

EXISTENCE OF *LISTERIA SPECIES* IN FRESHWATER FISH AND ITS CONTROL USING SANITIZED ICE

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ABSTRACT

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The present study was designed to investigate the occurrence of *Listeria spp.* in two of economically important freshwater fish species namely *Oreochromis niloticus* (tilapia) and *Clarias gariepinus* which were collected from commercial fish markets in Assiut, also to explore the efficiency of five ice formulations : tab water ice (control), three ices sanitized using trisodium phosphate (TSP) 2.5%, 5% and 10% in addition to an ice sanitized using sodium acetate (SA) 2.5% against *Listeria monocytogenes* (LM) artificially loaded on Tilapia fish at level of (5.5 log₁₀ CFU/g) and the effect of formulated ices on ice stability, sensory quality and pH of fish during storage at room temperature (25 °C). The microbiological analysis of sampled fish revealed that sampled fish represents health hazard to consumer and contact surfaces. *O. niloticus* were more contaminated with *Listeria spp.* compared with *C. gariepinus* samples (16% and 4%, respectively). The contamination with LM in both fish species was parallel (4%) while *Listeria innocua* was present in *O. niloticus* only (12%). Storage of Tilapia loaded with LM (5.5 log₁₀ CFU/g) on formulated crushed ices separately for 4h revealed that both (SA) 2.5% and (TSP) 10% ices had the efficacy (P<0.05) of reduction of LM on fish and in water from melted ice when compared with the control thus improves the safety and reduces the potential for cross contamination. Formulated ices were more stable (slow melting rate) when compared with the control. Storage of fish on formulated ices for 4h was not associated with any defects in sensory parameters (P>0.05). A desirable lowering of pH values of stored fish was noticed by application of (SA) 2.5% ice while (TSP) 10% ice resulted in a significant rise (P<0.05) of pH at the end of storage period. Despite this study demonstrates the effectiveness of (SA) 2.5% ice or (TSP) 10% ice application to control the growth of LM to enhance the microbiological safety of raw fish, (SA) 2.5% ice has the advantages of its organic origin and its desirable lowering pH of stored fish. The public health importance of the organism was discussed and the suggestive measures for control were outlined.

Key words: *Listeria spp.*, Freshwater fish, *O. niloticus*, Trisodium phosphate, Sodium acetate, Sensory pH.

INTRODUCTION

Fish is an excellent protein source (Jannat *et al.*, 2010) and provides many health benefits. One such benefit is its high level of omega 3 (n-3) fatty acids, which are known to reduce cholesterol level and the incidence of stroke, heart disease and preterm delivery, Willett (2005). Also fish is one of the most highly perishable food products, Ashie and Simpson (1996).

Normally fish muscle is sterile as its immune system prevents bacteria to proliferate easily whereas after death the fish's immune system collapses allowing easily penetration of microorganisms into the flesh, Huss (1995). This penetration increase in case of fish

caught from polluted area where there are high densities of bacteria, Howgate (1985). Microbial contamination can reduce the quality of fresh fish, cause economic loss and health hazards, Gram and Huss (1996).

Listeria species could be isolated from a wide range of fishery products, including freshwater and seawater fish as well as frozen and processed fish (Farber and Peterkin 1991, Gudbjornsdottir *et al.*, 2004 and Alves *et al.*, 2005). One particular *Listeria species* (*Listeria monocytogenes*) has been recognized as a food borne pathogen since 1981. The bacterium is ubiquitous, Gram positive, facultative anaerobic, non-spore-forming, rod-shaped (Jacquet *et al.*, 1992 and Jay 2000) and can grow over the temperature

range of about 1 °C to 45 °C and pH range 4.1 to around 9.6. It may be expected to survive in foods for long periods of time, Ryser *et al.* (1985).

Contamination of fish by *L.monocytogenes* (*LM*) may take place during harvest, processing and distribution owing to improper handling and storage of fish, Kim *et al.* (1999). The ingestion of *LM* in food can pose a significant health risk with reported mortality rate. Most healthy humans are not significantly affected by the intake of small numbers of *LM* in foods. However, elderly people, pregnant women and unborn infants are the risk groups that are affected by the pathogen, FAO (1999). In human. The pathogen causes listeriosis which may range from mild to severe sickness, Adzitey and Huda (2010). The severe form of human Listeriosis is present as meningoencephalitis followed by septic infections, Demetriou *et al.* (1996). Although an average of five to nine exposures to *LM* occur per person per year, Grif *et al.* (2003) listeriosis is a rather rare disease, Gerner-Smidt *et al.* (2005) but it is associated with a mortality rate of approximately 20% to 40%, Farber and Peterkin (1991), De Valk *et al.* (2005).

It has been reported that contamination of aquatic products with *LM* cannot be avoided totally, hence in order to inhibit the growth and development of this pathogen in the products and to ensure safety, antimicrobial additives are needed Nykanen *et al.* (2000). Antimicrobial activity of any preservative depends on its hydrophilic and hydrophobic properties i.e. solubility in water and fat, distribution in the model system, fat content pH and temperature, Glass and Doyle (1989). The antimicrobial additives must function the suppression of bacterial growth during storage with minor effects on the quality of the products, Zhu *et al.* (2005).

Trisodium phosphate (TSP) is generally recognized as safe by the US Food and Drug Administration and has been approved by the US Department of Agriculture-Food Safety and Inspection Service (USDA-FSIS) at levels of 8 - 12% as an antimicrobial agent. Trisodium phosphate has been evaluated by several investigators for its efficacy against pathogens and has been used as an effective compound in controlling the growth of Gram -negative pathogens, Ahmed and Abd El-Atti, (2012). Also, the efficacy of sodium acetate (SA) against *LM* has been investigated, Ghomi *et al.* (2011).

Research works have raised concerns about using solutions of TSP (Chang *et al.*, 1995; Kim and Marshall, 2002) or SA (Sallam, 2007a and Sallam, 2007b) to reduce the load of *LM* on fish. There is scarce in research papers concerning the use of TSP or SA to sanitize ice used in fish storage.

In the retail markets, fish is regularly displayed on ice to prevent spoilage and growth of pathogens. However if ice is made with sanitized water is used to

store fish, it has potential to be bactericidal to the organisms, Feliciano *et al.* (2010). Sanitized ice prepared with (TSP) or (SA) could help in achieving the goal. The aim of this study is to document the existence of *listeria spp.* in two economically, important freshwater fish species in Assiut markets (*O. niloticus* and *Clarias gariepinus*). A further goal is to evaluate the effectiveness of ices sanitized using TSP or SA in reducing populations of *LM* (on fish and in water from melted ice), their effect on the sensory quality and pH of stored fish as well as stability of ice.

MATERIALS and METHODS

1- Microbiological analysis of fish samples

Fifty samples of freshwater fish namely *Oreochromis niloticus* and *Clarias gariepinus* (25 each) were collected from fish markets with different sanitation levels in Assiut city. All the collected samples were transferred to the laboratory in an ice box without delay where they were prepared and examined.

Sampling

Samples were taken from the left hand side of each fish in the anterior dorsal region. 25 gram muscle and its covering skin, was aseptically transferred to sterile stomacher bag. (Scott *et al.*, 1992):

Isolation and identification of *Listeria spp.*: Tassou *et al.* (2004).

