*Abbassa Int. J. Aqua.* Spiceal Issue 2010
The third scientific Conference, Al Azhar University, Cairo 17 –18 October 2010

# Occurrence of *Listeria Monocytogenes* in Fermented Salted and Smoked Fish and Its Control by Some Organic Acids and Heating

***Neveen H. I. Abo El-Enaen***
*Food Hygiene Dept., Animal Health Research Institute, Zagazig*

## ABSTRACT

A total of 100 samples of both fermented 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 fermented salted and smoked fish were 8% and 4% respectively. On the other hand, the mean bacterial count per gm. in the positive fermented salted and smoked fish samples were 6.2 X $10^2$ ±1.6 X$10^2$ and 2.3 X$10^2$ ±1.1 X$10^2$ CFU/ gm. 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 fermented salted fish flesh 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 mentioned microorganism 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 (Faber and Peterkin, 1991). *Listeria monocytogenes* nowadays 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 had the ability to grow at refrigerator temperature (Walker et al., al., 1990) and on dry surfaces (Wong, 1998). Therefore, control of this bacterium is a significant challenge for the food manufacture. The frequent occurrence of *Listeria monocytogenes* in different foods may pose a potential risk for consumers, particularly for immuno - compromised peoples. In

human, the illness may range from mild flu like sickness to sever manifestation. The sever forms of human listeriosis are presented as meningo encephalitis followed by septic infections and occasionally isolated organ involvement (Mahmood et al., 2003). Groups at highest risk are pregnant women, neonates, adults with underlying diseases (cancer, AIDS, diabetes, hepatitis, transplant recipients), the elderly (> 65 years) and other immuno - compromised peoples (Mahmood et al., 2003). Death occur at a rate as high as 30% in persons at high risk as exhibited above (Demetrios et al., 1996).

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 and fermenting 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 fermented 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 as mentioned above, the aim of the present investigation is to determine the prevalence and levels of *Listeria monocytogenes* in fermented 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 fermented salted *Mygil capito* (feseakh) and smoked *Clupea harengus* (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.

### Bacterial analysis

#### 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) (Van Netten et al., 1989).

b- Enumeration

Counting of *L. monocytogenes* was achieved by direct planting of decimal dilutions of prepared samples (A.P.H.A., 1992), on to plates of palcam agar. The plates were incubated at 37°C for 24 hr. and typical colonies presumed to be *L. monocytogenes* were counted.

c- Identification:

Colonies suspected to be *L. moncytogenes* were identified (Konman,et al.,1996 and Quin et al., 2002) and characterized by Gram stain (Margolles, et al.,2000), 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 and described (Collee and Miles, 1989).

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 $10^7$ cuf/gm (Goncalves et al., 2005).

Sample inoculation

Eighty negative samples of *L. monocytogenes* resulted from the examined fish samples (40 from each fermented salted and smoked fish) were grounded. Each ground sample was mixed with *L. monocytogenes* at a ratio of 1 ml of culture per 100 gm of fish meat sample. The inoculation level for *L. monocytogenes* was about $10^7$ CFU/gm. Inoculated fish flesh samples were kept at 4°C for 30 min to allow bacterial cells attachment to flesh (Murphy et al, 2004).

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 (Goncalves et al., 2005).

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 (Dorsa et al., 1992).

Statistical analysis

Statistical analysis of data was conducted using "Statistic for animal and veterinary science (Petric and Watson 1999).

## RESULTS AND DISCUSSION

Table 1. The incidence and bacterial (CFU count per gm.) of *Listeria monocytogenes* in the examined fermented salted and smoked fishes.

| Type of samples | Sample No. | The incidence of positive samples | | Bacterial count CFU per gm. | | |
|---|---|---|---|---|---|---|
| | | No. | % | Max. | Min. | Mean ±S.E.* |
| Salted fish | 50 | 4 | 8 | $10 X10^2$ | $2.2 X 10^2$ | $6.2 X10^2 \pm 1.6 X10^2$ |
| Smoked fish | 50 | 2 | 4 | $3.5 X10^2$ | $1.2 X10^2$ | $2.3 X10^2 \pm 1.1 X10^2$ |

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

Table 2. The effect of organic acids treatment on the mean count ±S.E. (cfu/ gm.) of the inoculated *Listeria monocytogenes* in fermented salted fish (feseakh) flesh (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) | $1.2 \times 10^5 \pm 5.8 \times 10^4$ (b) | $2.5 \times 10^2 \pm 8.4 \times 10^{(c)}$ | $1.5 \times 10^3 \pm 5.9 \times 10^{2(c)}$ | $1 \times 10^2 \pm 2.8 \times 10^{(c)}$ |

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≤ 0.01).

