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Influence of food composition type on the microwave heating time in relation to the inactivation of *Salmonella enterica* serovar Enteritidis and Shiga-toxigenic *Escherichia coli* (STEC) O157

[1]New, C.Y., [2]Abdul Rahman, R., [3]Mohammed, A.S., [1]Ubong, A., [1]Chang, W.S., [1]Thung, T.Y., [1]Tan, C.W., [1]Lee, E., [4]Tang, J-Y-H. and [1,5]Son, R.

[1]*Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia*
[2]*Department of Food Technology, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia*
[3]*Department of Microbiology and Biotechnology, Faculty of Science, Federal University Dutse, Jigawa State, Nigeria*
[4]*Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, 22200 Besut, Terengganu*
[5]*Food Safety and Food Integrity, Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia*

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Abstract

The interaction of microwaves with the food molecules creates different volumetric heating effect. This research was aimed to study the influence of food composition type on the microwave heating time in relation to the inactivation of *Salmonella enterica* serovar Enteritidis and Shiga-toxigenic *Escherichia coli* (STEC) O157. Different food composition types, i.e. carbohydrate, protein and fats, were inoculated with 10^6 CFU/mL of bacteria cocktail and microwave heated. Food sampling was performed to enumerate the remaining surviving bacteria. The outcome of the research showed that fat food material had the shortest thermal inactivation time (<50s) compared to carbohydrate and protein food materials due to low specific heat of fat. In addition, *S. enterica* serovar Enteritidis exhibit a consistent inactivation profile compared to STEC O157. Both foodborne pathogens showed no signs of thermal resistance towards microwave heating as they were fully thermal inactivated at 60s of microwave heating for all food compositions. This study could provide an insight for further studies on the interaction of microwaves with mixture food compositions and other foodborne pathogens microwave heating inactivation profile in relation to microwave heating time.

1. Introduction

The microwave oven is now a highly sought-after equipment in the kitchen that almost every household, restaurants and convenience stores have this equipment. The quick reheating process from the volumetric heating effect of the complete interaction between microwave (300 MHz to 300 GHz), polar water molecules and charged ions in food (Puligundla *et al.,* 2013) is indeed the equipment's highest advantage which is time-saving regardless of the food heterogeneity (Coronel *et al.,* 2003). Microwave heating is generated through the molecular friction from the dipolar rotation of the water molecules leading to the breaking of hydrogen bonds as well as the migration of the ions of free salts in accordance to the electric field of rapid changing polarity (Fu, 2006; Puligundla *et al.,* 2013).

The efficiency of microwaves to interact with water and food is determined by several factors namely dielectric properties, electromagnetic penetration, food shape, volume, surface area and composition (Fu, 2006; Chaplin, 2015). To understand the properties of the interaction of microwaves with food, it is essential to know the dielectric properties of food (Sosa-Morales *et al.,* 2010). Dielectric properties of a material are described as the ability of the material to absorb, transmit and reflect electromagnetic effect (Puligundla *et al.,* 2013). A material that shows these abilities is categorized as 'lossy dielectric material'. Food materials generally consist of a mixture of organic materials, water, and salt (Chandrasekaran *et al.,* 2013), are neither poor electrical conductors nor good electrical conductors, but they have the ability to store and

*Corresponding author.
Email: *viviannew90@hotmail.com*

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dissipate energy, putting them under the group of lossy dielectric material (Buffler, 1993). To date, valuable technical literature data on the dielectric properties of various food have been published and continuous studies had been conducted to explore other food materials (Sosa -Morales *et al.,* 2010). These data are comprehensive in providing guidelines and the knowledge to describe the behaviour of the food materials when they are being submitted to electromagnetic heating. However, carefully constructed measurements are often conducted to determine the specific conditions for the particular application due to the variability composition of food materials. Dielectric properties of food vary with the composition of the food, particularly moisture and salt percentages whether of free or bound manner state in the food. Besides, the changing composition of carbohydrate, fat, and protein also contributes considerably. The distribution of these ingredients in the food exerts a strong influence on the mode of heating (Fu, 2006).

