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RESEARCH ARTICLE

STUDY THE EFFECT OF THERMAL TREATMENT ON *LISTERIA MONOCYTOGENES* ISOLATED BACTERIA IN THAMAR PROVINCE/YAMAN

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ABSTRACT

Study the effect of different thermal treatment on *Listeria monocytogenes* survival in applied studies. For this object, isolate was used from Red meat source. The preservation of meat at (4 ^{o}C) had no major effect on isolation of *Listeria monocytogenes*, also when we kept samples at a room temperature of (21^{o}C). Whereas the isolation of *Listeria monocytogenes* from preservation samples at (-18^{o}C) were stopped after the sixth weeks. The isolation rate was decreased in the infected cooked meat pieces at the temperature of (75^{o}C) for a period of 15 minutes, nonetheless the isolation was completely stopped in the infected meat pieces at (75^{o}C) for 5 minutes by cooking with Dry heat (Microwaves).

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INTRODUCTION

L. monocytogenes is a psychrotrophic bacterium with an optimum growth temperature between 30 and 37 °C, but it may grow within the range of minus 1.5 to plus 45 °C . However, the growth rate at low temperature is quite low. At 4°C, the level of *L. monocytogenes*, inoculated to Channel catfish, increased 2 log units during 15 days of storage (Lorentzen, 2010). However, the growth rate at low temperature at -1.5^{o} C is very slow **(**Lorentzen, 2010; Lunden, 2004). The limits of growth at refrigeration temperature are strongly dependent on medium pH. However, the overall food safety challenge with respect to *L. monocytogenes* is its ability to grow at refrigeration temperature (Lorentzen, 2010) The ability of *Listeria monocytogenes* to grow at refrigeration temperatures, coupled with the ubiquitous presence of the bacterium (e.g., in raw and processed meats, in unpasteurized milk, and in dairy products, as well as in poultry, vegetables, and seafood), makes this bacterium a serious threat to susceptible consumers (Goulet, 2007). Thermal treatment is one of the most reliable and commonly used methods to ensure food preservation and food safety. *L. monocytogenes* is a psychrotrophic bacterium with an optimal growth temperature is between 30 and 37°C, but it is able to grow at refrigeration temperatures as low as 0.5°C and can even survive freezing. Growth is limited by temperatures over 45°C, and *L. monocytogenes* is destroyed by pasteurization at 71.6°C in 15 seconds (Autio, 2003). *Listeria monocytogenes* can survive for 10-30 days in tap water at 28-30°C and for 7-110 days at 5-10°C. In pond water, *L. monocytogenes* has been reported to survive for more than 8 weeks (air temperature – 26 to 9°C). *Listeria monocytogenes* has been reported to survive for at least 3 weeks and probably significantly longer in sea water. Survival in sea water was dependent on sea water temperature and *L. monocytogenes* strain (Orndorff *et al*., 2006). Most bacteria grow poorly when temperatures fall below 4^{o}C, while listeria survives in temperatures from below freezing (-7^{o}C), to body temperatures and it not grow best at -81^{o}C to 10^{o}C, a temperature range including that used for refrigeration (Ramaswamy *et al*., 2007). They are resistant to freezing and drying temperature (AHW, 2011). Below the temperature of minus 1.5°C, *L. monocytogenes* is able to survive, although it is not able to grow. The ability to survive during freezing is partly dependent on the temperature, freezing rate and freezing menstruum. A low freezing temperature and rapid freezing appeared to be the

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most favorable to bacterial survival (Lorentzen, 2010). The relationship between duration of thermal treatment and log count of survivers is commonly referred to as the survival curve. Some components in food may protect *L. monocytogenes* against heat. When *L. monocytogenes* is exposed to sub lethal stress, i.e. thermal shock, acids, oxidants, starvation or high osmolality, it may develop an adaptive response to subsequent thermal treatments. Sub lethal thermal treatment includes slow heating, holding food in warm trays, inadequate thermal treatment or hot water washing. The mode of adaptive thermal tolerance is transient and thus, the potential of heat adaptation should be taken into account when planning the parameters of thermal processing (Shresh, 2013).