Two hundred and twenty five mL of sterile *Listeria* Selective Enrichment broth with antibioitic supplement (Difco Laboratories) was aseptically added to 25 g prepared sample and pummeled in a stomacher (Seward BA7021, England) then transferred to sterile flask and incubated at 30°C for 48h. After incubation the a loobfull of each broth was cultured onto Palcam Agar plates with antibioitic supplement (Difco Laboratories). After 48h of incubation at 35°C the colonies morphologically suspected to be *Listeria* (black colonies with black sunken) were sub- cultured onto Tryptic Soy agar supplemented with 0.6% of yeast extract, incubated at 37 °C for 24 h and subjected to confirmatory tests.

All isolates were subjected to Gram's stain, motility and standard biochemical tests including, catalase, acid production from glucose, manitol, rhamnose, zylose, α-methyl-Dmanoside, nitrate reduction, methyl red /Voges-Proskauer, β-hemolytic activity, and CAMP test, (Aygun and Pehlivanlar 2006).

2- Studying effect of ices formulated using TSP or SA on ice stability, *L. monocytogenes* load, sensory quality and pH of fish:

Preparation of ices: Feliciano *et al.* (2010).

Three concentrations, 2.5%, 5% and 10% (w/v) of TSP and one concentration (2.5%) of SA were

prepared by reconstitution of target chemical in tab water. After stirring, the solutions were transferred to separate domestic ice cube trays and stored at -20 °C for a week. Ices were weight into 700 g batches in polyethylene bags and stored at -20 °C until used. Tab water ice cubes was also made as a control and stored under the same conditions.

Ice stability:

For each of ice formulations, the speed of melting was determined by placing the ice cubes (of the same dimensions) in a tray that was left uncovered at room temperature (25 °C). At 30 min intervals the volume of water from melting ice was measured until the ice completely melted, Feliciano *et al.* (2010). All ices were tested at the same time and the test was repeated 3 times.

Preparation of bacterial inoculum: Ahmed and Abd El-Atti (2010).

One strain of *L. monocytogenes* (LM) previously isolated from the examined fish samples was used. The strain was maintained on 10 mL Tryptic Soya agar supplemented with yeast extract (Biolife). Tryptic soya broths (Difco Laboratories) were inoculated and incubated at 30 °C for 24h to achieve viable cell populations approximately of 10^9 cells/mL. Following incubation LM pool was prepared by diluting 10mL of the suspension with 90mL of sterile peptone water to yield a final inoculum approximately 10^8 cells/mL.

Fish used in the experiment:

Freshly caught *O. niloticus* (about 150 g each) were used. On arrival at the laboratory fish were gutted, washed with tab water, divided into two groups (GA = 20 fish and GB = 80 fish) and stored in the refrigerator until used (within 30 min).

Loading *L. monocytogenes* to fish:

The first group of fish (GA) was dipped for 60s in the LM pool (10^8 cells /mL). After dipping, fish left to drain for 10 min in sterile tray fixed in an insulated cabinet and immediately used in the experiment.

Effect of ice formulation on *L. monocytogenes* load in fish and in water from melted ice:

Five ice treatments were used for this experiment. Tab water ice (control), three concentrations (2.5%, 5% and 10%) TSP ices and 2.5% SA ice. Four fish were stored in each respective ice in a perforated sterile tray stand on sterile tray for collecting melted water. The fish were placed between two layers of crushed ice and left uncovered at 25 °C (ice:

fish=1:1w/w and still constant until end of the experiment).

For each treatment a fish was sampled every hour and its load of LM was tested. Where the fish was grinded in a sterile mortar and a 25 gram portion was aseptically sampled to sterile stomacher bag containing 225mL of 0.1 peptone water and pummeled for 1 min in a stomacher. Decimal dilutions were prepared and LM count was done following the technique recommended by Tassou *et al.* (2004). The counts were transferred to log₁₀ CFU/g. At the end of storage period (4h), melted water from each treatment was randomly sampled and its LM load was determined.

Effect of ice formulation on sensory quality and pH of fish:

The 2nd group of fish was subdivided into 5 subgroups (16 fish each). Each subgroup was stored between two layers of corresponding crushed ice treatment at 25°C. For each treatment, four fish were sampled every hour (3fish to be cooked for sensory evaluation and one for pH evaluation).

Effect of ice formulation on the sensory quality of cooked fish:

Treated fish were cooked separately for 15 min to an internal temperature of 75°C in a preheated conventional microwave oven adjusted to 180°C. One representative fish sample of the different treatments was individually presented in covered small porcelain dishes to each panelist. Panelists were asked to evaluate the overall acceptability with regard to appearance, odor intensity, flavor and aftertaste, tenderness, juiciness, off-odor and off-flavor according the score recommended by Sallam (2007b). Samples receiving overall scores of more than 4 were considered acceptable, while a score between 3 and 4 was considered the borderline of acceptability.

pH measurement:

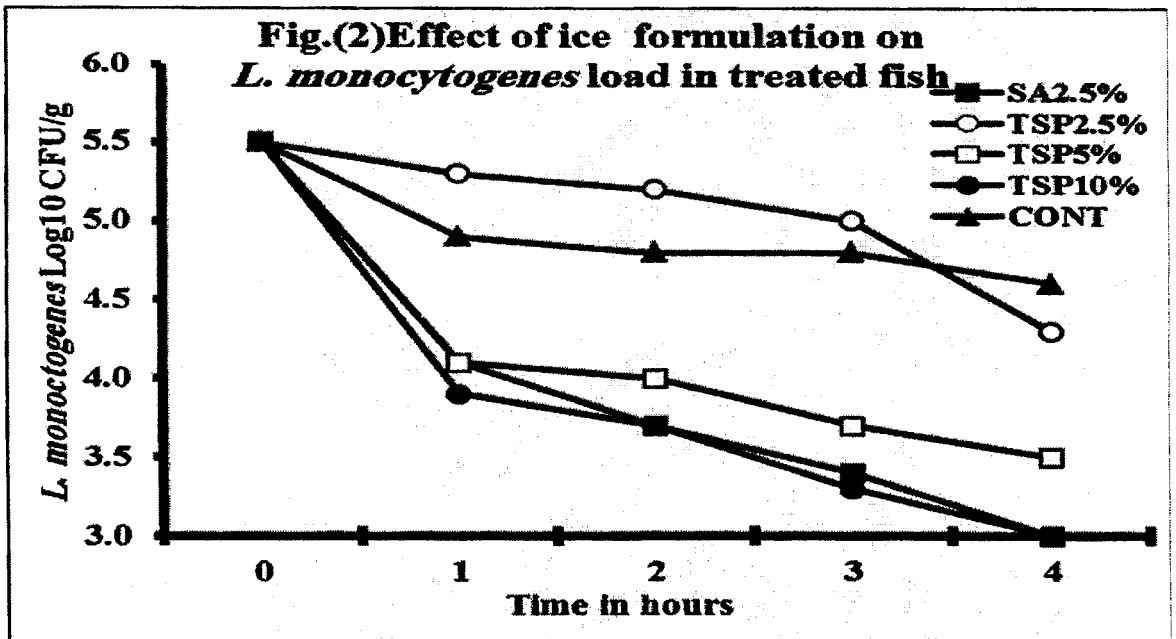
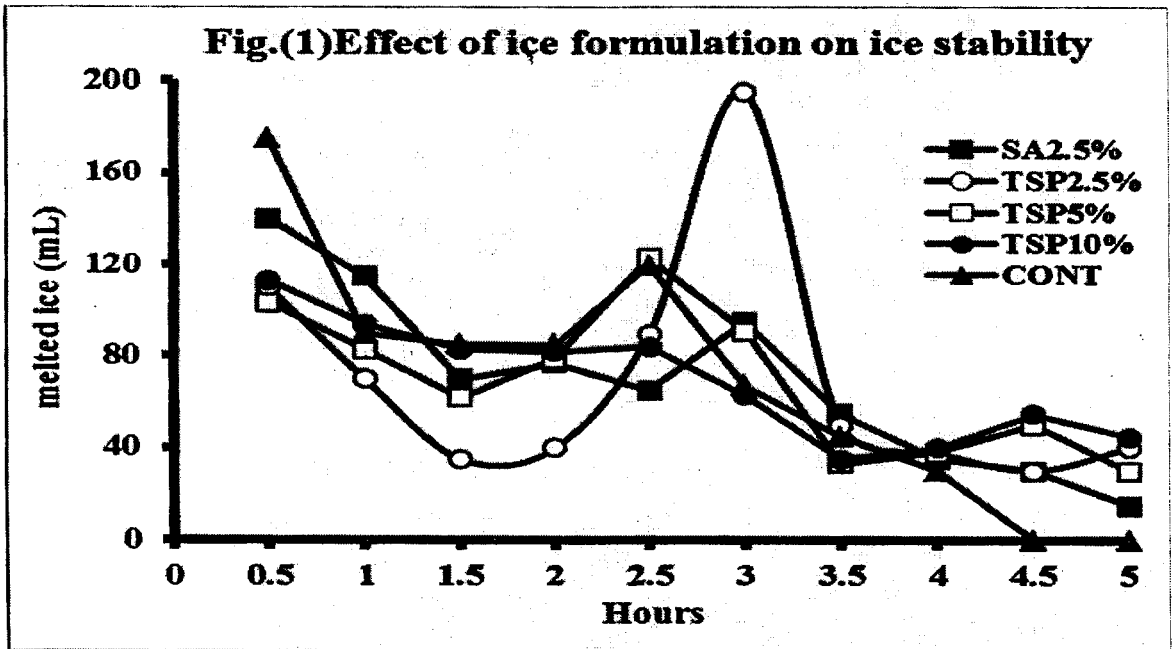
Ten grams of each ice treated samples was speredately blended with 20ml distilled water in a blender for 30 s and the pH of fish homogenate was measured by a digital pH-meter (Gallenhamp No.101284) standardized at pH 4 and 7, Sllam, (2007 b).

All experiments were repeated 3 times and the packaged SPSS program for windows version 12.0.1 was used for statistical analysis according to SPSS (2007). Data were expressed as mean ± standard error (SE). Differences between groups were determined by means of a Student "t"-test. Significance level was set at $P < 0.05$.

RESULTS

Table 1: Incidence of *Listeria spp.* in examined fish samples.

Fish spp.	No. of examined samples	No. of +ve <i>Listeria.spp.</i>		No. of +ve <i>L. monocytogenes</i>		No. of +ve <i>L.innocua</i>	
		No	%	No	%	No	%
<i>O. niloticus</i>	25	4	16	1	4	3	12
<i>C.gariepinus</i>	25	1	4	1	4	0	0
Total	50	5	10	2	4	3	6



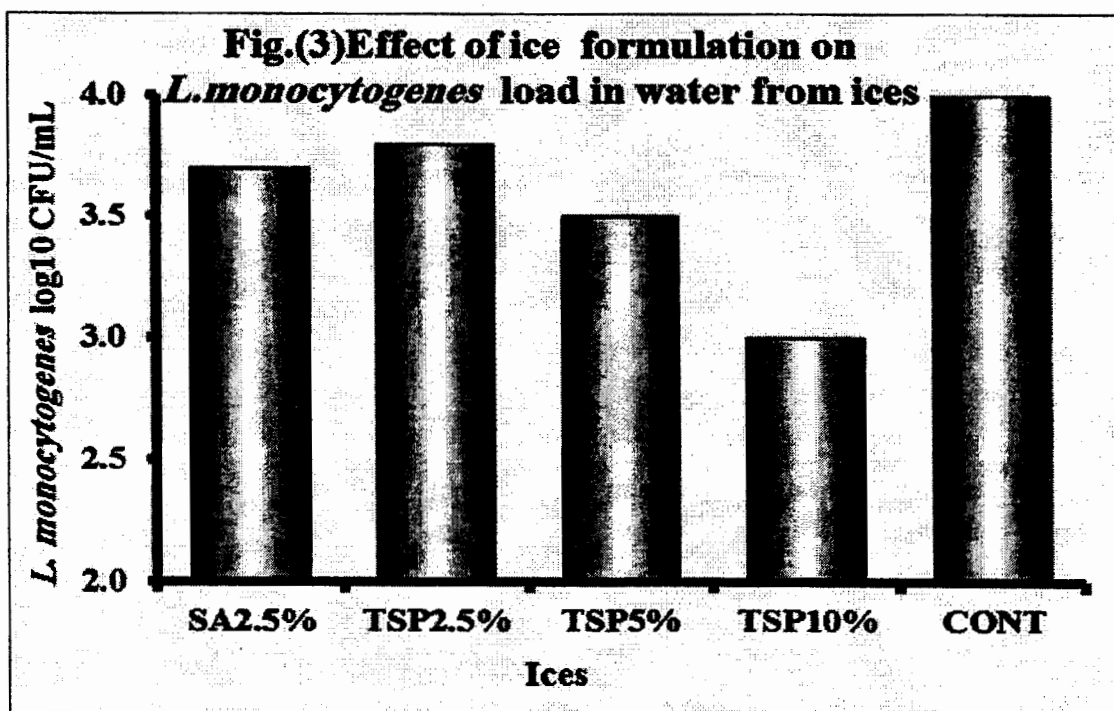


Table 2: Effect of ice formulation on the sensory quality of stored fish

Duration of storage	ices	Mean scores					Overall score
		Odor & Flav. Int.	Juiciness	Tenderness	Appearance	Off Odor & flav.	
1h	SA 2.5 %	7.7	5.7	6.0	6.7	6.0	6.4
	TSP 2.5%	7.3	5.7	5.7	6.7	5.7	6.2
	TSP 5%	7.7	5.6	6.0	7.0	6.0	6.5
	TSP 10%	7.7	7.0	6.0	7.7	5.7	6.8
	Cont.	8.0	7.0	6.0	8.0	6.0	7.0
2h	SA 2.5 %	5.7	6.0	6.7	6.3	6.0	6.1
	TSP 2.5%	6.7	5.7	7.0	6.0	6.0	6.3
	TSP 5%	6.7	5.7	7.0	6.0	6.0	6.3
	TSP 10%	6.7	7.0	6.7	6.3	6.0	6.5
	Cont.	6.3	6.7	6.3	6.3	6.0	6.3
3h	SA 2.5 %	6.7	6.7	6.7	6.3	6.0	6.5
	TSP 2.5%	6.7	6.3	6.7	6.3	6.0	6.4
	TSP 5%	6.3	6.7	6.7	6.3	6.0	6.4
	TSP 10%	6.7	6.0	7.0	6.0	6.0	6.3
	Cont.	7.3	7.3	7.6	6.6	6.3	7.0
4h	SA 2.5 %	6.7	6.0	6.7	6.3	6.0	6.3
	TSP 2.5%	6.3	6.3	6.3	6.3	6.0	6.2
	TSP 5%	6.3	6.7	6.3	6.7	6.0	6.4
	TSP 10%	6.7	6.7	6.7	6.7	6.0	6.6
	Cont.	7.3	7.3	7.3	6.7	6.0	6.9

