



Assessment of Bacterial Population and Enterotoxins Profile of Smoked Fish

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ABSTRACT

Smoked fish is a popular product that is widely consumed. Since fish spoil quickly after catching, various methods of processing and preservation have been developed to prevent spoilage. For the safety of fish consumers, this study was conducted to assess the microbial quality of smoked fish. One hundred samples of smoked fish were analyzed for different microbiological parameters, including the aerobic colony count and Enterobacteriaceae counts using the 3M™ Petri film™ technique. The total proteolytic count was determined using skim milk agar medium, while the total lipolytic count was assessed utilizing tributyrin nutrient agar media. *Staphylococcus aureus* was detected using mannitol salt agar, and its presence was confirmed by Gram staining and biochemical tests. Additionally, the presence of *Staphylococcus aureus* enterotoxins was detected. The results showed that the mean values of the total bacterial count were 1.4×10^4 and 3.0×10^3 cfu/g for hot-smoked and cold-smoked fish, respectively, and the mean total Enterobacteriaceae counts were 1.2×10^3 and 3.5×10^2 CFU/g for both types of samples, respectively. The mean values of total proteolytic counts were 8.4×10^5 and 1.8×10^4 , and the mean values of lipolytic counts were 3.9×10^4 and 6.6×10^4 cfu/g for hot and cold smoked fish, respectively. The prevalence of *Staphylococcus aureus* in hot and cold smoked fish was 14/50 (14%) and 12/50 (12%), respectively. Furthermore, out of 26 smoked fish samples tested for the presence of staphylococcal enterotoxins, 10/14 (71%) and 11/12 (92%) were positive in hot and cold smoked fish, respectively. The study revealed that hot and cold smoked fish are often contaminated with zoonotic bacterial agents and enterotoxins. These findings suggest that consuming contaminated smoked fish or food cross-contaminated with zoonotic bacteria may pose a threat to consumer health.

INTRODUCTION

Fish represent a highly nutritious food forming an excellent source of high-quality protein, essential amino acids, and unsaturated fatty acids. It is also a good source of most B-complex vitamins, lipo-soluble vitamins, and minerals such as cobalt, zinc, copper, iron, sodium, potassium, magnesium, phosphorus, iodine, and fluorine. Omega-3 fatty acids found in fish can help lower blood triglycerides, reduce abnormal heart rhythms,

reduce blood pressure, improve blood clotting regulation, and reduce the risk of cardiovascular diseases (NRC, 1998; Wang *et al.*, 2006).

In Egypt, smoked fish is a popular type of fish product made mainly from imported raw material of frozen herring fish (Salem, 2004; Elgazzaret *et al.*, 2005). However, fish are highly susceptible to spoilage after catching, so various methods of processing and preservation have been developed over time to retard the spoilage of fish (Hossain *et al.*, 2012).

Smoked products, such as cold-smoked herring, are a traditional way of consuming fish and can be a good carrier of pathogenic bacteria. Nevertheless, the smoke produced by the smoking process can contain antibacterial components like formaldehyde and phenols that reduce the water activity of the fish and cause microbial destruction, thus minimizing spoilage and increasing storage time (Gram, 2001). The duration of smoking determines the moisture content of the fish product, which in turn affects the rate of microbial growth and shelf-life. Preservation by drying can lower the water activity of the fish to a level where micro-organisms can no longer grow (Eyo, 2001).

The smoking process involves different preservative steps, including salting, drying, and smoking, as well as in the case of hot smoking, applying heat. Salting and drying are essential processing parts of the smoking process, and the temperature for drying is usually between 20- 26°C. The moisture content and temperature in the smoke are crucial factors that affect the quality of the final product (Krasemann, 2004). Cold smoking is processed at a temperature between 20- 30°C, while hot smoking is done between 70- 80°C (Berberoglu, 2004).

Therefore, this study aimed to evaluate the microbial quality of smoked fish by determining the total aerobic counts, Enterobacteriaceae counts, proteolytic counts, and lipolytic counts.

MATERIALS AND METHODS

1. Samples collection

A total of 100 smoked fish samples (Ringa) were purchased from various commercial fish retails and markets. The samples were conveyed to the laboratory in newly purchased polythene bags previously sterilized by 70% (v/v) ethanol and carefully transported without undue delay for microbiological analysis adopted from the methods of Booths (1971).

2. Bacteriological examination

2.1. Preparation of samples

The flesh of fish sample was transferred to a homogenizer to be minced using Moulinex Food Processor (La Moulinette, type D56, Paris, France), and 25g of homogenized sample was added to 225ml of 0.1% peptone water using plastic bag and put in a Seward stomacher (400R/UK) for 2 min.

