

Microbial Safety in Radio-frequency Processing of Packaged Foods

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ABSTRACT: Thermal resistance of *Clostridium sporogenes* (PA 3679) was determined at 115.6 °C, 118.3 °C, and 121.1 °C (240 °F, 245 °F, and 250 °F, respectively) in phosphate buffer (pH 7.0) and mashed potatoes (pH 6.3) using aluminum thermal-death-time (TDT) tubes developed at Washington State Univ. D-values were 1.8, 1.1, and 0.62 min in phosphate buffer and 2.2, 1.1, and 0.61 min in mashed potatoes at 115.6 °C, 118.3 °C, and 121.1 °C, respectively. Z-values were 12 °C and 10 °C in phosphate buffer and mashed potatoes, respectively. The thermal inactivation kinetic results were then used to validate a novel thermal process based on 27.12 MHz radio frequency (RF) energy. Trays of mashed potatoes inoculated with PA 3679 were subjected to 3 processing levels: target process ($F_0 \sim 4.3$), under-target process ($F_0 \sim 2.4$), and over-target process ($F_0 \sim 7.3$). The microbial challenge test data showed that microbial destruction from the RF process agreed with the calculated sterilization values. This study suggests that thermal processes based on RF energy can produce safe and shelf-stable packaged foods.

Keywords: dielectric heating, radio frequency, thermal resistance, *Clostridium sporogenes*, inoculated pack studies

Introduction

Clostridium sporogenes is a mesophilic spore-forming bacterium. A special strain of *C. sporogenes*, PA3679, has been widely used as a surrogate microorganism in canned food processing studies because of its nontoxicity and similar physiological requirements to the target microorganism, *Clostridium botulinum*, in the sterilization process of low-acid high-moisture food (Ocio and others 1994). Spores of *C. sporogenes*, in general, have a greater thermal resistance than that of *C. botulinum* (Larousse and Brown 1997). Therefore, smaller amounts of *C. sporogenes* can be used to validate a process for at least a 12 log reduction in *C. botulinum* population.

In conventional retort heating used in commercial food sterilization processes, the food is heated by steam or hot water at an elevated temperature beyond the boiling point of water by using high pressure of about 30 psi. In evaluating the effect of a thermal process on the safety and quality of the processed product, a sterilization value (F_0) is used to determine the thermal lethality of the process, while the cook value (C_{100}) is used to quantify food quality loss. Sterilization value accumulates at a much higher rate than the cook value when processing a food at high temperatures (>121 °C). The high temperature short time (HTST) process is, therefore, desirable in producing safe food while maintaining food quality. This strategy is used in aseptic processing of liquid foods. However, HTST processes for semisolid and solid foods are difficult to achieve with conventional retort heating because of slow conductive heat transfer. The food within the periphery of the container is often severely overheated by the time the cold spot in the center of the container reaches the desired sterility.

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Dielectric heating, such as microwave and radio frequency heating (RF), has the potential for fast heating in solid and semisolid foods. Dielectric heat is generated by direct interaction between electromagnetic waves and foods instead of by slow heat conduction as in conventional retort heating. RF heating refers to heating the dielectric materials with electromagnetic energy at frequencies between 1 to 300 MHz (Orfeuill 1987). According to Electromagnetic Compatibility (EMC) regulations (Rowley 2001), industrial, scientific, and medical (ISM) bands for RF heating are limited to 13.56, 27.12, and 40.68 MHz. The wavelength at these designated frequencies ranges from 22 to 360 times as long as that of the 2 commonly used microwave frequencies (915 and 2450 MHz), which allows RF energy to penetrate dielectric materials more deeply than microwave energy (Wang and others 2003). The deeper depths of RF energy penetration in foods and the simple uniform field patterns, as opposed to the complex nonuniform standing wave patterns in a microwave oven, make RF heating more suitable for processing large food trays (Zhao and others 2000; Wang and others 2003). Although it is known that RF heating has potential use in food sterilization, there have been no reported microbial challenge studies for RF sterilization of packaged foods.

