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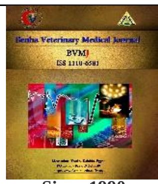

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Prevalence of sulfite-reducing clostridia in some salted marketed fish products

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ABSTRACT

Salted fish products are one of desirable commercial seafood in Egypt, especially in the spring season in the period of spring Eid as an Egyptian tradition. Besides that, however, some sulfite-reducing anaerobic bacteria do not potentiate a public health hazard, it can cause a marked spoilage of the salted fish proteins. Therefore, ninety random samples of salted Mugil cephalus (feseikh) and sardine, forty-five of each, collected from various retailers in Benha city to investigate the prevalence of sulfite-reducing clostridia focusing on Clostridium perfringens (C. perfringens) as a toxigenic food poisoning anaerobe. Out of the examined samples, 26.7% were contaminated with sulfite-reducing anaerobes; where, C. perfringens was the most detected species with incidence of 22.2% and 15.6% for feseikh and sardine samples with mean count (CFU/g) of 3.5x102 and 1.1x102, respectively; revealing feseikh samples of higher contamination level than sardine samples; whereas, Clostridium botulinum was not detected in any of the examined samples. Referring to the Egyptian standards, 81.1% of examined samples were fit for human consumption regarding with sulfite-reducing anaerobic count. So, high hygienic standards should be followed during collection of raw fishes all over the salting and preservation cycle..

1. INTRODUCTION

Fish is a great source of protein because it contains a wide variety of micro and macronutrients (such as calcium, phosphorus, fluorine, and iodine), unsaturated fatty acids, fat-soluble vitamins, and fats that are an excellent source of energy and hypocholesterolemia (a condition that prevents arteriosclerosis) (Maulu *et al.*, 2021).
Fish is one of the most perishable food items; the growth of aerobic spoilage microbes and the chemical effects of atmospheric oxygen reduce the product's shelf life when it is exposed to normal air. Food that has microorganism growth causes changes in color, odor and texture rendering it organoleptically unfit for human eating (Thabet *et al.*, 2016). However, a number of health risks linked to salted fish are caused by anaerobic and facultative anaerobic microorganisms, particularly because of the production of toxins produced by *C. botulinum* and *C. perfringens* as well as their additional putrefactive effects (Lorenzo *et al.*, 2018).
Salted fish products are popular food items in a lot of nations worldwide. One of the earliest methods of preserving fish is salting it. The main goal of this approach is to extend the product's shelf life by reducing the water activity of the fish by dehydration and salt absorption by the muscle. Furthermore, sodium chloride enhances flavor due to its impact on various biochemical pathways, which include decreasing or increasing the enzymatic activity of certain enzymes that are accountable for the production of distinct organoleptic characteristics (Albarracin *et al.,* 2011). But, consumers are more interested in salted fish for its flavor (Ali, 2012).
Numerous variables, including the type and size of fish, the salt used, and the environmental conditions, affect the salting process. Reducing the fish's moisture content to a degree where bacterial and enzyme activity are inhibited, accompanied by the toxic action of chloride ions on the microbial growth, preservation of salting is occurred (Gassem, 2019).
Microbial action, specifically the growth of halophilic bacteria or anaerobic bacteria and yeasts, is what causes spoilage of high salted fish products; which may result in alterations to the sensory and microbial qualities of the salted product (Tahiluddin *et al.*, 2022).
According to the Egyptian Organization of Standardizations (EOS) for microbiological aspects of fish products, salted fish, such as sardine and feseikh, should not contain anaerobic sulfite reducing spore formers counts greater than 10^2 CFU/g. An important and common food poisoning bacteria is *Clostridium perfringens (C. perfringens)*. Both polluted and unpolluted sea sediments, the bacterium has been isolated, even though *C. perfringens* is not a typical fish flora, it is known that other infections can contaminate fish that are harvested from contaminated water (Feldhusen, 2000).
Saad *et al.* (2015) discovered *C. perfringens* in their examined salted fish samples that included food poisoning potential, despite the Center of Disease Control and Prevention (CDC) did not mention fish as a typical source of the infection. In addition, it is one of the most common causes of food poisoning, with the CDC estimating that nearly one million foodborne illnesses occurred in the United States each year due to foodborne illnesses (CDC, 2023).
Therefore, the current study focused on the prevalence of some anaerobic bacteria in traditional salted sardine and *Mugil cephalus* (Feseikh) samples which were collected from different retailers in Benha city, Qalubiya governorate during spring season of 2023.