Table 3: Effect of ice formulation on the pH of stored fish

Ices	Initial pH	Duration of storage				
		pH values	1h	2h	3h	4h
SA 2.5%	6.03	mean	5.7	5.9	6.4	6.1
		P- value	0.002*	0.004*	0.04*	0.001*
TSP2.5%	6.03	mean	6.2	6.1	6.7	6.1
		P- value	0.288	0.021*	0.295	0.001*
TSP 5%	6.03	mean	5.9	6.4	6.2	6.5
		P- value	0.009*	1.000	0.009*	0.021*
TSP 10%	6.03	mean	6.2	6.7	6.6	7.1
		P- value	0.288	0.021*	0.308	0.021*
Cont.	6.03	mean	6.3	6.4	6.8	6.8
		P- value	-	-	-	-

*= significant (p<0.05)

DISCUSSION

From the summarized results given in Table (1) it is evident that 10 % of examined fish samples contained *Listeria spp.* (5 out of 50 samples of *O. niloticus* and *C. gariepinus* fish). The percentages of *Listeria sp.* in each type of examined fish was 16 and 4 % respectively, where 4 and 6% of analyzed fish contained LM and *L. innocua*, respectively. LM was found in a percentage of 4% in each of *O. niloticus* and *C. gariepinus*. While *L. innocua* could be detected in 12% of *O. niloticus* but failed to be recovered from *C. gariepinus*.

The percentage of *Listeria spp.* in examined fish samples in our study is higher than that recorded by Rodas (2006) and Nikolaos (2007) where their findings were (4.5%) and (4.2%) respectively. Our records was lower than that reported by Joanne *et al.* (2004) Panda and Garg (2003) Jallewer *et al.* (2007), Hussein *et al.* (2011) Siavash *et al.* (2011) and Mohamed (2012), where their findings were (23.3%), (20%), (17.1%) (18%), (26.7%), (16.7%), respectively. Some authors confirmed our results, they recorded nearly similar percentage of *Listeria spp.* as Adesiyum (1993) Modaresi *et al.* (2011) and Shole *et al.* (2013), where they recorded the presence of *listeria spp.* in 14.8%, 12.4% and 10.5% of examined raw fish samples, respectively.

The findings outlined in the same Table declared that the incidence of LM in raw fish samples was (4%). This trend was higher than that obtained by Panda and Garg (2003), Nikolaos (2007) and Rahimi *et al.* (2012) which were (1.9%, 1.7% and 0.8%), respectively. Our findings was lower than that reported by Rodas (2006), Jallewer *et al.* (2007), Modaresi *et al.* (2011), Mohamed (2012) and Shole *et al.* (2013), where their records were (22.7%), (10%), (67%), (21%) and (7.3%), respectively. On the other hand, the incidence of this organism was nearly agreed with that results obtained by Siavash

et al. (2011) Joanne *et al.* (2004) and Hussein *et al.* (2011) who recorded 4 %, 3.8% and 3.2 % respectively. Other studies have found that the prevalence of LM in raw fish is quite low, ranging from (0 to 1%) Autio *et al.* (1999) and Johansson *et al.* (1999). Jemmi and Keusch (1994) and Hartemink and Georgesson (1991) stated that in Iceland 56% of fresh fish on sale were contaminated with LM and other *listeria spp.* However many investigators as karunasagar *et al.* (1992), Kamat and Nair (1994) and Papadopoulos *et al.* (2010) could not isolated LM from any of fresh water fish samples. Many results suggest that the absence of LM in tropical fish is due to using inadequate methodology. Reliable and accurate isolation and detection techniques are important in the surveillance of LM, Shole (2013).

L. innocua as presented in Table (1) was recovered from the fish in a percentage of 6% which was higher than that reported by Shole *et al.* (2013) who recorded 0.9% while Panda and Garg (2003) and Siavash *et al.* (2011) recorded higher percentages of *L. innocua* than in our study (17% and 11%), respectively. On the other side the percentage of *L. innocua* was somewhat agreed with the result detected by Hussein *et al.* (2011) and Rahimi *et al.* (2013) which were (8.4% and 5.7%, respectively). Our study showed that *L. innocua* was predominant among *Listeria spp.* and this agreed with the result obtained by Adzitey and Huda (2010) Since both LM and *L. innocua* share the ecological niches, and the isolation of both bacteria is not surprising, where isolation of *L. innocua* in fishery products is considered as an indicator of possible contamination with LM.

The differences in prevalence of *Listeria spp.* may be due to the facts that: type of samples, methods of sampling, isolation techniques, geographical area and even climate of area which samples were collected, Hassan and Shole *et al.* (2013). In addition, contact with intestinal contents is risk factor for prevalence of

Listeria spp. in seafood samples, Ertas and Seker (2005).

Effect of ice formulation on ice stability, *L. monocytogenes* load (in fish and melted water), sensory quality and pH of fish:

Ice stability:

Results from the ice stability in showed that, tap water ice appeared to melt faster than other ices (Figure 1). For example, after 60 min storage time, the volume of water collected from tap water ice was 265 mL whereas 255, 180, 186 and 207 mL were collected from (SA) 2.5% (TSP) 2.5%, (TSP) 5% and (TSP) 10% ices, respectively. However, for most storage times after that, the volume collected from all treatments appeared to be relatively similar, except around the 3-2.5 h storage period. In general, formulated ices are more stable (700 g ice melted completely in 5 h versus 4h to tap water ice).

Efficacy of ice formulations in reduction of *Listeria monocytogenes* load in fish and in water from melted ice:

To evaluate the effectiveness of antimicrobial ices, viable *LM* on agar surface were enumerated following treatments. As shown in Figure 2, the initial *LM* count was 5.5 log₁₀ CUF/g. The initial count was reduced by 1.4, 0.2, 1.4, 1.6 and 0.6 log₁₀ CFU/g for (SA) 2.5%, (TSP) 2.5%, (TSP) 5%, (TSP) 10% and tap water ices at one hour sampling period, respectively. At two hours storage, the treatments were effective in achieving desired log₁₀ -unit reduction except TSP 2.5% and tap water ices. A reduction level of log₁₀-unit using (TSP) 2.5% ice was achieved after 4 h of storage. Whereas (SA) 2.5% ice was more effective at same concentration and storage time.

