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To Study the Effect of Microwave oven heating on Food Borne Pathogens

Neha.R. Bhalerao[1](B.Sc. ,M.Sc.), **Kaushiki.p.Pandey**[2](B.Sc., M.Sc.)
guddya_1992@yahoo.com, kaushiki143p@gmail.com
Dept. of Microbiology, Birla College Arts, Science & commerce University of Mumbai, India

Abstract

Microwave oven heating is currently the most modern technique being used for cooking food and inactivation of pathogens present in it. Microwave oven is widely used since 1971 as compared to conventional oven, as it reduces the time for cooking food. However, the major drawback of microwave heating is exposure of food to microwave radiation produced in microwave oven, because this reduces the nutritional value of the food and can cause skin, lungs infection on regular consumption of this radiated food to humans. This study shows the inactivation of pathogens that are artificially added to the food, when exposed to microwave radiation for 30sec, 60sec, 90sec & 120sec. Although the bacterial count in the food decreases when exposed for long duration, it simultaneously reduces the quality and nutritional level of the food by 75% as compared to tge food cooked by conventional heating. Thus, conventional heating ovens can be used for cooking as it does not affect the quality of the food and also in activates pathogens if present in food.

Keywords: Microwave heating; inactivation; high level of microwaves; low level of microwaves.

I. Introduction

Microwave heating is known to inactivate many micro-organisms; for instance, *Escherichia coli*, *Streptococcus faecalis*, *Staphylococcus aureus*, *Bacillus subtilis* spores, *Salmonella* species, *Lactobacillus plantarum*, *Listeria* spp., Saccharomyces cerevisiae and Clostridium perfringens (Woo *et al*., 2000). The microbial populations are mainly composed of mesophilic and spore forming bacteria, moulds and yeasts, among them are food spoiling and pathogenic genera like *Salmonella*, *Clostridium*, *Bacillus*, *Listeria* and *Staphylococcus* (Dabaneh, 2012). Food borne pathogens are a growing concern for human illness and death. There is increasing demands to ensure safe food supply. There is continuous development of methods for the rapid and reliable detection of food borne pathogens. Advent of biotechnology has greatly altered food testing methods. Improvements in the field of immunology, molecular biology, automation and computer technology continue to have a positive effect on the development of faster, more sensitive and more convenient methods in food microbiology (Mandal*et al.*, 2011). Food-borne diseases are of major concern worldwide. Food-borne diseases (FBD) are defined by the World Health Organization as "diseases of infectious or toxic nature caused by, or thought to be caused by the consumption of food or water" (Loir *et al*., 2002). Food borne diseases 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. The number of food borne diseases has increased globally, especially in developing countries where food safety interventions and sanitary control measures are not adequately implemented (Maureen *et al*., 2013).According to FDA, it is recommended that the use of microwave oven in houses for daily purpose must be avoided. From the study done, it is said that exposure of food to the radiation of microwave oven not only kills the micro organisms, but also affects the nutritional value and quality of food.So, in order to see the effect of microwave radiation, this study was conducted. The aim of this study was to analyze the effect of microwave heating on pre-existing and artificially added food borne pathogen by exposing the culture to radiations for different time intervals.

II. Method &Materials:

Study design

The Pretest-Posttest study design was applied where the mean counts of bacterial colonies formed in the food before exposure to microwave radiation were compared to the mean counts after exposure to microwave radiation. In addition, pre-existing organisms were isolated from the food.

Effect of radiations on pre-existing microbes in food samples

Sample collection & processing

Different food samples (≈10grams each)were collected from different food stalls of Kalyan. These included vegetable curry, chicken, dal, fish curry, egg curry, sambhar and rice. These particular foods were selected because they are very popular foods and are sensitive that can easily get spoiled due to their high protein and water content. Food samples were stored in sterile container and stored at 4^0C until examination. In order to see the effect of radiations on the pre-existing organisms in the food, the samples were exposed to MW radiations for different time period (0 sec, 30 sec, 60 sec, 90 sec, and 120 sec). Samples were then serially diluted up to 10^{-6}. Thereafter, 0.1ml of the samples was plated out using dilution 10^{-4}, 10^{-5}, 10^{-6} on sterile Nutrient Agar plate using Spread plate technique. The plates were incubated at 37 ^{0}C for 24 hours to enumerate the colonies. The morphological characteristics and biochemical tests were performed for isolation and identification of organisms. In addition to it, the physiology of food samples before and after exposure was checked.

