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ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

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# Efficacy of Manufacturer Recommended Microwave Time Against *Listeria monocytogenes* in Ready-to-Eat Chicken Products

A. Morey, M. Singh and S.R. McKee
Department of Poultry Science, 201 Poultry Science Building, 260 Lem Morrison Dr.,
Auburn University, Auburn, AL 36849, USA

**Abstract:** This study evaluated the effect of manufacturer-recommended microwave times on *Listeria monocytogenes* in Ready-to-Eat (RTE) poultry products. Three chicken products including battered and breaded chicken patties, fully cooked chicken breast fillets (both frozen) and chicken sausages (refrigerated) were inoculated with streptomycin-resistant (1500 µg/ml) *L. monocytogenes* (Brie 1) at low (ca. ~4 $\log_{10}$ CFU/ml) and high (ca. ~9 $\log_{10}$ CFU /ml) levels. Following 12 h of either frozen or refrigerated storage, random samples (n = 3 of each product) were individually microwaved for recommended time, undercooked and overcooked. Survival of *L. monocytogenes* after microwaving was determined by spread plating the samples and enumerating bacterial colonies on BHIA+streptomycin (1500 µg/ml). Analysis of variance with Duncans' grouping was conducted to determine significant differences in survival ($\alpha$ = 0.05). Microwaving the chicken patties inoculated with high levels of bacteria for a recommended time of 1 min increased surface temperature to 64°C-74°C leading to approximately 1-log reduction ($p \leq 0.05$) of *L. monocytogenes*. Chicken breast samples and sausages microwaved at 1100 W according to manufacturer's instruction reduced *L. monocytogenes* populations below detection limit at high as well as low levels. Although, recommended microwaving times might not always be effective in eliminating *L. monocytogenes*, its efficacy will depend upon factors such as product type and the level of *L. monocytogenes* contamination. Reconstitution of RTE foods below recommended times can make them a potential source of *Listeria*.

**Key words:** Microwave, *Listeria monocytogenes*, ready-to-eat chicken

## INTRODUCTION

*Listeria monocytogenes* has emerged as one of the most important and lethal foodborne pathogens resulting in a high rate of hospitalization (94%) and the highest fatality rate (19%) amongst all foodborne illnesses (Scallan *et al*., 2011). Although initial outbreaks of listeriosis were linked to consumption of coleslaw, raw vegetables, milk and Mexican-style cheese, the consumption of undercooked chicken and uncooked frankfurters has been strongly linked epidemiologically to an increased risk of listeriosis (Schwartz *et al*., 1988).

Consumption of *L. monocytogenes* contaminated foods can result in life threatening conditions in elderly individuals, pregnant women, newborns, immuno-suppressed and immunocompromised individuals (CDC, 2002). However, there are instances where apparently normal healthy individuals have exhibited listeriosis in both foodborne epidemics and sporadic illnesses (Farber and Peterkin, 1991). Hence, the United States Department of Agriculture (USDA) has a "zero tolerance" policy towards *L. monocytogenes* in Ready-to-Eat (RTE) foods. Although the industry applies strenuous efforts to eliminate *L. monocytogenes* in RTE foods, possible post-processing *Listeria* contamination has led to frequent recalls of RTE products as seen from the FSIS Recall Case Archive (http://www.fsis.usda.gov/fsis_recalls/Recall_Case_Archive/index.asp).

The ubiquitous nature of *Listeria* and its wide distribution in the environment along with its ability to survive in a wide range of temperatures (0 to 45°C), pH (4.4 to 9.4) and water activity of $\geq$0.92, makes it a pathogen of concern in food processing (FAO/WHO, 2004). *Listeria* enters the processing environment at low intensities and subsequently establishes itself in wet places like conveyor belts, floors, drains and other transporting equipment (Gubdjornsdottir *et al*., 2004). These niches act as a source for the dissemination of *L. monocytogenes* on cooked food (CDC, 2002).