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2.MATERIAL AND METHODS

This research was carried out after the approval of the Scientific Research Ethics Committee (BUFVTM07-03-24).

2.1. Collection of samples

A total of ninety random samples of salted marketed fish products represented by salted sardine and salted *Mugil cephalus* (Fesiekh) (45 of each) were collected from different retailers at Benha city, Qalubiya Governorate, Egypt. The samples were examined bacteriologically for the anaerobic spore forming bacterial count, and the prevalence of sulfite reducing clostridia during the spring season of 2023.

2.2. Preparation of samples: According to ISO 6887-1 (2017) which based on preparation of tenth fold serial dilution of the collected pectoral muscle of the selected sample. Each prepared sample was subjected for detection and counting of anaerobic bacteria directly.

2.3. Detection and counting of total anaerobic spore formers were performed according to NMKL (2017) using blood agar 5% (HIMEDIA, India), and incubation at 37ºC for 48h.

2.4. Detection of sulfite-reducing clostridia was performed according to ISO/DIS 15213-1 (2021) on Clostridium agar (HIMEDIA, India), followed by microscopical and biochemical identification of the isolated strains. The isolated strains were identified according to Quinn *et al.* (2004).

2.5. Compatibility of the examined samples for human consumption according the Egyptian standards (EOS 1725-p1 and p2 / 2005 a and b).

2.6. Statistical analysis

SPSS version 20 (2011) was used to analyze the data. The significance of the differences in the mean values of the groups under investigation was determined using independent sample T- test analysis. A significance level of $P < 0.05$ was deemed significant.

RESULTS

Referring to the recorded result in Table (1), sixty samples revealed positive anaerobic bacteria with the incidence of 66.7%; where feseikh samples revealed higher contamination level than sardine samples with the incidence of 71.1% (32/45) and 62.2% (28/45), respectively. Moreover, significant difference ($P \leq 0.05$) was detected between the mean counts of the total anaerobic bacteria (CFU/g) with the counts of 6.5×10^3 and 2.8×10^3 for feseikh and sardine, respectively.

Table (1) Statistical analytical counts of total anaerobes (CFU/g) of the examined salted marketed fish samples (n=90).

Samples	Positive samples		Count of CFU/g		
	NO.	%	Min.	Max.	Mean ± SE*
Feseikh	32	71.1**	5.0×10^2	2.3×10^5	$6.5\times10^3 \pm 0.7\times10^{3*}$
Sardine	28	62.2**	1.5×10^2	6.8×10^4	$2.8\times10^3 \pm 0.3\times10^{3*}$
Total	60	66.7***	-	-	-

* Superscript star means significant difference between the same column ($p \leq 0.05$). ** Incidence in relation to the sample number of each (45).
*** Incidence in relation to total number of samples (90).

Out of the positive samples, 24 samples showed positive sulfite-reducing clostridia (SRC) with the incidence of 26.7%. Feseikh samples represented 33.3% with the mean count of 15×10^2 CFU/g; while, sardine samples represented 20% out of the examined samples with mean count of 8.2×10^2 CFU/g; where 77.8 and 84.4% of the examined samples of feseikh and sardine were fit for human consumption in relation to the Egyptian standards referring to the permissible limits of sulfite-reducing clostridia count, respectively (Table, 2).

