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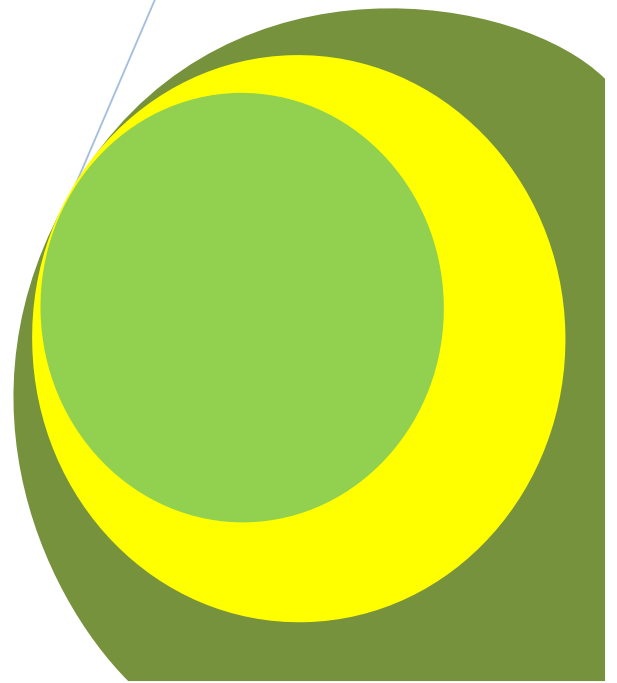
By

**Maureen Khatiera Luvanda**

**John Maingi**

**Paul Okemo**

**Yang E.**



*Research Article*

# Analysis of the Effects of Di-Electric Heating on Common Food Bacterial Pathogens: Evaluation Based on Antibiotic Susceptibility and Infective Doses

Maureen Khatiera Luvanda<sup>1\*</sup>, John Maingi<sup>2</sup>, Paul Okemo<sup>3</sup>,  
Yang E.<sup>4</sup>

<sup>1</sup>Department of Medical Laboratory Science, Kenyatta University, Nairobi, Republic of Kenya. P. O. Box 8528-00300, Nairobi, Kenya.

<sup>2,3</sup>Department of Plant and Microbial Sciences, Kenyatta University, Nairobi, Republic of Kenya. P.O. Box 43844 (00100).

<sup>4</sup>Department of Microbiology, Xi'an Jiaotong University, Xi'an, People's Republic of China.

<sup>2</sup>Email: maingijohn@gmail.com, Tel. +254-020-8710901 ext 57177, Fax +254-8711575

<sup>3</sup>Email: paulokemo@gmail.com, Tel: +254722942072

<sup>4</sup>Email: eyang@mail.xjtu.edu.cn, Tel: +8615029979505

\*Corresponding Author's Email: maureenluvanda@gmail.com, Tel: +254703590667

## ABSTRACT

In this study, various food samples (n=73) artificially infected by food borne disease pathogens (*Escherichia coli*, *Staphylococcus aureus*, *Shigella flexneri* and *Salmonella enterica* spp. paratyphi) were exposed to microwave radiation at different powers (P-00, P-20, P-40, P-60, P-80) and time periods (2 min, 4 min, 6 min, 8 min). The degree of inactivation was estimated by measuring the colony forming units formed in culture before and after exposure of samples to radiation. The data showed that microwave radiation produced a 1-2 log reduction when infected food samples were processed. Initial seeded bacterial numbers ( $>3.0 \times 10^4$ ) were reduced significantly ( $P < 0.05$ ). The most effective power-time combination for bacteria inactivation was 8 minutes at P-80 (1180W) where bacteria numbers were very low ( $<1.0 \times 10^3$ ); whereas the least effective power-time combination for bacteria inactivation was 2 minutes at P-20 (295W) where bacteria numbers remained at ( $>3.0 \times 10^4$ ). The study shows that microwave radiation reduces infective doses of bacteria but does not have any significant effect on their antibiotic susceptibility patterns.

**Keywords:** Di-electric Heating, Colony Forming Units, Inactivation, Multi-drug resistance, Infective dose, Bacterial Contaminants.

## INTRODUCTION

Food borne diseases (FBDs) are leading causes of illness and death in less developed countries killing approximately 7.2 million people annually, 1.9 million of whom are children (MPHS (K), 2011). WHO has estimated that each year 2.2 million people, including 1.9 million children, die because of diarrheal diseases (Vandepitte *et al.*, 2003; DeWaal *et al.*, 2009). Two to Four billion episodes of infectious diarrhoea have been estimated to occur annually in developing countries, resulting in 3 to 5 million deaths (Olaniran *et al.*, 2011). FBDs have been with us for centuries, however since 1977, new or newly characterized food borne pathogens have been recognized at a rate of approximately one every two years (Tauxe *et al.*, 2002). Therefore, the number of FBDs has increased globally, especially in developing countries where food safety interventions and sanitary control measures are not adequately implemented. FBDs remain responsible for high levels of morbidity and mortality in the general population but particularly for certain risk-groups such as infants, the elderly and the immune-suppressed (Manson-Bahr *et al.*, 1987; Mehata and Duan, 2011; Lampel *et al.*, 2012). Microwave heating is known to inactivate many micro-organisms; for instance, *Escherichia coli*, *Streptococcus faecalis*, *Staphylococcus aureus*, *Bacillus subtilis* spores, *Salmonella* sp., *Lactobacillus plantarum*, *Listeria* spp., *Saccharomyces cerevisiae* and *Clostridium perfringens* (Gedikli *et al.*, 2008). These effects of MW radiation on bacteria have been studied and debated for more than half a century (Shamis *et al.*, 2011) with most of these studies exposing micro-organisms to MW radiation in liquid suspensions and observing the effects. However, Jeng *et al.*, 1987 disputed this method of analysis by pointing out that a liquid suspension containing micro -