Frankfurters have a moderate risk of post processing contamination and growth of *L. monocytogenes* during extended shelf life. Therefore, they are relatively low risk products if properly reheated; however, if they are not reheated, frankfurters are considered a high risk product (Glass *et al*., 2001). Poultry products are being increasingly introduced as convenience foods and many are RTE. The reconstitution methods for such RTE meals generally recommended by the manufacturer include microwave, conventional oven and skillet cooking. Microwaves are used frequently for

**Corresponding Author:** M. Singh, Department of Poultry Science, 201 Poultry Science Building, 260 Lem Morrison Dr., Auburn University, Auburn, AL 36849

reconstituting convenience foods and consumers rely heavily on the simple directions provided by the food manufacturer to reheat these convenience foods. Heating due to microwaves is caused by molecular friction between the dipolar rotation of polar solvents like water and conductive migration of dissolved ions inducing, injury or death of bacteria in those foods (Farber *et al*., 1998; Heddleson and Doores, 1994). Although lethality of microwaves depend on type and load of target bacteria and the product type, non-uniform heating and inadequate cooking/reheating can lead to food safety concerns (Heddleson *et al*., 1996).

According to Oliveira and Franca (2002) the efficiency of microwaves to heat foods uniformly depends on the 1) type of food: (product density and constituents), 2) chemical composition of foods, 3) size and shape of the foods, 4) temperature of the food and 5) frequency of microwaves: Lower frequencies (915 MHZ) heat samples more effectively than higher frequencies (2450 MHZ) due to more efficient dissipation of electromagnetic energy into heat.

The potential of poultry products being exposed to *L. monocytogenes* and the popularity of microwaving as a reconstitution method prompted the need to conduct this study to determine the efficacy of manufacturer recommended microwave directions on different levels of *L. monocytogenes* in various poultry products. Since consumers tend to undercook or overcook the product in microwaves, one of our objectives was to determine the effect of undercooking and overcooking on *L. monocytogenes*.

## MATERIALS AND METHODS

**Bacterial culture preparation:** *Listeria monocytogenes* Brie 1 resistant to 1500 µg/ml streptomycin (Fisher Scientific, NJ) was cultured by growing the bacteria in Brain Heart Infusion (BHI, Acumedia, MI) broth supplemented with 1500 µg/ml streptomycin until it reached 9 $\log_{10}$ CFU/ml after 18 h of incubation at 37°C (experimental data not shown). High Concentration Levels (HL) of *L. monocytogenes* were obtained by inoculating samples with 9 $\log_{10}$ CFU/ml suspension, whereas the Low Populations Levels (LL) of 3 $\log_{10}$ CFU/ml were prepared by serially diluting the 18 h *L. monocytogenes* suspensions.

**Chicken products:** Fully cooked grilled chicken breast fillets, fully cooked chicken sausage habanero with monterey jack cheese and chicken breast breaded patties were obtained from a local grocery store. These products were stored as per manufacturers' recommendations under frozen (-18°C) or chilled (4°C) conditions (<24 h) until further use.

**Inoculation of poultry products:** Samples (five grilled chicken breast fillets and chicken breast patties at each inoculation level and trial) were aseptically removed from their commercial packaging and inoculated with 1 ml of either high (~9 $\log_{10}$ CFU/ml) or low levels (4 $\log_{10}$ CFU/ml) of bacteria and placed in a sterile laminar flow biosafety hood (Nuaire Inc., Plymouth, MN) for 30 min to allow bacterial attachment. Since the chicken sausage did not have an absorbing surface like the other two products, 0.01 ml of inoculum was spread on the surface. After inoculation, samples were transferred to whirlpak® bags and sealed by folding the top of the bags. Inoculated grilled chicken breast fillets and chicken breast patties were stored at -18°C whereas the chicken sausages were stored at 4°C for 12 h.

