Feasibility of Utilizing Bioindicators for Testing Microbial Inactivation in Sweetpotato Purees Processed with a Continuous-Flow Microwave System

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ABSTRACT: Continuous-flow microwave heating has potential in aseptic processing of various food products, including purees from sweetpotatoes and other vegetables. Establishing the feasibility of a new processing technology for achieving commercial sterility requires evaluating microbial inactivation. This study aimed to assess the feasibility of using commercially available plastic pouches of bioindicators containing spores of *Geobacillius stearothermophilus* ATCC 7953 and *Bacillus subtilis* ATCC 35021 for evaluating the degree of microbial inactivation achieved in vegetable purees processed in a continuous-flow microwave heating unit. Sweetpotato puree seeded with the bioindicators was subjected to 3 levels of processing based on the fastest particles: undertarget process (F_0 approximately 0.65), target process (F_0 approximately 2.8), and overtarget process (F_0 approximately 10.10). After initial experiments, we found it was necessary to engineer a setup with 2 removable tubes connected to the continuous-flow microwave system to facilitate the injection of indicators into the unit without interrupting the puree flow. Using this approach, 60% of the indicators injected into the system could be recovered postprocess. Spore survival after processing, as evaluated by use of growth indicator dyes and standard plating methods, verified inactivation of the spores in sweetpotato puree. The log reduction results for *B. subtilis* were equivalent to the predesigned degrees of sterilization (F_0). This study presents the first report suggesting that bioindicators such as the flexible, food-grade plastic pouches can be used for microbial validation of commercial sterilization in aseptic processing of foods using a continuous-flow microwave system.

Keywords: aseptic, bioindicators, continuous-flow, microwave, purees

Introduction

Continuous-flow microwave processing has potential in thermal processing of foods. A process for rapid sterilization and aseptic packaging of viscous products using a continuous-flow microwave system operating at 915 MHz has been successfully developed in the Dept. of Food Science at North Carolina State Univ. (Raleigh, N.C., U.S.A.). It has been demonstrated previously that this technology has the ability to produce a high-quality, shelf-stable sweetpotato puree with no detectable microbial growth based on 90 d of storage at ambient temperature (Coronel and others 2005). However, further research is needed to validate the process for use in commercial production with respect to microbial inactivation. For validation of commercial sterilization, it is necessary to quantitatively demonstrate adequate inactivation of *Clostridium botulinum* or a relevant surrogate spore former.

Studies have shown that microwave heating inactivates vegetative bacterial cells and spores in a manner indistinguishable from that of conventional heating (Welt and Tong 1994). Thus, the sporicidal activities of microwave energy are simply a function of thermal heat converted from the microwave energy (Jong and others 1987). Indeed, an equivalent degree of inactivation of the spores of *Bacillus subtilis* and other *Bacillus* species has been demonstrated using microwave as compared to conventional thermal processing (Wang and others 2003; Celandroni and others 2004).

Nonetheless, there are important considerations unique to microwave processing. For example, dielectric properties determine the extent of heating of a material subjected to electromagnetic waves. Dielectric properties consist of the dielectric constant (ϵ') and dielectric loss factor (ϵ''). Dielectric constant is a measure of the ability of a material to store electromagnetic energy whereas dielectric loss factor is a measure of the ability of a material to convert electromagnetic energy to heat (Metaxas and Meredith 1983). Loss tangent (tan δ), a parameter used to describe how well a product absorbs microwave energy, is the ratio of dielectric loss factor (ϵ') to the dielectric constant (ϵ''). A product with a higher loss tangent is heated faster under the microwave field as compared to a product with a lower loss tangent (Nelson and Datta 2001). Assuming no magnetic losses in the materials, the microwave power absorbed per unit volume (Q) in a material is given by the following equation (Metaxas and Meredith 1983):

$$Q = 2\pi f \varepsilon_0 \varepsilon'' E_{\text{rms}}^2 \quad (1)$$

where f is the frequency of the microwave in Hz, ϵ_0 is the permittivity of free space (8.86×10^{-12} F/m), ϵ'' is the dielectric loss factor, and E_{rms} is the root mean square value of the electric field.

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The exposure to electromagnetic energy to achieve a desired temperature is determined by the dielectric properties of the food, which significantly changes as the temperature of the food material changes (USFDA 2000). For microwave processing, the lethal kill for sterilization efficiency is defined as the time-and-temperature history at the coldest location of the product being processed. Within a microwave system, critical process factors affecting lethality include the chemical and physical properties of the food material (ionic content, moisture, density, and specific heat), temperature, microwave frequency, and the microwave applicator design.

Microbial validation has been widely used for various thermal processes, including retorting, high-temperature short-time (HTST) processes, and microwave heating (Pflug and others 1980; Smith and Kopelman 1982; Marcy 1997; Guan and others 2003). There are in fact many types of bioindicators and methods that can be used in this regard, the appropriateness of which depends on the process and the target pathogen. For example, Smith and Kopelman (1982) used the inoculated pack method with *B. subtilis*, *B. stearothermophilus*, and *C. sporogenes* spores as indicators to validate commercial sterility of sweetpotato puree processed by steam flash sterilization and aseptic filling. The inoculated pack technique was also used by Guan and others (2003) for validation of a pilot-scale 915 MHz microwave-circulated water combination heating system. However, the inoculated pack technique would not be suitable for the large volume of the material required in continuous-flow processes. Pflug and others (1980) have developed bioindicators made of plastic to validate sterilization processes for canned products. Several others have produced encapsulated spores for process validation of canned products (Pflug and Smith 1977; Jones and others 1980). A recent and popular trend has involved the immobilization of viable bacterial spores (usually *B. subtilis* or *B. stearothermophilus*) in calcium alginate gel beads, which are then seeded into the product preprocess and recovered postprocess. This approach has been used to evaluate ultra-high temperature (UHT) processing (Dallyn and others 1977) and microwave heating (Serp and others 2002). In the latter case, the immobilized spores were placed throughout the sterilization chamber for reliable multipoint mapping of the thermal treatment.

