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Evaluation of Hygienic Status of Some Ready to sale Salted Fishes

Wafaa, M.M. Eissa, Amany F. Zayed and Manal M. Said
Dept. of Food Hygiene, Animal Health Research Institute

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Abstract
A total of 100 random samples of salted fish represented by salted Sardine and Feseikh (50 of each) were collected randomly from different retail fish markets and shops in Alexandria governorate and they subjected to microbiological and chemical examinations. The obtained results indicated that the mean values of total *Coliform* counts (MPN) in the examined samples of salted Sardine and Feseikh were $1\times10^2\pm0.11\times10^2$ and $3.5\times10^2\pm0.46\times10^2$ (MPN/g), respectively. The average of total *Staphylococcus aureus* count were $0.67\times10^3\pm0.37\times10^3$ for salted Sardine and $2.6\times10^3\pm0.39\times10^3$ (cfu /g)for Feseikh samples. The results revealed that all samples were free from *Clotsridium perfringens*. Our results recorded the ratio of *Vibrio parahaemolyticus* in the examined samples of salted Sardine and Feseikh were30% and 40%,respectively results were $8.7\times10^2\pm1.3\times10^2$ for salted Sardine and $8.8\times10^3\pm1.2\times10^3$ for feseikh. Chemical examination revealed that sodium chloride percent were ranged from 6.2% to 7% for salted Sardine,6.5% to 7% for Feseikh samples. The mean value of Histamine level in the examined samples of salted Sardine and Feseikh were 18.1 ±0.43and 18.6±0.43 (mg/100g) ,respectively.

Key words: *Evaluation, Salted Fishes, Hygienic Status.*

Introduction

Salted fishes constitute important part of the diet of great portion of consumers and have subjected to many risks of contamination from various sources. **(Hobbs *et al.,* 1982).**

The salting process of fish is one of oldest methods in preservation of fish, and this process has still been used in many places in the world. The effect of salt are obstructed growth of the microorganism or they destroyed, and in this way the fish meat gets durability. The preservation period of product is linked to the amount of added salt to it. Between the amount salt used and preservation period are present straight proportion (**Filsinger, 1987).** That method is simpler and cheap for preservation of fish, in this way a source of protein is protected**.** The salt penetration will depend on fat content, temperature, amount of salt, salt composition, etc**. (Mol and Özden, 2004; Codex Alimentarius, 2009).** Therefore, risk and monitoring procedures for different health hazards depend on these conditions.

Despite the preserving aim of such traditional methods, salted fish are still under risk of several hazards due to following reasons: (i) these products have long maturation time, (ii) they are generally consumed without further cooking, (iii) changes in the original methodologies over the years such as decreasing salt content. Small and medium sized fish processing companies are usually suffering from preparing efficient Hazard Analysis Critical Control Points (HACCP) plan to prevent such hazards **(Köse *et al.,* 2010).**

Fesiekh is a semi-putrid form of salted and dried Grey Mullet species (*Mugilspp.)*, a saltwater fish that lives in both the

Mediterranean and the Red Seas. The traditional process of preparing it is to dry the fish in the sun before being preserved in salt. It has a distinctive stench to it that only its true lovers would appreciate. Fesiekh is traditionally eaten during Sham El Ne-seem ,which is a spring celebration from ancient times in Egypt **(Rakha, 1992).**

There are two types of Fesiekh on the Egyptian market, the first type having a low salt content and being suitable for consumption after 15–20 days of maturing, whilst the second has a high salt content and can be eaten after 2–3 months of storage. From the nutritional point of view, Fesiekh is a rich source of high quality protein, essential amino acids, vitamins, and minerals **(Rabie *et al.*, 2009).**

Since **1991, WHO/FAO** recorded the first documented outbreak of food poisoning in Egypt due to consumption of uneviscerated salted fish(Fesiekh) so pressure on the government, industry and the scientific community to seek new safe alternatives to control the microbial growth in uneviscerated salted fish**(Ahmed and Elkazzaz 2005).**

Pathogenic microorganisms can contaminate fish production in fish processing factors **(Hanna *et al.*, 2003).** Staphylocccus poisoning, caused by the ingestion of one or more preformed *Staphylocccus aureus* which give staphylococcal enterotoxins in food which is one of the most prevalent causes of gastroenteritis food poisoning in humans through the world **(Jablonski and Bohach, 1997) and (Hu *et al.*, 2007)**

Consumption of sea food containing spores of anaerobic bacteria will facilitate growth in the intestine and released toxins causing illness in human accompanied by high mortality rate **(Adams and Moss, 2008; Gracey *et al.*, 1999 and FDA 2001).**