**Treatment:** After 12 h of storage, samples (n = 3) were randomly reconstituted in a standard home microwave (Sharp Carousel R-305EW, 60 Hz, 1100 W, Sharp Electronics Corp., NJ) by heating the products (a) as per recommendations of the manufacturer, (b) less time than the recommendations of the manufacturer and © over the recommended time by the manufacturer (Table 1). Microwave times higher and lower than manufacturers' recommendations were chosen to determine effects of under and longer time than that recommended by the manufacturer for reconstitution on selected products. Products were microwaved individually on sterilized glass plates and at the half way point of each microwave time, the product was turned over for even exposure of the products to heat. Since *Listeria* is a surface contaminant in post-processed RTE foods, only surface temperatures of individual products was measured at the end of the microwave cycle using a temperature probe (Precision 0.01° Thermometer, Fisher Scientific, NJ).

Table 1: Microwave times used for reconstituting the products

| Product | Under-cook | Recommended time | Over-cook |
|---|---|---|---|
| Chicken breast | 2 min | 4 min | 6 min |
| Chicken sausages | 1 min | 1.5 min | 2 min |
| Chicken patties | 0.5 min | 1 min | 1.5 min |

**Microbiological enumeration:** After microwaving, individual samples were placed aseptically into a whirlpak® bag and a 4-fold dilution was made using a phosphate buffer solution (PBS; Fisher Scientific, NJ). Each sample was stomached (Seward Stomacher® 400 Circulator, England) for 1 min, serially diluted and spread-plated onto BHI agar + streptomycin (1500 µg/ml) plates. Colonies were then counted after an incubation period of 37°C for 24 h and reported as $\log_{10}$ CFU/g. To validate inoculation levels, BHI broth containing *L. monocytogenes* used to inoculate the products was spread-plated and incubated similar to the other plates, whereas the non-microwaved inoculated samples served as positive controls for our experiments.

**Statistical analysis:** Sampling for all three products was done in triplicate for each microwaving cycle and two replications of the experiment were performed in this study. Analysis of variance followed by Duncans' multiple comparison procedures between group means was done using SAS statistical software and significant differences were reported at $p \leq 0.05$.

Table 2: Recovery[1] of *Listeria monocytogenes* inoculated at two different levels from chicken breast, sausages and patties following microwaving

| Products | Microwave time | High level | Low level |
|---|---|---|---|
| Chicken breast | 0 min | 8.70±0.05$^{c}$ | 2.41±0.12$^{b}$ |
| | 2 min | 6.71±0.40$^{b}$ | ND$^{a}$ |
| | 4 min | ND$^{a}$ | ND$^{a}$ |
| | 6 min | ND$^{a}$ | ND$^{a}$ |
| Chicken sausages | 0 min | 6.60±0.31$^{b}$ | 2.95±0.06$^{b}$ |
| | 1 min | 5.52±0.05$^{b}$ | ND$^{a}$ |
| | 1.5 min | ND$^{a}$ | ND$^{a}$ |
| | 2 min | ND$^{a}$ | ND$^{a}$ |
| Chicken patties | 0 min | 7.50±0.10$^{c}$ | 2.56±0.20$^{b}$ |
| | 0.5 min | 7.19±0.08$^{c}$ | 2.67±0.27$^{b}$ |
| | 1 min | 6.41±0.09$^{b}$ | ND$^{a}$ |
| | 1.5 min | ND$^{a}$ | ND$^{a}$ |

[1]$Log_{10}$ CFU/g ± Standard deviation; ND: *L. monocytogenes* was below the detection limit (<1.3 $log_{10}$ CFU/g); Superscript a, b and c represents significant differences ($p<0.05$) within a column for each product individually

