

Health Risk Assessment of Smoked Herring

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Abstract:

A total of 100 random samples of herring fish were randomly collected from different supermarkets and shops in Zagazig and Menia El-kamh city, Sharkia Governorate, Egypt (50 of each). The collected samples were transferred in an insulated ice box under complete aseptic conditions, then directly transferred to the Food Control Laboratory, Faculty of Veterinary Medicine, Zagazig University without delay. All collected samples were subjected to bacteriological and chemical examinations. The mean count of coliform was 3.9 ± 0.129 and $3.3 \pm 0.082 \log_{10}$ cfu/g; Staphylococci count 7.3 ± 0.169 and $7.2 \pm 0.113 \log_{10}$ cfu/g and *S. aureus* was 6.6 ± 0.335 and $6 \pm 0.116 \log_{10}$ cfu/g in the examined smoked herring samples obtained from Zagazig and Menia El-kamh city, respectively. The mean content of histamine (mg %) was 11.08 ± 3.799 and 10 ± 3.827 , the moisture content was 58.6 ± 0.875 % and 58.1 ± 0.581 %, respectively and NaCl concentration was 7 ± 0.714 % and 6.9 ± 0.483 % in the examined smoked herring samples, respectively.

Key words: Smoked herring–Coliform–Staphylococci–Histamine.

Introduction:

Fish is a good source of proteins which are an excellent source of amino acids and mineral elements such as: zinc, phosphorus, iron and calcium. Fish is also a good source of riboflavin, vitamin A and D. So, fish is considered as a rich source of essential nutrients required for supplementing both infant and adult diets. In Egypt, the demand for fish consumption is increasing due to health benefits of eating fish primarily and secondarily to its low price. Because, fish protein is one of the most important animal proteins and it represents about 14% of all animal proteins in a global basis (Abolagba and Melle, 2008). Also, fish and fish products apart from satisfying the appetite and taste are essentially cheaper than other sources of animal protein.

Herring fish is the most abundant and commercially important species belong to the genus *Clupea*, found particularly in shallow, temperate waters of the North Pacific and North Atlantic Oceans. Three species of *Clupea* are recognised, and provide about 90% of all herrings captured in fisheries. Fish is highly susceptible to autolysis, oxidation and hydrolysis of fats and microbial spoilage. After the death of fish, spoilage bacteria enter the muscle from the skin and gills, disintegrate the muscle cells and take necessary energy to grow. So, different types of processing and preservation methods have to be followed as soon as possible after the catching of fish

to keep the freshness and nutritive value of fish flesh in a condition as near as possible to that of fresh fish (**Dutta et al. 2018**).

Fish smoking is one of the traditional processing methods, it is most widely practiced and recommended method of preservation aimed to prevent or reduce microbial load. Smoked fish are well accepted food items in our country. Smoking gives the product a desirable color, taste and odor, a longer shelf-life through its anti-bacterial and oxidative effect, lowering of pH and acts as antagonist to spoilage (**Olokor et al., 2007**). The smoked fish products have gained a popular market at commercial basis due to its attractive color, flavor and aroma and have a high potentiality as a processed item. Smoking of fish is generally done in two methods; cold smoking and hot smoking.

Smoked fish products can be a source of microbial hazards due to the unhygienic handling, marketing and storage or due to the partial removal of water activity during production. If the smoked fish are contaminated with pathogenic microbes, they can cause fatal diseases in the human body (**Akinwumi and Adegbehingbe, 2015**). For this reason, it is necessary to estimate the bacterial load (coliform) along with some pathogenic bacteria (*S. aureus*) smoked fish which are common cause of foodborne illnesses in many countries, including Egypt. Coliforms are present in the digestive tracts of animals, including humans, and are found in their wastes. They are also found in plant and soil material. Most coliform bacteria do not cause disease. *Staphylococcus aureus* is a leading cause of food poisoning worldwide due to the production of heat-stable enterotoxins causing an estimated 241,000 illnesses per year in the United States. A typical food borne-disease caused by *S. aureus* has a rapid onset following ingestion of contaminated food (usually 3–5 hours), characterized by vomiting and diarrhea (**Murray 2005**).