Clostridium perfringens is able to grow at concentration of 3-5% salt and can gain access during processing or food service operations **(ICMSF, 1986).**

Vibrio parahaemolyticus is a halophilic microorganism widely distributed in the marine environments.The pathogens grow well in temperature abused raw fish include *Vibrio parahaemolyticus*. Some other pathogens can contaminate through mishandling of fish. Such contamination can be eliminated by GHP and GMP starting from harvesting to consumer **(Sevim, 2010).**

Histamine as a biogenic amine are formed through the decarboxylation of specific free amino acids by exogenous decarboxylases release from microbial population associated with the seafood **(Rawles *et al.*, 1996; Lehane and Olley, 2000; Kim *et al.*, 2009).** Thus, mainly histamine has been proposed as a marker to evaluat fish freshness and serve as chemical indicator of fish spoilage **(Paleologos *et al.*, 2004**). High levels of histamine in food have important vasoactive effects in human **(Lehane and Olley, 2000**). The FDA established a guideline for histamine of 50mg/ kg- and fish with histamine above that level are prohibited from being sold for human consumption (**FDA, 2001)**

Finally,salted fish have not been produced under hygienic conditions or their preservation conditions were poor and considered as a risk foodstuff group. We must renew efforts for improving the quality of these products.

Materials and Methods

I-Collection of samples.

100 random samples of salted fish represented by salted Sardien and Fesiekh (50 of each) were collected from different retail markets in Alexandria governorate. Samples were obtained as they sold in sterile poly ethylene bags and transported immediately to the labo-ratory in an ice box, all samples for detection of bacteria contaminating such samples.

II-Bacteriological examination.

Preperation of samples (A.P.H.A., 1992).

Accurately, 25 gram of each samples were ho-mogenized in 225 ml of sterile peptone water under complete aseptic conditions. Further, decimal serial dilutions were prepared.

1) *Coliform* count (FDA, 2002).

Most probable number (MPN) the three tube method of MacConkey broth was used. The tubes showing acid and gas production were

considered positive. The MPN was estimated using table.

2) *Staphylococcus aureus* count and isolation.

Bairdparker agar with tellurie was used for counting *Staphylococcus* spp. (37±1°C 36- 48 h), the numbers of *Staphylococcus aureus* was determined. **(FDA, 2002)**

3) *Clostridium perfringens* count.

Clostridium perfringens was carried out according to the technique adopted by **(ISO 2004)** using cooked meat broth tubes incubated anaerobically at 37°C for 24 hours followed by subculturing onto tryptose sulfite cycloserine agar plates which were incubated in anaerobic jar at 37°C for 24 hours. *Clostridium perfringens* was black colonies.

4) *Vibrio parahaemolyticus* count (A.P.H.A., 1992).

The prepared homogenized samples were incubated at 35 ºC ± 2 ºC overnight. A loopul from incubated homogenate was streaked onto thiosulfate citrate bile salt sucrose agar (TCBS) and incubated overnight at 35 ºC ± 2 ºC. Rounded, green or bluish colonies 2-3 mm in diameter were recorded as a typical suspected colonies of *Vibrio Parahaemolyticus.*

III-Chemical examination.

1)- Determination of sodium chloride% in the samples were calculated according to method of **(AOAC**, **2003).**

2)- Determination of Histamine level in the samples were calculated according to Method **(Beljaars, *et al.*, 1998).**

Results

Table 1. Statistical analytical results of *Coliform* counts (MPN/g) in the examined samples of salted Sardine and Feseikh.

Samples	No. of samples	Positive samples		Total Coliform count (MPN/g)		
		No.	%	Min.	Max.	Mean± S.E.
Salted Sardine	50	20	40%	0.21×10^2	2.9×10^2	$1\times10^2\pm0.11\times10^2$
Feseikh	50	23	46%	0.93×10^2	1.1×10^3	$3.5\times10^2\pm0.46\times10^2$

S.E. = Standared error .

Table 2. Statistical analytical results of *Staphylococcus aureus* counts (cfu /g) in the examined samples of salted Sardine and Feseikh.

Samples	No. of samples	Positive samples		*Staphylococcus aureus count (cfu /g)*		
		No.	%	Min.	Max.	Mean± S.E.
Salted Sardine	50	40	80%	0.9×10^2	1.5×10^3	$0.67\times10^3\pm0.37\times10^3$
Feseikh	50	38	78%	2×10^2	7×10^3	$2.6\times10^3\pm0.39\times10^3$

Table 3. Statistical analytical results of *Vibrio parahaemolyticus* count (cfu /g) in examined salted Sardine and Feseikh samples.