To validate the RF sterilization system, mashed potatoes were selected as a model food. Mashed potatoes are simple, physically homogeneous, have a fairly uniform water distribution, and are easy to pack into the tray.

The objectives of this study were to determine the thermal resistance of *C. sporogenes* (PA 3679) in phosphate buffer (pH 7.0) and in mashed potatoes (pH 6.3) at temperatures of 115.6 °C, 118.3 °C, and 121.1 °C using novel aluminum TDT tubes and to validate a novel thermal process based on RF energy using inoculated pack studies.

Materials and Methods

Thermal kinetics determination

The thermal resistance of a microorganism is often characterized by the decimal reduction time (D-value) and thermal resistance

constant (z-value) (Larousse and Brown 1997). The values of D for *C. sporogenes* (PA 3679) in a M/15 phosphate buffer (pH 7.0) and mashed potatoes (pH 6.3, 7.8% butter, 14.8% instant potato flakes: Washington Potato Co., Warden, Wash., U.S.A., 24% whole milk, 53.4% water) were determined using novel aluminum TDT tubes developed at Washington State Univ. (Figure 1). PA 3679 spores in phosphate buffer with a concentration of 1.6×10^8 colony-forming units (CFU)/mL (NFPA nr S.C. 218) were obtained from the Technical Service Center of Natl. Food Processors Assn. (NFPA) in Dublin, Calif., U.S.A. The spores of PA3679 were verified under a microscope after receiving from NFPA. The spore suspension was enumerated and the initial concentration was verified. Vegetative cells that may be present in the spore suspension were inactivated during the activation of spores by heating at 90 °C for 10 min.

Mashed potatoes were inoculated with a spore suspension to approximately 1.0×10^6 CFU/g. The inoculated samples were mixed well and kept in an ice bath at 0 ± 0.2 °C before being transferred to sterile aluminum TDT tubes. The tubes were sealed with screw caps and then placed in a rack before being immersed completely in a 28-L circulating oil bath (Polyscience, Niles, Ill., U.S.A.) at the designated treatment temperatures (115.6 °C, 118.3 °C, and 121.1 °C). T-type thermocouples (Omega, Stamford, Conn., U.S.A.) through air-tight fittings in the caps were used to monitor the temperature (± 0.2 °C accuracy). Temperature data were collected using a data logger (Delta-T devices, Cambridge, U.K.). Immediately after the sample temperature reached within 0.2 °C of the set temperature, the heating time was recorded, and the 1st TDT tube was removed from the oil bath. The remaining samples were each subjected to 7 different exposure times ranging from 25 s to 270 s, depending on the treatment temperature. After heating, the tubes were immersed promptly into an ice bath at 0.0 ± 0.2 °C. The come-up time and cool-down time in mashed potatoes at 121.1 °C were 161.7 ± 7.5 s and 120 s, respectively. The unheated control samples were activated by heating in a test tube for 10 min in a water bath set at 90 °C. For spore suspension in phosphate buffer, dilutions were prepared in 0.2% peptone water, and survivors were enumerated by pour plating with Shahidi Ferguson Perfringens (SFP) agar (Difco Laboratories, Inc, Detroit, Mich., U.S.A.). For samples in mashed potatoes, the contents in the tube were aseptical-

ly removed with a sterile spatula to a stomacher bag and homogenized for 2 min in a Seward 400 circulator stomacher (Seward, Ltd., London, U.K.). Serial dilutions were made in 0.2% peptone water and 1 mL of the dilution was pour-plated with SFP agar. The plates were incubated at 37 °C for 48 h in anaerobic jars using Anaerogen (Oxoid, Ogdensburg, N.Y., U.S.A.) and GasPak (VWR Intl., Brisbane, Calif., U.S.A.), an anaerobic atmosphere generator and anaerobic indicator, respectively. Experiments were conducted in triplicate.