organisms is not an efficient system for the purpose of studies because of its reduction of the local electric field strength. This paper reports observations made based on whether the changes that occur on bacteria due to exposure to microwave radiation have any significant effect on their growth and multiplication, infective doses as well as their susceptibility to commonly prescribed antibiotics thus having an impact in the control and prevention of FBD outbreaks. The study was intended to measure the exact effect size of microwave radiation on the overall burden of FBDs thus validating the machines' contribution towards food safety.

## MATERIALS AND METHODS

### Study design and period

The Pretest-posttest study design was applied where the mean counts of bacterial colonies formed in food before exposure to microwave radiation were compared to the mean counts after exposure to microwave radiation. In addition, antibiotic susceptibility testing was performed on organisms before and after exposure to microwave radiation.

### Study area

The study was conducted in an accredited laboratory located in the Microbiology department of Xi'an Jiaotong University, Xi'an, Shaanxi district, People's Republic of China.

### Test Organisms and their Maintenance

*Staphylococcus aureus* ATCC 25922, *Escherichia coli* ATCC 25923, *Salmonella enterica* subsp. Paratyphi CMCC 50319 (Similar to ATCC 19430) and *Shigella flexneri* CMCC 51285 (Similar to ATCC 29903) were used as test organisms. Each reference culture was checked thoroughly upon receipt. Gram stain and biochemical testing was performed to confirm bacteria identities. Pure cultures were stored at -80°C in nutrient broth supplemented with 20% (v/v) of glycerol. The bacteria were routinely cultivated for 24 hours on nutrient agar. Working bacterial suspensions were freshly prepared for each independent experiment. Medium and incubation conditions were used as specified in the catalogue when first reviving strains to ensure optimal conditions for recovery. Drops of bacteria suspension were also transferred to an agar slant. Cultures were sub-cultured twice prior to testing each week.

### Data collection and processing

The food samples (n=73) included cooked beef, milk, chicken, eggs, gravies and sauces, mashed-potatoes, fish and cooked vegetables. These particular foods were selected because they are very popular foods plus they are sensitive foods that can easily get spoiled due to their high protein and water content. They are therefore classified as high risk foods (Kitagwa *et al.*, 2005; Bekker, 2003: Kenya Food, Drugs and Chemical Substances Act, CAP 254, 1992). 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.

Samples were autoclaved in order to ensure complete destruction of pre-existing pathogens at 121°C for 15 minutes. They were then inoculated with the specific bacteria in doses capable of causing infection (this was determined through comparison with 0.5 McFarland standard). Exposure to microwave radiation was then done at different power levels and time periods after which there was direct culturing of contaminated food samples into enrichment broths. The samples were incubated at 37°C for 24 hours. Thereafter, 100µl of the enrichment media was sub-cultured into agar media using spread plate technique for making total colony counts. The required physical and biochemical tests were performed for isolation, identification and analysis of antibiotic resistance characteristics.

### Statistical Analysis

The *Paired samples t-test* was then used and its non-parametric alternative *Wilcoxon Signed Ranks test* to the general sample statistics to address the question whether the average colony forming units for the two groups differ (exposed and unexposed). Relationship analysis between the two groups was done by constructing a *scatter plot* and calculating the number of *correlation coefficients*. Statistical analysis was done using SPSS V13.0.

## RESULTS

Table I: Table showing effect of microwave radiation on specific food-micro organism combinations in terms of mean colony forming units before exposure (Mean CFUB) and mean colony forming units after exposure (Mean CFUA).

