

Full Length Research Paper

The effect of short-time microwave exposures on *Escherichia coli* O157:H7 inoculated onto beef slices

A. Jamshidi^{1*}, H. A. Seifi² and M. Kooshan³¹Department of food hygiene, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Iran.²Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Iran.³DVM degree, Graduated from School of Veterinary Medicine, Ferdowsi University of Mashhad, Iran.

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Escherichia coli O157:H7 is an important human pathogen causing haemorrhagic colitis, hemolytic-ureamic syndrom and thrombotic thrombocytopenic purpura. In this study the effect of microwave irradiation of beef samples which were inoculated with *E. coli* O157:H7 were investigated. The portions of fresh beef slices weighting 200 g each and about 10 × 10 × 2 cm in size, were soaked in fully growth of *E. coli* O157:H7, in BHI broth. The swab samples were taken from the contaminated samples, after different times of radiation (10, 20, 30, 40 and 50 s), using a domestic microwave oven at full power. The bacterial counts were performed by using surface plating on sorbitol Mac Conkey agar supplemented with cifaxime and potassium tellurite. After each experiment the surface temperature of treated samples were measured. The experiment was carried out in triplicate and it was concluded that the microwave radiation which enhance the surface temperature more than 70°C, can eliminate the superficial contamination of cattle beef slices with *E. coli* O157:H7.

Key words: *Escherichia coli* O157:H7, microwave, beef.

INTRODUCTION

Meat is a rich nutrient matrix that provides a suitable environment for proliferation of meat spoilage micro-organisms and common food-borne pathogens, therefore adequate preservation technologies must be applied in order to preserve its safety and quality. Verocytotoxin-producing *Escherichia coli* (VTEC) strains are the most important recently emerged food-borne pathogens (Armstrong et al., 1996). VTEC may belong to many serotypes, but most severe human infections are caused by strains of *E. coli* O157:H7 (Mead and Griffin, 1998). Due to the severity of infection which *E. coli* O157:H7 causes and an infectious dose which may be as low as 10 organisms (Coia, 1998), it has emerged as an important food borne pathogen of considerable public health concern. It causes haemorrhagic colitis, Hemolytic-Ureamic Syndrom (HUS) and Thrombotic Thrombocytopenic Purpura (TTP) (Zhao et al., 1998; Nataro and Kaper, 1998).

Cattle especially the young ones have been implicated

as a principal reservoir of *E. coli* O157:H7 (Trevena et al., 1996). Cattle frequently excrete this bacteria in their feces (Molina et al., 2003; Van Donkersgoed et al., 1999). Feces and hides are significant sources of bacterial carcass contamination (Gun et al., 2003). Meat and environment become contaminated from intestinal content of cattle at the time of slaughter, and processing may introduce the organism when performed in non hygienic conditions (Mead and Griffin, 1998). Under-cooked beef is the major vehicle of food-borne outbreaks (Zhao et al., 1998; Oldfield, 2001).

High frequency energy includes microwave and radio-frequency energy belongs to the non-ionizing radiations,; microwaves lie between the infrared and radio frequency portions of the electromagnetic spectrum (Jay et al., 2007). In a microwave oven the heating of food results from molecular friction between water molecules under an oscillating electric field of specific frequency (Pucciarelli and Benassi, 2005). Heating by microwave (MW) energy is used for several purposes, e.g., cooking, pasteurization, sterilization and blanching of foods (Giese, 1992; Datta and Davidson, 2001). The safety of microwave cooking in relation to food borne pathogens is questioned. There are studies reporting in complete

*Corresponding author. E-mail: ajamshid@ferdowsi.um.ac.ir.
Tel: 0511-8763656. Fax: 0511-8763852.

inactivation of microorganisms including pathogens, in inoculated cooked foods or reheated in MW ovens (Heddleson and Doores, 1994; Datta and Davidson, 2001). The aim of the present study was, to investigate the effect of the microwave heating on the fate of *E. coli* O157:H7, inoculated onto cattle beef portions.

MATERIALS AND METHODS

Equipment and samples

Microwave irradiation was performed in a household microwave oven (Delonghi, type MW-675FI, with a rotating glass plate, a frequency of 2,450 MHz, and power of 850 watts. The microwave was used at full power for heating of beef portions. In each replicate of the experiment, six fresh portions from thigh meat, sold in wrapped packages, obtained from a supermarket were used. All samples were transferred to the laboratory within 1 - 2 h at 4°C in insulated boxes and stored at 4°C until use within 24 h after purchase. Slices of 50 cm² area and weighing 200 g from the samples were removed aseptically using a sterile scalpel. Prior to any further experimental procedure, the samples treated with H₂O₂ + Ag⁺ (sanosil) as a sanitizer then washed three times with sterile distilled water to remove the residuals. All samples were examined for any pre-existing contamination with *E. coli* O157:H7, following the method (specific detection of *E. coli* O157:H7, described by Roberts et al., 1995).