Table (2) Statistical analytical counts of sulfite-reducing clostridia (SRC) (CFU/g) of the examined salted marketed fish samples (n=90).

Samples	Positive samples		Count of CFU/g			EOS, 2005	Acceptability	
	NO.	%	Min.	Max.	Mean ± SE*		No.	%
Feseikh	15	33.3*	2.0x10	8.0×10^3	$15\times10^2 \pm 2.0\times10^{2*}$	$<10^2$	35	77.8**
Sardine	9	20.0*	6.0x10	3.0×10^3	$8.2\times10^2 \pm 0.03\times10^{2*}$	$<10^2$	38	84.4**
Total	24	26.7**	-	-	-	-	73	81.1***

* Superscript star means significant difference between the same column ($p \leq 0.05$). ** Incidence in relation to the sample number of each (45).
*** Incidence in relation to total number of samples (90).

Out of the isolated SRC strains, *C. perfringens* was detected in the incidence of 22.2 and 15.6% of the examined feseikh and sardine samples, respectively. In addition, *C. bifermentans* was detected only in feseikh samples with the incidence of 4.4%; *C. sporogenes* was detected in the incidence of 6.7% and 4.4% in feseikh and sardine samples, respectively. On the other hand, *C. botulinum* was not detected in any of the examined samples (Table, 3).

Table (3) Prevalence of different sulfite reducing anaerobic bacteria

Samples	SRC positive samples		*C. perfringens*		*C. bifermentans*		*C. sporogenes*		*C. botulinum*	
(n=90)	NO.	%	No.	%	No.	%	No.	%	No.	%
Feseikh (n=45)	15	33.3*	10	22.2*	2	4.4*	3	6.7*	ND	-
Sardine (n=45)	9	20.0*	7	15.6*	ND	-	2	4.4*	ND	-
Total	24	26.7**	17	18.9**	2	2.2**	5	5.6**	ND	-

* Incidence in relation to the sample number of each (45). ** Incidence in relation to total number of samples (90).

Clostridium perfringens, as a significant enterotoxin producing clostridium species, was detected in mean counts of 3.5×10^2 and 1.1×10^2 CFU/g in the positive samples of feseikh and sardine, respectively; revealing a significant higher count in feseikh samples than sardine samples (Table, 4).

Table (4): Statistical analytical counts of *Clostridium perfringens* (CFU/g) of the examined salted marketed fish samples.

Samples	Positive samples		Count of CFU/g		
	No.	%	Min.	Max.	Mean±SE*
Feseikh	10	22.2**	1.0x10	6.5x10^2	3.5x10^2±0.4x10^{2a}
Sardine	7	15.6**	2.0x10	5.0x10^2	1.1x10^2±0.2x10^{2b}
Total	17	18.9***			

* Superscript star means significant difference between the same column ($p \leq 0.05$). ** Incidence in relation to the sample number of each (45).
*** Incidence in relation to total number of samples (90).

4.DISCUSSION

Fish makes up around 60% of the protein that humans need and is an essential food source. Fish provides about one-third of the annual protein needs for the majority of underdeveloped nations (FAO, 2016).

In many cultures, salting fish is a customary processing technique. It is frequently combined with smoking and drying. It is an inexpensive method of preserving fishes and raising up their palatability and acceptability. There are two popular techniques for salting fish: brining, which involves submerging the fish in a salt/water solution, and dry salting, which involves applying salt directly to the fish's surface. Before being salted, fish are divided, sliced, or salted whole (if they are small) (Parvathy, 2018).

Feseikh is a type of salted fish product that is fermented and manufactured from mullet fish. It is regarded as an appropriate material for the fish salting industry due to its popular flesh, high nutritional content, versatility in cooking and preservation methods, high farm cultivation potential, and consumption on special occasions. Because of its straightforward procedure, inexpensive manufacturing costs, versatility in combining with other techniques, and capacity to meet consumer demands, salting is a traditional method of preservation (Ibrahim *et al.*, 2021).

