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Carol J. Valenzuela

University of Nebraska-Lincoln, valenzuelamartinez.carol@gmail.com

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VALIDATION OF MICROWAVE HEATING INSTRUCTIONS FOR THE
DESTRUCTION OF *Salmonella* spp. IN MICROWAVEABLE FOODS

by

Carol Jazmín Valenzuela Martínez

A DISSERTATION

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VALIDATION OF MICROWAVE HEATING INSTRUCTIONS FOR THE
DESTRUCTION OF *Salmonella* spp. IN MICROWAVEABLE FOODS

Carol Jazmín Valenzuela Martínez, Ph.D.

University of Nebraska, 2013

Advisor: Harshavardhan Thippareddi

Microwave heating instructions for three products (chicken nuggets, turkey pot-pies and mashed potato) were developed and validated based on end point temperatures using two microwave ovens (2,459 MHz; 700 W and 1,350 W). Heating instructions for chicken nuggets were validated using different configuration of product placement (edge or center of the carousel) and number of units (4, 6 and 8). *Salmonella* spp. reductions of 6.56 log CFU/g (700 W) were observed in chicken nuggets heated in groups of 4 and placed at the center of the carousel with 1 min 26 s of heating time with a target end point temperature of 73.8°C. Longer heating times (2 min 10 s) resulted in total *Salmonella* spp. reductions (7.22 log CFU/g) when chicken nuggets were placed in groups of 8. Similar *Salmonella* spp. reductions ($p>0.05$) were observed when chicken nuggets were placed at the center using shorter heating times (1350 W). Incorporation of standing time (2 min) eliminated *Salmonella* spp., regardless of the power of the microwave, location and the number of chicken nuggets. Heating instructions for turkey pot-pies were validated using inoculated product (at the geometric center or on the crust). *Salmonella* spp. reductions of 5.16 log CFU/g were observed following heating times of 9 min 31 s and 7 min 1 s for the low and high power microwave, respectively with a target end point temperature of 73.8°C. For the third product, different amounts of inoculated mashed potato (105 and 205 g)

were used to validate the microwave heating instructions. Destruction of *Salmonella* spp. (8.73 log CFU/g) in mashed potato (105 g) can be achieved with a target end point temperature of 70°C at the geometric center, regardless of the power of the microwave oven. *Salmonella* spp. destruction (8.73 log CFU/g) was observed in mashed potato (205 g) using the high power microwave oven using an end point temperature of 72.2°C to calculate heating times. *Salmonella* spp. destruction in mashed potato is dependent on the amount of the product and the power of the microwave oven.

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Introduction

Salmonella spp. is estimated to cause 1.0 million cases of illness, 19,587 hospitalizations and 378 deaths in the United States annually (Scallan et al., 2011). *Salmonella* spp. is widespread in the environment and is commonly isolated from swine, dairy, beef, and poultry farm environments (Rodriguez et al., 2006). However, poultry has been identified as a major reservoir of this pathogen as it has been commonly isolated from fresh poultry and poultry products. Typically, *Salmonella* spp. is eliminated in ready-to-eat poultry products (RTE) using conventional heating methods (Juneja et al., 2001). The USDA-FSIS performance standards require a 7.0 log reduction of *Salmonella* spp. in RTE poultry products; product temperatures 73.8°C (165°F) are recommended (USDA-FSIS, 1999). However, these performance standards may not be applicable for not-ready-to-eat foods (NRTE) that are prepared using microwave ovens. These types of products are combinations of meat or poultry with other components such as vegetables in which at least one of the components ingredients has not received adequate heat treatment for the elimination of pathogenic bacteria (GMA, 2008). Consumption of NRTE including chicken nuggets, chicken strips and pot-pies has been associated with *Salmonella* spp. infections when heated in microwave ovens (Kenny et al., 1999; MacDougall et al., 2004; Smith et al., 2008). Improper heating by the consumer and inadequate instructions on the package labels have been identified as the causes of the outbreaks.

Microwave ovens have been used for reheating food products (Heddleson et al., 1994) and are not designed for cooking purposes. The main advantage of microwave heating is the faster heating rate compared to conventional ovens. However, a major drawback of microwave heating is the non-uniform temperature distribution in the product that can

lead to hot and cold spots (Vadivambal and Jayas, 2010) that may allow survival of pathogens such as *Salmonella* spp. In the past, microwave heating instructions on the packages have failed to assure *Salmonella* spp. free NRTE foods. Factors such as the type of product (mashed potato, pot-pies, chicken nuggets), size and shape, configuration, power of microwave oven and the intrinsic characteristics of each product may result in differences in microwave heating (Pucciarelli and Benassi, 2005). The objective of this study was to develop science based heating instructions for microwave heating of mashed potato, chicken nuggets and pot-pies and validate the instructions using microbial challenge studies to evaluate *Salmonella* spp. destruction as affected by the power of microwave oven and different factors for each product. Microbial challenge studies of the developed microwave heating instructions should be used as a basis to validate final cooking instructions for NRTE products.

I. CHAPTER 1

Literature review

1. *Salmonella* spp.

1.1 General characteristics

Salmonella spp. is a Gram-negative, non-sporeforming, rod shaped facultative anaerobe and belongs to the *Enterobacteriaceae* family (Coburn et al., 2007). The size of *Salmonella* spp. cells ranges from 0.7 μm to 1.5 μm in width and from 2 μm to 5 μm in length. This microorganism is motile due to the presence of peritrichous flagella. The genus *Salmonella* spp. contains two species: *Salmonella enterica* and *Salmonella bongori*. Six different subspecies belong to the *Salmonella enterica* group (Table 1) with *Salmonella enterica* subspecies *enterica* being the most commonly implicated with foodborne illness (Agassan et al., 2002). The Judicial Commission of the International Committee of Systematic Bacteriology of the World Health Organization (WHO) Collaborating Centre has approved a third *Salmonella* spp. species (Table 1.1), however, the CDC has not officially adopted this new classification scheme (Su and Chiu, 2007).

The genus *Salmonella* spp. was named after the American bacteriologist D. E. Salmon who was the Director of the USDA research program when the bacteria was first isolated in 1884 (Evangelopoulou et al., 2010). Since then, numerous *Salmonella* spp. serovars have been identified and the nomenclature has become complex. The scientific community uses two nomenclature systems (Su and Chiu, 2007). For citation purposes, and for those serovars named before 1966, names may be written with the genus followed by the word serovar or ser. and then the serovar name (for example *Salmonella* serovar or ser. Typhimurium). When the same serovar is mentioned more than two times in the text, names can be written with the genus followed by the serovar name. For those unnamed

serovars described after 1966, antigenic formulae are used. When the antigenic formulae are used, the names include subspecies designation (subspecies I through VI), the somatic antigens (O) followed by a colon, the flagellar (H) antigens (phase 1) followed by another colon and the flagellar (H) antigens (phase 2) if present (Brenner et al. 2000). These two systems have been officially adopted by the CDC based on the recommendations of the World Health Organization Collaborating Centre for Reference and Research on *Salmonella* spp. at the Pasteur Institute, Paris, France (WHO, Collaborating Centre) (Su and Chiu, 2007).

Salmonella spp. is an important pathogen for humans and animals (Khakhria et al., 1997) and is of the leading causes of foodborne illnesses in the United States. Among other foodborne pathogens, *Salmonella* spp. ranks second and causes the most number of illnesses (1.0 million), hospitalizations (15,000) and deaths (600) in the United States (Scallan, 2011). Salmonellosis is defined as an infection caused by *Salmonella* spp. and occurs when people orally ingest contaminated food or water. Self-limiting gastroenteritis is the most common disease caused by *Salmonella* spp. (Shachar and Yaron, 2006) and the onset normally occurs 48 h after the consumption of the contaminated food. Symptoms of salmonellosis include abdominal pain, chills, diarrhea, fever, nausea and vomiting (MacDougall et al., 2004). However, *Salmonella* spp. can also cause typhoid fever, a systemic infection related with the capacity of certain serovars to invade the gut barrier, disseminate to systemic sites and replicate within the host cells (House et al., 2001). In complicated cases, typhoid fever can generate high fever, pneumonia, intestinal perforation and death (House et al., 2001; Santos et al., 2001)

Table 1.1. *Salmonella* spp. classification according to Kauffmann-White scheme (Popoff, et al. 2000).

Genus	Species	Subspecies	No. of serotypes
<i>Salmonella</i>	<i>enterica</i>	<i>enterica</i> (I)	1,450
		<i>salamae</i> (II)	489
		<i>arizonae</i> (IIIa)	94
		<i>diarizonae</i> (IIIb)	324
		<i>houtenae</i> (IV)	70
	<i>bongori</i>	<i>indica</i> (VI)	12
			20
		(V)	Total
			2,463
	<i>subterranea</i> *		

* New *Salmonella* species accepted by the World Health Organization Collaborating Centre for Reference and Research on *Salmonella* at the Pasteur Institute, Paris, France (WHO, Collaborating Centre). Not recognized by the Center of Disease Control (CDC).

1.2 *Salmonella* spp. growth conditions

Salmonella spp. can survive in the range of temperatures from 5°C to 47°C, with an optimum growth between 35°C and 37°C (Beuchat and Scouten, 2002; Matches and Liston, 1968). Some *Salmonella* serovars show increased heat resistance depending on

the environmental conditions or food matrix and is influenced by different intrinsic characteristics associated with food products. Among all the *Salmonella* serovars, *Salmonella* Senftenberg 775W is considered as the most heat tolerant (Ng, et al., 1969). The water activity (a_w) has also an impact on the heat resistance of *Salmonella* spp. (Mattick et al., 2001). *Salmonella* spp. growth occurs at high a_w levels (>0.94) but the organism can also survive in dry environments such as dry foods with a_w levels of <0.2 (Beuchat and Scouten, 2002). The heat resistance of *Salmonella* Typhimurium DT104 increases at temperatures $\geq 70^\circ\text{C}$ at low water activity, while lower heat resistance was observed at lower temperatures (below 65°C ; Mattick et al., 2001). It has been also reported that *Salmonella* Typhimurium was more heat resistant to dry heating compared to *Salmonella* Senftenberg 775W (Goepfert and Biggie, 1968). This information clearly indicates the importance of evaluating a heat process treatment using different *Salmonella* spp. strains at different conditions. The optimum pH for *Salmonella* spp. to growth is between 6.5 and 7.5 (Pui et al., 2011). However, the ability of *Salmonella* Typhimurium to survive in low pH environments has been demonstrated by the pre-exposure or pre-shock of this pathogen to mild pH conditions (5.5 to 6.0), by the occurrence of the acidification tolerance response (ATR; Foster, 1991).

1.3 *Salmonella* spp. in poultry products

Poultry meat is an important meat ingredient used for the manufacture of NRTE food products. Salmonellae are normal inhabitants of the gastrointestinal tract of poultry and it is a major reservoir for *Salmonella* spp. in the environment (Khan et al., 2003).

Limawongpranee et al. (1999) isolated *Salmonella* spp. from the cecal content of 14.3%

of 2345 broiler chickens from 12 farms in Canada. Similarly, Rostagno et al. (2006) reported the presence of *Salmonella* spp. in the cecal content of 33.3% of market-age turkeys sampled from 6 different farms in the United States.

Colonization of the gastrointestinal tracts of chickens with *Salmonella* spp. (Zhao et al., 2001) can lead to the contamination of processing facilities and raw meat when the slaughter process is inadequate. *Salmonella* spp. can be found in the environment of poultry facilities and can survive for long periods of time (Chaves et al. 2011). Rodriguez et al. (2006) reported the prevalence of *Salmonella* spp. in different environmental farm samples. *Salmonella* spp. prevalence of 16.2% was reported on poultry farms and the common serovars were *Salmonella* ser. Anatum, followed by *Salmonella* ser. Arizonae, and *Salmonella* ser. Javiana.

Although a number of foods including fruits and vegetables have been associated with *Salmonella* spp. contamination (Beuchat, 2002), poultry meat is considered as the main source of contamination of this pathogen (Whyte et al., 2002). Jorgensen et al. (2002) reported a *Salmonella* spp. prevalence of 25% in 241 raw chicken samples. The most prevalent serovars were *S. Hadar*, *S. Enteritidis*, and *S. Indiana*. Two chickens were positive for *Salmonella* spp. by direct plating methods with 3.8 and 4.5 CFU/carcass. Brichta-Harhay, et al. (2007) estimated *Salmonella* spp. population of 1.56 log CFU/mL in poultry carcass rinses. However, the prevalence of *Salmonella* spp. in chickens can change from region to region. Zhao et al. (2001) observed a prevalence of 4.2 and 2.6% for *Salmonella* spp. in raw chicken and turkey, respectively. Rose et al. (2002) reported

the prevalence of *Salmonella* spp. in raw poultry products collected from different slaughter establishments in the United States and reported a prevalence of 20%, 44.6% and 49.9% for broilers, ground chicken and ground turkey, respectively.

1.4 *Salmonella* spp. destruction in poultry products

Salmonella spp. lethality of 7D is specified for raw poultry used for the manufacture of RTE poultry meat products (USDA-FSIS, 1999). Heating is considered as the main step for the elimination of pathogenic bacteria. During heating, the destruction of pathogens is considered to follow first kinetics order (Juneja et al., 2001). USDA-FSIS proposed guidelines for RTE meat and poultry products that include lethality and stabilization performance standards for different meat and poultry products (USDA-FSIS, 1999). Based on the Performance Standards for the production of certain meat and poultry products, a 6.5 or 7.0 *Salmonella* log reduction is required for cooked beef, roast beef and corned beef. In the case of RTE poultry products, the performance standards specify a 7.0 *Salmonella* log reduction. The time-temperature schedules can be applied by the food processor in order to assure the safety of the final products. However, validation studies should be performed when time-temperature schedules not available in the Compliance Guidelines (USDA-FSIS, 1999) are used.

Thermal resistance of *Salmonella* spp. in poultry products has been reported in literature. Juneja et al. (2006) determined the D-values for *Salmonella* spp. in chicken broth and chicken meat and reported D-values of 4.87, 2.72, 1.30, and 0.41 min at 55, 58, 60 and 62°C, respectively in chicken broth. The authors reported that thermal resistance of *Salmonella* spp. significantly increased in chicken meat compared to the liquid system.

Mazzotta (2000) reported D-values for *Salmonella* spp. in ground chicken breast meat to be 3.2, 0.6, 0.31 and 0.18 min at 56, 60, 62 and 63°C, respectively.

Current methods for guideline development of microwave heating of foods include heating to a specific temperature based on the assumption that reaching that temperature is adequate to destroy foodborne pathogens (GMA, 2008). However, microwave heating results in non-uniform heating distribution in the product (Vadivambal and Jayas, 2010); achieving target temperatures at specific locations (geometric center) is not an indication of pathogen destruction, potentially resulting in foodborne illness. Therefore, it is important to develop time-temperatures guidelines for the destruction of *Salmonella* spp. in microwaveable, but NRTE foods, taking into account the occurrence of non-uniform heating distribution in the product.

2. Microwaveable Not-Ready-To Eat (NRTE) foods

2.1 Description of microwaveable foods

Microwaveable foods are defined as those aimed to be heated by the use of a microwave oven (George and Burnett, 2001). Microwaveable products are designed to satisfy the demand of faster life styles where products that can be cooked in a short time are more convenient. This is an advantage over conventional heating, where typical product preparation may require at least a couple of hours or more (Bertrand, 2005). In the case of microwaveable foods, the product can be cooked in shorter times while maintaining good quality characteristics such as texture and flavor, very similar to products cooked conventionally. Currently, microwaveable products occupy a significant proportion (87.5%) of the frozen foods section in most supermarkets and grocery stores. Domestic

microwave ovens have become an essential kitchen appliance (Thostenson and Chou, 1999) for most people in the US and are mainly used for reheating leftovers. It has been estimated that 93% of the population in the US owns a microwave oven and its popularity is due to the savings of heating times (Bertrand, 2005).

Microwaveable foods are categorized according to the required time for heating the product. Fully cooked and pre-cooked microwaveable products include foods that only need to be reheated during a short time in a microwave before consumption. Heating procedures applied during manufacturing of these foods eliminate foodborne pathogens such as *Listeria monocytogenes* and *Salmonella* spp. in the final product. Some examples are fully cooked snacks, precooked entrees, sandwiches, pizzas (Bertrand, 2005), one serving size meals (kids cuisine) and one serving size pot-pies among others. Typically, heating instructions for these products are developed by the manufacturer to assure that the quality of the final product is similar to the one for conventionally cooked food.

On the other hand, not-ready-to-eat (NRTE) foods are mixtures of meat or poultry with any other ingredient such as vegetables in which at least one ingredient has not received a heat treatment for the elimination of pathogenic bacteria (GMA, 2008). Similar in their external appearance to fully cooked products, NRTE foods include products such as frozen-stuffed chicken and raw chicken nuggets. The presence of raw ingredients, regardless of the component (meat, poultry or vegetables), implicates that the consumer must cook the product thoroughly (through the application of longer heating times) for the elimination of foodborne pathogens that may be present in the product. The U.S. Department of Agriculture's Food Safety Inspection Service (USDA-FSIS) classified

NRTE products as raw and they are considered as a potential source for the transmission of pathogenic bacteria such as *Salmonella* spp. For this reason, proper heating procedures should be applied to NRTE foods in order to reduce the risk of foodborne illness.

2.2 *Salmonella* spp. in NRTE foods

The consumption of not-ready-to-eat foods contaminated with *Salmonella* spp. led to the first outbreak associated with this type of products in Australia in 1998 (Kenny et al., 1999). “Flash fried” chicken nuggets contaminated with *Salmonella* Typhimurium phage-type 12 was identified as the etiologic agent. Frying of foods confers an external cooked appearance to the product (Gupta, 2005) with the internal product temperatures reached during the process being low, between 25°C and 30°C. During the outbreak two types of chicken nuggets were identified as the cause of the illnesses; fully cooked and flash fried chicken nuggets. These products were available in the supermarkets and were placed near to each other with similar external appearance on the packages. The recommended heating instructions were 15-20 min at 200°F for fully cooked nuggets and 20- 25 min at 200°F for raw flash fried nuggets, respectively with no microwave heating instructions. Despite, consumers assumed the product was fully cooked and heated the chicken nuggets in groups of six for 2 min in the microwave ovens.

The first *Salmonella* spp. outbreak associated with NRTE products occurred in 2003 in Canada. *Salmonella* ser. Heidelberg was associated with the illnesses. A case control investigation revealed that people who consumed chicken nuggets or strips were 11 times more susceptible to *Salmonella* spp. infections compared to people who did not. In total, 23 people became ill, two of which developed a systemic infection. Children under the

age of four or younger accounted for a significant number of the total salmonellosis cases as chicken nuggets in cartoon shapes are attractive to kids. The investigation revealed that most of the consumers used a microwave oven to heat the product, ignoring that the product was not fully cooked. Non-uniform heating was identified as the risk factor resulting in the survival of *Salmonella* Heidelberg during heating (MacDougall et al., 2004).

Four *Salmonella* spp. outbreaks linked to NRTE products occurred between 1998 and 2006 in Minnesota. The Minnesota Department of Agriculture (MDA) identified *Salmonella* Typhimurium subtype TM127 as the main cause of *Salmonella* spp. infections in thirty-three people in 1998 (Smith et al., 2008). A case-control study showed that Chicken Kiev, a pre-browned, breaded product with a cooked appearance was the vehicle of transmission and the consumers heated the product in a microwave oven. A second *Salmonella* spp. outbreak associated with for *Salmonella* Heidelberg occurred in 2005. Frozen, microwaveable products contaminated with *Salmonella* caused the illnesses. The third outbreak occurred from August 2005 to February 2006 and was associated with *Salmonella* Enteritidis SE43. Routine interviews showed that frozen, stuffed, pre-browned, microwaveable food products were consumed. A variety of stuffed chicken products including Cordon Bleu, Broccoli and Cheese, and Chicken Shrimp and Crab were linked to the outbreak. Of twenty-seven people who acquired salmonellosis, 70% used a microwave oven for heating the product and no internal temperatures were measured (Smith et al., 2008).

The fourth outbreak occurred during June 2006. In this case, *Salmonella* Typhimurium infections were associated with the consumption of stuffed chicken products from the same brand associated with the *Salmonella* Heidelberg infection that occurred in 2005. Three people acquired the infection and reported to have had eaten multiple varieties of products, which were also implicated within the 2005 outbreak. *Salmonella* Typhimurium was isolated from an intact package of Chicken Mushrooms in Wine Sauce purchased by a case patient. Once more, patients recalled heating the products using a microwave oven (Smith et al., 2008).

One of the largest outbreaks linked to NRTE products in the United States occurred in 2007. Epidemiological studies showed that the consumption of Banquet turkey, frozen, NRTE pot-pies was associated with 401 illnesses in 41 states (MMWR, 2008). Pot-pies are a complex mixture of different ingredients including pre-cooked poultry meat, a variety of vegetables and flour. Improper microwave heating by the consumers led to the *Salmonella* spp. outbreak. Although heating instructions were stated on the product package, some consumers were not sure of the power of their microwave ovens. The epidemiological study revealed that from 78 interviewed patients who used a microwave oven cooked the product, only 29% reported knowing the power of the appliance (CDC, JAMA, 2009). Additionally, sixty eight percent (48 out of 71 interviews) of patients mentioned that they did not apply the standing times recommended in the instructions and 19% cooked more than one pot-pie in the microwave oven (CDC, JAMA, 2009). Another important factor was the variety and quantity of ingredients used in the products. Different sizes and shapes of the ingredients may have resulted in different temperature

distribution during the heating process that allowed for the subsequent survival of *Salmonella* spp. Small pieces of vegetables may have been heated faster than larger pieces of poultry meat. This outbreak and the ones described above, highlight the importance of microbiological validation of microwave heating instructions for microwaveable, but NRTE foods products.

2.3 Risk factors associated with the consumption of NRTE foods

Food labeling plays an important role in the food industry and is intended to communicate to the consumers on the ingredients and the nutritional benefits of the product. In the food safety area, food labeling is used to inform the consumers about the safe methods for holding and preparing the product for consumption (Caswel, 1998).

Salmonella spp. outbreaks associated with NRTE foods indicate that labeling of the product and microwave heating have contributed significantly to the outbreaks (Smith et al., 2008). In the past, microwave ovens were mostly used for reheating foods and were not intended to cook foods. Another factor contributing to the outbreaks is the consumer attitude towards these food products when inappropriate heating methods are used.

Confusion over raw or cooked poultry products has led to the survival of pathogenic bacteria when products are heated in a microwave oven (MacDougall et al., 2004). Proper interpretation of food labels is a key factor to ensure that food is safe and free of pathogenic bacteria. In the case of the frozen turkey pot-pies implicated in the 2007 outbreak, the food labels placed in the package by the manufacturer were as follows (Fig. 1.1) (Powell, 2007).

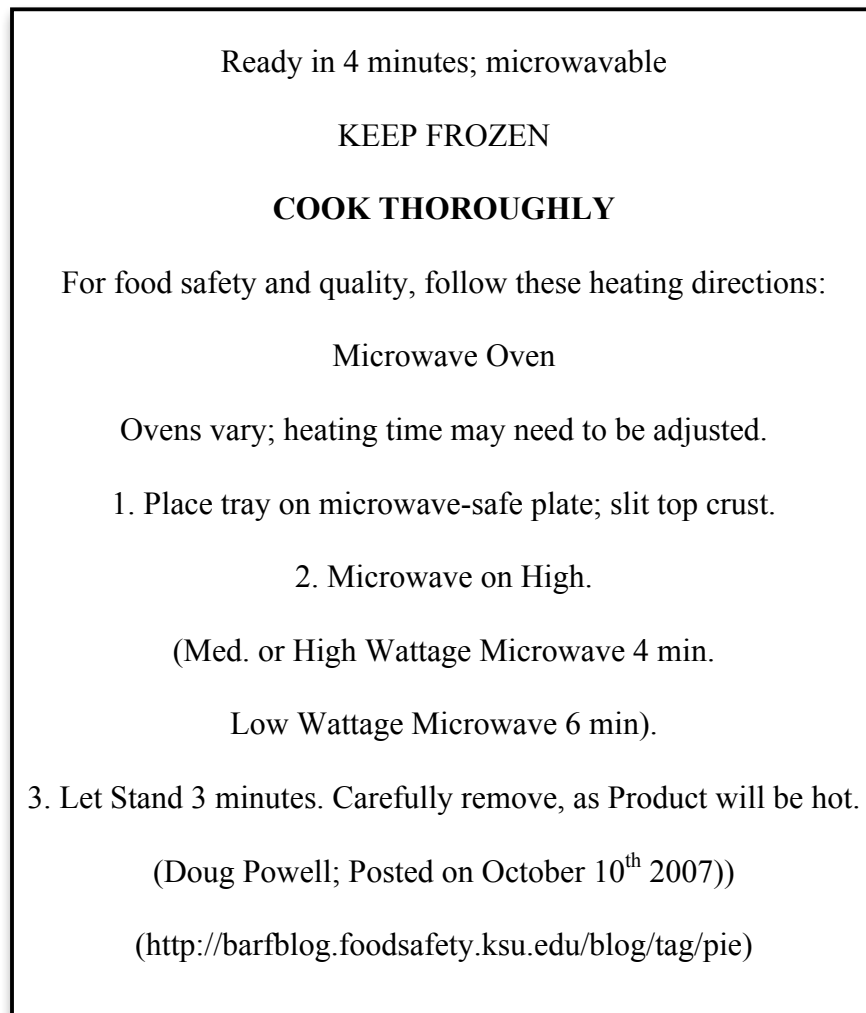


Fig. 1.1. Heating instructions for turkey pot-pies implicated in the 2007 *Salmonella* spp. outbreak.

