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 conditions of salted fish with E.O.S, and its hazards on public health. A total of 90 samples of fesiek, 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 chloride and histamine contents were 7.81 ± 1.62 x 10^6, 1.28 x 10^5 ± 0.19 x 10^5, 4.58 x 10^4 ± 0.24 x 10^4, 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, 5.43 x 10^4 ± 1.03 x 10^4, 1.03 x 10^4 ± 0.17 x 10^4, 6.24 ± 0.02, 5.96 ± 0.17 and 23.51 ± 1.21 in sardine, respectively and 2.16 x 10^4 ± 0.31 x 10^4, 8.92 x 10^4 ± 1.67 x 10^2, 6.79 x 10^4 ± 1.35 x 10^5, 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 than that in sardine (6.67%) and melloha (6.67%).

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

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 Sanjeczv, 2007). Vibrio infection results in one of three clinical syndromes: gastroenteritis, wound infections and/or primary septicemia (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 is evaluation 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 (ICMSF, 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)


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\(^{6}\) ± 1.62 x 10\(^{6+}\) cfu/g ,8.2 x10\(^{3}\) to 6.5 x 10\(^{6}\) with an average value of 9.95 x 10\(^{5}\)± 2.08 x 10\(^{5}\)cfu/g and 1.0 x10\(^{3}\) to 1.4 x10\(^{5}\) with an average value of 2.16 x10\(^{4}\)±0.31 x10\(^{4}\) 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 according 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).

<table>
<thead>
<tr>
<th>Salted fish</th>
<th>Min</th>
<th>Max</th>
<th>Mean± S.E**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fesiekh</td>
<td>$5.3 \times 10^4$</td>
<td>$8.9 \times 10^7$</td>
<td>$7.81 \times 10^6 \pm 1.62 \times 10^6$ ++</td>
</tr>
<tr>
<td>Sardine</td>
<td>$8.2 \times 10^3$</td>
<td>$6.5 \times 10^6$</td>
<td>$9.95 \times 10^5 \pm 2.08 \times 10^5$</td>
</tr>
<tr>
<td>Melloha</td>
<td>$1.0 \times 10^3$</td>
<td>$1.4 \times 10^5$</td>
<td>$2.16 \times 10^4 \pm 0.31 \times 10^4$</td>
</tr>
</tbody>
</table>

++ = 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)

<table>
<thead>
<tr>
<th>Salted fish</th>
<th>MPC/g*</th>
<th>Unaccepted samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fesiekh</td>
<td>100</td>
<td>16, 53.33</td>
</tr>
<tr>
<td>Sardine</td>
<td>100</td>
<td>11, 36.67</td>
</tr>
<tr>
<td>Melloha</td>
<td>100</td>
<td>8, 26.67</td>
</tr>
</tbody>
</table>

*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).

<table>
<thead>
<tr>
<th>Salted fish</th>
<th>EOS (2005)</th>
<th>Unaccepted samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fesiekh</td>
<td>absent</td>
<td>8, 26.67</td>
</tr>
<tr>
<td>Sardine</td>
<td>absent</td>
<td>5, 16.67</td>
</tr>
<tr>
<td>Melloha</td>
<td>absent</td>
<td>3, 10.00</td>
</tr>
</tbody>
</table>
Table (4): Acceptability of the examined samples of salted fish based on their contamination with *Vibrio parahaemolyticus* (n=30).

<table>
<thead>
<tr>
<th>Salted fish</th>
<th>EOS (2005)</th>
<th>Unaccepted samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Fesiekh</td>
<td>absent 5</td>
<td>16.67</td>
</tr>
<tr>
<td>Sardine</td>
<td>absent 2</td>
<td>6.67</td>
</tr>
<tr>
<td>Melloha</td>
<td>absent 2</td>
<td>6.67</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Salted fish</th>
<th>Allowable pH*</th>
<th>Unaccepted samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Fesiekh</td>
<td>6 - 6.5 12</td>
<td>40.00</td>
</tr>
<tr>
<td>Sardine</td>
<td>6 - 6.5 9</td>
<td>30.00</td>
</tr>
<tr>
<td>Melloha</td>
<td>6 - 6.5 14</td>
<td>46.67</td>
</tr>
</tbody>
</table>

* EOS (2005)

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

<table>
<thead>
<tr>
<th>Salted fish</th>
<th>Permissible limit*</th>
<th>Unaccepted samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Fesiekh</td>
<td>Not less than 6% 6</td>
<td>20.00</td>
</tr>
<tr>
<td>Sardine</td>
<td>Not less than 6% 5</td>
<td>16.67</td>
</tr>
<tr>
<td>Melloha</td>
<td>Not less than 6% 1</td>
<td>3.33</td>
</tr>
</tbody>
</table>

* EOS (2005)
Table (7): Acceptability of the examined samples of salted fish based on their histamine content (n=30).

