

Original Article

Impact of heat treatment on organoleptic and microbial quality of hotdog

Taslina Akter Shapna, Md. Muket Mahmud, Md. Shahab Uddin, Md. Maidul Islam, Shurya Khanam, Jafrul Hasan Ripon, Muhammad Tofazzal Hossain and K. H. M. Nazmul Hussain Nazir*

Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh, 2202, Bangladesh.

*Corresponding author: nazir@bau.edu.bd

ABSTRACT

This study determined the appropriate time of microwave oven treatment for destroying pathogenic organism of hotdog sold in a fast food restaurant at retail shop. 30 hotdog samples were collected from Masud Confectionary, at KR Market of Bangladesh Agricultural University. All hotdog samples were inoculated into Eosin Methylene Blue (EMB) agar, Salmonella Shigella (SS) agar, and Mannitol Salt (MS) agar. Colonies on MS agar were characteristics of *Staphylococcus* spp. Organisms were confirmed by sugar fermentation test, catalase test, coagulase test and polymerase chain reaction (PCR) assay. Organoleptic quality study was done by a panel of experts with standard protocol. 20 samples were positive to *Staphylococcus* spp. The total viable count (TVC) and total staphylococcal count (TSC) of premicrowave oven hotdog samples were 5.19 log CFU/g and 5.05 log CFU/g respectively. Similarly, for 30sec, 45sec, 1min and 2min post microwave oven treated samples, TVC and TSC were 4.93 log CFU/g and 4.78 log CFU/g; 3.76 log CFU/g and 3.56 log/g; 2.88 log CFU/g and 2.61 log CFU/g and nil respectively. Increased time of microwave heat treatment significantly reduced the TVC and TSC in the hotdog and 2min microwave oven treatment completely killed all bacteria. The organoleptic quality revealed that 1min microwave treated samples should be served for consumption. This study recorded that coagulase negative staphylococcus (CNS) resistance against Tetracycline, ciprofloxacin azithromycin, amoxicillin, and oxacillin. Data of this study suggest that hotdog sold in the fast food restaurant carried multidrug resistant CNS that might pose public health hazard.

Keywords: Coagulase negative *Staphylococcus* (CNS), Hotdog, Multidrug Resistant, Organoleptic quality.

INTRODUCTION

The term fast food refers to food sold in a restaurant or store with preheated or precooked ingredients and served in packages form for take-out /take –away (Harun et al., 2013). The unhygienic and unsafe treatment of food is seriously impacting public health by causing numerous chronic and non-chronic diseases (Ali, 2013). FDA reported believes that the considerable number of illnesses is was transmitted by food workers (Guzewich and Ross, 1999). The risk of an outbreak is high when food handlers with skin infections contaminate foods that are undercooked or left at room temperature (Boyce, 2012). The Codex Alimentarius Commission suggests that among the requirements for foods to be of good sanitary quality, they must be free of hazardous microorganisms or if present should be at a safe low level (Hubbert et al., 1996).

Hotdog is becoming one of the most popular fast food to the people of all ages (includes Children and adults) mainly for its nutritious value and delicious taste. The ingredients of Hotdog are long wheat yeast bread, chicken /beef, chilies, spices, egg, onion, ginger, garlic, tomato sauce, cucumber, mayonnaise. Chicken meat which is used in Hotdog may contain a lot of bacteria which includes: *Campylobacter spp.*, *Salmonella spp.*, *Listeria monocytogenes* *Staphylococcus aureus* etc. (Andrews 2013).

Staphylococcal food poisoning is one of the most common food borne diseases worldwide with high occurrence second to salmonellosis. In our country the use of microwave oven is gradually gaining popularity particularly in restaurants and fast food. But the time of microwave oven treatment of fast food shop offered to consumer is not appropriate time for destroying the pathogenic organism. No study has been done on the appropriate time of microwave oven treatment for destroying the pathogenic organism. The present study will therefore focus on the appropriate time of microwave oven treatment of the fast food and their impact on the potential health hazard arising from the consumption. The result will contribute in making suggestion to ensure establishment of the hygienic assessment of fast food items offered to the consumer. It is observed that the challenge is delivering safe food product to meet the demands of the consumers now and in future needs to be combated. The objectives of the research work were to measure the microbial load in Hotdog before and after different micro oven treatment, to determine organoleptic quality of microwave oven treated hotdog, and to know antimicrobial sensitivity profile of bacteria isolated from hotdog.

MATERIALS AND METHODS

Ethical approval: The work has been conducted by following international standard and ethical issues particularly during doing the organoleptic properties examination.

