

Effect of Trisodium phosphate on halotolerant bacteria in faseikh (salted mugil) sold at Dakahlia Governorate

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Abstract

One hundred and fifty mugil fish were collected as follows 30 salted mugil fish, the other 120 mugil fish were fresh fish randomly purchased from different markets with good keeping quality, divided into four groups each group were 30 fish before salting with the salt mixture. The first and the third group were mixed with the dry salt mixture in addition to 5 gm/kgm fish 0.5% trisodium phosphate (TSP), while the second and fourth group were mixed with the dry salt mixture then placed separately in a small barrels, filled with the dry salt mixture and closed. The first and second groups were kept aerobically at (22±1°C) while the third and fourth group were kept aerobically at 4±1°C. The barrels were rolled every week for the first six weeks of storage to obtain good ripening where the total ripening period were 60 days and examined for counting of total halotolerant bacteria, *Staph. aureus*, Sulphite reducing anaerobic bacteria and isolation of *Clostridium botulinum* and *Vibrio parahaemolyticus*. The results were reduced significantly ($p < 0.05$) by 1.6±0.3, 0.1±0.2, 2.4±0.6 and 1.2±0.4 log₁₀ cfu/g for total halotolerant bacteria, 0.5±0.3, 0.3±0.1, 1.2±0.1 and 0.7 ±0.2 log₁₀cfu/g for *Staph. aureus* 1.1±0.5, 0.7±0.3, 1.4±0.6 and 1.0±0.4 log₁₀cfu/g for sulphite reducing anaerobic bacteria respectively for group1, group2, group3 and group4, while *Clostridium botulinum* and *Vibrio parahaemolyticus* could not be detected. Finally the salted fish must be of good keeping quality with more care during processing and advice for addition of 0.5% trisodium phosphate as food additive to the product.

Key words: Trisodium phosphate, *Clostridium botulinum*, *Vibrio parahaemolyticus*

Introduction

Salt (NaCl) has been used for preservation and as a flavoring agent since ancient times, proper salting essential for processing the salted fish products to reduce moisture content, imparts essential flavors, work as a preservative or inhibitor of microbial growth and prolong the shelf life (Kassem, 1977). Nevertheless, outbreaks of food poisoning diseases due to consumption of the unevascerated salted fish products have been reported over 40 years ago (Heinitz and Johnson., 1998) & Heinitz, et al. (2000) in addition to bacterial halophiles are abundant in environments especially in the salted lakes, saline soils and salted food products (Ventosa et al. 1998).

Trisodium Phosphate (Na₃PO₄) have been approved for use in the United States by Food

and Drug Administration Department as food additives especially in a wide variety of meat products US Department of Agriculture, (1982) and Slavik et al. (1994), because trisodium phosphate kills microorganisms through permeabilizing and disrupting the cytoplasmic and outer membranes of the bacterial cell, due to its alkaline pH, which in turn leads to release of intracellular content and eventual cell death (Sampathkumar et al. 2003), so addition of 0.5% TSP significantly reduce the counts of halotolerant bacteria ($p < 0.05$) by 2.5 and 3.7 logs in the treated fish groups stored at 22 and 4°C respectively, also significant reduction to halotolerant bacteria in control group stored at 4°C by 1.55 log₁₀cfu/g, 0.5% TSP and storage at 4°C reduce completely halotolerant pathogenic bacteria (Ahmed and EL-Kazza,

2005). The mean counts of *Staph. aureus* and sulphite reducing anaerobic bacteria were 2.7 ± 0.4 and $3.29 \pm 0.70 \log_{10} \text{cfu/g}$ with incidence percent of 60 and 56% respectively, while red halophilic bacteria and *Clostridium botulinum* could not be detected in examined fish (**Hassan and Baher, 2011**) while, **Mousa (1986)** concluded that *E. coli* and *Staphylococci* considered as indicator organisms for sanitary quality of food. Pathogenic microorganisms can contaminate fish products during production in fish processing plants (**Hanna et al., 2003**) and Sometimes salted fish prepared under poor hygienic and unsafe condition resulting in human illness (**EL-Sheshnagui, 2006**).