Microwave heating is able to reduce the food processing time, but at the same time, microwave heating had some major drawbacks especially non-uniform temperature distribution which could not fully thermally inactivate foodborne pathogens if present in the food materials through contamination, thus making the food unacceptable for microbiological safety concerns. As aforementioned, the varying food composition influences the mode of microwave heating, the effects on the inactivation of the foodborne pathogens are currently being studied to maintain high quality. Thus, this study aimed to investigate the influence of food composition to the microwave heating time in relation to the inactivation of the two specific foodborne pathogens *Salmonella enterica* serovar Enteritidis and Shiga-toxigenic *Escherichia coli* (STEC O157). This study will provide an insight to understand how pathogens are thermally inactivated by the microwave heating under the influence of different food composition and to compare the thermal profiles of the pathogens descriptively. The study can be used to develop a safe microwave heating time qualitatively based on the time required to fully inactivate the foodborne pathogens contaminated in the ready-to-eat (RTE) food reheated using a microwave oven.

2. Materials and methods

2.1 Bacterial strains

The bacterial strains used for the experiment were *S. enterica* serovar Enteritidis ATCC 13076 and Shiga-toxigenic *E. coli* (STEC) O157 isolated previously from microwave heated ready-to-eat (RTE) foods according to the isolation procedure in New *et al.* (2018).

2.2 Preparation of bacterial strains

S. enterica serovar Enteritidis stock culture (-20°C) was inoculated in 10 mL of Nutrient Broth and incubated for 18 to 24 hrs at 37±2°C. The culture was confirmed by streaking onto Xylose Lysine Deoxycholate (XLD) agar and subsequently sub-cultured onto Nutrient Agar (NA) slants to be used as working culture. STEC O157 isolated was stored onto NA slants and were used as working culture. Working cultures were stored at 4°C

For this experiment, all bacterial strains were subjected to a 4-hour growth study to determine the total colony count. The bacteria growth at the 4^{th} hr is considered the mid-exponential log growth phase. Briefly, working cultures were inoculated into 10 mL NB and incubated for 4 hrs at 37±2°C. After incubation, cultures were serially diluted eight times with 0.85% saline solution and were plated out in duplicates for each dilution. XLD agar was used to plate out *S. enterica* serovar Enteritidis while Plate Count Agar (PCA) was used to plate out STEC O157. The total colony count was approximately at 10^6 CFU/ml regarded as the inoculation dose. All chemicals and media were purchased from Merck, Germany.

2.3 Food composition type microwave heating process

Ready-to-eat (RTE) food samples were purchased from a local restaurant and brought back to the laboratory immediately for the experiment. Four different RTE food samples were used to represent different food composition types (Table 1), notably; cooked rice represented food high in carbohydrate; roasted chicken breast represented food high in protein; deep fried nuggets represented food high in fat; and fried rice as the common microwave heated RTE food and representative food material of multi-composition. Food samples were aseptically weighed into UV sterilized microwaveable containers (172 × 120 × 57 mm). Roasted chicken breast and nuggets were weighed up to 100 g while rice samples were weighed up to 150 g.

Table 1. Proximate composition of cooked rice, roasted chicken breast, nuggets, and fried rice

Proximate Composition	Samples			
	Cooked Rice	Roasted Chicken Breast	Nuggets	Fried Rice
Carbohydrate (%)	30.47	-	20.4	30.11
Protein (%)	3.24	22.3	11.1	4.01
Fats (%)	0.15	6.4	16.1	2.8
Crude Fibre (%)	-	-	-	0.53
Ash (%)	0.14	0.8	2.82	1.13
Moisture (%)	65.99	70.5	49.58	61.42

Source: Ali *et al.,* 2008 and Tee, 1997

An aliquot (1 mL) of the 4-hour grown bacterial culture was randomly spiked into different spots of the food sample. The inoculated food samples were left to stand for 30 mins to attain better microbial distribution. Spiked food samples were then placed at the centre of the microwave cavity and reheated according to the times 10s, 20s, 30s, 40s, 50s, 60s, 90s and 120s using a 700W microwave oven [Elba, Malaysia]. Replicate food samples were prepared to cater to the different microwave heating times.

