Evaluation of Retiled Salted Fish according to Egyptian Standard

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A B S T R A C T

This study was conducted to confirm the bacterial and chemical conditionsof salted fishwith E.O.S, and its hazards on public health. A total of 90 samples of fesiekh , sardine and melloha (30 of each) were collected from different retail markets for bacteriological and chemical examination.The average of APC, Staphylococci ,*S.aureus* counts (cfu/g) ,pH ,sodium choride and histamine contents were $7.81x10^6$ ± 1.62 x 10^6, $1.28x10^5$±$0.19x10^5$,$4.58x10^4$±$0.24x10^4$, 6.39± 0.01^+, 5.45± 0.13 and 18.06 ± 0.99^+in fesiekh, respectively, 9.95 x 10^5± 2.08 x 10^5, $5.43x10^4$±$1.10x10^4$,$1.03x10^4$± $0.17x10^4$,6.24± 0.02,5.96±0.17 and 23.51±1.21 in sardine, respectively and $2.16x10^4$ ± 0.31 x 10^4, $8.92x10^2$±$1.67x10^2$, $6.79x10^2$±$1.35x10^2$,6.58± 0.01, 6.19± 0.22 and 14.79± 0.64 in melloha, respectively. The incidence of enterotoxins (A,B and C) produced by *S. aureus* were higher in fesiekh (13.33%) than sardine (10%) and melloha (3.33%). While , the incidence of isolated *E.coli* was higher in fesiekh(26.67%) than those isolated from sardine (16.67%) and melloha (10%).Also the incidence of *V.parahaemolyticus* in fesiekh(16.67%) was more that in sardine(6.67%) and melloha(6.67%).

Keywords: salted fish, *staph. aureus, E. coli ,vibrio parahaemolyticus* ,histamine content .

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1. INTRODUCTION

Fish acts as a vehicle for many types of microorganisms from its natural aquatic environment, sewage, soil, contaminated harvesting areas, contaminated utensils during handling, processing, distribution and storage (Shwewan, 1971). Regarding the external contamination of fish, it may be actively infected with human pathogens by exposure to contamination of water and may constitute a public health hazard (Janssen and Meyers, 1968).Faseikh a traditional Egyptian salted fish, has been considered as a popular part of the Egyptian diet especially in certain celebration times as spring day.The handling of fish products during the manufacturing process involves a risk of contamination by *S.aureus*, causing foodborne human intoxication (Ash, 1997). These bacteria are salt-tolerant and therefore can contaminate all cured preparations such as cold smoked fish and caviar (Shena and Sanjecv, 2007). *Vibrio* infection results in one of three clinical syndromes: gastroenteritis, wound infections and/or primary septicaemia (Hady and Klontz, 1996). The flesh of the fish become toxic because of bacterial contamination and once histamine is formed; it is carried over all products using contaminated fish (Hobbs, 1983). Moreover, Reilly and Santos (1985) claimed that a high level of histamine indicates poor handling and processing of fish products. They added that delays in the salting of fish resulted in higher histamine content.Histamine is heat stable; therefore cooking does not inactivate its effect (Morrow *et al*., 1991). The aim of this study isevaluation confirmation of retailed salted fish (Feseikh , Sardine and Melloha) with the Egyptian organization for Standardization and Quality Control either bacteriologically or chemically.

2. MATERIALS AND METHODS

2.1. *Collection of Samples*:

90 random samples of salted fish products (30 of each) represented by Fesiekh, Melloha, Salted sardine were collected from different markets in Qualuobia, Gharbia, Giza and Cairo governorates. The samples were transferred with minimum of delay to laboratory in ice box and all samples were subjected to bacteriological and chemical examinations.

2.2.Preparation of Samples:

The collected samples were prepared according to the technique recommended by (lCMSF, 1978) as follows: Ten grams from each sample were homogenized in a sterile polyethylene bag with 90 ml of 0.1% sterile peptone water for one minute using stomacher (Stomacherlab.Blender,400SewardLab., London) to provide a dilution of 10^{-1} The homogenate was then allowed to stand for 15 minutes at room temperature from the original dilution ,one ml was transferred aseptically with sterile pipette into a test tube containing 9 ml of sterile peptone water 0.1% and mixed well to produce a dilution of $10^{-2}$from which further decimal serial dilutions were prepared. The prepared samples were subjected to the following examination:

2.3.Determination of aerobic plate count: According to (APHA, 1992)

