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MICROBIOLOGICAL STUDIES ON SOME FISHERY PRODUCTS

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A B S T R A C T

A total of 125 random samples of fish products were collected after different periods from production, 25 each of vacuum- packed salted Mugil cephalus (Fesiekh) ; plastic jars containing salted Fesiekh; vacuum-packed cold smoked herring roe; plastic jars containing cold smoked herring fillets and plastic jars containing salted sardine .These products were produced by a single company where they were subjected to bacteriological examinations for aerobic plate count ,total *Enterobacteriaceae* count, total *Staphylococci* count, *Staphylococcus aureus* count and *Clostridium perfringens* count, as well as mycological examination for count, isolation and identification of moulds and yeasts. The results revealed that the plastic jars containing salted Fesiekh showed relatively higher values of aerobic plate mean count(5.3×10^5/g) than the other products. While the vacuum-packed cold smoked herring roe showed relatively the lowest values in *Staphylococcus aureus* mean count (1.7×10^2 /g). Moreover, Clostridium *perfringens* was absent in all products. *Candida albicans* was the only yeast genera isolated from Vacuumed packed feseikh, Feseikh in jars and Salted sardine fillets,But in vacuum-packed cold smoked herring roe and plastic jars containing cold smoked herring fillets couldn't isolate any yeast genera. While,the mould count was relatively higher in plastic jars containing cold smoked herring fillets. The isolated mould genera form these products were *A.niger, A.flavus, Alternaria,Cladosporium ,Pencillum , Fusarium* and *Mucor* species.

KEY WORDS: Bacteria, Fesiekh, Moulds, Salted sardine, Smoked herring.

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1. INTRODUCTIO

Fish is indispensable in the diet because of its high quality protein content [1]. It may be consumed either in the fresh state or after preservation. The preservation of fish aims to keep it as near its natural state as possible for relatively long time. The preservation of fish may be obtained by salting, smoking, pickling and/or canning of some kinds of fish [14]. Smoked products are traditionally consumed, and one of the most common smoked products is smoked herring. Cold smoked herring and other ready-to-eat fish products could be naturally contaminated with different microorganisms, therefore, they are good carriers of pathogenic bacteria [6]. Faseikh, a traditional Egyptian salted fish, has been considered as a popular part of the Egyptian diet especially in certain celebration times as spring day. Since 1991, the WHO/FAO recorded the first documented outbreak of food poisoning in Egypt due to consumption of uneviscerated salted fish, faseikh [2]. The handling of fish products during the manufacturing process involves a risk of contamination by *Staphylococcus aureus* causing food borne human intoxication [24]. Also may serve as a carrier for several zoonotic bacteria as *E. coli*; *Salmonella; Aeromonas*; *Pseudomonas*; *Proteus*; *Shigella* and *Staphylococcus aureus*, which are incriminated in food

poisoning, skin disorders and allergic conditions as well as other infections [17]. The most common isolates of fungus in smoked fish are *Aspergillus flavus* and *Aspergillus ochraceous* because of their high potential in producing aflatoxin and ochratoxin, respectively. Aflatoxin has been reported to cause acute hepatitis (aflatoxicosis) while ochratoxin is responsible for mitosis in animal kidney [4]. So, the aim of this paper is make attempts to determine the microbiological quality of these fish products.

2. MATERIAL AND METHODS

2.1. *Collection of samples*:
A total of 125 samples of five commercial products were collected from a single company and Fesiekh; vacuum-packed cold smoked represented by vacuum-packed salted Mugil cephalus (Fesiekh) ; plastic jars containing salted herring roe; plastic jars containing cold smoked herring fillets and plastic jars containing salted sardine(25 of each).

2.2. *Preparation of samples*:
Samples were prepared according to ICMSF [15]. Briefly, it was applied as 10g portion of each sample was aseptically weighed into 90 ml of 0.9% NaCl and 0.1% peptone water in a sterile plastic bag, and then blended in a Stomacher 400 Lab Blender (Seward Medical, London, UK) for 30 seconds. Ten-fold serial dilutions were used for microbiological examination.

2.3. *Microbiological examination*:
Aerobic plate count; *S. aureus* counting, isolation and identification were carried out according to APHA [3]. Total *Enterobacteriaceae* count was done according to Gork, [10]. Enumeration of total viable counts of *Clostridium perfringens* was done according to Harmon and Kautter [11]. Total mould and Yeast count was done according to Koburger and Farahat [18]. Identification of mould was done according to Samson et al. [23] and identification of Yeast according to Lodder and Kreger [19] were followed.