<table>
<thead>
<tr>
<th>Salted fish</th>
<th>Permissible limit</th>
<th>Unaccepted samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fesiekh</td>
<td>Not more than 20 mg%</td>
<td>13 43.33</td>
</tr>
<tr>
<td>Sardine</td>
<td>Not more than 20 mg%</td>
<td>10 33.33</td>
</tr>
<tr>
<td>Melloha</td>
<td>Not more than 20 mg%</td>
<td>6 20.00</td>
</tr>
</tbody>
</table>

4. DISCUSSION

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 *Mugil cephalus* (fesiekh) was $4.81 \times 10^6$/g, while Rashad (1986) recorded that sweat Fesiekh cured with either 10 or 15% salt had high total count ($10^5$-$10^6$/g), while Zeidan et al. (1983) found that the total viable count for 20 samples of locally produced salted sardines ranged from $4 \times 10^6$ to $80 \times 10^6$/g and El-Shorbagy (2005) stated that the mean colony counts in examined Feseikh samples was $51 \times 10^6$, finally the mean colony counts in examined salted sardine samples was $15.75 \times 10^6$. Lower results were obtained by El-kewaiey (2001) who revealed that the highest mean value of the total aerobic counts of Fesiekh sample was $1.3 \times 10^4$. The incidence of high viable counts in salted fish indicates cross 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 $15 \times 10^3$/gm and in sardine samples was $4.25 \times 10^3$/gm. Also nearly similar results were obtained by Morshdy (1980), Zeidan et al. (1983), 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 enterotoxins (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. aureus* 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). Bastiet 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 handling to decrease the contamination of the fish products to the minimum limits

5. REFERENCES


Evaluation of Retiled Salted Fish according to Egyptian Standard


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مدي تطبيق الأسماك الملمحة الموجودة في السوق المصري مع الموافقة المصرية

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المخصص العربي

يعتبر الأسماك الملمحة كالفسخ والسردين والملحة من الأكلات الحبيبة إلى الشعب المصري حيث أن تساعد على نطاق واسع في العديد من المناسبات لذا أجريت هذه الدراسة لمعرفة مدى تطبيق هذه المنتجات للمواصفة المصرية في الطريقة استبان الحالة البيوكيميائية والكيميائية لكل من الفسخ والسردين والملحة بواقع 30 عينة من كل منتج لفحصها بيوكيميائياً وكمياً وقد دلت النتائج على الآتي: متوسط العدد الكلي للميكروبات الهوانية في عينات الفسخ والسردين والملحة هذه 7.81 × 10⁹ و 9.95 × 10⁵ نجمات/عشرة 4/4 جم على التوالي. أما بالنسبة إلى ميكنوبوندنغ الذهبية فقد وجد متوسط العدد في عينات الفسخ والسردين الملمحة والملحة 4.58 × 10⁴ و 1.03 × 10⁴ و 6.79 × 10² نجمات/عشرة 4/4 جم على التوالي. كما تم عزل السموم الناتجة من ميكنوبوندنغ الذهبية من الفسخ والسردين والملحة بنسب 13.3% و 10% و 3.3% على التوالي. علاوةً على نهاية Calibration الممثيلة للأسماك الملمحة تنص على أن ميكنوبوندنغ الذهبية لا يزيد عن 100 خلية/جم وعلى أن تكون خالية من سمومها. كما تم عزل ميكنوبوندنغ كولي من الفسخ والسردين والملحة بنسب 26.67 و 10% على التوالي. وتم أيضًا عزل ميكنوبوند فابريباراهوميكلس من الفسخ والسردين والملحة بنسب 16.67% و 6.67% على التوالي. علاوةً على نهاية Calibration الممثيلة للأسماك الملمحة تتضمن أنها تكون خالية من ميكنوبوندنغ الكولي والفيبروبوند فابريباراهوميكلس. أما بالنسبة لنتيجة الفحص الكيميائي فقد تم تعديل قيمة الأس الهيدروجيني في الفسخ (من 6.06 إلى 6.92) ببمتوسط 6.39(سردين (من 5.51 إلى 7.20) ببمتوسط 6.24) والملحة (من 6.06 إلى 6.92) ببمتوسط 6.58(عملاً بأن الأس الهيدروجيني في الممواصفة المصرية (2005) للأسماك الملمحة تتراوح من 6 إلى 7.3) كما تم تقييم نسبة الملح في الفسخ (من 3.52% إلى 6.9% ببمتوسط 5.45%) والسردين (من 3.7% إلى 6.5%) والملحة (من 9.56% إلى 7.5% ببمتوسط 8.08%). علاوةً على نسبة الملح في الممواصفة المصرية للمالحة للأسموبلكس الملمحة لا تقل عن 6%. وتم أيضاً تقييم نسبة الهيسانتين في الفسخ (من 3.7 إلى 39.5 بتوصيل 18.0) السردين (من 5.3 إلى 49.1 بتوصيل 23.51) والملحة (من 2.4 إلى 32.2 بتوصيل 14.79). علاوةً بأن نسبة الهيسانتين في الممواصفة المصرية للأسموبلكس الملمحة 20/100 جم. وقد تم دراسة ونتيجة المنتجات الدراسات المتعلقة إجراء هذه الدراسات وسلا سما من إنتاج الأسماك الملمحة في السوق المصري مع الموافقة المصرية.