Sample collection and experimental design: 30 hotdog samples (n=30) were collected from Masud Confectionary, at KR Market of Bangladesh Agricultural University. Each sample was divided into 5 part, one part without oven treatment and rest 4 part were allowed for oven treatment for 30 sec, 45 sec, 1 min and 2 min, respectively. The whole experiment was divided into three steps. The steps included isolation of the bacteria from hotdog, identification of the *Staphylococcus* by cultural, morphological, biochemical characteristics and PCR. Finally antibiotic sensitivity test and organoleptic examination was done.

Isolation of bacteria in pure culture: Homogenized samples were enriched in nutrient broth and incubated at 37°C for overnight. Enriched culture was streaked duplicate onto MSA, EMB, and SS agars and incubated at 37°C for 24 h. Colony appeared onto the surface of MSA, EMB agars was further sub cultured on the same media until a pure culture was obtained.

Characterization of bacteria from hotdog: Bacteria was characterized by colony morphology, Gram's staining reaction, sugar fermentation tests, catalase test, coagulase test, Methyl Red, Voges-Proskauer, indole tests and Polymerase Chain Reaction (PCR) assay (Cheesbrough, 1985)

Polymerase Chain Reaction (PCR) for *Staphylococcus*: A genus specific PCR was performed to amplify *tuf* gene of *Staphylococcus spp.* using previously published primers (Martineau et al., 2001) (Table 1).

Table 1: PCR primers with sequence

| Primer | Sequence | Targeted gene | Size(bp) | References |
|----------|--------------------------|---------------|----------|-------------------------|
| Tseq271 | AAATGATACIGGIGCICARATGGA | <i>tuf</i> | 884 | Martineau et al. (2001) |
| Tseq1138 | CCIACIGTICKICCRCCYTCRCG | | | |

Antibiotic sensitivity test by the disc diffusion method: Antimicrobial susceptibility test was done against 10 different antibiotics by disc diffusion method according to the recommendation of Clinical and Laboratory Standards Institute (CLSI, 2016). Susceptibility of *Staphylococcus spp.* isolates to 10 mostly prescribed antimicrobial agents (Table 2) were measured in vitro by employing the modified Kirby-Bauer method (Bauer et al., 1966).

Organoleptic quality study: A panel of experts was first selected. The physical quality of the each hotdog i.e. flavor, taste, color, odor and appearance was checked by each experts.

RESULTS AND DISCUSSION

Among 30 Hotdog samples, 10 samples were found negative of bacterial growth after culture. The TVC of pre microwave oven hotdog samples (n=20) ranged between 4.72 to 5.19 log CFU/g (**Table 2**). On the other hand, the TVC of post microwave oven hotdog samples for 30 sec oven treated ranged from 3.43 to 3.58 log CFU/g for 45 Sec oven treated ranged from 2.88 to 3.76 log CFU/g for 01 min oven treated oven treated ranged from 1.94 to 2.88 log CFU/g (and the TVC of post microwave oven hotdog samples for 2 min oven treated oven treated detected nil. (**Table 2**).

Table 2: Total viable count (TVC) on five types of hotdog samples

| No | TVC (log CFU/g) Preoven | TVC (log CFU/g) 30sec treated | TVC (log CFU/g) 45sec treated | TVC (log CFU/g) 1min treated | TVC (log CFU/g) 2min treated |
|----|-------------------------|-------------------------------|-------------------------------|------------------------------|------------------------------|
| 1 | 5.04 | 4.56 | 3.62 | 2.31 | |
| 2 | 5.11 | 4.87 | 3.56 | 1.95 | |
| 3 | 5.19 | 4.34 | 3.76 | 2.45 | |
| 4 | 5.09 | 4.93 | 3.45 | 2.76 | |
| 5 | 4.99 | 3.87 | 3.48 | 2.88 | |
| 6 | 5.12 | 3.97 | 3.12 | 1.98 | |
| 7 | 5.14 | 4.05 | 3.72 | 1.94 | |
| 8 | 4.98 | 4.67 | 2.90 | 2.53 | |
| 9 | 5.03 | 3.94 | 2.98 | 2.59 | |
| 10 | 5.07 | 4.58 | 3.12 | 2.81 | No bacterial growth |
| 11 | 5.11 | 4.93 | 3.33 | 1.88 | |
| 12 | 4.95 | 4.42 | 2.88 | 1.86 | |
| 13 | 5.03 | 4.86 | 3.67 | 1.99 | |
| 14 | 4.72 | 3.87 | 3.41 | 2.76 | |
| 15 | 5.17 | 3.43 | 2.99 | 2.59 | |
| 16 | 5.12 | 4.53 | 2.83 | 2.09 | |
| 17 | 5.11 | 4.42 | 3.62 | 2.88 | |
| 18 | 5.05 | 4.78 | 3.76 | 2.01 | |
| 19 | 5.03 | 4.88 | 2.89 | 1.99 | |
| 20 | 5.13 | 3.98 | 3.45 | 2.92 | |