Survivor curves and TDT curves were plotted using Microsoft Excel software (Microsoft Corp., Redmond, Wash., U.S.A.) to determine D-values and z-values. D-value is the reciprocal of the slope of the survivor curve in semi-log coordinates and is calculated by Eq. 1 (Stumbo 1973).

$$D = \frac{U}{\log_{10} a - \log_{10} b} \quad (1)$$

where U is the heating time; a is the initial number of spores counted; and b is the number of spore survivors detected at the end of the heating.

Mathematically, z-value is the reciprocal of the slope of the TDT curve in semi-log coordinates and is calculated by Eq. 2 (Jay 2000).

$$z = \frac{T_2 - T_1}{\log_{10} D_{T1} - \log_{10} D_{T2}} \quad (2)$$

Water activity measurement

Water activity of mashed potatoes were determined using an Aqua Lab Series 3 TE water activity meter (Decagon Devices, Inc. Pullman, Wash., U.S.A.) at 25 ± 0.2 °C. The instrument has an accuracy of ± 0.003 .

RF sterilization system

A 6-kW pilot-scale RF sterilization system developed at Washington State Univ. based on a 27.12-MHz power supply (COMBI 6-S; Strayfield Fastran, U.K.) (Figure 2) and a water-conditioning system was used in this study. The RF system consisted of an RF feeder, 4 inductors, a pair of adjustable electrodes, and a pressure-proof vessel in which the food package was heated. The water conditioning system consisted of a high-temperature pump, a tank, and 2 plate heat exchangers, 1 of which used steam for heating, while the other used tap water for cooling. The circulating water temperature was controlled by the conditioning system to match the temperature of the heated food during the RF process to avoid the water to cool the food. One main purpose of the immersion water was to reduce fringe effects at the interface between the food package and the air in the RF applicators (Wang and others 2003). For that reason and from preliminary results, the conductivity of immersion water was adjusted using tap water and deionized water to approximately 110 $\mu\text{S}/\text{cm}$ at 21 °C to reduce edge heating of food. Because loss factor of circulating water was much lower than that of the food, the food absorbed most of RF energy during RF heating time.

Inoculated pack studies

The standard count-reduction method (Guan and others 2003) was used to evaluate the effectiveness of RF sterilization processes. An enrichment method was used to detect survival of inoculated PA 3679 spores under the detection limit of the count-reduction method.

Spore suspension of *C. sporogenes* (PA 3679) was inoculated into a liquid mixture of butter, milk, and water before being mixed with dried potato flakes. Inoculated mashed potatoes of 2726 g were