2.2. Microbiological analysis

The 3M™ petri film™ technique was used to determine the aerobic colony count. To do this, the top layer of the plate was lifted to expose the plating surface, and 1ml of the diluted sample was added using a pipette. The top film was then slowly rolled down, and the "spreader" was used to distribute the sample evenly. It took about a minute for the gel to form, after which the plates were incubated at 37°C for 24±2 hours. All red colonies, regardless of their size or color intensity, were counted using a standard Quebec-type counter, and the results were expressed as CFU/g. To enumerate the Enterobacteriaceae counts using the 3M™ petri film™ technique, the petri film plate was placed on a level surface, and 1ml of the sample homogenate was placed in the center of the bottom film. The top film was then slowly lowered to prevent air bubbles from forming, and the spreader was placed on top of the film over the inoculum. Gentle pressure was applied to distribute the sample over a circular area before the gel was formed, and the plate was left for at least one minute for the gel to solidify. The plates were then incubated with the clear side up for 24 hours ± 2 hours at 37°C ± 1°C. Petri film plates could be counted using a standard colony counter or other illuminated magnifiers.

The total proteolytic count was determined using the skim milk agar medium, which was inoculated at 37°C for 48 hours and examined for a clear zone around the growth. The countable plates were then flooded with 1% HCL for one minute to exclude acid from colonies. Organisms showing proteolytic activity were counted, and their activity was manifested by a clear halo zone around the colonies (APHA, 2002).

The total lipolytic count was enumerated by mixing 1ml of each dilution with tributyrin nutrient agar media. The medium included 10gm tributyrin (PM4, OXOID) and 28gm of NA (CM4, OXOID). The lipolytic activity was determined by measuring the clear zone around lipolytic colonies.

For the detection of *Staphylococcus aureus*, 0.1ml of previously prepared serial dilution was evenly spread over a dry surface of mannitol salt agar using a sterile bent glass spreader. The inoculated and control plates were incubated at 37±1°C for 24 hours. Yellowish colonies surrounded by a halo zone were found. Gram staining was used for confirmation, and the prepared film was examined for grape-like Gram-positive cocci. The catalase test was performed by adding one drop of hydrogen peroxide (3%) to a loopful from purified colonies on a dry glass slide, and a cover slide was applied. The appearance of gas bubbles was considered positive. The coagulase test was performed using two Wisserman tubes, each containing 1ml of 10-fold serially diluted rabbit plasma with sterile normal saline. Five drops of an overnight brain heart infusion broth culture were added to one tube, and the other tube was left as a control. Both tubes were incubated at 37°C for 24 hours and examined for coagulation of plasma. For the detection of hemolysis, suspected strains were streaked onto 5% sheep's blood agar (Oxoid) plates and incubated at 37°C for 24 hours. Most strains of *S. aureus* cause β- hemolysis, which is characterized by clearing of the medium and an increase in transparency around the

bacterial colonies. The detection of *Staphylococcus aureus* enterotoxins was performed using the Oxoid SET REPLA kit and the reversed passive Latex agglutination technique. The food extracts were diluted in a stepwise manner, with each dilution being half the concentration of the previous one. After approximately 16 hours of incubation, agglutination was observed using transmitted light from the bottom of the plate when high-density latex particles were used. To calculate the concentration of toxin in the sample, the toxin titer was multiplied by the detection limit of each toxin, which was 0.5ng/ ml. The recovery of toxin from the sample was determined by comparing the concentration of toxin detected in the sample (10 ml of food homogenate) to that in the control (10 ml of PB) using a ratio.. PCR identification was conducted using a marketed kit for DNA extraction (Presto Mini-DNA Bacteria Kit, Ltd. USA). DNA was prepared using 0.05 N NaOH and 0.25% sodium dodecyl sulfate. Primers were designed using 16S rDNA sequences shown in Table (1). The PCR product was analyzed using a 1% agarose substance by electrophoresis apparatus and observed under a UV transilluminator. Amplification and sequence determination of 16S rDNA were performed following the method described by **Al-Azawi et al. (2018)**.

The results were statistically analyzed by comparing the population data obtained using both methods for each dilution through the use of the Student's t-Test. This analysis was carried out using Graph Pad Instat 3 for Windows software. The average of each dilution for both methods was calculated to determine the presence of a significant difference between them. The Student's t-Test was performed using the same software and a *P*-value of less than 0.05 was considered statistically significant, while a *P*-value greater than 0.05 indicated that the observed difference was not significant.

