Microbiological Validation of Microwave-Circulated Water Combination Heating Technology by Inoculated Pack Studies

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ABSTRACT: A 915-MHz Microwave-Circulated Water Combination (MCWC) heating technology was validated for a macaroni and cheese product using inoculated pack studies. Before the tests, heat resistances of a *Clostridium sporogenes* (PA 3679) spore crop were determined in neutral phosphate buffer and macaroni and cheese product. Trays of macaroni and cheese products were subjected to 3 processing levels: target process (F_0 = 2.4), under target process (F_0 = 1.2), and over target process (F_0 = 4.8). The inoculated packs were evaluated by count-reduction method and end-point method. The microbial results showed that microbial destruction resulting from MCWC heating technology matched the calculated degree of sterilization (F_0 value). This study suggests that the MCWC heating technology has potential in sterilizing packaged foods.

Keywords: microwave heating, sterilization, validation, *Clostridium sporogenes*

Introduction

MICROWAVE HEATING REFERS TO THE USE of electromagnetic waves of frequencies between 300 MHz and 300 GHz to generate heat in a material (Metaxas and Meredith 1993). Research has been conducted to use microwave heating for food pasteurization and sterilization (Ayoub and others 1974; Mudgett 1982). These studies took advantage of volumetric heating resulting from the direct interaction between microwaves and foods to reduce process times (Ohlsson 1978). It is generally believed that the destruction of microorganisms during microwave heating is due to thermal effect (Fujikawa and others 1992). But achieving heating uniformity remains a major challenge in the research and development of microwave heating technologies. This nonuniform heating arises from the discontinuous dielectric properties between foods and the surrounding air (Ramaswamy and Pillet-Will 1992) as well as from the difference in the dielectric properties of different food constituents (Ryynänen and Ohlsson 1996). Several researchers have made use of a water immersion technique (Ohlsson 1981; Guan and others 2002) and 915-MHz microwaves (Lau and others 1998) to minimize non-uniform microwave heating. Ohlsson (1987) demonstrated good bacteriological safety of several microwave-sterilized products with a pilot scale 2450-MHz microwave–water immersion processing unit. The products exhibited sensory qualities that were superior to conventionally processed foods. A pilot scale 915-MHz Microwave-Circulated Water Combination (MCWC) heating system was developed at Washington State Univ. (WSU, Pullman, Wash., U.S.A.), which demonstrated a relatively uniform heat distribution within certain food products packaged in pouches and trays (Lau 2000) and produced high quality product (Guan and others 2002). However, the microbiological safety of 915-MHz microwave-processed foods was not validated.

Another challenge in developing a microwave sterilization process is how to monitor the processing history to ensure the microbiological safety of the processed foods. Fiber-optic sensors have been used to measure temperatures in microwave heating. They are particularly suited for use in high-temperature short-time processes for the following reasons: (1) Fiber-optic temperature probes do not interfere with microwave fields; (2) the probe sizes can be as small as 0.8 mm in dia, resulting in short response times (from 0.05 s to 0.2 s in most foods); and (3) they provide accuracy comparable to thermocouples in a normal heating medium (FISO Technologies, Inc., Que., Canada). At WSU, fiber-optic sensors have been used to monitor the temperature of products during microwave heating processes, and the data were used to calculate degrees of sterilization (F_0 values, unit = mm). But there was no evidence to support the hypothesis that these calculated F_0 values truly reflect the real lethality of the processes. Further efforts were required to confirm the reliability of using fiber-optic sensors to monitor MCWC heating processes with a pilot scale test unit.

The objectives of this study were to determine the sterilization effect of the MCWC heating test system by using inoculated pack studies and to determine the practicality of using fiber-optic temperature sensors for the MCWC heating processes.

Materials and Methods

Preparation of macaroni and cheese products

Margarine and 2% fat milk was purchased from local grocery stores. Box-type noodles and cheese powder were supplied by Kraft Foods (Glenview, Ill., U.S.A.). To prepare the macaroni and cheese samples, 166.1 g of dry noodles were precooked in 1.5 L of boiling water and stirred periodically for 6 min, then drained and cooled immediately with tap water. Cheese sauce was prepared separately by blending melted margarine, milk, and cheese powder together at 50 °C. Both the partially cooked noodles and sauce were prepared in a hygienic lab kitchen and were packaged immediately after preparation.

