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Research Paper

Radiofrequency inactivation of *Salmonella* Enteritidis PT 30 and *Enterococcus faecium* in wheat flour at different water activities

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Salmonella persistence in low-moisture foods creates a significant need for effective pasteurisation processes, but conventional thermal treatments for low-moisture products are challenged by long treatment times and insufficient information on inactivation kinetics. Radiofrequency (RF) heating can reduce heating time and inactivate *Salmonella* without inducing significant quality damage. The objectives were to study RF heating of organic wheat flour, and evaluate *Enterococcus faecium* as a surrogate for RF inactivation of *Salmonella*. Temperature profiles and uniformity of the top and cross-section surface of RF heated flour were obtained with an infrared camera, using different electrode gaps, platforms, and different materials that surrounded the sample to make the electromagnetic field uniform. The flour was inoculated with S. Enteritidis PT 30 or *E. faecium*, equilibrated to a specific a_w, and then RF heated for 8.5 (0.25 a_w) or 9 min (0.45 and 0.65 a_w) to reach ≈75 °C minimum temperature (no holding time); survivors were then enumerated. The best temperature uniformity was obtained using a 90 mm electrode gap, placing small polystyrene cylinders above and underneath the sample container, and using a platform of polystyrene Petri dishes. *Salmonella* reduction of 7 log was achieved at 0.45 and 0.65 a_w at room temperature, while 5 and 3 log reductions were reached for *Salmonella* and *E. faecium*, respectively, at 0.25 a_w. These data suggest that RF heating has potential as an inactivation treatment for *Salmonella*, and that *E. faecium* is a feasible surrogate to validate the efficacy of RF treatments.

1. Introduction

The new FDA Food Safety Modernization Act (FSMA) addresses food safety with a preventive focus. All manufacturers who provide food products or ingredients to the US market will need comply with pending rules (Food and Drug Administration, 2013). One of the requirements in section 204(d)(2) is for the FDA to designate high risk foods that require additional record keeping to protect the public's health. Due to the association with several *Salmonella* outbreaks, some low-moisture foods, like nuts and nut products,

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are included in the high risk food list (Food and Drug Administration, 2014; Food and Drug Administration, United States Department of Agriculture, & Homeland Security, 2011). This has forced the food industry and research community to study possible treatments to inactivate *Salmonella* in low-moisture foods. However, the efficacy of such treatments is challenging, because *Salmonella* becomes highly resistant to heat at low water activities (Archer, Jervis, Bird, & Gaze, 1998; Bari et al., 2009; Chen et al., 2009; Du, Abd, McCarthy, & Harris, 2010).

Heat treatments have been successfully implemented for both pasteurisation and sterilisation of high moisture products, and they show promise for low-moisture products. Jeong, Marks, and Orta-Ramirez (2009) observed that 1 log reduction of *Salmonella* on the surface of almonds could be achieved in 16 s if the air humidity was 70–90% (volume fraction) at 82.2 °C, and in 957 s if the air humidity was 5%. Radiofrequency (RF) heating is another promising technology to heat bulk low-moisture foods in short times and thereby inactivate pathogens in those products. Wang, Tiwari, Jiao, Johnson, and Tang (2010) compared hot air vs. RF-assisted heating for legumes (chickpeas, green peas, and lentils) as potential treatments for disinfestation. They found RF-assisted heating required less time (5–7.5 min) than did hot air (275–660 min) to reach the target temperature (60 °C) at the centre of the legume bed. Although changes in colour were not significantly different for either method compared to the untreated samples, hot air treated samples lost significant weight and moisture when compared to RF-treated or untreated samples. Kim, Sagong, Choi, Ryu, and Kang (2012) used RF heating to inactivate S. Typhimurium and *Escherichia coli* in spices, and found that 50 and 40 s treatments resulted in 2.8 and 4.3 log reductions in black pepper, and 3.4 and >5 log reduction in red pepper for S. Typhimurium and *E. coli*, respectively, without significant colour changes.

RF heating has potential as a heat treatment to control pests and pathogens (Alfaifi et al., 2014; Wang & Tang, 2004; Wang, Tang, Johnson, Mitcham, & Hansen, 2002); however, temperature uniformity is still a major challenge for this technology (Jiao, Tang, & Wang, 2014). Some parts of the food are being over treated, while others may be undertreated. Prolonging processing times to bring the cold spots to the minimum temperature required for pasteurisation increases the chances of quality damage due to extreme overtreatment of the hot spots.

Several studies have tried different approaches to improve heating uniformity of RF heating. Wang et al. (2010) studied the effect of forced hot air, shaking the container with a conveyor belt, and mixing food in the container during RF heating of legumes, as well as different combinations of all these. They found that using a combination of forced hot air and shaking the container reduced the standard deviation in temperature from 4.2 to 3.2 °C.

Previous studies reported that surrounding a peanut butter container with a plastic that has a similar dielectric constant to peanut butter reduced the temperature difference from 13 to 7 °C on the top surface and from 28 to 18 °C in the cross-section surface, with a similar effect reported for wheat flour (Jiao et al., 2014). However, there is no universal solution, and different products may require different approaches.

Once any process or technology is proposed for commercial application, validation of that process is essential, either via the use of microbial inactivation models (and appropriate dynamic product and process data) or via the use of a non-pathogenic surrogate inoculated onto the product and subjected to the actual process (Awuah, Ramaswamy, & Economides, 2007; Chen et al., 2009). However, there are very few established procedures to validate a process in low-moisture products. The Almond Board of California had documented procedures for the use of *Enterococcus faecium* NRRL B-2354 as a surrogate to validate thermal inactivation of *Salmonella* in almonds (Almond Board of California, 2007). *E. faecium* has also been studied to validate processes such as extrusion of carbohydrate—protein meal (Bianchini et al., 2014). However, the validity of *E. faecium* as an appropriate surrogate has been demonstrated for very few other products or processes.

The objectives of this research were to assess RF as an inactivation treatment for S. Enteritidis PT 30 in organic wheat flour and to evaluate the use of *E. faecium* NRRL B-2354 as a non-pathogenic surrogate for treatment validation.