FOOD SAMPLE	BACTERIA	MEAN CFUB	MEAN CFUA
Beef	<i>E. coli</i>	$>3.0 \times 10^4$	$>3.0 \times 10^4$
Beef	<i>S. paratyphi</i>	$>3.0 \times 10^4$	$>3.0 \times 10^4$
Fish	<i>S. flexneri</i>	$>3.0 \times 10^4$	$1.1235 \times 10^4$
Vegetables	<i>S. paratyphi</i>	$>3.0 \times 10^4$	$2.0917 \times 10^4$
Gravy	<i>S. paratyphi</i>	$>3.0 \times 10^4$	$1.3344 \times 10^4$
Vegetables	<i>S. flexneri</i>	$>3.0 \times 10^4$	$4.412 \times 10^3$
Beef	<i>E. coli</i>	$>3.0 \times 10^4$	$>3.0 \times 10^4$
Chicken	<i>E. coli</i>	$>3.0 \times 10^4$	$>3.0 \times 10^4$
Eggs	<i>S. paratyphi</i>	$>3.0 \times 10^4$	$7.444 \times 10^3$
Fish	<i>S. paratyphi</i>	$>3.0 \times 10^4$	$1.2547 \times 10^4$
Gravy	<i>S. paratyphi</i>	$>3.0 \times 10^4$	$7.444 \times 10^3$
Beef	<i>S. aureus</i>	$>3.0 \times 10^4$	$4.414 \times 10^3$
Chicken	<i>S. paratyphi</i>	$>3.0 \times 10^4$	$6.118 \times 10^3$
Eggs	<i>E. coli</i>	$>3.0 \times 10^4$	$8.250 \times 10^3$
Fish	<i>E. coli</i>	$>3.0 \times 10^4$	$5.265 \times 10^3$
Vegetables	<i>E. coli</i>	$>3.0 \times 10^4$	$4.412 \times 10^3$
Milk	<i>S. paratyphi</i>	$>3.0 \times 10^4$	$1.735 \times 10^4$
Milk	<i>E. coli</i>	$>3.0 \times 10^4$	$2.6822 \times 10^4$
Milk	<i>S. aureus</i>	$>3.0 \times 10^4$	$1.5872 \times 10^4$
Potato	<i>S. aureus</i>	$>3.0 \times 10^4$	$1.1776 \times 10^4$

Among all the infected food samples processed in this study, the least affected by microwave radiation at different power-time combinations were: beef inoculated with *Escherichia coli*, beef infected with *Salmonella Paratyphi* and chicken infected with *Escherichia coli*. The most affected were vegetables infected with *Shigella flexneri*, vegetables infected with *Escherichia coli* and beef infected with *Staphylococcus aureus*. All food samples did not produce an observable effect after exposure to microwave radiation for 2 minutes at power 20. The initial populations of viable *Shigella flexneri* microorganisms in fish sample (mean CFU counts for all samples before radiation was  $>300$ ) decreased by a  $1.5 \log \text{ unit}$  at power 40 for 4, 6 and 8 minutes, at power 60 for 4, 6 and 8 minutes and at power 80 for all time-periods. The mean CFU count after exposure to microwave radiation was 112.35 (Table I). The organoleptic properties for this sample were not affected by exposure to microwave radiation. The initial populations of viable *Salmonella Paratyphi* microorganisms in vegetables sample decreased by  $1.5 \log \text{ unit}$  as well at power 20 for 8 minutes, power 40 for 8 minutes and at power 80 for 4,6 and 8 minutes. Although the effect was much less than most of the other samples, after running the paired samples t-test statistic a P value of  $<0.05$  was generated which still indicated significance. The mean CFU counts after exposure to microwave radiation was 209.17. The organoleptic properties for this sample were also not affected by exposure to microwave radiation. The initial populations of viable *Salmonella Paratyphi* microorganisms in gravy sample decreased by a  $1.5 \log \text{ unit}$  at power 20 for 4, 6 and 8 minutes. The mean CFU counts after exposure to microwave radiation was 133.44. However, the organoleptic properties for this sample were affected by exposure to microwave radiation at the following time-power combinations: 2.60, 4.60, 8.60, 2.80, 4.80, 6.80 and 8.80. The initial populations of viable *Shigella flexneri* microorganisms in vegetable sample also decreased by a  $1.5 \log \text{ unit}$  at power 20 for 4 minutes as well as power 40, power 60 and power 80 for all time-periods. The mean CFU counts after exposure to microwave radiation was 44.12. There was no observable effect on this sample's organoleptic properties after exposure. The initial populations of viable *Salmonella Paratyphi* microorganisms in beef sample were not decreased at any time-power combinations. The mean CFU counts after exposure to microwave radiation remained at 300 and the paired samples t-test statistic could not be run because the standard error of the difference was 0. The organoleptic properties for this sample were also not affected by exposure to radiation. This was also the case for beef and chicken samples inoculated with *Escherichia coli*. However, organoleptic properties for chicken sample were affected by exposure at power 80 for 8 minutes. The initial populations of viable *Salmonella Paratyphi* microorganisms in eggs, gravy and milk samples decreased by a  $1.5 \log \text{ unit}$  at all time-power combinations except for 2 minutes at power 20. Their mean CFU counts after exposure to microwave radiation were 74.44 for both eggs and gravy and 173.50 for milk. The organoleptic properties for this sample were affected by exposure to microwave radiation at the following time-power combinations: 2.60, 4.60, 8.60, 2.80, 4.80, 6.80 and 8.80. This was the same case for eggs and milk samples inoculated with *Escherichia coli* and milk sample inoculated with *Staphylococcus aureus* whose mean C F U