Determination of heat resistance of PA 3679 in phosphate buffer (pH 7.0)

Clostridium sporogenes (PA 3679, NFPA NO. SC 218) spores were obtained from the Center for Technical Assistance of the National Food Processors Association (NFPA,

Dublin, Calif., U.S.A.). Thermal death time (TDT) tests were conducted at 3 different temperatures, 115.6 °C, 118.3 °C, and 121.1 °C using a thermoresistometer at NFPA (Dublin, Calif., U.S.A.). During the tests (115.6 °C: 3, 5, 8, 15, 25 min; 118.3 °C: 2.5, 3, 5, 8, 15 min; and 121.1 °C: 1, 2, 3, 5, 7 min), sample cups containing 0.01 mL of the diluted spore suspension (1.0×10^6 spores/mL, 1/15 *M* phosphate buffer) were placed in the carrier boats. After predetermined exposure times to saturated steam of constant temperature, sample cups were moved out of the pressurized heating chamber and fell directly into tubes containing culture media at ambient temperature. Sterile Vaspar (approximately 6 oz. of paraffin in 2 L Vaseline petrolium jelly, Chesebrough-Pond's USA Co., Greenwich, Conn.) was used to overlay the culture to provide anaerobic conditions in the tubes, which were then incubated at 30 °C for 3 at least wk. Positive growth was indicated by gas production and confirmed by characteristic odor and microscopic examination.

The D-values of PA 3679 spores were calculated using 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 spores count per sample multiplied by the number of replicates; and *b* (referred to as the most probable number of spores surviving the time–temperature relationship to which the samples were subjected) was calculated by Eq. 2:

$$b = x \times n \quad (2)$$

Here, n is the total number of replicates and x (referred to as most probable number of spores surviving per replicate sample) was calculated by Eq. 3:

$$x = 2.3026 \log_{10} (n/q) \quad (3)$$

where q is the number of sterile samples as evidenced by lack of growth in subculture medium.

Determination of heat resistance of PA 3679 in macaroni and cheese products

Thermal Death Time (TDT) mini-retorts (NFPA, Dublin, Calif., U.S.A.) were used to determine the heat resistance of PA 3679 spores in macaroni and cheese product. Six retort units were connected directly to a steam line with an automatic temperature controller. Mercury thermometers for the TDT retorts were graduated to within 0.28 °C (0.5 °F). The general description of the thermoresistometer and TDT mini-retorts was given by Townsend (Townsend and others 1956).

Macaroni and cheese product was prepared as described previously and made into a puree with a blender. 15.0 g of homogeneous and thick puree was weighed into a TDT can (208 × 006, or 6.4-cm dia × 0.95-cm height). The desired number of organisms, 10^4 spores in 0.1 mL de-ionized water, was pipetted into the center of each can. In total, 180 vacuum-sealed cans were heated at 3 temperatures: 115.6 °C, 118.3 °C, and 121.1 °C. After being heated for 5 time intervals selected for each temperature (115.6 °C: 3, 5, 8, 15, 25 min; 118.3 °C: 2.5, 3, 5, 8, 15 min; and 121.1 °C: 1, 2, 3, 5, 7 min), the TDT cans were incubated at 30 °C for at least 3 mo. Another 5 cans were used as controls, 3 of which were heat shocked (100 °C and 4 min) and 2 untreated. Positive growth was indicated by gas production and confirmed by characteristic odor and microscopic examination. Non-swollen cans and non-swollen cans in the next highest process level were also examined.

The D-values at the 3 temperatures were calculated using Eq. 1 and the corresponding z-values were obtained by plotting D-values on semi-log papers. Heating times were corrected for lag.

MCWC heating system

The 915-MHz MCWC heating system consisted of 3 major components: (1) a 5-kW 915-MHz microwave generating system (Microdry Model IV-5 Industrial Microwave Generator, Microdry Incorporated, Crestwood, KY., U.S.A.) and a multimode cavity (121.3 cm wide × 121.3-cm long × 151.1-cm high); (2) a pressurized microwave heating vessel; and (3) a water circulation heating and cooling system.

The 915-MHz microwave system was equipped with a circulator to protect the microwave generator from heat damage caused by reflected power. A directional coupler with appropriate sensors was used to measure forward and reflected powers. The output microwave power was calibrated and stabilized at 1.0 kW by regulating anode current to the magnetron.