The rate of reduction was dependent on the concentration for TSP ices. For example, reductions in *LM* cells were 1.2, 2.0 and 2.5 log₁₀ CFU /g for (TSP) 2.5% , TSP 5% and TSP 10% ices at the 4th sampling period, respectively. The results in Figure 2 also shows that (SA) 2.5% and TSP 10% ices were the most effective compared with the control (1.6 log₁₀ CFU/g additional reduction to control) followed by (TSP) 5% (0.6 log₁₀ CFU/g reduction additional to control) at the 4th hour of the storage period. Statistically, there were significant difference (data not shown) in the reductions between the ice treatments prepared from tap water and ices formulated using (SA) 2.5% or (TSP) 10%. Sodium acetate 2.5% or (TSP) 10% ices was efficient in reduction of *LM* in treated fish compared with control ice (P <0.05).

Figure 3 shows the amount of *LM* cells recovered in the waters collected from the ice treatments after 4h storage. The mean *LM* viable cells recovered were 3.7, 3.8, 3.5, 3.0 and 4.0 log₁₀ CFU / ml of melted ice for (SA) 2.5%, (TSP) 2.5%, (TSP) 5%, (TSP) 10% and tap water ices, respectively. Statistically, (TSP)

10% ice was the most efficient in reduction of *LM* population in the water from melted ice (P<0.001) compared with tap water ice while only a significant difference (P<0.05) for other formulated ices compared with tap water ice.

This study, confirmed that formulated ices especially (SA) 2.5% or (TSP) 10% ice can help to overcome the *LM* burden in the water when it used to store the fish. This thus reduced the potential for cross-contamination to contact surfaces and even to the environment of the market.

To evaluate sanitizers some factors should be taken into consideration before making any assumption. For example, animal products (fish and meat) are foods rich in proteins and/or lipids. It has been reported that even low amounts of proteins or fats are capable for reducing the antimicrobial efficacy of sanitizers) Vandekindern *et al.* (2009). In addition, it is documented that temperature also influences the efficacy of the sanitizer where Venkitanarayana *et al.* (1999) found that *LM* was more rapidly inactivated by sanitizers at 35 °C and 45 °C than at 4 °C or 23 °C. *LM* is also tolerant and survives in extreme conditions like a wide PH range (4.1-9.6), Adzitey and Huda (2010). Regarding TSP, Taormina and Beuchat (2013) revealed that *LM* survived at least 6 days when they were suspended in TSP at pH 9.0 and stored at 4 °C for 21 days. Capita *et al.* (2001) found that the rate of *LM* reduction was increased by increasing TSP concentration. The behavior of *LM* was significantly influenced by the origin of the strain and salt concentration in their experiment .

There is lake of researched papers about using of SA or TSP in ice formulations but the potential of using their solutions to reduce bacterial populations in fresh fishery products was explored. Kim and Marshall (2002) recorded a reduction of 0.6 to less than 1.8 log₁₀ CUF of *LM* on fish skin when treated for 10 min using TSP 2-6%. Whereas, Mu *et al.* (1997) mentioned that 10 or 20% solution treatments of TSP didn't significantly reduce *Listeria* populations in fish fillets after 6 days of storage.

Sodium acetate is widely available, economical and generally "recognized-as-safe" Sallam (2007 a). The available literatures focused on using its solutions for dipping of fish. Golden (1995) explored that *Listeria* inactivation was directly related to the concentration and incubation temperature and inversely related to pH. Kouassi and Shelf (1996) found that SA was effective antilisterial at concentration of 3% at 37 °C . Sallam (2007 a) noticed that aqueous solution of (SA) 2.5% was efficient against proliferation of various categories of spoilage microorganisms of fish under refrigerated storage.

Effect of ice formulation on sensory quality of treated fish:

The mean values for odor and flavor intensity, juiciness, tenderness, appearance and off odor and

flavor of cooked fish are illustrated in table (2). The analysis of these values showed non-significant difference between treated and control fish (data not shown). All treated fish were judged acceptable by the sensory panel. No off-odor or off-flavor could be detected in any of treated fish. The results regarding sensory evaluation of treated fish are in accordance with the finding of other researchers who treated fish with SA or TSP. Kilinc *et al.* (2009) found that dipping of fish in (TSP) 5% for 10 min did not affect the texture of fish fillets. Vyncke (1978) revealed that dipping of fish in a solution of sodium tripolyphosphate for 5 min improved the appearance of treated fish. Sodium salt of acetic acid has been used to improve sensory quality attributes and extend the shelf life of fish. Sallam (2007 b) noticed no difference in appearance, Juiciness and tenderness in fish dipped in 2.5% aqueous solution of SA compared with control. Manjua *et al.* (2007) found that SA treated fish (2% Sol.) still acceptable during refrigerator storage for 15 day. Kim *et al.* (1995) summarized that fish fillets treated with (SA) 1% had an appearance and odor scores similar to fresh controls up to 3 days of refrigerator storage. We can summarize that treating fresh fish with the studied SA or TSP ice formulations can maintain the sensory quality of the treated fish when stored at room temperature (25 °C).

Effect of ice formulation on pH of treated fish :

Values regarding the effect of ices on the pH of fish muscles during storage at room temperature (25 °C) are shown in table3. The initial pH value of fresh control fish (6.03) was significantly higher ($P < 0.05$) than those treated with (SA) 2.5% or (TSP) 5% ices but not tap water ice at the end of 1st hour of storage on ice. A significant decrease in the pH value of fish treated with (SA) 2.5% ice compared with the control was also observed at the 2nd, 3rd and 4th hour of storage ($P < 0.05$). At the end of storage time (4h) a significance reduction of pH was observed in samples treated with (SA) 2.5% (6.1), (TSP) 2.5% (6.1) and (TSP) 5% (6.5) compared with the control (6.8). Trisodium phosphate 10% ice resulted in a significance ($p < 0.05$) rising of pH of treated fish muscles at the 2nd and 4th sampling periods compared with the control, but still near the acceptable pH range of fish (6.6-6.8) recommended by Ross *et al.* (2000) The reductions of pH of fish muscles after treatment with SA in our experiment are consistent with those of Sallam (2007b) and Kim *et al.* (1995). In respect for TSP, Mu *et al.* (1997) recorded the rising in the pH values of fish fillets treated with TSP (10% solution) compared with the control.

We can summarize that SA ice treated fish had a significantly ($p < 0.05$) low pH values compared with the control along the storage period (4h). The reduction of pH is the main function of acetic acid and it is salts when added to foods. That reduction did not affect sensory attributes of fish as revealed in the

sensory evaluation. The relative high pH values in fish treated with (TSP) 10% ice compared with control may due to high skin pH value which recorded by Mu *et al.* (1997). The relatively high pH value (0.2 more than control) did not reflected in the sensory attribute tested by panels.

CONCLUSION

Chilling to about 0 °C is the most important means of fresh marketing in Egypt. The most common chilling media is ice. The finding during this study confirmed that Bolti (*O. niloticus*) and *C. gariepinus* from Assiut markets were contaminated by listeria (16% and 4%, respectively). Consequently raw fish and water from melting ice in contact can become a source of cross contamination if not dealt with properly. This potential could be significantly ($p < 0.05$) reduced by the use of (TSP) 10% or (SA) 2.5% ice. These ice formulations did not affect any of sensory quality parameters or the overall acceptability of fish compared with control ($p > 0.05$) and can be utilized as safe preservatives for fish under room temperature storage thus improving fish safety and protect public health.