Recently, commercial bioindicators (SGM Biotech Inc., Bozeman, Mont., U.S.A.), which contain high densities of purified bacterial spores encased in a thermoplastic polymer polypropylene pouch, have been released. The packaging material allows the indicators to tolerate high temperature processes. The bioindicators contain growth media (pH = 6.85) with a pH indicator that changes color to signal microbial growth, providing a single endpoint detection. Alternatively, the liquid spore suspensions can be recovered from the pouches and plated for enumeration using standard cultural procedures. Successful utilization of the bioindicators to monitor an array of thermal processes used in the medical and industrial sectors has been reported (Gillis and McCauley 2006). Although they have the potential for use in thermal process validation for foods, such studies have yet to be done. Accordingly, the objective of this study was to assess the feasibility of using the plastic self-contained bioindicators to evaluate spore inactivation using a continuous-flow microwave system as applied to processing of sweetpotato puree.

Materials and Methods

Sweetpotato puree

Sweetpotato puree was purchased from the Bright Harvest Sweet Potato Co. (Clarksville, Alaska, U.S.A.). The puree was made from the orange-fleshed Beauregard cultivar and had approximately 82% moisture content on a wet weight basis. The puree was packed in 20-lb bag-in-box containers, shipped frozen, and stored at −20 °C until use.

Bioindicators

The flexible, food-grade plastic pouches of bioindicators were manufactured by SGM Biotech Inc. and contained 0.1 mL of spore suspension of *Geobacillius stearothermophilus* ATCC 7953 or *B. subtilis* ATCC 35021 in sealed polypropylene (PP) tubing. These totally self-contained indicator pouches were 15 mm in length and 2 mm in thickness (Figure 1). The spore suspension had a pH of 6.85 and contained spores at populations sufficiently high enough to determine their log reduction for undertarget (126 °C), target (132 °C) and overtarget (138 °C) processing temperatures. Specifically, the *B. subtilis*

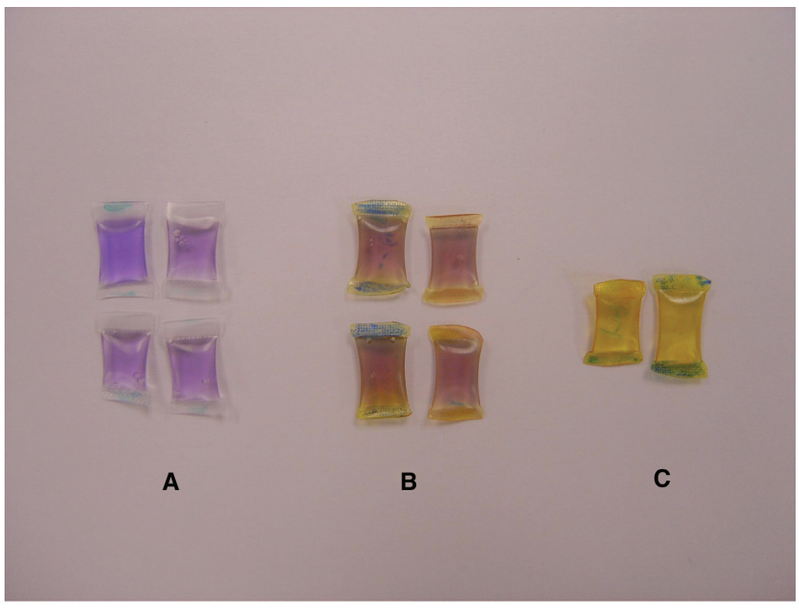

Figure 1 — Bioindicators containing spores of the tested microorganisms (A = unprocessed; B = processed with negative result; C = processed with positive result)

indicator contained approximately 4.85×10^6 viable spores and the *G. stearothermophilus* indicator contained approximately 1.8×10^6 viable spores per pouch. The indicator pouches were stored under refrigeration (4 °C) until used to prevent germination and outgrowth of the spores.

D and *Z* value determination

The determination of *D* and *Z* values was done by SGM Biotech Inc. and reported upon receipt of the indicators. The fraction negative (FN) and most-probable-number (MPN) analyses were used to calculate the *D* values based on the Stumbo–Murphy–Cochran (SMC) method (Pflug 2003). Briefly, 10 replicates of bioindicator units were subjected to lethal stress sufficient to inactivate all spores in some but not all replicate units. In the FN analysis, the units were subjected to different stress levels (heating times), all other factors held constant. Determining the fraction of replicate biological units that were negative assisted in estimating critical parameters of the surviving microbial population. Each stress level used 10 replicate pouches, and the various heating times differed from one another by a constant interval of time. The exposures were done in a steam bioindicator evaluator resistometer (BIER) vessel (Joslyn Corp., Macedon, N.Y., U.S.A.) at the specific temperatures. After completion of each exposure, test units were incubated for 7 d at 55 °C for *G. stearothermophilus* and 35 °C for *B. subtilis*. After incubation, spore units were evaluated for colorimetric endpoint (color change = positive for growth, no color change = negative for growth), and the fraction of replicate biological units negative and positive for growth was used to calculate the critical parameters of the surviving microbial population. During the incubation period, the spore test units were monitored for growth. After incubation, the suspensions were recovered from the pouches and evaluated to determine the MPN of survivors. Based on MPN data, the SMC procedure was used to calculate the *D* values at 115.6, 118, 121.1, 124, and 126.7 °C for *G. stearothermophilus* and at 110, 115.6, and 121.1 °C for *B. subtilis*. The data collected during the determination of the *D* values were used to calculate the *Z* values for both spores using log linear regression (Assn. for the Advancement of Medical Instrumentation 2006). For *B. subtilis* ATCC 7953, the *D* values at 121.1, 126, 132, and 138 °C were 0.4, 0.12, 0.029, and 0.006 min, and the calculated *Z* value was 9.5 °C. The *D* values for *G. stearothermophilus* ATCC 35021 at these temperatures were 2.0, 0.51, 0.097, and 0.018 min, respectively, and the *Z* value was 8.3 °C.