The pressurized microwave-heating vessel allowed treatment of a single meal tray at selected time intervals under an overpressure. The sidewall of the vessel was made of cylindrical aluminum tube (23.0-cm dia and 5.0-cm height). Its top and bottom plates were made of Tempalux material (Ultem Polyetherimide Resin, Lenni, Penn., U.S.A.) that had a high melting temperature (>150 °C) and was transparent to microwaves. An overpressure (about 34 to 35 psig) was provided by compressed air in a surge tank and used within the vessel to maintain the integrity of the food package. Fittings were provided to allow for temperature measurements and for concurrent circulation of pressurized water.

In the circulated-water control system, circulation water was maintained at the desired temperature by 2 plate heat exchangers and used to heat and cool the food package during MCWC processing. The exchangers were heated and cooled with steam and tap water, respectively. A Think & Do™ computer program (Entivity, Ann Arbor, Mich., U.S.A.) was used to control the modulating valves of the exchangers. The flow rate of the circulated water was 9.5 L/min.

Temperature measurement during MCWC processing

To measure the sample temperature using fiber-optic sensors, a 3.0-cm-long polyimide tubing (O.D.: 0.075 inch or 0.1905 cm; I.D.: 0.0710 inch or 0.18034 cm; thickness: 0.00200 inch or 0.00508 cm, Cole-Parmer, Ill., U.S.A.) was sealed at one end using silicone sealant (Dow Corning®, Dow Corning Corp., Midland, Mich., U.S.A.). The tube was inserted through a hole in the side of the tray such that the sealed tip was located in the center of a tray (10.0 cm wide × 14.0 cm long × 2.5 cm deep × 0.3 cm thick, Polypropylene and EVOH trays, Rexam™ Union, Mo., U.S.A.) before packaging. Two pieces of rubber (about 1-cm dia, 1/32-inch or 0.7938-cm thick, McMaster-CARR Supply Company, Santa Fe Springs, Calif., U.S.A.) adhered the tubing to both sides of the tray wall using silicone sealant, keeping it from shifting in the sample.

In this study, a fiber-optic sensor was inserted into the tubing to take the product temperature at the center of the tray. Due to the inherent nonuniformity in microwave heating, the center was not necessarily the coldest spot(s) in the tray. Not knowing exactly the location of cold spot(s) for the product packaged in the tray, we used the center as a reference location to measure the temperatures. Another reason for this selection is a chemical marker technique (Kim and Taub 1993) was used to understand the heating pattern of this MCWC heating technology for the same product packaged in pouches. The pouch had similar dimensions as the tray being used (Lau 2000). The least marker yield was obtained at the center of the pouches.

The F_0 values were calculated by Eq. 4 based on the temperature histories for different process procedures (Lopez 1987).

$$F_0^z = \int_0^t 10^{(T-T_r)/z}\,dt \quad (4)$$

where F_0 is the degree of sterilization at 121.1 °C for a certain z value; T is the actual temperature of the product (°C); T_r is the reference temperature (121.1 °C); the z-value in this test is 6.78 °C, which is obtained from the above heat resistance tests and t is heating time (min.).

During the MCWC process, the circulating water temperature for in-line heating and cooling as well as the product temperature at the center of the tray were displayed and recorded every 6 s. The degrees of sterilization (F_0 values) were also shown on the screen instantly.

Table 1—Processing procedures for 3 processing levels (unit: min)

Processing levels	Designed degree of sterilization (F_0 value)	Preheating time	Combination heating time	Holding time	Cooling time
Under target process	1.2	3.8	2.8	0	6.0
Target process	2.4	3.8	3.2	0	6.0
Over target process	4.8	3.8	3.2	1.2	6.0

Table 2—Thermal resistance of PA 3679 in phosphate buffer and macaroni cheese

Botulinum	Phosphate buffer	Macaroni cheese	Phosphate buffer*	pH range** (pH > 4.5)	*C. botulimun* (Type A, B)
D-value (min) at 121.1 °C	1.07	0.40	1.06	0.10~1.5	0.1~0.20
z-value (°C)	9.92	6.78	9.33	7.78~10	7.78~10

*From Nordsiden 1978.
**In low-acid and semi-acid foods, from Stumbo 1973.