Enumeration and characterization of colonies

After 24 hrs of incubation, the numbers of colonies were enumerated. Along with it, colony characteristics were studied followed by biochemical test for confirmation of the organisms survived before exposure (control) and after exposure to radiations (test). The effect of radiation on food physiology was also observed. The reduction in colony forming unit were recorded in tabular form according to time of exposure. The comparison between colony count before and after exposure to radiation was done represented in the form of charts. After enumeration, the isolated colony was further Examined using Gram staining and biochemical tests.

Effect of Microwave radiations on artificially infected food samples

a)Sample collection & processing

Samples collected in sterile container were stored under 4^0C until examination of sample. The Sample was autoclaved at 121^0C for 15minutes in order to kill the pre-existing bacteria in the food. Micro - organisms, namely, *Escherichia coli, Staphylococcus aureus*and*Salmonellatyphi*of cell density 10^4 were inoculated into each food sample separately (1ml of culture per 10g sample). These infected samples were incubated for 24hrs at 37^0C. The next day Samples were exposed to microwave for 30 sec, 60sec 90 sec, 120 sec and 0 sec (Control). Samples were diluted up to 10^{-7}. And 0.1ml of last three dilutions was plated out on Sterile Nutrient agar using spread plate technique. Dilution used was 10^{-4}, 10^{-5} and 10^{-6}. The plates were incubated at 37^0C for 24hrs to obtain the colony count.

b)Enumeration and characterization

After 24hrs of incubation, the colony forming unit was enumerated and the reduction after exposure was recorded in tabular form. The cultural characteristics of the organisms were examined followed by gram staining and Biochemical tests. The effect of radiation of texture and other physical parameters of food was observed. The colony count of the exposed samples was compared with the colony count of the unexposed samples. As the exposure time increased, the microbial count went on decreasing. The effect of long time exposure (more than 60 sec) on food physiology was also studied.

Tests done: Gram staining, Sugar fermentation test, Indole test, Methyl Red test, VogesPrausker (VP) test,

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Simmons citrate slant, TSI slant, Urease test, Oxidase test, Catalase test

III. RESULTS AND DISCUSSION

Microwave radiation affects the microbial flora present in the food. In this study, different food samples collected from different food stalls were treated with microwave radiations by exposing the samples to radiations for 30 sec, 60 sec, 90sec 120 sec and unexposed ones. The presence of pre-existing micro flora in the food was enumerated by plating out 0.1ml of sample on sterile nutrient agar plates using spread plate technique. After 24 hrs of incubation at 37^0C, colony count CFU/ml was recorded. The colony count before exposure was found to be > 1x 10^6 cfu/ml. After irradiation the colony count decreased and the count was < 1x 10^6cfu/ml (Table 1). This indicated that the microwave radiation affected the colony count and was found to be less.

Table 1 Colony count of pre-existing microbes before and after exposure to radiation (CFU/ml)

Sr.no	Sample	control	30sec	60sec	90sec	120sec
1	Vegetable Curry	260 x 10^6	16x10^6	**5x10^6**	**6x10^6**	-
2	Cooked dal	520 x 10^6	85x10^6	**18x10^6**	**15x10^6**	-
3	Egg curry	430 x 10^6	23x10^6	**3x10^6**	**1x10^6**	-
4	Chicken gravy	350 x 10^6	28x10^6	**2x10^6**	**20x10^6**	-
5	Fish curry	190 x 10^6	23x10^6	**5x10^6**	-	-
6	Sambhar	200 x 10^6	17x10^6	**56x10^6**	-	-
7	Rice	**-**	**-**	**-**	**-**	**-**

The CFU/ml in the unexposed samples of Vegetable curry was found to be 260x10^6, cooked Dal contained 520x10^6 CFU/ml, the count in egg curry and chicken was found to be 430 x 10^6CFU/ml&350x 10^6CFU/ml respectively, whereas Fish and sambhar count was 190x10^6 CFU/ml and 200 x 10^6CFU/ml. interestingly, no colonies were found in rice sample, since the sample was obtained from house.