Preparation of the *E. coli* O157:H7 inocula

E. coli O157:H7 (ATCC-35150) was used for inoculation in each experiments. Stock cultures of the strain were prepared in Tryptone Soya Agar (Himedia) slants, stored at 4°C and subcultured every 4 weeks. Pure cultures of *E. coli* O157:H7 were prepared by sub-culturing the test strain into 500 mL of Brain Heart Infusion Broth (Merck), following incubation at 37°C for 24 h. The concentration of the resulting culture of *E. coli* O157:H7 was determined by serial dilutions and viable counts by surface plating on CT-SMAC (sorbitol Mac-Conkey agar supplemented with cifaxime and potassium tellurite) agar (Himedia).

This culture media were used for inoculation of the beef samples. The absorbance of the cultured media were also determined in 600 nm wave length, using a spectrophotometer apparatus (Jenway 6105, Essex, England), in order to inoculate the same dose of bacteria in repeating the experiment.

Inoculation procedure and microbiological analysis

The six portions of fresh beef slices weighting 200 g each and about 10 × 10 × 2 cm in size, were immersed into 500 mL of the prepared *E. coli* O157:H7 suspension for 10 min, they were drained by dripping on absorbent sterile cheesecloth for another 10 min and they were placed in sterile glass Petri dishes. One sample was reserved for estimating the *E. coli* O157:H7 concentration of the tested portions as positive control. The remaining five samples were heated individually into the microwave oven operating at full power for 10, 20, 30, 40 and 50 s, respectively. Surface temperatures in the approximate center of the upper surface of the samples were measured immediately after exposures, using a thermometer. Following microwave heating, after about five minutes the upper surfaces of the samples were swabbed, by using a template (wet and dry swabbing method) (Vanderzant and Splittstoesse 1992). To determine the numbers of surviving *E. coli* O157:H7 cells, decimal

dilutions from each swabs containing tube were prepared and viable count were performed by surface plating on Sorbitol MacConkey Agar (Merck) following incubation at 37°C for 24 h,. The experiment was carried out in triplicate.

Statistical analysis

The statistical analysis was performed using SPSS statistical software (version 16). A non-parametric Kruskal-Wallis test at $p < 0.05$ was used to determine the effect of time duration of microwave exposure on *E. coli* O157:H7 viability. Pairwise comparison of viability of *E. coli* O157:H7 between positive control and other groups were investigated by the Mann-Whitney U-test considering Bonferroni adjustment. The relationship between inoculated bacterial population viability and temperature of samples due to microwave exposure was examined with Pearson correlation test.

RESULTS

Pre existing contamination with *E. coli* O157:H7 was not detected in beef samples. The concentration of cultured media inoculated with test strain was determined as 1.1×10^9 cfu mL⁻¹, using viable count and it is absorbance at 600 nm was determined as equal to 0.9. Destruction levels of inoculated *E. coli* O157:H7 at different times of microwave exposure are shown in Figure 1 the death curve is presented as the colony forming units concentration in cfu/cm² following the exposure in microwave. Final surface temperature of beef portions, after different time duration of microwave exposures are shown in Figure 2. Elimination of *E. coli* O157:H7 was observed after the end of 30 s exposure time, when the surface temperature was increased to 73°C.

Pearson correlation showed a very significant correlation between the bacterial population and temperature of samples due to microwave exposure ($p < 0.0001$, $r = 0.973$ and $r^2 = 0.947$).

DISCUSSION

Microwave ovens have become common household appliances in developed countries and, to some extent, in developing countries. This relatively inexpensive technology is commonly used to cook or warm foods in homes, offices, and some restaurants. With respect to consumer safety, the research reported here shows that microwave radiation can be used to control (to reduce or sometimes to completely eliminate) microbial potential pathogens in food. Evidence suggests that microwaves are being used more frequently than ever before to cook raw foods. Although, microwave reheating has been shown to be a generally reliable method of reducing microbiological pathogens, little research has been performed on its efficacy to promote microbiological safety in cooking raw foods (Farber et al., 1998). According to our study induction of 73°C superficial temperature in a slice of beef could eliminate the inoculated bacteria, which it's