counts were 82.50, 268.22 and 158.72 respectively. The initial populations of viable *Salmonella* Paratyphi microorganisms in Fish sample decreased by a  $1.5 \log$  unit at 6 minutes at power 40 all the way to 8 minutes at power 80. The mean CFU counts after exposure to microwave radiation was 125.47. The organoleptic properties for this sample were also not affected by exposure to microwave radiation. For *Staphylococcus aureus* microorganisms in Beef sample and *Escherichia coli* microorganisms in Vegetable sample, the initial populations decreased by a  $1.5 \log$  unit at all time-power combinations except 2 minutes at power 20. The mean CFU after exposure to microwave radiation was 44.14 and 44.12 respectively. The organoleptic properties for these samples were not affected by exposure to microwave radiation. The initial populations of viable *Salmonella* Paratyphi microorganisms in Chicken sample decreased by a  $1.5 \log$  unit at all time-power combinations except 2 minutes and 4 minutes at power 20. The mean CFU counts after exposure to microwave radiation was 61.18. The organoleptic properties for this sample were only affected by exposure to microwave radiation at power 80 for 8 minutes. The initial populations of viable *Escherichia coli* microorganisms in Fish sample decreased by a  $1.5 \log$  unit at all time-power combinations except 2 minutes at power 20 and 6 minutes at power 60. The mean CFU counts after exposure to microwave radiation was 52.65. The organoleptic properties for this sample were not affected by exposure to microwave radiation. Lastly, the initial populations of viable *Staphylococcus aureus* microorganisms in Potato sample was decreased by  $1.5 \log$  unit at various time-power combinations. However, the time-power combinations were not consistent for the duplicate experiments. The mean CFU after exposure to microwave radiation was 117.76. The organoleptic properties for this sample were also not affected by exposure to microwave radiation. As for the antibiotic susceptibility testing, there were no observable pattern changes in resistance or susceptibility of food bacteria before and after exposure of food to microwave radiation as shown in Table II)

Table II: Antibiotic Sensitivity Testing (Ast) Results

Table highlighting AST Results for *Escherichia coli* (E), *Salmonella enterica* (S<sup>1</sup>), *Shigella flexneri* (S<sup>2</sup>) and *Staphylococcus aureus* (S<sup>3</sup>) before and after exposure of Sample to Microwave Radiation

SAMPLE	AMPICILLIN	GENTAMYCIN	CIPROFLOXACIN	CEFOTAXIME	NITROFURANTOIN
CHICKEN (E)	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
GRAVY (E)	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
MILK (E)	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
VEGETABLES(E)	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
BEEF (E)	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
EGGS (E)	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
FISH(E)	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
MILK (S <sup>1</sup> )	Resistant	Sensitive	Sensitive	Resistant	n/a
EGGS (S <sup>1</sup> )	Resistant	Sensitive	Sensitive	Resistant	n/a
GRAVY (S <sup>1</sup> )	Resistant	Sensitive	Sensitive	Resistant	n/a
VEGETABLES(S <sup>1</sup> )	Resistant	Sensitive	Sensitive	Resistant	n/a
BEEF (S <sup>1</sup> )	Resistant	Sensitive	Sensitive	Resistant	n/a
CHICKEN (S <sup>1</sup> )	Resistant	Sensitive	Sensitive	Resistant	n/a
FISH (S <sup>1</sup> )	Resistant	Sensitive	Sensitive	Resistant	n/a
FISH (S <sup>2</sup> )	Sensitive	Sensitive	n/a	Sensitive	Sensitive
VEGETABLES(S <sup>2</sup> )	Sensitive	Sensitive	n/a	Sensitive	Sensitive
SAMPLE	OXACILLIN	VANCOMYCIN	ERYTHROMYCIN	AZITHROMYCIN	NITROFURANTOIN
BEEF (S <sup>3</sup> )	sensitive	sensitive	Sensitive	Sensitive	resistant
MILK (S <sup>3</sup> )	sensitive	sensitive	Sensitive	Sensitive	resistant
POTATOES(S <sup>3</sup> )	sensitive	sensitive	Sensitive	Sensitive	resistant