The aim this study was to evaluate the effect of 0.5% trisodium phosphate in controlling the growth of halotolerant bacteria in uneviscerated salted mugil fish.

Materials and Methods

Collection of samples: One hundred and fifty mugil fish weighting $120 \pm 30 \text{g}$ for each were collected as follows 30 salted mugil fish were collected from different markets at Dakahlia Governorate, wrapped in aluminium foils and sent to the laboratory in icebox for examination without delay (control group). The other 120 mugil fish were fresh fish randomly purchased from different markets with good keeping quality, washed and left to dry then divided into four groups, each group were 30 fish before salting with the following salt mixture (150 g sodium chloride + 65 g sugar + 10 g spices contain 10% sodium nitrate) / kgm fish.

The first and the third group were mixed thoroughly with the dry salt mixture in addition to 5gm/kgm fish 0.5% TSP while the second and fourth group were mixed thoroughly with the dry salt mixture then placed separately in a small barrels overnight to settle, then filled with the dry salt mixture and closed. The first and second groups were kept aerobically at room temperature ($22 \pm 1^\circ\text{C}$) as commercial methods while the third and fourth group were kept aerobically at $4 \pm 1^\circ\text{C}$. The barrels were

rolled every week for the first six weeks of storage to obtain good ripening where the total ripening period were 60 days.

Bacteriological examination:

a-preparation of the samples: according to the technique recommended by **APHA (2001)** 10 g each of examined mugil fish muscle samples were excised aseptically and homogenized with 90 ml 0.1% peptone water in a stomacher for 3 minutes at 3000 rpm and filtered through a sterile cheese cloth filter, followed by ten fold six serial dilutions in 0.1% peptone water and examined to determine the following:

Total halotolerant bacteria according to **APHA (2001)**

Isolation of *Staph. aureus* on baird parker agar according to **APHA(2001)**.

Isolation of sulphite reducing anaerobic bacteria on differential reinforced clostridial medium (**Oxoid CM149**).

Isolation of *Clostridium botulinum* according to **Dolman and Murakami (1961)**

Isolation of *Vibrio parahaemolyticus* bacteria on *Thiosulphate Citrate Bile Sucrose* agar (TCBS) (**Oxoid CM333**).

Detection of enterotoxigenic strains of *Staph. aureus*: it was done according to **Donnelly, et al. (1967)**; **Oda, et al. (1979)** and **Shingaki, et al. (1981)** using the SET-RPLA Kit for the detection of *Staphylococcal* enter toxins A, B, C and D.

Results

Table (1). Mean values of detectable bacterial count in salted mugil fish (faseikh) as log₁₀ cfu/g for salted mugil fish and treated groups

Salted fish Bacteria	Control group	Group1	Group2	Group3	Group4
Total halotolerant bacterial count	5.8±1.5	4.2±1.2	5.7±1.3	3.4±0.9	4.6±1.1
Staph. aureus count	2.4±0.8	1.9±0.5	2.1 ±0.7	1.2±0.7	1.7 ±0.6
Sulphite reducing bacterial count	2.5±1.1	1.4±0.6	1.8±0.8	1.1 ±0.5	1.5 ±0.7

Control group =salted mugil fish; Group 1=treated group with 0.5% TSP and kept at (25±10c); Group 2=non treated group with 0.5% TSP and kept at (25±10c); Group 3= treated group with 0.5% TSP and kept at (4±10c); Group 4=non treated group with 0.5% TSP and kept at (4±10c); SE=standard error and No= number.

Table (2). Means of reduction in counts between salted mugil fish (control group) and treated group as log₁₀ cfu/g.

Fish groups	Total halotolerant bacterial count	Staph. aureus count	Sulphite reducing bacterial count
	Reduction mean±SE	Reduction mean±SE	Reduction mean±SE
Group1	1.6±0.3	0.5±0.3	1.1±0.5
Group2	0.1±0.2	0.3±0.1	0.7±0.3
Group3	2.4±0.6	1.2±0.1	1.4±0.6
Group4	1.2±0.4	0.7 ±0.2	1.0±0.4

Table (3). Incidence of detectable bacteria in salted mugil fish (faseikh).