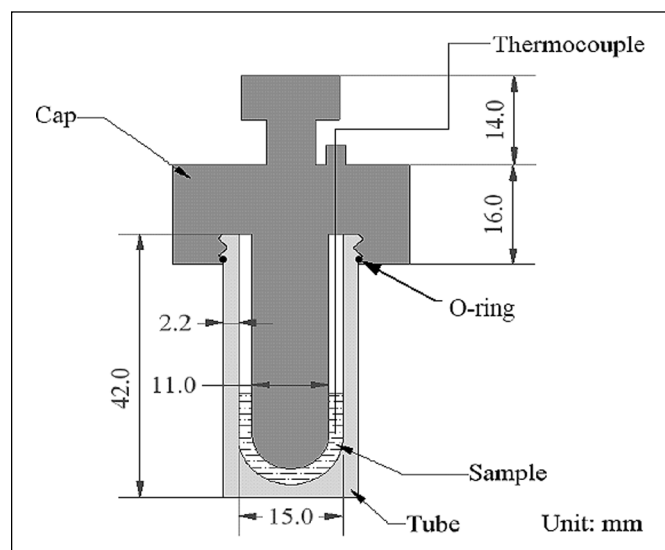


Figure 1—Schematic diagram of an aluminum TDT tube with a screwed-on cap

filled in the polymeric tray ($245 \times 235 \times 45$ mm) so that each tray contains approximately 1.0×10^7 viable spores before being vacuum sealed with a 0.15-mm-thick aluminum foil lid (Jefferson Smurfit, Dublin, Ireland) using a laboratory vacuum tray sealer (Reynolds Metals Co., Richmond, Va., U.S.A.). After inoculation, the tray was processed immediately in the pilot-scale 27.12 MHz RF sterilization system. Four fiber-optic temperature sensors (FISO technologies, Inc., Que., Can.) were inserted into 4 different locations where the least and most heated spots in the package based on previous chemical marker study (Wang and others 2003) as shown in Figure 3. A high correlation ($R^2 = 0.999$) was observed between the chemical marker M-1 yield and sterilization value (F_0) (Wang and others 2004), which confirmed the validity of this method to determine the cold spot. The time interval for measuring the temperature was set at 0.5 min. The recorded temperature profile was used to calculate the sterilization value (F_0) by Eq. 3 (Stumbo 1973).

$$F_0 = \int_0^t 10^{\frac{T-121.1}{z}} dt \quad (3)$$

where T is temperature ($^{\circ}\text{C}$), t is processing time (min), and z is 10°C .

Thermal processing was conducted in 3 different processing times on the basis of the temperature profile of a fiber-optic probe at the least heated spot. These 3 process times corresponded to (1) under-target process ($F_0 \sim 2.4$), (2) target process ($F_0 \sim 4.3$), and (3) over-target process ($F_0 \sim 7.3$). The target process is designed to inactivate all of the inoculated PA3679 population; using F_0 of 7 times the D-value to inactivate the population of 1×10^7 spores/tray.

Over-target process is aimed to inactivate the spores completely with F_0 greater than that of target process. After RF processes, portions of 100 g of mashed potatoes each were aseptically sampled with an alcohol flamed spoon from 5 different locations (Figure 3) and were then homogenized in 200 mL sterile 0.2% peptone water using a stomacher (Seward 400 Circulator Stomacher, Seward, Ltd., London, U.K.) at 260 rpm for 2 min. Four 2.5-mL portions of homogenate from each location were pour-plated with Shahidi Ferguson Perfringens (SFP) agar (Difco Laboratories, Inc.). The plates were incubated at 37°C for 48 h in anaerobic jars. To determine whether there was indeed no survival in target and over-target processes, enrichment tests were conducted on the rest part of mashed potatoes. The remaining mashed potatoes were divided into 6 portions, and each portion was mixed with the same amount (1:1 wt/wt) of Fluid Thioglycollate Medium (Difco Laboratories, Inc.) and incubated at 37°C for 48 h. After the incubation, a loopful ($10 \mu\text{L}$) of each portion was streak-plated on SFP agar, incubated under anaerobic condition at 37°C for 48 h. The experiments were conducted in triplicate.

Results and Discussion

Thermal resistance of *C. sporogenes*

The averaged D-values of *C. sporogenes* in phosphate buffer were 1.8, 1.1, and 0.62 min and 2.2, 1.1, and 0.61 min in mashed potatoes at 115.6°C , 118.3°C , and 121.1°C , respectively (Table 1). Figure 4 shows a typical linear correlated survival curve of *C. sporogenes* in phosphate buffer. The slight differences in D-value between phosphate buffer and mashed potatoes at 115.6°C are due to environmental influences. Because foods vary in their chemical and physical