2. Material and methods

2.1. Bacteria strains and wheat flour

S. Enteritidis PT 30 and *E. faecium* NRL B-2354 were acquired from Dr. Linda Harris at UC-Davis. They were kept in a stock solution of trypticase soy broth (TSB) supplemented with 0.6% (w/v) yeast extract (YE) and 20% glycerol at −80 °C until used. S. Enteritidis PT 30 was chosen because of its relation to a low moisture food outbreak (Isaacs et al., 2005), high resistance to thermal inactivation (Anderson, Keller, Gradl, Pickens, & Li, 2013) and various studies publish on its survival, persistence and inactivation kinetics in different matrixes with different technologies (Danyluk, Uesugi, & Harris, 2005; Harris, Uesugi, Abd, & McCarthy, 2012; Jeong et al., 2009; Jeong, Marks, Ryser, & Harte, 2012; Komitopoulou & Pen, 2009; Smith & Marks, 2015; Villa-Rojas et al., 2013). The studied food matrix was soft white wheat organic pastry flour (Eden Foods, Clinton, MI).

2.2. Radiofrequency-assisted heat treatment

2.2.1. Temperature uniformity and profile of RF treatments

A bench-top, 0.5 kW, 27 MHz RF heating unit (Thermail E0-1, W.T. LaRose & Assoc. Inc., Troy, NY) was used to heat treat the samples (Fig. 1 A and B). An infrared camera (Thermal CAMTM SC-3000, FLIR Systems, Inc., North Billerica, MA) was used to obtain temperature profiles of the top surface (Fig. 1C) and/or a cross-sectional (Fig. 1D) for all samples. The heating pattern of the RF equipment was evaluated by heating a foam slab made out of polyurethane, with the same dimension of the upper electrode (254 × 75 mm). The foam would show the location of the hot and cold spots within the equipment.

The effects of the electrode gap, platform, and surrounding material on the heating uniformity of the sample were also evaluated. The temperature uniformity was calculated with the uniformity index (UI) as explained by Alfaifi et al. (2014),

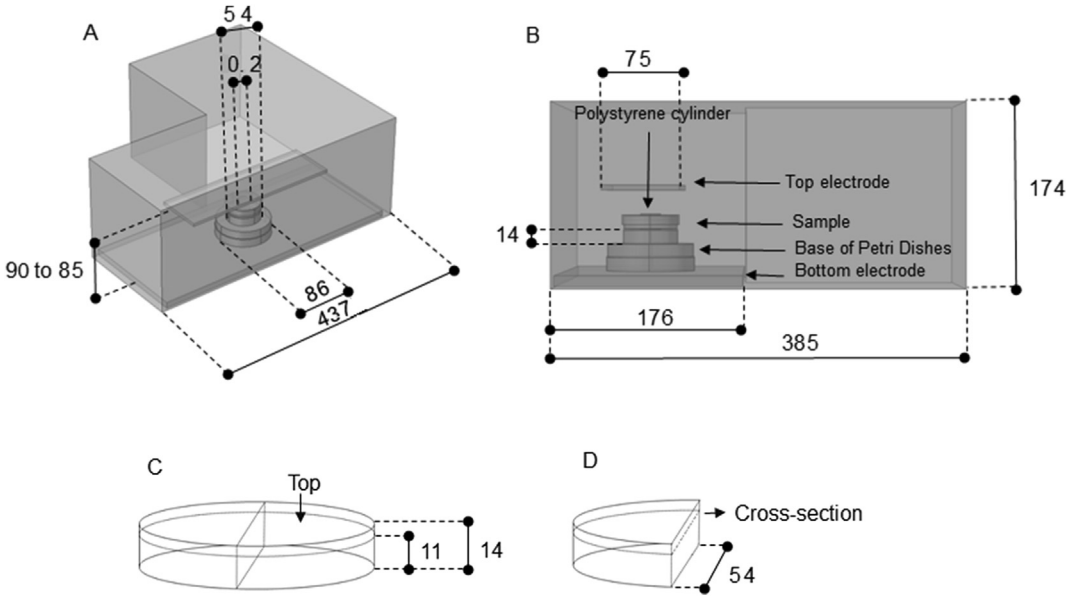

Fig. 1 – Wheat flour sample in bench scale radiofrequency equipment: (A) surface and (B) lateral view. (C) Top surface and (D) cross-sectional view of the samples in a Petri dish. Dimensions in mm.

and ΔT. The RF heating tests were run using 16 g of flour packed ($\rho = 868 \pm 5$ kg m^{-3}) in small polystyrene Petri dishes (53 mm diameter by 12 mm); the flour thickness in the dish was 9 mm (Fig. 1C and D).

$$UI = \frac{\frac{1}{S}\int \sqrt{(T - T_{av})^2}\, dS}{T_{av} - T_i} \quad (1)$$

where S is the surface area of the sample in m^2, T is the local temperature in °C, T_{av} is the average temperature in °C after RF treatment, and T_i is the initial average temperature in °C. A smaller UI indicate better temperature uniformity.

There were two restrictions for the treatment design; keeping the maximum temperature close to 100 °C to avoid excessive sample caking, and melting or deformation of the container. And the second restriction, was keeping the treatment time below 9 min so the equipment's electrical components would not overheat. The selection of the minimum temperature was based on a compromise between reaching a temperature that would achieve some level of inactivation in the time required to reach the target temperature and not violating the restrictions. The guidelines for the inactivation time required were obtained using the inactivation kinetics for S. Enteritidis PT 30 in organic wheat flour reported by Smith (2014). Using the parameters reported by Smith (2014) for the Bigellow-type relationship the calculated D-values at different temperatures for the three a_w. The calculated D-values at 75 °C are 37, 9 and 2 min for 0.25, 0.45 and 0.65 a_w, respectively. The D-values calculated for temperatures below 75 °C were too long for all a_w, and although D-values at temperatures above 75 °C were shorter, RF heating would place the maximum temperature well above 100 °C and would require heating times longer than 9 min. Therefore, our treatments would target to get a minimum temperature of 75 °C.

2.2.1.1. *Electrode gaps*. Four electrode gaps of 50, 60, 70 and 90 mm were evaluated on their influence on heating uniformity when reaching a minimum temperature 75 °C from an initial temperature of approximately 25 °C. The tests were done in triplicates for each electrode gap and the samples were on a Petri dish stack to hold the sample in the middle of the electrode gap.