Table 3. The effect of heat treatment on the mean count ±S.E. (cfu/ gm.) of the inoculated *Listeria monocytogenes* in smoked fish flesh (n = 10 for each treatment).

| Count Before treatment | At 55 °C | | At 70 °C | |
|---|---|---|---|---|
| | After 30 minutes | After 60 minutes | After 30 minutes | After 60 minutes |
| $10^7$ (a) | $3.4 \times 10^5 \pm 6.9 \times 10^4$ (b) | $8.4 \times 10^3 \pm 3.2 \times 10^{3(c)}$ | $3.6 \times 10^3 \pm 7 \times 10^2$ (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≤ 0.01).

The obtained results showed (Table 1) that the incidences of *Listeria monocytogenes* in the salted and smoked fish were 4 (8%) and 2 (4%) respectively These incidences were nearly similar with those obtained by Soultos et al., (2007) which estimated 3% of positive samples for *Listeria monocytogenes* in the examined Bogue (Boops boops) fish samples in Greece. Also, El Daly et al., (2008) recorded 5% of positive salted fish samples (feseakh) for *L. monocytogenes* in Egypt, while; they were not detect the tested microorganism in the smoked fish (Herring). Meanwhile, Hala and Hala (2006) recorded higher incidences of *Listeria monocytogenes* (9.3%) than our findings in some local fish types in Egypt. Furthermore, Johansson et al., (1999), Yucel and Balci (2010) and Chen et al., (2010) detected higher incidences of *Listeria monocytogenes* than our findings in smoked (17%) and salted fish (50%) in Finland, marine water fish in Turkey (10.4%) and in catfish fillet in U.S.A. (21.6%) respectively. On the other hand, the mean bacterial count per gm. in the positive fermented salted and smoked fish samples were 6.2 X $10^2$ ±1.6 X$10^2$ and 2.3 X$10^2$ ±1.1 X$10^2$ respectively. The obtained results were higher than those estimated by Uyttendaele et al., (2009) which detected *Listeria monocytogenes* counts lower than 100 CFU in smoked fish in Belgium. On contrast, Johansson et al., (1999) detected higher levels of the tested microorganism than those in the present study; they estimated 1.37 X$10^4$ (CFU/gm.) as maximum level of *Listeria monocytogenes* per gm. in the examined smoked and salted fish in Finland.

Regarding the fitness of the examined fermented 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 (EOSQC, 2005), which mentioned that the consumed fish must be free from *L. monocytogenes.*

The results illustrated in Table 2 revealed significant reduction of the mean count of *Listeria monocytogenes* in the examined fermented 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 and / or lactic acids on the *Listeria monocytogenes* count *(Hala and Hala, 2006* and *Akbas and Olmez, 2007).* Moreover the previous study in U.S.A. recorded that the acetic acid in concentrations 1-3% was potent as antilisterial and causes no adverse effect on the sensory properties of the meat *(Doyle 1999).* Although *L.*

*monocytogenes* is not remarkably acid tolerance and can not grow at pH below 4.5-4.6, however; due to a phenomenon called stress hardening, i.e. increase tolerance after adaptation to stressful environment, the organism may become highly resistant to even extremely acidic condition *(Koutsoumanis et al.,2003).*

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 *L. 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, *L. monocytogenes* 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 previously recoded in Egypt by *Hala and Hala (2006).*

From aforementioned results we could be concluded that the *L. monocytogenes* microorganism recorded low incidences in both fermented 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 fermented salted and smoked fish manufactures must be followed, the monitoring of fermented 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 fermented salted and smoked fish is recommended.

