1B	3.9 ± 1.0	2.2 ± 1.8B
	60	3.9 ± 0.7	4.1 ± 0.8A	4.0 ± 0.9	3.9 ± 0.9A	3.4 ± 0.9	3.1 ± 1.3A	3.0 ± 1.1	3.3 ± 1.5A
	80	3.2 ± 0.9	4.8 ± 0.9AB	3.1 ± 0.8	4.9 ± 0.8A	3.2 ± 0.7	3.5 ± 0.9B	2.4 ± 1.4	4.0 ± 1.3AB

[a] Same letters represent no significant differences in log reductions within rows ($P > 0.05$).
[b] Dried nuts represents the inoculum levels obtained from nuts prior to treatments.

infiltrated inside nuts, occurs during various stages like harvesting from ground after rainfall event, during conditioning treatment with contaminated water and others (Beuchat and Mann, 2010). Infiltrated cells exhibit higher resistance to thermal inactivation than the cells present on the surface of nuts as reported by Beuchat and Mann (2011b). Lower *Salmonella* reductions were observed from immersion inoculated pecans as compared to surface inoculated pecans exposed to hot water conditioning treatment (Beuchat and Mann, 2011b). In the current study, only the surface inoculation method was used to inoculate nuts to evaluate the suitability of *E. faecium* as a surrogate; should a submersion inoculation be desired to inoculate inshell pecans in a validation study, the suitability of *E faecium* would need to be evaluated under those inoculation conditions.

In the current study, all the inoculated nut samples were dried in a biosafety cabinet for 7 or 8 h to ensure moisture and water activity introduced through inoculation is reduced back to the original un-inoculated level, followed by refrigerated storage for the period of up to three weeks. Greater thermal tolerance of *Salmonella* was observed from inshell pecans dried and stored at 4 °C for 3–5 weeks than the samples which were not dried and stored after inoculation (Beuchat and Mann, 2011a). In addition, nuts were removed from refrigerator prior to treatments to warm the nuts back to ambient conditions. The thermal inactivation of *Salmonella* on nuts adjusted to ambient (21 °C) conditions prior to treatments did not differ from nut kept at refrigerated conditions (4 °C) (Beuchat and Mann, 2011a). However, the performance of *E. faecium* under the given conditions was not determined by Beuchat and Mann (2011a).

Under the dry heat treatments evaluated, *E. faecium* was a valid surrogate for *Salmonella* under most of the conditions of stagnant hot dry air treatment but not under forced dry air settings of the lab. Forced air equipment used in industry varies in terms of bed depth, air flow, and air distribution. Specifications of the equipment may impact the *Salmonella* reductions obtained. Further studies are required to determine the suitability of *E. faecium* in forced dry air setting. Industry members are encouraged to conduct experiments at pilot plant level with the same equipment used to provide forced dry air in order to obtain exact and accurate data for their specific settings (Poirier et al., 2014).

Previously, Beuchat and Mann (2011a) evaluated *Salmonella* inactivation on pecans during hot dry air heating. The reduction in *Salmonella* levels obtained during hot dry air treatments of pecan pieces and halves at 120 (2.4 log CFU/g for pieces and halves) and 130 °C (3.0 log CFU/g for pieces and 3.3 log CFU/g for halves) after 20 min exposure (Beuchat and Mann, 2011a), were higher than the results recovered in the current study under forced air setting (Fig. 2) and similar to the results from stagnant air setting (Fig. 1). However, in both studies on pecans or peanuts, the desired reductions (4–5 log CFU/g) were not achieved for *Salmonella* under the dry heat settings even after exposure of nuts to 130 °C for 20 min. After exposure to 140 °C for 30 min in the current study, greater than 4 log reductions of *S.* Seftenberg and Tennessee were observed on inoculated peanut kernels under stagnant air settings but not under forced air settings. Due to the variability between the experiments, it is recommended that each individual roaster is validated in their respective locations using a surrogate like *E. faecium*.

In oil heating treatments of pecan kernels, *E. faecium* was a suitable surrogate for *Salmonella* under all tested conditions. The higher thermal tolerance of *E. faecium* on pecan kernels exposed to hot oil treatments, indicate the potential use of the strain in the validation studies for oil heating. Unlike other oil roasting studies conducted on almonds (Du et al., 2010) and pecans (Beuchat and Mann, 2011a) where upwardly concave inactivation curves were obtained, here, our data fit in a linear model. Long exposure periods (up to 4 min) used in previous studies as opposed to the shorter exposure periods used in this study (2.5 min) could have impacted the inactivation curves; increasing exposure time in the current study from 2.5 min to 4.0 min may provide similar results. At 116, 121 and 127 °C, after 30 s exposure, *Salmonella* reductions observed by Beuchat and Mann (2011a) were 1.1, 1.2 and 1.4 log CFU/g for immersion inoculated pecan pieces and 1.9, 1.9 and 2.0 log CFU/g on immersion inoculated pecan halves, respectively. Du et al. (2010) observed higher *Salmonella* reductions of 2.9, 3.0 and 3.6 log CFU/g at 116, 121 and 127 °C after initial 30 s exposure, respectively. In the current study, *Salmonella* cocktail reduced by 0.82, 0.76 and 0.77 log CFU/g at these temperatures within 30 s exposure. Lower log reduction observed in the current study could be due to differences in inoculation