REFERENCES

- Adesiyun, A.A. (1993): Prevalence of *Listeria spp.*, *Campylobacter spp.*, *Salmonella spp.*, *Yersinia spp.* and toxigenic *E. coli* on meat and seafood in Trinidad. Food Microbiol.10: 395-403.
- Adzitey, F. and Huda, N. (2010): *Listeria monocytogenes* in foods. Incidences and possible control .Afri. J. of Microbial. Research 4: 2848-2852.
- Ahmed, A.M. and Abd El-Atti, Nashwa, A. (2012): Existence of *Listeria species* in broiler carcasses with an attempt to control *Listeria monocytogenes* using trisodium phosphate. Afri. J. of Food Sci.4 (2), 046-051.
- Alves, V.F.; De Martinis, E.C.; Destro, M.T.; Vogel, B.F. and Gram, L. (2005): Anti *Listeria* activity of *Carnobacterium piscicola* isolated from Brazilian smoked fish (*surubim pseudoplatystoma spp.*) and its activity against a persistent strain of *Listeria monocytogenes* isolated from surubim. J. Food Prot. 68: 2068-2077.
- Ashie, I.N.A. and Simpson, B.K. (1996): Application of high hydrostatic pressure to control enzyme related fresh sea food texture deterioration. Food Research International. 29 (5-6) 569-575.
- Aygun, O. and Pehlivanlar, S. (2006): *Listeria spp.* in the raw milk and dairy products in Antakya, Turkey, Food Control. 17: 676-679.
- Autio, T.; Hielem, S.; Miettinen, M.; Sjoberg, A.M.; Aarnisalo, K. and Bjork roth, J. (1999): Sources of *L. monocytogenes* contamination in a cold -smoked rainbow trout processing plant detected by pulsed-field gel electrophoresis

- typing. Appl. and Environ. Microbiol. 65, 150-155.
- Aygun, O. and Pehlivanlar, S. (2006): *Listeria spp.* in the raw milk and dairy products in Antakya, Turkey. Food Control. 17: 676-679.
- Ben Embarek, P.K. (1994): Presence, detection and growth of *Listeria monocytogenes* in seafood. A review. Int. J. Food Microbiol 23:17-34.
- Capita, R.; Alonso-Calleja, M.C. and Moreno, B. (2001): Influence of strain and trisodium phosphate concentration on growth parameters of *Listeria monocytogenes* in vitro. Lett. Appl. Microbiol. 32(6), 428-32.
- Chang, K.R.; James, H.O.; Amy, V.P.; Charles, W.H.; Marshall, H. and Douglas, L. (1995): Extending shelf life of refrigerated cat fish fillets using sodium acetate and monopotassium phosphate. J. Food Prot. 6: 597-708.
- Demetrios, K.; Bori, M. and Antonios, M. (1996): Growth of *Listeria monocytogenes* in the whey cheeses, Myzi theria, Anthotyros, and Manouri during storage at 5,12 and 22 C°. Food Prot. 59: 1193-1199.
- De Valk, H.; Jacquet, C.; Goylet, V.; Vaillant, V.; Perra, A.; Smon, F.; Desenclos, J.C. and Martin, P. (2005): Surveillance of *Listeria* infections in Europe. Eurosurveillance 10:251-255.
- Ertas, H.B. and Seker, E. (2005): Isolation of *Listeria monocytogenes* from fish intestines and RAPD analysis, Turkish J. of Vet. and Animal Scie. Vol. 29: 1007-1011.
- FAO (1999): Report of the FAO expert consultation on the trade impact of *Listeria* in fish products. FAO Fisheries Report No 64. FIIU/ESNS/R604, Amherst, MA, United States.
- Farber, J.M. and Peterkin, P.I. (1991): *Listeria monocytogenes*, a foodborne pathogen. Microbiol. Rev. 55: 476-511.
- Feliciano, L.; Lee, J.; Lopes, J. and Pascall, M.A. (2010): Efficacy of sanitized ice in reducing bacterial load on fish fillet and in the water collected from the melted ice. J. Food Sci. 75(4), 231-238.
- Gerner-Smidt, P.; Ethelberg, W.; Schiellerup, P.; Christensen, J.; Engeberg, J.; Fussing, V.; Jensen, A.; Petersen, A.M. and Bruun, B.G. (2005): Invasive listeriosis in Denmark 1994-2003: a review of 299 cases with special emphasis on risk factors for mortality. Clinic. Microbiol. Infect, 11: 618-624.
- Ghomi, M.R.; Nikoo, M.; Heshmatipour, Z.; Amir, J.A.; Ovissipour, M.; Hashemi, F.L.H.; Hasandoost, M. and Jadiddokhani, D. (2011): Effects of sodium acetate and nisin on microbial and chemical changes and fatty acid composition of grass carp *Ctenopharyngodon idella* during refrigeration storage. Food Saf.,Doi:10.1111/j.1745-4565.2010.00281.x.
- Glass, K.A. and Doyle, M.P. (1989): Fate of *Listeria* in processed meat products during refrigerated storage. Appl. Environ. Microbiol. 55: 1565-1569.
- Golden, M.H.; Buchanan, R.L. and Whiting, R.C. (1995): Effect of sodium acetate or sodium phosphate with EDTA and ascorbic acid on the inactivation of *L. monocytogenes* Food Safety Vol.15,1:53-56
- Gram, L. and Huss, H.H. (1996): Microbiological spoilage of fish and fish products. Int. J. Food Microbiol. 33: 121-137.
- Grif, K.; Patscheider, F. and Dierich, M.P. (2003): Incidence of fecal carriage of *Listeria monocytogenes* in three healthy volunteers: a one-year prospective stool survey. Eur. J. Clinic. Microbiol. Infect. Dis. 22: 16-20.
- Gudbjornsdottir, B.; Suiko, M.L.; Gustavsson, P.; Thorkelsson, G.; Salo, S. and Sjoberg, A.M. (2004): The incidence of *L. monocytogenes* in meat, poultry and seafood plants in Nordic Countries. J. Food Microbiol. 21:217-225.
- Hartemink, R. and Georgsson, F. (1991): Incidence of *Listeria species* in sea food and seafood salads. Int. J. of Food Microbiol. 12:189-195.
- Hassan, M. and Shole, Y. (2013): Molecular characterization of *Listeria monocytogenes* isolated from fresh seafood samples in Iran. <http://www.diagnosticpathology.org/content/8/1/149>.
- Howgate, P. (1985): The self-life of fish products. J. Sci. Food Agric. 36 (2), 126-127.
- Huss, H.H. (1995): Quality and quality changes in fresh fish. Food Agriculture Organization (FAO), Fisheries Technical Paper 348. Rome: FAO.
- Hussein, A.; Othman, R.E.E.; Sayed, S.M.A.; Hassanein, R. and Abushahahba, F.N.M. (2011): Occurrence of *Listeria monocytogenes* in poultry, fish and their products as well as its public health hazard on women. Animal hygiene and sustainable livestock production. Proceedings of the XVth International Congress of the International Society for Animal Hygiene, Vienna, Austria, 3-7, Vol.2: 987-992.
- Jacquet, C.; Aubert, S.; El Solh, N. and Rocourt, J. (1992): Use of rRNA gene restriction patterns for the identification of *Listeria species*. Systematic and Appl. Microbiol. 15: 42-46.
- Jallewar, P.K.; Kalorey, D.R.; Kurkure, N.V.; Pande, V.V. and Barbuddhe, S.B. (2007): Genotypic characterization of *Listeria spp.* isolated from freshwater fish. Internat. J. Food Microbiol. 114: 120-123.
- Jammi, T. and Keusch, A. (1994): Occurrence of *L. monocytogenes* in fresh water fish farms and