Fig 3 colony count of unexposed

fig 4 colony count of exposed sample

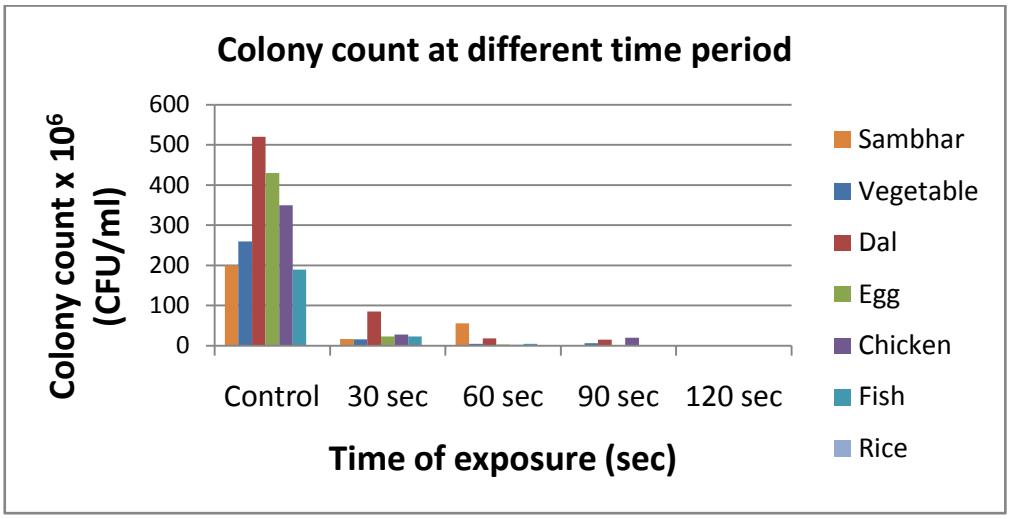

Figure 5 Time of exposure v/s colony count (CFU/ml)

The observation showed that most of the count was observed to be low after radiation for 30 sec, but no observable effect n the bacterial count was found in Dal sample when exposed for 30 seconds (fig. 5). Maximum irradiation of bacteria was observed for exposure more than 60 seconds. But this long time exposure leads to the depletion in nutrients and other nutritional factor which leads to low or decreased levels of nutritive value in food. Along with it they are harmful to human being leading to various disease relate to skin, eyes, lungs etc.The isolated colony was about 1mm in size, circular with entire margin, pale in color, with a Butyrous consistency. Further, its gram staining was performed and the colony was found to be gram negative rods (table 2). For further confirmation, the colony was streaked on Mac Conkeys agar and EMB agar. The suspected bacteria gave pink color colonies with zone of bile precipitation around the colony on Mac Conkeys agar (Fig 6) and pinpoint colonies with metallic sheen on EMB agar plate (Fig 7)

Fig 6 Mac Conkeys agar

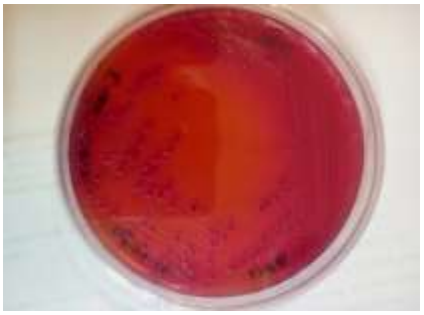

Fig 7 EMB Agar

From the cultural characteristics and observation on Mac and EMB plates (table 2, fig. 6&7); the isolated colony was suspected to be the species of *Escherichia.* Thus, to confirm the suspected agent biochemical tests were performed(Table).

In order to confirm the suspected species after cultural characteristics of the colony, biochemical tests were performed after 24hrs of inoculation. The tests included sugar fermentation test, Indole, Methyl red, Vp tests, citrate test, TSI, Catalase. The suspected agent fermented sugar by showing acid and gas production in the sugars glucose, mannitol, maltose, sucrose, lactose. Xylose sugar showed negative test. Indole was found to be positive as it showed red colour ring at the junction on addition of Kovac's reagent (fig. 9). On addition of methyl red reagent, red colour was observed in the tube indicating positive test (fig 10). While in TSI slant and butt showed acid production by yellowing of slant and butt, gas production was also observed. The suspected agent sowed Catalase test positive on addition of hydrogen peroxide to the culture. Effervescence was observed on addition of reagent (fig 8). Xylose, vogesprausker, citrate test, urease test were found to be negative. So, on comparing with the standard biochemical test, it was found that the isolate obtained from vegetable was *Escherichia coli* (ref. Bergey's Manual).