## DISCUSSION

Dielectric heating by microwave irradiation is well known to have an effect on killing both gram positive and gram negative micro-organisms (Tanaka *et al.*, 1998; Gedikli *et al.*, 2008; Dababneh, 2013) and the use of microwaves to reduce the population of micro-organisms in many different foods such as turkeys, meats, milk, corn on the cob, chickens, frozen foods and potatoes has been demonstrated in many studies (Valeschi *et al.*, 2004; Dumuta-Codre *et al.*, 2010). However, they failed to measure the exact effect size of these radiations on reducing the infective dose numbers of bacteria as well as determining their effect on antimicrobial susceptibility patterns. During this study, when the artificially infected food samples were irradiated for 2 minutes at power 20 (295W) the bacteria survival rate was 100% (bacteria numbers remained at  $>3.0 \times 10^4$ ) whereas when artificially infected food samples were irradiated for 8 minutes at power 80 (1180W), the bacteria survival rate was 3.33% (bacteria numbers were very low  $<1.0 \times 10^3$ ). This huge difference in reduction of seed numbers at higher time-power combinations is quite significant ( $P < 0.05$ ) and strongly indicates that there can be total destruction of bacteria in food samples and thus 100% reduction of their infective doses. When time-periods were kept constant, higher powers were shown to be very effective at reducing bacterial numbers and when the radiation power was kept constant, longer time-periods were shown to be more effective in the destruction of bacteria. The infected food samples that were not exposed to radiation served as the control and they all showed that the bacterial numbers remained at  $>3.0 \times 10^4$ . This showed that the inocula of test organisms used to infect food samples before exposure were sufficient to produce growth under the conditions used in the experiment and just like Sheen *et al.*, 2012 if no colonies were observed from a plate, the bacterial counts in the sample were treated as  $<1.0 \times 10^3$  because  $\log 0$  is mathematically meaningless. The highest mean CFU count after exposure of sample to microwave radiation was detected in beef sample processed for *Escherichia coli* and *Salmonella Paratyphi* ( $>3.0 \times 10^4$ ), whereas the lowest mean colony forming unit count after exposure of sample to microwave radiation was detected in vegetable sample processed for *S Shigella flexneri* and *Escherichia coli* as well as beef sample processed for *Staphylococcus aureus*. The above results were consistent with studies carried out by Gedikli *et al.*, 2008; Gomolka-Pawlicka *et al.*, 2013 and Sheen *et al.*, 2012 which showed that exposure of bacteria at higher powers during microwave radiation resulted in higher destruction than at lower powers. Sheen *et al.*, 2012 demonstrated that after 1 min with full power, approximately 1.5 to 2 log-reductions in the bacterial counts of *Salmonella*, *L. monocytogenes* and *E. coli* O157:H7 were achieved. Microwave power is a factor greatly influencing the rate of microwave heating. If a high value of power is applied, a high rate of temperature elevation in the heated body can be expected (Housova and Hoke, 2002). Therefore, the conceivable reason as to why the bacteria survived better at lower time-power combinations would be that not enough heat was generated during radiation whereas when it came to higher time-period combinations there was sufficient heat generated that may have been lethal to the micro-organisms. Other factors that could have affected the outcome after exposure to MWs may have been power fluctuation, probe location, varying dielectric properties in each food tested, sample placement within the microwave cavity, probe error or product density, ionic nature and thermal conductivity (Heddleson *et al.*, 1994; Gunasekaran *et al.*, 2005; Dumuta-Codre *et al.*, 2010). Another factor may be presence of an uneven electric field distribution inside the microwave cavity (Shamis *et al.*, 2011). The cavity in a domestic microwave oven is designed to have typically 3-6 different modes intended to provide a uniform heating pattern for general food items (Lidstrom, 2001). Heating uniformity has been a controversial topic when it comes to microwave radiation with some studies suggesting a non-uniform temperature distribution resulting in hot and cold spots in the heated product (Vadivambal and Jayas, 2008) and others suggesting that the temperature uniformity obtained by microwave heating is more than that of conventional heating (Valeschi *et al.*, 2004). We ensured heating uniformity in the study by placing samples in the exact same position inside the microwave during radiation treatment. The results were also consistent with a study by Dababneh, 2013; Morey *et al.*, 2012 and Sheen *et al.*, 2012 which showed that MW radiation produced a 1-2 log reduction when infected samples were processed. In the study by Park *et al.*, 2000 it was indicated that there was a 5-log reduction of viable cells of *Escherichia coli* and *Bacillus subtilis* after exposure to microwave radiation. It is assumed that this huge difference between our studies is due to the fact that Park *et al.*, utilized saline suspensions of test organisms while in this study infected food samples were utilized. Therefore, there was a difference in the local electric field strength (Jeng *et al.*, 1987). These results may be assumed to apply to other food pathogens that were not included in the study for example *Listeria monocytogenes*, *Vibrio cholera*, *Clostridium botulinum*, *Clostridium perfringens*, *Bacillus cereus*, *Brucella abortus*, *Brucella suis*, *Streptococcus pyogenes*, *Yersinia enterocolytica*, et cetera. From the statistical analytic procedures carried out on the data obtained, it was observed that the average exact effect size (Cohen's d) of reduction of food pathogens by microwave radiation was  $r = 2.82$ . This is a large effect size and therefore indicates a great practical significance (Cohen, 1988; Thalheimer and Cook, 2002). The specific effect sizes for each sample processed is highlighted in the last column of table 1.11 where beef infected with *Staphylococcus aureus* and vegetables infected with *Shigella flexneri* show the largest effect size and beef infected with *Escherichia coli* and *Salmonella Paratyphi* as well as chicken infected with *Escherichia coli* show the smallest effect sizes. Higher effect size indicates a higher magnitude of treatment effect. Thus concurs with Rodr'iguez-Marval *et al.*, 2009 in suggesting that when reheating, instructions are being formulated for microwave radiation of food, these instructions must be designed specifically for each type of food product

and consider variations in microwave appliance maximum output power, amount of food to be reheated, age of the product and the presence of antimicrobial compounds in the formulation.