Package integrity and sealing of products

Package integrity, critical to product stability, was visually observed after processing and during incubation. The products were sealed under vacuum to make rapid microwave heating and cooling possible. Nitrogen flush was applied during sealing and the overpressure was regulated throughout the process.

The sealing prototype unit, customize built by Rexam Containers (Model 1, Rexam™, Union, Mo., U.S.A.), consisted of a heating mechanism in an enclosed chamber. A pump and a nitrogen tank were connected to the chamber for vacuum seal and subsequent gas flushing. A metal "nest" holder secured trays containing the product in the sealing chamber. The holder was aligned with a thermostatically controlled heat-sealing head driven by a pneumatic cylinder. A control panel displayed the operation parameters including seal pressure (psig), sealing head temperature (°F), chamber vacuum (inches of mercury), and seal duration (s).

Before sealing the product, the partially cooked noodles (98.0 g) were placed into the tray in the laboratory kitchen and the inoculated liquid sauce (102.0 g) was poured onto the noodles. The tray filled with products was flushed with nitrogen and heat sealed (380 °F, or 193.3 °C) with a 0.1-mm lid stock (polypropylene/EVOH laminated) under vacuum (14 inches of mercury, or 58.69 kPa). A food tray prior to MCWC heat treatment is shown in Figure 2a.

MCWC heating process procedures

MCWC heating processes were similar to conventional steam or pressurized hot water retorting processes. It included 4 stages: preheating, combined heating, holding, and cooling. The product in the vessel was first preheated to 75 °C with circulating hot water at 100 °C. The combined heating started when the microwave power (1.0 kW) was turned on and the circulation water was set at 120 °C. The holding stage began by maintaining the circulated water at 120 °C while the microwave was turned off. After the desired holding period, the tray was cooled using circulating water at 80 °C (2 min) under pressure, then by 20 °C tap water at ambient pressure.

Before testing with inoculated packs, the product tray inserted with fiber-optic temperature sensors at the center was treated with the MCWC heating system. The lethality of the process was determined using Eq. 4. The procedures, as controlled by initial temperature of the product (around 45 °C), the time for preheating, heating time, and cooling, were repeated for the inoculated pack without the inserted tubing.

Three processing procedures (Table 1) were selected in this study aiming at 3 degree of sterilization (F_0 value). The target processing procedure was designed to eliminate the inoculated PA 3679 spore population (1.1×10^6 spore/tray, $D_{121.1} = 0.40$ min) with a sterilization value (log reduction value) of 6.04, corresponding to a degree of sterilization (F_0 value) of 2.4 min. Target processing was equivalent to an 8D process for *C. botulinum* (assuming a $D_{121.1}$ of 0.3 min). Overprocessing was designed to destroy the inoculated PA 3679 spores completely, aiming at a sterilization value (or log reduction value) of 12.1 or an F_0 value of 4.8; the underprocessing procedure was selected to allow a certain amount of spores to survive after processing and was designed to have a sterilization value of 3 or an F_0 value of 1.2.

Inoculated pack tests

The same batch of PA 3679 spore crop was re-enumerated at WSU just before the inoculated cheese sauce was prepared. The spore suspension was heat-shocked at 80 °C for 10 min, cooled in crushed ice-water, 10-fold serially diluted in 0.2% peptone water, and 100 mL was spread-plated onto duplicate SFP agar plates. Counts taken after 48 h of incubation at 37 °C indicated the initial concentration of the spore suspension. The diluted spore suspension containing approximately 1.1×10^6 viable spores/mL was added into the liquid cheese sauce, targeted to give the inoculated level of 1.1×10^6 spores/tray (200.0 g).

The survival of PA 3679 spores in MCWC-processed macaroni and cheese product were analyzed by the following 2 methods:

1. Count-reduction method. In this method, the log reduction (sterilization value, or SV) of PA 3679 spores in the processed products was determined by counting the survivors after incubation. All the MCWC-processed macaroni and cheese products (200 g) from 1 tray were divided into 2 100-g portions. They were homogenized with a Seward 400 Circulator Stomacher (Seward, Ltd., London, U.K.) in 200-mL sterile 0.2% peptone water at 260 rpm for 2 min. Four 2.5-mL portions of homogenate from each portion were pour-plated with *Clostridium*-selective SFP Agar Base (Difco, Detroit, Mich., U.S.A.) and incubated in Anaerobic Gas Pack Systems (BBL) at 37 °C. Colony counts were recorded after 48 h of incubation. Colony counts from both 100-g portions were added and expressed as viable CFU/tray. If no viable spores were detected in a processed tray, the survival numbers of PA 3679 spores in the tray was recorded as below the detection

limit (30 CFU/tray). Three trays subjected to each MCWC heating process level were evaluated.

