

Reduction of *Salmonella* Enteritidis in Fish by Microwave Cooking

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Abstract

The effect of microwave cooking on the survival of *Salmonella* Enteritidis was investigated. Inoculated whiting and salmon fillets (6-7 log cfu/cm²) were cooked in microwave either packed or unpacked at two internal temperatures (50 and 70°C). When the samples were cooked up to the internal temperature of 50°C, the reductions were 1.82 log cfu/cm² (29%) for packed and 0.69 log cfu/cm² (11%) for unpacked whiting. For the same cooking temperature, the reductions were 2.39 (33%) and 0.73 log cfu/cm² (10%) for packed and unpacked salmon, respectively. When the internal temperature was 70°C, the reductions in *S. Enteritidis* counts were 2.89 (45%) and 3.90 cfu/cm² (54%) unpacked whiting and salmon, respectively. However, the reductions were higher in packed samples of both fish cooked to 70°C internal temperature than that of unpacked samples and counts of the pathogen were below the detectable level (<1.00 log cfu/cm²). These results suggested that packaging increased the *S. Enteritidis* reduction during microwave cooking and the reductions were higher in salmon than that of whiting. Microwave-cooking instructions must be included in the MW operating manuals. The foods must be cooked in microwave not lower than 360 W and 70°C.

Introduction

In recent years, the microwave oven has become an essential appliance in most kitchens. The percentage of homes possessing microwave ovens is approximately 93-95% in the United States, 97% in Japan and 90-95% in the United Kingdom (Horikoshi et al., 2018). The use of oven is remarkably popular in commercial and home cooking, since it is faster and more practical than conventional methods (Giese, 1992; Tassinari & Landgraf, 1997; Thostenson & Chou, 1999). In addition to the increasing use of microwave oven at home, it has also been used in industrial food processing systems. Efficient operating conditions, compatibility with other equipment, and increased product quality are the advantages of microwave to traditional heating

methods. Microwave heating is a sustainable and eco-friendly technology that reduces adverse environmental impact and power consumption (Komarov, 2021). Delivering high temperature in a very short time results in nutritional and sensorial advantages over the traditional cooking techniques (Aymerich et al., 2008; Orsat et al., 2017).

In conventional thermal processing, energy is transferred from surface to the inner parts of the food via convection, conduction, and radiation. In contrast, microwave energy is delivered directly to the food through molecular interaction with the electromagnetic field (Thostenson & Chou, 1999). In a microwave oven molecular friction between water molecules, under an oscillating electric field of specific frequency, increases the temperature of food (Pucciarelli & Benassi, 2005).

Microwave energy is used for heating, cooking, pasteurization, and sterilization of foods (Datta & Davidson, 2001; Giese 1992). It is known that microorganisms in food are inactivated by the thermal effect of microwave (Vela & Wu, 1979). However, incomplete inactivation of pathogens in foods, cooked or reheated in microwave ovens, has been reported (Burdick et al., 1983; Coote et al., 1991; Farber et al., 1998; Harrison & Carpenter, 1989; Hollywood et al., 1991; Huang et al., 1993). Microwave cooking promises to reduce the time spent on cooking. However, cold spots in microwave-cooked foods pose a food safety risk for the consumer. It is challenging to detect hot and cold spots that occur in packaged foods during microwave cooking and to evaluate the temperature distribution within the food (Das & Banik, 2021). Foods have complex natures, and electromagnetic distribution is not homogenous in the cavity. These properties have important effects on power absorption during MW cooking and the temperature distribution (Bedane et al., 2021). It is estimated that 9% diarrheal illnesses that occur worldwide annually are associated with *Salmonella*. Although *Salmonella* is the fifth most common pathogen, the mortality is high and 41% of all diarrheal disease-associated deaths can be attributed to *Salmonella* (Besser, 2018). Outbreaks associated with *Salmonella* resulting from the consumption of undercooked food are of much concern throughout the world (Evans et al., 2000). Although there are some studies on microwave-induced destruction of *Salmonella* in meat, poultry, and milk (Aleixo et al., 1985; Bookwalter et al., 1982; Evans et al., 1995; Levre & Valentini, 1998), data for seafood is limited. Since it is an internationally important human pathogen and *Salmonella* Enteritidis outbreaks are generally related to undercooked foods (Humphrey et al., 1995), it is necessary to determine whether microwave cooking is sufficient to eliminate this pathogen in fish (Heddleson & Doores, 1994; Datta & Davidson, 2001). Whenever validating the cooking directions of a microwave oven, it is necessary to standardize the equipment and its set up to provide objective, repeatable use for the consumer. Validation is mandatory to ensure food safety and desired food quality (Vlock, 2020).