Samples	No. of samples	Positive samples		*Vibrio parahaemolyticus count(cfu /g)*		
		No.	%	Min.	Max.	Mean± S.E.
Salted Sardine	50	15	30%	$4.2x10^2$	$2.5x10^3$	$8.7x10^2 \pm 1.3x10^2$
Feseikh	50	20	40%	$1.3x10^3$	$2.1x10^4$	$8.8x10^3 \pm 1.2x10^3$

Table 4. Statistical analytical results of *Sodium chlorid %* in the examined samples of salted Sardine and Fe seikh.

Samples	No. of samples	*Sodium chlorid %*		
		Min.	Max.	Mean± S.E.
Salted Sardine	50	6.2	7	6.5±0.15
Feseikh	50	6.5	7	6.7±0.15

Table 5. Statistical analytical results of *Histamine* level in the examined samples of salted Sardine and Fe seikh.

Samples	No. of samples	*Histamine level (mg/100g)*		
		Min.	Max.	Mean
Salted Sardine	50	17	18.5	18.1±0.43
Feseikh	50	18	19.2	18.6±0.43

Discussion

Fish receive now a great attention in term of their recognized zoonotic role. Pathogenic microorganisms can contaminate fish products during production in fish processing factories **(Hanna *et al.*, 2003)** .

Results achieved in table (1) indicated the average of total *Coliform* counts (MPN*)* in the examined samples of salted Sardine and Feseikh were$1\times10^2\pm0.11\times10^2$ and $3.5\times10^2\pm0.46\times10^2$ (MPN/g), respectively.

The test of *Coliform*s considered of much great value in assessing hygienic quality of food (**Cruickshank *et al.*, 1975)**. *Coliform* group are referred as general indicator organisms to measure the potential presence of enteric pathogens in food, also measure the fecal contamination of the food **(El-Tabiy, 2008).**

National Academy of Sciences (1985) stated that fish is subjected to many risks of contamination from different sources during fishing and marketing till reaching to consumers. The chief sources of fish contamination are water, soil, sewage, working hands, and equipment. Such contamination may render the product unfit for human consumption resulting in economic losses or public health hazard to consumer. The pathogen can be introduced into the process through raw materials, handling and unsanitary procedures and equipment, thus the product′s safety needs to assured. Sea foods are susceptible to all common food poisoning organisms **(Nickelson and Finne, 1992)** .

On the other side, the mean values of total *Staphylococcus aureus* counts in the examined samples of salted Sardine and Feseikh were shown in table (2) $0.67\times10^3\pm 0.37\times10^3$ and $2.6\times10^3\pm0.39\times10^3$,respectively.

The natural reservoir for *Staphylococcus aureus* is human skin, hair and superficial mucous membranes (nose), while it is not a part of the normal flora on fish and fish products **(Jay, 1992).** Therefore, small numbers are to be expected in products handeled by human **(Jablonski and Bohach, 1997)**

It is difficult to control growth of *S. aureus* at temperatures over 7°C, especially at ambient temperatures **(Huss *et al.*, 2003).** Fish were responsible for 5.7% of reported *Staphylococcus aureus* outbreaks **(Moussa, 1986)**

The growth of *S.aureus* in food products is a potential public health hazard since many strains, in favorable growth conditions produce thermostable enterotoxins which cause intoxication food poisoning if ingested as well as neurotoxins which act on vomiting centers in the brain via the vagus nerve **(Adams and Moss, 2008).**

However, *S.aureus* was the leading cause of foodborne intoxication, and the minimum infection dose was 10^5-10^7/g or 1-20µg enterotoxin /person **(Luca *et al.*, 1997)**.

Bashir and Agab (1987) recorded that the bacterial isolates in fesiekh were *Staphylococci and Clostridia*. The *staphylococcal* count was ranged from 10^4-10^6 **/g.** which was attributed to mishandling during processing.

The handling of fish products during the manufacturing process involves a risk of contamination with *Staphylococcus aureus* .These bacteria are salt-tolerant and therefore can contaminate fish **(Vernam and Evans, 1991).**

Egyptian Organization for standardization and Quality Control (E.O.S.Q.C.) (1651/2005) recorded that salted fish must be free from the pathogenic bacteria and their toxins. Also, the counts of *S.aureus* must be $\leq 10^2$ cfu/gm. On the other side, our current results indicated that all samples under observation were free from *Clostridium perfringens*. Similar results were obtained by **El-Tahan *et al.*, (1998)**. While, **Kassem (1996)**stated that *Clostridium* spp. could be isolated from feisekh.