Measurement of dielectric properties

An open-ended coaxial probe (HP 85070B, Agilent Technologies, Palo Alto, Calif., U.S.A.) equipped with an automated network analyzer (HP 8753C, Agilent Technologies) was used to determine the dielectric properties of the spore suspension and sweetpotato puree. The calibration of the system was performed using a short block, air, and water. The spore suspension was heated in an oil bath (Model RTE111, Neslab Instruments Inc., Newington, N.H., U.S.A.) at a range of temperatures (20, 75, 90, 100, 110, 120, 125, and 130 °C) with 915 MHz frequency. The sweetpotato puree was heated in an oil bath (Model RTE111) from 15 to 145 °C in 5 °C intervals with 915 MHz frequency. The dielectric constant and dielectric loss factor were calculated using the software provided with the probe based on the phase shift and magnitude of the reflected signal. Three repetitive measurements were performed for each duplicate sample.

Measurement of viscosity

Viscosity of the puree was determined using a controlled stress rheometer (Stress Tech Rheological Instruments AB, Lund, Sweden) equipped with a pressurized sealed cell and a cuvette bob and cup. Compressed air was applied to the sealed cell at 29 psi to prevent boiling and excessive moisture loss of the sweetpotato puree. All measurements were performed in duplicate. Samples were presheared for 30 s at 50 s^{-1} and allowed to equilibrate for 25 s before testing began. Shear rate sweeps were performed on the samples at 70 and 130 °C, with shear rate ramped up and down from 1 to 250 s^{-1}.

Sixty-kilowatt continuous-flow microwave heating unit

A pilot scale 60 kW continuous-flow microwave heating unit (Industrial Microwave Systems, Research Triangle Park, N.C., U.S.A.) operating at 915 MHz was used in this study. The temperatures were measured with thermocouples positioned at the inlet of the system, the inlet and exit of each applicator, and the holding tube, as shown in Figure 2. The temperatures were recorded using a Datalogging system (HP 3497A, Agilent Technologies). The power supplied to the microwave system was adjusted to control the experimental temperatures of 126, 132, and 138 °C at the center-point of the holding tube exit. The system was preheated by pumping hot water at 130 °C and recirculating it for approximately 30 min. The sweetpotato puree was then loaded into the system and the bioindicators were injected when the temperature at the center of the holding tube exit reached the experimental temperature. The bioindicators were mixed with the puree, filled in a tube, and released into the puree stream through the hopper or the injection tubes A/B as illustrated in Figure 2. The puree was heated to each processing temperature, with a residence time of 25 s in the holding tube, followed by rapid cooling to 70 to 80 °C in a tubular heat exchanger. Bioindicators were collected at the end of the cooling tubes, placed in ice slurry, and immediately taken to the laboratory for microbiological analysis.

Microbiological tests

Bioindicators (3 to 10 units) were placed in sterile Whirl-Pak bags (Nasco, Fort Atkinson, Wis., U.S.A.) and immediately incubated for 48 h at 55 °C for *G. stearothermophilus* and 35 °C for *B. subtilis*, after which they were evaluated colorimetrically as described above. After incubation of the indicators, an unchanged purple color indicated that all spores were inactivated. For bioindicators containing surviving spores, acid production associated with bacterial growth caused a change in the pH, resulting in a color change from purple to yellow.

A 2nd set of indicators was used for enumeration. Specifically, bioindicators obtained postprocess were pooled (2 sets of 5 each, that is, duplicate evaluations) for each processing run. Indicators were placed in a 10% hypochlorite solution for 1 min to decontaminate the pouch surface, and then removed and air-dried for 30 s. Dissecting scissors sterilized with 70% ethanol and flamed were used to cut open the indicator pouches. A P200 micropipette was used to remove 75 μL of the indicator spore suspension from each of the 5 pouches, and these aliquots were pooled in a 1.5-mL Eppendorf microcentrifuge tube to achieve a 375 μL volume of spore suspension for a pooled sample set. The suspensions were spread plated on brain heart infusion (BHI) agar (Becton Dickinson Co., Franklin Lakes, N.J., U.S.A.) undiluted and after 10-fold serial dilution up to 10^{-5} using fluid D (Millipore, Billerica, Mass., U.S.A.) as the diluent. Plates were incubated for 48 h at 55 °C for *G. stearothermophilus* and 35 °C for *B. subtilis* prior to manual counting. Untreated indicators were processed for colorimetric endpoint detection of growth and by plating to establish the baseline microbial population prior to thermal treatment.