## REFERENCES

Akbas, M.Y. and H. Olmez, 2007. Inactivation of Escherichia coli and *Listeria monocytogenes* on iceberg lettuce by dip wash treatment with organic acids. Lett. Appl. Microbiol. Vol. 44, No(6), 619-24.

2-A.P.H.A.(American Puplic Health Association) 1992. Compendium of Methods for the Microbial Examination of Foods.3rd ed. American Puplic Health Association,Washington,D.C.,USA.

Chen, B.Y., R. Pyla, T.J. Kim, J.L. Silva and Y.S.Jung. 2010. Prevalence and contamination patterns of Listeria monocytogenes in catfish processing environment and fresh fillets. Food Microbiol.;Vol.27 No(5), 645-52.

4-Collee, J. G. and R.S. Miles, 1989. Tests for identification of bacteria Mackia and McCarteny practical medical microbiology, J.P.Collee, J.P. Duguid, A.G. Fraser

and B.P. Marion(ed.) Vol 11,Bed,Churchill living stone Edin burg, London, PP 141-159.

5- Demetrios, K; M. Bori, and M. Antonios, 1996. Growth of *Listeria monocytogenes* in the whey cheeses, Myzitheria, Anthotyros and Manori during storage at 5, 12 and 22 °C. J. Food Prot. 59: 1193- 1199.

6-Dorsa, W.J.: L.M. Douglas, W. Michael, W. moody; and R.H.Cameron, 1992. low temperature growth and thermal inactivation of *Listeria monocytogenes* in precooked Crawfish tail meat. J. food protect. Vol. 56 No(2), 106-109.

7- Doyle M.E., 1999. Use of organic acids to control Listeria in meat. Food Research Institute, University of Wisconsin – Madison- FRI Briefings. In web site: www.wisc.edu/fri/briefs.htm.

8- EL- Daly, E.; H. El- Kelish,; A. EL- Gaml, and S. Shafik, ,2008. Prevalence of *Listeria* organisms in fresh and processed fish and shell fishes. 9th Vet. Med. Zag. Conference 20-22, August, 2008, Port – Said. PP: 289-300.

E.O.S.Q.C. 2005. 'Egyptian Organization for standardization and Quality Control': The permissible limits for fish. 1-889/2005.

Faber, J.M. and P.L. Peterkin, 1991. *Listeria monocytogenes*, a food borne pathogen. Microbiol. Rev. Vol. 55: 476- 511.

Goncalves, A.C., R.C.C.; R.C.C.Almeida, M.A.O. Alves, and P.F. Almeida, 2005. Quantitative investigation on the effects of chemical treatments in reducing listeria monocytogenes population on chicken breast meat. Food control Vol. 16, 617-622.

Hala, S.I. and F.H. Hala, 2006. Contamination of some local fish with *Listeria monocytogenes* and studying its characterization and control. Assiut Vet. Med. J. Vol. 52 No. (108), 109- 127.

Johansson, T; L Rantala, ; L. Palmu, and T. Honkanen-Buzalski . 1999. Occurrence and typing of Listeria monocytogenes strains in retail vacuum-packed fish products and in a production plant. Int. J. Food Microbiol. Vol. 47 No.(1-2),111-119.

14-Konman,E.W.; S.D.Allen,; W.M. Janda, P.C. Schrecken berger, and W.C. Winn, 1996. Introduction of Diagnostic Microbiology Microbiology 6th ed., Lippincott company, Philadelphia, USA.

15- Koutsoumanis, K.P., P.A. Kendall, , and J.N. Sofos, 2003. Effect of food processing – related stresses on acid tolerance of *Listeria monocytogenes* .Applied and environmental Microbiology, Dec. p. 7514-7516.

16- Mahmood, M.S.; A.N. Ahmed, and I. Hussain, 2003. Prevalence of *Listeria monocytogenes* in poultry meat, poultry meat products and other related inanimates at Faisalabad. Pakistan Journal of Nutrition Vol. 2 No.(6),346- 349.