As part of the label, COOK THOROUGHLY is intended to inform the consumer that the food product must be fully cooked in order to assure food safety. A minimal internal temperature of 165°F must be achieved when raw ingredients are present in the product in order to eliminate pathogenic microorganisms (HealthLinkBC, 2009). Although microwave heating instructions were stated in the pot-pie package and the most relevant factors were included for proper heating, the epidemiological study indicated that improper interpretation of this information contributed to the outbreak (CDC, 2009). As a result of these findings, new specifications in terms of microwave oven power, number of pot pies per heating cycle and final temperature, were included as part of the heating instructions (Fig. 1.2).

Subsequent to the numerous salmonellosis outbreaks for the consumption of NRTE frozen food products, the USDA-FSIS issued a letter to all food processors that manufacture these types of products requiring the re-evaluation and validation of the heating instructions for lethality of *Salmonella* spp. using microwave ovens (Smith et al., 2008). The Grocery Manufacturer's Association (GMA) and the American Frozen Food Institute (AFFI) developed guidelines for food processors on the proper labeling of heating instructions and their validation (GMA, 2008).

KEEP FROZEN; DO NOT THAW

Ovens and wattages vary. Adjust heating times as needed.

Product must be cooked thoroughly.

Read and follow these heating instructions:

Microwave Oven. Cooked only one product at a time.

1. Place pot-pie on microwave-safe plate; slit top crust.

2. Microwave on High. 1100 watts oven or more. 4 to 5 min.

DO NOT COOK in microwave ovens below 1100 watts as pot-pie may not cook thoroughly. Conventional oven preparation is recommended.

3. Let Stand 3 minutes in microwave to complete heating. CAREFULLY REMOVE as product will be hot.

4. CHECK that pot-pie is cooked thoroughly. Internal temperature needs to reach 165°F as measured by a food thermometer in several spots. Crust is golden brown and steam rises from filling.

Fig. 1.2. Adjusted heating instructions for turkey pot-pies after the 2007 *Salmonella* spp. outbreak.

2.4 Guidelines for the labeling of microwave heating instructions

The American Frozen Food Institute (AFFI) developed guidelines for food processors on developing heating instructions for microwaveable food products (AFFI, 2008). These guidelines are recommendations and are not specific for all the products. However, food processors have to develop instructions for specific products considering intrinsic

characteristics (volume, size, composition). The AFFI gives recommendations regarding heating statements. Some of the examples are:

- “For food safety, cook thoroughly to X °F (internal temperature).”
- “Follow the heating instructions carefully.” OR, if food safety is not mentioned prior to this “Food Safety, follow these HEATING instructions carefully.”

In order to warn the consumer and provide recommendations of how the food product needs to be handled, the AFFI has also developed some recommendations that the food processor may consider as supportive statements:

- “Do not eat the product without heating.”
- “Do not allow the product to thaw.”
- “When using a turntable, place [food name] to one side to help it heat more evenly.”
- “Stand [ing] time is important for safety and quality.” (Can be used as a footnote to a standing time in the instructions.)
- “Check for cold spots and continue cooking, if needed.”

Principal display panel call-outs to be used singly or in combination, as appropriate:

- “Cook thoroughly”
- “Contains raw/uncooked ingredients”
- “Not ready to eat. Cook thoroughly”

In the ingredient statement, label those ingredients that may pose risk as “raw” or “uncooked,” i.e., “raw chicken” instead of “chicken.”

The AFFI recommended the specification of the number of units that can be cooked using instructions provided and to advise against heating multiple units simultaneously. Also, it mentioned provision of graphics whenever possible.

2.5 Guidelines for validation of consumer heating instructions for Not-Ready-to-Eat (NRTE) products

Heating instructions for microwaveable foods were inadequate to eliminate pathogenic microorganisms such as *Salmonella* spp. as indicated from several outbreaks (MMWR, 2008). Validation of microwave heating instructions that support the elimination of pathogens should be performed by the food processor in order to provide adequate lethality after the product has been cooked (GMA, 2008). The Grocery Manufacturer Association (GMA, 2008) issued guidelines that would help to develop microwave heating instructions that can contribute to the elimination of pathogenic bacteria if present in the product. The guidelines cover the key factors that should be considered by the manufacturer when validating microwave heating instructions for NRTE food products.

The determination of the heat resistance of the pathogen in specific product is necessary as it varies with the product characteristics such as moisture, pH, ingredients, etc. The thermal destruction parameters for *Salmonella* spp. in different broths and food matrices have been documented (Juneja et al., 2001). The higher heat resistance of *Salmonella* spp. in meat systems compared to chicken broth has been attributed to the presence of more solids in the meat (Juneja et al., 2001). Differences in the heat resistance of *Salmonella* spp. was observed among meat species, which have been attributed to differences in fat content between the meat systems and other food components such as

proteins and salts that may contribute to the protection of microorganisms (Jay, 1986). Although this data may be useful for the determination of a time-temperature schedule for any process, it is recommended to validate the destruction of this pathogen in specific products (Juneja et al., 2001), which may include food ingredients that could affect the heat resistance of the pathogen. Although the thermal destruction of pathogens based on the D and z-values have been applied successfully for conventional heating, it may be possible that this approach may not be applicable for microwaveable NRTE food products. For fully cooked products the target internal temperature of 160°F should provide adequate lethality to assure the safety of product cooked by consumers, whereas for product containing poultry that is not fully cooked an internal temperature of 165°F is required (GMA, 2008). These temperatures are based on the Lethality Requirements guidelines for RTE meat and poultry products (USDA-FSIS, 1999). However, the GMA advises that the lethality requirements may be different for NRTE products.

Microbial challenge studies should be performed to validate the microwave heating instructions. The objective of the validation is to determine if microwave heating times are adequate to eliminate pathogenic bacteria, intentionally introduced, in microwaveable, but NRTE products (GMA, 2008). For *Salmonella* spp., a 7 log CFU/g reduction is required to assure the food safety of RTE poultry products (USDA-FSIS, 1999). In order to determine the efficacy of the heating instructions, the guidelines recommend the use of a minimum number of samples to evaluate the variability and performance of microwave heating. As the evaluation of the adequacy of time-temperatures is performed, the food processor will identify the factors affect on microwave heating and the ones that contribute to the variability of the temperature of the

product. This will depend on the type of study performed by the food processor. For food products that contain a mixture of several ingredients, the variability could be more extensive. Other factors that may have an impact on microwave heating such as food packaging configuration or product type should also be evaluated. Microwave heating instructions can also be developed for those microwaveable foods that only need to be reheated (GMA, 2008).

3. Microwave Heating of Foods

3.1 General principles

Microwaves belong to the electromagnetic spectrum with wavelengths ranging between 300 MHz to 300 GHz (Fig. 1.3). In the United States, the Federal Communications Commission has designated two microwave frequencies for food processing and industrial microwave heating; 915 MHz and 2450. Domestic microwave ovens are designed at a frequency of 2,450 MHz (Datta and Davidson, 2000). The application of microwaves for pasteurization (Lau and Tang, 2002), thawing of frozen foods (Bengtsson and Ohlsson, 1974), baking of foods (Sumnu, 2001) and frying of bacon and potatoes (James et al., 2006; Oztop et al., 2007) has been studied. The potential application of microwave sterilization of foods has also been evaluated (Bengtsson and Ohlsson, et al., 1974) due to the high temperature, short time that can be achieved in a food product. For domestic purposes, microwave ovens have been used for reheating foods (Heddleson et al., 1994). However, the increasing popularity of the microwaveable foods has increased the use of microwave ovens for heating frozen microwaveable foods (Bertrand, 2005).

In microwave heating, electromagnetic energy is directly applied to the food material which results in the generation of heat throughout the volume of the food (Thostenson and Chou, 1999). This form of heat generation is known as volumetric heating, meaning that the material can internally absorb microwave energy and convert it into heat (Vadivambal and Jayas, 2010). The generation of heat in food products by microwaves involves two primary mechanisms; dielectric and ionic mechanisms (Heddeson and Doores 1994). Dielectric heating is influenced by the presence of water molecules in a food product. When the electromagnetic field, which is comprised of an electric and magnetic field, is applied to the food system, water molecules are aligned to the electric field as it oscillates at very high frequencies. The oscillations of the water molecules within the electric field result in generation of heat (Datta and Davidson, 2000). Ionic heating is related to the presence of ionic compounds in a food sample. Heat is produced by the migration of ionic compounds through the oscillatory electric field in the food product (Datta and Davidson, 2000). The increase in the concentration of the ions by addition of a saline solution in marinated shrimp resulted in higher temperatures compared to a non-marinated sample. The presence of more ions in foods increases the energy dissipation and power absorption, which is the result of dipolar rotation of ions (Oliveira and Franca, 2000).

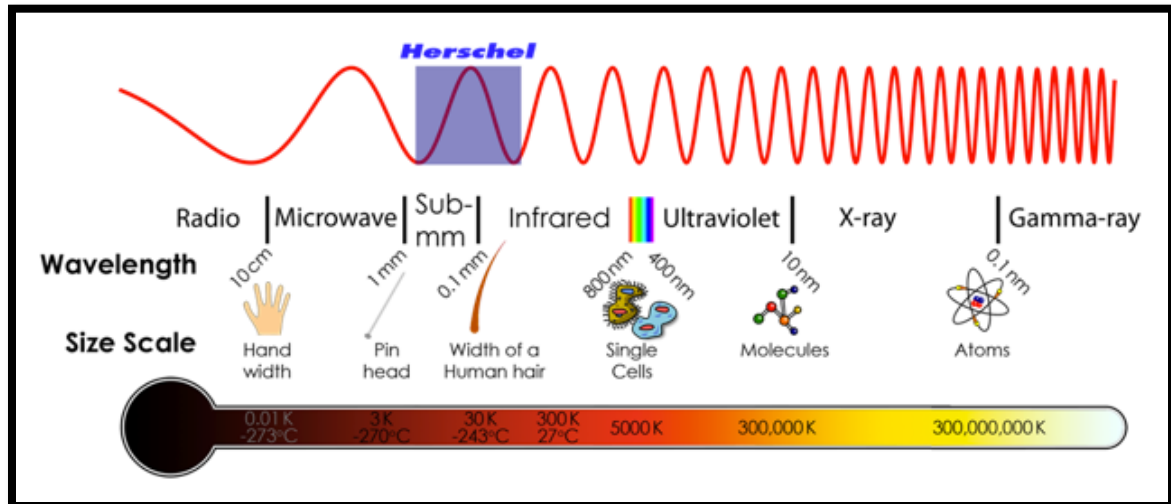


Fig. 1.3 Electromagnetic spectrum.

3.2 Dielectric properties

Dielectric properties of foods describe the interactions between the microwave energy and the foods to be heated (Tulasidas, et al., 1995) and they are defined in terms of the dielectric constant (k') and the dielectric loss factor (k''). While the dielectric constant is responsible of the electric energy of the microwaves, the dielectric loss is an imaginary component that describes the ability of the material to dissipate the electrical energy into heat. These two factors will determine the amount of energy that will be reflected, transmitted and absorbed by the material (Heddleson and Doores, 1994). Salt and water content are the main components that determine the dielectric properties of the food (Ryynanen, et al., 2004) and changes in these two parameters greatly affect the dielectric properties (Tulasidas et al., 1995).

Water is the main component of foods and responsible for the generation of heat during microwave heating. Tulasidas et al. (1995) evaluated the dielectric properties of grapes at

different moisture contents as a function of temperature. The measurement of the dielectric properties of grapes indicates that high moisture levels (80 and 60%) decreased the dielectric constant when the temperature of the product is increased. However, low moisture levels (40 or 15%) resulted in an increase in the dielectric constant with increasing temperature. A decrease in the dielectric loss was reported for grapes with high and intermediate moisture level. However, for grapes containing 15% moisture, the loss factor increases when the temperature of the product increases (Tulasidas et al., 1995). The presence of salt in food products greatly influences microwave heating. High salt concentrations in foods result in poor penetration depth of microwaves. In such, cases food products tend to heat more on the surface than in the middle or bottom of the products (Heddleson and Doores, 1994). Products containing ingredients with different dielectric properties result in different heating patterns and excessive or rapid heating as well as superheating (Schiffmann, 1992).

3.3 Major factors influencing microwave heating

The microwave frequency approved for domestic microwave ovens in the United States is 2,450 MHz (Heddleson and Doores, 1994). Oliveira and Franca (1997) reported that microwave ovens with lower frequencies resulted in a rapid increase in temperature and greater energy penetration depth in food samples compared to microwave ovens of higher frequency. Higher temperature values were observed in beef samples exposed to microwave irradiation at 915 MHz compared to 2,450 MHz (Oliveira and Franca, 2002). However, commercial microwave ovens designed with high frequencies result in greater control of heating (Oliveira and Franca, 2002; Copson, 1970).

Size and shape of the heated product affect microwave heating (Oliveira and Franca 2002). Larger food masses will take longer time to be heated compared to smaller masses (Heddleson and Doores, 1994). This aspect plays a relevant role in those products that contains portions or pieces of different sizes. Differences in product geometry are a major drawback with microwave heating: the non-uniform temperature distribution. Non-uniform energy absorption leads to uneven temperature distribution in the food products (Vadivambal and Jayas, 2010). This phenomenon results in cold and hot spots in the product that could affect the final quality and safety. The cold spots formed in food product can lead to the survival of pathogenic bacteria. Non-uniform microwave heating is caused due to differences in thermal and dielectric properties of the components (Ryynanen et al., 2004; Manickavasagan et al., 2009).

3.4 Destruction of *Salmonella* spp. by microwave heating in different food products.

Culkin and Fung (1975) studied the effect of microwave heating on the destruction of *Salmonella* Typhimurium in several liquid products (tomato soup, vegetable soup, and beef broth). Liquid products were exposed to different microwave heating times using a microwave oven at a frequency of 915 MHz. Survival of *Salmonella* Typhimurium was determined at different locations of the products (top, middle and bottom). For any given time, *Salmonella* spp. survival in the top sections of the product was observed even when lethal temperatures ($>70^{\circ}\text{C}$) were recorded. The temperatures at the middle and bottom sections of the product were 69°C and 71°C , respectively. However, the warmest sections (middle) resulted in greater *Salmonella* spp. survival compared to the bottom section.

Haddleson and Doores (1994) evaluated the destruction and injury to *Salmonella* spp. in ultrahigh-temperature (UHT) processed whole milk and commercially sterile beef broth heated by microwave at a frequency of 2,450 MHz. The microwave was set at the highest power level and the products were heated to at temperatures ranging from 66°C to 74°C and from 64°C to 72°C for milk and beef broth, respectively. After heat treatment, both food systems were stirred after 0, 5 or 10 min to equilibrate the temperature in the product. The greater destruction of *Salmonella* spp. in milk and beef broth was observed when the products reached the mean final temperature and were immediately stirred to eliminate the temperature gradient. Microwave heating of milk and beef broth that were not stirred immediately after heating resulted in greater survival of *Salmonella* spp. even when temperatures above 66°C and up to 72°C were achieved. Post-heating holding time 10 min did not affect the destruction of *Salmonella* spp. in non-stirred in milk or beef broth. The level of injury of *Salmonella* spp. was also minimal in product that were heated and sampled immediately after treatment. The data indicates that microwave heating causes non-uniform temperature distribution in the product.

In another study, UHT processed whole milk was heated with a microwave oven (700 W) operated at a frequency of 2,450 MHz (Heddleson et al., 1994). The temperature of the product was measured at different depths and the liquid product was heated at different times to a mean final temperature of 60°C. *Salmonella* spp. was able to survive in milk with a final temperature of 61°C with significantly lower destruction at a product temperature of 57°C. The power level (low, medium and high) affected microbial destructions with increasing power level resulted in greater microbial destruction. The

authors reported similar destruction of *Salmonella* spp. in product heated to the same mean final temperature regardless of the power level. These data indicate that the destruction of microorganisms is dependent. Post-heating times contributed to the destruction of *Salmonella* spp. Increasing holding times up to 6 min significantly affected *Salmonella* spp. destruction probably due to equilibration of the temperature throughout the product. Size and shape of the containers did not influence the destruction of *Salmonella* spp. in UHT milk.

Dos Reis Tassinari and Landgraf (1997) reported that reheating contaminated foods using preset programmed (700 W) and traditional microwave (750 W) ovens allowed the survival of *Salmonella* Typhimurium in different food products (baby food, mashed potato and beef stroganoff). Although the highest time-temperature exposure resulted in the highest *Salmonella* Typhimurium destruction, positive samples were observed even at maximum average temperatures of 74.4°C and 79.8°C for both, the preset programmed and the traditional microwave oven, respectively. Although measurements of high temperatures in the product were recorded, they are not representative of the whole sample. The lowest temperatures obtained could have had an impact on the survival of *Salmonella* Typhimurium. Also, it is mentioned that the product was taken out of the microwave and stirred for 10 s. Stirring could have affected the exposure time of the bacteria to high temperatures and the stabilization of the temperature throughout the product could have been lower. Even when food samples reached temperatures as high as 70°C survival of the microorganism could be observed. Although it is possible that some *Salmonella* spp. destruction could have occurred at specific locations in the product that

reached temperatures above 70°C, the microorganism could have survived the cold spots where low temperatures were recorded.

Jamshidi et al., (2009) evaluated the effect of microwave on superficial contaminated raw chicken drumettes with *Salmonella* Typhimurium. A microwave oven with a frequency of 2.450 MHz and power level of 850 W at full power was used. The exposure of chicken drumettes for 35 s resulted in a superficial temperature in the chicken of 72°C. This time-temperature profile resulted in a complete inactivation of *Salmonella* Typhimurium in the chicken product. Interestingly, the complete inactivation of the pathogen could not be tightly related to the high temperature obtained at the end of the process, but to the time that the product was exposed to before sampling. The authors reported that the chicken drumettes were subjected to a 5 min of standing time before sampling. This standing time could have had an effect on the complete inactivation of the pathogen. It could be also possible that some survival would have been observed if the product had been sampled right after the regular heating time. Injured *Salmonella* cells could have been present after the heating process, but they were not able to grow in a selective agar medium such as XLD.

Pucciarelli and Benassi (2005) studied the effect of microwave heating on the inactivation of *Salmonella* Enteritidis using a microwave oven (800 W) at two power levels, medium and high. Higher power levels resulted in higher *Salmonella* Enteritidis destruction compared to lower power level. Microwave heating times of 95 and 140 s at high and medium power levels resulted in 6.4 and 5.0 log CFU/g reductions, respectively.

Salmonella Enteritidis was not detected after 110 s for the high power microwave oven, however, at 140 s of heating the pathogen was still present for the medium power microwave oven.

3.5 Microbial inactivation mechanisms of microwaves

The inactivation of microorganisms by microwaves has been explained in terms of thermal and non-thermal destruction (Culking and Fung, 1975; Heddleson and Doores, 1994). A group of researchers have indicated that the heat generated by microwaves is the only form of destruction of microorganisms (Heddleson and Doores, 1994). Other group have reported that not only the heat generated in a food matrix is involved in the destruction of microorganisms, but microwave irradiation have an effect on microbial survival (Culking and Fung, 1975).

The non-thermal effect of microwaves on microorganisms has been reported (Olsen et al., 1966; Culkin and Fung, 1975; Cunningham 1978). Tomato soup, vegetable soup and beef broth inoculated with *E. coli* and *Salmonella* Typhimurium were heated for different time periods using a microwave oven at a 915 MHz frequency (Culkin and Fung, 1975). For any given time, the middle area of the liquid product was the warmest, the bottom the intermediate and the top area resulted to be the coolest part. A greater decline in the population was observed in the coolest regions of the product even at temperatures that supported the survival of microorganisms. Culkin and Fung (1975) concluded that the heat generated was not sufficient for the thermal effect and microwave irradiation was involved. Cunningham (1978) also reported the non-thermal effects of microwaves. Short time exposure (10-40 s) of raw chicken patties to microwaves resulted in reduction of

microorganisms. The reductions were related to microwave irradiation as the temperatures reached after heating were in the range of 40°C to 56°C for the middle and top of the chicken patty, respectively, and these temperatures should allow the survival of microorganisms.

The non-thermal effects of microwave radiation on microorganisms have also been studied at the protein level. Porcelli et al. (1997) studied the effect of microwave radiation at a 10.4 GHz frequency oven on two thermophilic and thermostable enzymes isolated from *Sulfolobus solfataricus*, a thermophilic microorganism belonging to the Archaea. The exposure of both enzymes at 10.4 GHz of microwave irradiation causes a loss on the enzymatic activity as a function of the exposure time but they retained their stability even in a range of temperature of 70-90°C. These authors concluded that the enzymatic inactivation could be ascribed to non-thermal microwave effects as both enzymes were active after 90 min at 70°C or 30 min at 90°C.

Despite the information about the non-thermal microwave effects on the destruction of microorganisms, the most accepted inactivation mechanism of microwaves is the thermal effect. Goldblith and Wang (1967) studied the effect of microwaves at 2450 MHz on the destruction of *E. coli* and *Bacillus subtilis* spores. Suspensions of both microorganisms exposed to microwave radiation resulted in similar microbial reduction as compared to the same time-temperature exposure to conventional heating. The exposure of *E. coli* to microwave radiation at 2,450 MHz did not affect the destruction of this microorganism when held at temperatures below 51°C. In general, these authors concluded that the

inactivation of *E. coli* was solely due to thermal effects and no microwave radiation effects can be attributed.

Vela and Wu (1978) studied the lethal effect of microwaves at 2,450 MHz on microbial cells at different conditions. Soil samples containing bacteria, actinomycetes or fungi were inactivated as a function of moisture content in the soil samples. It was noted also that lyophilized microorganisms exposed to microwave radiation survived in a dry state but were killed when suspended in water. All the organisms tested were unable to absorb sufficient microwave energy in the dry state to decline the microbial population even for prolonged periods of time.

Although there is information regarding the effect of microwave heating in different food systems, it is important to understand the effect of microwave heating as affected by the nature and composition of specific food systems, especially in those that are frozen. Validation studies that support the efficacy of microwave heating are still needed.