- fish smoking plants. *Food Microbiol.* 11:309-316.
- Jannat, A.H.; Shabanpoor, B.; Shabani, A. and Sadeghi, A. (2010): Effects of cooking methods on physico-chemical and nutritional properties of Persian sturgeon *Acipenser persicus* fillet. *Int. Aquat. Res.*, 2: 15-23.
- Jay, J.M. (2000): *Modern Food Microbiology*. An Aspen Publication and Aspen publishers, Inc. Gaithersburg, Maryland.
- Joanne, T.; Kendra, K.N.; Ken, G.; Virginia, N.S. and Martin, W. (2004): Tracking of *Listeria monocytogenes* in smoked fish processing plants. *J. of Food Prot.* Vol.67 (2), 328-341.
- Johansson, T.; Rantala, L.; PaLMu, L. and Honkanen-Huzalski, T. (1999): Occurrence and typing of *L. monocytogenes* strains in retail vacuum-packed fish products and in a production plant. *Internat. J. Food Microbiol.* 47:111-119.
- Kamat, A.A. and Nair, P.M. (1994): Incidence of *Listeria species* in India sea foods and meat. *J. Food Safety* 14: 117-130.
- Karunasagar, I.; Segar, K.; Karunasagar, I. and Goebel, W. (1992): Incidence of *Listeria spp.* in tropical seafoods. in: *Listeria*. Abstract No. 155. Eleventh international Symposium on problems of Listeriosis (ISOPOL XI), May 11th -14th, Copenhagen, Denmark.
- Kilinc, B.; Cakli, S.; Candun, A. and Sen, B. (2009): Effects of phosphate dip treatments on chemical, microbiological, color, textural and sensory changes of *Rainbow trout* fillets during refrigerate storage. *Aquatic Food Product Teechnology* (24), 110-119.
- Kim and Marshall (2002): Influence of catfish skin mucus on trisodium phosphate inactivation of attached *Salmonella typhimurium*, *Edwardsiella tarda*, and *Listeria monocytogenes*. *J. Food Prot.* 65(7), 1146-1151.
- Kim, C.R.; James, O.H; Vickery, A.P.; White, C.H. and Marshall, D.L. (1995): Sodium acetate and Bifidobacteria increase shelf life of refrigerated catfish fillet. *Food Sci.* 60 (1), 15-27.
- Kim, J.M.; Huang, T.S.; Marshall, M.R. and Wei, C.I. (1999): Chlorine dioxide treatment of seafood to reduce bacterial loads. *Food Sci.* 64: 1089-1090.
- Kouassi, Y. and Shelf, L.A. (1996): Metabolic activities of *L. monocytogenes* in the presence of sodium propionate, acetate, lactate and citrate. *Appl. Bacteriol.* 81(2), 147-153.
- Modaresi, R.; Mardani, K.; Tukmechi, A. and Ownagh, A. (2011): Prevalence of *Listeria spp.* in fish obtained from Urmia fish markets. *African J. Microbiol.* 5(30), 5398-5401.
- Manjua, S.; Ravishankar, C.N. and Lalithac, K.V. (2007): Effects of sodium acetate dip treatment and vacuum- packaging on chemical, microbiological, textural and sensory changes of (Pearlspot) *Eetroplus saratensis* during chill storage. *Food Chemistry.* 102(1) 27-37.
- Mohamed, G.M. (2012): Comparative study between raw and cooked fish sold in Assiut city on the incidence of some foodborne pathogens. *Assiut Vet. Med., J.* Vol. 58, 133: 99-108.
- Mu, D.; Huang, Y.; Gates, K. and WU, W. (1997): Effect of trisodium phosphate on *L. monocytogenes* attached to *Rainbow trout* and shrimp during refrigerated storage. *Food Safety.* 17(1) 37-46.
- Nikolaos, S.; Amin, A.; Konstantinon, P. and Vasikios, S. (2007): Incidence of *Listeria spp* in fish and environment of markets in Northern Greece. *Food Control* 18: 554-557.
- Nykanen, A.; Weckman, K. and Lapvetalainen, A. (2000): Synergistic inhibition of *Listeria monocytogenes* on cold-smoked *Rainbow trout* by nisin and sodium lactate. *Internat. J. Food Microbiol.* 61: 63-72.
- Pand, A.K. and Garg, S.R. (2003): Prevalence of *Listeria* in foods of animal origin. *Indian J. Anim. Sci.* 73: 967-968.
- Papadopoulos, bTh.; bAbraham, A.; Sergelidis, D.; Kirkoudis, I. and Bitchava, K. (2010): Prevalence of *Listeria spp.* in freshwater fish (*Oncorhynchus mykiss* and *Carassius gibelio*) and the environment of fish markets in Northern Greece. *J. Hellenic Vet. Med. Soc.* 61(1), 15-22.
- Rahimi, E.; Shakerian, A. and Raissy, M. (2012): Prevalence of *Listeria species* in fresh and frozen fish and shrimp in Iran," *Annals of Microbiol.* 62:37-40.
- Rodas, O.R.; Flores, J.F.; Betancourt, J.M.; Quinones, E.I. and Vazquez, C. (2006): Occurrence and antibiotic Sensitivity of *Listeria monocytogenes* strains isolation from oysters, fish, and estuarine water. *Appl. Environ. Microbiol.* 72(11), 7410-7412.
- Ross, T.; Dalgaard, P. and Tienungoon, S. (2000): Predictive modeling of the growth and survival of *Listeria* in fishery products. *Internat. J. Food Microbiol.* 62: 231-245.
- Ryser, E.T.; Marth, E.H. and Doyle, M.P. (1985): Survival of *Listeria monocytogenes* during manufacture and storage of cottage cheese. *J. Food Prot.* 48: 746-750.
- Sallam, K.I. (2007 a): Chemical, sensory and shelf life evaluation of sliced salmon treated with salts of organic acids. *Food Chem.* 101 (2), 592-600.
- Sallam, K.I. (2007b): Antimicrobial and antioxidant effects of sodium acetate, sodium lactate and sodium citrate in refrigerated sliced salmon. *Food Control.* 18(5), 566-575.
- Scott, D.; Fletcher, G.C.; Charles, J.C. and Wang, R.J. (1992): Spoilage changes in the deep-water fish *Smooth oreodory* during storage in