3. RESULTS AND DISCUSSION

The results recorded in table (1) showed that the mean values of the aerobic plate count /g of vacuumed packed feseikh, feseikh in jar, vacuumed packed herring, herring fillet in jar and salted sardine fillet in jar were 4.9×10^5, 5.3×10^5, 3.2×10^5, 4.9×10^5 and 4.3×10^5, respectively. These results were nearly similar to those obtained previously [22]. Higher results were reported previously [20]. Lower results were obtained previously [7]. The presence of high viable counts in salted fish indicates cross contamination from different sources such as fresh fishes, the kind of used salt, human and animal wastes, inadequately cleaned equipments and exposure to unsuitable environmental conditions [15]. *Clostridium perfringens* was not detected in all examined samples.
The results in table (2) showed that the mean values of *Enterobacteriaceae* count /g of vacuumed packed feseikh, feseikh in jar, vacuumed packed herring, herring fillet in jar and salted sardine fillet in jar were 13×10^2, 10×10^2, 7.5×10^2, 3.4×10^2 and 8×10^2 , respectively. Such results were nearly similar to those obtained previously [5]. Higher results were recorded previously [7]. The presence of *Enterobacteriaceae* in fish may be related to fecal pollution of surface water or aquatic environment of fish or to improper handling. From zoonotic point of view, it constitutes a public health hazard [21].
The results in table (3) showed that the mean values of total *Staphylococci* counts /g of vacuumed packed feseikh, feseikh in jar, vacuumed packed herring, herring fillet in jar and salted sardine fillet in jar were 4.5×10^3, 3.1×10^3, 1.1×10^3, 1.3×10^3 and 1.4×10^3, respectively.

Table 1 Aerobic plate counts (CFU/g) for the examined fish products samples (n=25).

Samples	No of Positive samples		Min	Max	Mean ± SE
	N	%			
Vacuumed packed Feseikh	25	100	10×10^4	$9.8X10^5$	$4.9\times10^5 \pm 5.3\times10^{4b}$
Feseikh in jar	25	100	3×10^4	9.9×10^5	$5.3\times10^5 \pm 6.2\times10^{4a}$
Vacuumed packed herring	25	100	1×10^4	8.8×10^5	$3.2\times10^5 \pm 5.7\times10^{4b}$
Herring fillet in jar	25	100	20×10^4	8.4×10^5	$4.9\times10^5 \pm 4.2\times10^{4b}$
Sardine fillet in jar	25	100	16×10^4	6.8×10^5	$4.3\times10^5 \pm 2.9\times10^{4b}$

Means (±S.E) with a-c different letters within the same column differ significantly at $P < 0.05$.

Table 2 *Enterobacteriaceae* counts (CFU/g) for the examined fish products samples (n=25)

Samples	No of Positive samples		Min	Max	Mean ± SE
	N	%			
Vacuumed packed Feseikh	4	16	10×10	40×10^2	$1.3\times10^3 \pm 9\times10^{2a}$
Feseikh in jar	3	12	6×10^2	16×10^2	$1.0\times10^3 \pm 2.9\times10^{2\,a}$
Vacuumed packed herring	9	36	10×10	32×10^2	$7.5\times10^2 \pm 3.2\times10^{2a}$
Herring fillet in jar	5	20	1.2×10^2	6×10^2	$3.4\times10^{2\,a} \pm 9.1\times10^{a}$
Sardine fillet in jar	2	8	6×10^2	10×10^2	$8\times10^2 \pm 20\times10^{a}$

Means (±S.E) with a-c different letters within the same column differ significantly at $P < 0.05$.

Table 3 *Total Staphylococci* counts (CFU/g) for the examined fish products samples (n=25)

Samples	No of Positive samples		Min	Max	Mean ± SE
	N	%			
Vacuumed packed Feseikh	20	80	2×10^2	12×10^3	$4.5\times10^3 \pm 7.1\times10^{2a}$
Feseikh in jar	14	56	20×10	8.8×10^3	$3.1\times10^3 \pm 8.4\times10^{2b}$
Vacuumed packed herring	19	76	9×10	7×10^3	$1.1\times10^3 \pm 3.6\times10^{2b}$
Herring fillet in jar	17	68	6×10	7.4×10^3	$1.3\times10^3 \pm 4.4\times10^{2b}$
Sardine fillet in jar	18	72	3.5×10	6.7×10^3	$1.4\times10^3 \pm 4.3\times10^{2b}$

Means (±S.E) with a-c different letters within the same column differ significantly at $P < 0.05$.