RESULTS

Microbial quality

The statistical analysis of the microbiological quality in smoked fish, as shown in Table (2). The results suggest that hot smoked fish had higher mean values of total bacterial count, total Enterobacteriaceae counts, total proteolytic counts, and total lipolytic counts compared to cold smoked fish. The minimum and maximum values varied for each type of count for both hot and cold smoked fish. The statistical analysis showed that the difference in microbial counts between hot and cold smoked fish was significant. This indicates that cold smoked fish may be a safer option as it had lower microbial counts compared to hot smoked fish.

Prevalence of *Staphylococcus aureus* and enterotoxins

The results in Table (3) reveal the prevalence of *staphylococcus aureus* in the examined smoked fish samples. The prevalence of *S. aureus* in hot smoked and cold smoked fish were 14/50 (14%) and 12/50 (12%), respectively. Out of 26 smoked fish samples tested for presence of staphylococcal enterotoxins, 10/14 (71%) and 11/12 (92%) were positive in hot and cold smoked fish, respectively.

Table 1. Primers sequences of *S. aureus* enterotoxins

Target gene	Primers sequences	Reference
<i>Sea</i>	GGTTATCAATGTGCGGGTGG CGGCACTTTTTTCTCTTCGG	Mehrotra <i>et al.</i> , 2000
<i>Seb</i>	GTATGGTGGTGTAACTGAGC CCAAATAGTGACGAGTTAGG	
<i>Sec</i>	AGATGAAGTAGTTGATGTGTATGG CACACTTTTAGAATCAACCG	
<i>Sed</i>	CCAATAATAGGAGAAAATAAAAAG ATTGGTATTTTTTTTCGTT	
<i>See</i>	AGGTTTTTTCACAGGTCATCC CTTTTTTTTCTTCGGTCAATC TTCACGACTACTACTATGCGG	

Table 2. Statistical analytical results of microbial quality of the smoked fish samples

Item	Total aerobic counts (log ₁₀ cfu/g)		Total <i>Enterobacteriaceae</i> counts (log ₁₀ cfu/g)		Total proteolytic counts (log ₁₀ cfu/g)		Total lipolytic counts (log ₁₀ cfu/g)	
	Hot smoked	Cold smoked	Hot smoked	Cold smoked	Hot smoked	Cold smoked	Hot smoked	Cold smoked
Min.	4.0×10	<10	2.2×10 ²	<10	1.0×10 ³	6.0×10 ²	5×10 ³	<10
Max.	7.3×10 ⁴	2.0×10 ⁴	5.7×10 ⁴	1.2×10 ⁴	5.7×10 ⁶	1.0×10 ⁵	3.0×10 ⁶	3.6×10 ⁵
Mean	1.4×10 ⁴	3.0×10 ³	1.2×10 ³	3.0×10 ³	8.4×10 ⁵	1.8×10 ⁴	3.9×10 ⁴	6.6×10 ⁴
±S.E	±5.3×10 ²	±1.4×10 ²	±3.8×10 ²	±8.4×10 ²	±3.8×10 ⁴	±6.4×10 ³	±2.0×10 ³	±1.3×10 ³

Table 3. Prevalence of *Staphylococcus aureus* in the smoked fish samples

Item	<i>Staphylococcus aureus</i>				Enterotoxins	
	+ve samples		-ve samples		No.	%
	No.	%	No.	%		
Hot smoked	14	70%	6	30%	14	0%
Cold smoked	12	60%	8	40%	12	0%
Total	26	65%	14	35%	26	0%

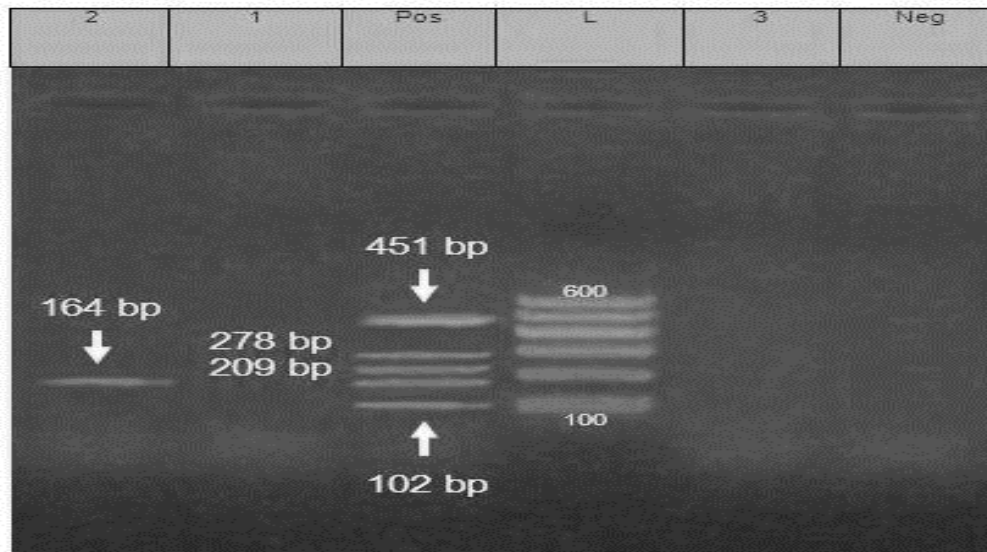


Fig. 1. Agarose gel electrophoresis of specific dose-dependent amplification of *Staph. aureus* pathogenic genes