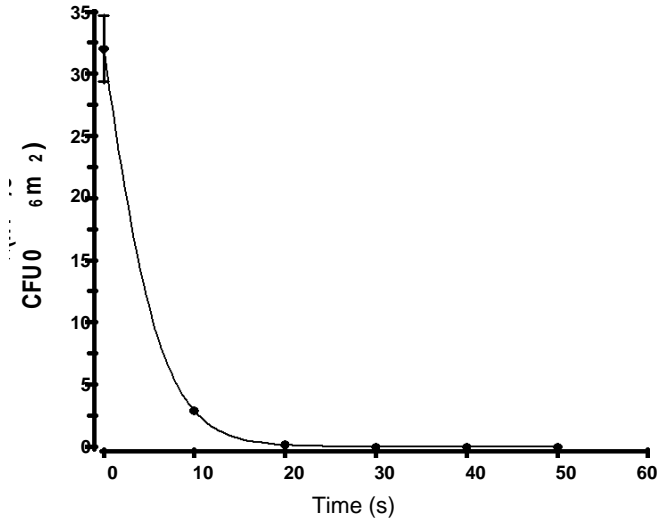


Figure 1. Destruction of *E. coli* O157:H7 as a function of microwave exposure time of beef portions.

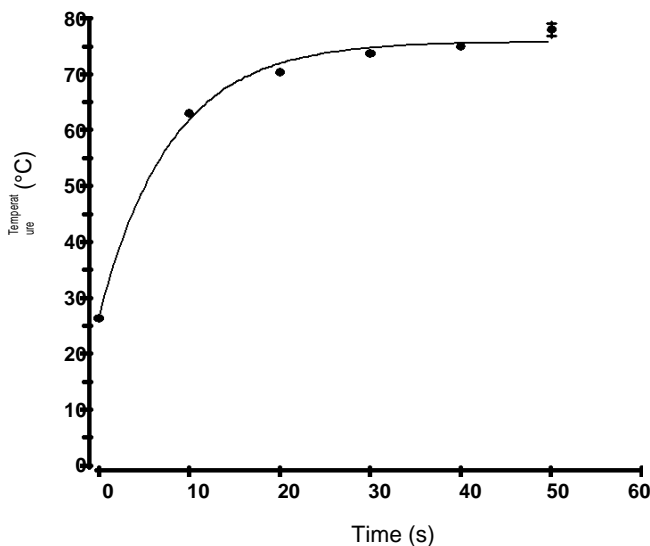


Figure 2. Final surface temperature of beef portions after different time of microwave exposure.

primary contamination rate with *E. coli* O157:H7 was 3.2×10^7 cfu/cm². Duration of radiation with full power to produce this temperature was 30 s. A 5- log reduction of the viable count was also reported for *E. coli* suspension exposed to full power of microwave radiation (600 watts) in 80 s (Woo et al., 2000). In another study microwave radiation which produced an internal temperature of 85°C in fresh whole roasting chickens, was shown to eliminate *Salmonella typhimurium* (Schnepf and Barbeue, 2007). It seems that other parameters like as size and shape of the irradiated beef may influence the elimination of inoculated bacteria, because survival of pathogens such as *Salmonella* spp. (Schnepf and Barbeau, 2007) and *L.*

monocytogenes (Farber et al., 1998), in foods heated in microwave ovens, is attributed to the non-uniform heating and their asymmetrical form. In another study although, elimination of *E. coli* O157:H7 in chicken breast portions occurred after 35s of microwave exposure at 73.7°C, but when whole chickens were exposed to microwave radiation, even with 92°C in some area, viable cells of *E. coli* O157:H7 were recovered from all samples (Apostolou et al., 2005).

In our study only surface temperature measurements were taken from the sample's centre to avoid the extreme variability of surface and subsurface temperatures observed by other researchers in meat samples heated by microwaves, and because the central area is where the least temperature increase is expected to occur (Farber et al., 1998; Goksoy et al., 1999) and also to prevent the "edge- heating effect" which is overheating of corners and edges of foods in a microwave field, caused primarily by the uneven energy distribution during microwave heating (Huang and Sites, 2007).

In our study the swabbing of samples were performed after five minutes of microwave exposure, because it was reported that post- heating holding times of two or more minutes, increases bacterial destruction (Heddison et al., 2006). Survival of some inoculated pathogens in meat portions after microwave exposure may be due to immediate sampling. The primary concern associated with microwave cooking is uneven heat distribution, which results in the formation of hot and cold spots in the food (Farber et al., 1998). To guarantee microbiological safety it has been recommended to cover the food with wax paper and checking temperature in at least three different sites (Farber et al., 1998).

In conclusion, consumers can use microwave ovens to significantly reduce microbial pathogens in foods like beef. Microwave irradiation is a cost-effective, practical, fast, easy and safe method of decontaminating foods.

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