Thermal death time and *D* value calculations

The thermal death time or *F* value was determined to evaluate the efficiency of the processing method. Using Equation 2, the recorded temperature profile was utilized to calculate the F_0 to establish the sterilization value, where *T* is temperature (°C) and *t* is the processing time (min). The sterilization value is used to ensure safety for low-acid (pH ≥ 4.6) foods processed to commercial sterility. Sweetpotato puree has a pH of 5.8 to 6.0. The reference temperature used was 121.1 °C with a *Z* value of 10 °C for *C. botulinum* (Heldman and Hartel 1997; Eszes and Rajko 2004).

$$F_0 = \int_0^t 10^{\frac{(T(t)-T_0)}{z}} dt \quad (2)$$

Using a reference temperature (T_2) and the thermal resistance of the specific microorganism (*Z*), the D-value at another processing temperature (T_1) can be estimated using Eq. (3).

$$D_1 = D_2 \times 10^{\frac{(T_2-T_1)}{z}} \quad (3)$$

Results and Discussion

Dielectric properties and apparent viscosity of the processing materials

The dielectric properties of the spore suspension and sweetpotato puree were measured at 915 MHz in a temperature range from 20 to 130 °C (Figure 3). Dielectric constant and dielectric loss factor were similar to the values reported by Fasina and others (2003) and Coronel and others (2005) for sweetpotato purees. Similar trends were reported by Guan and others (2004) for mashed potatoes and for other food materials by Nelson and Datta (2001). The dielectric constant for the spore suspension and the puree had similar values. However, dielectric loss factor values for sweetpotato puree were higher than those of the spore suspension, which is desirable because we did not want the indicators to receive a more extensive heat treatment than that applied to the carrier fluid during microwave processing. The apparent viscosity was measured at 70 and 130 °C (Figure 4). As expected, the puree viscosity decreased as temperature increased, and the results are in agreement with those of Grabowski and others (2006). These changes in viscosity would be among the factors affecting the dielectric properties of the sweetpotato puree during microwave heating.

Injection of the bioindicators into the microwave system

In an initial experiment, 40 bioindicator units were added to the puree (60 to 70 °C) and loaded into a tube (1.7 cm in diameter, 110 cm in length). The tube was then dipped to the base of the hopper to release the bioindicators into the sweetpotato puree stream, which was being pumped at a rate of 3.8 L/min. The intent was to collect the indicators at the end of a cooling tube with a side-entry strainer (W25E15SCC420B, Waukesha Cherry Burrell, Delavan, Wis., U.S.A.). Unfortunately, very few of the initial indicators injected into the process (20% from 3 replicated runs) were collected at the end of the runs. Several problems were noted: (1) indicator pouches tended to become trapped at the bottom of the hopper and were never processed; (2) pouches tended to cluster, causing blockage of the system; and (3) pouches adhered to the side of the pipe walls and blades of the static mixers.

An improved method was designed for injecting the bioindicators into the system. This included the use of 2 removable tubes connected to the continuous-flow microwave system, which allowed injection of the units into the process stream without interrupting the puree flow (Figure 2). Each tube was connected to the system with inlet and outlet valves, which were tightened using sanitary

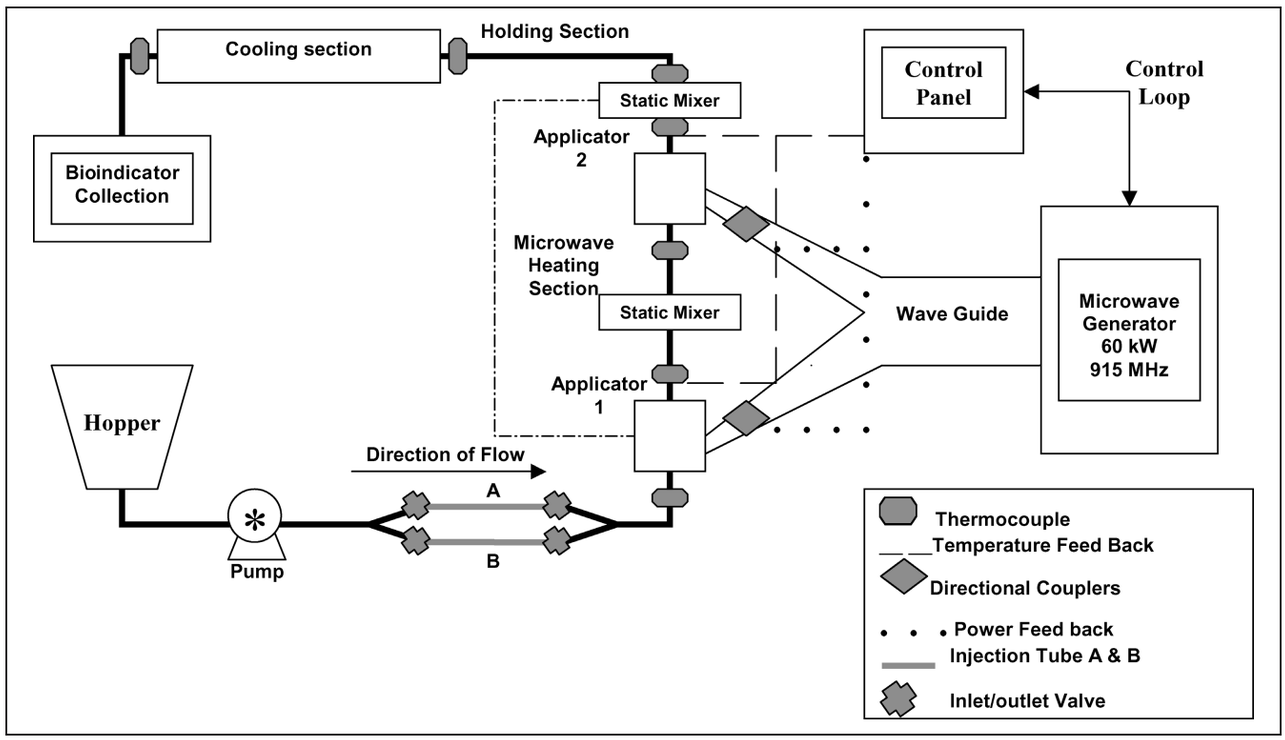

Figure 2—Schematic diagram of the processing system (adapted from Coronel and others 2005, with modifications)