PCR amplification of the enterotoxins; (*Seb*) 164 bp, (*Sea*) 278 bp, (*Sec*) 209 bp, (*Sed*) 451 bp, (*See*) 102 bp products of DNA extracted from *Staph. aureus*.

DISCUSSION

It is widely recognized that the quality of the microbiological aspects of food is the result of a series of interconnected events. In 1989, world fish production was estimated to be 100 million tons, with 15% of it processed in some way. Among the processed fish, one-third was smoked. To meet consumer demand, it is essential to manufacture safe and high-quality smoked seafood products.

The statistical analysis results of the total bacterial count in smoked fish products indicated that the mean values for the total bacterial count were 1.4×10^4 and 3.0×10^3 cfu/g for hot smoked and cold smoked fish, respectively. The minimum values were 4.0×10 for hot smoked fish and <10 for cold smoked fish, while the maximum values were 7.3×10^4 and 2.0×10^4 cfu/g, respectively. Furthermore, the statistical analysis showed a significant difference ($P=0.05$) in the mean total bacterial count between hot smoked and cold smoked fish. The aerobic bacterial plate count is an essential indicator of the microbiological status of food. A high viable count often indicates contaminated raw materials, inadequate sanitation, unsuitable time/temperature conditions during production or storage or a combination of these factors. Additionally, high counts indicate the likelihood of spoilage because most foods contain 10^6 to 10^8 microorganisms per gram when decomposition becomes apparent. Although heating food before consumption can eliminate pathogenic microbes, it does not necessarily eliminate any toxins that may have been produced. According to the Egyptian Organization for

Standardization and Quality Control, this is important to consider (EOS, 2005); 100% of the examined samples of hot and cold smoked Ringa were within the acceptable limits stipulated by these standards. According to this standards limit, all examined smoked fish samples are considered fit for human consumption from the microbiological point of view. The same results were obtained by El-Dengawyet *et al.* (2012). For hot smoked samples, higher results were obtained by Abolagba and Igbinevbo (2010), Omoyemi (2012) and Adegunwaet *et al.* (2013). While, much lower total bacterial count was reported by Debrah *et al.* (2011), Obodaiet *et al.* (2011) and Salama and Ibrahim (2012). For cold smoked samples, a high total bacterial count was recorded in the study of Dondero *et al.* (2004), while a lower count was determined in the investigation of Espe *et al.* (2004). Despite that hot smoking is more effective in reducing the initial bacterial load of fish due to higher temperature used, the examined cold smoked fish samples have a lower total aerobic count due to post smoking contamination of the hot smoked fish by poor handling conditions and improper storage, contrary to the cold smoked fish samples that are sold packaged in a vacuumed condition and kept in a low temperature.

The *Enterobacteriaceae* group of bacteria is commonly used to evaluate the overall hygiene of food products. This group comprises various species that originate from the intestinal tracts of animals and humans, as well as plants and environment.. The mean total *Enterobacteriaceae* counts values of hot and cold examined smoked fish samples were 1.2×10^3 and 3.5×10^2 cfu/ g, respectively, while the minimum values were 2.2×10^2 , <10 cfu/ g, respectively, and the maximum values were 5.7×10^4 and 1.2×10^4 cfu/g, respectively. In addition, the statistical analytical results revealed that the mean total *Enterobacteriaceae* counts were significantly different ($P < 0.05$) between cold and hot smoked fish samples. According to the Egyptian Organization for Standardization and Quality Control (EOS, 2005), there is no standard limit for the proteolytic count in smoked fish. The same results were obtained by Tirloniet *et al.* (2015). Higher results were assessed by Leroi *et al.* (2001). While, the *Enterobacteriaceae* counts were not detected in the study of Fentie and Emire (2013). Remarkably, the presence of *Enterobacteriaceae* is used as an indicator of fish quality since it can be related to various aspects of fish storage, washing and evisceration. Gram and Huss (1996) noted that lightly preserved fish products may have high levels of *Enterobacteriaceae*, which can lead to smoked fish deterioration. According to Ababouch (1995), the presence of contamination in food products indicates non-compliance with good manufacturing practices and hygiene rules. Among the vendors interviewed, *Enterobacteriaceae* contamination was linked to poor hygiene practices during smoking and washing, as well as the poor microbiological quality of the utensils and water used. Additionally, failure to eviscerate and salt the fish before smoking also increases the risk of contamination by fecal coliforms. The presence of *Enterobacteriaceae* in fish can be an indication of poor hygiene practices during processing and post-processing, such as handling fish by the producer and customers after smoking. *Enterobacteriaceae* in fish may be related to fecal

