Role of traditional cooking methods in controlling *S. aureus* and *L. monocytogenes* in shrimp

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A B S T R A C T

This study was planned out to evaluate the effectiveness of different traditional cooking methods on the viability of *L. monocytogenes* and *S. aureus* by contaminating unpeeled whole shrimp samples with 2.4x 10^6 cfu/g for *L. monocytogenes* and 4x10^6 cfu/g for *S.aureus*. Also the effect of refrigeration at 4c°on the viable count of *S. aureus* and *L. monocytogenes*, was studied, *S. aureus* and *L. monocytogene* counts were 1x$10^6$and 3x10^6 cfu/g after one day and 2.7x10^7 and 2x10^5 cfu/g after two days of refrigeration, respectively. This indicated that the refrigeration temperature decreased *S. aureus* count but increased *L. monocytogenes* count. While, the traditional cooking by boiling for inoculated shrimp revealed that, the boiling in different stages at zero day, first and second day of refrigeration decreased the count to <10 cfu/g for both. Microwave oven treatment of the samples resulted in destruction of both *S. aureus* and *L. monocytogenes* cells decreasing the count from about 10^6cfu/g to <10cfu/g. With the exposure of inoculated shrimp to different traditional cooking temperatures within one min for each temperature of 60°c decreased *L. monocytogenes* count to be 1.7x10^2 cfu/g, and *S. aureus* count to 2x10^4 cfu/g. At 80°c temperature effect on L. *monocytogenes* count decreased to be <10 cfu/g and *S. aureus* count to be 3x10 cfu/g.

Key words: *L. monocytogenes, S. aureus*, Inoculation, Microwave oven, traditional cooking.

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1. INTRODUCTION

Sea food is considered as one of the most healthful diet and it's consumption has been linked to a number of health benefits (Genevieve, 2012). A number of factors resulted in contamination of sea food products, as improper handling, processing and preparation (contaminated by an infected food handler or cross contamination through mishandling),as these stages may be linked to improper storage and preparation (Iwamoto et.al., 2010). Aqua cultures and imported seafood are considered a source of unsafe seafood (NOAA, 2010). Heat treatment usually in the form of cooking plays a major role in the safety and sensory aspects. Cooking is defined as "The exposure of food to heat treatment to improve its quality and to meet sensory expectations of consumers as well as to reduce its microbial load, which improves its safety and may extend its shelf life (NACMCF, 2008). Heat is the most practical and effective mean to destroy microbial cells. The rate of death as well as injured cells will be increased markedly as the temperature is raised, while chilling to a temperature below growth range will assist in stopping reproduction and kills few cells (Food Technology and processing, 2008). Consumers uses boiling of shrimps until they float to the water surface, and determine that it is done when observing its color changing to gray , pink which proves that shrimp is thoroughly cooked (Genevieve, 2012). Different factors such as thermal resistance of microorganism

and storage conditions will affect the cooking time and temperature to ensure destruction of pathogens in a product, generally consumers store shrimp at refrigerator temperature (2-4°c) for up to two days before cooking as recommended by the FDA, Certain organisms such as *Listeria Monocytogenes* can grow and multiply in optimum conditions while growth under stress could promote development of heat resistance in some pathogens, products may also be stored in refrigerator after cooking by the consumer (Edwards et. al., 2013). Food contaminated with pathogenic microorganism usually do not look odd , taste bad or smell bad, it is impossible to determine whether a food is contaminated with pathogenic microorganism without microbiological testing to avoid potential problems in food, it is very important to control or eliminate these microorganism from food products (Julie and Susan 1992). Cooking is a heat treatment applied to fishery products (as cooked shrimp) that are distributed either refrigerated or frozen (FDA, 2011). Storage of meat requires low temperature to make sure that the growth of microorganism will be retarded (chilling between one to four c°), cooking meat requires high temperature starting from 55°c to denature microorganism, by generally higher temperature is applied up to 100°c (FAO, 1990). The most common food poisoning is bacterial, such as *Staphylococcal aureus*, and *Listeria monocytogenes* they are implicated in more than 90%of food poisoning cases yearly. These bacteria are commonly found in many raw foods. Food poisoning illness can be prevented by destroying the bacteria by proper cooking .When *S. aureus* is allowed to grow in food it can produce toxins that cause illness. *S. aureus* can grow in a wide range of temperature (6 to 46 c°) but grow best at 37c° (Public health agency of Canada, 2001). Although cooking destroys the bacteria the toxin produced is heat stable (Wagner, 2008). Heat treatment inactivate *S. aureus* but the preformed *S. aureus* enterotoxin remained active (Olimpia Pepe et. al., 2006). The optimum temperature for *S. aureus* is 40°c - 45°c and the inactivation temperature of *S. aureus* is 60c° (Susan, 2012) but the enterotoxin of *S. aureus* was not produced at 15°cand produced at 30c°and37c° (Brigid Herten et.al., 1989). refrigeration temperature does not stop growth of *L.monocytogenes* even its capable of doubling in number every 1.5 day at 39.5°c, since high temperature greater than 170°f will inactivate *L.monocytogenes* (Wagner, 2008). Cellular injury of *L.monocytogenes* increased with time exposure to 60c°and the refrigerated storage did not decrease the potentiality of Listeria to survive (John and Vizay, 2003). Although *L.monocytogenes* can grow in temperature 4°c to 37°c pasteurization and sufficient cooking, kill Listeria (Wikipedia-Listeria 2014). Food borne illness is an ever-present threat that can be prevented by proper care and handling of food products with proper processing, which needs to be evaluated.