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2. CHAPTER 2

5. Destruction of *Salmonella* spp. in Microwaveable Mashed Potato

5.1 ABSTRACT

Mashed potato is included as a side dish in microwaveable, but not-ready-to-eat foods (NRTE). NRTE foods have been associated with several outbreaks of salmonellosis. Two household microwave ovens (2,450 MHz frequency) of low (700 W) and high (1,350 W) power were used. Commercial mashed potato flakes were obtained and prepared following manufacturer's instructions. Two cylindrical containers (small and large; 7 and 10 cm in dia., respectively) with total weight of mashed potato of 105 and 205 g, respectively, were heated using the microwave ovens. Twelve individual temperature profiles were obtained during heating of mashed potato for each microwave oven and container size. Based on a linear regression of each temperature profile, times required to reach 70, 72.2 and 73.8°C at the geometric center of the mashed potato were calculated and heating times were selected based on 90, 95 and 99% upper confidence limits (UCL) and chi-square value. The adequacy of the heating times to eliminate *Salmonella* spp. was validated by placing a portion (0.3 g) of mashed potato inoculated with *Salmonella* spp. (8.73 log CFU/g) at the geometric center of the container and heating for the specified times in each microwave oven. *Salmonella* spp. survival after microwave heating was determined by plating and enrichment methods. Microwave heating of mashed potato to a target temperature of 70°C resulted in the elimination of *Salmonella* spp. (8.73 log CFU/g reduction) in mashed potato when heated in either of the microwave ovens and container sizes. The destruction of *Salmonella* spp. in mashed potato placed in the small container can be achieved at 90% UCL with a target temperature of 70°C after heating, regardless of the microwave oven. However, the heating times for mashed potato heated in the high

power microwave oven and placed in the large container should be calculated at 90% UCL with a target end temperature of 72.2°C to eliminate *Salmonella* spp. The destruction of *Salmonella* spp. in mashed potato is dependent on the amount of the product and the microwave oven.

Key words: Mashed potato, *Salmonella* spp., heating instructions and microwave oven.

5.2 Introduction

Salmonella spp. is estimated to cause one million foodborne illnesses, 19,587 hospitalizations and 378 deaths in the United States annually (Scallan et al., 2011). *Salmonella* spp. is a Gram negative, rod-shaped bacterium with over 2,500 different serovars (Brenner et al., 2000) and is widely distributed in the environment (Rodriguez et al., 2006). Elimination of *Salmonella* spp. can be achieved by thermal processing methods traditionally used for the manufacture of fully cooked or RTE meat and poultry products (Juneja et al., 2001). During heating, heat diffuses from the outside to the inside of the product until temperatures lethal to *Salmonella* spp. are achieved. The USDA-FSIS compliance guidelines specify a target temperature of 73.8°C (165°F) to achieve 7.0 log reduction of *Salmonella* spp. in RTE poultry products.

NRTE products are defined as foods that contain at least one ingredient that has not received a lethal heat treatment for the elimination of pathogenic bacteria (GMA, 1998). Microwaveable, but NRTE foods can be found in the market as individual meals such as frozen pot-pies or multi-compartment meals where one main dish such as chicken pieces or chicken nuggets is accompanied with two or more side dishes such as mashed potato or mixed vegetables. Consumption of NRTE pot-pies prepared using microwave ovens resulted in over 300 salmonellosis cases in the United States (CDC, 2009). In addition, microwaveable chicken nuggets have been associated with several *Salmonella* spp. outbreaks in Australia and Canada (Kenny et al., 1999; MacDougall et al., 2004). Improper microwave heating and uneven heating of the product when heated using microwave ovens were identified as the main factor contributing to *Salmonella* spp. outbreaks.

Mashed potato has been implicated as the main source of infection in some outbreaks of *Salmonella* spp. (Lee et al., 2009; Khuri-Bulos et al., 1994). Cross contamination of mashed potato by food handlers or other foods prior to heating as well as abusive storage have been identified as risk factors in these outbreaks. Survival of foodborne pathogens during dehydration of mashed potato has been identified as a risk factor (Doan and Davidson, 2000) as reheating pre-cooked foods products such as mashed potato can result in the survival of *Salmonella* spp. The advantage of microwave heating is the shorter preparation times needed to achieve high product temperatures compared to the conventional ovens. However, the major drawback of microwave heating is the non-uniform temperature distribution within the product, resulting in hot and cold spots leading to the survival of *Salmonella* spp. (Vadivambal and Jayas, 2010).

Mashed potato can be used as a food matrix model to study destruction of foodborne pathogens during microwave heating as it is easy to prepare and has a homogeneous chemical and physical composition (Burfoot et al., 1996; Liu et al., 2013). Although time-temperature schedules recommended by the USDA-FSIS (USDA FSIS, 1999) are effective in destroying *Salmonella* spp. in RTE meat and poultry products, their use for developing heating instructions for microwaveable, but NRTE food products such as mashed potato must be evaluated for temperature uniformity and microbial destruction during microwave heating.

The objective of this study was to develop and validate microwave heating instructions for the destruction of *Salmonella* spp. in mashed potato during preparation using microwave ovens of different power and product configurations.

5.3 Materials and Methods

Microwave ovens: Two household microwave ovens (2,450 MHz frequency) of different power were used in this study: (1) 700 W (Model R-9470, SHARP Electronics, Mahwah, NJ) and (2) 1,350 W (Model JE51451DN1BB, General Electric Co., Louisville, KY).

Determination of microwave heating times: Commercial dehydrated mashed potato flakes (IDAHOAN) were obtained from a local grocery store and stored at room temperature until use. Mashed potato flakes (400 g) were mixed with 1,861 mL of deionized, boiling water in a bowl mixer (Model K5SSWH, Kitchen Aid, Troy, OH) and mixed at high speed for 5 min. Two cylindrical pyrex glass containers (7 and 10 cm dia., and 5 cm height) were used. Mashed potato (105 or 205 g) was placed in the containers to a height of 3 cm and covered with a polypropylene lid. Temperature profiles of the mashed potato were obtained by heating the product in the microwave ovens. Fiber optic probes (1.1 mm, Model T1S-2M. FOT, FISO Technologies Inc., Quebec, Canada) were placed at the geometric center, and 1.0 and 3.5 cm from the center at the same depth (1.5 cm) of the mashed potato on the same axis. Mashed potato was heated in the microwave oven and the temperatures were recorded until all the probes reached 90°C. From the temperature profiles, a linear regression was obtained and the times required to reach 70.0, 72.2 or 73.8°C at the geometric center of the mashed potato were calculated and heating times were selected based on the best best-fit distribution and chi-square value using the @Risk program (Ver. 5.7, Palisade Corporation, Ithaca, NY) at upper

confidence limits of 90, 95 and 99%. Infrared imaging camera (640x480 Pixels, FLIR systems Model # SC640. North Billerica, MA) was used to obtain color images to record the product appearance after heating.

Bacterial cultures: Five *Salmonella* spp. serotypes/strains (FSIS, *Salmonella* Thompson; CDC, *Salmonella* Enteritidis, phage type 4 (H3502); FSIS, *Salmonella* Hadar; *Salmonella* Enteritidis B2; *Salmonella* Enteritidis 11) were used in the study. The cultures were maintained as glycerol stocks at -18°C. Before each experiment, each serotype/strain was thawed at room temperature, grown individually in tryptic soy broth (TSB, Becton, Dickinson and Co., Sparks, MD) and incubated for 24 h at 35°C. Two subsequent transfers into fresh TSB were performed every 24 h. Five mL of from each culture was transferred into a sterile centrifuge tube and mixed (final volume 25 mL) with the other serotypes/strains. The cocktail was centrifuged for 10 min at 4 °C, 6,000×g (Model GS-15R; Beckman Instruments, Palo Alto, CA). The supernatant was discarded and the cells in the pellet were re-suspended in 500 µL of sterile water.

Inoculum preparation and inoculation: Five-hundred µL of the *Salmonella* spp. cocktail were added to 50 g of the prepared mashed potato to obtain an initial population of *ca.* 8.5 log CFU/g. The inoculated material (0.3 g) was placed in the middle of a fabric wick (length of 3 cm) sealed at one end, and filled between two portions of non-inoculated mashed potato. The wick containing the inoculated mashed potato was placed at the center of the container and filled with non-inoculated mashed potato amount (105 or 205 g for the small and large container, respectively). The surface of the sample was flattened and the container was covered with a polypropylene lid as described before.

Microwave heating of mashed potato: Mashed potato in the container was placed at the center of the microwave carousel and heated for the selected times. Product temperature was determined by placing a fiber optic probe at the geometric center of the container to verify the temperature.

***Salmonella* spp. enumeration and enrichment:** After heating, the inoculated portion was transferred to a sterile tube containing 20 mL of chilled 0.1% buffered peptone water (BPW, Becton, Dickinson and Co., Sparks, MD) stop additional lethality. The samples were transferred to a stomacher filter bag (BagFilter, Spiral Biotech, Norwood, MA) and homogenized for 2 min in a stomacher (NEUTEC, Albuquerque, NM), serially diluted in BPW and plated on tryptic soy agar (TSA; Becton, Dickinson and Co., Sparks, MD) and TSA supplemented with ferric ammonium citrate (3.4/0.5 L) (Becton, Dickinson and Co., Sparks, MD) and sodium thiosulfate (0.4 g/05 L) (Fisher Scientific, Fair Lawn, NJ) for enumeration of *Salmonella* spp. Typical *Salmonella* spp. colonies were enumerated and reported as log CFU/g after incubation for 24 h at 35°C. The rest of the sample was incubated for 18 h at 35°C for enrichment and detection of *Salmonella* spp. After incubation, aliquots (100 µL) from the enriched samples were transferred into Rappaport Vassiliadis medium (RV, Fisher Scientific, Fair Lawn, NJ) and incubated for 18 h at 35°C. A loop from the RV medium showing turbidity was streaked on xylose lysine deoxycolate agar (XLD, Becton, Dickinson and Co., Sparks, MD) and incubated for 18 h at 35°C. Development of typical black colonies on XLD agar from specific samples were reported as positives for *Salmonella* spp. Three independent replications as identified by day of product preparation and *Salmonella* spp. cocktail were performed for each experiment.

Water activity, moisture and pH measurement: Five-g portion of mashed potato were homogenized with 25 ml of deionized water for 1 min in a stomacher blender, and the pH of each sample was measured by immersing the pH electrode (Accumet-Basic/AB15, Fisher Scientific, Bridgewater, NJ) in the sample homogenate. The water activity of the samples was measured using an Aqua Lab 3TE water activity meter (Decagon Devices, Inc., Pullman, WA) following the manufacturer's instructions. The moisture analysis was performed by weighing 5 g of mashed potato and heat dry in a aluminum dish using a heating oven with mechanical convection for 18 h at 100°C (FD 53, BINDER. Bohemia, NY) following the AOAC method.

5.4 Results and Discussion

The water activity and pH of mashed potato were >0.997 and 5.85 ± 0.01 , respectively.

The a_w and pH of the mashed potato can support the survival of *Salmonella* spp.

(Blackburn et al, 1997). The moisture content of the prepared mashed potato was $81.74 \pm 0.09\%$ and is similar to the moisture content (86.4 %) reported in the literature (Regier et al., 2006).

The calculated heating times to achieve the target temperature of 70°C in the mashed potato at a 90% UCL were 2 min 8 s and 5 min 1 s for the small and large containers heated in the low power microwave oven, respectively. Longer heating times were required for heating larger amounts of mashed potato (large containers) regardless of the type of microwave oven used (Figs. 2.1-2.4). Larger mass of product requires longer times to heat a product in microwave ovens as the energy per mass of the product decreases with an increase in mass of the product. This may be due to the time that is

required for the heat to equilibrate throughout the product (Heddleson and Doores, 1994). Vilayannur et al. (1998) reported that increasing the volume of potato (from 75 to 105 cm³) resulted in longer heating times to reach 80°C for potatoes with different shapes. Similarly, Oliveira and Franca (2002) reported that the amount of absorbed power at the center of the product decreases as the size of the samples increases. The effect of sample size should be considered when designing food package for microwaveable food products and for developing of heating instructions for these products.

Non-uniform temperature of mashed potato during microwave heating was observed (based on the standard deviations). Heating the mashed potato in the large container resulted in a more uniform temperature distribution across the product compared to the small container when heated in the low power microwave oven (Fig. 2.2). However, greater temperature uniformity was observed when mashed potato was heated in high power microwave oven, with the product placed in the small container. Vilayannur et al. (1998) reported greater temperature non-uniformity with an increase in the diameter of cylindrical product. Pitchai et al. (2012) reported that temperature uniformity is increased with an increase in the power of the microwave oven. These results indicate that the power of the microwave oven and other factors that affect internal temperature distribution must be considered in the validation of microwave heating instructions.

The calculated heating times for mashed potato placed in the small or large container were sufficient for elimination of *Salmonella* spp. (8.73 log CFU/g) when heated in the low power microwave oven. The mean final internal temperature achieved was $72.7 \pm$

4.6°C and $79.3 \pm 2.1^\circ\text{C}$ for the small and large container, respectively. Heating the mashed potato in the small container (1 min 46 s) in the high power microwave oven resulted in 8.73 log CFU/g of *Salmonella* spp. reductions in all the samples. However, *Salmonella* spp. survival was observed in one (of three) mashed potato samples heated in the large container, with *Salmonella* spp. reductions of 2.93 log CFU/g observed in one sample. The internal temperatures achieved during heating of mashed potato in the high power microwave oven were $71.9 \pm 0.5^\circ\text{C}$ and $69.1 \pm 2.5^\circ\text{C}$ for the small and large containers, respectively. For the large container, increasing the heating time to 2 min 38 s to reach a target temperature of 72.2°C (Table 2.1) resulted in the elimination of *Salmonella* spp. in all the samples. For this heating time an internal temperature of 69.8 ± 3.05 was achieved. Heating the mashed potato for 2 min 38 s did not affect the quality of the product (Figs. 2.5 and 2.6).

Survival of *Salmonella* spp. in mashed potato reheated in a microwave oven has been reported. Tassinari and Landgraf (1997) reported that *Salmonella* Typhimurium was able to survive in mashed potato reheated for 75 s in microwave ovens of different power (750 W and 700 W). The lowest and highest temperatures reported for 750 W microwave oven were 74.0 and 84.5°C , respectively for the 700 W microwave oven and 59.0 to 82.5°C for the 750 W microwave oven. Our data showed that *Salmonella* spp. can be eliminated when mashed potato temperatures of $\geq 70^\circ\text{C}$ are achieved. Survival of *Salmonella* spp. in the mashed potato reported by Tassinari and Landgraf (1997) could be attributed to several factors such the sample and composition of the mashed potato (potatoes, margarine, milk, grated parmesan cheese and salt). Although the container size used by

Tassinari and Landgraf (1997) was larger than the one used in this study, difference in heating times can be the main factor contributing to larger differences in survival or destruction of the pathogen. The reported temperatures should be adequate to eliminate the organism in the mashed potato suggesting that non-uniform temperature distribution in the product may be contributing to the survival of this organism. Based on our heating profiles, heating the mashed potato using the low power microwave oven in the large container for 75 s resulted in final end temperatures of 36.3 ± 1.0 , 32.5 ± 1.0 , and 85.2 ± 10.0 for the geometric center, 1.0 cm and 3.5 cm from the center, respectively. Heating the mashed potato in the small container for 75 s resulted in temperatures of 60.3 ± 2.1 , 50.5 ± 4.1 and 93.3 ± 10.8 for the geometric center, 1.0 cm and 3.5 cm from the center, respectively. For the high power microwave oven, our heating profiles showed that heating the mashed potato for 75 s resulted in temperatures of 52.6 ± 0.1 , 63.2 ± 3.0 , and 91.3 ± 4.0 and 41.1 ± 1.6 , 48.5 ± 2.3 and 80.7 ± 22.9 for large container at the geometric center, 1.0 cm and 3.5 cm from the center, respectively. Our data suggest that the temperatures reported by Tassinari and Landgraf (1997) could have been measured only at one location of the product, probably near the edge of the container resulting in high temperature measurements after heating the product for 75 s. However, the specific locations of temperature measurements are not reported by Tassinari and Landgraf (1997). Other cold spots may have been present in the product where the temperature was not measured leading to the survival of *Salmonella* spp. This may explained the high percentage of *Salmonella* survival.

Salmonella spp. destruction in the mashed potato may have been influenced by the high moisture content of the mashed potato ($81.7 \pm 0.1\%$). Water is a major component of most food products and the main source for microwave interactions due to its dipolar nature (Oliveira and Franca, 2002). The electric field of the microwaves plays an important role in microwave heating (Heddleson and Doores, 1994) due to its interactions with water molecules resulting in heat generation from molecular friction (Oliveira and Franca, 2002). Therefore, high moisture content in a food product will cause a faster rise in temperature. Additionally, a higher degree of damage to microbial cellular components, and thus greater microbial destruction can occur when high moisture levels are present in a food product. This occurs due to the molecular vibration of water produced by microwaves affect proteins and other components of the cell (Ernshaw et al., 1995)

5.5 Conclusions

Microwave heating instructions for mashed potato were developed and validated for destruction of *Salmonella* spp. Heating times for mashed potato using microwave ovens of different power were calculated and adequate elimination of *Salmonella* spp. was obtained at UCL of 90% for end target temperatures of 70°C, using the low power microwave oven. Higher target temperatures (72.2°C) were needed to achieve the destruction of *Salmonella* spp. in mashed potato placed in the large container and heated in the high power microwave oven. Heating mashed potato to temperatures $\geq 70^\circ\text{C}$ with longer heating times (slow heating) were sufficient to eliminate the organism in the mashed potato. Power of the microwave ovens, sample size and product composition

should be considered when developing and validating heating instructions for microwaveable foods.

5.6 References

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5.7 List of Tables

Table 2.1. Heating times of mashed potato based on microwave power, container size and target end temperature with an upper confidence limit of 90, 95 and 99%.

Microwave power	Container size	Temperature (°C)	Heating times (min) at three UCL (%)		
			90	95	99
Low	Small	70.0	128	132	139
		72.2	135	138	146
		73.8	140	144	152
	Large	70.0	301	308	321
		72.2	314	322	334
		73.8	328	341	369
High	Small	70.0	106	111	122
		72.2	110	115	126
		73.8	115	120	132
	Large	70.0	151	152	153
		72.2	158	162	169
		73.8	162	164	168

5.8 Legend to the Figures

Fig. 2.1. Temperature profiles of mashed potato during heating in the small container (7 cm dia. and 5 cm in height) using microwave oven (700 W). Fiber optic probe locations: geometric center, 1.0 and 3.5 cm radial distance with the probes at 1.5 cm depth from the top.

Fig. 2.2. Temperature profiles of mashed potato during heating in the large container (10 cm dia. and 5 cm in height) using microwave oven (700 W). Fiber optic probe locations: geometric center, 1.0 and 3.5 cm radial distance with the probes at 1.5 cm depth from the top.

Fig. 2.3. Temperature profiles of mashed potato during heating in the small container (7 cm dia. and 5 cm in height) using microwave oven (1,350 W). Fiber optic probe locations: geometric center, 1.0 and 3.5 cm radial distance with the probes at 1.5 cm depth from the top.

Fig. 2.4. Temperature profiles of mashed potato during heating in the large container (10 cm dia. and 5 cm in height) using microwave oven (1,350 W). Fiber optic probe locations: geometric center, 1.0 and 3.5 cm radial distance with the probes at 1.5 cm depth from the top.

Fig. 2.5. Distributions for the selection of the heating times for mashed potato heated to a target temperature of 70.0 (left side), 72.2 (middle) and 73.8°C (right side) in the small container using the low power microwave oven with upper confidence limits of 90, 95, and 99%.

Fig. 2.6. Distributions for the selection of the heating times for mashed potato heated to a target temperature of 70.0 (left side), 72.2 (middle) and 73.8°C (right side) in the large container using the low power microwave oven with upper confidence limits of 90, 95, and 99%.

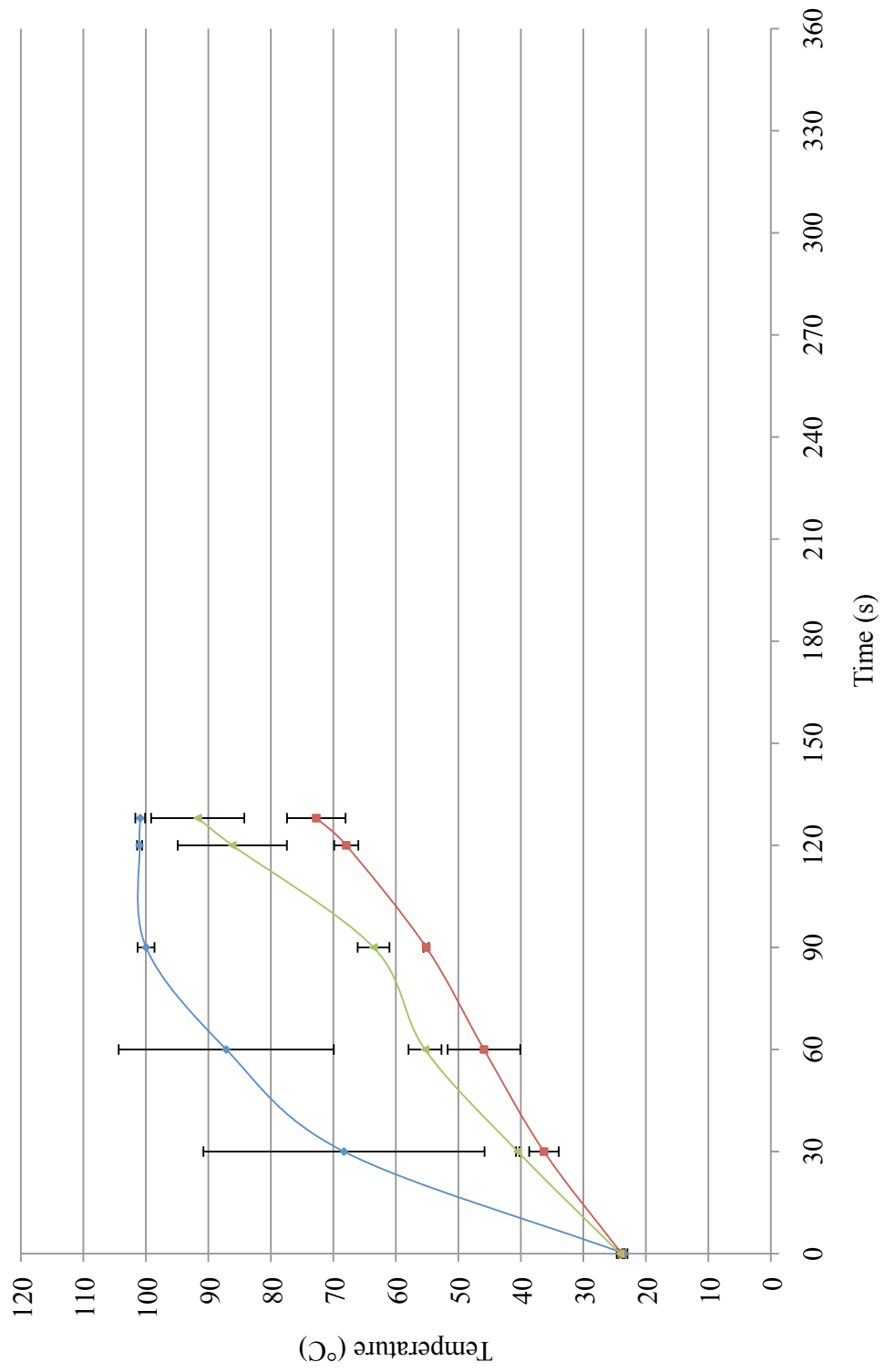
Fig. 2.7. Distributions for the selection of the heating times for mashed potato heated to a target temperature of 70.0 (left side), 72.2 (middle) and 73.8°C (right side) in the small container using the high power microwave oven with upper confidence limits of 90, 95, and 99%.