pollution of surface water or aquatic environments or improper handling. Fish and fish products are globally consumed and are an essential source of high-quality protein for over one billion people (**Obodaiet al., 2011**). The texture of fish and shellfish is a crucial factor as it affects consumer acceptance and market price. During storage or distribution in ice, fish and shellfish can become soft or mushy due to muscle protein degradation caused by proteolytic activity. The mean total proteolytic counts values of hot smoked fish and cold smoked examined samples were 8.4×10^4 and 1.8×10^4 , respectively, while the minimum values were 1.0×10^3 , 6.0×10^2 cfu/ g, respectively, and the maximum values were 5.7×10^6 and 1.0×10^5 cfu/ g, respectively. The proteolytic bacteria reported as one of important spoilage microorganisms which can grow as psychrophiles. The packing under low and high vacuum inhibited development of proteolytic bacteria. Very little information is available concerning the proteolytic bacteria in smoked fish; however, since the smoked fish produced in Egypt is prepared from imported frozen uneviscerated fish, many authors show interest in studying enzyme producing bacterial flora isolated from fish digestive tract. Fish distributed in ice are susceptible to a textural problem called “softening or mushiness”. This deterioration is usually influenced by the activity of digestive enzymes during iced storage which accordingly limits a shelf-life up to a week (**Pornratet al., 2007; Sriket et al., 2010**). Table (2) displays the statistical analytical results of total lipolytic counts in smoked fish products. The mean total lipolytic counts values of hot smoked fish and cold smoked examined samples were 3.9×10^4 and 6.6×10^4 cfu/ g, respectively, while the minimum values were 5×10^3 , <10 cfu/ g, respectively, and the maximum values were 3.0×10^6 and 13.6×10^5 cfu/ g, respectively. Eminently, there is no standard limit for the proteolytic count in smoked fish. It is worth noting that, hydrolysis of fats by lipolytic microorganisms produce volatile components including acids, alcohols, esters, ketones, aldehydes, lactones and furans, leading to off flavor in fatty foods (**Forss, 1972**). Lipase production in large amount is associated with increasing the virulence of the pathogen (**Ciborowski & Jelzszewicz, 1985**). According to **Obodai et al. (2011)**, the smoking achieves preservation by four different means including that the hot smoking melts down the fat in the fish which drips away, and subsequently an increase is sustained in lipolytic bacteria indicating an improper smoking process.

The occurrence of *Staphylococcus aureus* in the tested smoked fish samples was noted, with 30% (or 6 samples) and 40% (or 8 samples) prevalence in hot-smoked and cold-smoked fish, respectively. *S. aureus* is a major bacterial agent responsible for foodborne diseases globally, as cited by the European Food Safety Authority (**EFSA, 2010**). These bacteria can withstand salty environments and thus have the potential to contaminate all types of cured food, including cold-smoked fish (**Le-Loir et al., 2003**). *Staphylococcal* food poisoning typically resolves within 24 to 48 hours and is often not reported to healthcare providers, leading to a higher actual incidence than what is reported. According to **Vishwanath et al. (1997)** and **El-Dengawy et al. (2012)**, *S. aureus*

was found in vacuum-packaged smoked fish. *Staphylococcal* food poisoning (SFP) is a type of food poisoning that results from consuming food containing one or more preformed enterotoxins (Dinges *et al.*, 2000). Symptoms of SFP appear quickly (within 2–8 hours) and include nausea, severe vomiting, and abdominal cramps, with or without diarrhea (Balaban & Rasooly, 2000). Some *S. aureus* strains produce a range of protein toxins and virulence factors that are believed to contribute to the organism's pathogenicity. The staphylococcal enterotoxins have been classified into several types, the most common of which are SEA to SEE. These enterotoxins are heat-resistant and can withstand digestive enzymes. Out of the smoked fish samples, 14% (or 14 out of 50 samples) of hot smoked fish and 12% (or 12 out of 50 samples) of cold smoked fish were found to be positive for *S. aureus*. Among the positive samples, 71% (or 10 out of 14 samples) and 92% (or 11 out of 12 samples) of hot and cold smoked fish, respectively, were found to be positive for *staphylococcal* enterotoxins.