Isolation, identification of *S.aureus* and it is enterotoxin**:** According to (ICMSF, 1978), (Cruickshank et al. 1975), (Macfaddin, 1976), (Collins and Lyne, 1984), (Bailey and Scott, 1978), (APHA, 1984), (Lachia et al. 1971) and (Ewalid, 1988). Isolation and identificationof Escherichia coli. (ISO, 2001, Cruickshank et al. 1975, Collins, 1984, MacFaddin, 2000, Cheesbrough, 1985 and Varnam and Evans, 1991)**.** Isolation and identification of *V. parahaemolyticus*: According to (ISO, 2007), (Thacter and Clark, 1978), (Baillary and Scott, 1978 and MacFaddin, 1978). Determination of histamine content by using HPLC: According to (Moret and Conte, 1996). Determination of sodium chloride percentage: According to (AOAC, 2000 and E.O.S, 2007). Determination of pH: According to (AOAC, 2000 and E.O.S, 2006)

3. RESULTS

It is evident from the result recorded in table (1) that APC in the examined samples varied from $5.3x10^4$to $8.9x10^7$ with an average value of $7.81x10^6 \pm 1.62 \times 10^{6++}$ cfu/g ,8.2×10^3 to 6.5×10^6 with an average value of $9.95 \times 10^5 \pm 2.08 \times 10^5$cfu/g and 1.0×10^3 to $1.4 \times10^5$with an average value of $2.16 \times10^4 \pm 0.31 \times10^4$ cfu/g for the examined samples of fesiekh, sardine and melloha ,respectively. There was highly significant difference of APC between the examined fesiekh *(P< 0.01).* Table (2) showed that 53.33% ,36.67% and 26.67% were unaccepted based on their *S. aureus* count /g according to E.O.S (2005) of examined samples of` fesiekh ,sardine and melloha ,respectively. Results achieved in table (3) indicated that *E.coli* was isolated from 26.67%, 16.67%, 10.00% of fesiekh , sardine, melloha ,respectively. It is evident from the results recorded in table (4) that prevalences of unaccepted samples of salted fish based on their contamination with *V.parahaemolyticus were* 16.67%, 6.67%, 6.67% of fesiekh, sardine and melloha, respectively. Moreover, the results in table (5) showed that 40% , 30%, 46.67% of fesiekh, sardine , melloha , respectively, were unaccepted accorrting to E.O.S (2005). The results achieved in table (6) showed that 20%, 16.67%, 3.33% of fesiekh, sardine and melloha, respectively were unaccepted according to E.O.S (2005). Table (7) showed that the prevalence of unaccepted samples according to histamine content were 43.33%, 33.33 % and 20% in examined fesiekh, sardine and melloha, respectively.

Table (1): Statistical analytical results of Aerobic Plate Count/g (APC) in the examined samples of salted fish (n=30).

Salted fish	Min	Max	Mean± S.E**
Fesiekh	5.3×10^4	8.9×10^7	$7.81\times10^6 \pm 1.62\times10^{6\,++}$
Sardine	8.2×10^3	6.5×10^6	$9.95\times10^5 \pm 2.08\times10^5$
Melloha	1.0×10^3	1.4×10^5	$2.16\times10^4 \pm 0.31\times10^4$

++ = High significant differences (*P<0.01*)

Table (2): Acceptability of the examined samples of salted fish based on their *S.aureus* count/g (n=30)

Salted fish	MPC/g*	Unaccepted samples	
		No.	%
Fesiekh	100	16	53.33
Sardine	100	11	36.67
Melloha	100	8	26.67

*MPC: Maximum Permissible Count stipulated by EOS (2005)

Table (3): Acceptability of the examined samples of salted fish based on their contamination with Enteropathogenic *E. coli* (n=30).

Salted fish	EOS (2005)	Unaccepted samples	
		No.	%
Fesiekh	absent	8	26.67
Sardine	absent	5	16.67
Melloha	absent	3	10.00

Table (4): Acceptability of the examined samples of salted fish based on their contamination with *Vibrio parahaemolyticus* (n=30).

Salted fish	EOS (2005)	Unaccepted samples	
		No.	%
Fesiekh	absent	5	16.67
Sardine	absent	2	6.67
Melloha	absent	2	6.67

Table (5): Acceptability of the examined samples of salted fish based on their pH values (n=30).

Salted fish	Allowable pH*	Unaccepted samples	
		No.	%
Fesiekh	6 - 6.5	12	40.00
Sardine	6 - 6.5	9	30.00
Melloha	6 - 6.5	14	46.67

* EOS (2005)

Table (6): Acceptability of the examined samples of salted fish based on their sodium chloride % (n=30).