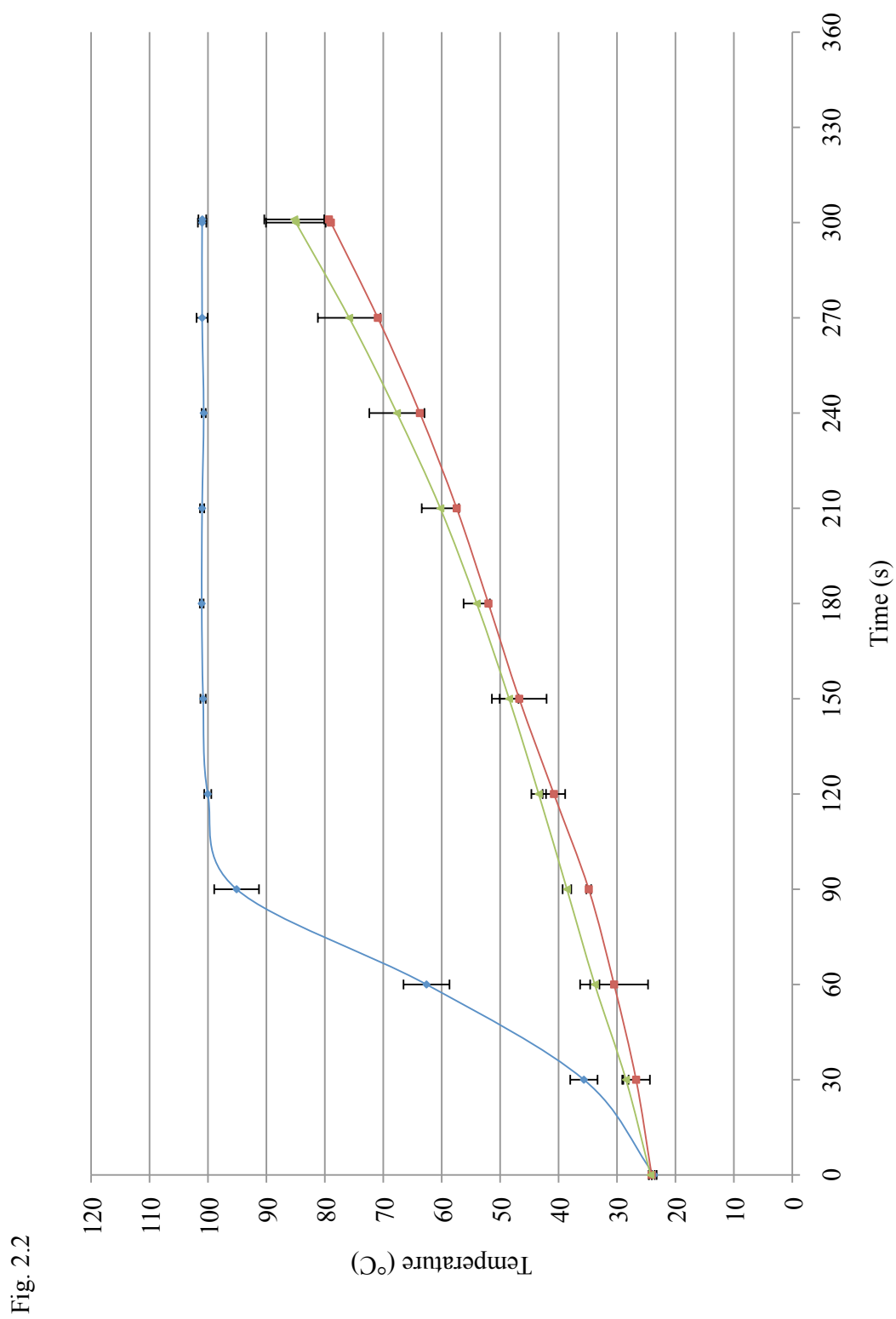
Fig. 2.8. Distributions for the selection of the heating times for mashed potato heated to a target temperature of 70.0 (left side), 72.2 (middle) and 73.8°C (right side) in the large container using the high power microwave oven with upper confidence limits of 90, 95, and 99%.

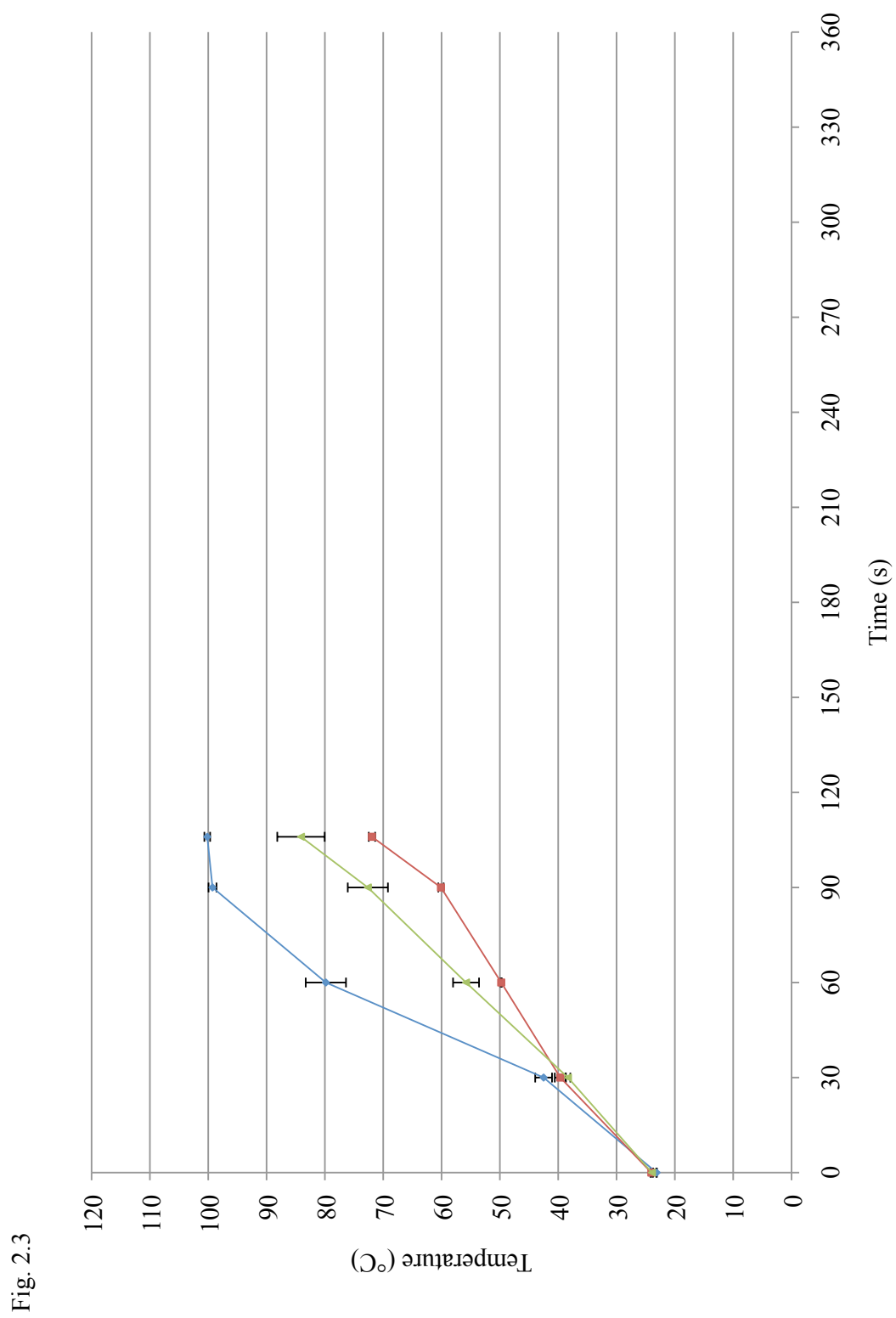
Fig. 2.9. Color images of the mashed potato heated in the large container in the low power microwave oven a) not heat treatment b) heat treatment (surface part) c) heat treatment (bottom part).

Fig. 2.10. Color images of mashed potato microwave heated with the high power oven in a large container: a) after heat treatment (surface part) b) after heat treatment (bottom part).