Histamine is one of the biogenic amines; naturally occur in many species of fish with dark meat, especially of the family Clupeidae. Histamine formation in fish relates to the free histidine content of the fish muscle, the presence of bacterial histidine decarboxylases and certain environmental conditions. The production of histamine is largely induced by post-harvest exposure of fish to high temperatures that enhance the multiplication of histidine decarboxylase producing bacteria (**Economou et al., 2007**). High concentration of Histamine in fish and fish products may cause food poisoning in humans particularly, Scombroid fish poisoning. Another problem related to consumption of herring fish, is high salt or sodium content which lead to many health problems. Sodium chloride is the most used food additive in the fish processing industry, mainly for preserving but also for improving the taste of the product. In fact, the current demand for salted fish is driven more by the aroma and flavour of the product than for preservation purposes (**Mujaffar and Sankat, 2006**). Excessive intake of sodium has been linked to hypertension which in turn increases the risk of stroke and premature death from cardiovascular diseases.

Consequently, this study was conducted to identify the bacteriological and chemical hazards associated with smoked herring fish available for human consumption in Sharkia governorate.

2. Materials and methods:

A total of 100 random samples of herring fish were randomly collected from different supermarkets and shops in Zagazig and Menia El-kamh city, Sharkia Governorate, Egypt (50 of each).

1. Bacteriological examination:

1.1. Preparation of fish samples: According to APHA (2002):

1.2. Determination of total Coliform count (MPN):

Three tubes most probable number (MPN) method recommended by (ICMSF 1978) was adopted as follows:- One ml of decimal dilution was inoculated separately into each of three MacConkey broth tubes (HiMedia, Mumbai) with inverted Durham's tubes. The inoculated tubes were incubated at 37° C, and then examined after 24 hours and 48 hours. Positive tubes showing acid and gas productions in inverted Durham's tubes were recorded. The most probable number of coliforms was calculated.

1.3. Enumeration of total *S. aureus* count (ISO, 1999):

A quantity of 0.1 ml of each previously prepared dilution was spread over the dried surface of duplicate Baird –parker agar plates supplemented with egg – yolk tellurite using glass spreader. Inoculated plates and control one were incubated at 37°C for 24 hours. Suspected colonies (jet black, shining, convex colonies, 1-1.5 mm in diameter with a narrow white margin and surrounded by a wide clear area with a one Opacity around them) were recoded counting was repeated after reincubated at 37°c for a further 24 hours. Staphylococcal count/cm² was calculated as presumptive count.

1.4. Isolation of *Staphylococcus aureus* (AOAC, 2000) & *S. aureus* enterotoxins

Five suspected colonies from *S. aureus* colonies on Baird- Parker medium were picked up, purified and then cultured on slope agar tubes for preservation and further identification.

Table (1): primers specific for demonstration of *S. aureus* enterotoxins

Target gene	Oligonucleotide sequence (5' → 3')	Product size (bp)	References
<i>sea</i> (F)	5' TTGGAAACGGTTAAAACGAA'3	120	
<i>sea</i> (R)	5' GAACCTTCCCATCAAAAACA '3		
<i>seb</i> (F)	5' TCGCATCAAACGACAAACG '3	478	
<i>seb</i> (R)	5' GCGGTACTCTATAAGTGCC '3		
<i>sec</i> (F)	5' GACATAAAAGCTAGGAATTT '3		

<i>sec</i> (R)	5' AAATCGGATTAACATTATCC '3	257	Rall et al. (2008)
<i>sed</i> (F)	5' CTAGTTTGGTAATATCTCCT '3	317	
<i>sed</i> (R)	5' TAATGCTATATCTTATAGGG '3		

Detection of *S. aureus* enterotoxins by PCR: Different enterotoxin primers (Pharmacia Biotech) specific for demonstration of *S. aureus* enterotoxins (A, B, C & D) as virulence genes were applied as shown in **Table (1)**.