Staphylococcosis, a common cause of gastroenteritis worldwide, can be transmitted to humans either by consuming contaminated fish or through unhygienic practices. This disease manifests rapidly, typically between 2 to 4 hours after ingesting the heat-resistant Staphylococcal enterotoxins (SEs). Despite the fact that these SEs prove more stable than the *S. aureus* bacteria, it is still possible to yield negative *S. aureus* cultures while receiving positive results for SEs from food samples (El-Jakee *et al.*, 2013). Research indicates a higher prevalence of *S. aureus* in fish meat than previously thought, with findings exceeding those reported by Osman *et al.* (2015), who noted a 15% prevalence. A study by Kumar *et al.* (2011) documented a 10.8% prevalence of *S. aureus* toxins in South Asian fish. In contrast, Khalifa *et al.* (2014) reported a significantly higher prevalence of 63%, while Gwida and Elgohary (2015) found a 22% prevalence in fish meat from Mansoura, Egypt. A survey conducted by Mathenge *et al.* (2015) discovered that 37.4% of fish meat products in Kenya, tainted with *S. aureus*. SEs, are responsible for approximately 95% of all human food poisoning cases from a public health viewpoint. This risk escalates in environments where rigorous hygiene measures are not strictly enforced (Clarisse *et al.*, 2013; Al-Jumaily *et al.*, 2014; Mathenge *et al.*, 2015). Furthermore, *S. aureus* has the potential to contaminate food via contact with infected hands, materials, surfaces, and even the air due to coughing. Therefore, to ensure the utmost safety for consumers, it is imperative that meat and meat products are kept free from such harmful pathogens.

CONCLUSION

The results of the survey indicate that zoonotic bacterial agents and enterotoxins are frequently found in hot and cold smoked fish products sold in Egypt. In addition, several bacterial strains tested positive for virulence markers and enterotoxin genes. These findings suggest that consuming contaminated smoked fish products or food that has been

cross-contaminated with zoonotic bacteria can be a significant risk to consumer health. Therefore, it is crucial to take additional measures to prevent foodborne illnesses. Proper handling, storage, and effective processing methods can help reduce the risk of bacterial contamination and ensure the safety of fish products.

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REFERENCES

- Ababouch, L.** (1995). Assurance in fishing industry. Scientific and Quality Technical Handbooks. Actes Ed., Rabat.
- Abolagba, J. and Igbinevbo, E.** (2010). Microbial load of fresh and smoked fish marketed in Benin Metropolis, Nigeria. *Research journal of fisheries and hydrobiology*, 5(2): 99-104.
- Adegunwa, M.; Adebowale, A.; Olisa, Z. and Bakare, H.** (2013). Full length research paper: chemical and microbiological qualities of smoked herring (*sardinella*, valenciennes 1847) in Odeda, Ogun state, Nigeria. *International journal of microbiology research and review*, 1(5): 85-87.
- Al-Azawi, I. H., Al-Hamadani, A. H., and Hasson, S. O.** (2018). Association between biofilm formation and susceptibility to antibiotics in *Staphylococcus lentus* isolated from urinary catheterized patients. *Nano Biomed. Eng*, 10(2): 97-103.
- Al-Jumaily, E., Saeed, N., Hussain, H and Khanaka, H.** (2014). Detection of enterotoxin types produce by coagulase positive *Staphylococcus* species isolated from mastitis in dairy cows in Sulaimaniyah region. *Appl Sci Report*. 2(1): 19-26.
- APHA (American Public Health Administration)** (2002). *Methods for microbiological examination of food*. 4th ed., Technical Committee on Washington, D. C., USA.
- Balaban, .and Rasooly, A.** (2000). Staphylococcal enterotoxins. *Int J Food Microbiol*. 61: 1-10.
- Berberoglu, H.** (2004). Smoked fish, Food reference website (FRW), online: <http://www.foodreferance.com/html/artsmokedfish.html>
- Booth C.** (1971): *Methods In Microbiology*, Chapter I, Introduction to General Methods. 1- 47.
- Ciborowski, P. and Jezaszewicz, J.** (1985). Staphylococcal enzyme and virulence. In: "Bacterial enzyme and virulence". I. A. Holder (Ed.), P. 146. CRC press, Inc. Boca Raton, Fl.