MATERIALS AND METHODS

Isolation of *L. monocytogenes* from Red Meat

Red meat samples were cut into small pieces by sterile blades for liberation of adherent bacteria to the enrichment broth. This step was done under sterile conditions according to (Nader, 1998; Aodah, 2009; Al-Dulaimi, 2013). Bacterial isolation from the red meat samples was carried out by suspending a representative fragment of each sample in a sterile saline solution. 25 gm of red meat (as the optimal sample size) in 225 ml of Listeria Enrichment Broth (LEB) and incubated for 48h at 37°C . As 0.1 ml of the LEB was dispensed onto Oxford Medium Base (OXA) plate. The culture was then incubated at 37°C for 48h in microaerophilic atmosphere (Derra, 2007; Abdelgadir *et al*., 2009). Sometime used anther media but made selective by the addition of acriflavine hydrochloride (10 mg/L), nalidixicacid sodium salt (40 mg/L) and cycloheximide (50 mg/L) (Gracey *et al*., 1999; Roberts and Greenwood, 2003).

Purification of *Listeria monocytogenes* Isolates

After growth of *listeria monocytogenes* Colonies on selective media, make subculture on anther same media and incubate plates at 37°C for 24 to 48h (Saleh, 2010 ; AL-Dulaimi, 2013).

Identification of *L. monocytogenes* isolates from Red meat

Isolates were cultivated on growth medium Listeria Oxford Medium Base (OXA) and Listeria Enrichment Broth (LEB) and incubated at 37C for 24 and 48hr. After incubation period, all plates were examined to determine the relative proportion of various typical colony types of *Listeria monocytogenes*. These colonies are stained with Gram stain examined by check the colonies size, shape, color and texture. Further study including biochemical tests (Catalase, Oxidase, Indole, Urease, H2S production, Methyl red, Simmon,s citrate and Voges- proskaur) were studied for isolates identification. Isolates are small, smooth and appear pale blue-green when viewed from the side (45 angle) with a beam of white light, gram positive with exposure the smear to Crystal violate for 1 min, slightly curved, tiny rods with rounded ends, often occurring in pairs at an acute angle (Cheesbrough, 2009) But old cultural may appear gram negative (Gracey *et al*., 1999). Biochemical tests (Table 1) confirmed the identification of isolate as *Listeria monocytogenes.*

Table 1. Biochemical Tests of *L. monocytogenes*

Test		Reaction
Catalase		+
Oxidase		-
Indole		-
Urease		-
Gram		+
Motility	25°C	+
	37°C	-
H_2S production		-
Hemolytic (β)		+
TSI		A/A
Methyl red		+
Simmon's citrate		-
Voges- proskaur		+

Preparation of *Listeria monocytogenes* Isolates

For the purpose of testing on the impact of methods which use for red meat keeping from growth of *Listeria monocytogenes*. Two strains were selected: first one isolated from blood , and the other one from red meat. These strains are cultured on Listeria Oxford Medium Base under micro aerobic conditions and incubated at 37 °C for 48h, then examined to make sure of purity. Then added to each dish 10 ml of sterile phosphate buffer saline (PBS) and harvested colonies by sterilized loop and then collected and mixed well. After that work series of dilution, then calculated the number of bacterial cells by compared with 0.5 McFarland tube, and using a spectrophotometer in optical wavelength 475 nm, where the number 10^8 cell / ml used in the contamination of the Red meat pieces by 1 ml for each piece (Nader, 1998 ; AL-Dulaimi, 2013).

Preparation of Cattle Meat Samples

A 35 g of *L. monocytogenes* free, fresh, and raw Cattle meat was weighed in a sterile polyethylene bag and inoculated with 1 ml of broth containing 10^8 cells on the surface and inside each piece of meat by sterile disposable syringe (Nassir, 1997 ; FSSAI, 2012 ; AL-Dulaimi, 2013).

Study the Effect of Thermal Treatment on *L.monocytogenes* (Applied Study)

Cooling Temperature

Inoculated control Cattle meat samples were kept in the Refrigeration at 4°C. The number of Colony Forming Unit (CFU) was determined at zero time and after 1, 2, and 3 days of incubation by serial dilution and plate count techniques in duplicate using OX agar at 37°C for 48h (Nassir, 1997; Nader, 1998; AL-Dulaimi, 2013).