DNA Extraction: By using QIA amp kit (Shah et al., 2009)

Amplification of *S. aureus* enterotoxin genes: According to Rall et al. (2008).

2. Chemical examination:

The examined samples of fish were analyzed for determination of their contents of moisture and sodium chloride contents by using the standard methods recommended by Association of Official Analytical Chemists "AOAC" (2016) as follow:

2.1. Determination of moisture %:

A dish was dried in an oven and cooled in the desiccator. Approximately 2 g of sample were weighed into the dish and dried in the oven at 102°C with the lid alongside for 2 hours. The dish was covered with the lid, and transferred to the desiccator and when the dish completely cooled, it was weighed, then heated in the oven half-an-hour and re- weighed. This technique was repeated several times until successive weights did not differ.

$$\text{Moisture \%} = \frac{\text{Weight lost} \times 100}{\text{Weight of sample}}$$

2.2. Determination of sodium chloride %:

To 1 gram of the sample, 40 ml of silver nitrate solution N/10 were added to precipitate all the chloride as silver chloride, then 5 ml nitric acid was added. The contents were then gently boiled on hot plate until all solids except silver chloride were dissolved (about 15 minutes). After cooling, 50 ml of distilled water and 2 ml of saturated solution of ferric ammonium sulphate were added. The excess of silver nitrate was titrated against N/10 ammonium thiocyanate solution using ferric indicator. The amount of standard ammonium thiocyanate exhausted in the titration (R) was recorded. The same technique was repeated using 0.5 ml of the brine solution. The sodium chloride % was calculated according to the following formula:

$$\text{Sodium chloride \%} = (R - 10 \times 0.00585 \times 10)$$

3.2. Determination of histamine by ELISA:

This enzyme immunoassay is for the quantitative determination of histamine in plasma and urine. In combination with supplementary kit (available for purchase separately, cat. no. BA E-1100), the assay is performed for the determination of histamine release in heparinized whole blood.

Statistical analysis:

Statistical analysis of data was done by using the statistical package for social sciences (SPSS Inc.; Chicago, IL, USA) software. One Way ANOVA at 95% level of confidence was done to determine significant differences ($P < 0.05$ was considered as significant).

3. Results and discussion:

Smoking is a method of preservation but most of the time, spores of bacteria were not destroyed at the time of smoking due to the use of inappropriate or low temperature and then this spores multiplied during storage period. Smoking can control the microbial contamination in fish at adequately high temperatures ($>600^{\circ}\text{C}$), although, sometimes the use of high temperature might not be sufficient enough to kill all the microbial contaminants such as spores (Hwang et al., 2009)

Coliform organisms are indicator organisms that indicate the possibility of fecal contamination. Coliforms are called 'Sanitary Index' organisms whose presence in food in a large quantity indicates the probability of culturing the organism in unhygienic condition or the usage of polluted water during processing. Results illustrated in **Table (2)** revealed that the mean count of coliform was 3.9 ± 0.129 and $3.3 \pm 0.082 \log_{10} \text{ cfu/g}$ in the examined smoked herring samples, respectively. This finding corroborates the report of Akinwumi and Adegbehingbe (2015) and Dutta et al. (2018). Higher counts of coliform were recorded by Nyarko et al. (2011) ($4.3 \log_{10} \text{ cfu/g}$) and Majumdar et al. (2014) ($5.3\text{--}6.6 \log_{10} \text{ cfu/g}$). But lower count was recorded by Adegunwa et al. (2013) ($0\text{--}3.3 \log_{10} \text{ cfu/g}$).

The statistical analysis revealed a significant difference ($P < 0.05$) between the examined herring samples. It was found that samples obtained from Zagazig markets had a high count of coliform than the samples obtained from Menia El-kamh markets. This may be due to the environmental condition and also due to the fact that the place is highly congested with traffic, which may create dust, from which the fish may be contaminated. Contamination of the examined smoked herring fish by coliform may be due to failure of food handlers to observed basic sanitary rules. Detection of coliform bacteria is used as an indicator of water sanitation or as a general indicator of sanitary condition of the culture area as well as the food-processing environment (Feng et al., 2002). Therefore, fecal coliforms are considered more accurate indicator of food contamination by animal or human feces than the total coliforms.

