Prevalence of *Listeria Monocytogenes* in Salted and Smoked Fish and Its Control by Some Organic Acids and the Heating

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ABSTRACT

A total of 100 samples of both salted (feseakh) and smoked (herring) fish (50 of each) were collected from different shops and markets in Zagazig city, Egypt for examining the incidence and levels of *Listeria monocytogenes*, and studying its control by heating and some organic acids. The obtained results revealed that the incidences of *Listeria monocytogenes* in the salted and smoked fish were 4 (8%) and 2 (4%) respectively out of 50 examined samples from each products. On the other hand, the mean bacterial count per gm. in the positive salted and smoked fish samples were $6.2 \times 10^2 \pm 1.6 \times 10^2$ and $2.3 \times 10^2 \pm 1.1 \times 10^2$ respectively.

Regarding the fitness of the examined salted and smoked fish for the human consumption, all the positive fish for *Listeria monocytogenes* were unfit for the human consumption according to the Egyptian standard.

The obtained results revealed a significant reduction of the mean count of *Listeria monocytogenes* in the examined salted fish meat after exposure to acetic acid 5% for 2 minutes compared with those before treatment. Furthermore, the treatments with acetic acid 5% for 5 minutes and lactic acid 5% for 2 and 5 minutes recorded significant lower bacterial counts compared with those with acetic acid 5% for 2 minutes only. On the other hand, our findings showed a significant reduction of the mean count of *Listeria monocytogenes* after exposure to 55 °C for 30 minutes compared with those before heat treatment. On the other hand, the mean counts of *Listeria monocytogenes* were significantly decreased after exposure to both 55°C for 60 minutes and 70 °C for 30 minutes compared with those with the first heat treatment (55 °C for 30 minutes). Moreover, the organism could not be detected after heat treatment at 70 °C for 60 minutes. The suitable recommendations were suggested upon the results of the present study.

INTRODUCTION

The gram positive bacterium *Listeria monocytogenes* is recognized as a food born pathogen with significance for the food consumers *(1)*. *Listeria monocytogenes* today is a major concern to the food manufactures worldwide due to the high mortality rate of listeriosis in susceptible populations and the resistance of the pathogen to the number of food preservation practices. This microorganism has the ability to grow at refrigerator temperature *(2)* and on dray surfaces *(3)*. Therefore, control of this bacterium is a significant challenge for the food manufacture. The frequent occurrence of *Listeria monocytogenes* in different foods may consitute a potential risk for consumers, particularly for immuno - compromised peoples. In human, the illness may range from mild flu like sickness to severe manifestation. The severe forms presented as meningo encephalitis followed by septic infections and occasionally isolated organ involvement *(4)*. Groups at highest risk are pregnant women, neonates, adults with underlying diseases (cancer, AIDS, diapetes, hepatitis, transplant recipients), the elderly (> 65 years) and other immuno - compromised peoples *(4)*. Death occur at a rate as high as 30% in persons at high risk as exhibited above *(5)*.

In Egypt, the consumption of salted and smoked fish distributed widely since long time. The more common salted fish in Egypt is the feseakh which manufactured by salting the mullet fish since several thousand years. Meanwhile, the smoked fish usually prepared by smoking of the herring fish. Improper salting or smoking of these fish leads to unlimited contamination by pathogenic bacteria. Moreover, unhygienic handling and / or storage causes the same bacterial contamination.

Generally; although the governmental efforts, the salted and smoked fish manufactures sometimes suffered from hygienic problems rather than the most food manufacture activities. Because of the major concern of *Listeria monocytogenes* on the public health, the aim of the present investigation was to determine the prevalence and levels of *Listeria monocytogenes* in salted fish (feseakh) and smoked fish (herring) and study its control by some organic acids and heat treatment.

MATERIAL AND METHODS

Collection of samples

A total of 100 samples of both salted (feseakh) and smoked (herring) fish (50 of each) were collected from different shops and markets in Zagazig city for examining the presence of *Literia monocytogenes*. Each sample was wrapped separately and aseptically in sterile polyethylene bag, then identified and transferred as quickly as possible to the laboratory.