The results in table (4) showed that the mean values of *Staph. aureus* count /g of vacuumed packed feseikh, fseikh in jar, vacuumed packed herring, herring fillet in jar and salted sardine fillet in jar were 6.1×10^2 , 2.1×10^2, 1.7×10^2 , 3.2×10^2 and 2.4×10^2 , respectively. Higher results were recorded previously [8]. Presence of *Staphylococcus aureus* in a food indicates its contamination from the skin, mouth and/or nose of food handlers. Inadequately cleaned equipment may be also a source of contamination [25]. The results in table (5) showed that the mean values of total yeast count /g of vacuumed packed feseikh, feseikh in jar, vacuumed packed herring, herring fillet in jar and salted sardine fillet in jar were 3.9×10^2, 5.2×10^2, 0, 0 and 3×10^2, respectively. These results were nearly similar to those obtained by [13]. Higher results were reported previously [9]. Moulds and yeasts are widely distributed in nature and commonly contaminate fish during processing, storage, handling and exposure to other unhygienic environmental factors, and thus become responsible for deterioration of a major portion of such foods in developing countries [12]. The results in table (6) showed that the mean values of total mould count /g of vacuumed packed feseikh, feseikh in jar, vacuumed packed herring, herring fillet in jar and salted sardine fillet in jar were 8.7×10^3, 3.8×10^4, 1.7×10^4, 9.8×10^3 and 9.2×10^3, respectively. These results were nearly similar to those obtained previously [16]. Lower results were reported previously [13].

Table 4 *Staphylococcus aureus* counts (CFU/g) for the examined fish products samples (n=25)

Samples	No of Positive samples		Min	Max	Mean± SE
	N	%			
Vacuumed packed Feseikh	5	20	8.8×10	9.9×10^2	$6.1\times10^2\pm 1.7\times10^{2a}$
Feseikh in jar	6	24	2.2×10	6.6×10^2	$2.1\times10^2\pm 1.0\times10^{2a}$
Vacuumed packed herring	8	76	2×10	6×10^2	$1.7\times10^2\pm$ 7.7×10a
Herring fillet in jar	10	40	3.3×10	8.8×10^2	$3.2\times10^2\pm 1.0\times10^{2a}$
Sardine fillet in jar	6	24	3×10	7.5×10^2	$2.4\times10^2\pm 1.1\times10^{2a}$

Table 5 Total mould counts (CFU/g) for the examined fish products samples (n=25)

Samples	No of Positive samples		Min	Max	Mean± SE
	N	%			
Vacuumed packed Feseikh	19	76	2.2×10^3	6.2×10^4	$8.7\times10^3\pm 2.9\times10^{3a}$
Feseikh in jar	14	56	3.0×10^3	9×10^4	$3.8\times10^4\pm 8.2\times10^{3c}$
Vacuumed packed herring	13	52	2.1×10^3	8×10^4	$1.7\times10^4\pm 6.4\times10^{3ab}$
Herring fillet in jar	17	68	1.0×10^3	9×10^4	$9.8\times10^3\pm 5.0\times10^{3c}$
Sardine fillet in jar	18	72	1.2×10^3	4.4×10^4	$9.2\times10^3\pm 2.6\times10^{3c}$

Table 6 Total yeast counts (CFU/g) for the examined fish products samples (n=25)

Samples	No of Positive samples		Min	Max	Mean± SE
	N	%			
Vacuumed packed Feseikh	11	44	2.2×10	8×10^2	$3.9\times10^2\pm1.0\times10^{2a}$
Feseikh in jar	7	28	6.6×10	9×10^2	$5.2\times10^2\pm1.1\times10^{2a}$
Vacuumed packed herring	0	0	0	0	0
Herring fillet in jar	0	0	0	0	0
Sardine fillet in jar	3	12	6.3×10	6×10^2	$3\times10^2\pm1.6\times10^{2a}$