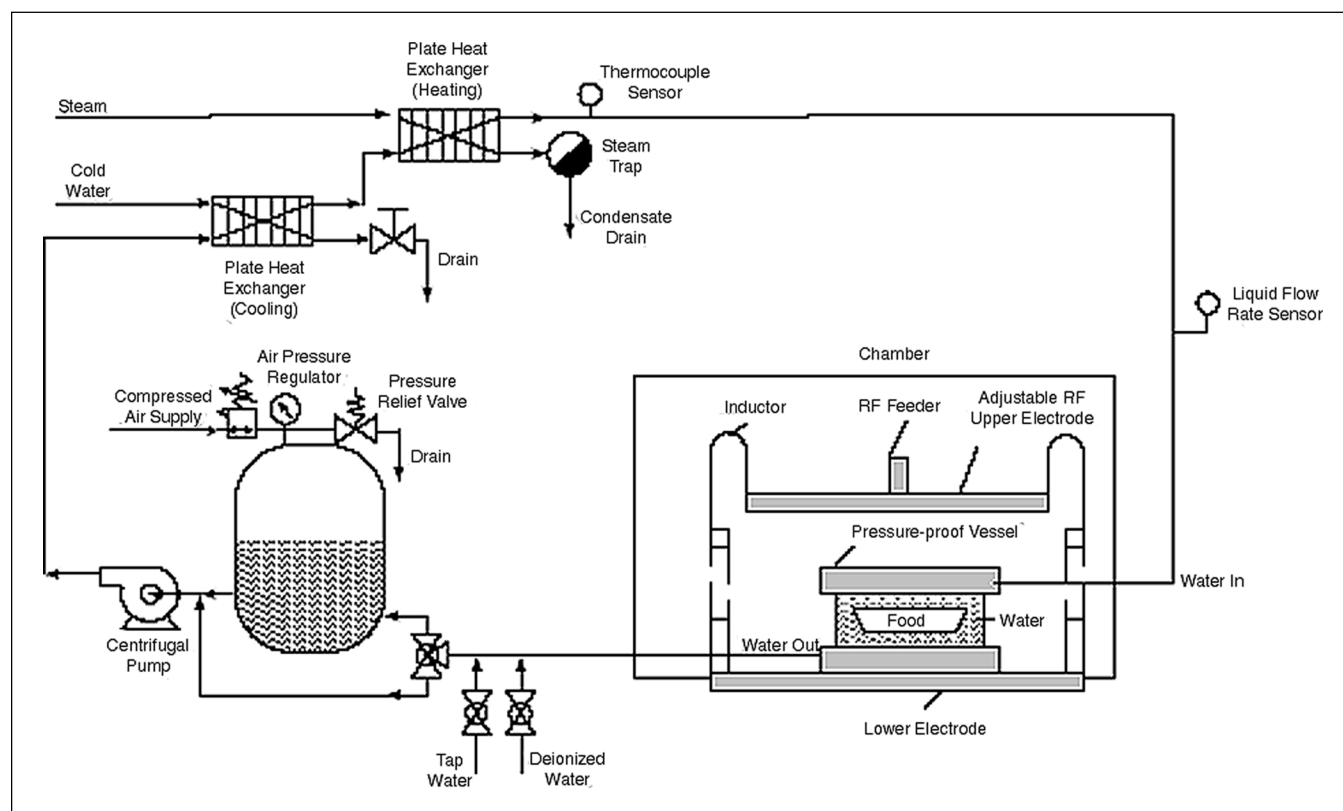


Figure 2—Simplified schematic diagram of the Washington State Univ. Radio frequency sterilization unit with a water-circulating system

Table 1—D-values of *Clostridium sporogenes* at different temperatures in phosphate buffer and mashed potato

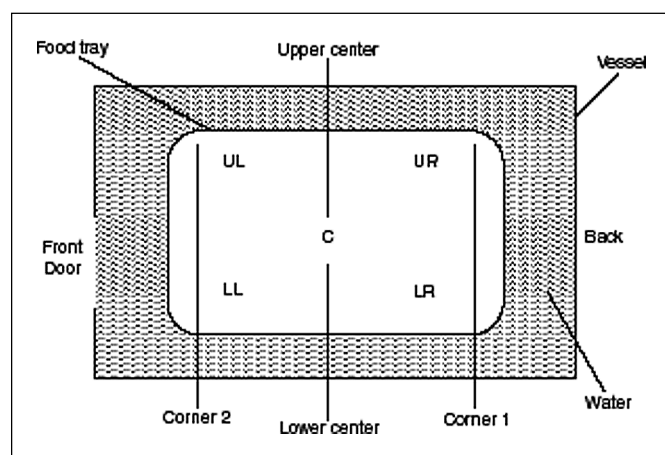
	D-value (min) \pm SD ^a			
	115.6 °C	118.3 °C	121.1 °C	z-value (°C)
Phosphate buffer ^b	1.83 \pm 0.22	1.10 \pm 0.03	0.62 \pm 0.03	12 ($R^2 = 0.99$)
Mashed potatoes ^c	2.16 \pm 0.05	1.09 \pm 0.01	0.61 \pm 0.05	10 ($R^2 = 0.88$)