In this study, determining the efficiency of microwave cooking on *Salmonella* Enteritidis in salmon and whiting was aimed, as well as to determine the effect of packaging on destruction of this pathogen during microwave cooking.

Material and Methods

Materials

Frozen salmon (*Salmo salar*) and whiting (*Merlangius merlangus*) blocks were obtained from a local market in Istanbul, Turkey, and transferred to the laboratory in a chilled box within 30 minutes. Totally 120 fish pieces were used for each fish species. The shape of

the fish blocks was a rectangular prism (6 × 7 cm) with 2 cm in thickness. The upper and undersides of fish blocks were used as inoculation area and the total inoculation area was 84 cm² for each of them. Fish blocks were thawed in the refrigerator (10 hours at 4 ±1°C), before analysis.

Preparation of *Salmonella* Inoculum

Salmonella Enteritidis (*Salmonella enterica* serovar. Enteritidis ATCC 13076) pure culture was obtained from the culture collection of the Food Engineering Department of Sakarya University. *Salmonella* Enteritidis was activated twice in Tryptic Soy Broth (TSB; Merck, Darmstadt, Germany) at 37°C for 18-24 h. A tube containing 10 mL TSB was inoculated with active culture and incubated at 37°C for 24 h. Following incubation, cells were harvested by centrifuging (Centromix, Selecta, Barcelona, Spain) at 3354 × g for 10 minutes. The pellet was washed three times with sterile peptone water (0.1%). Following washing, the supernatant was discarded and the pellet was resuspended in 10 mL of sterile peptone water (0.1%).

Inoculation of Fish Blocks

Samples were inoculated with *S. Enteritidis* at a level of 6-7 log cfu/cm². A volume of 0.25 mL inoculum was deposited on one side of each block and was spread by a sterile bent glass rod to obtain an even distribution of the cells. The inoculated fillets were left for 15 minutes for inoculum attachment. The same procedure was repeated for the other side of each fillet. Environmental temperature was 10°C during the processes.

Microwave Cooking

Inoculated whiting blocks were separated into two groups (60 pieces). The first group was packed with a film (Polinas Polibarr Y10C1B, 90µ, the oxygen transmission rate of 160 cc/m²/day, Manisa, Turkey), while the other group left unpacked. The same procedure was carried out for the salmon samples. All samples, whether packed or unpacked, were cooked in 2450 MHz microwave oven at medium power (360 W, 30 L capacity, with a 34 cm diameter rotary plate, Bosch, Germany) to internal temperatures of 50 and 70°C. To determine the risk that may occur in the case of inadequate cooking or preferring very rare fish, cooking at low temperature such as 50°C was studied as well as 70°C. The internal temperatures were measured using a digital thermocouple (Multi, China, range -50/+150°C) placed into the geometric center of fish blocks, immediately after cooking, as suggested by Vela and Wu (1979). The cooking times needed to reach to the target temperatures (50 and 70°C) for packed/unpacked fish were presented in Table 1.

Microbiological Analyses

Each fish block was transferred to a stomacher bag with 90 mL of sterile peptone water (0.1%) and homogenized using a stomacher (IUL Masticator, Barcelona, Spain) for 1 min. Serial dilutions were prepared with peptone water (0.1%) and 0.1 mL of appropriate dilutions were spread onto Xylose Lysine Tergitol4 Agar (XLT4; Merck, Darmstadt, Germany) for *Salmonella* enumeration. Additionally, 1 mL from the first dilution (10^{-1}) was spread onto three XLT4 Agar plates. Thus, the lowest detection limit was 1 log cfu/cm². After incubation at 37°C for 24 h, black or red colonies with black center were counted manually as *S. Enteritidis*. When the black colonies were not determined the samples were tested for the presence or absence of *Salmonella* (Andrews et al., 2020).

Statistical Analyses

Two replicated experiments were conducted and two samples per treatment group were analyzed before and after microwave cooking ($n=4$). Data relating to microbial counts were subjected to ANOVA, using SPSS version 16.0 for Windows (SPSS, Chicago, Illinois, USA) and the Duncan test was performed for multiple comparison ($P<0.05$).

Results and Discussion

Salmonella Enteritidis Reduction After Microwave Cooking

S. Enteritidis counts of fish samples before and after microwave cooking are shown in Table 2. The initial counts of the pathogen on whiting and salmon samples were 6.37 and 7.23 log cfu/cm². *S. Enteritidis* counts reduced by microwave cooking depending on target internal temperature and cooking conditions. When cooked to the internal temperature of 50°C, the reduction rates of *S. Enteritidis* in unpacked and packed whiting, were 0.69 log cfu/cm² (11%) and 1.82 log cfu/cm² (29%) (Figure 1), respectively ($P<0.05$). The reduction rate was 2.89 log cfu/cm² (45%) for unpacked, while higher than 5.37 log cfu/cm² (84%) in packed whiting cooked at 70°C. A similar result was observed for salmon samples. When cooked to an internal temperature of 50°C, the reduction rates of *S. Enteritidis* in unpacked and packed salmon, were 0.73 log cfu/cm² (10%) and 2.39 log cfu/cm² (33%), respectively ($P<0.05$).