The results in table (7) revealed that the incidence of the *A.niger* isolated from herring fillet in jar was 64% higher than the other products, while *Mucor* species was absent in vacuumed packed feseikh samples and feseikh in jar samples. *Candida albicans* as one of yeast genera was isolated from the examined vacuumed packed feseikh, feseikh in jar and salted sardine fillet in jar which isolated with percentages of 10%, 13 % and 5%, respectively. While vacuumed packed herring and herring fillet in jar samples were free from any yeast genera. In conclusion, the present study demonstrated that unhygienic handling during harvesting, transportation, salt processing, smoking, storage, handling and packaging techniques such as the use of old news prints, cement papers and polyethylene bags are all sources of contamination of fish which constitute a public health hazard.

Table 7 Incidence of mould and yeast genera isolated from the examined samples of fish products

Samples	A.niger		A.flavus		Alternaria		Cladosporium		Pencillum		Fusarium		Mucor		Candida. albicans	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Vacuumed packed Feseikh	8	32	7	28	5	20	8	32	1	4	6	24	0	0	11	44
Feseikh in jar	8	32	5	20	2	8	9	36	7	28	2	8	0	0	6	24
Vacuumed packed herring	13	52	10	40	0	0	13	52	5	20	3	12	13	52	0	0
Herring fillet in jar	16	64	13	52	2	8	9	36	9	36	3	12	6	24	0	0
Sardine fillet in jar	8	32	9	36	4	16	10	40	2	8	4	16	6	24	3	12

Minimizing contamination of raw material and avoid recontamination of final suitable amount of salt to produce efficiently salted products, shortening the duration of swelling stage and packaging of salted fish in suitable boxes like other products will be helpful to ensure the quality of the different types of salted fish.

4. REFERENCES

1. Abidemi-Iromini, O.A., Olawusi-Peters, O.O., Fadeyi, A. and Bello-Olusoji, O.A. 2011. Smoking impact on the microbial load of Clarias gariepinus. *EJESM* **4**: 3-5.
2. Ahmed, A.M. and Elkazzaz, W.M. 2005. Control of halotolerant Bacteria in salted fish (faseikh) using trisodium phosphate *Pakistan. J. Biol. Sci*. **8**: 882-887.
3. American Public Health Association (APHA) 1992. Compendium of methods for the microbiological examination of food. 3rd ed.
4. Bastaurous, A.F., Abo-EL-Alla, A.A., Sayed, A.M. and Abdel-Sater, M.A. 2000. Microbiological quality of smoked herring fish in Assuit City. *Assiut Vet. Med. J.* **43**: 110- 123.
5. Ayolabi, C.I. and Fagade, E.O. 2010. Mycological evaluation of smoked fish (Ethmalosa fimbriata) from retail outlets in Ago-Iwoye, Ogun state. *Nigeria acta SATECH* **3**: 64 - 68.
6. Dabrowski, W., Różycka-Kasztelan, K., Medrala, D. and Skotarczak, B. 2000. Occurrence of Listeria spp. in vacuum-packed smoked fish. *Folia Universitatis Agriculturae Stetinensis Piscaria* **26**: 23-28.
7. El-kewaiey I.A. 2001. Quality assessment of some locally manufactured and retailed meat and fish products. PhD, Fac. Vet. Med., Kafr El-Shiek, Tanta Univ.
8. El-Shorbagy, I.M. 2005. Some food poisoning microorganisms in salted fish Animal Health Research Institute, Food Hygiene 4th Int. Sci. Conf., Mansoura, 5-6 April. Pp. 55-90.
9. El-Sayed, Y.S.A. 1995. Mycological studies on locally produced smoked fish with special reference to toxigenic strains. PhD, Fac. Vet. Med., Zagazig University.
10. Gork, F.P. 1976. Uber die ursachen Von aualit atsmangeln beitiefgefroten auf fleisch boaicin der fluggast verp flegung. Ding. Dis. Tu-Berlin.
11. Harmon, S.M. and Kautter, D.A. 1978. Media for confirming *Clostridium perfringens* from food and feaces. *J. Food Prot*. **41**: 626-630.
12. Hassan, A.A. and Abdel-Dayem, R.H. 2004. Prevalence of fungi and mycotoxins in fresh and salted fish. *J. Egypt. Vet. Med. Assoc*.**64**: 59-68.
13. Hoda A. Awad, Ragheb, R.R., El-Sharnoby, R. and Zienab Niazi 1998. Mycological study on some kinds of processed fishes with special reference to Penicillium species. *J. Egypt, Vet. Med. Ass.* **58**: 667-678.
14. Ismail, M.A., Nassar, A., Ahmed, A. and Youssef, H. 1994. Mycological status of ready to eat salted fish. *Assiut Vet. Med. J.* **32**: 74-81.
15. International Committee on Microbiological Specification for foods (ICMSF) 1978. Micro-organisms in foods. Their significance and methods of enumeration. 2nd ed., Univ. of Toronto press. Toronto, Canada.
16. Ibrahim, H.A.M. 2000. Incidence of fungal contaminants in fish and fish products. M.V.Sc., Fac. Vet. Med., Zagazig Univ. (Benha branch).
17. Janssen, W.A. 1970. Fish as a potential vector of human bacterial diseases. Symposium on diseases of Fish and Shellfish. Edited by S.F. Snieszko, AFS Special Publication. No.5, Washington, D.C. Pp.284-290.
18. Koburger, J.A. and Farahat, B.Y. 1975. Fungi in foods. V.I. A comparison of media to enumerate yeasts and moulds. *J. Milk and Foods Technol*. **38**: 466-468.
19. Lodder, J. and Kreger-Van, R.I.J. 1970. The yeasts, A taxonomic study. 2nd ed Amsterdam North-Holland publishing Co. Pp. 555-718.
20. Morshdy, A. 1980. Studies on the sanitary condition of salted fishes marketed in Sharkia. PhD, Zagazig University.
21. Mohamed, M.E.M., Maysa A.I. Awadallah, Magda A. Amin and Rasha M. M. Abou-Elez 2001. Role of Atherina Species in Transmitting some Bacterial Diseases to Human. *J. American Science* **7**: 1-20