2.2.1.2. *Platforms*. Previous studies have shown that placing the samples in the middle of the electrode gap improves temperature uniformity (Tiwari, Wang, Tang, & Birla, 2011). Preliminary tests also showed that platform materials used to hold the sample influenced the temperature uniformity of the wheat flour. The platforms used were a tripod made of polypropylene (Fig. 2A), a glass container (Fig. 2B), and a stack of Petri dishes made of polystyrene (Fig. 2C). We RF-heated the flour samples on each platform in a 90 mm electrode gap for the time necessary to obtain a minimum temperature of 75 °C without exceeding a 9 min treatment or a T_{max} of 100 °C; tests were done in triplicate.

2.2.1.3. *Surrounding material*. Three types of plastics as surrounding materials were used during RF treatment: polyetherimide (PEI), polyethylene terephthalate (PET) and polystyrene (PS), which have dielectric constants ($\varepsilon' \approx 3.0$, 3.2 and 2.6 at 1 MHz, respectively, Lampman, 2003) similar to that of wheat flour ($\varepsilon' \approx 3.3$ at 27 MHz and 8.8% moisture content w. b., Tiwari et al., 2011). Three different approaches were tested: a box that surrounded the whole sample (Fig. 3), a layer that surrounded only the periphery of the sample (Fig. 4 A), and

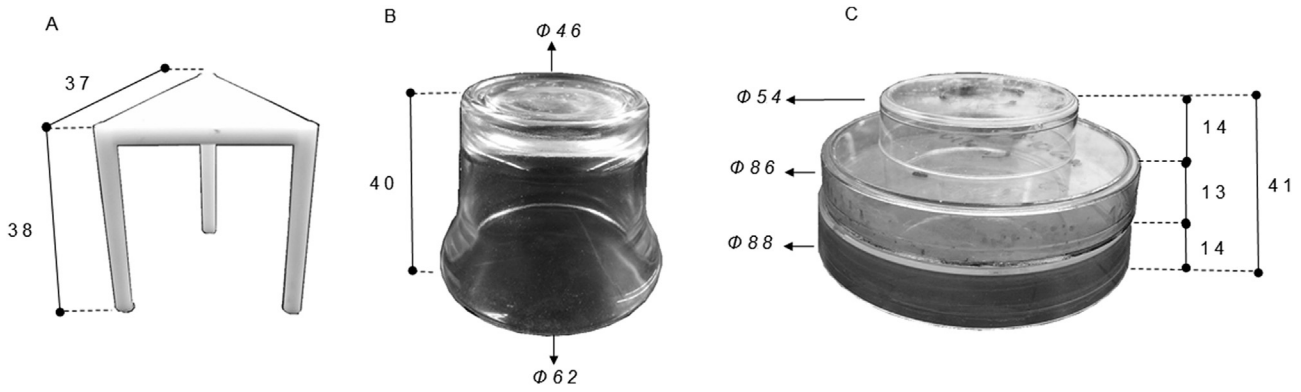

Fig. 2 – Different platforms used to assess the influence of different shapes and materials on the heating pattern: (A) tripod (polypropylene), (B) glass, and (C) polystyrene Petri dish stack. Dimensions in mm.

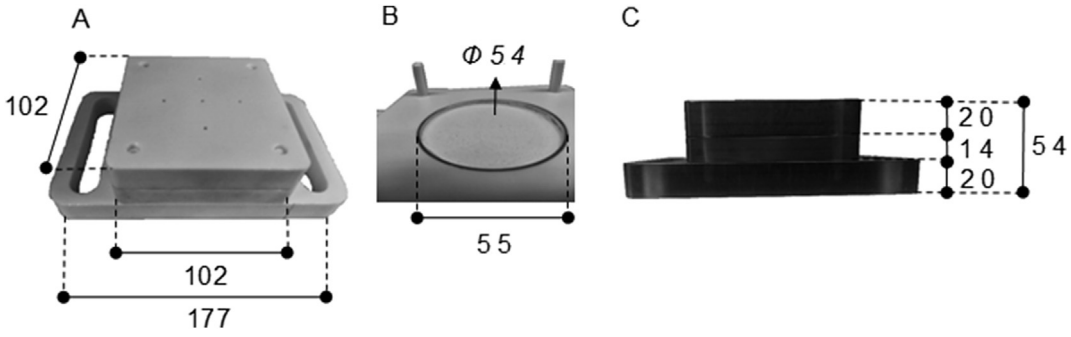

Fig. 3 – Plastic boxes used to improve temperature uniformity in samples: (A) PET box, (B) inside PET boxes and (C) PEI (polyetherimide) box. Dimensions in mm.

different size cylinders over and under the sample container at sample's center (Fig. 4 B). The boxes and side cover help concentrate more energy on the food sample area and also enhance the electromagnetic field uniformity around the sample. The cylinders would only help concentrate energy on the spot they would cover, so we placed them above and below the sample container to cover the cold spot in the centre. The cylinders had a 20 mm diameter, were made of two different materials, and had different thickness. One pair was PS 1 mm thick; the other pair was PEI 7 mm thick. Each sample was heat treated, using a 90 mm electrode gap and a Petri dish stack as a platform, from 25 °C to a T_{min} 75 °C, without exceeding a 9 min treatment or having a T_{max} over 100 °C. Tests were conducted in triplicate.

2.2.2. *RF* Salmonella *and* Enterococcus *inactivation treatments*

2.2.2.1. a_w influence on RF heating. Heat treatment of the inoculated samples was conducted using RF conditions that gave the best temperature uniformity (as described in the results section). We used a 90 mm electrode gap, with a Petri dish stack platform, and added two small polystyrene cylinders, one above and one below the sample container. Then we evaluated the time required to heat from 25 °C to a T_{min} of

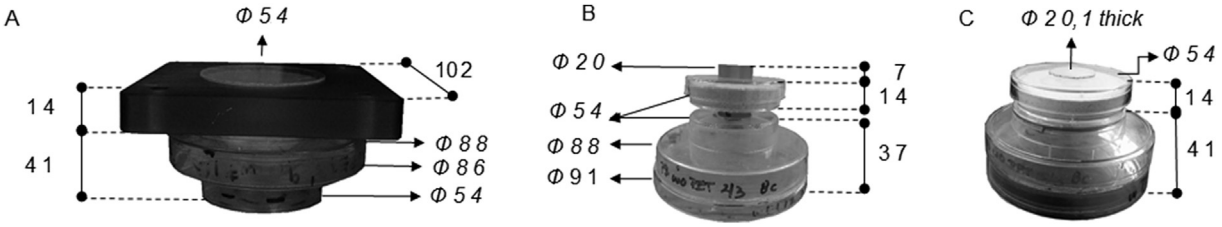

Fig. 4 – Different surrounding materials used to assess their influence on the heating pattern: (A) PEI (polyetherimide) surrounding the periphery, (B) PEI cylinder above and below the sample, and (C) Polystyrene cylinders above and below the sample. Dimensions in mm.