### Effect of Microwave Radiation on Infective Doses

By using the McFarland standard to standardize the initial number of bacteria seeded into the food samples, it is assumed that the initial seed range was  $1-2 \times 10^8$  CFU /MI (CLSI, 2006). This was in excess of the known infective doses of each specific bacteria included in the study (*Staphylococcus aureus* ATCC 25922 Infective Dose =  $>10^4$  CFU/mL, *Escherichia coli* ATCC 25923 Infective Dose =  $>10^2$  CFU/mL, *Salmonella enterica* CMCC 50319 Infective Dose =  $10^1$  - should not be detected in 25g and *Shigella flexneri* CMCC 51285 Infective Dose =  $<500$  - should not be detected in 25g (Roberts and Greenwood, 2003; Greig *et al.*, 2010). Reduction of each of the specific bacterial infective doses by 97.67% would theoretically result in the following numbers (*E. coli*  $<= 0.333 \times 10^1$ , *S. flexneri*  $<= 1.665 \times 10^1$ , *S. aureus*  $<= 3.33 \times 10^2$ , *S. enterica*  $<= 3.33 \times 10^1$ ). These numbers are lower than their infective doses and are therefore unlikely to cause food borne disease.

### Effects of Microwave Radiation on Organoleptic Properties of Foods

Changes in food such as colour, texture and any other physical properties were evaluated subjectively. Evaluations were carried out before and after treatment. As highlighted by Morey *et al.*, 2012 in their study, exposure of food samples to microwave radiation at longer periods of time leads to reduction of microbial populations at significant levels ( $p < 0.05$ ) but also leads to the product quality being severely compromised. This unwanted effect of MW radiation was also highlighted by Sheen *et al.*, 2012 who stated that MW heating induces texture damage, poor yield due to loss of moisture and poor appearance. It was also seen in our study that treatment at higher power and longer periods of time also resulted in alterations in the organoleptic properties of the samples thus rendering them inedible and therefore the affected samples were disqualified from the experiment. Samples such as eggs, milk and gravy were the most affected by changes in organoleptic properties. They could not withstand exposure at both power 60 (885W) and power 80 (1180W) at any time-period. In all foods (especially those with little moisture), moderate power and time combinations would appear to be required to reduce populations of contaminating micro-organisms while avoiding the undesirable alterations in organoleptic properties.

### Effect of Microwave Radiation on Viability, Growth and Multiplication Ability of Bacteria

Enrichment media was used to favour the proliferation of the bacteria in food through a selective repression or inhibition of the growth of competing micro-organism thus we were able to note that the surviving colonies had viability, growth and multiplication abilities.

### Effect of Microwave Radiation on Antimicrobial Susceptibility

Antimicrobial resistance has been a major challenge in the management of FBDs (Nyenje and Ndip, 2013). Studies have also shown that antimicrobial drugs are not the only selective pressure that results in emergence of resistant organisms (Chao *et al.*, 2007); this is why this study included assays to determine if exposure to microwave radiation had any effect on the antimicrobial susceptibility patterns of the test micro-organisms. There were no observable differences between antibiotic susceptibility patterns before and after exposure of infected food samples to microwave radiation (Table II). The results were similar at the 95% Confidence Interval. However, Al-Mayah and Ali, 2010 demonstrated that exposure of *Staphylococcus aureus* to mobile microwave radiation for long periods of time affected their antimicrobial susceptibility patterns tremendously. All strains showed more antibiotic resistance development after long period of exposure which they speculated may have occurred due to slight changes in the DNA structure of the micro organism. Japoni *et al.*, 2010 proposed that to preserve the effectiveness of antibiotics, rational prescription and concomitant application of preventive measures such as these against the spread of bacteria are recommended.

The destruction mechanisms of microwaves required in a system to effect microbial cells has been proved to be due to heat generated in a medium; in a solution and/ or a solid substrate. But it is not until recently, it was thought otherwise that microwaves themselves have a direct destructive effect on the microbial cells with contribution from the substrate heat generated (Barnabas *et al.*, 2011). In addition to reducing the living cell numbers of bacteria, microwave radiation has also been shown to lower acid resistance and verocytotoxin productivity of enterohaemorrhagic *E. coli* O157: H7 (Tsuji and Yokoigawa, 2011). The decrease in the verocytotoxin productivity suggests that the bacteria that may have survived exposure to microwave radiation may not be as virulent / pathogenic as the unexposed bacteria. Microwave radiation has also been shown to have a significant reduction effect on fungal micro-organisms (Gorny *et al.*, 2007; Senna *et al.*, 2012) and complete inhibition of aflatoxin production in *Aspergillus flavus* (Dholiya *et al.*, 2012).

## CONCLUSION

As indicated by numerous studies, the possibility of survival of various pathogens in food products subjected to microwave heating exists; therefore, undertaking studies concerning the widely understood influence of microwaves on micro-organisms (particularly the bacteria - the presence of which in food has direct influence on food safety) seems fully justified. From the results observed, it can be concluded that the least effective time-period when it comes to killing food pathogens in food samples is 2 minutes and the least effective power is P-20 (295W). Whereas, the most effective time-period when it comes to killing food pathogens in food samples is 8 minutes and the most effective power is 80 (1180W –full power). However, the latter power-time combination is detrimental to the organoleptic properties of most foods thus making them inedible. The reduction of bacteria infective doses is dependent on the power-time combinations with longer time periods and exposure to higher powers being more effective. However, it seems likely combining moderate powers (P-40 – 590W, P-60 – 885W) with moderate time (4 min, 6 min) is effective in decreasing the number of micro-organisms while also avoiding unwanted effects on organoleptic properties of the foods.