2.4 Recovery of survival bacteria

After microwave heating, 25 g portion of the stirred food sample was weighed into a stomacher bag [Interscience, France] and added with 225 mL of ice-cold Buffered Peptone water [BPW, Merck, Germany]. The mixture was then plunged for 2 mins. Serial dilution was carried out with 0.85% saline solution and each dilution was plated out onto the respective selective agar for the growth of bacteria tested. The agar plates were then incubated at 37±2°C for 18 to 24 hrs and the presumptive colonies formed were counted. Bacterial counts were converted to $\log_{10}$ CFU/g. Each experiment was repeated at least three times.

3. Results and discussion

The thermal effect from the heat generation of microwave heating is the main contributor to the inactivation of the microorganisms in the food being reheated using a microwave oven as observed from this study. Studies performed by Atmaca *et al.* (1996), Levre and Valentini (1998), Woo *et al.* (2000), and Sheen *et al.* (2012) suggested the same. The mechanisms of thermal microbial inactivation via microwave heating includes the denaturation of enzymes, proteins, nucleic acids or other vital components, as well as disruption of membranes as proposed by Erkmen and Bozoglu (2016). Woo *et al.* (2000) added that the disruption membrane led to the leakage of nucleic acids, such as pyrimidines and purines, and protein from cells. Possible non-thermal effects of microwaves on microorganisms were discussed in several reports but, there was no satisfactory mechanism proposed to explain the bioeffects (Rougier *et al.,* 2014).

Figure 1 to 4 shows the survival curve patterns of the pathogens for different microwave reheated food compositions according to time. As observed in the figures, the inactivation pattern of both *S. enterica* serovar Enteritidis and STEC O157 was observed to have a curve with a shoulder survival pattern, which started with a shoulder followed by a linear decline of reduction.

Both pathogens' survival curve of carbohydrate and protein food materials were observed to have a shoulder of 30 s before beginning to decline sharply after 30 s of microwave heating time (Figures 1 and 2). For carbohydrate food material, the log-reduction achieved at the shoulder was approximately 0.2 to 0.5 for both pathogens. The bacterial counts of *S. enterica* serovar Enteritidis and STEC O157 indicated that approximately 5.4 log-reduction and 5.2 log-reduction was achieved at 60s and 50s respectively with no formed colonies on the agar plate. For protein food material, 0.4 to 0.5 log-reductions were achieved at the shoulder and both pathogens achieved 5.3 log reduction at 60s of microwave heating. The pathogens' survival curve for fat food materials showed gradual decline at 10s to 30s (1.5 to 1.8 log-reduction) before plunging into a sharp decline after 30s of microwave heating (Figure 3) and achieving 5.4 log-reduction at 40s for *S. enterica* serovar Enteritidis and 5.6 log-reduction at 50 s for STEC O157 respectively. As for fried rice that had a proximate composition of 30.11% of carbohydrate, 4.01% of protein and 2.80% of fat as shown in Table 1, the survival curve of the pathogens showed a longer shoulder time up till 40s with 0 to 1.5 log-reductions achieved (Figure 4). The longer shoulder time could be inferred that the food material contained moderate moisture (61.42%) and salt (1.13%), therefore decreasing the dielectric properties of the food and thus, the microwave heating rate. On the other hand, the clumping of the microorganisms in certain parts of the food could cause a drag in the shoulder of the survival curve which had occurred in the suspension when it was spread onto the food causing the microorganisms unevenly distributed in the food (Adams and Moss, 1997). The viable cell counts for *S. enterica s*erovar Enteritidis and STEC O157 at 60s of microwave heating time indicated that approximately 5.4 and 4.9 log-reduction was achieved respectively (Figure 4).