## RESULTS AND DISCUSSION

Chicken breast inoculated with low levels (ca. ~4 $log_{10}$ CFU/mL) of *L. monocytogenes* and microwaved for 2, 4 and 6 min resulted in reducing ($p \leq 0.05$) the bacterial populations to below detection limits, while the chicken breast inoculated with high levels (ca. ~9 $log_{10}$ CFU/mL) of *L. monocytogenes* and microwaved for 2 min (undercooked) significantly ($p \leq 0.05$) reduced the populations of the bacteria by 2-logs (Table 2). When microwaved for the recommended time of 4 min, the chicken breast inoculated with high levels of *L. monocytogenes* yielded reductions ($p \leq 0.05$) of bacteria to below the detection limit (<1.3 $log_{10}$ CFU/g) and although overcooking (6 min) yielded no surviving *L. monocytogenes*, the product quality was severely compromised. The reduction in bacterial counts is probably due to increased absorption of microwaves by the free water available in the sample thus increasing surface temperatures (Heddleson and Doores, 1994) to 60-76°C after microwaving. McCormick *et al.* (2003) have reported $D_{61}$ and $D_{65}$ values of 124 and 16.2 s respectively during surface pasteurization of *L. monocytogenes*-inoculated turkey bologna using a water bath. In contrast to the present study, Harrison and Carpenter (1989) reported that an internal temperature as high as 82.2°C was insufficient to eliminate approximately 6-log *L. monocytogenes* on fresh raw chicken breast.

The control samples of chicken sausage inoculated with low levels (ca. ~ 4 $log_{10}$ CFU/ml) of *L. monocytogenes* showed approximately 1-log reduction after 12 h of refrigerated storage that can be attributed to the presence of antilisterial compounds such as sodium phosphate, salt, sorbic acid, sodium lactate, sodium nitrite and sodium erythroborate in the sausages (Lado and Yousef, 2007). The sausage had a natural pork casing, potentially inhibiting bacterial attachment to the product and also the possibility of the inoculum being lost to the sample bags. Microwaving for 1, 1.5 and 2 min resulted in surface temperatures ranging from 65 to 90°C which might have in combination with the above stated reasons contributed to the absence of viable cells ($p \leq 0.05$) on sausages inoculated with low levels of *L. monocytogenes*. Chicken sausages inoculated with high levels of *L. monocytogenes* showed a reduction (~ 1 log; $p>0.05$) in bacterial populations following 1 min of microwaving (Table 2) and further microwaving for 1.5 and 2 min resulted in reducing ($p \leq 0.05$) the populations of *L. monocytogenes* below the detection limit.

Breaded and battered poultry products present a unique surface with niches for bacterial colonization and protection; therefore, it is challenging to decontaminate such surfaces. At the same time, the presence of oil on the surface of such par fried products can increase the surface temperature quickly leading to bacterial injury or death. Since the product used in our study was in a frozen state, undercooking of the inoculated (low or high levels) chicken patties for 0.5 min did not change *L. monocytogenes* counts ($p>0.05$) relative to the control samples in both experiments (Table 2). Microwaving the samples for 1 min (recommended time) increased the surface temperature to 64-75°C, leading to approximately a 1-log decrease ($p \leq 0.05$) in bacterial counts, on chicken patties inoculated with high levels of *L. monocytogenes* during both trials, whereas, no recoverable growth was observed on patties inoculated with low levels of *L. monocytogenes* after 1 min of microwaving. Treatment of chicken patties for 1.5 min increased the product surface temperature to 86°C at the point where the temperature probe was placed.

Results from the experiment with patties suggested that 1 min of recommended microwaving is insufficient to kill a high load of *L. monocytogenes*. Further research needs to be conducted to determine safety of reconstitution methods for RTE products by taking into consideration factors such as the presence of oil and water, presence of salt and the non-uniform distribution of temperature on the products during microwaving that have been reported to help in the survival of *L. monocytogenes* in further processed and RTE meat and poultry products (Heddleson *et al.*, 1996). As evident from our study, recommended microwaving times might not be effective to eliminate *L. monocytogenes* especially when present at high levels. Moreover,

unequal and inconsistent heating of foods due to the presence of salt (Heddleson *et al*., 1996), size and shape of the product, its position in the microwave and the make and model of microwave (Farber *et al*., 1998; Harrison and Carpenter, 1989) are important factors that can affect the microbicidal effect of microwaves. Further research can be conducted to study the listericidal efficacy of microwaves with different wattages with use of thermocouple for temperature measurement during microwaving. Hence, microwave reconstitution methods of poultry products should be carefully recommended without compromising the product quality.

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