17- Margolles, A.; B.Mayo, and G.Clara, 2000. Phenotypic Characterization of L. monocytogenes and L. Innocua stain c isolated from short-ripened cheese. Food Microbiol. Vol. 17, 461-467.

Murphy, R.Y.; T.Osaili,; L.K. Duncan, and Marcys 2004. Thermal inactivation of Salmonella and *Listeria monocytogenes* in ground chicken thigh/leg meat and skin. Poultry Scince Vol. 83, 1218-1225.

19-Petric A. and P. Watson 1999. Statistics for Veterinary and Animal science. 1st Ed., pp. 90- 99. The Blackwell science Ltd, United Kingdom.

Quin, P.J.; M.Carter, B.K.; Donnelly, W.J.C. and Leonard, F.C.(2002): Veterinary Microbiology and Microbial Diseases, Great Britain by MOG, book ltd, Bodmen, corn wall, U.K.

Soultos, N; A. Abrahim,; K. Papageorgiou, and V. Steris, 2007. Incidence of *Listeria Spp.* in fish and environment of fish markets in northern Greece. Food Control. Vol. 18, 554- 557.

Uyttendaele, M; P Busschaert,; A. Valero, . A.H. Geeraerd, Vermeulen, AL.; K.K.Jacxsens, Goh; A. De Loy; J.F. Van Impe, and F. Devlieghere, 2009. Prevalence and challenge tests of *Listeria monocytogenes* in Belgian produced and retailed mayonnaise-based deli-salads, cooked meat products and smoked fish between 2005 and 2007. International Journal of Food Microbiology. Vol. 133, Issues, 1-2: 94- 104.

Van Netten, P.; B. Van Gaal, and D.A. Mossel, 1989. selection, differentiation and counting of haemolytic listeria species on PALCAM medium. Lett. App. Microbial. Vol. 12, No. (1), 20-22.

Walker, SJ; P. Archer, and J.G. Bank, 1990. Growth of *Listeria monocytogenes* at refrigeration temperature. J. applied Bacteriol. Vol. 68, 157- 162.

Wong, A.C.I. 1998. Biofilm in food processing environment. J. dairy Sci. Vol. 81, 2765-2770.

Yücel N. and S. Balci, 2010. Prevalence of Listeria, Aeromonas, and Vibrio species in fish used for human consumption in Turkey. J. Food Prot., Vol. 73 No. (2),:380-384.

# تواجد ميكروب الليستيريا مونوسيتوجينز في الأسماك المملحة المخللة و المدخنة و التحكم فيها باستخدام بعض الأحماض العضوية و المعاملة الحرارية

**نيفين حسن إسماعيل أبو العينين**

قسم صحة الأغذية- معهد بحوث صحة الحيوان – الزقازيق

## الملخص العربي

تم تجميع مائة عينة من الأسماك المملحة و المدخنة (٥٠ من كل نوع) من أسواق مختلفة 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 ٨% من عينات الأسماك المملحة المخللة بالإضافة لعدد 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 المخللة 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 المعاملة، في حين كان تأثير نفس الحامض المذكور و بنفس التركيز لمدة خمس دقائق بالإضافة إلي أن استخدام حمض اللاكتيك ٥% لمدة دقيقتان و لمدة خمس دقائق في تقليل أعداد البكتيريا محل الدراسة أكبر بشكل معنوي من حامض الخليك ٥% لمدة دقيقتان.

أما المعاملة الحرارية للأسماك المدخنة فقد وجد أن تعرض العينات المحقونة بالبكتيريا لدرجة حرارة ٥٥ درجة مئوية لمدة ٣٠ دقيقة لها أثر معنوي في تقليل أعداد بكتيريا الليستيريا مونوسيتوجينز مقارنة بما قبل المعاملة، في حين أدي تعرض العينات محل الدراسة لدرجة ٥٥ مئوية لمدة ٦٠ دقيقة و لدرجة ٧٠ مئوية 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 مئوية لمدة ٦٠ دقيقة. و قد تمت مناقشة النتائج و اقتراح التوصيات الملائمة في ضوء ما توصلت إليه الدراسة.