Bacteriological examination

a- Isolation

Twenty-five grams of each sample were homogenized with Listeria enrichment broth in sterile moulinex type blender equipped with metallic flask for 1 min. and incubation at 37°C for 48 hrs. After incubation one loopful was subcultured on Listeria selective medium (Palcom agar) *(6)*.

b- Enumeration

Counting of *L. monocytogenes* was achieved by direct planting of decimal dilutions of prepared samples *(7)*, on to plates of palcam agar. The plates were incubated at 37°C for 24-48 hr. and typical colonies presumed to be *L. monocytogenes* were counted.

c- Identification:

Colonies suspected to be *L. moncytogenes* were identified *(8,9)* and characterized by Gram stain *(10)*, tumbling motility, V.P., catalase, oxidase, haemolysis on horse blood agar and CAMP test, for further confirmation of *L. monocytogenes*, the isolates were inoculated into 10% aquas stock solution of Mannitol, L. Rhamnose and D. xylose *(11)*.

Preparation of bacterium inoculum

A strain of *L. monocytogenes* isolated from the examined fish from our study was used. *L. monocytogenes* strain was subcultured at least twice by loop inoculation of 10-ml volumes of trypticase soy broth, which was incubated at 30°C for 18hr to achieve viable cell population of 10^{10} cfu/ml. An inoculum of *L. monocytogenes* was prepared by diluting 1.2 ml of the suspension with 1200 ml sterile 0.1 (w/v) peptone water to yield 10^7 cfu/ml *(12)*.

Sample inoculation

Eighty negative samples of *L. monocytogenes* resulted from the examined fish samples (40 from each salted and smoked fish) samples in the present study were grounded. Each ground sample was mixed with *L. monocytogenes* at a ratio of 1 ml of the diluted suspension per 100 gm of fish meat sample. The inoculation level for *L. monocytogenes* was 10^7 CFU/gm. Inoculated fish meat samples were kept at 4°C for 30 min to allow bacterial cells attachment to meat *(13)*.

Treatment 1

Forty of the inoculated salted fish samples were dipped in acetic acid 5% and lactic acids 5% for 2 and 5 min. (10 samples for each time in each acid). Then all samples were tested microbiologically for estimation the count of *L. monocytogenes* after organic acid treatment *(12)*.

Treatment 2

Forty of the inoculated smoked fish samples were treated by submersion in thermostatically controlled water bath at 55°C and 70°C for 30 and 60 min (10 samples for each time in each temperature). Samples were removed from heated water bath, cooled immediately in an ice water bath. All samples were tested microbiologically for obtaining the count of *L. monocytogenes* after heat treatment for 30 and 60 minutes *(14)*.

Statistical analysis

Statistical analysis of data was conducted using "Statistic for animal and veterinary science" *(15)*.

RESULTS AND DISCUSSION

The obtained results showed in Table 1 exhibited that the incidences of *Listeria monocytogenes* in the salted and smoked fish were 4 (8%) and 2 (4%) respectively out of 50 examined samples from each products. These incidences were nearly similar with those obtained in a previous study in Greece ***(16)***, in this mentioned study; 3% of positive samples for *Listeria monocytogenes* were detected in the examined Bogue *(Boops boops)* fish. Also, in a previous Egyptian investigation ***(17)***, *L. monocytogenes* was isolated in 5% of salted fish samples (feseakh), while; it was not detected in all the examined smoked fish samples (Herring). Meanwhile, higher incidences of *Listeria monocytogenes* (9.3%) than our figures were estimated in some local fish types in Egypt ***(18)***. Furthermore, higher incidences of *Listeria monocytogenes* than our findings were detected in smoked (17%) and salted fish (50%) in Finland ***(19)***, marine water fish in Turkey (10.4%) ***(20)***, and in catfish fillet in U.S.A. (21.6%) ***(21)***. On the other hand, the mean bacterial count per gm. in the positive salted and smoked fish samples in the current study (Table 1.) were $6.2 \times 10^2 \pm 1.6 \times 10^2$ and $2.3 \times 10^2 \pm 1.1 \times 10^2$ respectively. Lower levels of *Listeria monocytogenes* than those in the present study (< 100 CFU) were estimated in a previous investigation in Belgium ***(22)***. On contrast, higher level (1.37×10^4 per gm as maximum level) of *Listeria monocytogenes* than our findings was estimated in the examined smoked and salted fish in Finland ***(19)***.