and drying methodology of all three studies. Where Beuchat and Mann (2011a) used immersion inoculation method, Du et al. (2010) used surface inoculation as used in the current study, but dried the inoculated nuts at room temperature for the period of 24 h. Drying in biosafety hood with the blower could possibly inactivate the loosely attached cells of *Salmonella* and leave the more resistant cells on the surface of nuts. This hypothesis is supported by the *Salmonella* reductions of 4.0 log CFU/g after drying in the current study and by 2.2 log CFU/g in almond study (Du et al., 2010). Also, drying at room temperature possibly did not reduce the moisture content levels thoroughly to the initial levels. The moisture content of untreated inoculated almonds used in Du et al. (2010) study were 4.4 ± 0.09%, and in the current study, the moisture content of *Salmonella* inoculated pecan kernels was 3.9 ± 0.6%. Exposing the pecan kernels to 127 °C for 1.5 min, resulted in 4.7 ± 0.5 log CFU/g reduction of *Salmonella*, which is in agreement with the results obtained by Beuchat and Mann (2011a), where 4.31 log CFU/g reduction was observed on surface inoculated pecan halves. The z-values obtained in this study, 44.8 and 45.2 °C for *Salmonella* and *E. faecium*, respectively, are high. Similarly high z-values can be seen in a study conducted on milk chocolate, where the z-values obtained for *Salmonella typhimurium* and *Salmonella seftenberg* 775W were 34.2 and 32.4 °C, respectively (Geopfert and Biggie, 1968).

Heat tolerance of salmonellae increases with decreasing a_w values (Barrile and Cone, 1969; Villas-Rojas et al., 2013). A recent study investigated the impact of reduced a_w on the thermal resistance of *Salmonella* and *Enterococcus faecium* at a pre-selected temperature setting and concluded a log-linear increase in the D-values for both the organisms at that temperature (Liu et al., 2018). The significance of reduced a_w on bacterial thermal tolerance was not evaluated in the current research, however the evaporation of water and a significant reduction of moisture content was observed in the dry heat settings (Table 2). Reduction of *Salmonella* on peanut kernels under hot dry air conditions in the current study, depends both on process parameters and water activity or moisture content of peanuts. Loss of water activity and moisture content was also observed for pecan pieces and halves exposed to hot dry air conditions of 60–120 °C for up to 20 min (Beuchat and Mann, 2010). No further modeling of data was conducted on the basis of time and temperature conditions only. Whereas in oil roasting experiments, the moisture content did not decrease significantly (Table 4), indicating the process parameters to be the sole factor affecting the *Salmonella* reductions. Oil roasting data fit in a linear model (Table 5) and further calculations were conducted for the data.

E. faecium is a suitable surrogate, with significantly higher thermal resistance than *Salmonella,* in most of the hot water conditions, evaluated. Initial 20 s exposure of inshell pecans to 75, 80, 85, 90 and 95 °C resulted in 1.9, 3.0, 2.7, 4.0, 3.2 log CFU/g reductions of *Salmonella* levels; similarly, *Salmonella* Enteritidis PT 30 reductions obtained from almond kernels exposed to 60, 70, 80, 88 °C for 30 s were 0.9, 1.1, 2.9, 4.7 log CFU/g (Harris et al., 2012). *Salmonella* reductions after exposure of inshell pecans to 75, 80, 85, 90, 95 °C for 120 s were 3.8, 3.7, 4.0, 4.8, 4.8 log CFU/g, respectively, in the current study. The results obtained from a study conducted by Beuchat and Mann (2011a) were in agreement with the current study, where, 4.4, 3.8, 3.9, 4.5, 4.9 log CFU/g reductions were obtained after exposure of inshell pecans to hot water treatments of 75, 80, 85, 90, 95 °C after 120 s exposure. In the current study, a 4 log reduction of *Salmonella* was observed at 85 °C, following 80 and 120 s exposure times; and for all exposure time periods at 90 and 95 °C, except at 95 °C, 20 s exposure, where 3.2 log CFU/g reduction was obtained. Similar to the current study, research conducted in 1975 resulted in 4 log CFU/g reduction of *S.* Seftenberg on immersion inoculated inshell pecans treated at 99 °C hot water conditions for 2 min (Beuchat and Heaton, 1975). A recent study evaluated thermal inactivation of different foodborne pathogens inoculated onto in-shell pecans during hot water treatment conditions and obtained a 5-log reduction after 4 min of treatment at 80 and 90 °C (Kharel et al., 2018). In the study, in-shell pecans were submerged in the inoculum for 1 h and were air dried for 20 min prior to hot water treatment, while the current study used surface inoculated pecans that were dried under forced air conditions for 8 h.

In complex food matrices; the composition of food, treatment temperature, a_w, and local microenvironment based on the type of contaminated ingredient present in the food are some of the factors that affect thermal inactivation of *Salmonella* (Jin et al., 2018; Li et al., 2013). In the current study, thermal inactivation of *Salmonella* on the surface of peanuts and pecans was studied in comparison with *Enterococcus spp.* under different time temperature treatment conditions after bringing the water activity to original levels after inoculation. *E. faecium* (ATCC 8459) is a suitable surrogate of *Salmonella* under most of the treatment conditions of stagnant dry heating of peanuts and all the treatment conditions of oil roasting pecan kernels and hot water treatment of in-shell pecans. To validate processes and equipment under the new FSMA regulations, industry should use *E. faecium* (ATCC 8459) to ensure the desired log reduction of pathogens on the product is obtained under specific plant conditions.

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Disclosure statement

No authors have any financial or professional conflict of interest to disclose pertaining to the research described in this manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fm.2018.12.006.

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