75 °C for wheat flour at 0.25, 0.45 and 0.65 a_w and compared the temperature distribution and other parameters (T_{min}, T_{max}, ΔT, $T_{average}$ and SD) to ensure the treatments would be equivalent, in order to test only the a_w influence on *Salmonella* inactivation.

2.2.2.2. *Inoculation and sample preparation*. Background flora was obtained for five random 1 g samples diluted in 9 mL of 0.1% peptone water, plated on Trypticase Soy Agar (TSA, Difco, Sparks, MD) and incubated for 48 h at 37 °C. Estimated number was $10^{2.20 \pm 0.45}$ colony forming units (CFU) g^{-1} [sample], low enough that it wouldn't interfere with target microorganism enumeration in the RF treated samples.

The inoculation method used was previously shown to be repeatable, and to give a sufficiently high *Salmonella* population (Hildebrandt et al., 2014). Briefly, a loopfull (~10 μL) was taken from the stock strains stored in 20% glycerol at −80 °C and transferred into 9 mL of TSBYE and incubated for 24 h at 37 °C. Then, 100 μL of activated microorganisms were transferred to 9 mL of TSBYE and incubated for 24 h at 37 °C. One mL of that culture was spread plated onto a 150 × 15 mm Petri dish with TSA supplemented with 0.6% (w/v) yeast extract (TSAYE) and incubated for 24 h at 37 °C to create a bacterial lawn. The lawn was harvested with maximum recovery diluent (MRD, Fisher Scientific, Pittsburg, PA), pelleted by centrifugation (15 min at 6000 g; 4 °C), and re-suspended in 3 mL of MRD. Then, 10 g of wheat flour were inoculated with 1 mL of the suspension and hand massaged ~3 min, until all clumps were eliminated. The inoculated 10 g were then added to 90 g of flour and stomached for 3 min at 230 rpm in a Stomacher® (400, Seward, West Sussex, UK).

The initial bacterial inoculation level in the flour was ~10^8 CFU g^{-1}. Inoculated samples were spread in plastic trays to form a 1–2 mm thick layer. The trays were placed inside an equilibration chamber at room temperature until sample a_w had reached the target, which took approximately 4–5 days. The equilibration chamber consisted of a small glove box (EW-34788-00, Cole Parmer, Vernon Hills, IL) with a humidity control system custom-designed and built at Michigan State University. The target a_w levels were 0.25, 0.45, and 0.65 at 25 °C for flour with *Salmonella*, and only 0.25 a_w for flour with *E. faecium*.

All a_w was measured with a water activity meter (Aqualab serious 3TE, Decagon Devices Inc., Pullman, WA, USA) at 25 °C before treatment; only samples within ±0.02 of the target a_w were used for RF inactivation treatments. All a_w reported was measured at 25 °C, recognising that a_w increases as temperature increases during RF heating, which impacts thermal resistance of *Salmonella* and dielectric properties of the wheat flour. The a_w could not be measured at treatment temperatures, because there is no commercial equipment available that can perform a_w measurements above 60 °C during treatment.

2.2.2.3. *RF inactivation treatment*. Inoculated and equilibrated flour samples, 16 g, were placed in a Petri dish and compressed with a stainless steel expresso tamper (51 mm diameter) to obtain an even surface and density (leaving a 2 mm headspace). The conditions giving the best temperature uniformity described in Section 2.2.2.1 were used for the inactivation treatments. The treatment was 8.5 min for samples with 0.25 a_w, and 9 min for 0.45 and 0.65 a_w samples, to achieve a minimum temperature of 75 °C. After the RF treatment, the Petri dish with the sample was closed with tape, placed in a Ziploc bag and the sealed bag was partially submerged (enough to cover the Petri dish) in a water-ice bath for 12 min to cool samples to ~25 °C (the process took ~20 s). Three biological replicates (batches inoculated with independently grown inoculums) and two technical replicates (samples from the same batch) of each were used in the experiments.

To enumerate surviving colonies after RF treatment, whole samples were placed in Whirl-Pak bags with 144 mL of MRD and then stomached for 3 min at 230 rpm and tenfold serially diluted in 9 mL MRD blanks. Three dilutions of all samples were duplicate-plated onto TSAYE supplemented with 0.05% (w/v) ferric ammonium citrate, and 0.03% (w/v) sodium thiosulfate, and incubated for 48 h at 37 °C. Colonies with the characteristic black centre for *Salmonella* and all *E. faecium* colonies were counted after incubation and converted to log values.

An untreated inoculated sample was used as a control to obtain the survivor fraction (log S) by subtracting the log of the initial population count in the control (log N_0) from the log count at the end of the treatment (log N).

2.3. Statistical analysis

All statistical tests were conducted using Minitab 14.1, with a standard test criterion of $\alpha = 0.05$.

To assess the difference in temperature distribution due to electrode gaps, platforms, or surrounding material, we used analysis of variance (ANOVA) with a Tukey pairwise comparison. The effects of surrounding materials on ΔT, were analysed with Kruskal–Wallis and a Bonferroni pairwise comparison due to the non-normality distribution of the data.

In order to compare the temperature distributions of wheat flour samples at different a_w after RF treatments, we ran temperature distribution identification with Minitab's "Individual distribution identification" tool. We tested for distribution fitness of 14 different models: normal, lognormal, 3-parameter lognormal, exponential, 2-parameter exponential, Weibull, 3-parameter Weibull, largest extreme value, smallest extreme value, gamma, 3-parameter gamma, logistic, log-logistic and 3-parameter log-logistic. Since no distribution fit, we used the non-parametric Kruskal–Wallis test to assess differences between temperature parameters (T_{max}, T_{min}, ΔT, $T_{average}$ and SD) across different a_w levels after RF treatment.