Results found in **Table (3)** revealed that the prevalence of *S. aureus* in the examined herring samples was 14 (14%), with 8 (16%) in the examined samples obtained from Zagazig markets and 6 (12%) in the examined samples obtained from Menia El-kamh markets. The mean count of *S. aureus* was 6.6 ± 0.335 and $6 \pm 0.116 \log_{10} \text{ cfu/g}$ with minimum counts 5 and $5.3 \log_{10} \text{ cfu/g}$ and maximum count 7.3 and $6.3 \log_{10} \text{ cfu/g}$, in the examined smoked herring samples obtained from Zagazig and Menia El-kamh markets, respectively (**Table 2**).

Table (2): Statistical analytical results of coliform, total staphylococci and *S. aureus* count in the examined herring samples (n= 50 of each) log₁₀ cfu/g

Samples	Coliform count (MPN)		<i>S. aureus</i>	
	Min-Max	Mean± S.E	Min-Max	Mean± S.E
Herring (Zagazig)	3-5.4	3.9 ^A ± 0.129	5-7.3	6.6 ± 0.335
Herring (Menia Elkamh)	2.5-5	3.3 ^B ± 0.082	5.3-6.3	6 ± 0.116

n= Number of examined samples (50 of each). cfu/g: Colony Forming Unit per gram

S.E: Standard error of mean. Min: Minimum. Max: Maximum.

Means within the same column bearing different superscript letters are significantly different (P< 0.05).

Nearly similar results were recorded by **Kasozi et al., (2016)**. While, lower counts were reported by **Ayuba et al. (2013)** (3.1 log₁₀ cfu/g). The results of multiplex PCR given in **photograph (1)**, revealed that 4 out of 12 (33.3%) of the isolated *S. aureus* strains were enterotoxigenic, while 8 (66.7%) of the isolated *S. aureus* strains were non-toxin producing.

It was found that two strains of *S. aureus* (16.7%) harbored SEA, one strain (8.3%) harbored SEC and one strain (8.3%) harbored SEC and SED genes. This result was in line with **Chiang et al., (2006)**. Some strains of *S. aureus* has the ability to produce one or more enterotoxins resulting in many cases food poisoning symptoms, these toxins are classified according to the antigenic properties into five SEs including SEA, SEB, SEC, SED and SEE, which are heat stable enterotoxins and resistant to proteolysis by enzymes. Consequently, it is very important to detect the level of smoked fish contamination with enterotoxigenic strains of *S. aureus*.

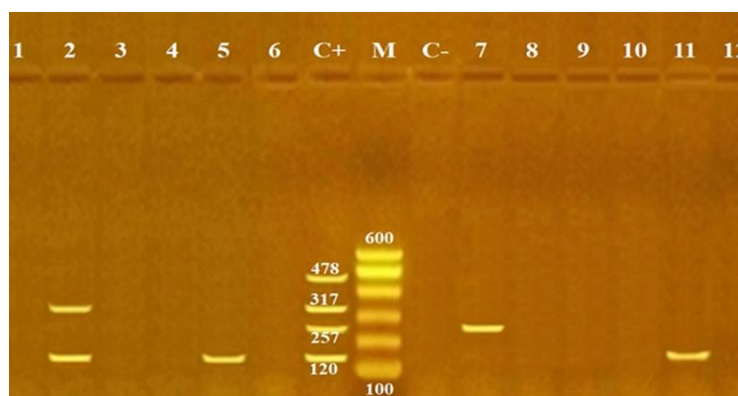
Table (3): Prevalence of *S. aureus* in the examined herring samples (n= 50)

Samples	Positive		Negative	
	No.	%	No.	%
Herring (Zagazig)	8	16%	42	84%
Herring (Menia Elkamh)	6	12%	44	88%
Total	14	14%	86	86%

n= Number of examined samples (50 of each)

No.: Number of positive or negative samples

The disease caused by SEs has a short incubation period (4.4 hours), nausea, vomiting, abdominal cramps, headache, and diarrhea. Although this disease is usually a self-limiting, death may occur among more susceptible peoples, as children and the elderly (Tarekne et al., 2016).