2. MATERIALS AND METHODS

2.1. Microbial culture used:

The pathogens used are *Listeria Monocytogenes* (ATCC 35152) and *Staph aureus* (ATCC6538) (TCS, 2014) and are obtained from the reference strain bank of food hygiene department (FHD), Animal Health Research Institute(AHRI) .

2.2.Inoculum preparation:

Each strain was deep frozen stored in a cryo protective vial containing preservative solution at -70c°, Cryobead (inoculums)of each strain was cultivated in tryptic soy broth over night at 35c°. Then cells were centrifuged for 10 min at 8000rpm. Supernatant was discarded and cells were washed three times and re-suspended in sterile 0.1% peptone water. The cells were diluted in peptone water adjusted to obtain the desired inoculums level (10^7 cfu/ml) (Osman, 2008).

2.3.Sample preparation:

Unpeeled whole large size shrimp was pre-examined to be free from *S.aureus* and *L.monocytogenes* (the individual shrimp weight approximately 16±2 grams). The shrimp samples were soaked in the previous prepared culture solution for 30min. and then allowed to air dry for two minutes .in class II biological safety cabinet .This procedures are followed for each of the two bacterial species used in this study .The final concentration of bacteria in shrimp samples was 10^6 cfu/g. After inoculation, the shrimp samples were divided into three groups and packed in polyethylene bags. This experiment was conducted using the mentioned two organisms under test as follows: count of the inoculated microorganisms were conducted at zero day and first then second day of refrigeration in group (1) (as control), and in group (2) after exposure to heat treatment as follow. The shrimp samples were applied in to hot boiling water bath containing one liter of tap water and a thermometer of digital probe was used for monitoring the water temperature to adjust it at 100c° till the shrimp float to the water surface. While in group (3) the inoculated samples were heat treated with different temperature cooking in microwave oven for one min exposure time of 2450 MH and by applying samples in to hot water bath similar to the boiling one but adjusted temperature till 60°c and 80c°for one minute sample exposure then counting of the inoculated microorganism was applied.

2.4. Assessment of microbial growth:

Analysis was conducted on the artificially contaminated shrimp within determined time of analysis after bacterial inoculation and refrigeration then after heat treatment. Counting of bacterial load was applied for *staph aureus* according to (FDA, 2001) and for *Listeria monocytogenes* as (FDA,2011). Determination of *S. aureus* enterotoxins was applied as (Olempia et. al., 2006).

2.5.Statistical analysis:

For statistical analysis, average count of colonies on duplicate plates was transformed in to Log cfug-1.

3. RESULTS:

Fig. (1). Count of *L.monocytogenes* and *S.aureus* in inoculated shrimp samples during refrigeration storage at4c°:

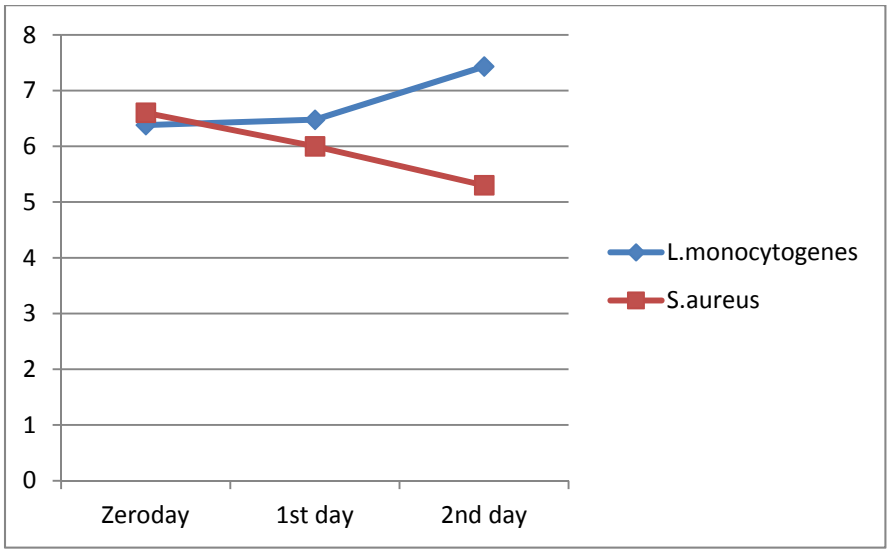

Table (1). Count of *L.monocytogenes* and *S.aureus* in shrimp after cooking (boiling) at100c°:

Day M.O.	*L.monocytogenes* (*cfu/g)	*S. aureus* (cfu/g)
Zero day	<10	<10
1st day	<10	<10
2nd day	<10	<10

(cfc/g) colony forming unit/gram

Table (2). Effect of different exposure temperatures for one min on *L. monocytogens* and *S. aureus* count

Temperature/1min	*L. monocytogenes count* *(cfu/g)	*S. aureus count (cfu/g)*
60c°	$1.7x10^2$	$2x10^4$
80c°	<10	3x10
Microwave oven temp.	<10	<10

*(cfu/g) colony forming unit/gram

Table 3. *S. aureus* enterotoxin production in shrimp samples:

Detection Day	*Initial count (cfu/g)*	*Toxin production*	
		Refrigerated shrimp	*Boiled shrimp*
Zero day	$4x10^6$	*ND	ND
1st day	$1x10^6$	ND	ND
2nd day	$2x10^5$	ND	ND

*ND (not detected)

4. Discussion

Food borne diseases and food poisoning are common worldwide, this problem can be minimized by proper food preparation .Cooking by boiling is considered a common method used by consumer which plays a role in food safety and sensory aspects as it improves the shelf life.

In this study, the effect of boiling and heat treatment on two of the most hazardous micro-organisms, *staphylococcus aureus* and *Listeria monocytogenes* on public health were studied. As cultured bacterial cell of both *S.aureus* and *L.monocytogenes* were of a count 10^6cfu/ml prepared to form a dipping solution where whole shrimp samples were dipped in this bacterial suspension and socked for 30 minuets And was divided to groups for each micro-organism then several studies were performed on them. The contaminated shrimp samples with *L.monocytogenes* were counted at zero day and kept at refrigerator and then recounted at 1st and 2nd day of storage. At the inoculation day(zero day) the count was $2.4x10^6$ cfu/g ,in the first day of examination the count was $3x10^6$ cfu/g then in the 2nd day the count increased with one log to be $2.7x10^7$as seen in (fig1). The same technique was carried out with contaminated shrimp samples with S.aureus at zero day, 1st and 2nd day of refrigeration . The recovered count was $4x10^6$cfu/g, $1x10^6$cfu/g and,$2x10^5$ cfu/ respectively (fig 1). From the obtained results ,it can be concluded that *L.monocytogenes* grows in refrigerator temperature 4°c and this growth was more obvious in the 2nd day of examination ,while *S.aureus* count decreased from the day of inoculation slightly in the first day and obvious decrease in the 2nd day with one log reduction which indicates that *S.aureus* is affected by refrigeration temperature. Similar investigations was noticed as the slowest growth of *S.aureus* was found at 7c°,and the organism started to grow after four days at 8°cin rate about 5.5 times higher than the growth at 7°c (Alizbeta Medvedova et.al., 2009). Refrigeration of raw food will retard the growth of *S.aureus* (Wagner et.al., 2008).In this respect, Julie and Susan (1992), Illustrated that micro-organisms needs time to grow and multiply under favorable conditions as it grows within certain temperature range. besides *S.aureus* can grow in a wide range of temperature (6 to 48°c) with optimum temperature 37°c (Public health agency of Canada 2001). On the other hand *L.monocytogenes* was found to grow at temperature from 4°cto 37°c (Wikipedia –Listeria, 2014). A study conducted by Katarina Pintar et.al.(2007)could be observed that the *L.monogytogenes* count in raw chicken in zero day was lower than it's count after five or eight day of refrigeration storage. *L.monocytogenes* can grow at refrigeration temperature mean while it could not stop the growth of *L.monocytogenes* (Wagner, 2008). After the contamination of pork samples with *L.monocytogenes* and preserved at 4°c for 10 days ,the bacterial population increased by two log cfu/gm (Membre´ et.al., 2004). Cooking eliminates bacterial pathogens in fishery products, cooking products considered one of the main causes to eliminate vegetative cells of pathogenic bacteria or reduce them to an acceptable level, so proper processing needed to be evaluated.