Differences in mean log reductions between *Salmonella* and *E. faecium* at 0.25 a_w were evaluated via Student's t-test.

3. Results and discussion

3.1. RF equipment heating pattern

The position of the hot spot in the foam slab was in the geometric centre of the gap, while the cold spots were located on the periphery (Fig. 5). Therefore, the best positioning for the samples would be in the geometric centre of the electrode gap, as suggested by previous studies (Tiwari et al., 2011).

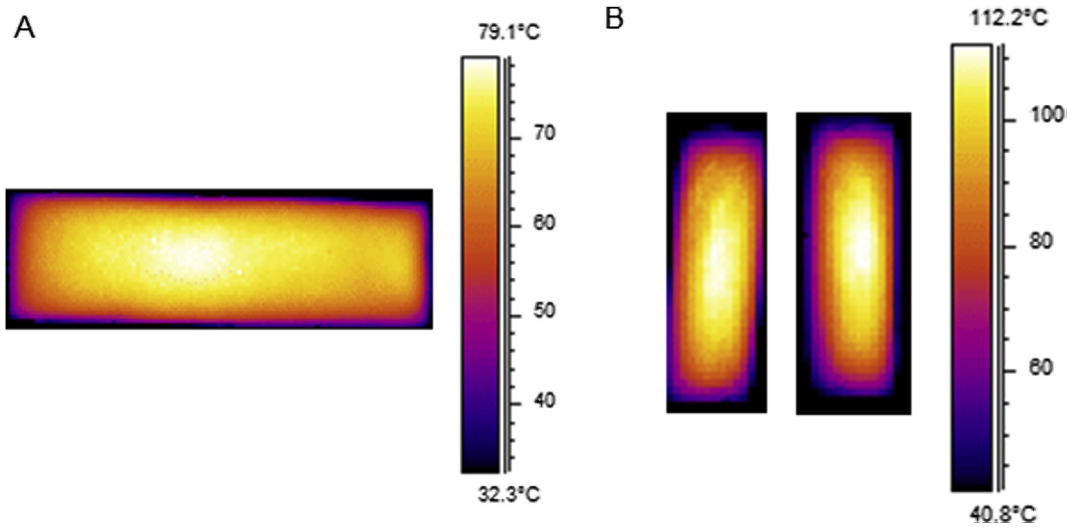

Fig. 5 – Heating pattern at: (A) the top surface and (B) cross-sectional view of the RF bench equipment demonstrated with a foam slab subjected to RF heating.

3.2. *Influence of electrode gap on heating uniformity*

Smaller electrode gaps resulted in less time to reach a minimum temperature of 75 °C (Table 1); as the gap increased, treatments took longer to reach the same temperature. Shorter heating times also led to larger temperature variation, as reflected by ΔT in Table 1. The UI also decreased significantly as the electrode gap increased, showing that increasing the gap and therefore treatment times helped improve temperature uniformity. The ΔT obtained for a 50 mm electrode gap was higher ($p < 0.05$) than for all other gaps, while UI for 50 mm gap was higher ($p < 0.05$) than other gaps.

The results show that short time treatments may not be ideal, as they solely rely on RF heating with little influence of internal heat conduction. This means that hot and cold spots are larger in short time treatments. Longer treatment times allow heat conduction to improve temperature distribution within the samples, leading to a more uniform temperature profile. Therefore, the best choice would be an electrode gap that yields the longest treatment time possible with a relatively low ΔT and UI, and high $T_{average}$, which was a 90 mm gap for our experiments.

3.3. *Influence of platforms on heat uniformity*

Treatment time to reach the target T_{min} for the sample on the Petri dish stack was 9 min, whereas the sample on the tripod did not reached the target T_{min} within the maximum allowed time (9 min), and the sample on the glass platform only took 6 min (Table 2). There was no significant difference in T_{min} between samples on the glass and Petri dish platforms. Samples on the tripod had a lower T_{min} ($p < 0.05$) than did samples on the other platforms (i.e., 10 °C lower than the targeted 75 °C). T_{max} values were significantly ($p < 0.05$) different among all platforms. Samples on the glass platform had the highest T_{max}, while the samples on the tripod had the lowest. There were no differences in ΔT between the samples on the tripod and those on the Petri dish stack, but the samples on the glass platform had a comparatively higher ($p < 0.05$) ΔT. The UI was higher ($p < 0.05$) for the tripod than the other platforms.

The results show that platform material and its dielectric properties can impact temperature uniformity and final temperature of the wheat flour. Samples on platforms made out of plastics with dielectric constants similar to that of the wheat flour had a lower ΔT temperature than did the samples on glass. However, the dielectric constant of PP (tripod) is lower than that of PS (Petri dish), and therefore the energy concentrated on the sample area was smaller when using the tripod as platform than when using the Petri dish stack. This was reflected in the lower temperatures reached by the samples on the tripod compared to the temperatures from the samples on the Petri dish stack. On the other hand, samples placed on the glass platform reached a higher T_{max} than the samples on other platforms in a shorter time (6 min). This was because glass has a higher dielectric constant (ε′ 4.7–15 at 1 MHz from

Table 1 – Influence of electrode gap on temperature uniformity of the cross-section surface of RF heated wheat flour.[a]

Gap (mm)	Time (min)	T_{min} ± SD (°C)	T_{max} ± SD (°C)	ΔT ± SD (°C)	$T_{average}$ ± SD (°C)	UI ± SD
50	3.0	75.3 ± 3.2^{A}	120.9 ± 5.5^{A}	45.8 ± 8.8^{A}	105.1 ± 4.7^{A}	0.09 ± 0.02^{A}
60	3.0	76.2 ± 2.4^{A}	103.6 ± 1.4^{B}	27.4 ± 3.4^{B}	92.1 ± 0.7^{B}	0.05 ± 0.00^{B}
70	4.5	75.3 ± 1.7^{A}	99.0 ± 2.2^{B}	23.4 ± 1.5^{B}	91.1 ± 0.5^{B}	0.05 ± 0.00^{B}
90	9.0	76.2 ± 3.3^{A}	101.7 ± 3.7^{B}	25.5 ± 0.8^{B}	94.6 ± 3.6^{B}	0.05 ± 0.00^{B}

[a] Means with a common capital letter superscript within a column are not significantly different ($\alpha = 0.05$).