2. End-point method. Because of the inherent detection limit (> 30 CFU/tray) of the count-reduction method, the end-point method was used to further confirm the lethality of the processed products. In this method, 10 trays processed under each process level were incubated at 37 °C for 3 mo to check the survival of PA 3679 spores. The trays were checked every 2 to 3 d during incubation. Bulged trays were indicative of viable *C. sporogenes* spores, which were further confirmed by the presence of the characteristic putrefactive odor. Nonswollen trays and nonswollen trays in the next highest process level were also examined. Trays that showed no signs of bulging after 3 mo were scored as having zero viable spores.

Results and Discussion

Thermal resistance of the PA 3679 spores

The D-values at 121.1 °C and z-value for spores in neutral phosphate buffer (pH = 7.0) and macaroni and cheese product (pH = 5.7) are listed in Table 2. At 121.1 °C, the D value (1.06 min) and z-value (9.33 °C) of the spore crop in phosphate buffer were very close to literature values (Nordsiden and others 1978). But the D-value (0.40) in macaroni and cheese product was at the lower end of the value range (about 0.10 to 1.5 min) for low-acid foods (pH > 4.5), and the related z-value (6.78 °C) was off the corresponding range (about 7.78 °C to about 10 °C) (Jay 2000).

Many factors affect the heat resistance of bacteria: inherent genetic and environmental factors during the growth of bacteria, heating of the bacterial suspension, the pH value of the medium (Santos and others 1993) and the salt and fat/lipid content (Molin and Snygg 1967). Inherent resistance varies not only with species but also with different strains of the same species. Different strain of the same species grown in the same medium and heated in the same menstruum might show widely different resistance (Stumbo 1973). On the other hand, lowering pH of a medium or increasing salt content typically reduces the thermal resistance of spores (Stumbo 1973). In this study, certain ingredients in the macaroni and cheese products obviously decreased the heat resistance of PA 3679 spores. However, it is not clear which ingredients led to this reduction of heat resistance.

MCWC processing and integrity of packaging

Figure 1 shows a typical MCWC heating history with a degree of sterilization (F_0 value) of 2.4. In microwave processing, cooling, rather than heating, is said to set the process speed limit (Stenström 1970). According to Figure 1, the cooling time takes 6 min, about one half the total processing time. This should be considered in the industrialization of MCWC heating technology. Package integrity was visually examined after the MCWC processing and incubation period (90 d at 37 °C). It appeared that the tray wall was slightly softened upon removal from the process vessel after processing. The package expanded slightly, with stretching of the material. But the package integrity was maintained during the microwave sterilization process and over the 3-mo storage period (Figure 2b). The seals held well in trays that were under-processed as shown in Figure 2c.

Inoculated pack studies

The initial concentration of the spore suspension was 1.1×10^8 spores/mL. The re-