The second group of contaminated shrimp with both *L.monocytogenes* and *S.aureus* were exposed to cooking by boiling till shrimp floats on water surface and it's color turned to pink. After cooking, the infected shrimp samples at zero day, 1st and 2nd day of refrigeration were examined for counting both organisms. The results revealed that *L.monocytogenes* count was <10 cfu/gm in all three phases (zero,1st and2 nd) as shown in (Table1) .Similar investigations were revealed the same results after heat treatment, the bacterial load of *L.monocytogenes* was reduced to non detectable level <1cfu/25g, (Genevieve Anne Edwards, 2012). In this aspect

Edwards, et al (2013) found that Initial *L.monocytogenes* count of 10^5cfu/g in shrimp when exposed to boiling the bacterial count was declined to non detectable level (<10 cfu/g). The same results were observed in cooked shrimp samples previously contaminated with *S.aureus* as the count declined to a non detectable level (<10 cfu/g) at zero,1st and 2nd day of storage(Table 1). This indicates the effectiveness of cooking by boiling on *S.aureus* and *L.monocytogenes* count (10^6cfu/g) decreasing to non detectable count. The obtained results agreed with that recorded by (Public Health Agency of Canada, 2001), (FDA Hazards Guide, 2011)and (Wagner, 2008). The effect of different exposure temperature on inoculated shrimp samples individually with both S. *aureus* and *L. monocytogenes* of initial count about 10^6 cfu/g was studied in group (3), which divided into three parts; the first part for both organisms of this group was cooked by Microwave oven of 2450 MH for one minute exposure time, while the second and third parts were dropped in adjusted water bath at 60c°(for 2nd part) and 80c°(for 3rd part) each for one minute duration. All the three parts were examined after exposure for *L.monocytogenes* and *S.aureus* counts. As shown in table (2), the obtained results revealed that the counts of both microorganisms in shrimp samples after cooking by Microwave oven decreased to be<10cfu/gm, while the counts of exposed shrimp samples to 60c° for both *S.aureus* and *L.monocytogenes* were decreased to $2x10^4$ and $1.7x10^2$cfu/g respectively, while the counts of exposed samples to 80c° were 3x10 and<10cfu/gm respectively (Table2). Aliyer Fouladkhah et. al. (2012) showed similar results as illustrated that microwave oven reheating of inoculated cooked chicken breast with *L.monocytogenes* for 90s reduced *L.monocytogenes* to <0.4-2.6 log cfu/g. Moreover, Walecka et al. (2011) mentioned that heat exposure of *L.monocytogenes* to 54c° for 20 min reduced its count) while Susan et.al. (2012) illustrated that the inactivation temperature was 60°c, also cellular injury of *L.monocytogenes* increased with increasing time of exposure to 60°c. Presence of *S.aureus* enterotoxins was determined in this study in the contaminated whole shrimp samples at zero day, after the 1st and 2nd day of refrigeration storage and after boiling of samples. The results revealed that, the different types of enterotoxins were not detected in any of the examined samples as shown in (Table 3).These results illustrated that neither the time of shrimp exposure to *S.aureus* during 30 min dipping nor the temperature of refrigeration in the 1st and 2nd day is enough to *S.aureus* enterotoxin to be produced . These results approved the evaluation of Susan et.al. (2012) on *S.aureus* optimum temperature for toxin production to be at 40-45°c.Also enterotoxin were found not to be produced at 15c°and produced at 30°c and 37°c. Toxin production appeared to be influenced more by growth temperature (Brigid Herten et.al., 1989) while the maximum amount of *S.aureus* enterotoxins (SE) in ready to eat sandwiches did not exceed 0.5 ng/g after 30.5 h and 52 h. at 30c° and was not produced at 17c°. The use of cooking by boiling for shrimp preparation (till floats on water turning to pink color) for consumption is considered to be safe in destroying pathogenic *L.monocyogenes* and *S.aureus* ,while improper cooking and exposure to lesser heat treatment will resulted in public health hazards as it is not enough to destroy both pathogenic micro-organism completely . While the use of refrigeration for raw food material is variable as it stops *S.aureus* growth while it is not enough for controlling *L.monocytogenes* growth, which acts as public health hazard, besides refrigeration could help in limiting or even stopping *S.aureus* toxin production. Meanwhile, the use of microwave seems to be suitable method for shrimp preparation as it was proper to destroy the microorganism under examination. Finally the high temperature treatment may be good in controlling the infected shrimp with *S.aureus* and

L.monocytogenes while chilling refrigeration temperature may be good method in controlling *S.aureus* growth and it's toxin production in food but contaminated food with *L.monocytogenes* needs another proper method for preservation as freezing may be safer for consumer, leading to safe food and public health protection.

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