clamps. The injection tube was 91 cm in length with an internal diameter of 2.3 cm. The larger diameter facilitated the loading of the puree–bioindicator mixture. In an effort to avoid cold slug, the tube was held in a warm bath to maintain the temperature of the puree at between 60 and 70 °C. In addition, the puree flow rate was increased from 3.8 to 5.7 L/min in order to push the indicators more effectively through the static mixers. The increased flow rate also assisted in overcoming the increased viscosity of sweetpotato puree in the cooling tube, which occurred when the puree temperature was dropped from 130 to 70 °C (Figure 4); this also reduced the number of bioindicators trapped during the cooling phase of the process. It was also necessary to decrease the total number of indicators used per replicate run from 40 to 30, which provided additional distance between individual units and reduced their tendency to associate with one another and clump. This also reduced warping of the indicators, which occurred as processing temperatures approached the 160°C melting point of polypropylene (Kissel and others 2003). Taken together, these improvements allowed us to retrieve about 60% of the input indicator units (Table 1 and 2). Most of the remaining bioindicators were distributed along the static mixers during the tests and they did not appear to hinder the movement of the pouches through the system.

The temperature of the sweetpotato puree was measured with thermocouples positioned at the inlet of the system, the inlet and exit of each applicator, and the inlet and exit holding tube (Figure 2). Figure 5A and 5B show time–temperature profiles recorded during the microwave processing of sweetpotato puree containing bioindicators for the target temperature of 132 °C. The inlet temperature of the puree fluctuated between 60 and 70 °C (Figure 5A). The temperature was approximately 100 °C at the exit of the first applicator, and was the target processing temperature after the exit of the second applicator. The outlet temperature of the puree after the cooling tube was cooled to between 70 and 80 °C is detailed in Figure 5B. For each injection, 2.3 L of sweetpotato puree carrying bioindicators passed through the 2 microwave applicators and entered a 2.4 m long holding tube with an internal diameter of 22.9 mm and a

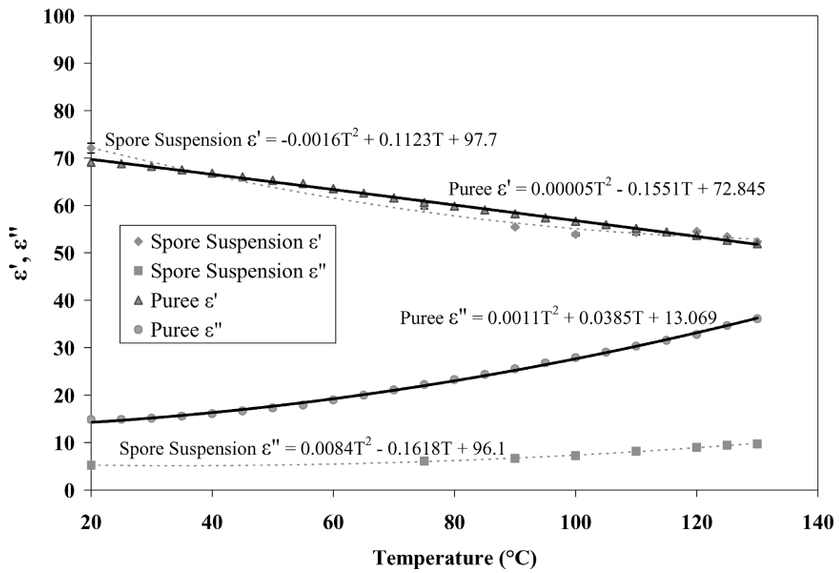

Figure 3 — Dielectric properties of the bioindicators spore suspension and sweetpotato puree at 915 MHz

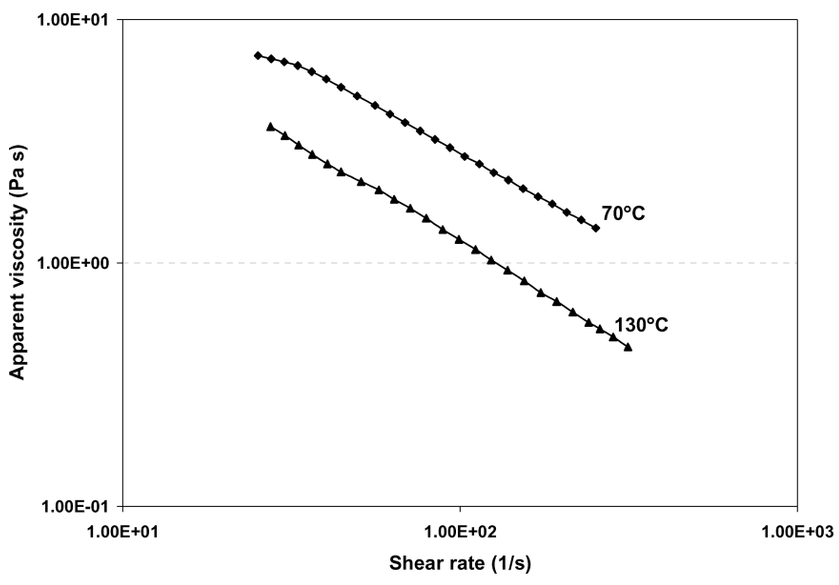

Figure 4 — Apparent viscosity of Beauregard sweetpotato puree

residence time estimated at 25 s. The radial temperature distributions for processing temperatures of 132 and 138 °C were narrow, which is in accordance with the temperature profile reported by Coronel and others (2005). The average hold time within the holding tube for the volumetric flow rate was 25 s, and that of the fastest particles (center of the tube) was 12.5 s. The F_0 value was calculated based on the hold time required to achieve the specific log reduction in the population at the set of target processing temperatures used in this study. The fastest fluid elements (center, about 2 times of the mass mean residence time) at the undertarget (126 °C), target (132 °C), and overtarget (138 °C) processing temperatures received a thermal treatment equivalent to $F_o = 0.65$, 2.80, and 10.10 min, respectively (Table 1 and 2).