^aSD = standard deviation^bPhosphate buffer, M/15 with pH 7.0^cMashed potatoes contained 7.8% butter, 24% milk, 53.4% water with pH 6.3

characteristics such as pH and composition (carbohydrate, fat, salt, and moisture content), the heat resistance of a microorganism would vary when suspended in different foods (Banwart 1989). *C. sporogenes* are most resistant to heat at their optimum growth pH of 7.0 (Hersom and Hulland 1980). On the basis of our results, D-values in mashed potatoes at 118.3 °C and 121.1 °C were 0.01 min apart from those in phosphate buffer, although the pH of mashed potatoes (pH 6.3) was lower than that of phosphate buffer (pH 7.0). At high temperatures with short heating times, the effect of pH is not as obvious as in longer heating times at lower temperatures (Ocio and others 1994). The water activity of freshly prepared mashed potatoes was 0.998 ± 0.003 , which is close to that of phosphate buffer. D_{121} of *C. sporogenes* in low-acid foods normally is in the range of 0.1 to 1.5 min with a z-value of 7.8 °C to 10 °C (Stumbo 1973). Z-values of *C. sporogenes* in phosphate and mashed potatoes were 12 °C and 10 °C, respectively. Our results in general agreed with these literature values. For example, Ocio and others (1994) determined the D-values of PA3679 in mushroom extracts with different pH values using the capillary tube method. The D-values at 121 °C are 0.51 and 0.67 min at pH 5.34 and 6.70, respectively. The z-value of PA3679 in M/15 phosphate buffer at pH 7.0 and mushroom extract with pH 5.34 were 10.26 °C and 9.79 °C, respectively.

RF heating of foods in polymeric trays with aluminum foil lid

Figure 5 illustrates typical temperature distribution and histories at 4 different positions in the mashed potatoes during a RF sterilization process. It took 25 min for the least heated part of the mashed

**Figure 3—Positions of fiber-optic probes in mashed potatoes inside the trays. The probes were secured half height from the bottom of the tray****Table 2—Inoculated pack study results of radio frequency heated mashed potatoes inoculated with *Clostridium sporogenes***

Process level	F ₀ (min) ^a	Designed sterilization value (SV) ^b	Log reduction per tray	Enrichment result ^c
Under-target process	2.4	4.0	3.10 \pm 0.08	Positive
Target process	4.3	7.0	>4.10	Negative
Over-target process	7.3	12.0	>4.10	Negative

^aF₀, SV \times D_{121.1}^bSV, Log₁₀ reduction for *C. sporogenes*^cEnrichment was done by 1:1 (wt/wt) of Fluid Thioglycollate Medium at 37 °C for 48 h.

potatoes to reach the desired temperature of about 121 °C. In a conventional retort process, it requires about 90 min for the least heated part of the product to reach a similar temperature because of slow conductive heat transfer in the product (Wang and others 2003).

It is clear from this figure that RF energy penetrated well through the aluminum foil lid of the tray between the metal plates of the pressurized vessel in the applicators (Figure 2). This is one of the important differences between RF and microwave heating. In microwave heating, a metal film shields electromagnetic wave. It was discussed in our earlier studies (Wang and others 2003) that the aluminum foil lid on top of the tray and parallel to the 2 electrodes did not block the RF energy from coupling in the food. The polarization of positive and negative charges in the metal film formed 2 pairs of electrodes: the 1st between the top plate of the vessel and the aluminum foil lid; the 2nd between the aluminum foil lid and the lower electrode, which is the bottom of the vessel. The food was heated between the 2nd pair of electrodes.