Regarding the fitness of the examined salted and smoked fish for the human consumption, all the positive fish samples for *Listeria monocytogenes* were unfit for the human consumption according to the Egyptian Standard ***(23)***, which mentioned that the consumed fish must be free from *Listeria monocytogenes*.

The results achieved in Table 2 revealed significant reduction of the mean count of *Listeria monocytogenes* in the examined salted fish meat after exposure to acetic acid 5% for 2 minutes compared with those before treatment. Furthermore, the treatment with acetic acid 5% for 5 minutes and lactic acid 5% for 2 and 5 minutes recorded significant lower mean bacterial count compared with those with acetic acid 5% for 2 minutes only. These results agreed with those obtained in another studies which examined the effects of acetic or lactic acids on the *Listeria monocytogenes* count ***(18,24)***. Moreover the previous study in U.S.A. recorded that the acetic acid in concentrations 1-3% is potent as antilisterial and causes no adverse effect on the sensory properties of the meat ***(25)***. Although *Listeria monocytogenes* is not remarkably acid tolerance and can not grow at PH below 4.5-4.6, however; due to a stress hardening phenomenon, i.e. increase tolerance after adaptation to stressful environment, the organism may become highly resistant to even extremely acidic condition ***(26)***.

Table 1. The incidence and bacterial count per gm. of *Listeria monocytogenes* in the examined salted and smoked fish.

Type of samples	Sample No.	The incidence of positive samples		Bacterial count per gm.		
		No.	%	Max.	Min.	Mean ±S.E.*
Salted fish	50	4	8	10×10^2	2.2×10^2	$6.2 \times 10^2 \pm 1.6 \times 10^2$
Smoked fish	50	2	4	1.2×10^2	3.5×10^2	$2.3 \times 10^2 \pm 1.1 \times 10^2$

*: In the mean ±S.E. calculation, only the positive samples were estimated.

Table 2. The effect of organic acids treatment on the inoculated *Listeria monocytogenes* (mean ± SE) in salted fish (feseakh) meat (n = 10 for each treatment).

Count Before treatment	Acetic acid 5%		Lactic acid 5%	
	After 2 minutes	After 5 minutes	After 2 minutes	After 5 minutes
10^{7} (a)	1.2×10^{5} $\pm 5.8 \times 10^{4}$ (b)	2.5×10^{2} $\pm 8.4 \times 10^{(c)}$	1.5×10^{3} $\pm 5.9 \times 10^{2(c)}$	1×10^{2} $\pm 2.8 \times 10^{(c)}$

N.B.: Different letters within the same category (before treatment and after treatments by acetic and lactic acid for 2 and 5 minutes) mean significant variations between the values of *Listeria monocytogenes* count ($P \leq 0.01$).

Concerning the effects of heat treatment on the *Listeria monocytogenes* count, Table 3 showed a significant reduction of the mean count of this microorganism after exposure to 55 °C for 30 minutes compared with those before heat treatment. On the other hand, the mean counts of *Listeria monocytogenes* were significantly decreased after exposure to both 55°C for 60 minutes and 70 °C for 30 minutes compared with those the first heat treatment (55 °C for 30 minutes). Moreover, the mentioned microorganism could not be detected after heat treatment at 70 °C for 60 minutes. Similar results of the thermal reduction of *Listeria monocytogenes* in the fish meat were recorded in the previously Egyptian study *(18).*

From aforementioned results we could be concluded that the *Listeria monocytogenes* microorganism recorded low incidences in both salted and smoked fish in the present study. Meanwhile, because the serious hazardous effects of the tested microorganism on the public health, hygienic control of salted and smoked fish manufactures must be followed, the monitoring of salted and smoked fish in the markets must be continue. Moreover, using of organic acids and heat treatment to avoid or reduce the probability of *L. monocytogenes* infection in the salted and smoked fish respectively is recommended.