The reduction rates were 3.90 log cfu/cm² (54%) for unpacked, and higher than 6.23 log cfu/cm² (86%) in packed salmon, cooked at 70°C ($P<0.05$). As seen in Table 2, *Salmonella* was not completely inhibited in whiting and salmon samples as a result of the presence/absence test. These were reflected in the results as '<1.00' in the table. Microwave cooking resulted in the reduction of *Salmonella* Enteritidis, especially in packed samples at higher degrees, but did not eliminate this pathogen.

Huang et al. (1993) cooked catfish fillets in a microwave oven to internal temperatures varied from 55 to 70°C, and reported incomplete reduction of *Listeria monocytogenes* and *Aeromonas hydrophila*. Incomplete inactivation of *Salmonella* spp. in microwave-cooked poultry feed (Burdick et al., 1983) and chicken (Lindsay et al., 1986) has also been reported. Likewise, cooking in microwave oven to 76.6°C did not destroy *Salmonella* Typhimurium, *Staphylococcus aureus* and *Clostridium perfringens* in turkey meat (Aleixo et al., 1985). Tassinari and Landgraf (1997) evaluated the destruction of *Salmonella* Typhimurium during reheating of foods in microwave oven and reported the percentage of food samples positive for this pathogen as 47.8%. Since a single *Salmonella* cell may constitute a human infectious dose (D'Aoust, 2000), we suggest that heating contaminated fish in microwave may not ensure safety.

As it was shown in Table 2, cooking to an internal temperature of 70°C resulted in lower ($P<0.05$) *S. Enteritidis* counts than that of 50°C for both fish species. Higher inactivation rates have also been reported for microwave-cooked red meat when temperature increased (Paterson et al., 1995; Yilmaz et al., 2005). Mendes-Oliveira (2020) studied the inactivation of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium by microwave and reported increased reduction up to 7 log cfu/g, with the higher temperature, time and power. In another study, enriched corn-soy-milk blends were inoculated with *Salmonella* Senftenberg, and submitted to microwave heating. The higher reduction rates were observed at higher cooking temperatures and *S. Senftenberg* was reduced 2-log to 5-log after processing at 56.7°C through 82.2°C, respectively (Bookwalter et al., 1982). Likewise, 5-6 log reduction has been reported for *Salmonella* in popcorns, cooked at higher temperatures (Anaya et al., 2008). Woo et al. (2000) have studied the inactivation patterns of the microwave-radiated cells using cell suspensions of *E. coli* and *B. subtilis* and reported 5-log reduction when

Table 1. Cooking times (min) required to reach to the target internal temperatures (50°C and 70°C) for unpacked/packed fish.

Fish species	Cooking Conditions			
	50°C		70°C	
	Unpacked	Packed	Unpacked	Packed
Whiting	2.4	1.3	5.0	3.4
Salmon	1.4	1.1	3.3	2.3

cooked at 80°C. In another study on the survival of *Salmonella* on half carcasses of chicken broilers during microwave cooking showed that long cooking times and higher temperatures were needed for the entire destruction of the pathogen (Gomółka-Pawlicka et al., 2014). Microwave cooking of fish reported to be effective to reduce *E. coli* O157:H7, *Staphylococcus aureus* and *Listeria monocytogenes*, when their internal temperature is 50°C or 70°C (Ulusoy et al., 2019). It is clear that higher cooking temperatures increase the reduction rates.

Effect of Packaging on Reduction Rates

The reduction rates were higher (P<0.05) for both fish species when cooked in a package (Table 2, Figure 1). In a study by Aleixo et al. (1985), microwave cooking in bags increased the destruction of *Salmonella* Typhimurium in whole turkeys. Likewise, Huang et al. (1993) cooked *L. monocytogenes* and *A. hydrophila* inoculated channel catfish fillets either uncovered or covered with polyvinylidene chloride films in a microwave oven and reported that covering fillets increased lethality at each temperature (55, 60, and 70°C).

Effect of Fish Species on Reduction Rates

Muscle foods are complex systems, mainly due to the presence of fat and proteins (Aymerich et al. 2008).