22. Nayel, M.S. 2007. Microbiological status of some marketed canned and pickled fish M.V.Sc., Fac. Vet. Med., Benha Univ.
23. Samson, R.A., Stollc, A. and Hadlok, R. 1976. Revision on subsection fasciculate of *pencillium* and some allied species. *Stud. Mycol*. **11**:1-47.
24. Shena, S.S. and Sanjecv S. 2007. Prevelence of enterotoxigenic *Staphylococcus aureus* in fishery products and fish processing factory work. *Food Control. J*. **18**: 1565-1568.
25. Thatcher, F.S and Clark, D.S. 1978. Microorganisms in food. 1st Int. Comm. Microbial Specific foods. Toronto. Univ. Press. Toronto and Buffalo, Canada. Pp. 5-15.

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مجلة بنها للعلوم الطبية البيطرية

دراسات ميكروبيولوجية علي بعض منتجات الاسماك

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الملخص العربى

تعتبر الأسماك المدخنة كالرنجة والمملحة كالفسيخ والسردين من الأكلات المصرية القديمة المحببة للشعب المصري خاصة في أعياد الربيع، لذلك أجريت هذه الدراسة لتقييم الحالة الميكروبيولوجيه لخمسه منتجات(25 عينه لكل منتج) وهما فسيخ معبأ بالتفريغ و رنجة معبأة بالتفريغ وبرطمانات بلاستيكيه تحتوي علي شرائح رنجة مدخنه وبرطمانات سردين وبرطمانات فسيخ.وقد وجد ان العد الكلي للميكروبات الهوائية والميكروبات المعوية في برطمانات الفسيخ اعلي نسبيا من المنتجات الاخري. بينما خلت جميع المنتجات من ميكروب الكلوستريديم برفرنجنز. وسجلت عينات الفسيخ المعبأة بالتفريغ اعلي نسبه تواجد للميكروب العنقودي الذهبي و اعلي نسبه تواجد للخميرة بينما خلت عينات الرنجة المعبأة بالتفريغ وبرطمانات شرائح الرنجة المدخنة من الخمائر. وكان نوع الخميرة المعزولة هو الكانديدا البيكانز. وسجلت برطمانات شرائح الرنجة المدخنة اعلي نسبه من وجود الفطريات وكانت الفطريات المعزولة من المنتجات من أنواع الاسبراجيلس والبنسليوم والميكور والالترناريا وفيوزيريم وكلادوسبوريم.

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