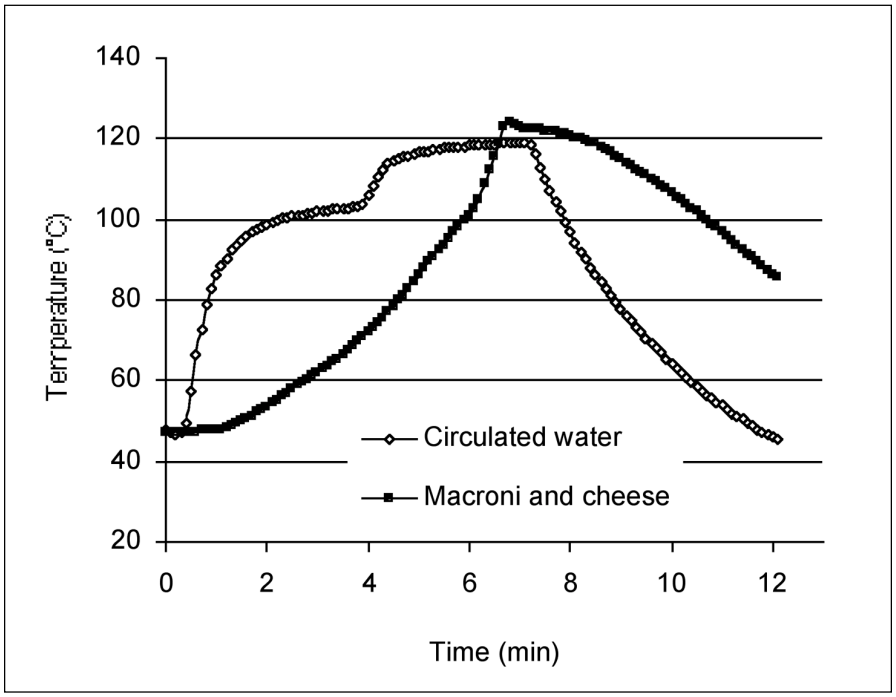

Figure 1—A typical temperature–time heating history for the MCWC heating process (equivalent to a processing procedure with degree of sterilization of 2.4)

Figure 2—Visual observation of package integrity of typical food trays. (a) Food tray prior to MCWCV heat treatment. (b) Properly processed tray after 3-mo incubation at 37 °C. (c) Underprocessed tray stored for 3 mo at 37 °C.

sults from the inoculated pack studies were summarized in Table 3 and 4.

1. Count-reduction method. The number of surviving spores in processed food trays was counted after incubation; the process values equivalent to log reduction (PA 3679) were shown in Table 3. No viable spores were detected in macaroni and cheese product from trays subjected to the target process and the over target process. The surviving numbers of PA 3679 spores in these trays were recorded as below the detection limit (30 CFU/tray). The corresponding log reduction values (4.56) of the detection limit were given as the actual sterilization values for these processes. The actual sterilization value from the trays subjected to the under target process was slightly more than the designed value.

2. End-point method. All 10 controls subjected to no heat treatment swelled within 2 wk due to gas production caused by the growth of PA 3679. Gas production also occurred in the 10 trays subjected to the under target process. The 20 (10 × 2) inoculated trays subjected to the target process and the over target process showed no evidence of gas production and lacked characteristic odor. These results suggest that these processing levels could destroy the targeted PA 3679 spores adequately, which agreed with the designed degree of sterilization (Table 4).

As mentioned earlier, one of the major concerns in developing a microwave sterilization processes is how to monitor the nonuniform microwave heating. The designed degrees of sterilization (F_0 values) in this study were based on the temperature history obtained through fiber-optical sensors inserted at the center of the trays, whereas the microbial inoculation test indicated the possible biological safety for the whole tray. The fact that the results of endpoint studies matched the calculated degree of sterilization (F_0 value) suggests the practicality of using fiber-optic sensors to monitor the microwave sterilization process for this 915-MHz MCWC heating test system. However, further studies are needed to determine the real location of the coldest spots for various kinds of products. We are designing a 915-MHz MCWC heating system that could process more than one tray at a time. One of the trays can be used to monitor the heating processes along with other trays that contain no sensors.

Table 3—Result from count-reduction method

Inoculated levels (spores/200 g)	Process levels	Designed degree of sterilization (F_0 value)*	Designed sterilization value (SV)**	Actual sterilization value (SV)
1.1×10^6	Under target process	1.2	3.0	3.3
1.1×10^6	Target process	2.4	6.04	>4.56
1.1×10^6	Over target process	4.8	12.1	>4.56

*F_0 value = SV × $D_{121.1}$ (unit: min)
**Equivalent Log_{10} reduction for PA 3679

Table 4—Results from end-point method

Inoculated levels (spores/200 g)	Process levels	Designed sterilization value (SV)*	Number of processed trays	Number of positive trays**
1.1×10^6	Controlled process	N/A	10	10
1.1×10^6	Under target process	3.0	10	10
1.1×10^6	Target process	6.04	10	0
1.1×10^6	Over target process	12.1	10	0

*Equivalent Log Reduction
**Indicated by gas production and characteristic odor; storage period: 3 mo

Conclusions

THE INOCULATED PACKS STUDIES EVALUATED by inoculated pack studies suggest that microbial destruction by a pilot-scale Microwave-Circulated Water Combination (MCWC) heating system matched with designed degrees of sterilization (F_0 value). This study suggests that the MCWC heating technology has potential in sterilizing packaged foods and it is practical to use fiber-optic sensors to measure the temperature for the 915-MHz MCWC heating test unit.

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