Salted fish in Bacteria	Control group	Group1	Group2	Group3	Group4
Total halotolerant bacterial count	100%	100%	100%	100%	100%
Staph. aureus count	60%	40%	50%	30 %	43.3%
Sulphite reducing bacterial count	53.3%	33.3%	46.7%	26.7%	40%

Table (4). Distribution of enterotoxins produced by some strains of *Staph. aureus* isolated from the examined samples.

Product	No of isolated	No of strains producing		Types of Produced			
	No.	No.	%	A	B	C	D
Control group	3	1	33.3	-	-	-	-
Group 1	-	-	-	-	-	-	-
Group 2	2	1	50	-	1	-	-
Group 3	1	1	100	1	-	-	-
Group 4	1	-	-	-	-	-	-

Discussion

Halophilic bacteria constitute a heterogeneous physiological bacterial group which include spoilage microorganisms as *Bacillus* sp. and *Micrococcus* sp. (Morshdy, *et al.* 1982), in addition to food borne pathogens as *Clostridium botulinum*, *Staph. aureus* and *Vibrio parahaemolyticus* (Abdel-Rahman, *et al.* 1988). The antimicrobial effect of TSP has been examined in several types of food including fish (Kim and Marshall 2002) meanwhile, halotolerant bacteria can grow in salted fish under a wide range of salt concentration, where the initial load of bacteria, hygienic measures during manufacturing and storage temperature are the main factors affecting halotolerant bacterial count of fish (Nassar and Ahmed 1997). The average count of halotolerant bacteria were 5.8 ± 1.5 , 4.2 ± 1.2 , 5.7 ± 1.3 , 3.4 ± 0.9 and 4.6 ± 1.1 log₁₀cfu/g for control group and the tested four groups: group1 (salted with addition of 0.5% TSP and kept at 22°C for 60 days), group2 (salted without addition of 0.5% TSP and kept at 22°C for 60 days), group 3 (salted with addition of 0.5% TSP and kept at 4°C for 60 days) and group 4 (salted without addition of 0.5% TSP and kept at 4°C for 60 days). These results were in accordance with Ahmed and Saad (1999), they recorded the mean total halophilic count of 4.53×10^4 cfu/g while Baze (1995) mentioned that the halophilic count ranged from 10^4 - 10^6 cfu/g in salted fish products meanwhile, Ahmed and EL-Kazzaz (2005) obtained nearly similar results. The results in table (1) were statistically compared between the market group (control group) and the treated groups and we found that addition of 0.5% TSP significantly reduced the halotolerant bacteria ($p < 0.05$) by 1.6 ± 0.3 , 0.1 ± 0.2 , 2.4 ± 0.6 and 1.2 ± 0.4 log₁₀cfu/g as recorded in tables (2) respectively. All groups were positive for halotolerant bacteria table (3), while the results of *Staph. aureus* counts were 2.4 ± 0.8 , 1.9 ± 0.5 , 2.1 ± 0.7 , 1.2 ± 0.7 and 1.7 ± 0.6 log₁₀cfu/g table (1) respectively and addition of 0.5% TSP significantly reduced *Staph. aureus* count ($p < 0.05$) by 0.5 ± 0.3 , 0.3 ± 0.1 , 1.2 ± 0.1 and 0.7 ± 0.2 log₁₀cfu/g for the tested groups

table (2), all groups were positive for *Staph. aureus* with different percentages table (3) these results were nearly similar to Ahmed and EL-Kazzaz (2005) and Hassan and Baher (2011). The results in table (4) showed that the enterotoxigenic strains of *Staph. aureus* were affected by salting and addition of 0.5% TSP. There are limited reports about the effect of TSP on the enterotoxigenic strains of *Staph. aureus* in salted mullet fish. The total counts of sulphite reducing anaerobic bacteria were 2.5 ± 1.1 , 1.4 ± 0.6 , 1.8 ± 0.8 , 1.1 ± 0.5 and 1.5 ± 0.7 log₁₀ cfu/g table (1) respectively and addition of 0.5% TSP significantly reduced the counts ($p < 0.05$) by 1.1 ± 0.5 , 0.7 ± 0.3 , 1.4 ± 0.6 and 1.0 ± 0.4 log₁₀cfu/g table (2) respectively, with incidence percent of 53.3%, 33.3%, 46.7%, 26.7% and 40% table (3) respectively. These results were nearly similar to that reported by Ahmed and EL-Kazzaz (2005) and Hassan and Baher (2011). There were limited reports about the effect of TSP on bacteria in salted fish.