Table 2 – Influence of different platforms on temperature uniformity of the cross-sectional surface of RF treated wheat flour (90 mm electrode gap).[a]

Platform (treatment time, min)	T_{min} (°C)	T_{max} (°C)	ΔT (°C)	$T_{average}$ (°C)	UI ± SD
Petri dish stack (polystyrene) (9)	76.2 ± 3.3^{A}	101.7 ± 3.7^{A}	25.5 ± 0.8^{A}	94.6 ± 3.6^{A}	0.05 ± 0.00^{A}
Plastic tripod (polypropylene) (9)	65.9 ± 0.2^{B}	91.1 ± 2.6^{B}	25.2 ± 2.6^{A}	84.8 ± 2.6^{B}	0.07 ± 0.01^{B}
Glass (6)	79.5 ± 1.5^{A}	111.8 ± 1.8^{C}	32.3 ± 3.0^{B}	101.4 ± 1.8^{A}	0.06 ± 0.00^{A}

[a] Means with a common capital letter superscript within a column are not significantly different (α = 0.05).

Bansal and Doremus (1986)) and concentrated a larger amount of energy to the food volume during RF heating. In summary, the Petri dish stack platform yielded the best temperature uniformity in wheat flour for our RF treatments.

3.4. *Influence of surrounding material*

Surrounding materials in different conformations affected sample heating patterns (Table 3). In RF heating of wheat flour samples without surrounding material, the cold spot was localised in the centre, while the hot spot was in the periphery (Fig. 6A and D). When wheat flour samples were inside the plastic boxes, the location of the cold and hot spots shifted (Fig. 6B, C, E, and F). The hot spot moved to the centre, while the periphery became the cold spot. Having a cold spot in the periphery makes it easier to use other methods, such as hot air, to improve heating uniformity by bringing the periphery to the target temperature. We also reduced the heating time from 9 min to 8 or 7 min, because the boxes helped concentrate more energy onto the food sample volume. However, the PEI and PET boxes increased the temperature difference to around 36.9 or 32.5 °C, respectively, and also increased UI (Table 3).

The samples that had only their sides surrounded with the plastic material did not reach the targeted minimum temperature after 9 min of RF heating. They also had higher ($p < 0.05$) UIs than samples not surrounded. The ΔT for samples with their sides surrounded by plastic (PEI 31.2 °C, and PET 29.6 °C) were not significantly different from those of the samples with no surrounding material or surrounded by boxes. In contrast to our study, Jiao et al. (2014) reported a decrease in ΔT when RF-heated peanut butter and wheat flour were surrounded with PEI. However, they did not report whether the difference was statistically significant.

When the thicker cylinder was placed over the cold spot of the sample, the treatment time was reduced to 8.5 min, and the UI was higher ($p < 0.05$) than that of the sample without any surrounding material, although the ΔT was not significantly different. The ΔT improved when we used a thin PS cylinder (1 mm thick), decreasing from the original 25.5 to 20.9 °C. Jiao (2014) also reported that placing a pair of cylinders above and below the sample's cold spot reduced ΔT and UI, although they did not report if the difference was significant.

Results show that placing the wheat flour inside plastic with a dielectric constant similar to that of the food sample, or just surrounding the sides of the sample with that plastic, focuses too much energy on the sample area and does not improve the temperature uniformity.

3.5. *RF treatment temperature uniformity at different a_w*

The minimum wheat flour temperature achieved with an initial a_w of 0.25 after 9 min of RF heating was 77.1 °C, with a maximum of 99.9 °C. Wheat flour samples with initial a_w of 0.45 and 0.65 reached minimum temperatures of 77.9 and 75.6 °C, respectively, after 8.5 min of RF heating, while the corresponding maximum temperatures were 98.7 and 101.6 °C, respectively (Tables 4 and 5). There were no significant differences between the temperature parameters (T_{max}, T_{min}, $T_{average}$, ΔT or SD) of wheat flour at the different a_w levels for these treatments. The lack of significant difference among temperature parameters indicates temperature distribution of heat treated wheat flour at different a_w is equivalent and therefore the thermal inactivation treatments would be equivalent for all flour samples.

3.6. *Inactivation of* Salmonella *and* Enterococcus *during RF treatment*

The initial populations of *Salmonella* inoculated into wheat flour at the different a_w were not significantly different. There

Table 3 – Influence of surrounding material on temperature uniformity of the cross-sectional surface of RF heated (90 mm electrode gap) wheat flour.[a]

Surrounding material (treatment time, min)	T_{min} (°C)	T_{max} (°C)	ΔT (°C)	$T_{average}$ (°C)	UI ± SD
None (9)	76.2 ± 3.3^{A}	$101.7 \pm 3.7^{A,C}$	$25.5 \pm 0.8^{A,C}$	$94.6 \pm 3.6^{A,C}$	0.05 ± 0.00^{A}
PEI box (8)	75.6 ± 2.2^{A}	112.4 ± 0.7^{B}	36.9 ± 1.9^{B}	102.1 ± 1.9^{B}	$0.08 \pm 0.00^{B,C}$
PET box (7)	75.5 ± 3.0^{A}	$101.6 \pm 0.7^{A,B}$	$32.5 \pm 3.5^{A,B}$	$98.6 \pm 0.5^{B,C}$	$0.08 \pm 0.00^{B,C}$
PEI surrounding sides (9)	64.1 ± 2.3^{B}	95.2 ± 5.0^{C}	$31.2 \pm 6.9^{A,B}$	83.8 ± 2.1^{D}	0.09 ± 0.02^{B}
PET surrounding sides (9)	65.5 ± 0.7^{B}	95.1 ± 3.0^{C}	$29.6 \pm 3.5^{A,B,C}$	83.9 ± 3.2^{D}	0.09 ± 0.00^{B}
PEI cylinders (8.5)	75.2 ± 2.1^{A}	$107.1 \pm 2.7^{A,B}$	$31.9 \pm 1.0^{A,B}$	$93.3 \pm 2.4^{A,C}$	0.10 ± 0.00^{B}
Polystyrene cylinders (9)	77.9 ± 1.4^{A}	98.7 ± 1.9^{C}	20.9 ± 1.0^{C}	92.1 ± 2.3^{A}	$0.06 \pm 0.00^{A,C}$

[a] Means with a common capital letter superscript within a column are not significantly different (α = 0.05).