Salted fish	Permissible limit*	Unaccepted samples	
		No.	%
Fesiekh	Not less than 6%	6	20.00
Sardine	Not less than 6%	5	16.67
Melloha	Not less than 6%	1	3.33

* EOS (2005)

Table (7): Acceptability of the examined samples of salted fish based on their histamine content (n=30).

Salted fish	Permissible limit*	Unaccepted samples	
		No.	%
Fesiekh	Not more than 20 mg%	13	43.33
Sardine	Not more than 20 mg%	10	33.33
Melloha	Not more than 20 mg%	6	20.00

4. DISSCUSION

It is evident from the results recorded in table (1)that the total APC in the examined samples nearly similar to those obtained by Nayel (2007) who revealed that 60% of the examined samples of salted sardine had frequency range 10^5 to 10^6, also he found that 12% of examined samples of (Fesiekh) were 32% at frequency range 10^5 to 10^6. Higher results were reported by Morshdy (1980)who concluded that the total colony counts in salted *Mugilcephalus* (fesiekh) was 41.81 x 10^6/g, while Rashad (1986) recorded that sweat Fesiekh cured with either 10 or 15% salt had high total count (10^7-10^8/g), while Zeidan et al. (1983) found that the total viable count for 20 samples of locally produced salted sardines ranged from 4x10^6 to 80x 10^6/g and El-Shorbagy (2005) stated that the mean colony counts in examined Feseikh samples was 51 x 10^6, finally the mean colony counts in examined salted sardine samples was 15.75 x 10^6. Lowerresults were obtained by El-kewaiey (2001) who revealed that the highest mean value of the total aerobic counts of Fesiekh sample was 1.3 x 10^4. The incidence of high viable counts in salted fish indicates corss contamination from different sources such as fresh fishes, kind of the salt used, human and animal wastes, inadequately cleaned equipment and exposure to unsuitable environmental conditions (ICMSF, 1978). Although, the aerobic plate counts of any food articles are not a sure indicative of their safety for consumption, yet it is of supreme importance in judging the hygienic conditions which food has been produced, handled and stored (Levine, 1987). It is evident from the results recorded in table (2) nearly similar results were obtained by El-Shorbagy (2005) who found that *S. aureus* count in fesiekh samples was 15x 10^3/gm and in sardine samples was 4.25 x 10^3/gm , Also nearly similar results were obtained by Morshdy (1980), Zeidan*et al*. (1983) and Abdel Rahman *et al*. (1988) and lower results were obtained by El-Kewaiey (2001). Actually, *Staphylococcus aureus* is still a major cause of food poisoning due to ingestion of enerotoxins (Stenge, 1990);the ability to produce such enterotoxins in food is more likely when competing microorganisms were absent (Frazier and Westhoff, 1984).Presence of *S.arueus* in food indicates its contamination from the skin, mouth and / or nose of food handlers. Inadequately cleaned equipment may be considered a source of contamination (Thatcher and Clark, 1978). Staphylococci can grow best in salty and low water activity-containing foods in which the competing organisms are in reduced numbers (Vishwanath*et al* 1998). Basti*et al*. (2006) showed that the *S.aureus* was the most important genus identified from heavy-salted fish and was due to the contamination of fish during capture and

subsequent unhygienic handling and processing. The results in table (5) were higher than those recorded by Patir*et al.*,(2006) who found *Escherichia coli* in 3% of the examined samples. *Escherichia coli* was frequently encountered in fish produced under poor condition of sanitation (Surkiewicz*et al.* 1968). Pathogenic strain of *E.coli* causes gastro intestinal illness in healthy humans Ewing (1986). The results in table (4) were higher than those recorded by Baffone *et al.* (2000) who isolated *V.parahaemolyticus* from 5% of the examined marine fish samples. Isolation of *V.parahaemolyticus* from the examined fish samples could be attributed to the fact that *V. parahaemolyticus* is mainly related to sewage pollution in addition to this organism is commonly found in fish and shellfish during the warmer summer months. It is evident from the result of pH recorded in table (5) were nearly similar to those reported by Sedak (1971) and Ahmed (1976). The pH of fish ranged from 6 to 7 depending on the species and the age **(**Bardach and Prise, 1978), and due to the formation of large amount of nitrogenous bases during the fish spoilage, the pH of flesh becomes more alkaline (Zitseve*et al*., 1969**)**. The results of sodium chloride table (6) were lower than those recorded by Salama (1969),Sedik (1971), Ahmed (1976) and Morshdy (1980). Spoilage condition characterized by slime formation occurred in light salt-curing cod (12%) during initial drying period (Dussault, 1953). The results of histamine content recorded in table (7) were higher than those reported by Samaha *et al*. (1997) and Azudine and Sarri (1988). Histamine poisoning incidents have occurred after consumption of fish containing high levels of histamine (Murry, 1982). Information given by the obtained results allowed concluding that salted fishes are contaminated with various types of bacteria; this due to neglected sanitary measures adopted during handling of fish during salting processes and could be attributed to improper sanitation during catching, handling, processing, storage transportation, distribution and fish marketing. Therefore, a concerted effort should be made to maintain sanitary condition in processing, preparation and handlingto decrease the contamination of the fish products to the minimum limits