As described in the Materials and Methods section, the *D* values for *B. subtilis* (0.4 min) and *G. stearothermophilus* (2.0 min) were much higher than those of *C. botulinum*, which generally has been reported to be 0.25 min at 121.1 °C (Holdsworth 1997). Thermal process guidelines stipulate the need to achieve a 12 D drop in *C. botulinum* for commercially available sterile low-acid foods, a process usually equivalent to a full exposure of 121.1 °C for approximately 3 min (Pflug and others 1990; Holdsworth 1997). In this study, at the target process temperature of 132 °C, a 8.55 D inactivation of *B. subtilis* and 2.50 D inactivation of *G. stearothermophilus* were equivalent to a 12 D inactivation of *C. botulinum*.

Microbiological data

Microbiological results are detailed in Table 1 and 2. The *B. subtilis* indicators receiving undertarget, target, and overtarget processes demonstrated log reductions exceeding 4.69 as evaluated using enumerative assays (Table 1). Furthermore, the *B. subtilis* units

Table 1—Growth indicators and count reduction of bioindicators containing *Bacillus subtilis*

Initial spore count	Process level	Degree of sterilization		Reps	Log reduction value[c]	Indicators	
		F_0[a]	F_0[b]			Positive	Negative
4.85×10^6	Undertarget process	1.29	0.65	1a	>4.69	4	14
				1b	>4.69		
4.85×10^6		1.29	0.65	2a	>4.69	2	13
				2b	>4.69		
4.85×10^6		1.29	0.65	3a	>4.69	4	16
				3b	>4.69		
4.85×10^6	Target process	5.13	2.80	4a	>4.69	0	12
				4b	>4.69		
4.85×10^6		5.13	2.80	5a	>4.69	0	16
				5b	>4.69		
4.85×10^6		5.13	2.80	6a	>4.69	0	13
				6b	>4.69		
4.85×10^6	Overtarget process	20.41	10.10	7a	>4.69	0	9
				7b	>4.69		
4.85×10^6		20.41	10.10	8a	>4.69	0	10
				8b	>4.69		
4.85×10^6		20.41	10.10	9a	>4.69	0	10
				9b	>4.69		

[a] F_0, based on volumetric holding time (Unit: min).
[b] F_0, based on fastest particle/element (Unit: min).
[c] Log_{10} reduction for *B. subtilis* based on enumerative assay; inactivation was calculated on the basis of the difference between the log of the initial counts (4.85×10^6) and the log of the final counts. The limit of detection of the assay was 100 CFU/mL, so the absence of colonies after processing constituted a >4.69 log inactivation (i.e., $6.69 - 2.0 = >4.69$).

Table 2—Growth indicators and count reduction of bioindicators containing *Geobacillus stearothermophilus*

Initial spore count	Process level	Degree of sterilization		Reps	Log reduction value[c]	Indicators	
		F_0[a]	F_0[b]			Positive	Negative
1.8×10^6	Undertarget process	1.29	0.65	1a	1.34	14	0
				1b	>4.26		
1.8×10^6		1.29	0.65	2a	>4.26	11	3
				2b	1.57		
1.8×10^6		1.29	0.65	3a	1.68	4	5
				3b	2.99		
1.8×10^6	Target process	5.13	2.80	4a	>4.26	7	3
				4b	>4.26		
1.8×10^6		5.13	2.80	5a	>4.26	5	8
				5b	>4.26		
1.8×10^6		5.13	2.80	6a	>4.26	3	4
				6b	>4.26		
1.8×10^6	Overtarget process	20.41	10.10	7a	>4.26	0	9
				7b	>4.26		
1.8×10^6		20.41	10.10	8a	>4.26	0	1[d]
				8b	>4.26		
1.8×10^6		20.41	10.10	9a	>4.26	0	10
				9b	>4.26		

[a] F_0, based on volumetric holding time (min)
[b] F_0, based on fastest particle/element (min)
[c] Log_{10} reduction for *G. stearothermophilus* based on enumerative assay; inactivation was calculated on the basis of the difference between the log of the initial counts (1.8×10^6) and the log of the final counts. The limit of detection of the assay was 100 CFU/mL, so the absence of colonies after processing constituted a >4.26 log inactivation (i.e., $6.26 - 2.0 = >4.26$).
[d] 1 bioindicator was removed from the run because of surface adherence.