Fig:8 catalase Fig:9 Indole Fig:10 Methyl Red

Similar colony characterization was done of the colonies isolated from Dal. Its other characteristics were also studied along with gram nature (table 2). The obtained isolated were found to be gram negative rods (D_2) and the other was gram positive cocci in clusters (D_1).

The biochemical test performed for D_1 were sugar fermentation, indole, urease, coaagulase, starch hydrolysis, methyl red, nitrate reductase, citrate, TSI, Catalase, Oxidase. D_1 was fermented glucose and mannitol without gas production. It gave Catalase test positive i.e. effervescence were observed. The coaggulase, urease and starch hydrolysis test were found to be positive (Fig 11, 12). Negative test: lactose, sucrose, IMViC.D_2 showed observable acid gas production in sugar fermentation, with indole and methyl red test positive, TSI slants and butt were acidic and gas production was observed and was found to be Catalase positive.On comparing with the standard results, the colony D_1 was *Staphylococcus*, *aureus*, and D_2 was found to be *Escherichia coli* (table 2).


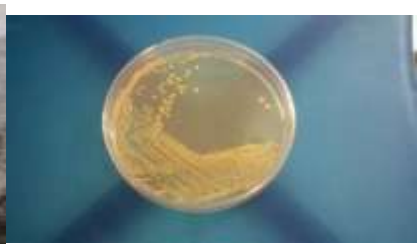

Fig 11 starch hydrolysis Fig 12 isolation of s.aureusfom sample

Table 2 Biochemical test of colonies from Food samples Colony characterization was done of the colonies isolated from Egg. Its other characteristics were also studied along with gram nature (table 2). The obtained isolated were found to be gram positive rods (E_1) and the other gram negative rods(E_2). Further confirmation was done with biochemical test.

Biochemical test	glucose	mannitol	maltose	lactose	sucrose	indole	Methy red	vp	Citrate	xylose
E^1	Acid	Acid	Acid	Acid	Acid	-	-	+	+	NA
E^2	Ag	Ag	Ag	Ag	Ag	+	+	-	-	NA
C^1	Acid	Acid	Acid	Acid	Acid	-	-	+	+	NA
F^1	Ag	Ag	Ag	Ag	Ag	-	+	-	+	NA
F^2	Ag	Ag	Ag	-ve	Ag	-	+	-	-	NA
S^1	Acid	Acid	Acid	Acid	Acid	-	-	+	+	NA
D^1	Acid	Acid	NA	-Ve	-	-	-	-	-	NA
D^2	Ag	Ag	NA	Acid	Acid	+	+	-	-	NA
V^1	Ag	Ag	NA	Ag	Ag	+	+	-	-	-Ve
TSI Test	C	slant	Butt	H_2S	Gas	oxidase	catalase	SH	Motility	urease
E^1		Acid	Acid	-	-	-	+	+	+	NA
E^2		Acid	Acid	-	+	-	+	-	-	NA
C^1		Acid	Acid	-	-	-	+	+	+	NA
F^1		Acid	Acid	-	+	-	+	NA	NA	NA
F^2		Alkaline	Acid	-	+	-	-	NA	NA	NA
S^1		Acid	Acid	-	-	-	+	+	+	NA
D^1	+	Acid	Acid	-	-	-	+	+	NA	+
D^2	NA	Acid	Acid	-	+	-	-	NA	NA	NA
V^1		Acid	Acid	-	+	-	+	NA	NA	-

KEY- AG: Acid Gas production; +ve: positive test; -ve: negative test

Set B: artificially infected food with organisms

Table 3 Effect of microwave radiation on infected food

Sample	Bacteria	Mean CFU B	Mean CFU A
Vegetable curry	*E. coli*	31×10^7	23×10^6
Cooked dal	*E. coli*	27×10^7	17×10^6
Egg curry	*E. coli*	33×10^7	17×10^6
Chicken	*E. coli*	20×10^7	17×10^6
Fish	*E. coli*	19×10^7	14×10^6
Sambhar	*E. coli*	17×10^7	62×10^6
Vegetable curry	*S. aureus*	11×10^8	14×10^6
Cooked dal	*S. aureus*	23×10^7	14×10^6
Egg curry	*S. aureus*	30×10^7	28×10^6
Chicken	*S. aureus*	29×10^7	12×10^6
Fish	*S. aureus*	20×10^7	8×10^6
Sambhar	*S. aureus*	21×10^7	15×10^6
Vegetable curry	*S. typhi*	27×10^7	16×10^6
Cooked dal	*S. typhi*	24×10^7	11×10^6