5. REFERENCES

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مدي تطابق الاسماك المملحة الموجودة في السوق المصري مع المواصفة المصرية

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الملخص العربي

تعتبر الأسماك المملحة كالفسيخ والسردين والملوحة من الآكلات المحببة إلى الشعب المصري حيث أنها تستهلك على نطاق واسع فى العديد من المناسبات لذا أجريت هذه الدراسة لمعرفة مدى تطابق هذه المنتجات للمواصفة القياسية المصرية عن طريق استبيان الحالة الميكروبيولوجية والكيميائية لكل من الفسيخ والسردين والملوحة بواقع 30 عينة من كل منتج لفحصها ميكروبيولوجيا وكيميائياً وقد دلت النتائج على الآتي: متوسط العدد الكلي للميكروبات الهوائية فى عينات الفسيخ والسردين والملوحة هذه 7.81×10^{6} و 9.95×10^{5} و 2.16×10^{4} /جم على التوالي. أما بالنسبة إلى ميكروب العنقود الذهبي فقد وجد متوسط العدد فى عينات الفسيخ والسردين المملح والملوحة 4.58×10^{4} و 1.03×10^{4} و 6.79×10^{2}/جم على التوالي. كما تم عزل السموم الناتجة من ميكروب العنقود الذهبي من الفسيخ والسردين والملوحة بنسب 13.33 % و 10% و 3.33% على التوالي. علماً بأن المواصفة القياسية المصرية للأسماك المملحة تنص على أن ميكروب العنقود الذهبي لا يزيد عن 100 خلية / جرام وعلى أن تكون خالية من سمومها. كما تم عزل ميكروب الاشريشيا كولاي من الفسيخ والسردين والملوحة بنسب 26.67 و 16.67 و 10% على التوالي. وتم أيضا عزل ميكروب فيربوباراهيموليتكس من الفسيخ والسردين والملوحة بنسب 16.67 % 6.67% و 6.67 % على التوالي علماً بأن المواصفة القياسية المصرية للأسماك المملحة تنص على انها تكون خالية من ميكروب الاشيرشيا كولى و الفيبريوبارهيموليتكس. أما بالنسبة لنتيجة الفحص الكيميائي فقد تم تقدير قيمة الأس الهيدروجيني في الفسيخ (من 5.64 إلى 7.3 بمتوسط 6.39)و السردين(من 5.51 إلى 7.20 بمتوسط 6.24) والملوحة (من 6.06 إلى 6.92 بتوسط 6.58).علماً بأن الأسس الهيدروجيني فى المواصفة القياسية المصرية (2005) للأسماك المملحة تتراوح من (6 إلى 6.5).كما تم تقدير نسبة الملح فى الفسيخ(من 3.52 % إلى 6.9% بمتوسط 5.45%) والسردين (من 3.7% إلى 7.5 % بمتوسط 5.96%.) والملوحة(من 5.93 % إلى 7.08%بمتوسط 6.19%.)علماً بأن نسبة الملح فى المواصفة القياسية المصرية للأسماك المملحة لا تقل عن 6%. وتم ايضا تقدير نسبة الهيستامين في الفسيخ (من 3.7 إلى 39.5 بمتوسط 18.0)والسردين (من 5.3 إلى 49.1 بمتوسط 23.51)والملوحة (من 2.4 إلى 32.2بمتوسط 14.79).علماً بأن نسبة الهيستامين فى المواصفة القياسية المصرية للأسماك المملحة 20مجم/100جم. وقد تم دراسة ومناقشة الأهمية الصحية للميكروبات المعزولة ومصادر تلوث السماك المملحة التي تم فحصها بالإضافة إلي اقتراح التوصيات اللازمة لجودة هذه المنتجات وسلامتها.

(مجلة بنها للعلوم الطبية البيطرية: عدد 27(2):168-176 , ديسمبر2014)