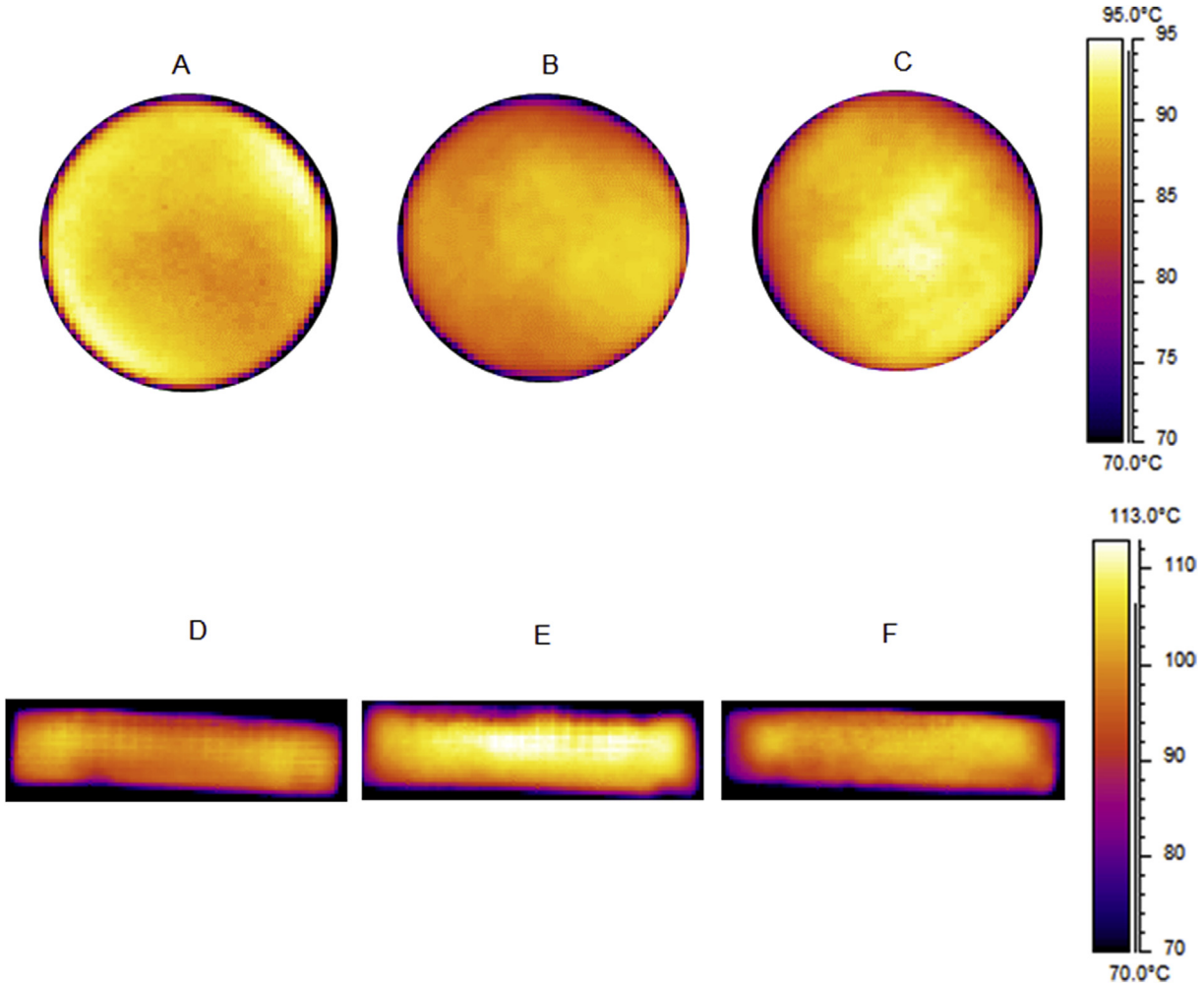

Fig. 6 – Top and cross-section surface temperature profile comparison between RF treated flour using a 90 mm gap with: (A and D) no box and (B and E) PET box and (C and F) PEI (polyetherimide) box, respectively.

was also no difference between the inoculated level of *Salmonella* and *Enterococcus* at 0.25 a_w (Fig. 7A and B).

After RF heating (9 min) of wheat flour samples conditioned to 0.45 and 0.65 a_w, measured at room temperature, we were unable to recover any *Salmonella* from the treated flour at a dilution of 10^{-1} (Fig. 7A). Since no *Salmonella* population could be recovered from wheat flour at 0.45 and 0.65 a_w, it was decided to only compared RF inactivation treatment for *E. faecium* at 0.25 a_w, since only that sample had *Salmonella* survivors after RF treatment. At 0.25 a_w, the RF heating (8.5 min) resulted in survivor counts of 3.08 and 4.95 $\log_{10}$ CFU g^{-1} for *Salmonella* (Fig. 7A) and *E. faecium* (Fig. 7B), respectively. In other words, using RF heating, we achieved an inactivation level of ~7 $\log_{10}$ CFU g^{-1} for *Salmonella* in wheat flour preconditioned at 0.45 and 0.65 a_w, measured at room temperature, and ~5 and 3.2 log reductions for *Salmonella* and *E. faecium* at a_w of 0.25 (Fig. 7C).

Table 4 – Temperature profile of the top surface of RF heated wheat flour at different a_w.

Temperatures (°C)/a_w[a]	0.25	0.45	0.65
Minimum	88.2	86.6	90.8
Maximum	99.7	96.9	101.6
Difference	11.5	10.3	8.8
Average	96.1	92.3	96.5
SD	1.5	1.7	1.6
Variance	4.1	3.1	3.4
Treatment time (min)	8.5	9	9

[a] Averages of five replicates. No significant ($p > 0.05$) differences relative to a_w were found within temperature parameters.

Table 5 – Temperature profile of the cross-section surface of RF heated wheat flour at different a_w.