The results showed that addition of 0.5% TSP to fish groups stored at 4°C was significantly reduce ($p < 0.05$) the halotolerant bacterial count, additionally combining of chilling and salting has great inhibitory effect on microbial growth during storage (Kraft 1992) with other factors include raw fish and salt quality. The use of good quality fish and salt during processing with 0.5% TSP could also be interpreted with the obtained results by Huss, (1995) and Korber, *et al.* (2002).

While *Clostridium botulinum* and *Vibrio parahaemolyticus* could not be detected in the examined salted mullet samples which were in agreement with Krieg and Holt (1984); Gram and Huss (1996); Basti, *et al.* (2004) and Hassan and (Baher 2011).

Conclusion

New regulations for salted fish products are required in order to establishing microbiological performance standards for halotolerant bacterial contamination and advice for addition of 0.5% trisodium phosphate as a food additive to the product.

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تأثير ثلاثى فوسفات الصوديوم على البكتيريا المقاومة للتملح فى الفسيخ المباع فى محافظة الدقهلية حاتم فتحى احمد السوقي* ، شيرين سامى مصطفى**

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المخلص العربى

اجريت هذه الدراسة على عدد ١٥٠ سمكة من لسمك البورى (الفسيخ) المملحة تتكون من ٣٠ سمكة من اسماك البورى المملحة والمباعة بمحافظة الدقهلية وعدد ١٢٠ سمكة من اسماك البورى الطازجة ذات جودة عالية حيث تم تقسيمها الى اربع مجموعات ومعالجة المجموعة الاولى والثالثة باضافة ٥ جم ٥٠ ٪ ثلاثى فوسفات الصوديوم لكل كجم سمك مع مخلوط الملح وتمليحها جميعاً اما المجموعتين الثانية والرابعة تم تمليحها بدون ثلاثى فوسفات الصوديوم وحفظ المجموعتين الاولى والثانية عند درجة حرارة ٢٢ °م اما المجموعتين الثالثة والرابعة يتم حفظها عند ٤ °م وبعد ٦٠ يوما من بداية التملح ثم فحص جميع العينات لمعرفة العد الكلى للبكتيريا المقاومة للملحة وعد ميكروب الاستافيلوكوكس اوريس والعد الكلى للبكتيريا المختزلة للكبريت ومعرفة مدى تواجد ميكروب الفيبريوبا راهيموليتكس وميكروب الكلوستريديم بوتوليم وقد اسفرت النتائج عن ان اضافة ٥٠ ٪ ثلاثى فوسفات الصوديوم ادى الى اختزال العد الكلى للبكتيريا المقاومة للملحة بمقدار ١,٦±٠,٣ ، ١,٠±٠,٢ ، ٠,٤±١,٢ و ٠,٢±٠,٤ لوج ١٠ / جم واختزال ميكروب الاستافيلوكوكس اوريس بمقدار ٥,٠±٠,٣ ، ٣,٠±١,١ ، ٢,٠±١,١ و ٠,٧±٠,٢ لوج ١٠ / جم واختزال العد الكلى للبكتيريا المختزلة للكبريت بمقدار ١,١±٠,٥ ، ٧,٠±٠,٣ ، ٤,٠±١,٦ و ٠,٩±١,٠ لوج ١٠ / جم على الترتيب واسفرت النتائج ايضا عن خلو العينات جميعا من ميكروب الفيبريوبا راهيموليتكس وميكروب الكلوستريديم بوتوليم مما سبق يتضح ان اختيار اسماك طازجة تتمتع بالجودة واطافة ٥٠ ٪ ثلاثى فوسفات الصوديوم لها تأثير واضح على الاقلال من الحمل البكتيرى للاسماك المملحة وينصح باضافته الى المواد المستخدمة فى التملح.