Fish are extremely vulnerable to a variety of microbial pathogens, particularly bacteria, which can primarily be aerobic gram-negative Bacillus species. However, there are very few known anaerobic bacteria that can cause harm to fish products, making it difficult to determine their potential role as fish pathogens (Pękala-Safińska, 2018). The most well-known genus of anaerobic bacteria, Clostridium, includes the species *C. botulinum* and *C. perfringens*, which cause food poisoning symptoms, gas gangrene, and botulism toxicity, respectively (Maikanov *et al.*, 2019).

It is common knowledge that food-borne illnesses in humans are largely caused by fish and fish products. Food that has not been thoroughly cooked or the handling of fish can introduce these microbiological pathogens into humans. Therefore, it is crucial to look into the presence of fish infections in order to guarantee the safety of fish, fish products, and fish habitats (Novoslavskij *et al.*, 2016).

The kind of salt used and the length of the process have an impact on the salting procedure and the final product's properties. Depending on the species and salt/moisture content, salted fish can last up to six months on the shelf (Andrés *et al.*, 2005).

Regarding with the present results, feseikh samples showed higher anaerobic bacterial contamination levels than the examined salted sardine samples, which came in agreement with the recorded results of Saad *et al.* (2015) and Ghanem (2017); whereas, El-Sheshnagui (2006), Edris *et al.* (2017) and Amer (2018) found higher anaerobic bacterial contamination in the examined salted sardine samples than feseikh samples. Higher feseikh level contamination may be referred to higher initial bacterial load of the used mugil fish in feseikh preparation, their culture environment and their added anaerobic fermentation process other than the salted sardine.

Referring to the recorded findings of sulfite-reducing anaerobes in the examined samples (Table, 2), it came in line with those of El-Sheshnagui (2006) who found that the mean values (CFU/g) of sulfite reducing clostridia (SRC) in feseikh and salted sardine samples were 2.9×10^3 and 7.2×10^2, respectively; and Hassan (2011) who found that the mean values (CFU/g) of SRC in feseikh and salted sardine samples were 1.9 ×10^3 and 2.1×10^2, respectively.

Sulfite-reducing anaerobe (clostridia) spores are widely distributed in the environment. They can be found in soil, waste water, and the excrement of both humans and animals. Because they are more resilient to the effects of chemicals and physical elements than vegetative forms, the spores can endure prolonged periods of time submerged in water. They may serve as a sign of contaminated drinking water and groundwater. Within this species, *C. perfringens* is the most significant organism. It frequently corresponds with fecal contamination (García-Prieto et al., 2022).

In Table (3), the detected sulfite-reducing strains could be identified majorly to *C. perfringens,* followed by *C. sporogenes* and *C. bifermentans*, respectively; whereas, *C. botulinum* was not detected in any of the examined samples. At the same line, Saad *et al.* (2021) recorded superiority of *C. perfringens* in their examined fish product samples; where, *C. bifermentans, C. subterminal, C. sporogenes, C. sordelli* were isolated from such examined samples at different percentages. On the contrast, Hamad *et al.* (2022) could detect *C. botulinum* in 41.6% out of the examined fermented and salted fish samples.

Food poisoning from salted fish can be difficult to diagnose because of its wide range of symptoms and potential overlap with other gastrointestinal problems. Spores are dormant forms of *C. perfringens* that aid in the bacterium's resistance to heat, dryness, and other environmental factors. When food is stored at a hazardous temperature (between 40°F and 140°F), for example, *C. perfringens* spores have the ability to become live bacteria that proliferate within the food. Food poisoning can result from eating food contaminated with *C. perfringens* because it can generate a toxin, or poison, which can cause symptoms like nausea, vomiting, diarrhea, stomach discomfort, fever, and dehydration in severe cases (FAO, 2016).