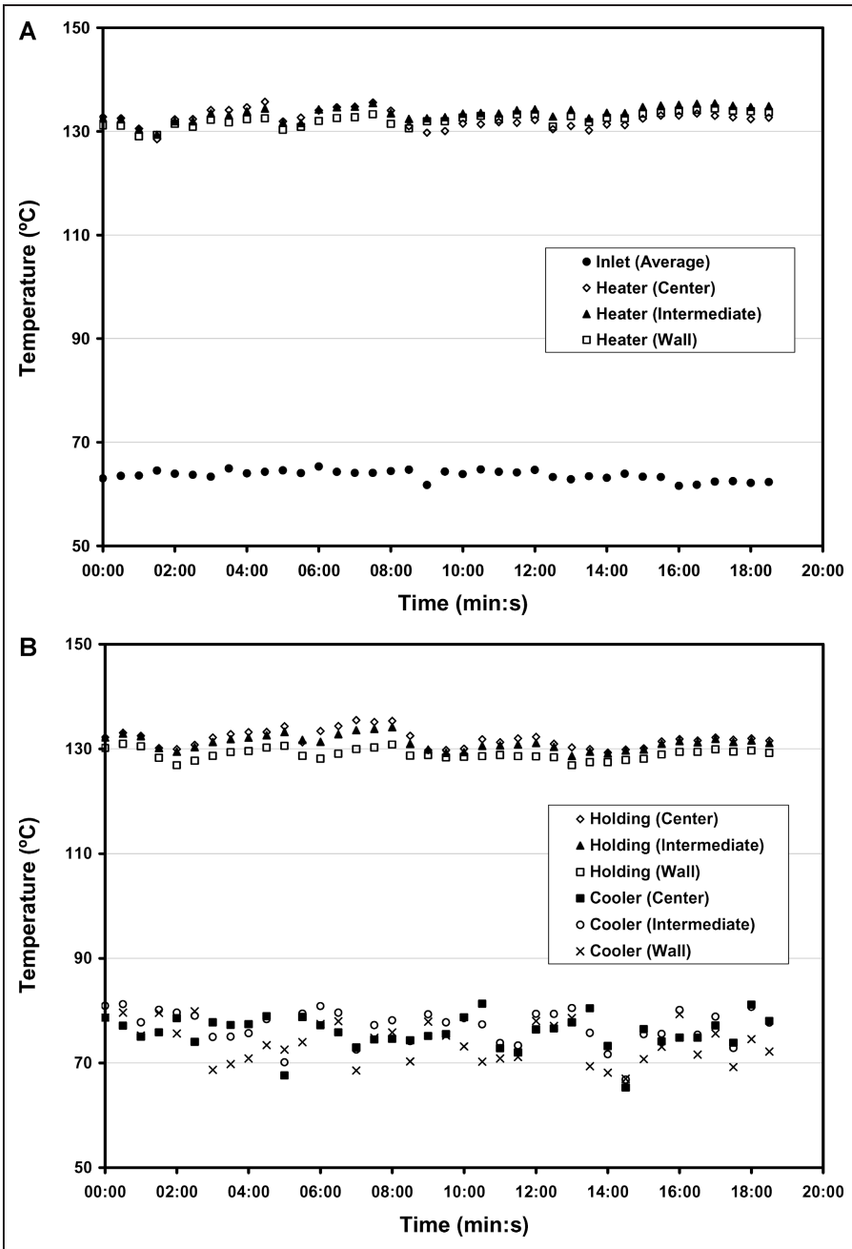

Figure 5 — Typical temperature–time history at the (A) inlet of the microwave heating section and exit of second microwave heating applicator and (B) exit of holding and cooling section

subjected to target and overtarget process temperatures showed no indication of spore survival in endpoint detection by colorimetric assays, confirming a log reduction > 4.69 and in agreement with the predicted F_0 value. However, the *B. subtilis* bioindicators subjected to the undertarget process demonstrated color changes in 10/53 pouches, suggesting that viable spores survived the 126 °C thermal treatment. These results were anticipated because the calculated average degree of sterilization for a 126 °C process was 10.5 D reduction for the fluid element and a 5.32 log reduction for the fastest particle using *C. botulinum* as the target.

For the undertarget process, the *G. stearothermophilus* indicators demonstrated log reductions ranging from 1.34 to >4.26 (Table 2), with positive color changes in 29/37 processed pouches (Table 2). Consistent with predictions, *G. steraothermophilus* spores, which are quite heat resistant, should have displayed approximately 1.3 to 2.5 log reductions when exposed to undertarget process conditions. The >4.26 $\log_{10}$ reduction of *G. stearothermophilus* in some pouches exposed to the undertarget process was not expected. It was possible that several indicator pouches adhered to the surface of the static mixers and the tubes throughout the system, and in so doing, received longer heat treatment. For target and overtarget processes, log reductions exceeding 4.26 were seen for the *G. stearothermophilus* indicators (Table 2), which agrees with the expected log reductions for these processes. The indicators processed at target displayed color changes in 50% (15/30) of the pouches (Table 2), suggesting that the color-based assessment of growth is more sensitive than the enumerative assay. This is logical because plating detection limits were 100 CFU/mL, while with the colorimetric endpoint, we could detect as few as 1 CFU per 0.1 mL of spore suspension per pouch. No color change was noted for the overtarget process indicators, confirming the destruction of all viable spores and consistent with the predicted sterilization (F_0) value.

Conclusions

This study demonstrates a potential of using polypropylene-packaged bioindicators to evaluate thermal inactivation efficacy as applied to a continuous-flow microwave process intended for viscous food materials. Engineering the system was achieved, and we were able to recover approximately 60% of the input indicators injected into the system. Viability of the spores in the pouches could be evaluated by an endpoint colorimetric method or by cultural enumeration, and the results obtained were consistent with predicted thermal inactivation kinetics. In fact, the log reduction results for the *B. subtilis* indicators were equivalent to the predesigned degrees of sterilization (F_0). Further refinements to the system are necessary to improve the postprocess recovery of the indicators and to facilitate monitoring their residence time within the holding tube. Overall, the use of bioindicators for verification of spore inactivation in continuous-flow microwave processing of sweetpotato puree is feasible. This approach may be useful in evaluating microbial inactivation in aseptically processed viscous food products.