Egg curry	*S. typhi*	98 x 10^7	24 x 10^6
Chicken	*S. typhi*	33 x 10^7	76 x 10^6
Fish	*S. typhi*	18 x 10^7	11 x 10^6
Sambhar	*S. typhi*	19 x 10^7	1 x 10^7

Key: Mean CFU B: mean colony count of sample before exposure; Mean CFU A: mean colony count of sample after exposure;

Table 3 shows effect of microwave radiation on specific food microorganism combinations in terms of mean colony forming units before exposure (Mean CFUB) and mean colony forming units after exposure (Mean CFUA). Samples were autoclaved to kill pre existing microbes and were artificially infected by various organisms. The food samples were exposed to radiation for 30sec (control), 30sec, 60sec, 90sec, and 120sec. the above table enlists the colony count before and after exposure.From the above tables, the results show that among all the infected food samples, the least affected by microwave radiations at different temperatures were: chicken inoculated with *S. typhimurium*andSambhar infected with *Escherichia coli*. The most affected were vegetable and fish inoculated with *S. aureus*. All food samples when exposed for 30 seconds to microwave radiations did not produce observable results. The initial count of the food samples before exposure was as follows:

Table 4 Colony count of infected sample before exposure (Control)

Food samples	E. coli (CFU/ml)	S. aureus (CFU/ml)	S. typhi (CFU/ml)
Vegetable curry	31 x 10^7	11 x 10^8	27 x 10^7
Cooked dal	27 x 10^7	23 x 10^7	24 x 10^7
Egg	33 x 10^7	30 x 10^7	98 x 10^7
Chicken	20 x 10^7	29 x 10^7	33 x 10^7
Fish	19 x 10^7	20 x 10^7	18 x 10^7
Sambhar	17 x 10^7	21 x 10^7	19 x 10^7

Table 5 Colony count after exposure for different time (CFU/ml)

Vegetable curry infected with				
	30 sec	60 sec	90 sec	120 sec
E. coli	49 x 10^6	18 x 10^6	4 x 10^5	-
S. aureus	33 x 10^6	9 x 10^6	1 x 10^5	-
S. typhi	29 x 10^6	3 x 10^6	-	-

Cooked dal infected with				
	30 sec	60 sec	90 sec	120 sec
E. coli	31 x 10^6	17 x 10^5	-	-
S. aureus	38 x 10^6	51 x 10^5	1 x 10^5	-
S. typhi	21 x 10^6	17 x 10^5	-	-

Egg curry infected with

	30 sec	60 sec	90 sec	120 sec
E. coli	27×10^6	58×10^5	-	-
S. aureus	43×10^6	12×10^6	-	-
S. typhi	56×10^6	16×10^6	2×10^5	-

Chicken infected with				
	30 sec	60 sec	90 sec	120 sec
E. coli	43×10^6	73×10^5	1×10^5	-
S. aureus	23×10^6	18×10^5	-	-
S. typhi	19×10^6	56×10^5	4×10^5	-

Fish infected with				
	30 sec	60 sec	90 sec	120 sec
E. coli	23×10^6	5×10^6	-	-
S. aureus	16×10^6	12×10^5	-	-
S. typhi	21×10^6	10×10^5	-	-

Sambhar infected with				
	30 sec	60 sec	90 sec	120 sec
E. coli	12×10^7	37×10^5	-	-
S. aureus	26×10^6	41×10^5	-	-
S. typhi	14×10^6	48×10^5	-	-

Table 5 shows the decrease in the colony count after exposure to 30 sec, 60 sec 90sec and 120 sec. It was observed that maximum destruction of organisms was found when samples were exposed for more than 60 seconds. Very few or no growth was observed when food sample radiated for 90 seconds. This indicates that all organisms in the food are destroyed when exposure of sample is more than 60 seconds. But as the radiation time increases, the quality and texture of the food decreases. Liquid food becomes dry in texture. Thus it will lead to decrease in the nutritional value of food. The samples retain their physiology when exposed up to or less than 60 seconds. But it does not destroy all the microbes present in it.