When comparing all types of food material, it was observed that fat food material had the shortest thermal inactivation time. This was due to the low specific heat of fat which causes fat food materials to reheat quickly in the microwave (Fu, 2006, Chaplin, 2015). The rapid and improved heating, therefore, inactivated the microorganism effectively which was observed in the results of this study (Figure 3). According to Fakhouri and Ramaswamy (1993), the addition of fat also improves the temperature uniformity of microwave heating which aided the thermal inactivation of the microorganisms. Carbohydrate is a complex structure of monosaccharides, scientifically known as polysaccharide that provides the source of energy to humans. The structure of monosaccharide consist of the hydroxyl groups are the groups that react to the electromagnetic

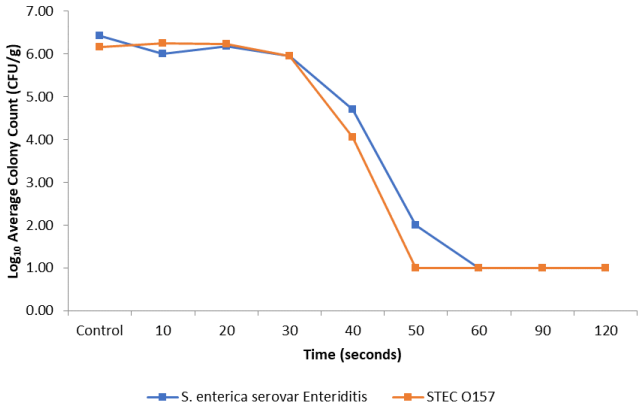

Figure 1. Survival curves of *S. enterica* serovar Enteritidis (Initial count:6.44 log CFU/g) and STEC O157 (Initial count:6.16 log CFU/g) in white rice reheated at different heating times

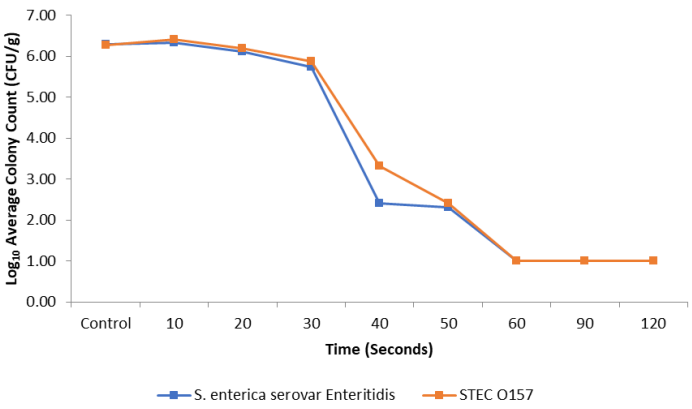

Figure 2. Survival curves of *S. enterica* serovar Enteritidis (Initial count:6.28 log CFU/g) and STEC O157 (Initial count:6.26 log CFU/g) in roasted chicken breast reheated at different heating times

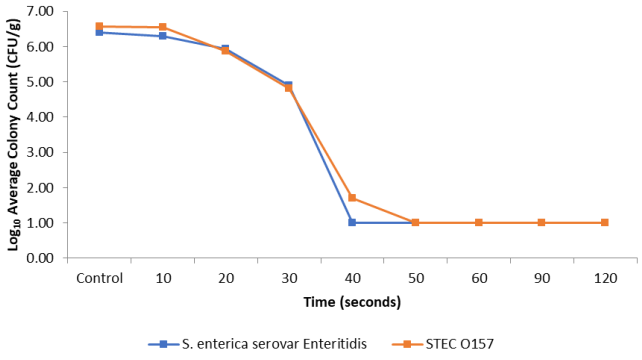

Figure 3. Survival curves of *S. enterica* serovar Enteritidis (Initial count:6.40 log CFU/g) and STEC O157 (Initial count:6.57 log CFU/g) in deep fried nuggets reheated at different heating times