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- Clarisse, T.; Michèle, S.; Olivier, T.; Valérie, E. and Vincent, M.** (2013). Detection and quantification of staphylococcal enterotoxin A in foods with specific and sensitive polyclonal antibodies. *Food Control*.32: 255- 261.
- Debrah, J.; Obodai, E.; Aheto, D.; Ameworwor, D. and Sarpong-Baidoo, M.** (2011): Coliform and Escherichia Coli levels in the yellow fin tuna (*Thunnus albacares*) landed at the dixcove beach in Ghana (2011). *Journal of microbiology and biotechnology research*, 1(4): 198-205.
- Dinges, M.M.; Orwin, P.M. and Schlievert, P.M.** (2000). Exotoxins of *Staphylococcus aureus*. *Clin Microbiol Rev* 13: 16-34.
- Dondero, M.; Cisternas, F.; Carvajal, L. and Simpson, R.** (2004). Changes in quality of vacuum-packed cold-smoked salmon (*Salmosalar*) as a function of storage temperature. *Food chemistry* 87: 543-550.
- EFSA, European Centre for Disease Prevention and Control**, (2010). The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in the European Union in 2008. *EFSA Journal*. 8 (1):1496.
- El-Jakee, J.; Marouf, S.A.; Nagwa S.; Eman H.A.; Sherein I.; Abd El-Moez, A.A. and El-Dengawy, A.; El-Shehawy, M.; Kassem, M.; El-Kadi, M. and Zeinab, F.** (2012). Chemical and Microbiological evaluation of some fish products samples. *Journal of agricultural, chemical and biotechnology, Mansoura University*, 3(8): 247-259.
- Elgazzar, M.; Hassan, A.; Farghaly, M. and Sallam, A.** (2005). Mycological and related studies on smoked fish. 4th International Science Conference Mansoura. 762-793.
- EOS** (2005). Egyptian Organization for Standardization and Quality. Smoked Fish, Arab Republic of Egypt. No. 288.
- Espe, M.; Kiessling, A.; Lunestad, B.; Torrissen, O. and Røra, A.** (2004). Quality of cold smoked salmon collected in one french hypermarket during a period of 1 year. *Lebensm-Swiss society of food science and technology*. 37: 627-638.
- Eyo, A.** (2001). Fish processing technology in the Tropics. National Institute for freshwater fisheries research, Newbussa, Nigeria, ISBN-13: 9781770457: 403.
- Fentie, E. and Emire, S.** (2013). Effect of hot smoking process parameters on microbiological and shelf stability of Nile tilapia (*Oreochromis niloticus*) fillets. *Journal of food process technology*, 4(1).
- Forss, D.A.** (1972). Odour and flavor compound from lipids. In: "progress in the chemistry of fats and other lipids". V.12, P. 181, Ed., Holman, R. T. Pergamon press. New York.
- Gram, L.** (2001). Potential hazard in cold-smoked fish: *Clostridium botulinum* type E. *Journal of Food Science*, 66: S1082-S1087.
- Gram, L. and Huss, H.H.** (1996). Microbiological spoilage of fish and fish products. *International Journal of Food Microbiology*, 33:121-137.