Inoculated pack studies

The results from the inoculated pack studies are summarized in Table 2. There was no growth of *C. sporogenes* observed from direct plating above the detection limit (150 CFU/tray) in the target and over-target processes. This corresponds to more than a 4.1 log reduction in PA3679. The log reduction was determined to be 3.10D for the under-target process, slightly less than the calculated sterilization value (4D). The test results from the enrichment check (with streak-plating of the processed sample) for the under-target

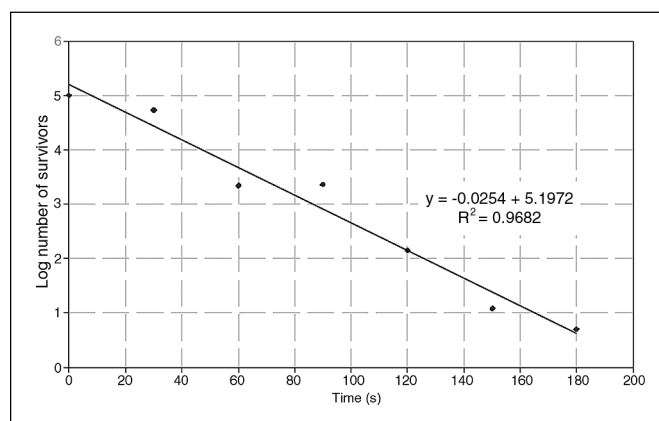
**Figure 4—A typical survivor curve of *Clostridium sporogenes* in phosphate buffer at 121.1 °C**

Table 3—Sterilization value (F_0) and cook value (C_{100}) of radio frequency heated mashed potatoes from 3 levels of processing

Location	Experiment nr.	Under-target process (4D)			Target process (7D)			Over-target process (12D)		
		1	2	3	1	2	3	1	2	3
Left (Corner 1)	F_0 (min)	2.8	2.8	2.2	4.0	4.9	18.4	6.7	8.1	8.1
	C_{100} (min)	45	48	53	60	56	90	75	64	69
Right (Corner 2)	F_0 (min)	4.3	20.2	10.0	9.6	10.1	23.9	7.1	43.2	16.6
	C_{100} (min)	50	88	81	76	69	111	67	120	82
Upper center	F_0 (min)	8.0	10.7	16.6	20.5	18.0	4.1	13.1	21.3	12.9
	C_{100} (min)	54	79	82	105	82	54	85	83	80
Lower center	F_0 (min)	4.3	15.4	59.8	27.3	6.0	8.0	27.0	14.0	9.4
	C_{100} (min)	74	98	155	117	59	78	121	96	84
Minimum log reduction for PA3679 ^a		4.54	4.56	3.67	6.49	8.07	6.77	10.93	13.31	13.31

^aMinimum log reduction; the minimum sterilization value among 4 locations = $\frac{F_0}{D_{21}}$

process were also positive (detecting survivors). For the target and over-target processes, however, no growth of *C. sporogenes* was detected in the enrichment tests. The microbial challenge studies showed that the RF process indeed delivered the desired lethality. This indicates that RF heating can be used to produce safe shelf-stable foods. Compared with microwave energy, theoretically, RF energy provides relatively more uniform heating over the product geometry because of deeper wave penetration into the product (Wang and others 2003).

Uniformity of sterilization and cook values in polymeric tray

Sterilization values (F_0) and cook values (C_{100}) of RF heated mashed potatoes of under-target (4D), target (7D), and over-target processes (12D) were summarized in Table 3. Theoretically, foods were heated relatively uniformly by RF energy because of its deep penetration depths and simple uniform field patterns (Zhao and others 2000; Wang and others 2003). In the heating stage, the greatest temperature difference among 4 locations at 121 °C was less than 5 °C (Figure 5). However, during the cooling stage, part of the mashed potatoes at the upper center was cooled faster than the rest because of the greater heat transfer rate of the aluminum lid foil. The nonuniform flow rate of the cooling water caused the differences in cooling rate of the 2 corners. Therefore, corner 2 cooled faster than corner 1. In this study, the values of F_0 indicated that the left part of the tray was the cold spot in almost all cases, which was