Fig. 2.1







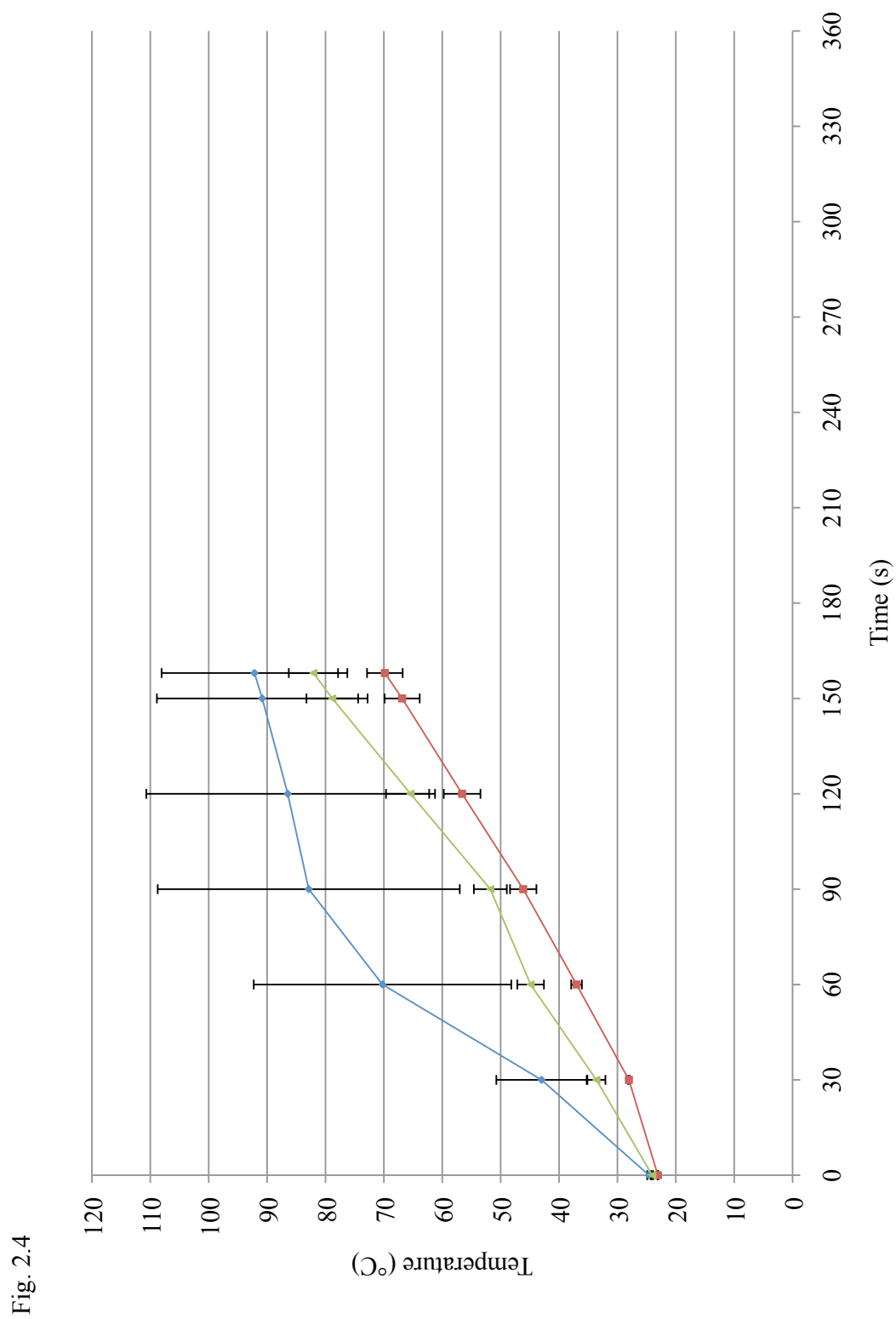


Fig. 2.5

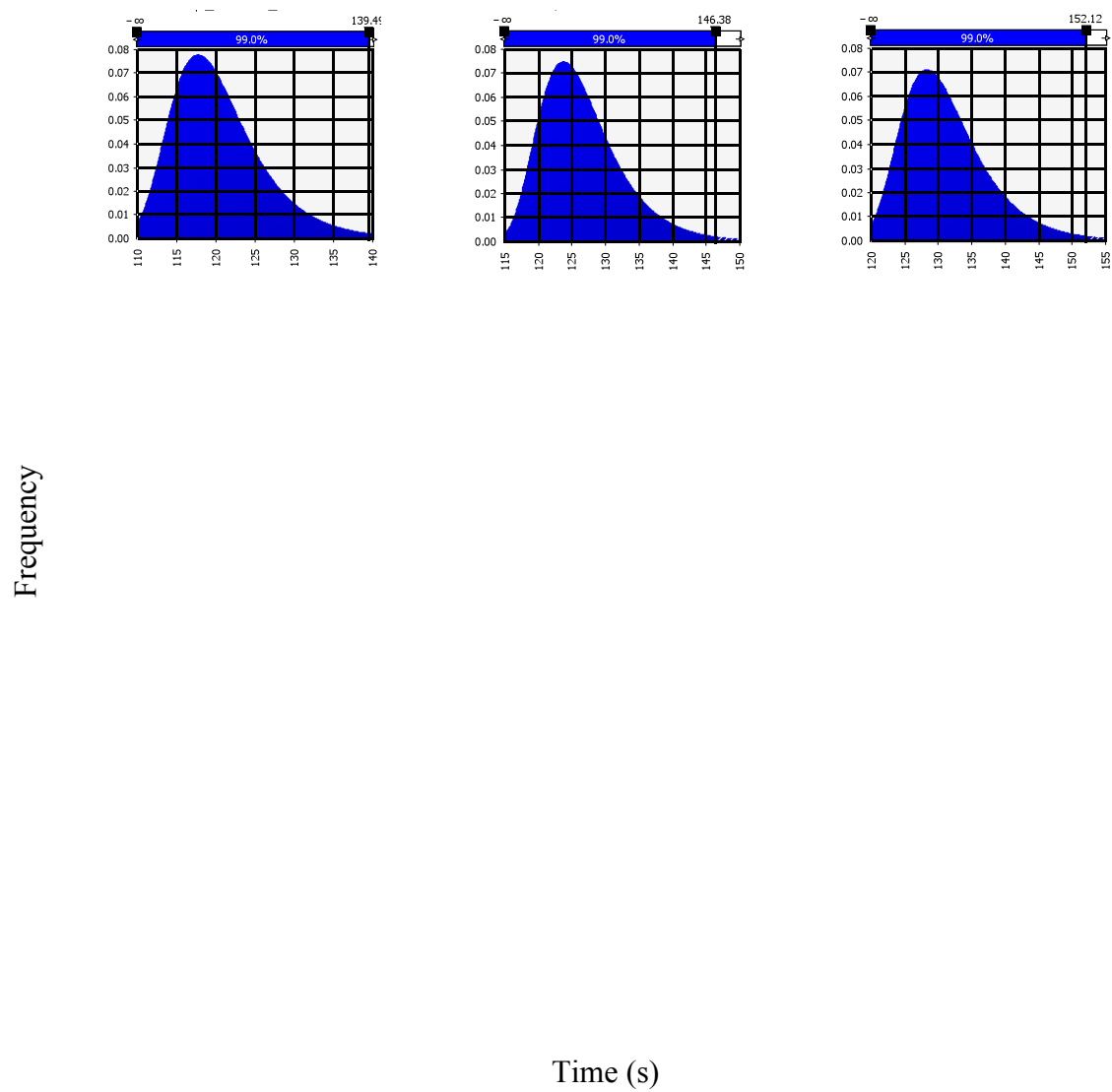


Fig. 2.6

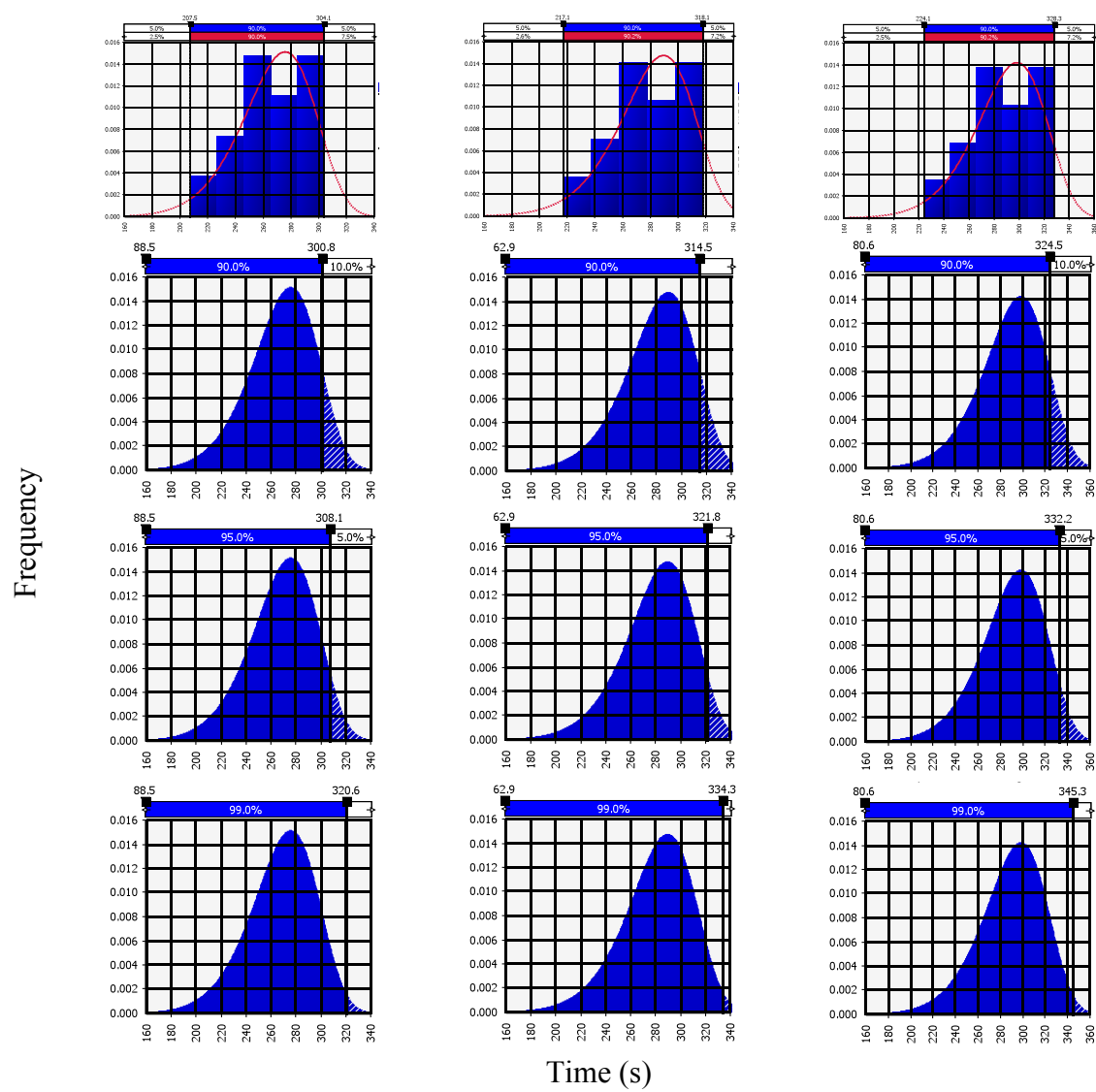


Fig. 2.7

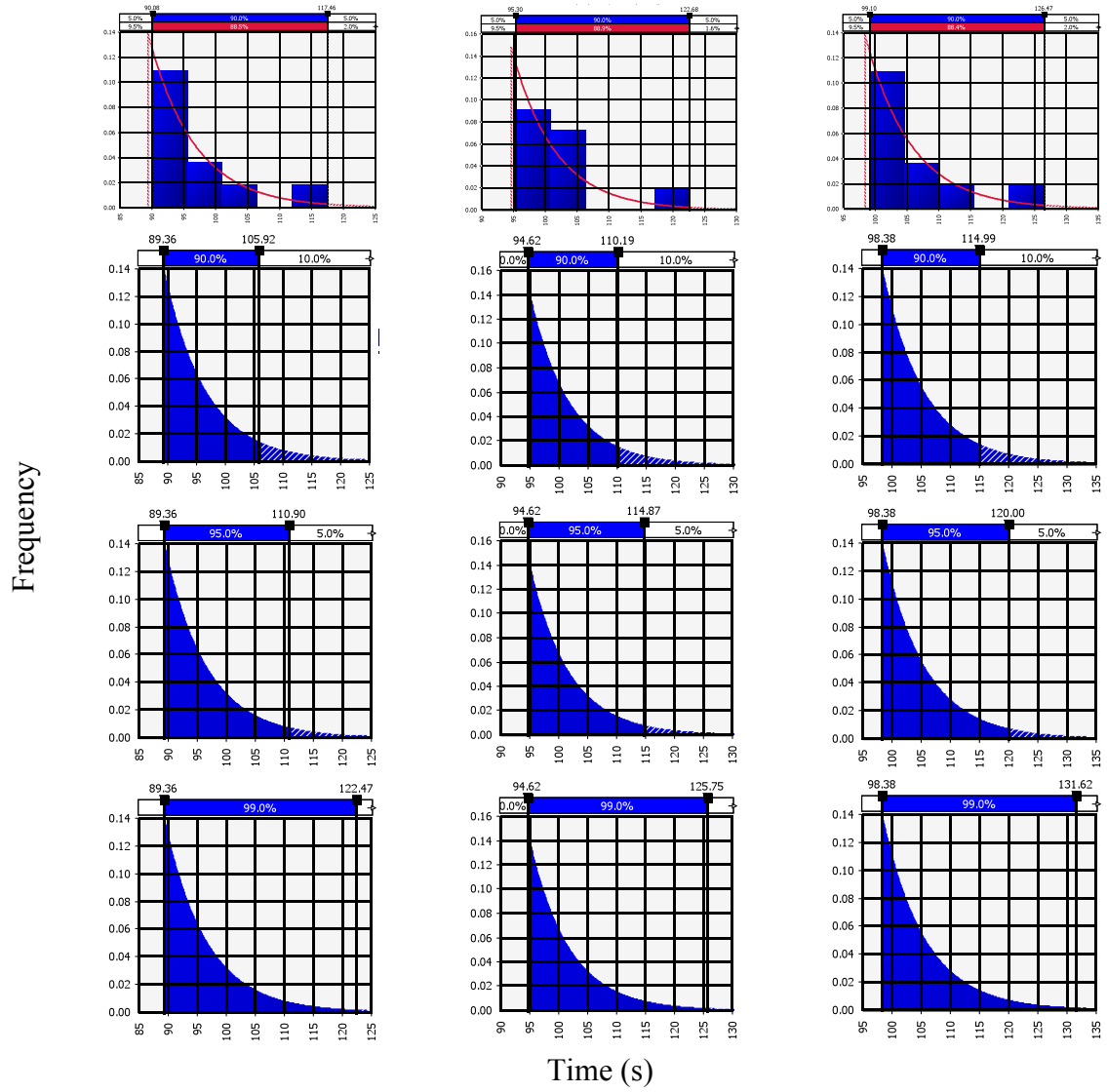


Fig. 2.8

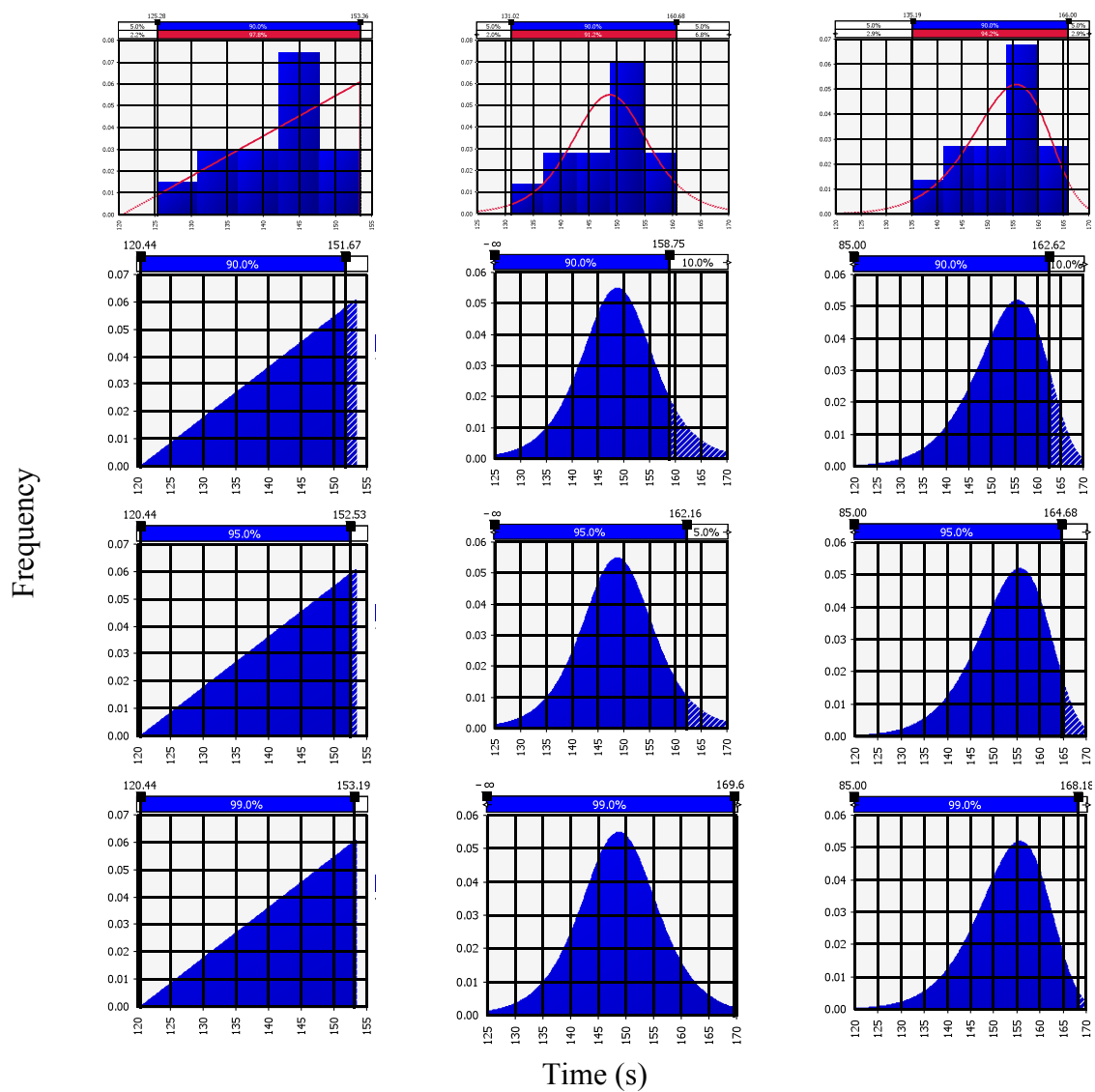


Fig. 2.9

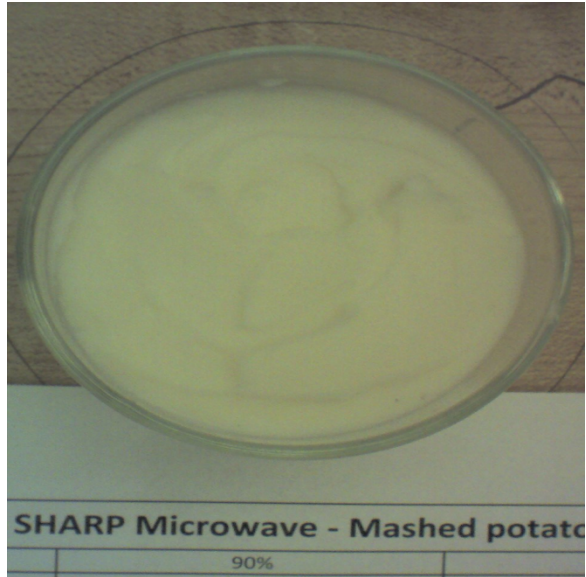


Fig. 2.10





3. CHAPTER 3

6. Development and Validation of Microwave Heating Instructions for Chicken Nuggets

6.1 ABSTRACT

Outbreaks of salmonellosis resulting from the consumption of microwave heated chicken nuggets were attributed to non-uniform heating of these products. Microwave heating instructions were developed using two microwave ovens (2,459 MHz; 700 W and 1,350 W) and various configurations of product placement (chicken nuggets) in the microwave ovens. Temperature profiles of frozen chicken nuggets during heating were obtained by placing different numbers (4, 6 or 8) of chicken nuggets at either the edge or the center of the carousel and heated for specific times for each microwave. Twenty-four temperature profiles for each combination of number (3) and position (2) were obtained and times to reach 70.0, 72.2 and 73.8°C at the geometric center of the chicken nuggets were calculated based on the linear regression of the temperature profiles. Microwave heating times were selected based on the best-fit distributions at 90, 95 and 99% upper confidence limits (UCL) and the chi-square value for the distributions. The adequacy of microwave heating times were validated by heating chicken nuggets inoculated at the geometric center with a five-strain cocktail (*ca.* 7.22 log CFU/g) of *Salmonella* spp. and heated in each microwave for the calculated heating time. Survival of *Salmonella* spp. was determined by plating and enrichment methods subsequent to heating and after 2 min of standing time. For the low power microwave oven, *Salmonella* spp. reductions of 6.56 log CFU/g were observed in chicken nuggets heated in a group of 4 and placed at the center of the carousel with 1 min 26 s of heating time at 99% UCL with an target end point temperature of 73.8°C (final temperature achieved 100.2°C). Longer heating times

(1 min 26 s) resulted in 7.22 log CFU/g *Salmonella* spp. reductions when chicken nuggets were placed in a group of 8. Heating chicken nuggets in a microwave of higher power resulted in similar *Salmonella* spp. reductions ($p>0.05$) when chicken nuggets were placed at the center even with shorter heating times. Incorporation of standing time in addition to the heating time eliminated *Salmonella* spp., regardless of the power of the microwave, location and the number of chicken nuggets.

Key words: Chicken nuggets, *Salmonella* spp., heating instructions and microwave oven.

6.2 Introduction

Salmonella spp. is a major cause of foodborne illness and is estimated to cause 1.0 million cases of illness, 19,587 hospitalizations and 378 deaths in the United States annually (Scallan et al., 2011). *Salmonella* spp. is a Gram-negative, rod shaped bacterium with over 2,500 different serovars (Brenner et al., 2000). *Salmonella* spp. is widespread in the environment and is commonly isolated from swine, dairy, beef, and poultry farm environments (Rodriguez et al., 2006). However, poultry has been identified as a major reservoir of this pathogen as it has been commonly isolated from fresh poultry and poultry products. Jorgensen et al. (2002) reported prevalence of *Salmonella* to be 25% in whole chickens and can be present at a concentration of 3.80 and 4.50 log CFU. Brichta-Harhay et al. (2007) estimated *Salmonella* population of 1.56 log CFU/mL on poultry carcasses. Typically, *Salmonella* spp. is eliminated in ready-to-eat poultry products (RTE) during cooking following conventional heating methods (Juneja et al., 2001). The USDA-FSIS performance standards require a 7.0 log reduction of *Salmonella* spp. in RTE poultry products; the target temperatures to achieve this log reduction is 73.8°C (165°F; USDA-FSIS, 1999). Although the time-temperature schedules recommended by the USDA-FSIS compliance guidelines are effective for the elimination of *Salmonella* spp. using conventional heating methods in RTE poultry products, those time-temperature schedules may not be applicable for microwaveable, but not-ready-to-eat (NRTE) food products due to non-uniform temperature distribution leading to cold and hot spots resulting in the survival of *Salmonella* spp.

Not ready-to-eat foods are combinations of meat or poultry with other components such as vegetables in which at least one of the components ingredients has not received

adequate heat treatment for the elimination of pathogenic bacteria (GMA, 2008). Raw, frozen chicken nuggets and chicken strips have been identified as sources of foodborne pathogens such as *Salmonella* spp. (Bucher et al., 2007). Consumption of these products has been associated with *Salmonella* spp. infections when heated in microwave ovens. Foodborne illness outbreaks associated with the consumption of NRTE chicken nuggets and other chicken products have occurred in Australia, Canada and the United States (Kenny, Hall and Cameron, 1999; MacDougall et al., 2004; Smith et al., 2008). Improper microwave heating by the consumer and inadequate heating times recommended on the package have been identified as the cause of these outbreaks.

Microwave ovens have been traditionally used for reheating foods (Heddleson et al., 1994) and are not designed for the preparation or cooking the NRTE food products (Smith et al., 2008). The main advantage of microwave heating is the higher heating rate compared to conventional heating. However, heating with microwaves could result in non-uniform temperature distribution in the food product (Vadivambal and Jayas, 2010) which results in the formation of hot and cold spots that may allow the survival of pathogenic bacteria such as *Salmonella* spp. Pucciarelli and Benassi (2005) reported that heating raw poultry in a microwave oven resulted in differences in temperature profiles at different locations of the product (under the skin and 1.5 cm inside the chicken thigh). These differences in temperatures could result in the survival of *Salmonella* spp. in food products heated in a microwave oven.

The objective of this study is to develop science based heating instructions for microwave heating of chicken nuggets and validate the instructions using microbial challenge studies

to analyze *Salmonella* spp. destruction as affected by the number and configuration of chicken nuggets and the power of microwave oven.

6.3 Materials and Methods

Microwave ovens: Two household microwave ovens (2,450 MHz frequency) were used in this study: (1) low power (700 W; Model R-9470, SHARP Electronics, Mahwah, NJ) and (2) high power (1,350 W; Model JE51451DN1BB, General Electric Co., Louisville, KY).

Determination of microwave heating times: Commercial frozen breaded chicken nuggets (Tyson) with an average weight of 20.7 ± 0.3 were obtained from a local grocery store and stored frozen (-11°C) until use. Each chicken nugget was drilled from the side to the geometric center of the chicken nugget using a mechanical drill (Model 47158, Central Machinery, Camarillo, CA). Twenty-four temperature profiles were collected for each microwave oven by individually heating the chicken nuggets in groups of 4, 6 or 8 (placed on paper towels) placed either at the edge of the carousel or at the center. The heating time was started when at least two chicken nuggets reached a temperature of -9°C . Product temperature was measured during heating by placing fiber optic probes (1.1 mm, Model T1S-2M. FOT, FISO Technologies Inc., Quebec, Canada) through the holes made in at least 2 chicken nuggets. Chicken nuggets were heated until every probe recorded a maximum temperature of 90°C . Time to reach 70.0, 72.2 and 73.8°C at the geometric center of the chicken nuggets was calculated and heating times were selected based on 90, 95 and 99% upper confidence limits (UCL) of the best-fit distribution and chi-square value (@Risk program, Ver. 5.7, Palisade Corporation, Ithaca, NY). Infrared

imaging camera (Model # SC640, FLIR Systems, North Billerica, MA) was used to obtain images of the product after heating.

Bacterial culture: Five different *Salmonella* spp. serotypes/strains (FSIS, *Salmonella* Thompson; CDC, *Salmonella* Enteritidis, phage type 4 (H3502); FSIS, *Salmonella* Hadar; *Salmonella* Enteritidis B2; *Salmonella* Enteritidis 11) were used. The cultures were maintained as glycerol stocks at -80°C. Before each experiment, each serotype/strain was thawed at room temperature and grown individually in tryptic soy broth (TSB, Becton, Dickinson and Co., Sparks, MD) and incubated for 24 h at 35°C. Two subsequent transfers into fresh TSB were performed every 24 h. Five mL from each culture were transferred into a sterile centrifuge tube and mixed with the other serotypes/strains using a vortex (final volume of 25 mL). The cocktail was centrifuged for 10 min at 4°C at 6,000×g (Model GS-15R; Beckman Instruments, Palo Alto, CA). The supernatant was discarded and the cells in the pellet were re-suspended in 5 mL of sterile water.

Inoculum preparation and inoculation: The chicken nuggets were thawed in a refrigerator (10°C) and 20 µL of the *Salmonella* spp. cocktail was injected from the side of the chicken nuggets to the geometric center of the product to obtain an initial population of either 7.0 log CFU/g (high inoculum level) or 3.0 log CFU/g (low inoculum level). To prevent the dispersion of *Salmonella* spp. cells outside the geometric center, the chicken nuggets were inoculated by inserting a Glass Syringe for Chromatography (NS Target, Rockwood, TN) into the geometric center of the chicken nuggets through a wider needle that was previously inserted in the product (3 mm before the geometric

center). After inoculation, chicken nuggets were refrozen and heated in the microwave oven as described below.

Microwave heating of chicken nuggets: Chicken nuggets were placed in groups of 4, 6 or 8 either at the center or edge of the microwave carousel and heated for times obtained from the heating profiles. Each group included two inoculated chicken nuggets placed equidistant from each other. During heating, product temperature was measured by placing a fiber optic probe at the geometric center of the non-inoculated chicken nuggets as described. Standing time was applied to treatments (99% UCL, target temperature 73.8°C) that resulted in the survival of *Salmonella* spp. and with a standard deviation ≥ 1.0 log CFU/g *Salmonella* spp. reductions. When *Salmonella* spp. survival was observed subsequent to heating and the standing time, the chicken nuggets were inoculated at lower levels (3 log CFU/g) and heated to evaluate the potential elimination of *Salmonella* spp. in the chicken nuggets. The heating times for the low inoculum *Salmonella* spp. level consisted of the longest times (end target temperatures of 73.8 with an UCL of 99%) of either the edge or center positions for chicken nuggets placed in groups of 4 or 8. The chicken nuggets were heated in the low and high power microwave followed by standing time (2 min) or without standing time.

***Salmonella* spp. enumeration and enrichment:** After heating, two chicken nuggets (high inoculum) were transferred to a filter bag (BagFilter, Spiral Biotech, Norwood, MA) containing 40 mL of chilled 0.1% buffered peptone water (BPW, Becton, Dickinson and Co., Sparks, MD) to stop the lethality of *Salmonella* spp. After cooling, samples were homogenized for 2 min in a stomacher (NEUTEC, Albuquerque, NM), serially diluted in BPW and plated on tryptic soy agar (TSA; Becton, Dickinson and Co., Sparks, MD) and

TSA supplemented with ferric ammonium citrate (0.4 g/0.5 L; Fisher Scientific, Fair Lawn, NJ) and sodium thiosulfate (3.4 g/0.5 L; Fisher Scientific, Fair Lawn, NJ). Typical *Salmonella* spp. colonies were enumerated and reported as log CFU/g after incubation for 24 h at 35°C. The bag containing the BPW and the sample was incubated for 18 h at 35°C for enrichment. Aliquots (100 µL) from the enriched samples were transferred into Rappaport Vassiliadis media (10 mL; RV, Becton, Dickinson and Co., Sparks, MD) and incubated for 18 h at 35°C. A loop from the RV tubes showing turbidity was streaked into xylose Lysine deoxycolate agar (XLD, Becton, Dickinson and Co., Sparks, MD) and the plates were incubated for 18 h at 35°C. XLD plates showing typical *Salmonella* spp. colonies were considered positive for the organism. Three independent replications as identified by day of sample preparation, *Salmonella* spp. cocktail and lots of chicken nuggets were conducted for each experiment.

Water activity, moisture and pH measurement: Three chicken nuggets were homogenized with 25 mL of deionized water for 1 min in a stomacher, and the pH of each sample was measured by immersing the pH electrode (Accumet-Basic/AB15, Fisher Scientific, Bridgewater, NJ) in the sample homogenate. The water activity of the chicken nuggets was measured using an Aqua Lab 3TE water activity meter (Decagon Devices, Inc., Pullman, WA) following the manufacturer's instructions. The moisture analysis was performed by weighing 5 g of minced chicken nugget in an aluminum dish and dried using a heating oven (FD 53, BINDER, Bohemia, NY) with mechanical convection for 18 h at 100°C following the AOAC method.

6.4 Results and Discussion

The pH and water activity (a_w) of chicken nuggets were 6.53 ± 0.06 and 0.98 ± 0.01 , respectively, with moisture content of 62.54 ± 3.68 . Blackburn et al. (1997) reported that the optimum pH for the survival of *Salmonella* spp. ranges from 5 to 7. The pH value reported for turkey breast is *ca.* 6.09 and is similar to the pH of the chicken nuggets (Owens et al., 2000). Although the low a_w and moisture content of the chicken nuggets were low, water activity level as low as (0.500) can support the survival of *Salmonella* spp. in food products (Shachar and Yaron, 2006).

Heating times to achieve target temperature of 73.8°C at different UCL for the low and high power microwave oven are presented in Table 3.1. Longer heating times were needed for chicken nuggets heated in the low power microwave oven compared to the high power microwave oven. Pitchai et al. (2012) reported that microwave ovens with higher rated power result in faster heating rates compared to lower rated power microwave ovens. Longer heating times were needed when the product was placed at the center of the turntable in all configurations of the product (except for chicken nuggets placed in groups of 4) when heated in the low power microwave oven. Similar trend was observed for the high power microwave oven with longer heating times needed for chicken nuggets placed at the center of the microwave turntable compared to those placed on the edges of the turntable. Pitchai et al. (2012) also reported that the ideal location for heating foods in the microwave oven is at the edge of the turntable and not at the center as this position results in a faster and more uniform heating. The number of chicken nuggets present in the microwave oven also affected the heating times to reach a specific target temperature. Increasing the number of chicken nuggets placed in a microwave

oven resulted in longer heating times for both the low and high power microwave ovens. Ayappa and Davis (1992) reported that the power absorbed and the distribution uniformity during microwave heating is better for smaller samples compared to larger samples. Vilayannur et al. (1998) also reported that the volume of the product affects microwave heating, with higher volumes resulting in longer heating times compared to smaller volume products to reach target temperatures.

The recommended microwave heating instructions for the commercial chicken nuggets were between 1 min and 1.5 min or between 2 min and 2.5 min for heating 5 or 10 chicken nuggets, respectively. In this study, increasing the number of chicken nuggets to 8 resulted in longer heating times to achieve the target temperature (73.8°C) than the ones recommended time for 10 chicken nuggets. The heating times for 8 chicken nuggets were 3 min and 11 s and 2 min and 28 s for the low and high power microwave ovens, respectively for achieving the target temperature of 73.8°C. These results indicate that the heating instructions for heating 10 chicken nuggets may not be sufficient to heat the product thoroughly and may result in the survival of *Salmonella* spp. This may increase the food safety risk if the consumer does not use the appropriate microwave oven.

Heating 4 chicken nuggets (high inoculum) in the low power microwave oven to a target temperature of 73.8°C (99% UCL) resulted in *Salmonella* spp. log reductions of 6.56 ± 1.03 and 5.59 ± 1.50 CFU/g when the chicken nuggets were heated in position A (edge) and B (center), respectively (Figs. 3.1 and 3.2). However, increasing the number of chicken nuggets to 8 resulted in *Salmonella* spp. log reductions of 6.32 ± 1.43 log CFU/g and >7.22 log CFU/g for positions A and B, respectively after heating (73.8°C) (Figs. 3.3

and 3.4). Similarly, heating 8 chicken nuggets to reach a target temperature of 73.8°C (99% UCL) using the high power microwave oven resulted in *Salmonella* spp. log reductions of >7.22 and 6.72 ± 0.63 CFU/g when the chicken nuggets were heated in position A (edge) and B (center), respectively (Figs. 3.5 and 3.6). Heating the chicken nuggets in groups of 4, 6 or 8 resulted in temperatures $\geq 100^{\circ}\text{C}$, sufficient for the destruction of the *Salmonella* spp. in meat and poultry products. Thermal inactivation of *Salmonella* spp. in ground chicken breast and liquid medium (0.1% peptone) has been reported previously (Murphy et al., 2000). The D-values at 70.0°C for *Salmonella* spp. were 0.23 min and 0.15 min for chicken meat breast and liquid medium, respectively (Murphy et al., 2000). Mazzota (2000) also reported a D-value at 63°C of 0.18 min for *Salmonella* spp. in ground chicken breast. Thermal inactivation of *Salmonella* spp. reported in literature has been traditionally conducted under isothermal conditions, which may not be applicable for foods heated in microwave ovens. *Salmonella* spp. survival in chicken nuggets heated in a microwave oven might be explained by the non-uniform temperature distribution in the product. The uniformity of temperature distribution in foods heated by microwaves is affected by factors such as the dielectric properties of the food, frequency and power of the incident microwave energy, and the geometry and dimensions of the product (Vilayannur and Anantheswaran, 1998). Although temperatures above 100°C were observed in the chicken nuggets, it is possible that *Salmonella* spp. was able to survive in areas (microscopic) where lethal temperatures were not achieved uniformly throughout the product. Hedderson and Doores (1994) reported that *Salmonella* spp. destruction was achieved when milk or beef broth were heated to 68 and 70°C , respectively, and stirred after the heat treatment, whereas

Salmonella spp. survival was observed in the products that were not stirred. This indicates that heating food in a microwave oven may result in the survival of pathogenic bacteria when uniform temperatures are not achieved in the food products.

Including standing time (2 min) after heating was not sufficient for elimination of *Salmonella* spp. in chicken nuggets (high inoculum) regardless of the power of the microwave oven used (Table 3.2). To simulate the performance of microwave heating under real life scenario, the destruction of *Salmonella* spp. using a lower inoculum level was evaluated. *Salmonella* spp. reductions of >3.00 log CFU/g were observed in all the chicken nuggets heated in the low and high power microwave ovens. However, *Salmonella* spp. survival was observed subsequent to microwave heating. Heating the chicken nuggets in a group of 4 without standing time using the low power microwave oven resulted in the survival of *Salmonella* spp. after 1 min 40 s of heating. Increasing the number of chicken nuggets to 8 with longer heating time (3 min 7 s) resulted in the elimination of *Salmonella* spp. in the chicken nuggets even without standing time. In the case of the high power microwave, the position of the chicken nuggets in the microwave oven affected the survival of the pathogen after the standing time. Heating of chicken nuggets placed in position A (edge) resulted in *Salmonella* spp. survival, whereas the microorganism was eliminated when the chicken nuggets were placed near the center of the microwave plate.

Other food characteristics such as the lower water content in the product may have led to the survival of *Salmonella* spp. in chicken nuggets. Water is a major component of most

food products and the component responsible for heating due to its dipolar nature (Oliveira and Franca, 2002). Low water activity and/or moisture content of the food products have been associated with the higher in heat resistance of *Salmonella* spp. (Podolak, et al., 2010). This phenomenon occurs due to the low water content that results in the protection of bacterial cells from thermal injury (Mattick et al., 2001).

Color images of chicken nuggets were taken after heating the chicken nuggets in the low and high power microwave ovens (Figs. 3.10 and 3.11). Eight chicken nuggets were heated in intervals of 30 s for a total time of 3 min 30 s to evaluate the quality (color) of the chicken nuggets. As heating times increased, the color of the chicken nuggets changed from light brown to a darker brown color. The color change was more pronounced for the chicken nuggets heated in the low power microwave oven where the chicken nuggets tend to shrink resulting in a harder product. As the final heating time used (3 min 30 sec s) is shorter than the one necessary for the elimination of *Salmonella* spp. in chicken nuggets (3 min and 7 s) in the low power microwave oven, this data may be useful for the selection of proper microwave heating time and development of instructions as long heating times (needed for *Salmonella* spp. destruction) may affect the quality of the product.

6.5 Conclusions

Microwave heating instructions were developed and validated for chicken nuggets heated in low and high power microwave ovens. Position of the chicken nuggets, number and the power of the microwave oven affected *Salmonella* spp. destruction. Inclusion of standing time subsequent to heating resulted in the elimination of *Salmonella* spp.

Microwave heating instructions on the packages may not be sufficient to eliminate *Salmonella* spp. and methods to develop package instructions should be revised, followed by microbial challenge studies to verify the adequacy of the heating instructions to assure the food safety and quality of the product.

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6.7 List of tables

Table 3.1. Heating times of chicken nuggets placed in the low or high power microwave ovens at two different positions to achieve an end point temperature of 73.8°C at a 90, 95 and 99% upper confidence limit (UCL).

Number of chicken nuggets	Position	Heating times of chicken nuggets heated in a low and high microwave oven (s) at three UCL (%)					
		90		95		99	
		LP	HP	LP	HP	LP	HP
4	Edge	84	63	89	65	100	68
4	Center	83	69	84	73	86	79
6	Edge	104	82	110	83	119	84
6	Center	127	92	132	97	143	111
8	Edge	124	105	126	110	130	120
8	Center	157	121	166	130	187	148

Table 3.2. *Salmonella* spp. survival after heating the chicken nuggets (high inoculum level) subsequent to standing time (2 min).

Number of chicken nuggets	Position	Heating times of chicken nuggets heated in a low or high microwave oven (s) at a 99% UCP		
		Power of the Microwave	Upper Confidence Limit (99% UCL)	Number of positive samples after heating
4	Edge	Low	99	6/6
4	Center	Low	99	5/6
8	Edge	Low	99	6/6
8	Center	High	99	4/6

Table 3.3. *Salmonella* spp. destruction in chicken nuggets with a low inoculum level (3.0 log CFU/g) applying the longest heating time for each location.

Number of chicken nuggets	Position	Low power microwave oven		High power microwave oven	
		Without ST	With ST	Without ST	With ST
4	Edge	3/3 ^a	0/3 ^a	3/3 ^c	2/3 ^c
4	Center	3/3 ^a	0/3 ^a	3/3 ^c	0/3 ^c
8	Edge	0/3 ^b	0/3 ^b	3/3 ^d	3/3 ^d
8	Center	0/3 ^b	0/3 ^b	3/3 ^d	0/3 ^d

a: Heating time of 1 min 40 s; b: Heating time of 3 min 7 s; c: Heating time of 1 min 19 s; d: Heating time of 1 min 28 s.