Room Temperature

Inoculated meat samples were kept at 21°C for 3 days and examined for the recovery of *L. monocytogenes* in meat at zero time and after 1, 2, and 3 days of incubation in duplicate using listeria selective agar (Nassir, 1997; Nader, 1998; AL-Dulaimi, 2013).

Freezing Temperature

Inoculated samples were held at -18°C in polyethylene bag and samples were withdrawn at zero, 1, 2, 3, 4, 5, 6 and 7 weeks of

storage. Colony Forming Unit (CFU) were determined in duplicate using of *L. monocytogenes* selective agar (Nassir, 1997; Nader, 1998; AL-Dulaimi, 2013).

Moist Temperature

Putted the pieces of contaminated red meat in a polyethylene bag, then put it in sterile flask containing of 300 ml of sterile distilled water and closed top by folic paper, and put with water bath separately the one from another. A special thermometer passed through a wire up to cheek internal temperature of meat. Samples was withdrawn and one from the moment of the start and thus upon the arrival of the temperature inside the contaminated pieces to (65, 70 and 75) °C and time for each temperature degree respectively for (5, 10 and 15) min. The CFU was calculated in duplicate in *L.monocytogenes* selective agar at 37°C for 24h (Nassir, 1997; Jay *et al*., 2005; AL-Dulaimi, 2013).

Dry Temperature

Three pieces of contaminated red meat with 10^8 cell/ml are putted in sterile polyethylene bags. Then exposed each piece for short radiation inside the microwave. Specified periods of time (1, 3 and 5) min, and the temperatures of these pieces are (65, 70 and 75) respectively. The CFU was calculated in duplicate in *L. monocytogenes* selective agar at 37°C for 24h (Nassir, 1997; Jay *et al*., 2005; AL-Dulaimi, 2013).

RESULTS

Show in (Figure 1), little decrease during incubation period at the Cooling Temperature (4°C) in the number of *Listeria monocytogenes*

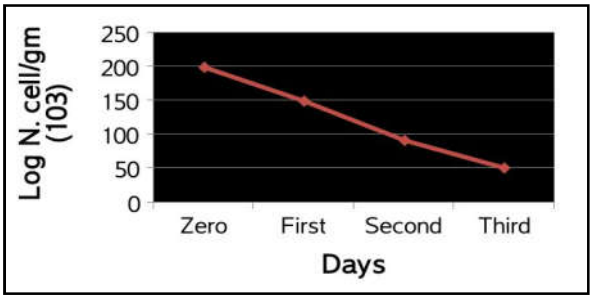

Figure 1. Effect of Cooling Temperature (4°C)

At the room temperature (21°C) show in (Figure 2), constant in the number of *L. monocytogenes* in first and second days, hereas showed declined during third day in *L.monocytogenes* number.

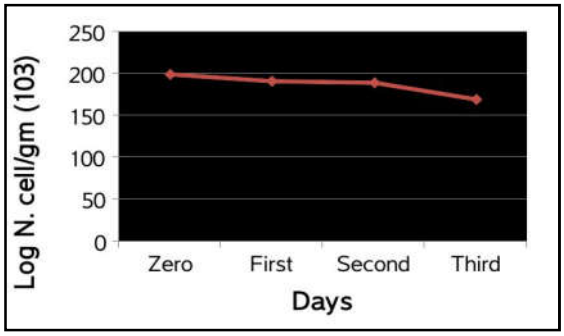

Figure 2. Effect of Room Temperature (21° C)

At the meantime, *L.monocytogenes* count declined during the six weeks of storage at (-18°C), and by the end of storage period the colonies cannot be recovered (Figure 3).