Table 3. The effect of heat treatment on inoculated *Listeria monocytogenes* (mean ± SE) in smoked fish meat (n = 10 for each treatment).

Count Before treatment	Temperature			
	At 55 °C		At 70 °C	
	After 30 minutes	After 60 minutes	After 30 minutes	After 60 minutes
10^{7} (a)	3.4×10^{5} $\pm 6.9 \times 10^{4}$ (h)	8.4×10^{3} $\pm 3.2 \times 10^{3(c)}$	3.6×10^{3} $\pm 7 \times 10^{2}$ (c)	Not detected

N.B.: Different letters within the same category (before treatment and at 50 and 70 °C for 30 and 60 minutes) mean significant variations between the values of *Listeria monocytogenes* count ($P \leq 0.01$).

CONCLUSION AND RECOMMENDATIONS

1- The nutritional quality and palatability of the examined fish must be always considered. Therefore, if the treatment by some organic acids or heating exhibit adverse effect on the fish quality, it was not recommended.

2- Although the incidence of *L. monocytogenes* in the examined samples is obviously low, the continuous monitoring of salted and smoked fish is recommended.

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الملخص العربي

تواجد ميكروب الليستيريا مونوسيتوجينز في الأسماك المملحة و المدخنة و التحكم فيها ببعض الأحماض العضوية و الحرارة

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تم تجميع مائه عينة من الأسماك المملحة و المدخنة (٥٠ من كل نوع) من الأسواق بمدينة الزقازيق بمحافظة الشرقية و ذلك لاستبيان مدى تواجد ميكروب الليستيريا مونوسيتوجينز بالإضافة لدراسة تأثير المعاملة الحرارية و بعض الأحماض العضوية علي حيوية البكتيريا محل الدراسة، و قد أسفرت الدراسة عن النتائج التالية.

تواجدت الليستيريا مونوسيتوجينز في عدد ٤ (٨%) من عينات الأسماك المملحة بالإضافة لعدد ٢ (٤%) من عينات الأسماك المدخنة، و ذلك بمتوسط عدد يبلغ ٦,٢ ×١٠^{٢} ±١,٦ ×١٠^{٢} ، ٢,٣ ×١٠^{٢} ±١,١ ×١٠^{٢} خلية/ جرام و ذلك في الأسماك المملحة و المدخنة علي التوالي.

من ناحية أخري أثبتت الدراسة التأثير الفعال لحامض الخليك بتركيز ٥% لمدة دقيقتان في التقليل من أعداد ميكروب الليستيريا مونوسيتوجينز في الأسماك المملحة بشكل معنوي مقارنة بما قبل المعالجة، في حين كان تأثير نفس الحامض المذكور و بنفس التركيز لمدة خمس دقائق وكذلك لحمض اللاكتيك ٥% لمدة دقيقتان و لمدة خمس دقائق في تقليل أعداد البكتيريا محل الدراسة أكبر بشكل معنوي من حامض الخليك ٥% لمدة دقيقتان.

أما المعالجة الحرارية للأسماك المدخنة فقد وجد أن تعرض العينات المحقونة بالبكتيريا لدرجة حرارة ٥٥ درجة منوية لمدة ٣٠ دقيقة لها أثر معنوي في تقليل أعداد بكتيريا الليستيريا مونوسيتوجينز مقارنة بما قبل المعالجة، في حين كان أدي تعرض العينات محل الدراسة لدرجة ٥٥ منوية لمدة ٦٠ دقيقة و لدرجة ٧٠ منوية لمدة ٣٠ دقيقة لتقليل أعداد البكتيريا المذكورة بشكل معنوي مقارنة بالمعالجة الحرارية الأولي (٥٥ درجة لمدة ٣٠ دقيقة)، و من ناحية أخري لم تتواجد بكتيريا الليستيريا مونوسيتوجينز عند تعرض العينات لدرجة حرارة ٧٠ درجة منوية لمدة ٦٠ دقيقة. و قد تمت مناقشة النتائج و اقتراح التوصيات الملائمة في ضوء ما توصلت إليه الدراسة.