In the current study, *C. perfringens* were isolated from 22.2 and 15.6% of the examined feseikh and sardine samples, with mean values (CFU/g) of 3.5×10^2 and 1.1×10^2 revealing significant higher contamination levels in feseikh than sardine samples, respectively. This came in line with the recorded results of Saad *et al.* (2015) who found *C. perfringens* in the mean count of 2.0×10^2 and 5.5×10^2 CFU/g for sardine and feseikh samples, respectively; whereas, lower incidence was recorded (3.3 and 10.0% for the same samples, respectively); as well as, Nayel (2007) (24 and 36% of the examined salted sardine and feseikh samples, respectively), while Lela (2012) failed to detect *C. perfringens* from all the examined salted samples.

Variation between different authors may be referred to the difference in the initial bacteriological quality of raw fish, season of collection, and the hygienic procedures followed during processing and storage.

5. CONCLUSIONS

After all, in reference to the obtained results, feseikh samples showed higher risk factors regarding with higher prevalence of anaerobic bacterial contamination. High contamination levels may be referred to the time of collection and incomplete period of salting; therefore, application of strict hygienic conditions during salting and storing of such products is essential for keeping consumer's health.

6. REFERENCES

1. Albarracin, W., Sanchrz, I.C, Grau, R, Barat, J.M., 2011. Salt in food processing; usage and reduction; a review. Int. J. of Food Sci. and Technol., 46, 1329-1336.
2. Ali, M., 2012. Shelf life determination of the brined Golden mullet during vacuum refrigerated storage using some quality aspect; Acta Scientiarum Polonorum– Technologia Alimmetaria, 11, 37- 43.
3. Amer, W., 2018. Quality assurance of some fish products. Thesis, Ph.D. of Vet. Med. (Meat Hygiene), Alex. Univ., Egypt .
4. Andrés, A., Rodriguez-Barona, S., Barat, J., Fito, P.J., 2005. Salted cod manufacturing: Influence of salting procedure on process yield and product characteristics. J. Food Engin. 69, 467-471.
5. CDC, 2023. Prevent illness from C. perfringens. Foodborne germs and illnesses. https://www.cdc.gov/foodsafety/diseases/clostridium-perfringens.html
6. Edris, M.A., Hassanien, F.S., Shaltout, F.A., ELbaba, A.H., Adel, N.M., 2017. Microbiological evaluation of some frozen and salted fish products in Egyptian markets. BVMJ, 33, 317-328.
7. El-Sheshnagui, S., 2006. Sanitary evaluation of some salted fishes in Alexandria. Assiut Vet. Med. J., 52, 96-110 .
8. EOS, 2005a. Egyptian Organization for Standardization and Quality No. 1725- part 1: Salted fishes, Feseikh.
9. EOS, 2005b. Egyptian Organization for Standardization and Quality No. 1725- part 2: Salted fishes, Sardine.
10. FAO, 2016. The State of World Fisheries and Aquaculture. https://www.fao.org/3/i5555e/i5555e.pdf
11. Feldhusen, F., 2000. The role of seafood in bacterial foodborne diseases. Microb. Infect., 2, 1651-1660.
12. García Prieto, J., Núñez Núñez, C., Proal Nájera, J., García Roig, M., 2022. Study of coliforms and Clostridium bacteria inactivation in wastewaters by a pilot photolysis process and by the maturation lagoons of a low cost nature based WWTP. Env. Sci. Pollut. Res., 29, 35484–35499.
13. Gassem, M.A., 2019. Microbiological and chemical quality of a traditional salted-fermented fish (Hout-Kasef) product of Jazan Region, Saudi Arabia. Saudi J. Biol. Sci., 26, 137-140.
14. Ghanem, N., 2017. Incidence of pathogenic bacteria in fish products. Thesis, Ph.D. of Vet. Med. (Meat Hygiene), Alex. Univ., Egypt .