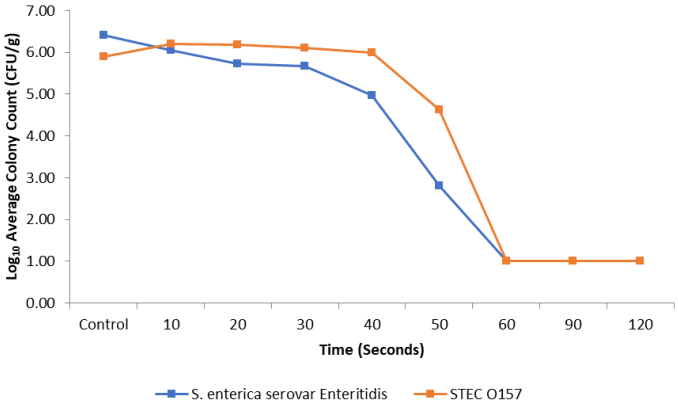

Figure 4. Survival curves of *S. enterica* serovar Enteritidis (Initial count:6.41 log CFU/g) and STEC O157 (Initial count:5.91 log CFU/g) in fried rice reheated at different heating times

field when being exposed to microwaves. According to Chaplin (2015), the hydroxyl groups in sugars and polysaccharides behave similarly to water dipole in the attempt to re-orient in electromagnetic radiation's oscillating electric field. However, the large structure of the molecule and the orientation creates a high shear environment which could increase the microwave heating (Shukla and Anantheswaran, 2001) and possibly change the heating pattern. Similarly, protein showed the same properties when being microwave heated as carbohydrate due to the similar molecular structure of protein and carbohydrate in which both had hydroxyl groups. However, Fakhouri and Ramaswamy (1993) added that addition of more protein into a food material will show high non-uniform temperature distribution with a lower heating rate in a study they conducted. It was possible to conclude that the heating pattern of fried rice had similar food composition distribution as described by Fakhouri and Ramaswamy (1993) which decreased the microwave heating rate and therefore, reduced the rate of inactivation of the pathogens. When repetitive tests were conducted, there were instances where the inactivation period to attain zero bacterial counts was at 90s for STEC O157 while *S. enterica* serovar Enteritidis attained zero bacterial counts constantly at 60s of microwave heating. The uneven temperature distribution of the microwave heating could also contribute to this matter, providing the pathogen a chance of survival. Additionally, other factors could be included as well in the reduction of the rate of inactivation such as the concentration of the pathogens and the variability of food composition.

By comparing based on the different pathogens, *S. enterica* serovar Enteritidis had a consistent time to be thermally inactivated by microwave heating which was at 60s except for fat food materials to be thermally inactivated at 40s (Figure 5). No visible viable growth of the bacteria was observed on the agar plates indicated no residual resistant bacteria. In contrast, STEC O157 had a variation of microwave heating time to be thermally inactivated at 50s for carbohydrate and fat food materials and at 60s for protein food material and fried rice (Figure 6). The results showed similarity with Sheen *et al.* (2013) who reported that *E. coli* O157: H7 was the most sensitive pathogen in comparative to *Salmonella* and *L. monocytogenes* with *Salmonella* survived better when testing the survivability of catfish fillets. It is no wonder

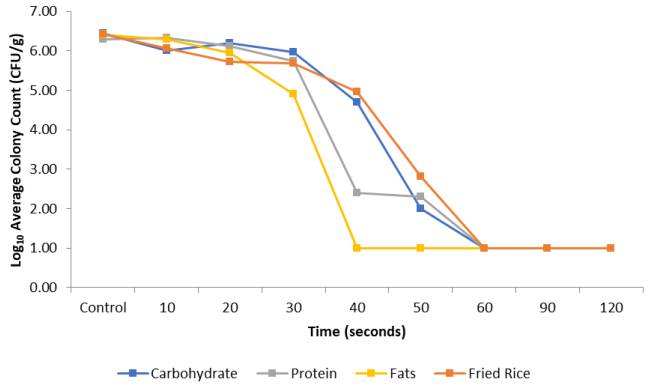

Figure 5. *S. enterica* serovar Enteriditis survival in various food composition types in accordance to microwave heating times