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Nomenclature

D = thermal death time (min)
F_0 = sterilization value (min)
T = temperature (°C)
t = time (s)
V = volume (m^3)
z = thermal resistance of microorganisms (°C)
ΔT = change in temperature (°C)
ϵ' = dielectric constant
ϵ'' = dielectric loss factor
Q = microwave power absorbed per unit volume
ϵ_0 = permittivity of free space
f = frequency (Hz)
E_{rms} = root mean square value of the electric field

References

Assn. for the Advancement of Medical Instrumentation. 2006. Sterilization of healthcare products—biological indicators. Part1—general requirements. Arlington, Va.: Assn. for the Advancement of Medical Instrumentation p 53.
Celandroni F, Longo I, Tosoratti N, Giannessi F, Ghelardi E, Salvetti S, Baggiani A, Senesi S. 2004. Effect of microwave radiation on *Bacillus subtilis* spores. J Appl Microbiol 97(6):1220–7.
Coronel P, Truong V-D, Simunovic J, Sandeep KP, Cartwright GD. 2005. Aseptic processing of sweetpotato purees using a continuous flow microwave system. J Food Sci 70(9):E531–6.
Dallyn H, Falloon WC, Bean PG. 1977. Method for immobilization of bacterial spores in alginate gel. Lab Practice 26:773–5.
Eszes F, Rajko R. 2004. Modelling heat penetration curves in thermal processes. In P. Richardson, editor. Improving thermal processing of foods. Boca Raton, Fla.: CRC Press, LLC. p. 351–9.
Fasina OO, Stewart H, Farkas BE, Fleming HP. 2003. Thermal and dielectric properties of sweetpotato puree. J Food Prop 6(3):461–72.
Gillis JR, McCauley K. Feb 21–23, 2006. A glass-free self-contained biological indicator for use in monitoring the thermal sterilization of foods. SGM Biotech Inc. Presented at Inst. for Thermal Processing Specialists Conference. Orlando, Fla.
Grabowski JA, Truong V-D, Daubert CR. 2006. Spray-drying of amylase hydrolyzed sweetpotato puree and physicochemical properties of powder. J Food Sci 71(5):E209–17.
Guan D, Gray P, Kang DH, Tang J, Shafer B, Ito K, Younce F, Yang TCS. 2003. Microbiological validation of microwave-circulated water combination heating technology by inoculated pack studies. J Food Sci 63(4):1428–32.
Guan D, Cheng M, Tang J. 2004. Dielectric properties of mashed potatoes relevant to microwave and radio-frequency pasteurization and sterilization processes. J Food Sci 69(1):30–7.
Heldman DR, Hartel RW. 1997. Principles of food processing. New York: Chapman & Hall. 289 p.
Holdsworth SD. 1997. Thermal processing of packaged foods. London: Chapman & Hall. p 109.
Jones AT, Pflug IJ, Blanchett R. 1980. Performance of bacterial spores in a carrier system in measuring the F_0 value delivered to cans of food heated in a steritort. J Food Sci 45:940–5.
Jong DK, Kaczmarek KA, Woodworth AG, Balasky G. 1987. Mechanism of microwave sterilization in the dry state. Appl Environ Microbiol 53(9):2133–7.
Kissel WJ, Han JH, Meyer JA. 2003. Polypropylene: structure, properties, manufacturing processes and applications. In: Karian HG, editor. Handbook of polypropylene and polypropylene composites. New York: Marcel Dekker Inc. p 15–37.
Marcy JE. 1997. Biological validation. Food Technol. 51(10):48–52.
Metaxas AC, Meredith RJ. 1983. Industrial microwave heating. London: Peter Peregrinus Ltd. p 357.
Nelson SO, Datta AK. 2001. Dielectric properties of food materials and electric field interactions. In: Datta AK, Anantheswaran RC, editors. Handbook of microwave technology for food applications. New York: Marcel Dekker. p 69–114.
Pflug IJ. 2003. Microbiology and engineering of sterilization processes. Analysis of microbial-survivor data (calculating d-value). Minneapolis, Minn. Environmental Sterilization Laboratory. p 7.1–7.41.
Pflug IJ, Smith GM. 1977. The use of biological indicators for monitoring wet-heat sterilization processes. In: Gaughran ERL, Kereluk K, editors. Sterilization of Medical Products. Montreal: Multiscience. p 193–222.
Pflug IJ, Smith G, Holcomb R, Blanchett R. 1980. Measuring sterilizing values in containers of food using thermocouples and biological indicators units. J Food Protect 43(2):119–23.
Pflug IJ, Berry MR, Dignan DM. 1990. Establishing the heat-preservation process for aseptically-packaged low-acid food containing large particulates, sterilized in a continuous heat-hold-cool system. J Food Protect 53(4):312–21.
Serp D, Stockar UV, Marison IW. 2002. Immobilized bacterial spores for use as bioindicators in the validation of thermal sterilization processes. J Food Protect 65(7):1134–41.
Smith GM, Kopelman M. 1982. Effect of environmental conditions during heating on commercial spore strip performance. Appl Environ Microbiol 44(1):12–8.
[USFDA] U.S. Food and Drug Administration. 2000. Kinetics of microbial inactivation for alternative food processing technologies. A report of the Inst. of Food Technologists for the Food and Drug Administration of the U.S. Dept. of Health and Human Services. Available from: http://www.cfsan.fda.gov/~comm/ift-toc.html. Accessed Oct 8, 2006
Wang JC, Hu SH, Lin CY. 2003. Lethal effect of microwaves on spores of *Bacillus* spp. J Food Protect 66(4):604–9.
Welt BA, Tong CH. 1994. Effects of microwave radiation on inactivation of Clostridiu, Sporogenes (PA 3679) spores. Appl Environ Microbiol 60(2):482–8.