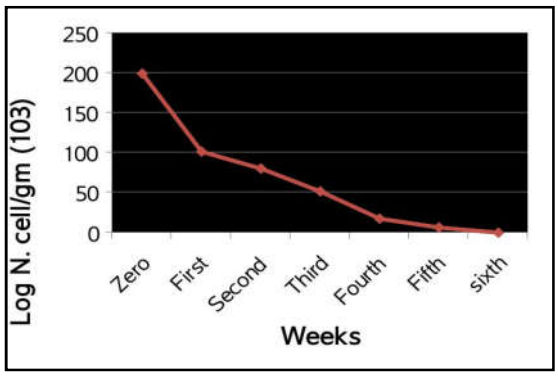

Figure 3. Effect of Freezing Temperature (-18°C)

The moist temperature (cooking temperature) where influential on survival growth of *L. monoytogenes*in in red meat, when the internal temperature had reached to (75°C) for 15 minutes no organism can be recovered (Figure 4).

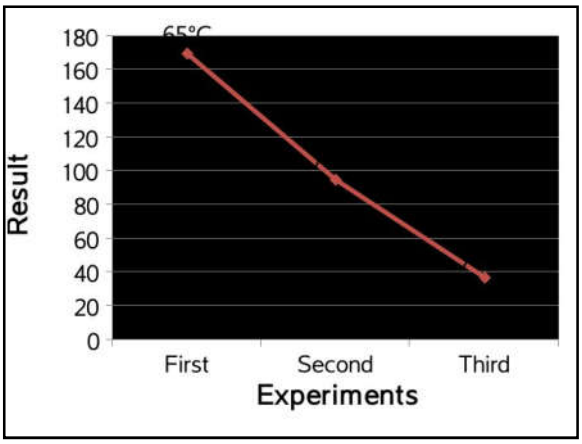

Figure 4. Effect of Moist heat Temperature

Dry temperature in oven microwave was more affected than moist temperature, when the internal temperature had reached to (75°C) for 5 minutes no organism can be recovered (Figure 5).

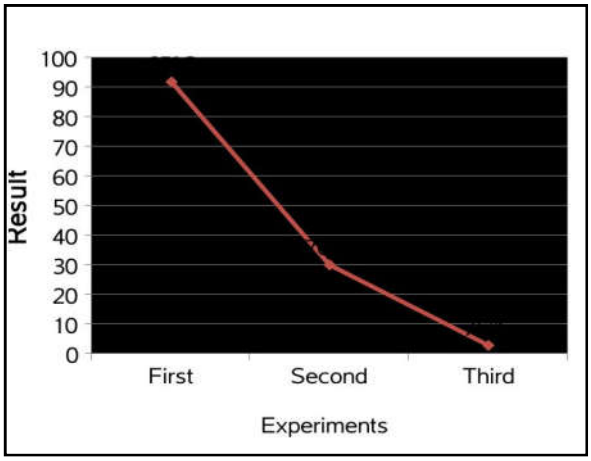

Figure 5. Effect of Dry heat Temperature

DISCUSSION

Show in (Figure 1), *L. monocytogenes* grow in Cooling temperature (4°C) was accompanied by a decrease in the counts of *L. monocytogenes* gradually until the end of the experiment period. Studies of the cell membrane show that the membrane phospholipids must remain in a liquid-crystalline state to maintain membrane fluidity and thereby be able to grow at low temperatures. The fatty acid composition determines whether the membrane phospholipids are in the liquid-crystalline state or not. In *L. monocytogenes*, the level of branched-chain fatty acids in the cell membrane is more than 95%. At 37°C, the major fatty acids are (41-52%), (24-51%), and (2-18%). While grown at 5°C increases to 65-85% of the total membrane fatty acids . The reduction of long aliphatic chains and the increase in asymmetric branching reduce Vander Wals bonds among membrane constituents. Hence, the tight packing of membrane phospholipids at low temperature is reduced and the membrane fluidity of the pathogen is maintained (Lorentzen, 2010). According to (Lefoka, 2009), *L. monocytogenes* grows in pasteurized milk with the numbers increasing 10-fold in 7 days at 4°C. Therefore temperature abuse may further enhance the multiplication of bacterial cells. In relation to the above condition, noted that the organism exhibits a surprising resistance to the heat and it has been suggested that it survives the minimum pasteurization heat treatment of 72°C for 15 seconds required by many countries for raw milk.