6.8 Legend to the Figures

Fig. 3.1. *Salmonella* spp. log reductions and final temperatures achieved after heating 4 chicken nuggets at position A (edge) and B (center) in a low power (LP) microwave. *Salmonella* spp. log reduction CFU/g; end point temperature of the chicken nugget; n=6.

Fig. 3.2. *Salmonella* spp. log reductions and final temperatures achieved after heating 8 chicken nuggets at position A (edge) and B (center) in a low power (LP) microwave. *Salmonella* spp. log reduction CFU/g; end point temperature of the chicken nugget; n=6.

Fig. 3.3. Fig. 3.3. *Salmonella* spp. log reductions and final temperatures achieved after heating 8 chicken nuggets at position A (edge) and B (center) in a high power (HP) microwave. *Salmonella* spp. log reduction CFU/g; end point temperature of the chicken nugget; n=6.

Fig. 3.4. Distribution of microwave heating times for chicken nuggets heated in a group of 4 in position A (edge; left side) and B (center; right side) to a target temperature of 73.8°C using the low power microwave oven with an upper confidence limit of 99%: with the data points collected (above) and calculated time to achieve 73.8°C at a geometric center of the nugget (below).

Fig. 3.5. Distribution of microwave heating times for chicken nuggets heated in a group of 6 in position A (edge; left side) and B (center; right side) to a target temperature of 73.8°C using the low power microwave oven with an upper confidence limit of 99%: with the data points collected (above) and calculated time to achieve 73.8°C at a geometric center of the nugget (below).

Fig. 3.6. Distribution of microwave heating times for chicken nuggets heated in a group of 8 in position A (edge; left side) and B (center; right side) to a target temperature of 73.8°C using the low power microwave oven with an upper confidence limit of 99%: with the data points collected (above) and calculated time to achieve 73.8°C at a geometric center of the nugget (below).

Fig. 3.7. Distribution of microwave heating times for chicken nuggets heated in a group of 4 in position A (edge; left side) and B (center; right side) to a target temperature of 73.8°C using the high power microwave oven with an upper confidence limit of 99%: with the data points collected (above) and calculated time to achieve 73.8°C at a geometric center of the nugget (below).

Fig. 3.8. Distribution of microwave heating times for chicken nuggets heated in a group of 6 in position A (edge; right side) and B (center; left side) to a target temperature of 73.8°C using the high power microwave oven with an upper confidence limit of 99%: a) with the data points collected b) calculated time to achieve 73.8°C at a geometric center of the nugget.

Fig. 3.9. Distribution of microwave heating times for chicken nuggets heated in a group of 8 in position A (edge; left side) and B (center; right side) to a target temperature of 73.8°C using the high power microwave oven with an upper confidence limit of 99%:

with the data points collected (above) and calculated time to achieve 73.8°C at a geometric center of the nugget (below).

Fig. 3.10. Color images of chicken nuggets heated in the low power microwave oven. Upper row from left to right: 0, 30, 60 and 90 s of heating. Lower row from left to right: 120, 150, 180 and 210 s of heating.

Fig. 3.11. Color images of chicken nuggets heated in the high power microwave oven. Upper row from left to right: 0, 30, 60 and 90 s of heating. Lower row from left to right: 120, 150, 180 and 210 s of heating.

Fig. 3.1

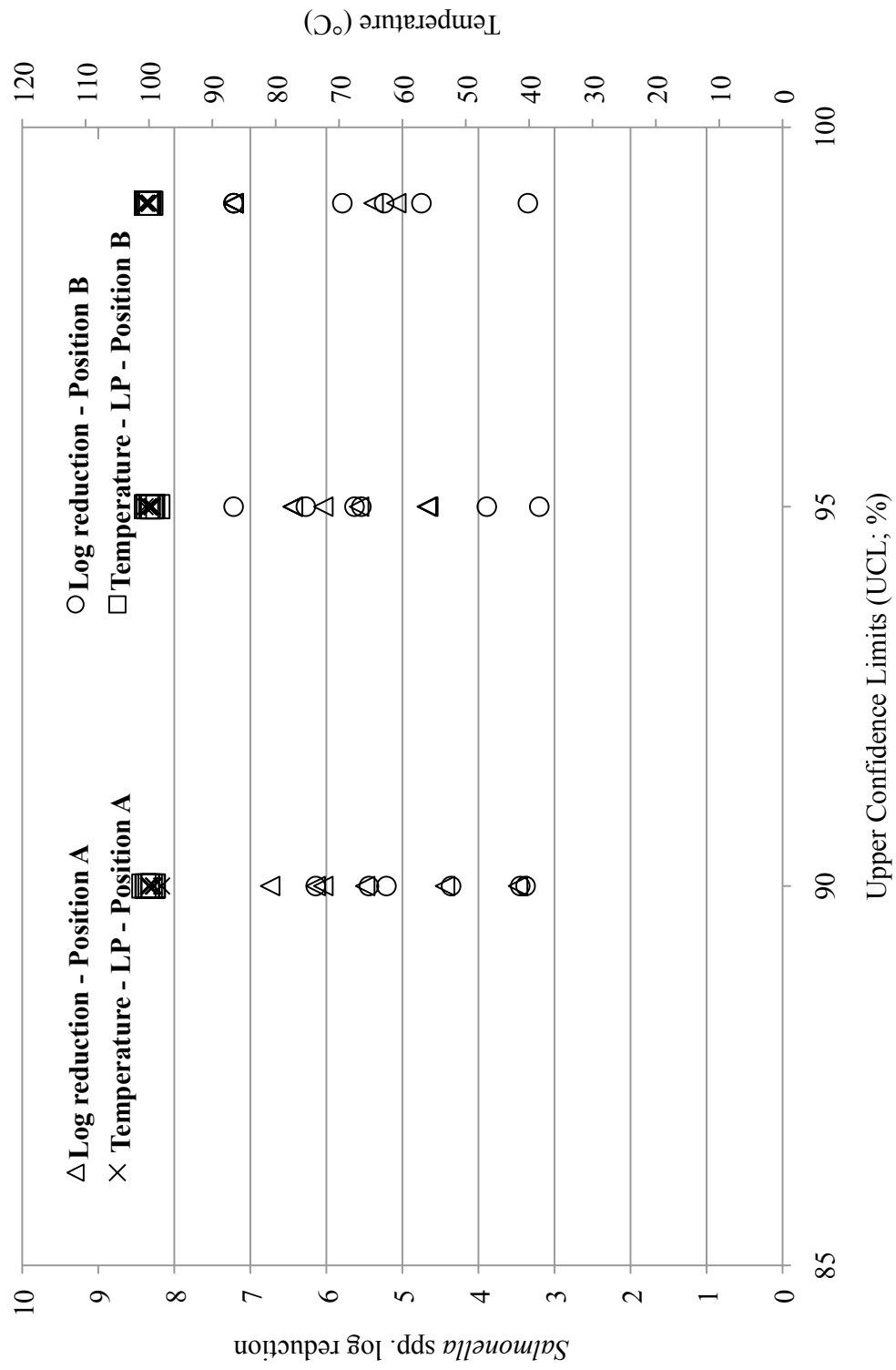


Fig. 3.2

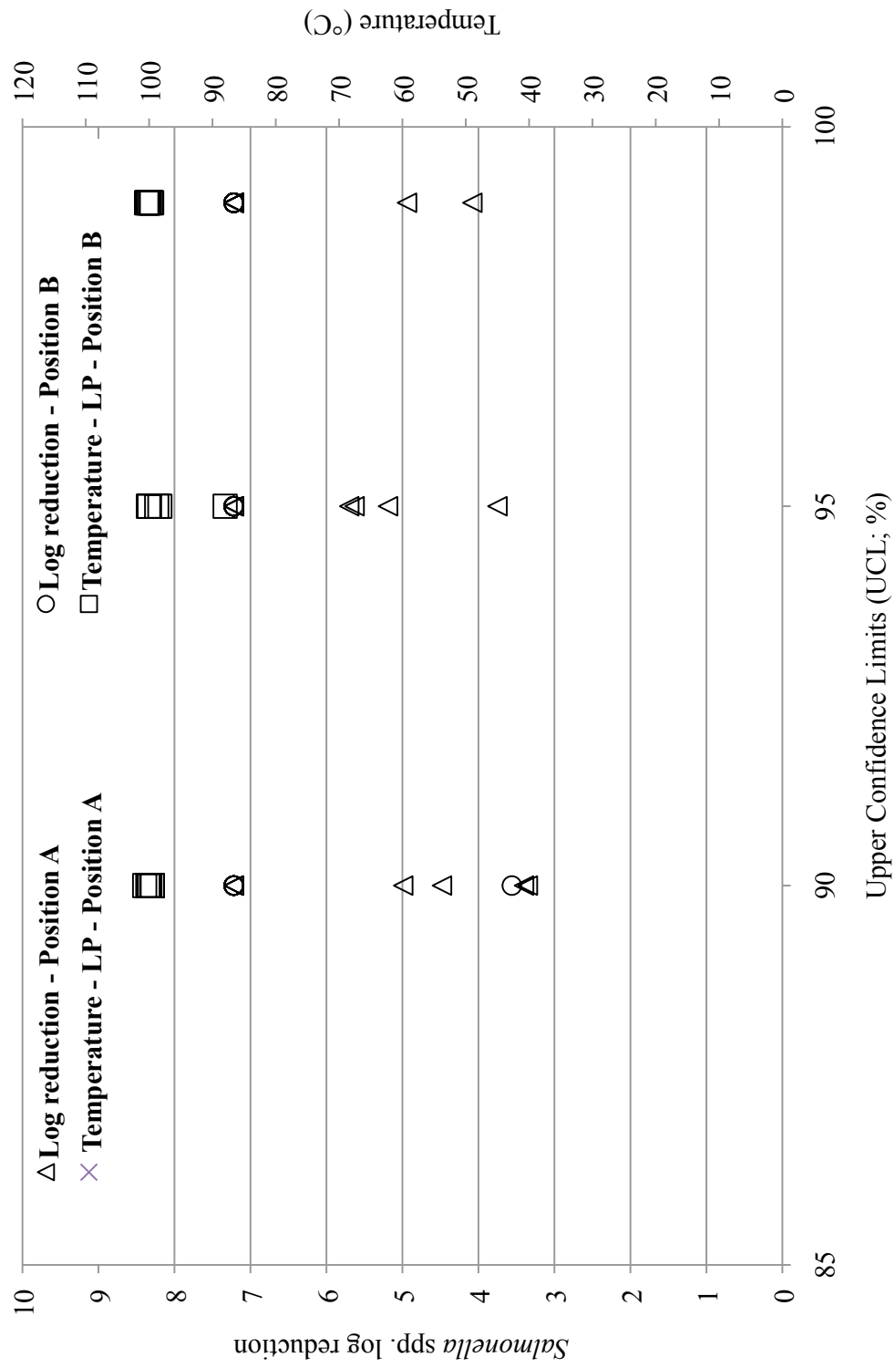


Fig. 3.3

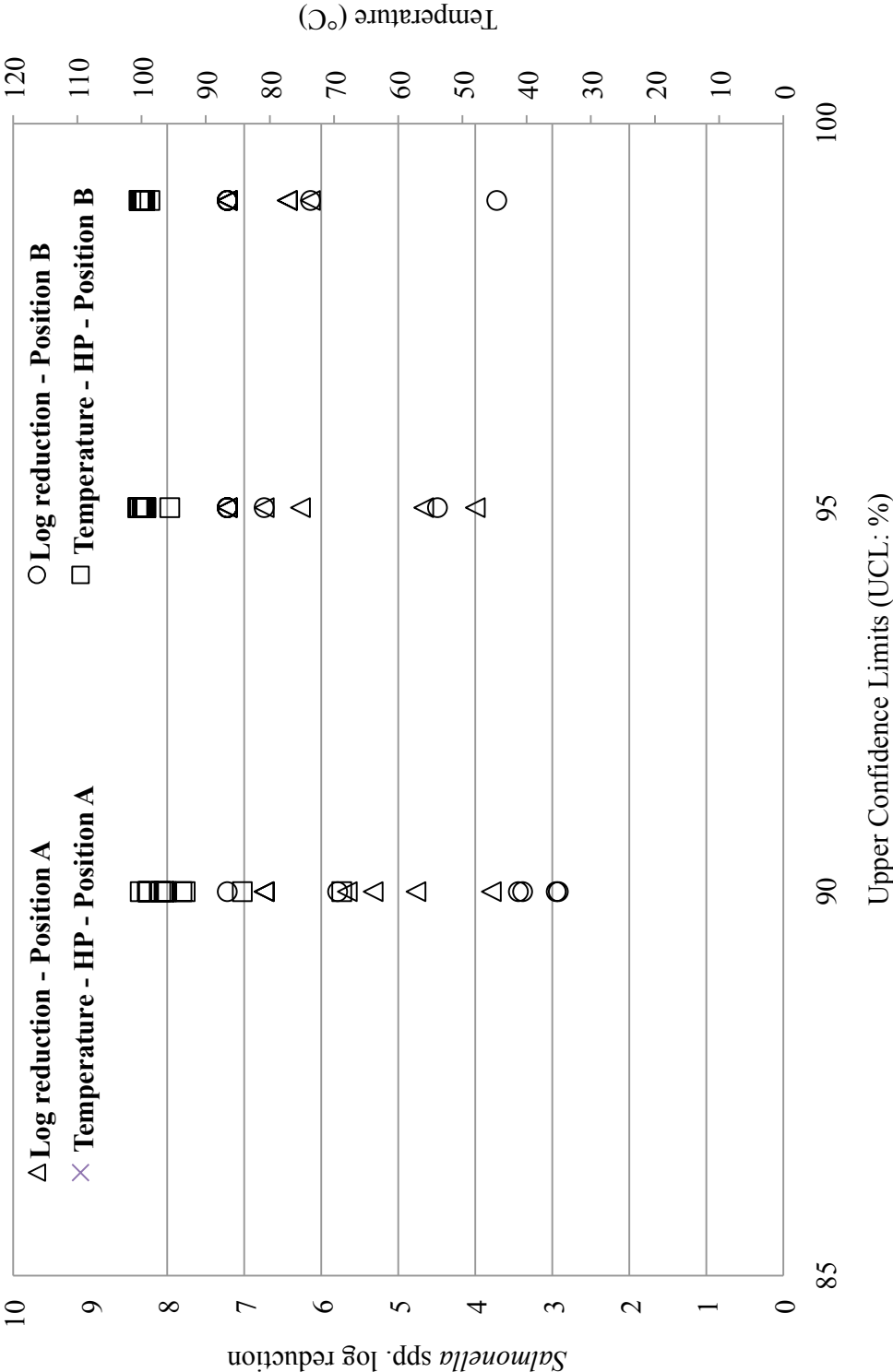
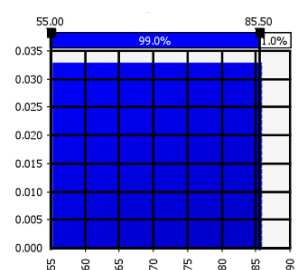
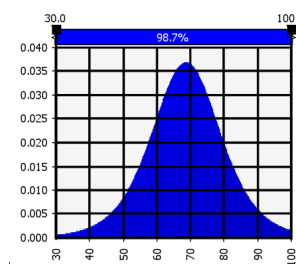


Fig. 3.4

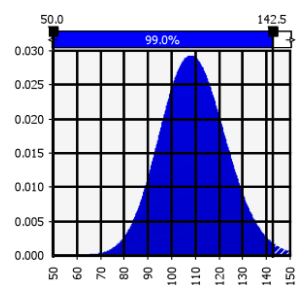
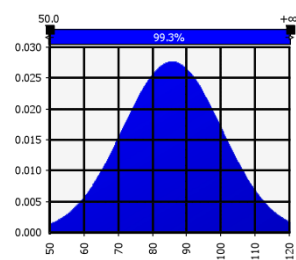
Frequency



Time (s)

Fig. 3.5

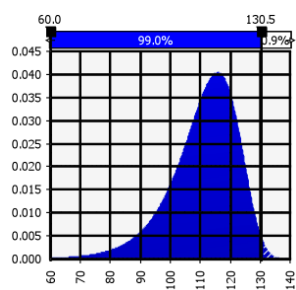
Frequency



Time (s)

Fig. 3.6

Frequency



Time (s)

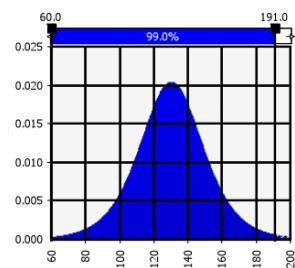
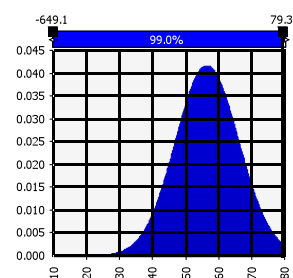
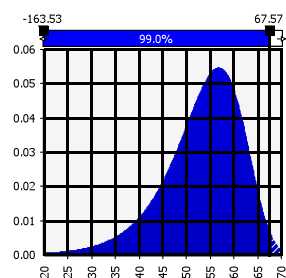


Fig. 3.7

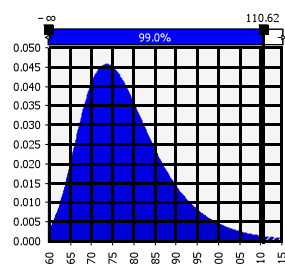
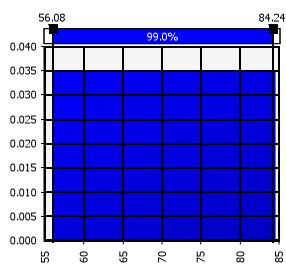
Frequency



Time (s)

Fig. 3.8

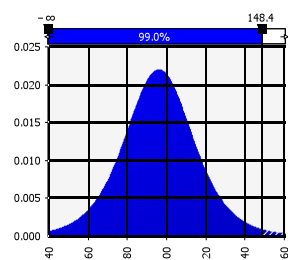
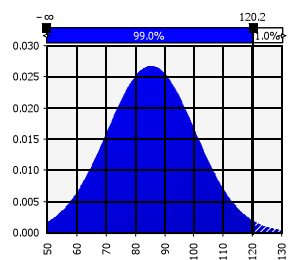
Frequency



Time (s)

Fig. 3.9

Frequency



Time (s)

Fig. 3.10

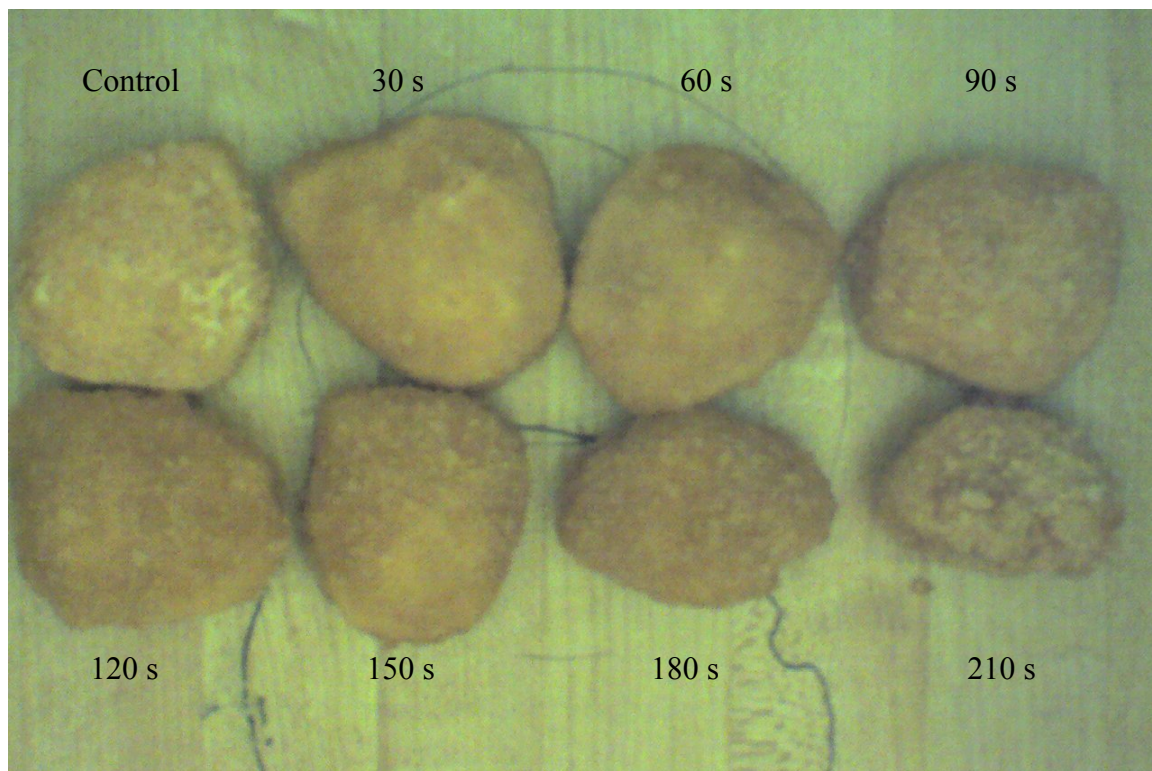
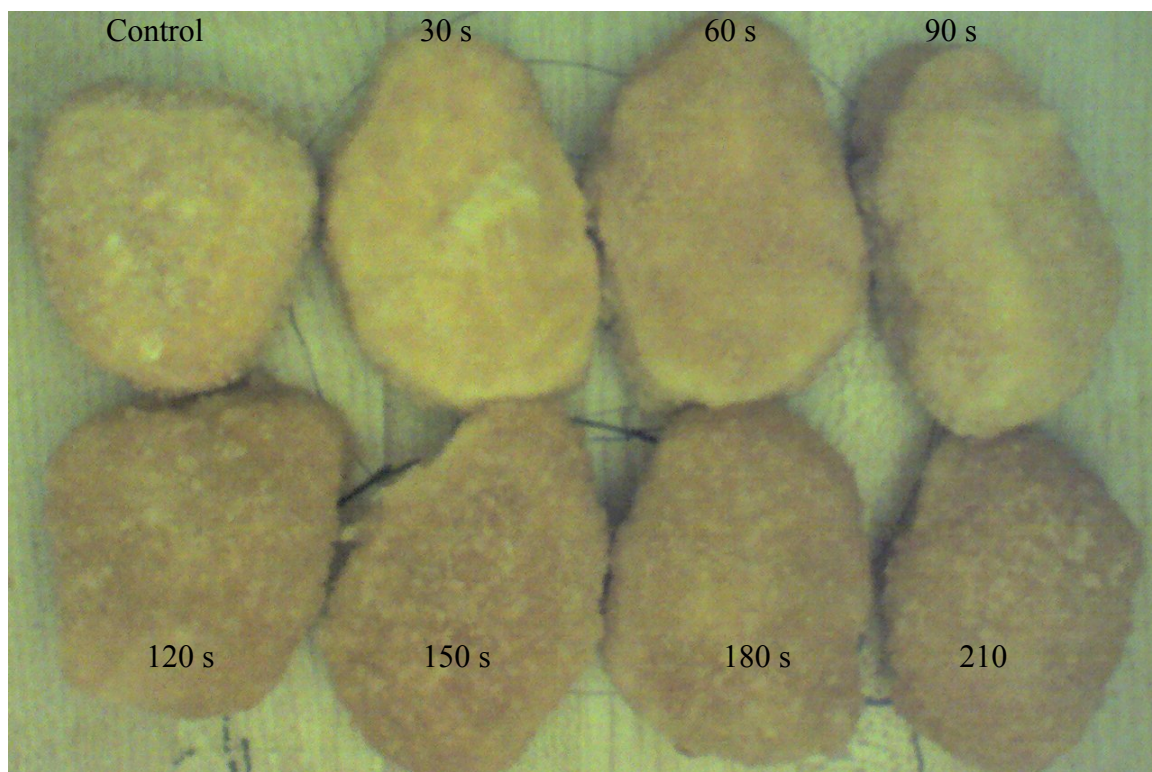


Fig. 3.11



4. Chapter 4

7. Development and Validation of Microwave Heating Instructions for Pot-Pies to Assure Food Safety

7.1 ABSTRACT

Not-ready-to-eat (NRTE) microwaveable foods have been associated with salmonellosis outbreaks. Current method for development of microwave heating instructions may have not been adequate to assure elimination of *Salmonella* spp. Microwave heating instructions were developed based on end point temperatures obtained at multiple locations in the product subsequent to heating. The heating instructions were validated using microbial challenge studies. Two household microwave ovens (2,450 MHz frequency) of low (700 W) and high (1,350 W) power were used. For each oven, twenty-four individual temperature profiles were obtained by heating turkey pot-pies. Time to reach 73.8°C was calculated for each pot-pie and end point microwave heating times were selected based on the best-fit distribution based on chi-square value at 99% upper confidence limit (UCL). Validation of the microwave heating instructions was conducted by inoculating the turkey pot-pies with a five-strain *Salmonella* spp. cocktail to attain *ca.* 5.0 log CFU/g at the geometric center (filling) and on the crust (center or edges). Destruction of *Salmonella* spp. was determined subsequent to the heating time and after 3 min of standing time. *Salmonella* spp. populations were enumerated and enrichment method was followed for samples with *Salmonella* spp. population below the detection limit. *Salmonella* spp. reductions of 5.16 log CFU/g were observed following the heating time of 9 min 31 s and 7 min 1 s for the low and high power microwave, respectively, at 99% UCL to attain 73.8°C. Inclusion of a 3 min standing time subsequent to microwave heating resulted in elimination of *Salmonella* spp. in the pot-pies regardless of the power

of the microwave oven. Minimal reduction in *Salmonella* spp. population was observed when two pot-pies were heated in the microwave ovens following the heating times for a single pot-pie. *Salmonella* spp. destruction in microwaveable pot-pies was affected by the location of the inoculum (geometric center vs. crust) in the pot-pie as well as the number of samples placed in the oven. Inclusion of standing times subsequent to heating resulted in elimination of *Salmonella* spp. in the pot-pies.