- ice. Internat. J. Food Sci. Technol. 27: 577-587.
- Shole, Y.; Hassan, M.M.D. and Elahe, T.B. (2013): *Listeria monocytogenes* serotypes in fresh fish, shrimp, crab and lobster in Isfahan and Shahrekord, Iran. *Inte. J. of Advan. Biological and Biomedical Research*, Volume 1, Issue 5: 493-504.
- Siavash, M.; Ali, F. and Samhra, E. (2011): Incidence of *Listeria* Species in farmed tropical fish in Khuzestan, Iran. *World J. of Fish and Marine Scie.* 3(3), 206-209.
- SPSS (2007): Sample Power Statistic, SPSS, 12.01 Syntax Reference Guide for SPSS Base. SPSS Inc, 233 South Wacker Drive, Chicago, IL, pp111-119.
- Taormina, P.J. and Beuchat, L.R. (2003): Survival and heat resistance of *Listeria monocytogenes* after exposure to alkali and chlorine. *Appl. Environ. Microbiol.* 79: 24-29.
- Tassou, C.C.; Lambropoulou, K. and Nychas, G.J.E. (2004): Effect of prestorage treatment and storage condition on the survival of *Salmonella enteritidis* PT4 and *Listeria monocytogenes* on fresh marine and freshwater aquaculture fish. *Food Protect.* 67(1), 193-198.
- Vandekinderen, I.; Devlieghere, E.; Van Camp, J.; Kerhaert, B.; Cucu, T.; Ragaert, P.; De Bruyne, J. and De Meulemaer, B. (2009): Effects of food composition on the activation of foodborne microorganisms by chlorine dioxide. *Internat. J. Food Microbiol.* 131: 38-44.
- Venkitanarayanan, K.S.; Ezeike, G.O.; Hung, Y.C. and Doyle, M.P. (1999): Efficacy of electrolyzed oxidizing water for inactivation of *Escherichia coli* O157:H7, *Salmonella enteritidis* and *Listeria monocytogenes*. *Appl. Environ. Microbiol.* 65: 4276.
- Vyncke, W. (1978): Influence of sodium tripolyphosphate and citric acid on the shelf life of thornback ray (*Raja clavata* L.). *Zlebensum Unters* 28, 166(5)284-286.
- Willett, W.C. (2005): Fish balancing health risks and benefits. *Amer. J. Prev. Med.* 29: 320-322.
- Zhu, M. Du. M.; Cordray, J. and Ahn, D.U. (2005): Control of *Listeria monocytogenes* Contamination in Ready-to-Eat Meat Products. *Comprehensive Rev. Food Saf.*, 22: 34-42.

تواجد ميكروبات الليستيريا في أسماك الماء العذب والسيطرة عليها باستخدام الثلج المطهر

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تم تصميم هذه الدراسة لمعرفة مدى تواجد ميكروبات الليستيريا في نوعين من أسماك الماء العذب هما البلطي والقرموط لما لهما من أهمية اقتصادية في أسواق محافظة أسيوط. كما تهدف هذه الدراسة أيضاً إلى إستبيان مدى كفاءة اربعة تركيبات من الثلج المطهر (الثلج المحضر من محلول خلاص الصوديوم بتركيز ٢.٥ % ، الثلوج المحضرة من محاليل ثلاثي فوسفات الصوديوم بتركيزات ٢.٥ % ، ٥ % ، ١٠ %) علي تواجد ميكروب الليستيريا مونوسيتوجينز المحملة اصطناعياً علي اسماك البلطي وكذلك تأثيرها علي تواجد الميكروب في الماء الناتج عن الثلوج المستخدمة مقارنة بثلج الماء النقي (الثلج الضابط للتجربة وكذلك معرفة تأثير هذه الثلوج علي الجودة الحسية ، الاس الهيدروجيني (pH) ومدى ثبات الثلج اثناء معاملة اسماك البلطي بهذه الثلوج عند درجة حرارة الغرفة (٢٥ درجة مئوية). بالتحليل الميكروبيولوجي لخمسين عينة (٢٥ عينة من كل نوع) تم جمعها من اسواق مختلفة بمحافظة أسيوط ان ميكروبات الليستيريا تواجدت في اسماك البلطي بنسبة أكبر عن تلك التي في اسماك القرموط (١٦ % ، ١٤ % علي التوالي). كما تواجد ميكروب الليستيريا مونوسيتوجينز بنسبة ٤ % في كلاً من اسماك البلطي والقرموط بينما تواجد ميكروب الليستيريا انوشي في اسماك البلطي فقط (١٢ %) مما يجعل هذه الاسماك تمثل خطورة علي صحة المستهلك وكذلك علي الاسطح التي تلامس هذه الاسماك. بمعاملة اسماك البلطي المحملة اصطناعياً بميكروب الليستيريا مونوسيتوجينز بالثلوج المختلفة لمدة اربعة ساعات ان لكل من ثلج خلاص الصوديوم ٢.٥ % وكذا ثلج ثلاثي فوسفات الصوديوم ١٠ % الكفاءة في إختزال اعداد ميكروبات الليستيريا مونوسيتوجينز في كل من الاسماك والماء الناتج عن انصهار الثلج المستخدم وكان الفارق معنوياً مقارنة بالثلج الضابط للتجربة ($P < 0.05$) مما يساعد علي تحسين الحالة الصحية لهذه الاسماك وكذلك الحد من تلوث الاسطح الملامسة لهذه الاسماك وبينه تسويق هذه الاسماك بميكروبات الليستيريا وخصوصاً الليستيريا مونوسيتوجينز. كما تبين أن الثلوج المحتوية علي ثلاثي خلاص الصوديوم او ثلاثي فوسفات الصوديوم اكثر ثباتاً وبطيئة الانصهار مقارنة بثلج الماء النقي. وبالتقييم الحسي للاسماك المعاملة بالثلوج المختلفة لمدة اربعة ساعات تبين انه لم ينتج عن المعاملة أي تأثير سلبي علي الجودة الحسية للاسماك ($P > 0.05$) مقارنة بالثلج الضابط للتجربة في حين أظهر ثلج خلاص الصوديوم ٢.٥ % إنخفاضاً معنوياً في الأس الهيدروجيني للاسماك المعاملة به بينما أظهر الثلج المحتوي علي ثلاثي فوسفات الصوديوم ١٠ % إرتفاعاً معنوياً في الأس الهيدروجيني للاسماك المعاملة. أثبتت هذه الدراسة كفاءة الثلج المحتوي علي خلاص الصوديوم ٢.٥ % او الثلج المحتوي علي ثلاثي فوسفات الصوديوم ١٠ % في الحد من تواجد ميكروب الليستيريا مونوسيتوجينز بالاسماك المعاملة وكذلك في الماء الناتج عن انصهار الثلج المستخدم مع عدم تآثر الجودة الحسية للاسماك المعاملة إلا أن الثلج المحتوي علي خلاص الصوديوم ٢.٥ % يفضل علي الثلج المحتوي علي ثلاثي فوسفات الصوديوم ١٠ % لانه عضوي ولما له من تأثير خافض للاس الهيدروجيني للاسماك المعاملة به مما يؤخر فسادها. هذا وقد تم مناقشة خطورة ميكروب الليستيريا مونوسيتوجينز علي صحة المستهلك والطرق المقترحة للحد من تواجد بلاسماك.