The TSC of pre microwave oven hotdog samples (n=20) ranged between 4.31 to 5.05 log CFU/g (**Table 3**). On the other hand, the TSC of post microwave oven hotdog samples for 30 sec oven treated ranged from 3.43 to 4.78 log CFU/g, for 45 Sec oven treated ranged from 2.33 to 3.56 log CFU/g for 1 min oven treated oven treated ranged from 1.69 to 2.61 log CFU/g for 2 min oven treated oven treated detected nil (**Table 3**).

Bacteria were recovered only from samples inoculated onto MS agar. The results are identical to those by Paludi et al. (2011), Madueke et al. (2014), Furlaneto et al. (2010) and Borges (2011). The cultural characteristics of *Staphylococcus* spp were similar to the findings of other authors (Sharada et al., 1999; Thomas et al., 2005; Konuku, 2012). Small whitish colonies appeared on MS agar, which were characteristics of *Staphylococcus* spp. Gram positive cocci, were arranged in grape-like clusters, characteristics of *Staphylococcus* spp.

Staphylococcus spp fermented all five basic sugars without gas production Catalase, Methyl Red and Voges-proskaur tests were positive but indole and coagulase tests were negative. The results are identical to those by Martin et al. (2009), Abdezzak et al. (2012), Adejuwon et al. (2004) and Freeman (1979).

DNA extracted from *Staphylococcus spp* were used in PCR assay. PCR primers targeting *tuf* gene of *Staphylococcus spp*. Amplified 884-bp fragments of DNA confirmed the identity of *Staphylococcus spp*. This result are similar to those by Kucukoglu et al. (2013), Brizzio et al. (2013) and Brakstadt et al. (1992) (**Figure 1**).

Staphylococcus spp. resistant to Tetracycline, Ciprofloxacin, Amoxicillin, Oxacillin and Azithromycin (**Table 4**).The result are identical to those by Thong and Modarressi (2011), Singh et al. (2011) and Tagoe et al. (2011).

Table 3: Total *Staphylococcal* count (TSC) on five types of hotdog samples

| No | TSC (log CFU/g) Preoven treated | TSC (log CFU/g) 30 sec treated | TSC (log CFU/g) 45 sec treated | TSC (log CFU/g) 1min treated | TSC (log CFU/g) 2 min treated |
|----|------------------------------------|-----------------------------------|-----------------------------------|---------------------------------|----------------------------------|
| 1 | 5.01 | 3.97 | 3.32 | 2.59 | |
| 2 | 4.92 | 4.65 | 3.47 | 1.64 | |
| 3 | 5.05 | 3.87 | 3.12 | 2.61 | |
| 4 | 5.06 | 3.97 | 2.56 | 2.12 | |
| 5 | 4.79 | 3.97 | 2.81 | 2.31 | |
| 6 | 5.02 | 4.63 | 2.33 | 2.45 | |
| 7 | 5.04 | 4.53 | 2.62 | 2.09 | |
| 8 | 4.93 | 4.66 | 3.45 | 1.87 | |
| 9 | 4.83 | 3.94 | 3.56 | 2.58 | |
| 10 | 5.20 | 4.78 | 2.67 | 2.59 | |
| 11 | 5.01 | 4.05 | 2.35 | 2.06 | No bacterial growth |
| 12 | 4.35 | 4.86 | 3.41 | 2.61 | |
| 13 | 4.83 | 3.43 | 3.12 | 2.45 | |
| 14 | 4.82 | 4.78 | 3.01 | 2.59 | |
| 15 | 4.67 | 4.05 | 3.56 | 2.61 | |
| 16 | 5.01 | 3.98 | 3.16 | 2.39 | |
| 17 | 4.31 | 4.78 | 3.12 | 2.48 | |
| 18 | 5.05 | 3.97 | 3.48 | 2.45 | |
| 19 | 5.03 | 4.87 | 3.72 | 2.60 | |
| 20 | 4.88 | 4.93 | 3.33 | 2.34 | |

Table 4: Antimicrobial profile *Staphylococcus spp*.

