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Destruction of Surface-Inoculated *Escherichia coli*, *Vibrio cholerae* and *Salmonella typhi* in Rohu (*Labeo rohita*) Steaks during Microwave Cooking

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The destruction of bacteria during microwave cooking of fish was studied. Fish steaks were surface-inoculated with *Escherichia coli, Vibrio cholerae* and *Salmonella typhi* and cooked in a microwave oven. The bacteria were enumerated by plating on selective and non selective media and by MPN technique. The vegetative cells of *S. typhi* and *V. cholerae* were totally destroyed from an initial count of 10^7 cfu/g to a non detectable level and *E. coli* was destroyed from an initial count of 10^7 cfu/g to 1 cfu/10g after microwave treatment. Cooking for a longer duration at low power output level was found to ensure the killing of pathogens and minimize bumping without any distortion of shape.

Key words: Microwave, fish steak, pathogen, *E. coli, V. cholerae, S. typhi*

Microwave heats food materials by dielectric heating mechanism, which is an entirely different way of heating, compared to conventional heating. It is independent of atmospheric pressure and thus of the boiling point of the concerned material. It is 40% more efficient and causes less nutrient and vitamin loss as compared to conventional heating. Microwave-cooked food hardly gets an internal temperature of about 80°C. It is still a controversy if there is some non-thermal killing effect of microwave on the microorganisms, because of very small heat exposure time at low internal temperature.

After microwave cooking became familiar to the common people, an urgent need to establish its safety was of great concern to the research workers. Scientists have been giving several controversial theories about the destruction of food poisoning and pathogenic microorganisms in microwave-cooked food (Brown & Morrison, 1954; Campbell *et al.*, 1958; Dessel *et al.*, 1960; Greez *et al.*, 1964; Lacey *et al.*, 1965; Baldwin *et al.*, 1968; Delaney *et al.*, 1968). The studies so far with inoculated microorganisms show that the lethality of microwave heating to several food poisoning and pathogenic bacteria is specific to both the food system and pathogens. Hence, there is an urgent need to study the potential health hazards that may arise from the consumption of microwave-cooked foods, in respect of different food systems and different pathogens.

Baldwin *et al.* (1971) reported the reduction of *Salmonella tyhpimurium* in surface-inoculated carp (*Cyprinus carpio*) by microwave heating for 195 sec. under a frequency of 2450 MHz. Reduction of *Escherichia coli* and *Staphylococcus aureus* in fried fish steaks (Mudson *et al.*, 1971) and *Listeria monocytogenes* and *Aeromonas hydrophila* in catfish fillets (Huang *et al.*, 1993) after microwave treatment have been reported.

In this work, the degree of destruction of surface-inoculated *E. coli, Vibrio cholerae* and *Salmonella typhi* in the dorsal steaks of rohu (*Labeo rohita*) was studied.

Materials and Methods

The microwave oven used for this experiment was (T-23 model Kelvinator

brand), with 23 litres capacity, working on 50 Hz power supply and 1200 W power input with a power output of 700 W and a microwave frequency of 2450 MHz.

Rohu (*L. rohita*), weighing approximately 1.0±0.2 kg, was obtained from the local market and the steaks from the dorsal side, each weighing approximately 40.0±2.0 g, were used for the study.

S. typhi, V. cholerae and *E. coli* were isolated from sewage water following routine biochemical tests and maintained in the laboratory by subculturing every fortnight. Each of the three pathogens under study was grown in 100 ml nutrient broth for 16-18 h. The cultures were then centrifuged at 8000 rpm for 10 min, the cells were collected, washed and resuspended in 100 ml sterile saline. The inoculum was taken in a 250 ml beaker and two fish steaks were inoculated by dipping them in it, for 2-3 min. One of the inoculated steaks was used for enumeration before microwave treatment and the other after the microwave cooking.

The inoculated fish steak was kept at the center of a sterile petridish and the petridish was placed in the center of the microwave oven and then cooked for pre-standardised cooking time (7 min with 10% power). The cooking time was standardized by slowly reducing the power output level with increasing time to minimize bumping (the abrupt bursting of flesh with audible sound) and to obtain adequate cooking.

Enumeration of bacteria was done before and after cooking by spread plate technique using both selective and non-selective media (AOAC, 1984).

After inoculation with respective bacteria, fish was homogenized in 160 ml sterile saline and plated on both selective and non-selective agar media by spread plate technique (APHA 1980). Thiosulfate citrate bile salt agar (TCBS), Bismuth sulfite agar (BSA) and Eosin methylene blue (EMB) agar were used as selective media for *V. cholerae, S. typhi* and *E. coli* respectively. Nutrient agar was used as non-selective media for all samples.