C. perfringens bacteria are the third most common cause of foodborne illness **Warrell *et al.*, (2003).** *C. perfringens* need presence of high amount of organic pollutions of human and animal origin and strict anaerobic condition to grow and become pathogenic**(Marzouk *et al.*, 2005).**

Table (3) recorded the ratio of *Vibrio parahaemolyticus* in the examined samples of salted Sardine and Feseikh were 30% and

40%,respectively. $8.7\times10^2\pm1.3\times10^2$ for salted Sardine and $8.8\times10^3\pm1.2\times10^3$ for feseikh.

V. parahaemolyticus is found on seafoods, and requires the salt environment of sea water for growth. *V. parahaemolyticus* is very sensitive to cold and heat. Proper storage of perishable seafoods below 40 degrees F, and subsequent cooking and holding above 140 degrees F, will destroy all the *V. parahaemolyticus* on seafoods. food poisoning caused by this bacterium is a result of insufficient cooking and/or contamination of the cooked product by a raw product, followed by improper storage temperature **(Wagner, 2011)**.vibrios are able to respond adverse environmental conditions by entering a viable , but non-culturable phase .

Egyptian Organization for standardization and Quality Control (E.O.S.Q.C.) (1651/2005) recorded that salted fish must be free from *Vibrio parahaemolyticus.*

Results recorded in Table (4) showed that the sodium chloride percent were ranged from 6.2% to 7% for salted Sardine,6.5% to 7% for Feseikh in examined samples.

The Minimum Permissible Limit of salt % in salted fish is (6%) stipulated by **"E.O.S.Q.C." (1651/2005).**

There are three types of salting of fish: dry salting, wet salting and a combination of the two methods Length of salting period as well as salt concentration depends on the expected final product **(Barat *et al.,* 2002**). Dry salting is more rapid and gives a higher salt content after the same salting period. However, this is inverted during drying and brined sardines have a lower water content at the end of the drying process. both salting methods give dried sardines of acceptable shelf- life since their water activity are low. However, even though microbial quality was similar for all samples, sensory assessment and acceptability appear to be higher for 21% brined sardines **(Bellagha *et al.,* 2002).**

The present results recorded in Table (5) revealed that the mean value of Histamine level in the examined samples of salted Sardine and Feseikh were 18.1**±0.43** and 18.6**±0.43** (mg/100g), respectively

"E.O.S.Q.C." (1651/2005) state that the maximum permissible limit of Histamine in salted fish is 20mg/100g from the final product.

Development of biogenic amines in salted fish occurs at maturation stage during salting or fermenting, poor handling of the raw materials and improper storage conditions **(Sevim, 2010)**. Histamine was found at a high level (521 mg/kg) in Feseikh **(Mohamed *et al.,* 2009) maximum tolerance level (200 mg/kg).** In most cases, histamine levels in illness-causing fish have been above 200 ppm, often above 500 ppm. A hazardous level of histamine for human health has been suggested as 500 mg/kg although low levels as 50 mg/kg (50 ppm) have been reported in histamine poisoning **(FDA, 2001; Huss *et al.,* 2003; Lehane and Olley, 2000). Shalaby (1996)** suggested the following guideline levels for histamine content of fish as regards to health hazard as; (i) <5 mg/100 g (safe for consumption), (ii) 5-20 mg/100 g (possibly toxic), (iii) 20-100 mg/100 g (probably toxic), (iv)>100 mg/100 g (toxic and unsafe for human consumption).

Conclusion and suggested measures.

1- Egyptian salted-fermented fish (Feseikh) can be consumed without any health risks between 20 and 40 days, but it could be hazardous after 60 days, due to the increased biogenic amines content of Feseikh during ripening and storage**.** While the (Feseikh) must not be consumed before 30 days from salting process on other hand salted Sardien must not be consumed before 3 weeks from salting .

2-Addition of 0.5% of Trisodium phosphate as a preservative agent to salted fish control the growth of halotolerant bacteria and increase the shelf-life

3-Periodical medical examination of persons sharing in handling, transporting and salting of fishes.

4-In case of salting of fish, raw fish with better physical condition, intact skin and belly should be used.

5- The usage of salts of poor quality must be avoided and the fish must be allowed to take suitable time for salting.

6-Utensils and equipments used in processing

should be cleaned and sterilized.

7- The growth of microorganisms must be prevented by manipulation and control of one or more of several factors such as storage temperature, pH and preservatives.

8- Storage food should be at certain time and temperature that prevent changing of non- ineffective number of organisms into Clinical doses.

9- Minimizing contamination of raw material and avoid recontamination of final suitable amount of salt to produce efficiently salted products

10-Food handlers must have adequate knowledge of proper food ,handling producers, preparation and storage.

11-Licences should only be given to well hygienic fish.

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