different from what Wang and others (2003) reported. They observed that the upper center of the tray was the least heated part. In this study, mashed potatoes at upper center were found to be the least heated part in only 1 of the target processes (7D). The discrepancy might have been caused by (1) the shape of the trays: we used flat-bottom trays in this study compared with the dome-shaped bottom tray used in Wang’s studies. In that study, the thickness of the food in the center of the dome-shaped bottom tray was slightly less than in the rest of the tray. The electric conductivity of the immersion water (approximately 110 μ S/cm) was less than the mashed potatoes (approximately 3000 μ S/cm) in the tray. As a result, the electric field intensity was less in the thinner part of the food than that in the thicker part, resulting in less RF heating in the center of the tray. Circulating water in cooling stage removed heat from the upper part of the tray faster than the rest parts of the tray resulting in the cold spot as observed by Wang and others (2003). (2) Asymmetric electrode: because of engineering limitation, the distance from the end of the electrodes to a tray at front door side (left side of the tray in Figure 3) was slightly greater than that of the back side (right side of the tray in Figure 3). This resulted in the slightly greater electromagnetic field concentration at the right than the left of the tray. Therefore, the left part of the tray received less heat.

The cook values (C_{100}) were calculated based on recorded temperature profiles by Eq. 4 (Lund 1986).

$$C_{100} = \int_0^t 10^{\frac{[T-100]}{z}} dt$$

(4)

The z-values are in the range of 25 °C to 47 °C according to the quality parameters, that is, texture and sensory attributes. The value of 33 °C is generally used to calculate the over all quality loss (Lund 1986).

Sterilization values (F_0) in the over-target process (12D) were in the range from 6 min to 43 min (Table 3) resulted in cook values in the range of 60 to 120 min, which were in the same range of RF heated macaroni and cheese reported by Wang and others (2003). The cook values of retort heated macaroni and cheese were in the range of 154 to 277 min when the product received sterilization value in the range of 7 to 33 min. The cook values of RF heated products were half of that of the conventional retort process while delivering approximately the same sterilization value (F_0) (Wang and others 2003). RF energy can be used for HTST sterilization to produce safe food while maintaining food quality.

Use of symmetrical electrodes to minimize temperature nonuniformity and validation of RF sterilization with different type of

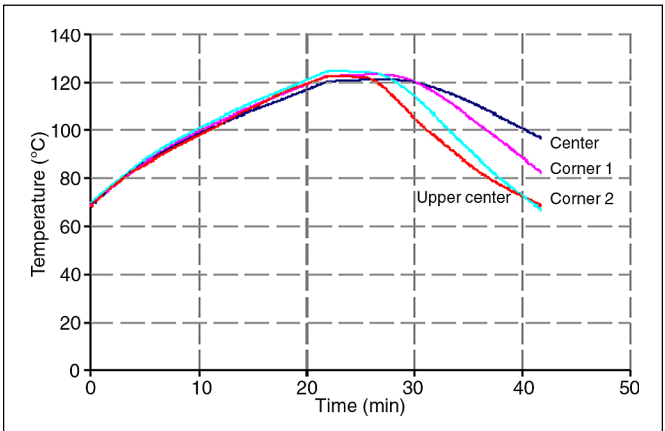


Figure 5—Time-temperature profile for mashed potatoes thermally processed by radio frequency heating

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foods would provide needed data for future development of the RF heating process.

Conclusions

The results demonstrated that aluminum TDT tubes can be used to determine thermal resistance at high temperatures. The results of inoculated pack studies suggest that microbial destruction of packaged foods in a 27.12 MHz RF sterilization system matches with the pre-designed degrees of sterilization (F_0) based on temperature measurement. Thermal processes based on RF energy have the potential as an alternative to conventional retort heating in the sterilization of packaged foods for civilian and military uses, especially for heat-sensitive and high value foods (for example, eggs, seafoods, al dente pasta).

Acknowledgments

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