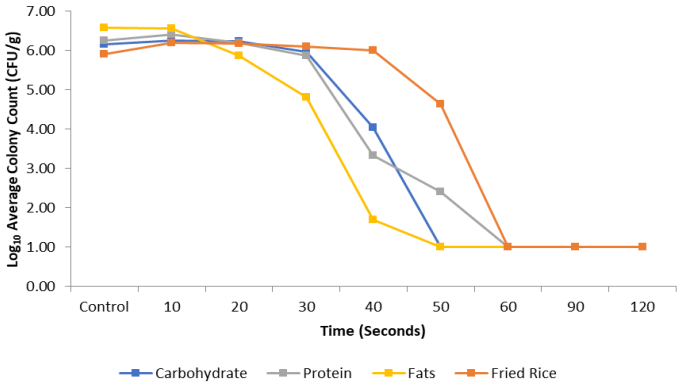

Figure 6. STEC O157 survival in various food composition types in accordance to microwave heating times

that *Salmonella* was the causative agent of most outbreaks reported related to microwave heating (Gessner and Beller, 1994; Evans *et al.,* 1995; Smith *et al.,* 2008; Meyer *et al.,* 2008; Rounds *et al.,* 2013). The stable thermal profile of Salmonella was the probable reason. Fain *et al.* (1991) previously reported that the thermal resistance of bacteria in food appeared to increase with its fat content which differed from this study possibly due to the low specific heat of fat and the higher water activity generated while microwave heating. Goepfert and others (1970) reported that thermal resistance of the organisms decreased if the water activity of the heating menstruum increased. Likewise, Beuchat and Scouten (2002) reported the rate of inactivation of the organisms increased at higher water activity and temperature. High water activity is generated during the microwave heating from the evaporative heat loss due to the large difference in temperature between the hot product and cooler surroundings (Fakhouri and Ramaswamy, 1993) which favoured the inactivation of the pathogens.

There was no indication of the pathogens were showing signs of thermal resistance towards microwave heating. All pathogens subjected to microwave heating were fully thermal inactivated at 60s for all food composition types regardless of the microorganisms' initial concentration spiked into the food material (in the range of 10^6 – 10^7 CFU/g). Dąbrowski *et al.* (2009) reported the same observation for the study conducted on the survivability of *Campylobacter* spp. in poultry nuggets being heated using a microwave oven. This provides a substantial evidence that microwave heating was indeed capable of thermal inactivating pathogens. The volumetric heating effect of the microwave was inferred to be the probable reason for this condition. Heat generated by the microwave will rise the temperature of the food in food causing the thermal attacks on the microorganisms from multiple areas. In comparison to conventional heating, the heat is transferred via convection and conduction means, and thus, the thermal attacks on the microorganisms could be focused on a single area. Higher surface area thermal exposure will eventually efficiently thermally inactivate the microorganism present in the food. It is no doubt that microwave heating could thermally inactivate the pathogens, however, the necessity to understand the different microwave heating effects on the pathogens in relation to different food composition types will provide better quality and safety of the food. This study qualitatively indicated that food should be reheated to a minimum of 60s to ensure the microbiological safety of the food, having observed that the pathogens are thermally inactivated at 60s with no viable cell counts. However, it is suggested that further quantitative measures should be taken to verify and validate the proposed safe microwave heating time.

4. Conclusion

Different food composition types were observed to have influence in microwave heating in relation to the thermal inactivation of microorganism. Fat food materials had the highest influence due to its low specific heat compared to carbohydrate and protein food materials as all the pathogenic bacteria was thermally inactivated below 50s. All pathogens were fully thermally inactivated at 60s which indicated 60s microwave heating time will be ascribed as the microbiologically safe microwave heating time qualitatively. No pathogenic bacteria were observed to gain thermal resistance towards microwave heating.

Conflict of Interest

The authors declare no conflict of interest.

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