These findings indicate that exposure of foods to higher time-power combinations in a domestic microwave oven (2450 MHz) plays a role in reducing the spread of food borne diseases especially in those that are immune-compromised. However, the same radiation does not play any role in the increase/decrease of bacterial antibiotic susceptibility. Another concern is that colonies that survive exposure to microwave radiation seem to maintain their ability to grow, multiply and remain viable. The major parameters included in this study were infective doses, time, power, food palatability and antibiotic susceptibility. This ensured that the results were more conclusive. It is not guaranteed that the recommended microwaving power-time combinations in this study will always be effective in eliminating all the pathogenic bacteria in food. We believe its efficacy will depend upon factors such as product type and the level of bacterial contamination.

## ETHICAL CONSIDERATION

There were no breaches of integrity executed while conducting this study and no conflicts of interest declared.

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## REFERENCES

- Al-Mayah, A. and Ali, E. (2010). Mobile microwave effect on bacterial antibiotic sensitivity. *Basrah Journal of Veterinary Research*, 10 (2), 89-102.
- Barnabas, J., Mamatha, T. and Vidyaranya, V. (2011). Cell population reduction of *Salmonella Typhimurium* using non-thermal microwaves. *International Journal of Genetic Engineering and Biotechnology*, 2 (3), 233-242.
- Bekker, J. (2003). *Food safety and analysis laboratory manual*. Pretoria: Technikon Pretoria Press
- Chao, G., Zhou, X., Jiao, X., Qian, X. and Xu, L. (2007). Prevalence of antimicrobial resistance of food borne pathogens isolated from food products in China. *Food borne Pathogens and Diseases*, 4 (3), 277-284.
- Clinical and Laboratory Standards Institute. (2006). *Approved Standard: Performance standards for antimicrobial disk susceptibility tests*, 9th ed. Clinical and Laboratory Standards Institute, Wayne, Pa. Document M2-A9, 2006.
- Cohen, J. (1988). *Statistical power analysis for the behavioural sciences* (2<sup>nd</sup> edition), Hillsdale, NJ: Erlbaum
- Dababneh, B. (2013). An innovative microwave process for microbial decontamination of spices and herbs. *African Journal of Microbiology Research*, 7(8), 636-645.
- DeWaal, C., Robert, N., Witmer, J. and Tian, X. (2009). A comparison of the burden of food borne and water borne diseases in three world regions, 2008. *Food Protection Trends*, 30(8), 483-490.
- Dholiya, K., Patel, D. and Kothari, V. (2012). Effect of low power microwave on microbial growth, enzyme activity and aflatoxin production. *Research Biotechnology*, 3 (4), 28-34.
- Dumuta-Codre, A., Rotaru, O., Giulescu, L., Boltea, F., Crisan, L. and Neghelea, B. (2010). Preliminary researches regarding the microwaves influence on the milk microflora. *Analele Universitatii din Oradea-Fascicula Biologie*, 17(1), 103-107.
- Gedikli, S., Tabak, O., Tomsuk, O. and Cabuk, A. (2008). Effects of microwaves on some gram negative and gram positive bacteria. *Journal of Applied Biological Sciences*, 2(1), 67-71