During leaving of experimentally contaminated Cattle meat with *L .monocytogenes* at room temperature (21°C), was accompanied by constancy in the counts in experiment start, then decrease in the counts in the end of experiment period. The isolation of these bacteria was continuing until the end of experiment (Figure 2). In this part of our result, I did not find any other researches about growth of *L. monocytogenes* at in room temperature, but other studies about growth of *Campylobacter jejuni* (Nassir, 1997) in Iraq, noticed that the growth was affected by temperature (4 and 20° C) our result were agreement with these result. Also our result agreement with (AL-Dulaimi, 2013) in Yemen, who found the growth of *campylobacter* was affected by room temperature. The freezing process was very influential on the growth of *L.monocytogenes*, where it was observed a clear reduction in counts of *L.monocytogenes* from the first week of storage, and not isolated after the sixth week (Figure 3).

Ramaswamy *et al*. (2007) in India, agreement with our result, whom explained the most bacteria grow poorly when temperatures fall below (4°C), while *L. monocytogenes* survives in temperatures from below freezing (-7°C) to body temperatures and it not grows best at (-18 to 10°C), a temperatures range including that used for refrigeration. Below the temperature of minus 1.5 °C, *L. monocytogenes* is able to survive, although it is not able to grow. The ability to survive during freezing is partly dependent on the temperature, freezing rate and freezing menstruum. A low freezing temperature and rapid freezing appeared to be the most favorable to bacterial survival . Freezing and storage at minus 18 °C inactivated 1 to 2 log units and injured >50% of the pathogen population. Multiple procedures of freezing and thawing are more detrimental to the survival of Listeria than a single cycle of freezing , due to the rupturing of the cell wall that involves leakage of cytoplasmic content. In general, freezing followed by storage causes limited inactivation of the pathogen. Thus, contamination of frozen food should be prevented (Lorentzen, 2010). Heat (in two types: moist and dry) was drastic effect on the survival of *L. monocytogenes*in meat samples. The moisten heat temperature were very influential on growth of *L. monocytogenes* in cattle meat. In this experiment found that the compatibility relationship between increasing temperature and speed of bacterial number reduction. When the internal temperature had reached to (75°C) for 15 min no organism can be recovered (Figure 4). Our result agreement with (Nader, 1998), who observed that the isolation rate of *Yersinia Enterocolitica* from experimentally contaminated cattle meat was reduced, she noticed that when increased temperatures above 65°C no organism can be recovered after 15 min.

Hitchins and Jinneman, 2011. recorded that the inactivation temperatures of *L. monocytogenes* in experimentally contaminated Borger were 65°C, 70°C and 75°C in time 9.3, 2.0 and 0.4 minutes respectively. Dry heat temperature was more affected than moist heat temperature. In this experiment found that the compatibility relationship between increasing temperature and speed of bacterial number reduction. When the internal temperature had reached to (75°C) for 3 min no organism can be recovered (Figure 5). Bacteria require relatively high levels of moisture for their growth, Because most bacteria require Water activity ^{a}w values above 0.90 for growth, they play no role in the spoilage of dried foods (Jay, 2000). In New Zealand (Bremer *et al*., 2003) by recorded thermal death times of *L. monocytogenes* during heat shucking at temperature 60°C for 7.46 min, 62°C for 3.24 min, 68°C for 16 seconds and 72°C for 3 seconds. While thermal death times of *L. monocytogenes* during hot smoking at temperature 60°C for 5.49 min and 62°C for 1.85 seconds. Thermal treatment is one of the most reliable and commonly used methods to ensure food preservation and food safety. However, thermal treatment can alter the quality of food by affecting the liquid loss and thereby influencing texture and content of water soluble nutrients. Thermal treatment may also modify amino acids making them less available as nutrients Survival and thermal resistance of *L. monocytogenes* under different conditions in a variety of foods have been investigated. Thermal resistance of *L. monocytogenes* is influenced by many factors such as strain variations, growth phase, growth conditions, exposure to thermal shock, acid and the composition of the heating menstruum. After the thermal treatment, the number of surviving cells detected depends on the ability to recover, recovery method, recovery medium and incubation conditions used (Lorentzen, 2010).

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