- Gwida, M. and El-Gohary, A.** (2015). Prevalence and Characterization of Antibiotic Resistance Food Borne Pathogens Isolated from Locally Produced Chicken Raw Meat and their Handlers. *Journal of Dairy, Veterinary & Animal Research*. 3(1): 00062. DOI: 10.15406/jdvar.2015.03.00062.
- Hossain, M.; Adhikary, R.K.; Mahbub, K.R.; Begum, M. and Islam, M.R.** (2012). Effects of 10% concentrations of Salt, Garlic and Coriander on the Quality of Smoked Hilsa Fish (*Tenualosailisha*). *American Journal of Food Technology*, 7 (8): 501-505.
- Khalifa, M.; Abdel-Rhman, SH.; Abd El Galil, H.; Habib, E. and Barwa, R.** (2014). Occurrence and Characterization of *Staphylococcus Aureus* in Meat and Meat Products Marketed in Mansoura, Egypt. *Egyptian Journal of Medical Microbiology*, 23(3): 47-56.
- Krasemann, S.** (2004). Smoling seafood “A History of Smoke Preservation”, on line: <http://www.3men.com/history.htm>
- Kumar, R.; Yadav, R.; Anand, K. and Singh, S.** (2011). Molecular surveillance of putative virulence factors and antibiotic resistance in *Staphylococcus aureus* isolates recovered from intramammary infections of river buffaloes. *Microbial Pathogenesis* 51: 31-38.
- Law, B.A. and Kolstad, J.** (1983). Proteolytic systems in lactic acid bacteria. *Antonie van Leeuwenhoek*. 49(3): 225-245.
- Lawrynnowick-Paciorek, M.; Kochman, M.; Piekarska, K.; Grochowska, A. and Windyga, B.** (2007): The distribution of enterotoxin and exotoxins-like genein *Staphylococcus aureus* strains isolated from nasal carriers and food samples. *Inyernational Journal of Food Microbiology*. 117: 319-323.
- Le-Loir, Y.; Baron, F. and Gautier, M.** (2003). *Staphylococcus aureus* and food poisoning. *Genetics and Molecular Research*. 2(1): 63-76.
- Leroi, F.; Joffraud, J.; Chevalier, F. and Cardinal, M.** (2001). Research of quality indices for cold-smoked salmon using a stepwise multiple regression of microbiological counts and physico-chemical parameters. *Journal of applied microbiology*, 90: 578-587.
- Mathenge, J.; Okemo, P.; Ng'ang'a, P.; Mbaria, J. and Gicheru, M.** (2015). Identification of enterotoxigenic *Staphylococcus aureus* strains from meat and dairy products by multiplex PCR and reverse passive latex agglutination test in Nairobi, Kenya. *East and Central Africa Medical Journal*. 2: 97-103.
- Mehrotra, M.; Wang, G. and Johnson, W.M.** (2000). Multiplex PCR for detection of genes for *Staphylococcus aureus* enterotoxins, exofoliative toxins, toxic shock syndrome toxin 1, and methicillin resistance. *J ClinMicrobiol*. 38: 1032–1035.
- NRC (National Research Council)** (1998). *Improving Fish Stock Assessments*. National Academy Press, Washington, D.C
- Obodai, N.; Boamponsem, E.; Coomson, L. and Aniwe, Y.** (2011). Microbial profile of smoked sardine (*Sardillellaaurita*) AT smoking sites and market centres of Tema, Ghana *Archives of Applied Science Research*, 3 (3):443-453.

-
- Omoyemi, J.** (2012). Microorganisms associated with smoked fish (*Sardinella*) in selected market locations in Abeokuta zone of Ogun state, Nigeria. In the partial fulfillment for Bachelor degree of agriculture and fisheries management, Federal university of agriculture, Abeokuta, Ogun state.
- Osman, M.; Amer, M.; Badr, M. and Saad, S.** (2015). Prevalence and antimicrobial resistance profile of *Staphylococcus* species in chicken and beef raw meat in Egypt. *Foodborne Pathog Dis* 12(5): 406-413.
- Pornrat, S.; Sumate, T.; Rommanee, S.; Sumolaya, K. and Kerr, W.L.** (2007). Changes in the ultrastructure and texture of prawn muscle (*Macrobrachium rosenbergii*) during cold storage. *LWT - Food Science and Technology*. 40(10): 1747-1754.
- Salama, M. and Ibrahim, A.** (2012). Changes in the quality properties of Grass Carp (*Ctenopharyngodon idella*) fillets smoked during chilled storage. *Journal of Arabian Aquaculture Society*, 7(2): 165-184.
- Salem, A.; Shawky, N. and Abo-Hussein, L.** (2018). "Microbiological Profile of Some Meat Products in Menofia Markets." *Benha Veterinary Medical Journal*. 34(2): 1-7.
- Salem, I.** (2004). Microbiological profile of local smoked fish. Master degree of Veterinary Science Thesis, (meat Hygiene), Faculty of Veterinary Medicine Moshtohor, Zagazig University. Benha Branch, Egypt.
- Sriket, C.; Benjakul, S. and Visessanguan, W.** (2010). Postmortem changes of muscle from fresh water prawn (*Macrobrachium rosenbergii*) as influenced by spawning stages. *Lebensmittel-Wissenschaft und Technologie - Food Science and Technology*. 43, 608-616.
- Tirloni, E.; Stella, S. and Bernardi, C.** (2015). An Efficient and Tasty Use of Atlantic Salmon Trimming: Microbiological and Chemical-Physical Evaluation of Salmon Frankfurters. *Turkish Journal of Fisheries and Aquatic Sciences* 15: 111-117.
- Vishwanath, W.; Lilabati, H. and Bijen, M.** (1997). Biochemical, nutritional and microbiological quality of fresh and smoked mud eel fish *Monopterus albus*- a comparative study. *Food chemistry*, 61(1/2): 153-156.
- Wang, C.; Harris, W.S.; Chung, M.; Lichtenstein, A.H.; Balk, E.M.; Kupelnick, B.; Jordan, H.S. and Laun, J.** (2006). Fatty acids from fish or fish-oil supplements, but not α -linolenic acid, benefit cardiovascular disease outcomes in primary- and secondary-prevention studies: a systematic review. *American Journal of Clinical Nutrition*, 84: 5-17.