- Gomolka-Pawlicka, M., Uradzinski, J., Migowska-Calik, A. and Pastuszczak-Frak, M. (2013). Effect of short-time microwave heating on survival of *Escherichia coli* 0157 in beef hamburgers. *African Journal of Microbiology Research*, 7 (5), 361-366.
- Górny R., Mainelis G., Wlazło A., Niesler A., Lis D., Marzec S., Siwińska E., Łudzeń-Izbińska B., Harkawy A. and Kasznia-Kocot J. (2007). Viability of fungal and actinomycetal spores after microwave radiation of building materials. *Annals of Agriculture and Environmental Medicine*, 14, 313-324.
- Greig J., Todd E., Bartleson C. and Michaels B. (2010). Infective doses and pathogen carriage. In 2010 Food safety education conference, Atlanta, Georgia, USA.
- Gunasekaran, N., Mallikarjunan, P., Eifert, J. and Sumner, S. (2005). Effect of fat content and temperature on dielectric properties of ground beef. *American Society of Agricultural Engineers*, 48(2): 673–680
- Heddleson, R., Doores and S., Anantheswaran, R. (1994). Parameters affecting destruction of *Salmonella* spp. by microwave heating. *Journal of Food Science*, 59(2), 447-451.
- Housova, J and Hoke, K. (2002). Microwave heating –the influence of oven load parameters on the power absorbed in the heated load. *Czech Journal of Food Science*, 20 (3), 117-124.
- Japoni, A., Ziyaeyan, M., Jmalidoust, M., Farshad, S., Alborzi, A., Razaatpour, N. and Badiee, P. (2010). Antibacterial susceptibility patterns and cross resistance of methicillin resistant and sensitive *Staphylococcus aureus* isolated from the hospitalized patients in Shiraz, Iran. *Brazilian Journal of Microbiology*, 41, 567-573.
- Jeng, D., Kaczmarek, K., Woodworth, A. and Balasky, G. (1987). Mechanism of microwave sterilization in the dry State. *Applied and Environmental Microbiology*, 53(9), 2133-2137.
- KENYA. (1992). Food, drugs and chemical Substances Act, CAP 254. Nairobi: Government Printers
- Kitagwa, W., Bekker, J. and Onyango, R. (2005). The influence of knowledge, attitudes and practices on food kiosk hygiene - Eldoret, Kenya. *Environment and Health International. The Magazine of International Federation of Environmental Health (On-line version) www.ifeh.org*, 8 (2), 19-29. ISSN: 1726-9210.
- Lampel, K., Al-Khalali, S. and Cahill S. (2012). *Bad Bug Book: Food borne pathogenic microorganisms and natural toxins handbook (2<sup>nd</sup> Edition)*. United States of America. Food and Drug Administration. pp 13
- Lidstrom, P., Tierney, J., Wathey, B. and Westman, J. (2001). August 29. [Microwave assisted organic synthesis – a review]. *Tetrahedron* 57, 9225-9283.
- Manson-Bahr, C. and Bell, R. (1987). *Manson's Tropical Diseases* (pp 3-29, 292-297). London: Bailliese Tindall Ltd.
- Mehata, S. and Duan, G. (2011). Molecular mechanism of multidrug resistance in *Shigella* isolates from rural China. *Nepal Medical College Journal*, 13 (1), 27-29.
- Morey, A., Singh, M. and McKee, S. (2012). Efficacy of manufacturer recommended microwave time against *Listeria Monocytogenes* in ready-to-eat chicken products. *International Journal of Poultry Science*, 11 (3), 177-180.
- Nyenje, M. and Ndip, R. (2013). The challenges of food borne pathogens and antimicrobial chemotherapy: a global perspective. *African Journal of Microbiology Research*, 7 (14), 1158-1172.
- Olaniran, A., Naicker, K. and Pillay, B. (2011), April. [Review of Toxigenic *Escherichia coli* and *Vibrio cholera*: Classification, Pathogenesis and Virulence determinants]. *Biotechnology and Molecular Biology*, 6(4), 94-100.
- Park, H., Rhee, I., and Woo, I. (2000). Differential damage in bacterial cells by microwave radiation on the basis of cell wall structure. *Applied and Environment Microbiology*, 66, 2243-2247
- Roberts D and Greenwood M. (2003). *Practical Food Microbiology* (pp 168-174). Oxford, UK: Blackwell Publishing Ltd.
- Rodríguez-Marval, M., Geornaras, I., Kendall, P., Scanga, J., Belk, K. and Sofos, J. (2009). Microwave oven heating for inactivation of *Listeria monocytogenes* on frankfurters before consumption. *Journal of Food Science*, 74 (8), 453-460.
- Senna, P., Jose de Silva, W. and Del Bel Cury, A. (2012). Denture disinfection by microwave energy: influence of *Candida albicans* biofilm. *Gerodontology*, 29, e186 – e191.
- Shamis, Y., Taube, A., Mitik-Dineva, N., Croft, R., Crawford, R. and Ivanova, E. (2011). Specific electromagnetic effects of microwave radiation on *Escherichia coli*. *Applied and Environmental Microbiology*, 3017-3023.
- Sheen, S., Huang, L. and Sommers, C. (2012). Survival of *Listeria monocytogenes*, *Escherichia coli* 0157:H7 and *Salmonella* spp. on Catfish Fillets exposed to microwave heating in a continuous mode. *Journal of Food Science*, 77 (8), E209-214.
- Tanaka, Y., Fujiwara, S., Kataoka, D., Takagaki, T., Takano, S., Honda, S., Katayose, M., Kinoshita, Y. and Toyoshima, Y. (1998). Warming and sterilizing towels by microwave irradiation. *Yonago Acta Medica*, 41, 83-88.
- Tauxe, R. (2002). Emerging Food-borne pathogens. *International Journal of Food Microbiology*, 78(1-2), 21-41.
- Tsuji, M. and Yokoigawa, K. (2011). Acid resistance and verocytotoxin productivity of Enterohaemorrhagic *Escherichia coli* 0157:H7 Exposed to microwaves. *Journal of Food Science*, 76 (6), M445- M449.
- Vadivambal, R. and Jayas, D. (2008). August 26. Non-uniform temperature distribution during microwave heating of food Materials – A Review. *Food Bioprocess Technology*, 3, 161-171.

- Valeschi, O., Horii, J., Angelis, D. (2004). The effects of microwave radiation on organisms. *Arquivos do Instituto Biologico*, 71(3), 399-404.
- Vandepitte, J., Verhaegen, J., Engbaek, K., Rohner, P., Piot, P. and Heuck, C. (2003). *Basic laboratory procedures in clinical biotechnology*. Geneva, World Health Organization.