Photograph (1): Agarose gel electrophoresis of multiplex PCR of *sea* (120 bp), *seb* (478 bp), *sec* (257 bp) and *sed* (317 bp) enterotoxin genes for characterization of *S. aureus*. Lane M: 100 bp ladder as molecular size DNA marker. Lane C+: Control positive for *sea*, *seb*, *sec* and *sed* genes. Lane C-: Control negative. Lane 2: Positive *S. aureus* strain for *sea* and *sed* genes. Lanes 5 & 11: Positive *S. aureus* strains for *sea*. Lane 7: Positive *S. aureus* strain for *sec* gene. Lanes 1, 3, 4, 6, 8, 9, 10 & 12: Negative *S. aureus* for enterotoxins.

Histamine is the end product of histidine decarboxylation by microbial decarboxylase enzyme which is produced by various species of bacteria that grow on protein-rich food particularly fish. The level of histamine formation depends mainly on the bacterial species, the temperature and the exposure time. It can be present mainly in fishes which contain high levels of free histidine such as Scombridae (Tuna, Mackerel) and Clupeidae (Herring, Sardine) (Prestor, 2011). Histamine is a causative agent of histamine fish poisoning (scombroid poisoning); consumption of fish containing high concentration of histamine can cause symptoms of allergies (Petrovic et al., 2016). The illustrated data in Table (4) revealed that the mean content of histamine (mg %) was 11.08 ± 3.799 and 10 ± 3.827 in the examined smoked herring samples obtained from Zagazig and Menia El-kamh city, respectively. Nearly similar results were reported by Ekici and Alisarli (2008). Meanwhile, higher level of histamine was reported by Erkan et al. (2001). But, lower level was reported by Hussein (2014). The EOS (2005) established 20 ppm as a permissible limit for histamine in smoked salted and frozen fish products, it appears that level of histamine in all examined groups not constitutes a hazard for human health.

Histamine is related to scombroid fish poisoning when its concentration exceeds 200 ppm, often above 500 ppm. Meanwhile, Shalaby (1996) reported guideline concentrations for histamine content in seafood as following; < 5 mg/100 g is considered safe for consumption; 5-20 mg/100 g is possibly toxic; 20-100 mg/100 g

is probably toxic; and > 100 mg/100 g is considered toxic and unsafe for human consumption. The main characteristic symptoms of scombrototoxin poisoning are nausea, vomiting, diarrhea, burning in or around the mouth and throat, rashes on the upper part of the body, decrease the blood pressure, headache, dizziness, itching of the skin, respiratory distress and heart palpitation. These symptoms occur within a few minutes to a few hours of consumption and last from 12 hours to a few days.

Table (4): Statistical analytical results of Histamine (mg %), Moisture (%) and NaCl (%) content in the examined herring samples (n= 50 of each)

Samples	Histamine (mg %)		Moisture (%)		NaCl (%)	
	Min-Max	Mean \pm S.E	Min-Max	Mean \pm S.E	Min-Max	Mean \pm S.E
Herring (Zagazig)	2.7-24	11.08 \pm 3.799	56.5-61.8	58.6 \pm 0.875	5.6-9.4	7 \pm 0.714
Herring (Menia kamh)-El	1.2-20.9	10 \pm 3.827	56.1-59.4	58.1 \pm 0.581	5.4-8.3	6.9 \pm 0.483

n= Number of examined samples (50 of each)

S.E: Standard error of mean. Min: Minimum. Max: Maximum.

Means are not significantly different (P> 0.05).

The results in **Table (4)** declared that the moisture content in the examined smoked herring samples obtained from Zagazig and Menia El-kamh city ranged from 56.5- 61.8 % and 56.1-59.4%, respectively. These results were in a line with **Usydu Osheba et al. (2013)** who found nearly similar results. While, lower result was reported by **Akinwumi and Adegbehingbe (2015)**. But, higher result (50.68-77.75%) was recorded by **Adegunwa et al. (2013)**.