Key words: Microwave heating, pot-pies, *Salmonella* spp.

7.2 Introduction

Salmonella spp. causes most number of foodborne illnesses (1.0 million), hospitalizations (19,587) and deaths (378) in the United States annually (Scallan et al., 2011). *Salmonella* spp. is a Gram negative, rod-shaped bacterium with over 2,500 different serovars (Brenner et al., 2000) and is a common inhabitant of the gastrointestinal tract of animals and birds and in the environment of poultry farms (Rodriguez et al., 2006), poultry and poultry products being the primary reservoir. Ready-to-eat foods (RTE) undergo a heating lethality process that imparts adequate lethality to reduce *Salmonella* spp. population by ≥ 7 log CFU/g or render the food free of the pathogen (Murphy et al., 2000). However, in not-ready-to-eat (NRTE) products *Salmonella* spp. can survive for longer periods during frozen storage conditions and if not adequately or heat treated can potentially cause foodborne illness.

NRTE food products are defined as foods that contain at least one ingredient that has not received a lethal heat treatment for the elimination of pathogenic bacteria. NRTE products include mixtures of vegetables and poultry meat that can be contaminated with *Salmonella* spp. Performance standards for the production of RTE poultry products (USDA-FSIS, 1999) specify a target *Salmonella* spp. reductions of 7 log for RTE poultry meat (FSIS, 1999). Although time-temperatures schedules for lethality of *Salmonella* spp. are available, they may not be applicable for microwaveable, but NRTE foods as microwave heating does not heat the product uniformly. These NRTE products have been associated with several salmonellosis outbreaks in the United States, Canada, Australia, and other countries. Turkey pot-pies were identified as the source of *salmonellosis* resulting in 401 cases in 41 states in the United States in 2007 (CDC, 2009) resulting from

inadequate microwave heating. Although microwave-heating instructions are part of the main display on the packages and refer to the microwave power, several of the patients were not aware of the power of their microwaves (CDC, 2009). This could have led to the lower product temperatures than required to eliminate *Salmonella* spp. in the product.

Microwave ovens have traditionally been used for reheating or warming cooked, ready-to-eat food products. The advantage of microwave ovens is the shorter time needed to achieve relatively high temperature compared to the conventional ovens. However, a major drawback of microwave heating is the non-uniform temperature distribution in the product (Vadivambal and Jayas, 2010) leading to hot and cold spots in the product and resulting in the survival of *Salmonella* spp. or other pathogens. Additionally, heating patterns of NRTE products could be affected by the power of the microwave oven used. Heating of foods in a low power microwave oven (700 W) resulted in greater degree of non-uniformity in product temperatures compared to higher power microwave ovens (1,100 and 1,200 W; Manickavasagan, et al., 2009). Some NRTE microwaveable foods are a complex mixture of various ingredients and combinations of foods (multi component product) that may interact differently (due to differences in dielectric properties) during microwave heating. This may enhance the non-uniform heating pattern in the food product as well. As a consequence, cold spots may be created, increasing the risk of *Salmonella* spp. survival. Science based methods to develop microwave-heating instructions for products containing multiple ingredients are not available in the literature. The objective of this study was to develop and validate microwave-heating instructions to assure the destruction of *Salmonella* spp. in microwaveable, but NRTE pot-pies.

7.3 Materials and Methods

Microwave ovens: Two household microwave ovens (2,450 MHz frequency) of different power were used in this study: (1) 700 W (Model R-9470, SHARP Electronics, Mahwah, NJ) and (2) 1,350 W (Model JE51451DN1BB; General Electric Co., Louisville, KY).

Determination of microwave heating times: Commercial turkey pot-pies were obtained from a local grocery store and stored frozen (-11°C) until use. Temperature profiles were collected by individually heating twenty-four pot-pies using each microwave. Four holes, 3.1 mm in dia. and 1.25 cm deep from the top of the product (one at the center, one at the top from the center and two on the bottom from the center) were drilled using a mechanical drill (Model 47158, Central Machinery, Camarillo, CA). Product temperature was measured during heating by placing fiber optic probes (1.1 mm, Model T1S-2M, FOT, FISO Technologies Inc., Quebec, Canada) in each hole. Pot-pies were heated until 90°C was recorded at each location. Time needed to reach 73.8°C was calculated based on the regression line for each temperature profile, microwave oven and the heating times were calculated (@Risk program, Ver. 5.7, Palisade Corporation, Ithaca, NY) based on the best-fit distribution at 99% upper confidence limit (UCL). Infrared imaging camera (Model # SC640, FLIR systems, North Billerica, MA) was used to determine surface temperature of the pot-pies after heating.

Bacterial cultures: Five *Salmonella* spp. serotypes/strains (FSIS, *Salmonella* Thompson; CDC, *Salmonella* Enteritidis, phage type 4 (H3502); FSIS, *Salmonella* Hadar; *Salmonella* Enteritidis B2; *Salmonella* Enteritidis 11) were used. The *Salmonella* spp. cultures were maintained as glycerol stocks at -18°C. Before each experiment, each serotype/strain was thawed at room temperature and grown individually in tryptic soy broth (TSB, Becton, Dickinson and Co., Sparks, MD) incubated for 24 h at 35°C. Two subsequent transfers

into fresh TSB were performed every 24 h to obtain cultures in the stationary phase. Five μL from each culture were transferred into a sterile centrifuge tube and mixed with the other strains (final volume of 25 ml). The five strains cocktail was centrifuged for 10 min at 4 °C, 6,000 $\times g$ (Model GS-15R; Beckman Instruments, Palo Alto, CA). The supernatant was discarded and the cell pellet was re-suspended in 500 μL of sterile water.

Inoculum preparation and pot-pies inoculation: Five-hundred μL of the *Salmonella* spp. cocktail were added to 5 g of pot-pie filling to obtain an initial population of *ca.* 5.0 log CFU/g. A small hole (3.1 mm dia.; 1.50 cm deep at the center and three holes 1.25 cm deep (around the center) from the top of the product were drilled as described. Pot-pies were inoculated at the geometric center with the inoculated pot-pie filling (20 μL) to attain an initial *Salmonella* spp. population of *ca.* 5.0 log CFU/g. The crust of the pot-pie was also inoculated at the top center or edges of the product using the inoculated pot-pie filling (liquid part of the mixture) to attain an initial population of 5.0 log CFU/g. After inoculation, pot-pies were stored at -15°C overnight and used the following day.

Microwave heating of pot-pies: Inoculated pot-pies were placed individually at the center of the microwave carousel or in groups of two (the edge of each pot-pie was placed in the center of the microwave plate) and heated for the times obtained from the heating profiles to attain a final end target temperature of 73.8°C. For the experiments using crust inoculated pot-pies, one pot-pie was placed per heating cycle. For experiments with standing time, the pot-pies were removed from the microwave oven and allowed to stand for 3 min. Standing time of 3 min was applied after heating the pot-pies for 9 min 31 s and 7 min 1 s of heating time, for the low and high power microwave oven, respectively for the experiments using one pot-pie. During this experiment, product

temperature was determined by placing three fiber optic probes in the holes that are 3 mm apart from the center of the crust (forming a triangle).

***Salmonella* spp. enumeration and enrichment:** After heating, a portion of the inoculated pot-pie (*ca.* 105 g) was transferred to a stomacher bag (BagFilter, Spiral Biotech, Norwood, MA) containing 70 mL of chilled 0.1% buffered peptone water (BPW, Becton, Dickinson and Co., Sparks, MD) and massaged manually to allow the sample to cool rapidly and stop the lethality to *Salmonella* spp. The samples were homogenized for 2 min in a stomacher (NEUTEC, Albuquerque, NM), serially diluted and plated on tryptic soy agar (TSA; Becton, Dickinson and Co., Sparks, MD) and TSA supplemented with ferric ammonium citrate (0.4 g/0.5 L; Fisher Scientific, Fair Lawn, NJ) and sodium thiosulfate (3.4 g/0.5 L; Fisher Scientific, Fair Lawn, NJ) for the enumeration of *Salmonella* spp. Typical black *Salmonella* spp. colonies were enumerated and reported as log CFU/g. For enrichment, additional BPW was added to the stomacher bags to obtain a final volume of 325 ml and the samples were incubated for 18 h at 35°C. After incubation, aliquots (100 µL) from the enrichment were transferred into Rappaport Vassiliadis tubes (RV, 10 mL; Fisher Scientific, Fair Lawn, NJ) and incubated for 18 h at 35°C. A loop from the RV tubes showing turbidity was streaked into xylose lysine deoxycolate Agar (XLD, Fisher Scientific, Fair Lawn, NJ) and incubated for 18 h at 35°C and XLD plates with typical *Salmonella* spp. colonies were considered positive for the organism. Three independent replications as identified by day of sample preparation, *Salmonella* spp. cocktail and lots of chicken nuggets were conducted for each experiment.

Moisture and pH measurement: Five-g portions of the pot-pie crust or filling were homogenized with 25 mL of deionized water for 1 min in a stomacher, and the pH of each sample was measured by immersing the pH electrode (Accumet-Basic/AB15, Fisher Scientific, Bridgewater, NJ) in the sample homogenate. The moisture analysis was performed following the AOAC protocol.

7.4 Results and Discussion

The pH of the filling and the crust was 6.62 ± 0.03 and 6.18 ± 0.02 , respectively. The pH of the filling and crust can support the survival of *Salmonella* spp. The filling of the pot-pie used in the present study contained cooked turkey, carrots and peas immersed in a turkey broth mixture covered by a wheat flour-based crust. Owens et al. (2000) reported that the pH of turkey breast is approximately 6.09, a value that is consistent with the samples used in the present study where turkey meat is used as the main ingredient. Low moisture content was observed for the crust ($22.52\% \pm 12.38$). *Salmonella* spp. can survive for long time periods in low moisture foods (Podolak et al., 2010).

Based on the temperature profiles, the cold spot was located at the geometric center of the pot-pies (Figs. 4.1 and 4.2), while other areas such the edges were heated to higher temperatures. The same pattern was observed for ready-to-eat chicken pot-pies where the cold spot was also located at the geometric center of the product (Manickavasagan et al., 2009). The heating times necessary to attain target temperature of 73.8°C at the cold spot (99% UCL) were of 9 min 31 s and 7 min 1 s, for the low power and high power microwave oven, respectively (Table 4.2). The heating instructions recommended for

pot-pies were 4 to 5 min using a microwave ovens of $\geq 1,100$ W. Temperature of $38.1 \pm 3.7^{\circ}\text{C}$ and $66.8 \pm 6.5^{\circ}\text{C}$ would be achieved following the package instructions of heating for 4 and 5 min. The manufacturer recommended heating instructions recommended by the manufacturer may not be adequate and lower temperatures may be expected using the recommended microwave oven of lower power (1,100 W).

Placing one pot-pie at the center and heating for 9 min 31 s and 7 min 1 s for the low and high power microwave oven, respectively, resulted in *Salmonella* spp. populations below the detection limit (0.22 log CFU/g) regardless of the type of microwave oven used. The observed mean internal temperature was 100°C at the geometric center after heating for both microwave ovens (Figs. 4.3 and 4.4). The D-values of *Salmonella* spp. in ground turkey and ground chicken breast are 0.09 and 0.23 min, respectively (Murphy et al., 2004; Murphy et al., 2000). Although frozen pot-pies were inoculated using the filling of the product, the inoculum may be spread in other parts surrounding the geometric center interacting with the solid components including turkey during heating. Therefore, D-values of *Salmonella* spp. may be used as a reference to compare the differences in the destruction of this organism when different heating methods are used. Despite the high temperatures reached in the pot-pies, *Salmonella* spp. survival was observed in one (of three) pot-pie for each microwave oven (Table 4.1). Heddleson and Doores (1994) reported *Salmonella* spp. survival after heating milk and beef broth to 74°C and 72°C , respectively. It may be possible that *Salmonella* spp. cells were located at microscopic locations of the pot-pie where lethal temperatures were not achieved even when temperatures of 100°C were recorded at the geometric center. The presence of cold spots

away from the fiber optic probes could explain *Salmonella* spp. survival subsequent to microwave heating, even though reported temperatures should eliminate the organisms in the product.

Non-uniform temperature distribution has been identified as a result of microwave heating in a single product at different positions. Manickavasagan et al. (2009) observed internal temperatures of 64.8, 86.8 and 75.7°C in cooked chicken pot-pies after heating for 5 min in a household microwave oven (700 W). Similarly, Culkin and Fung (1975) observed that temperature distribution in beef broth heated using a microwave oven (915 MHz) was dependent on the location of measurement with the middle portion of the broth being the warmest and the bottom region, the coolest.

Incorporation of standing time (3 min) subsequent to heating resulted in elimination of *Salmonella* spp., regardless of the power of the microwave oven (Table 4.1). Heddleson et al. (1994) reported that post-heating holding times are crucial for the elimination of microorganisms. The heating instructions for turkey-pot-pies involved in the 2007 *Salmonella* spp. outbreak specify a heating time of 4 to 6 min depending on the power of the microwave oven used, with longer times specified for lower power ovens (however, the power levels of the microwave ovens was not specified) (Powell, 2007). The recommendation also included a standing time of 3 min after heating. The case study revealed that consumers misunderstood the microwave heating instructions, with 68% of consumers responding that the recommended heating times were not followed (CDC, 2009). The microwave heating instructions for the turkey pot-pies were specified in more

detail subsequent to the 2007 outbreak (CDC, 2007). Heating times of 4 to 5 min were recommended for microwave ovens of $\geq 1,100$ W, along with a standing time of 3 min. In the current study, a longer heating time (7 min 1 s) than the one recommended for the commercial product was calculated for the high power microwave oven (1,350 W). Heating to the target temperature of 73.8°C resulted in survival of *Salmonella* spp. in turkey pot-pies (Table 4.1), indicating that the recommended heating times by the manufacturer may not be adequate for the elimination of *Salmonella* spp. and to assure microbial safety.

Heating more than one pot-pie was reported by consumers involved in the pot-pie outbreak (CDC, 2009). Hence, the temperature of the pot-pies was obtained heating two turkey pot-pies simultaneously in the microwave oven and *Salmonella* spp. survival was evaluated. Placing two pot-pies in the microwave oven resulted in *Salmonella* spp. reductions of 0.41 and 0.11 log CFU/g when the pot-pies were heated in the low and high power microwave ovens, respectively. Based on the temperature profiles, the mean internal temperatures of the pot-pies were 49.2°C and 55.9°C for the low and high power microwave oven, respectively (Figs. 4.3 and 4.4). Tassinari and Landgraf (1997) showed that heating mashed potatoes and beef stroganoff in a microwave oven (750 W) resulted in higher temperatures at the end of heating compared to a microwave oven of lower power (700 W). In the current study, heating pot-pies in a higher power microwave oven also resulted in higher temperatures at the end of the heating process compared to the low power microwave. However, heating baby food in a low power microwave oven (750 W) resulted in lower temperatures at the end of heating compared to a lower power (700 W)

microwave oven (Tassinari and Landgraf, 1997). However, the study did not show the locations where the temperature was measured resulting in differences in temperature depending on the location of the product. These differences may be due to the size and shape of the food product. The data generated from a case study of the 2007 multistate outbreak of *Salmonella* spp. indicated that 16 patients recalled heating more than one pot-pie in the microwave oven (CDC, 2009). The efficacy of microwaves is reduced with an increased number of pot-pie units per heating cycle as the microwave radiation is dissipated in a food matrix of a larger size resulting in lower product temperature.

Microwave heating resulted in *Salmonella* spp. survival after enrichment in two out of three replications tested for each microwave oven type when the product was inoculated on the crust (center inoculation) (Table 4.1). The survival of *Salmonella* spp. inoculated on the crust could be explained in terms of the low moisture content of the crust compared to the gravy. The heat resistance of *Salmonella* spp. in low moisture foods is significantly higher (Podolak et al., 2010). Hiramatsu et al. (2005) reported minimal reductions in *Salmonella* spp. and *E. coli* O157:H7 in desiccated paper disks heated for 5 h at 70°C. Similarly, *Salmonella* spp. survival has been reported in dry corn flour with moisture levels of 10% or 15% after heating at 49°C (VanCauwenberge et al., 1981). Low water content promotes bacterial survival in dry foods, as few water molecules are available to vibrate and damage proteins during exposure to ultrasound waves and high pressure (Ernshaw et al., 1995). However, the heat resistance of *Salmonella* spp. in dry foods depends on additional factors such as the temperature of storage, formulation of the product and the *Salmonella* serotypes studied (Podolak et al., 2010).

Incorporation of 3 min standing time subsequent to heating resulted in elimination of *Salmonella* spp. from the center and edges of the crust for the both microwave ovens. Thermal images of the surface of the crust showed differences in temperature distribution with cold and hot spots at various locations for both microwave ovens (Figs. 4.3 and 4.4). Cold spots were observed on the pot-pie surface for each microwave with temperatures between 60 and 75°C, which may allow the survival of *Salmonella* spp. if the crust of the pot-pie was contaminated. However, these cold spots did not correspond to the inoculated locations on the pot-pies where the temperatures were > 90°C (center or edges of the pot-pie) were achieved. Incorporation of standing time subsequent to heating food products in a microwave oven can add an additional measure of safety to food products heated in a microwave oven.

The mechanism of destruction of microorganisms by microwaves has been studied extensively. Thermal and non-thermal effects of microwaves have been reported as mechanisms responsible for microbial destruction. However, thermal effect of microwaves is recognized as the main mechanism of microbial destruction in foods. Heddleson and Doores (1994) studied the effect of temperature gradients in milk and beef broth using a microwave oven of 700 W at a frequency of 2,450 MHz. They reported elimination of *Salmonella* spp. destruction when the product was immediately mixed after heating whereas survival of the organisms was observed with 10 min of standing time in non-stirred beef broth and milk. These data suggest that heating in a microwave oven is not sufficient in achieving a uniform temperature throughout the product and

additional steps by the consumers such mixing the product after microwave heating can enhance the equilibration of temperature throughout the product resulting in greater destruction of *Salmonella* spp. in microwaved food products.

Pot-pies heated with the low power microwave oven resulted in a darker brown color compared those heated in the high power microwave oven. This could be due to the longer heating times (9 min 31 s) used when heating in the low power microwave oven. Heating in the low power microwave oven also resulted in shrinkage of the crust.

7.5 Conclusions

Salmonella spp. can survive in pot-pie filling and on the crust when the pot-pies were heated to a target temperature of 73.8°C (9 min 31 s and 7 min 1 s for the low and high power microwave ovens, respectively). Incorporation of standing time (3 min) after heating pot-pies eliminated *Salmonella* spp. (5.0 log CFU/g) regardless of the location of the organism (filling or crust). Heating more than one pot-pie simultaneously following heating instructions for one pot-pie resulted in inadequate heating and survival of *Salmonella* spp. with minimal reduction (≤ 0.5 log CFU/g). Lethal effects of microwaves on microorganisms are caused mainly by the effect of the temperature; however, the survival of the organism is affected by temperature distribution throughout the product and the composition of the food matrix (water content). Heating instructions for commercially available NRTE microwaveable products may be inadequate as longer periods of heating time were necessary to eliminate *Salmonella* spp. even though microwave ovens of high power were used.

7.6 References

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7.7 List of Tables

Table 4.1. *Salmonella* spp. survival in NRTE turkey pot-pies after microwave

Location of Inoculation	No. of pot-pies per heating cycle	Microwave power	Positive samples after heating ^a	Positive samples after 3 min of ST ^b
Filling	1	Low	1/3	0/3
Filling	1	High	1/3	0/3
Crust (center)	1	Low	2/3	0/3
Crust (center)	1	High	2/3	0/3
Filling	2	Low	3/3 ^c	NA
Filling	2	High	3/3	NA

heating as affected by location of inoculum and number of pot-pies.

^aPositive samples after enrichment procedure when *Salmonella* spp. counts were below the detection limit of the plating technique (0.22 log CFU/g).

^bST: Standing Time

^cNo enrichment procedure was applied for experiments using two pot-pies as *Salmonella* spp. counts were reported in all the samples.

Table 4.2. Heating times required for pot-pies to reach a final end temperature of 73.8°C with an upper confidence limit (UCL) of 90, 95 and 99% for the low and high power microwave ovens.

Upper confidence limit (UCL; %)	Heating times (MM:SS)	
	Low power (700 W)	High power (1,350 W)
90	8:49	5:53
95	9:05	6:14
99	9:31	7:01

7.8 Legend to the Figures

Fig. 4.1. Temperature profiles of pot-pies heated in a low power microwave oven showing the hot and cold spots of the product.

Fig. 4.2. Temperature profiles of pot-pies heated in a high power microwave oven showing the hot and cold spots of the product.

Fig. 4.3. Temperature profiles of NRTE turkey pot-pies pot heated with a low power microwave oven (700 W).

Fig. 4.4. Temperature profiles of NRTE turkey pot-pies pot heated with a high power microwave oven (1,350 W).

Fig. 4.5. Temperature profiles of twenty-four pot-pies at the geometric center of the product.

Fig. 4.6. Thermal image of the surface of the crust of the pot-pies when heated with the low power microwave oven (700 W) after 3 min of standing time.

Fig. 4.7. Thermal image of the surface of the crust of the pot-pies when heated with the high power microwave oven (1,350 W) after 3 min of standing time.

Fig. 4.8. Fit comparison for the selection of the heating times with a) all the data points from temperature profiles to achieve 73.8°C with upper confidence limit (UCL) at b) 95% and c) 99% for pot-pies heated in a low power microwave oven.

Fig. 4.9. Fit comparison for the selection of the heating times with a) all the data points from temperature profiles to achieve 73.8°C with upper confidence limit (UCL) at b) 95% and c) 99% for pot-pies heated in a high power microwave oven.

Fig. 4.10. Color image frozen turkey pot-pie without the application of heat treatment.

Fig. 4.11. Color image of pot-pie after heated in a low power microwave oven.

Fig. 4.12. Color image of pot-pie after heated in a high power microwave oven.