Microwaving food, in effect, potentially destroys and depletes the life energy, rendering the food completely dead and lifeless. In addition, the food's nutritional value is lost and it becomes nearly useless in terms of providing any real health benefit.Much research is under way on microwaves and how they might affect the human body. It is known that microwave radiation can heat body tissue the same way it heats food. Exposure to **high levels of microwaves** can cause a painful burn. The lens of the eye is particularly sensitive to intense heat, and exposure to high levels of microwaves can cause cataracts. Likewise, the testes are very sensitive to changes in temperature. Accidental exposure to high levels of microwave energy can alter or kill sperm, producing temporary sterility. But these types of injuries - burns, cataracts, temporary sterility - can only be caused by exposure to largeamounts of microwave radiation, much more than the 5mW limit for microwave oven leakage. Less is known about what happens to people exposed to **low levels of microwaves**. Controlled, long-term studies involving large numbers of people have not been conducted to assess the impact of low level microwave energy on humans. Much research has been done with experimental animals, but it is difficult to translate the effects of microwaves on animals to possible effects on humans. For one thing, there are differences in the way animals and humans absorb microwaves. For another, experimental conditions can't exactly simulate the conditions under which people use microwave ovens. However, these studies do help us better understand the possible effects of radiation.Dielectric

heating by microwave radiation has a good effect on killing gram positive and gram negative organisms. This was studied by Dabaneh (2013). In this study when the samples vegetable curry, cooked dal, egg curry, chicken gravy, fish curry and sambhar infected with *E. coli, S. aureus and S. typhi* were radiated to microwave radiation for 0sec (control), 30 sec, 60 sec, and 120 sec; it was found that mean cfu/ml of the vegetable curry before exposure was >1 x 10^7 cfu/ml. But when exposed to radiation, decrease in the colony count of the food sample was observed. The count after exposure was <1 x 10^7cfu/ml. Similar study was done by Luvanda*et al.*,(2013). The results of this study showed decrease in the colony count when sample exposed to long duration (more than 60 sec). Least reduction was observed in the combination of chicken infected with *S. typhimurium* and Sambhar infected with *E. coli*. *S. typhi*and *E. coli* were found to be unaffected by the radiations.Along with the decrease in the count, as the time of exposure increased, the quality of food was found to be decreasing with the increase in the time. The quality of food was found to be low. The similar problem was observed by Yuan *et al., (2009)* in the study of Broccoli. It was found that after radiation Broccoli's nutrient value was decreased by 95% while convention heating decreases only 9- 10 % of its nutritive value.

IV. CONCLUSION

Microwaves are now-a-days used domestically for cooking food in houses as well as industries. Thus, this study was conducted to see the effect of radiation on the organisms present in the food. The main objective of this study was to observe the decrease in the organisms count after radiation. In this study, the various food samples collected from different food stalls were examined for the presence of microbes pre-existing in the food and to see the effect of radiation of food microbes after exposure to radiations for 30, 60, 90 and 120 seconds. In the set 1,samples with the pre existing microbes were exposed to radiations. Exposure for 60 sec or more showed more decrease in the count as compared to exposure for 30 sec. Though there was observable decrease in the colony count, the radiations also affected the texture of food samples. Later, the isolates obtained were examined by cultural characteristics, gram staining and confirmed with biochemical tests. The organisms present in the food: *E. coli, S. aureus, S. typhi, Klebsiella pneumonia* and *Bacillus* species. The above organisms may be due to improper handling of food, lack of cleanliness in case of utensils, surroundings and may be because of aerial contamination due to long exposure to environment.

In set B, food samples were artificially infected with E. coli, S. aureus, and S. typhi. These samples, after incubation of 24hrs, were irradiated for 30 sec, 60 sec, 90 sec, and 120 sec. it was found that *E. coli* infected sambhar, *S. aureus*infected Vegetable were least affected to radiation at 30 sec. Maximum decrease in the colony count was found at exposure for 60sec and more. Along with it, the food quality was unaffected until exposure for 60 sec. Later the texture was found to be dry.

From the study, it is concluded that though microwave radiations kill all the organisms when exposed to more than 60 sec, it lowers the quality of food. Food must be exposed for less than 60 sec; this will not only reduce the count but also retain the nutritional quality of food.

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