15. Hamad, G., Ombarak, R., Eskander, M., Mehany, T., Anees, F., Elfayoumy, R., Omar, S., Lorenzo, J., Abou-Alella, S., 2022. Detection and inhibition of Clostridium botulinum in some Egyptian fish products by probiotics cell-free supernatants as bio-preservation agents. LWT, 163, 113603.
16. Hassan, A., 2011. Chemical and microbiological evaluation of some marketed salted fish in Mansoura. Assiut Vet. Med. J., 57(131), 1-12.
17. Ibrahim, A.I., Rabie, M., Siliha, H., Salama, M., 2021. Physicochemical and microbiological properties of salted fermented fish (feseikh) consumed in Egypt. Zagazig J. Agric. Res., 48, 761-778.
18. ISO, 2017. International Organization for Standardization. No.6887-1. Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1: General rules for the preparation of the initial suspension and decimal dilutions.
19. ISO, 2021. International Organization for Standardization. No.15213-1. Microbiology of the food chain Horizontal method for the detection and enumeration of Clostridium spp. Part 1: Enumeration of sulfite-reducing Clostridium spp. by colony-count technique
20. Lela, R.A.M., 2012. Microbiological studies on some fish products. Thesis, Master of Vet. Med. (Meat Hyg.), Fac. Vet. Med., Benha Univ., Egypt.
21. Lorenzo, J.M., Munekata, P.E., Dominguez, R., Pateiro, M., Saraiva, J.A., Franco, D., 2018. Main groups of microorganisms of relevance for food safety and stability: General aspects and overall description. Inn. Technol. Food Preserv., 2018, 53–107.
22. Maikanov, B., Mustafina, R., Auteleyeva, L., Wiśniewski, J., Anusz, K., Grenda, T., Kwiatek, K., Goldsztejn, M., Grabczak, M., 2019. Clostridium botulinum and Clostridium perfringens occurrence in Kazakh Honey samples. Toxins, 11, 472.
23. Maulu, S., Nawanzi, K., Abdel-Tawwab, M., Khalil, H.S., 2021. Fish nutritional value as an approach to children's nutrition. Front. Nutr., 8, 780844.
24. Nayel, M.S., 2007. Microbiological status of some marketed canned and pickeled fish. Thesis, Master of Vet. Med. (Meat Hyg.), Fac. Vet. Med., Benha Univ., Egypt.
25. NMKL, 2017. Aerobic and anaerobic microorganisms or bacterial spores. 2nd Ed., No. 189. WWW.nmkl.org
26. Novoslavskij, A., Terentjeva, M., Eizenberga, I., 2016. Major foodborne pathogens in fish and fish products: A review. Ann. Microbiol., 66, 1–15.
27. Parvathy, U., 2018. Drying and salting of fish. Indian Council of Agricultural Research. Available on: https://krishi.icar.gov.in/jspui/bitstream/123456789/20503/1/Drying%20and%20salting%20of%20fish.pdf
28. Pękala-Safińska, A., 2018. Contemporary threats of bacterial infections in freshwater fish. J. Vet. Res., 62, 261-267.
29. Quinn, P.J., Carter, M.E., Markey, B., Carter, G.R., 2004. Clostridium species. In: Clinical Veterinary Microbiology, 6th Ed., MOSBY, p. 191.
30. Saad, M.S., Islam, Z., Islam, I.S., Ibrahim, I.A., 2021. Bacteriological evaluation of imported canned fish with special reference to Clostridium perfringens. BVMJ, 40, 56-60.
31. Saad, S.M., Edris, A.M., Salem, A.M., Hassan, E.A., Mostafa, E.M., 2015. Incidence of some food poisoning microorganisms in salted fish. BVMJ 29: 225-229 .
32. Tahiluddin, A., Marıbao, I., Amlani, M., Sarri, J., 2022. A review on spoilage microorganisms in fresh and processed aquatic food products. Food Bulletin, 1, 21-36 .
33. Thabet, M.G., Selim, A.O., Osman, M., 2016. Improvement the shelf life of tilapia fillets stored at chilling condition. BVMJ, 31, 45-55.