Fig. 4.1

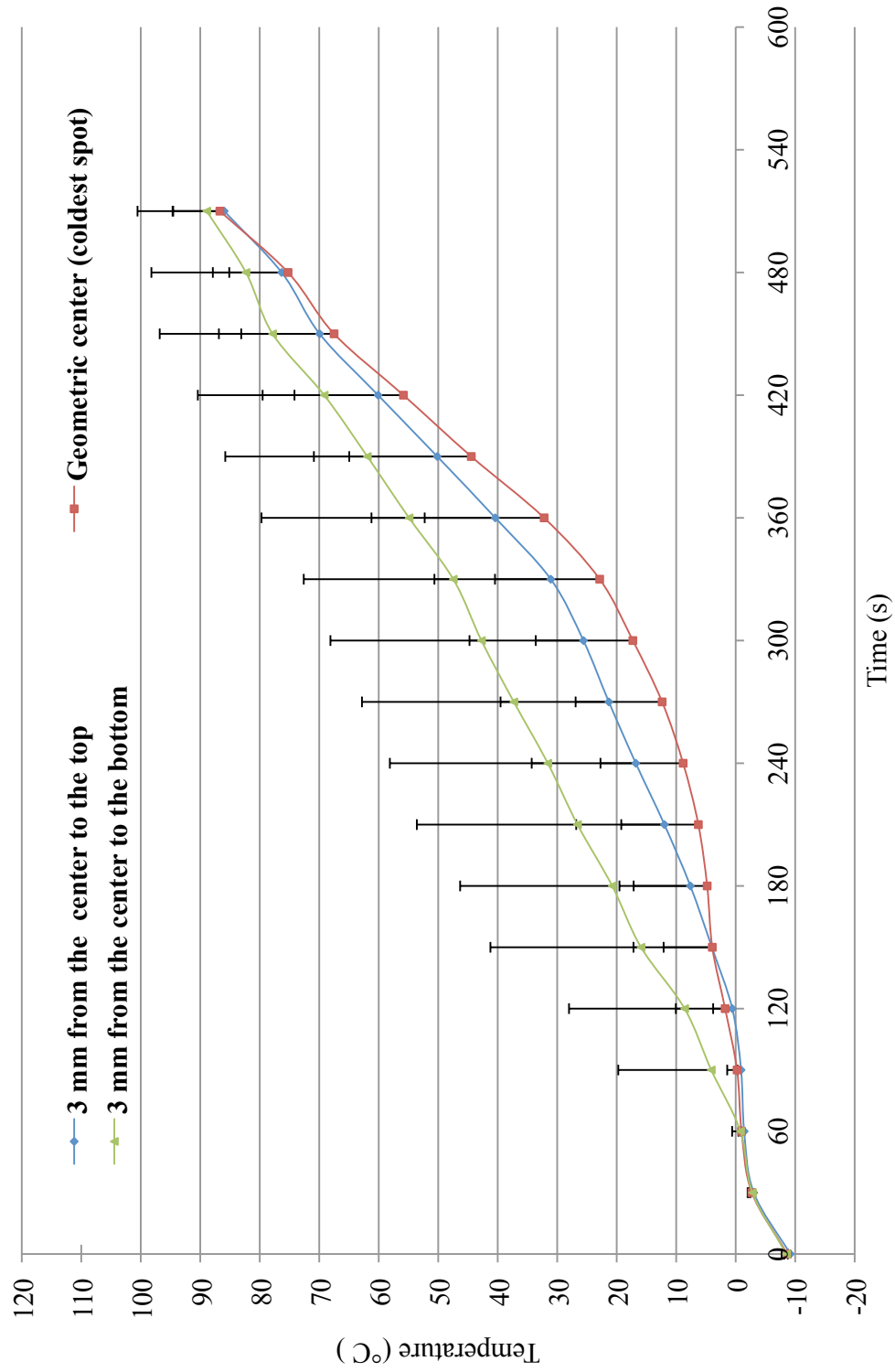


Fig. 4.2

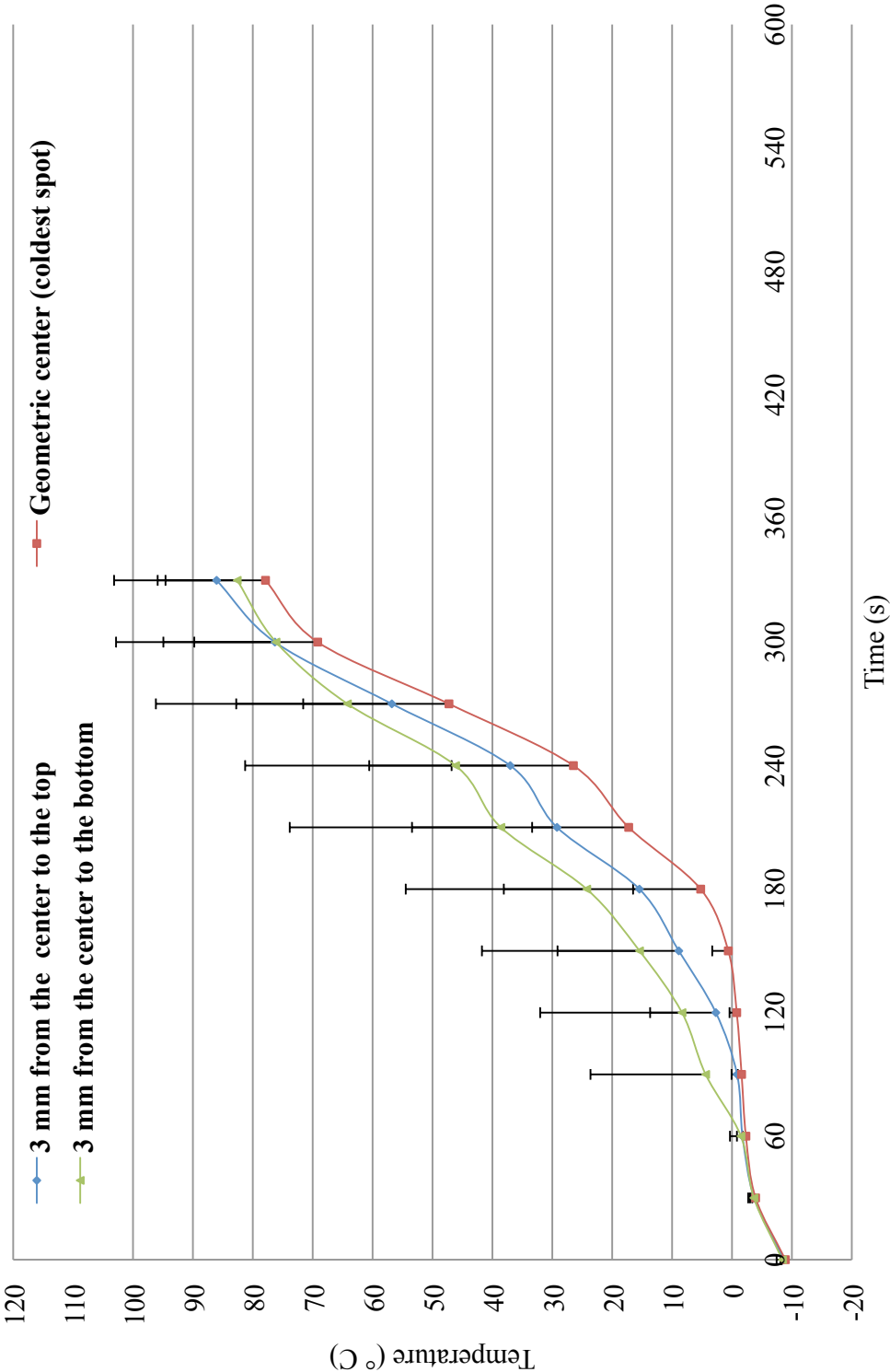


Fig. 4.3

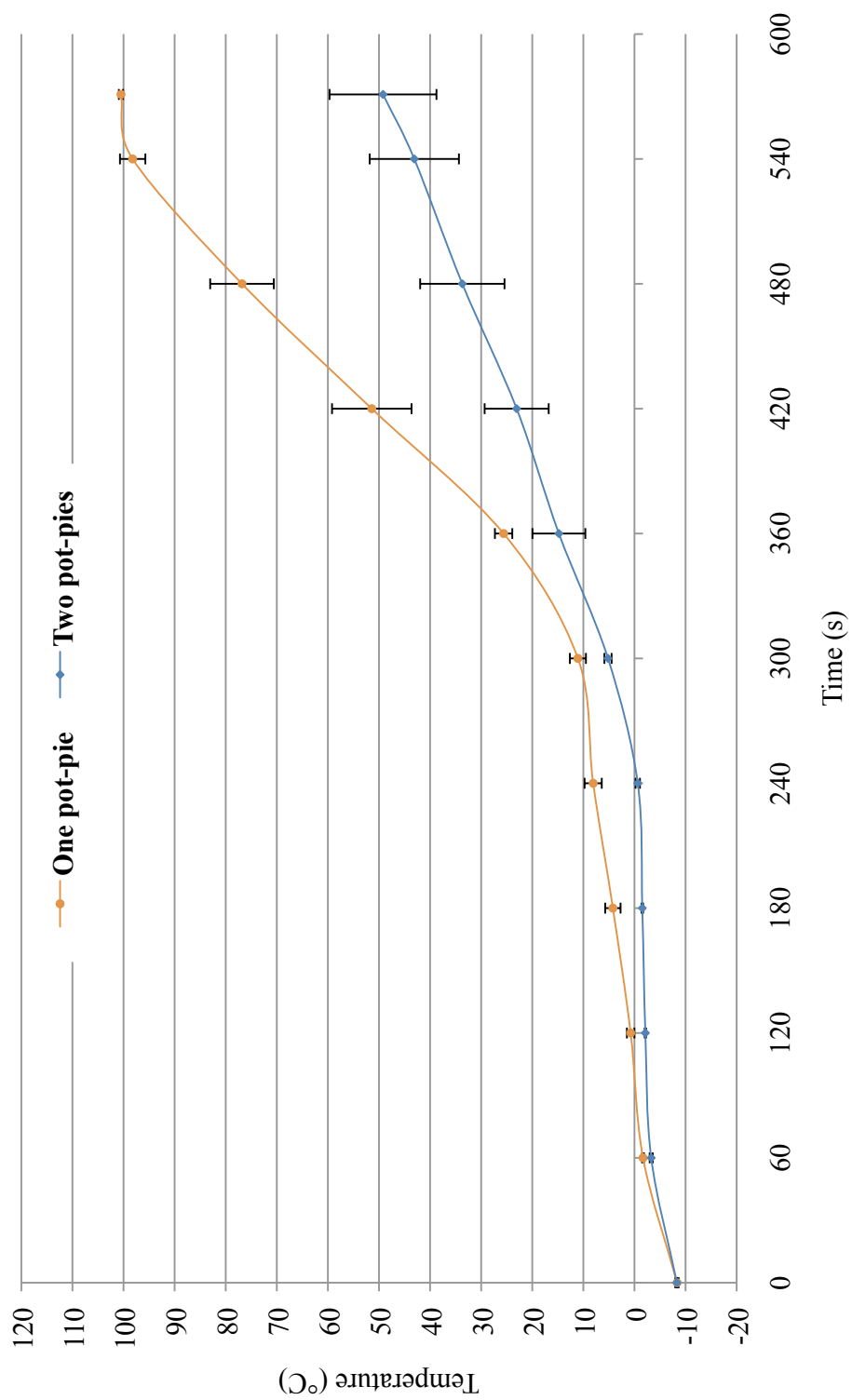


Fig. 4.4

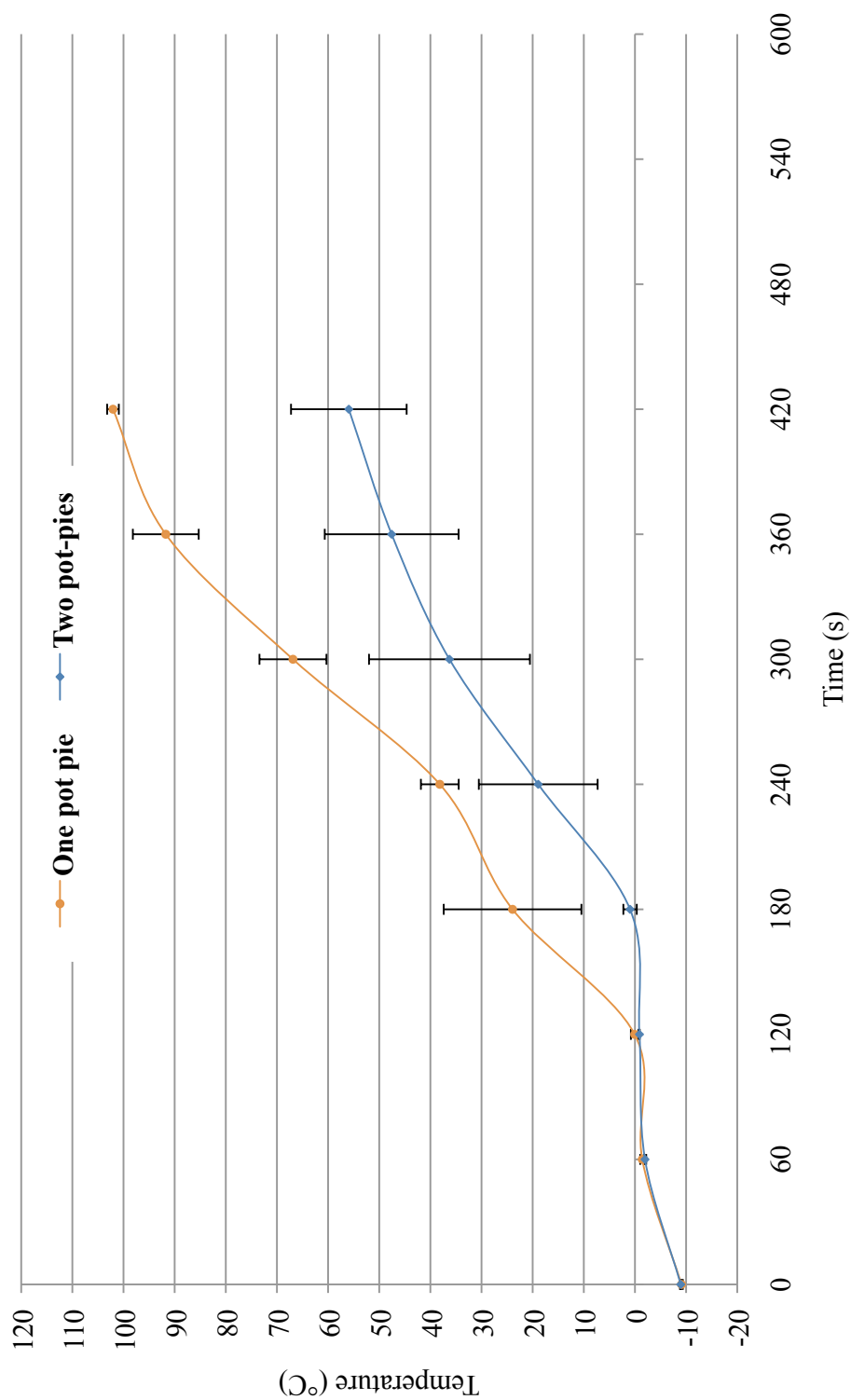


Fig. 4.5

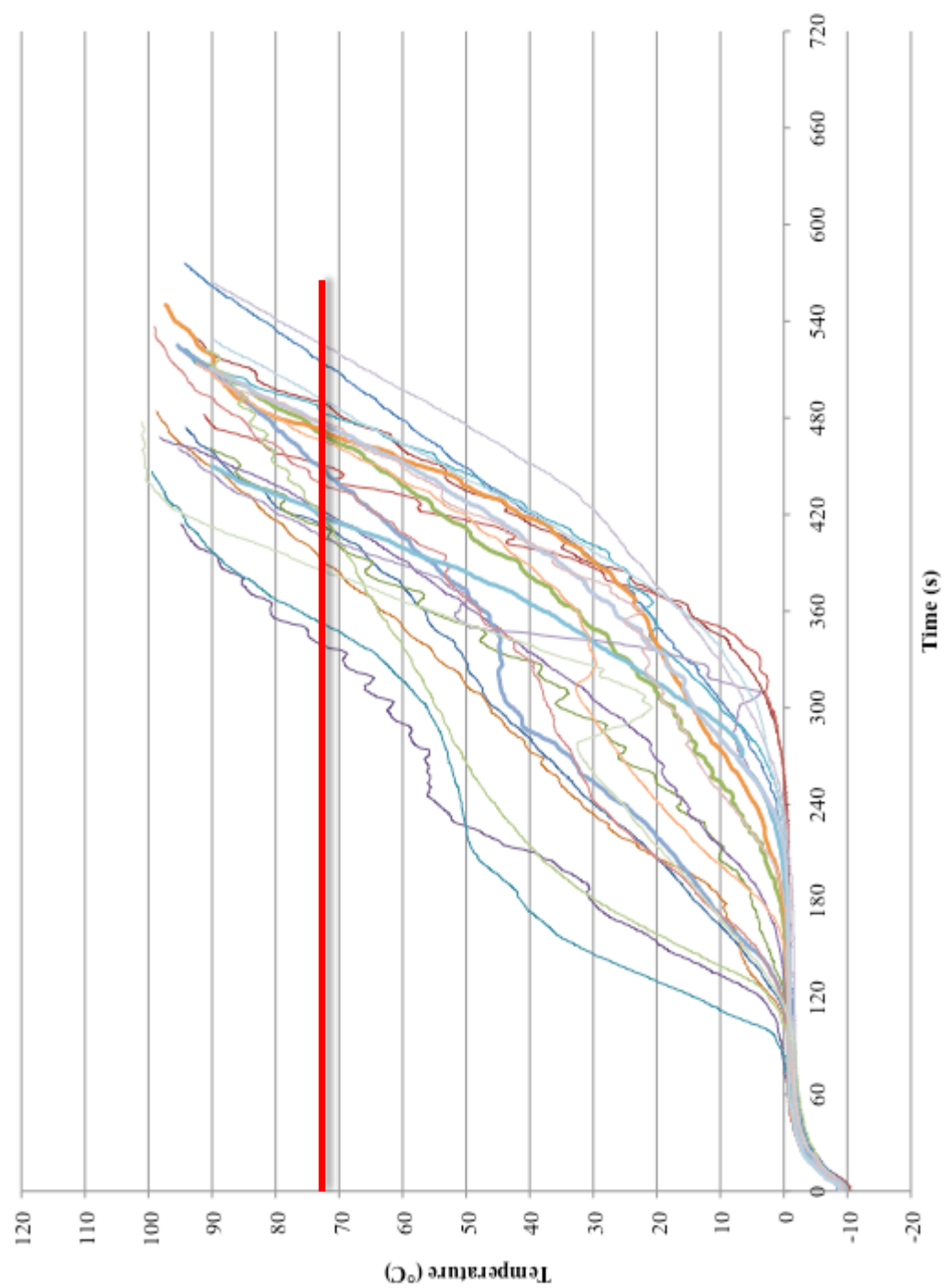


Fig. 4.6

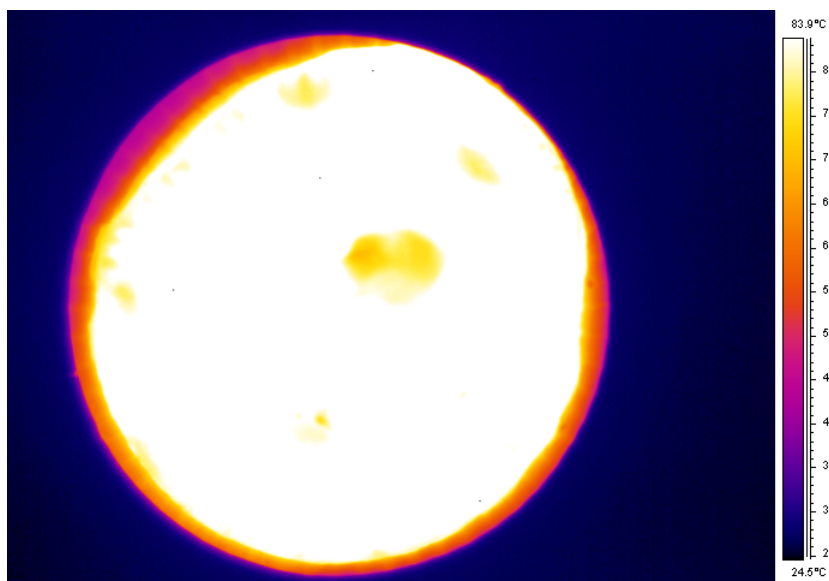


Fig. 4.7

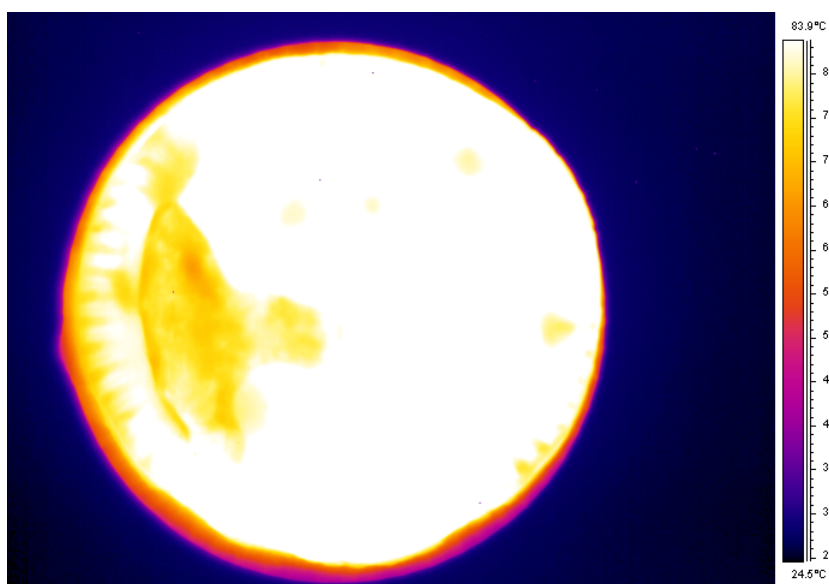
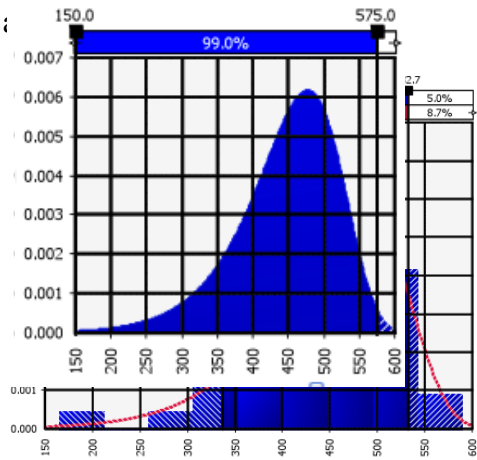
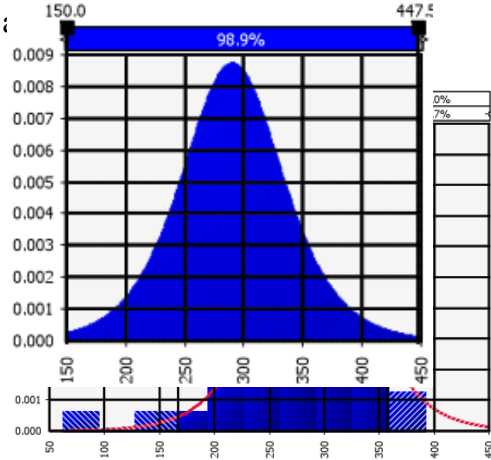


Fig. 4.8



b)

Fig. 4.9



b)

Frequency

c)

c)

Time (s)

Fig. 4.10



Fig. 4.11



Fig. 4.12



5. RECOMMENDATIONS FOR FUTURE RESEARCH

According to the observations made in the present study, some recommendations can be made for future research on the way microwave ovens can be studied for the validation of heating instructions:

- As just two microwave power levels were used in this study (700 and 1,350W) further studies should include the analysis of microwave ovens with intermediate power levels. Microwave ovens with intermediate power level may be used in a significant number of households in the United States.
- Results from this research demonstrated that microwave heating instructions are highly dependent on the type and configuration of product used. Future research can incorporate the validation of heating instructions of other frozen products as affected by product composition, shape and package.
- Microwave heating instructions for other pathogenic microorganisms of concern must be validated. Other studies can include the destruction of organisms such as *E. coli* O157:H7, *Listeria monocytogenes* and sporeformers like *Bacillus cereus*.
- The development of proper analytical and mathematical tools to predict the level of destruction of microorganisms after microwave heating was identified as a current need in the present study. Future research on predictive models for microwave heating will represent a valuable tool for food industries when assessing the risk associated with different NRTE microwaveable products.