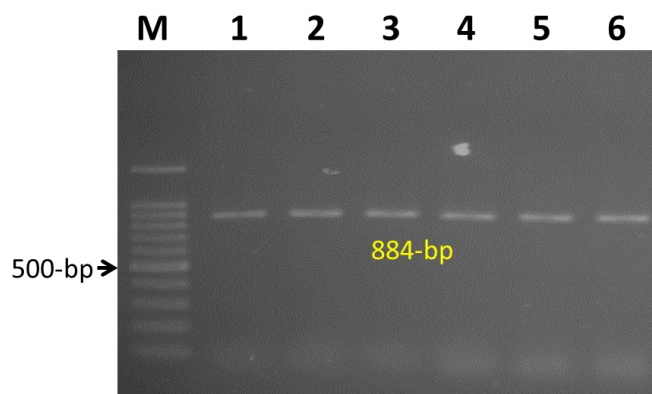
| Name of antibiotic disc | Diameter of Zone of inhibition (mm) | Interpretation |
|-------------------------|-------------------------------------|----------------|
| Streptomycin | 25 | S |
| Tetracycline | 8 | R |
| Ciprofloxacin | 9 | R |
| Gentamicin | 23 | S |
| Amoxicillin | 9 | R |
| Vancomycin | 19 | S |
| Oxacillin | 0 | R |
| Ceftriaxone | 18 | S |
| Azithromycin | 0 | R |
| Meropenem | 14 | I |

On organoleptic examination it is clearly evident that the minutes post microwave oven treated hotdogs are secured the highest score 57 marks that 95% marks. On the other hand the 30 Sec microwave oven treated hotdogs secured the lowest score 30 marks that are 50% of marks (**Table 5**).

Though the microwave oven treatment reduce the number of the microorganism to but the time of microwave treatment of fast food offered by retail fast food shop keepers and it is not enough to kill all pathogenic microorganism. The present study mainly highlighted on tentative time of microwave heat

Table 5: Sensory characteristics representing panel scores of hotdog of different microwave oven treatment from the retail shop in Mymensingh.

| Characters | Microwave Oven Treated | | | |
|--------------------------|------------------------|-----------|-----------|-----------|
| | 30 Sec | 45 Sec | 1 Min | 2 Min |
| Appearance | 10 | 8 | 9 | 6 |
| Flavor | 4 | 8 | 10 | 5 |
| Color | 2 | 6 | 9 | 7 |
| Taste | 6 | 7 | 10 | 10 |
| Odor | 4 | 8 | 9 | 8 |
| Overall acceptability | 4 | 7 | 10 | 9 |
| Total Grand Score | 30 | 44 | 57 | 45 |

**Figure 1.** Lane M 100bp DNA ladder and Lane 1-6 isolated sample of staphylococcus spp. This figure represents the PCR amplification of the culture positive 6 staphylococcus spp. using genus specific primer Tseq271, tseq 1138 showing amplification of 884-bp.

treatment of hotdog without changing food taste and this evidence from the analysis of the above data that the samples of five microwave oven treatment differs among themselves with regard to their microbiological quality. Total counts were taken as an index of quality then samples of 2 min microwave oven treatment revealed no microorganism count. On the other hand the sample of pre microwave oven treatment demonstrated to have the highest microbial count. In the present study coagulase negative Staphylococci (CNS) were isolated from all the hotdog samples of pre-microwave oven and post-microwave oven. In this study Staphylococcus spp. were found to be multidrug resistant and the antibiotics were Tetracycline, Ciprofloxacin, Amoxicillin, Oxacillin, and Azithromycin.

Microbial growth propagation can lead to the development of undesirable organoleptic quality changes in food. In the present study it have been observed that the one minute micro wave oven treated hotdog secured the highest mark of 95% given by the panelist. On the contrary, the 30 sec micro wave oven treated hotdog got the lowest scoring marks of 50%. It is clearly evident that there was no change in hotdog after 01 min microwave oven treated.

CONCLUSION

From the findings of the study, it may be concluded that, increased time of microwave oven treatment there is significant reduction of pathogenic microorganism. The organoleptic quality revealed that 1 min microwave treated samples should be served for consumption. *Staphylococcus spp.* resistant to Tetracycline, Ciprofloxacin, Amoxicillin, Oxacillin and Azithromycin was identified. They may be transmitted to human through the consumption of contaminated hotdog.

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CONFLICT OF INTERESTS

The authors has no conflict of interests in terms of authorship,

AUTHORS' CONTRIBUTION

TAS conducted the actual researches. MMM and MSU helped in sample collection. MMI, SK, and JHR helped in analysis of data. MTH and KHMNH supervised the work and finally approved the manuscript.

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