Temperatures (°C)/a_w[a]	0.25	0.45	0.65
Minimum	77.1	77.9	75.6
Maximum	99.9	98.7	97.8
Difference	22.8	20.9	22.2
Average	92.2	92.1	89.7
SD	4.5	4.4	4.3
Variance	19.9	18.1	16.2
Treatment time (min)	8.5	9	9

[a] Averages of four replicates. No significant ($p > 0.05$) differences relative to a_w were found within temperature parameters.

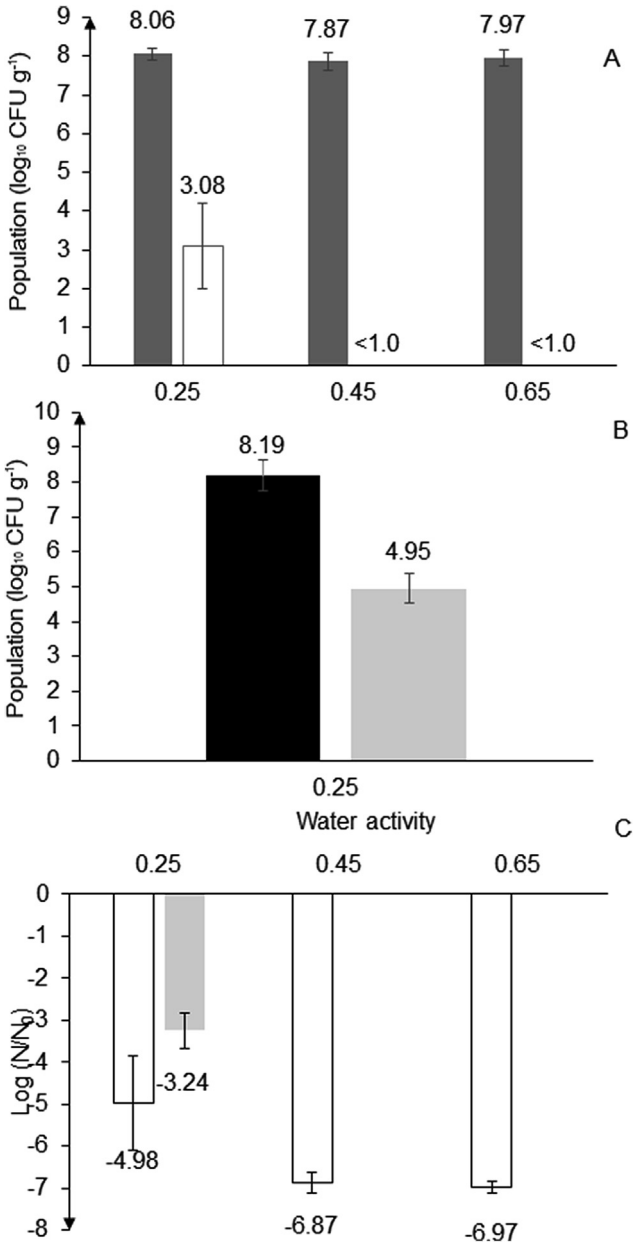

Fig. 7 – (A) Population counts before (dark grey) and after (white) RF heating for *S.* Enteritidis PT 30, (B) Population counts before (black) and after (light grey) RF heating for *E. faecium* B-2354 and (C) log reduction after RF heating for *S.* Enteritidis PT 30 (white) and *E. faecium* B-2354 (light grey). Microorganisms were inoculated in organic wheat flour (6 replicates). The population after RF treatment was significantly higher ($p < 0.05$) for *E. faecium* B-2354. The log reduction after RF treatment was significantly lower ($p < 0.05$) for *E. faecium* B-2354.

This study shows that RF treatments have the potential to pasteurise wheat flour. Similar conclusions have been drawn for other commodities in the literature. Kim et al. (2012) reported that a 50 and 40 s RF treatments can achieve log reductions of around 2.8 and 4.3 for black pepper and 3.4 and >5 log for red pepper, for *S.* Typhimurium and *E. coli*, respectively. Another study on inactivation of *S.* Typhimurium and *E. coli* O157:H7 in fishmeal reported that 2–3 min of RF achieved from 2 to more than 6 log reductions, depending on the target temperature (60–90 °C), with no significant impact on quality attributes (Lagunas-Solar et al., 2005).

With respect to the assessment of *E. faecium* as a potential surrogate for the *Salmonella* during RF heating of wheat flour, the FDA and USDA define a surrogate as "a non-pathogenic species and strain responding to a particular treatment in a manner equivalent to a pathogenic species and strain" (Institute of Food Technologists, 2015). This means that in order for *E. faecium* to qualify as an appropriate surrogate for *Salmonella* inactivation with RF heating, its non-pathogen status should be proven and its thermal resistance would have to be equal or higher to that of *Salmonella* under the same conditions.

Genomic sequence analysis has shown that *E. faecium* B-2354 is a safe surrogate for validation of thermal treatments (Kopit, Kim, Siezen, Harris, & Marco, 2014). Furthermore, *E. faecium* B-2354 has already been established as a suitable surrogate for thermal inactivation of *Salmonella* in almonds (Almond Board of California, 2007), and a recent study by Liu et al. (2015) demonstrated that *E. faecium* B-2354 is also a suitable surrogate for thermal treatments of *Salmonella* in wheat flour. The results of our study reinforce the suitability of *E. faecium* B-2354 as a surrogate for thermal treatment of *Salmonella* in wheat flour, given a greater reduction of *Salmonella* than *E. faecium* subjected to an equivalent treatment (albeit at a single a_w). This information is crucial for any future scale up and validation of wheat flour pasteurisation with RF heating.

4. Conclusions

RF heating seems to be a promising technology for heat treating dry products in a short time. Placing a thin layer of a material with a dielectric constant similar to that of the product helped concentrate more energy in that spot and improved temperature uniformity. Furthermore, RF appears to be an acceptable method to pasteurise *Salmonella* in wheat flour, and *E. faecium* B-2354 may be an adequate surrogate for future evaluation of RF inactivation on a larger scale. Holding times appear not to be necessary as long as RF treatments attain a minimum temperature that is high enough to ensure sufficient pathogen inactivation. However, this study does not include any evaluation of the sample quality, which should be addressed in future studies.

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