The fish steaks after microwave treatment were homogenized in 200 ml sterile saline and then plated on nutrient agar with simultaneous inoculation into nutrient broth. The broth was streaked on nutrient agar plates after every hour. Simultaneously, the broth was also smeared and stained for spores. The staining was continued until vegetative cells could be observed.

In order to enumerate bacterial population after microwave treatment, a homogenate was prepared as described before and a five-tube MPN method was followed using enrichment broth for different pathogens. Alkaline peptone water (APW), Selenite cystine broth (SCB) and Lauryl sulfate tryptone broth (LSTB) were used as enrichment broth for *V. cholerae, S. typhi* and *E. coli* respectively. Only the

Table 1. Standardization of microwave cooking time for fish steaks : Steak weight = 40 ± 2 g.

Power %	Time (min)	End Temperature (°C)	State of cooking	Bumbing	Shape
100	1	90.0	Over cooked	Severe	Destroyed
70	2	91.0	Cooked	Severe	Destroyed
50	3	91.0	Cooked	Severe	Destroyed
40	3	92.0	Under cooked	Mild	Destroyed
30	3	91.0	Under cooked	Mild	Destroyed
20	3	90.0	Under cooked	Little	Almost intact
10	3	59.5	Under cooked	Nil	Intact
10	7	87.5	Cooked	Nil	Intact

positive tubes were further streaked on selective plates. Growth in APW and SCB was recorded as positive reaction, while growth with gas production in LSTB was recorded as positive reaction in enrichment tubes. The inoculums from positive tubes were further streaked on respective selective agar plates. MPN tubes giving rise to typical colonies on selective agar were recorded as positive after biochemical tests. One set of MPN tubes with nutrient broth was also inoculated simultaneously and were streaked on nutrient agar after 8h of enrichment. The enrichment was done only for 8 h to avoid the germination of spores in the nutrient broth MPN tubes.

The native population of bacteria in the fish samples was determined by homogenizing 10g of the tissue in 90 ml of sterile saline and plated on nutrient agar.

Results and Discussion

The cooking time was standardised at 10% power out put to get an adequate doneness (state of cooking at which the rawness of the material disappears) with an intact shape. Higher levels of power resulted in bumping and consequent loss of shape of the steak. Though piercing the food (Instruction Manual) could reduce bumping, it was avoided, because it affected shape of the fish steak, thus affecting the main aim of the experiment. Therefore, at 10% power output level, the cooking time was slowly increased. The taste panel suggested 7 min cooking at 10% power output level to be optimum.

The natural bacterial load was approximately 10^6 cfu/g which was reduced to a non-detectable level after the standardized cooking. When incubated in enrichment tubes for 11 h, all tubes showed growth. This indicated that the vegetative cells were destroyed completely, but the spores and probably a few injured cells might have germinated during nutrient broth enrichment after a longer incubation period.

Among the three pathogens, *E. coli* showed more resistance to microwave heating compared to other two bacteria i.e. *S. typhi* and *V. cholerae*. *E. coli* could not be reduced to less than 1 cfu/10g, but the other two could be reduced to an undetectable level (Table 2). It shows that microwave heating has species specific lethality. Fung & Cunningham (1979) reported this phenomenon and they observed that among ten bacteria, *Streptococcus faecalis* had highest resistance to microwave heating, followed by *E. coli* (survival rate 7.9 and 0.93% respectively). Page & Martin (1978) showed that in rehydrated samples of cooked tomato soup, the time taken for a reduction of 8 log cycle for *Stapylococcus aureus* was 30 sec., for *E. coli* 45 sec and for Bacillus subtilis spores, 10 min. White & Hobbs (1963) have also observed that vegetative cells of *E. coli* and *Salmonella* were completely destroyed in beef stew after microwave treatment, but *B. subtilis* was not affected.

Microwave cooking has capacity to kill the vegetative cells of certain pathogens effectively. But microwave heating is more 'food dependent' than conventional heating, because of the mechanism of generation of heat by microwaves. Therefore, emphasis should be given to the understanding of the heating profiles of each class of food. Many researchers suggested the use of microwaves

Table 2. Destruction of different pathogens after pre-standardized cooking (by microwave) of fish steak (steak weight 40 ± 2 g)

Inoculated pathogen	Initial count (TPC) C fu / g	Count after cooking (TPC) Cfu / g	MPN cell / 100 ml.
Salmonella typhi	1.5×10^8	$<5 \times 10$	Nil
Vibiro cholerae	6.1×10^7	$<5 \times 10$	Nil
Escherichia coli	9.8×10^5	$<5 \times 10$	2 (1 / 10 g)

in combination with conventional heating of foods for destruction of bacteria. Microwaves exert different killing effects in different bacterial species. Therefore, it is important to know the microbial profile of food under investigation. Thus, microwave heating is acceptable from the standpoint of food spoilage and food safety as long as users understand its limitations and possibilities.

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