Fat accelerates the microwave heating rate and increases the effect of temperature (Jeong et al., 2006; Picouet et al., 2007). Juneja and Eblen (2000) have reported that increased fat levels in beef resulted in lower D-values, i.e. reduced heat resistance. Likewise, the time, needed to achieve the desired temperature is shorter in salmons, than that of whiting blocks in our study (Table 1). As it may be seen in Figure 1, reduction of *S. Enteritidis* was higher in salmon than whiting, especially when the samples were cooked to 70°C. Garcia-Linares et al. (2004) reported fewer microbial counts in fatty species than that of lean fish after cooking. Salmon has been known as a fatty fish, while whiting has been known as a lean species (Tulsner, 1994). Our results are well correlated with these literatures, since the reduction rates of salmon were higher (P<0.05) than whiting.

Conclusions

It was concluded that packaging increased the reduction of *S. Enteritidis* on fish during microwave cooking. Higher reduction was achieved in salmon samples than that of whiting. Therefore, these results suggested that the fish species were also important in terms of the risk of *S. Enteritidis* survival during microwave cooking. Cooking in microwave to higher internal temperatures increased reduction rates significantly (P<0.05), but *S. Enteritidis* could not be eliminated completely in both fish species. Therefore,

Table 2. *Salmonella* Enteritidis counts on fish samples before and after cooking (log cfu/cm²)

Fish species	Before Cooking	Cooking Conditions			
		50°C		70°C	
		Unpacked	Packed	Unpacked	Packed
Whiting	6.37±0.06 ^a	5.68±0.07 ^c	4.55±0.68 ^b	3.48±0.26 ^d	<1.00 *
Salmon	7.23±0.73 ^a	6.50±0.13 ^c	4.84±0.30 ^b	3.33±0.24 ^d	<1.00 *

^{a-d}= Different letters in the same row show the significant differences (P<0.05) between the groups
 * *Salmonella*-positive after enrichment

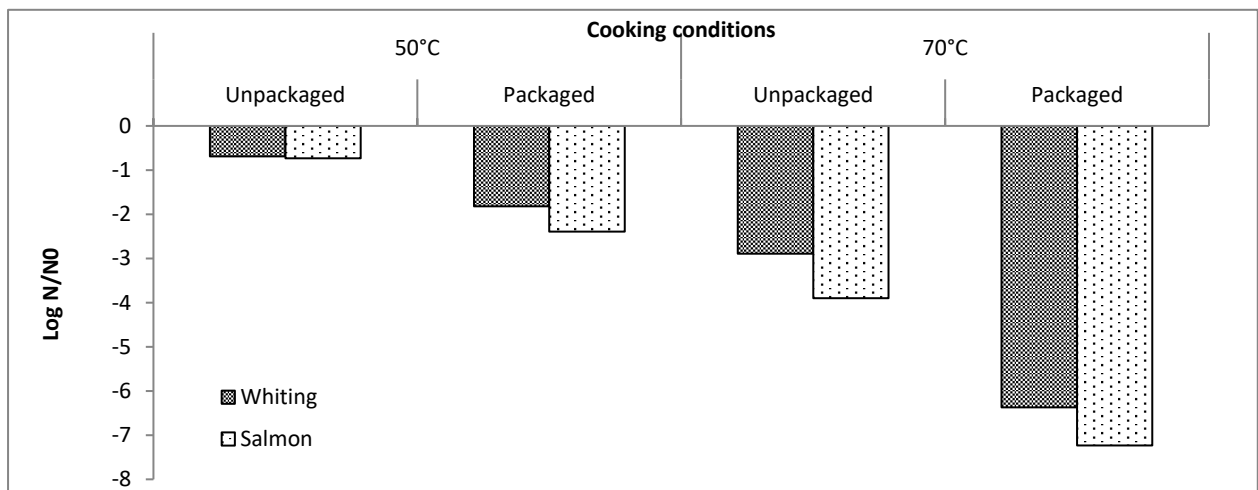


Figure. 1. The log reductions of *Salmonella* Enteritidis (log cfu/cm²) counts on fish samples after microwave cooking.

even packed or unpacked cooking the fillets to an internal temperatures of 50°C and 70°C was considered inadequate to destroy *S. Enteritidis*.

Considering the popularity of microwave cooking, results of this study will be important regarding food safety. Microwave cooking instructions for fish should be prepared considering the risk of uneven heating and bacterial survival. The data presented in this paper may be interesting either for the standardization of microwave ovens for domestic use, or for ready to eat food producers, when preparing HACCP plans.

Microwave-cooking guidelines for the domestic purposes and /or food service facilities must be included into the MW operating manuals. The MW-cooking guidelines and HACCP plan for food service facilities must be determined accordingly. The foods must be cooked in microwave not lower than 360 W and 70°C. Microwave cooking foods in package is recommended.

Ethical Statement

This article does not require IRB/IACUC approval because there are no human and animal participants.

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Author Contribution

First Author and second Author: Data curation, Writing - review & editing; Third Author: Methodology, Writing - review and editing; and Fourth Author: Conceptualization, Methodology, Writing - review & editing.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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