Sodium chloride is the most used food additive in the fish processing industry, mainly for preserving but also for improving the taste of the product. In fact, the current demand for salted fish is driven more by the aroma and flavor of the product than for preservation purposes (**Mujaffar and Sankat, 2006**). As illustrated in **Table (4)**, the mean value of NaCl concentration was 7 \pm 0.714 % and 6.9 \pm 0.483 % in the examined smoked herring samples obtained from Zagazig and Menia El-kamh city, respectively. Higher results (8.35% and 8.7%) were reported by **Osheba et al. (2013)** and **Nader et al. (2016)**. Excess sodium consumption has also been associated with numerous other negative health effects, including gastric cancer (**Tsugane et al., 2004**), decreased bone mineral density and possibly obesity (**He and MacGregor, 2008**). In addition to high blood pressures which may in turn increase the risk of stroke and premature death from cardiovascular diseases (**Osheba et al., 2013**).

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المخاطر الصحية لأسماك الرنجة المدخنة

عادل إبراهيم العتباتي، وجيهه صبحي درويش، عبدالله فكرى محمود و سارة عبدالله متولي

قسم مراقبة الأغذية - كلية الطب البيطري - جامعة الزقازيق - مصر - صندوق بريد ٤٤٥١١

تم تجميع عدد مائة عينة عشوائية من أسماك الرنجة المدخنة المتداولة في محافظة الشرقية، بواقع خمسين عينة من كل من أسواق مدينتي الزقازيق ومنيا القمح، محافظة الشرقية، مصر. تم نقل العينات التي تم تجميعها دون تأخير في صندوق الثلج إلى معمل الدراسات العليا لمادة صحة وتكنولوجيا اللحوم، كلية الطب البيطري جامعة الزقازيق لتقييم الحالة الصحية لهذه العينات من خلال إجراء الفحص البكتيريولوجي والكيميائي لها. أظهرت النتائج أن أسماك الرنجة المدخنة المسوقة في مدينتي الزقازيق ومنيا القمح كانت ملوثة بكتيريا البكتيريا القولونية وأيضاً المكورالعنقودي الذهبي. كان متوسط عدد الميكروبات القولونية 3.9 ± 0.129 و 3.3 ± 0.082 لوغاريتم مستعمرة بكتيرية/جرام ؛ و كان متوسط قيم عدد المكور العنقودي الذهبي 6.6 ± 0.335 و 6 ± 0.116 لوغاريتم مستعمرة بكتيرية/جرام في عينات الرنجة المدخنة التي تم الحصول عليها من الزقازيق ومنيا القمح على التوالي. كان متوسط قيم محتوى الهستامين (ملغ/%) 11.08 ± 3.799 و 10 ± 3.827 ، وكان محتوى الرطوبة 58.6 ± 0.875 % و 58.1 ± 0.581 % ، على التوالي وكان متوسط قيم تركيز ملح الطعام 7 ± 0.714 % و 6.9 ± 0.483 % في عينات الرنجة المدخنة التي تم الحصول عليها من مدينة الزقازيق ومنيا القمح على التوالي. لذلك توصي هذه الدراسة بالتالي بضرورة متابعة أسواق الأسماك من الجهات المختصة للتأكد على سلامة الرنجة المدخنة وإتباع طرق العرض المناسبة للحد من التلوث البكتيري، التخلص الصحي من النفايات بعيداً عن المجارى المائية للحد من التلوث البكتيري للأسماك ومنتجاتها، إختيار الأسماك الطازجة حديثة الصيد لعملية التملح والتدخين وإتباع الطرق الصحيحة في إعداد وتجهيز الأسماك المدخنة وعملية التخزين والعرض، التدريب المستمر للعاملين على إجراءات النظافة الشخصية للحد من التلوث بالإضافة الى شراء الأسماك المدخنة من أماكن تتوافر بها الظروف الصحية المناسبة للعرض والتداول.