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MICROBIOLOGICAL QUALITY OF SMOKED HERRING FISH IN ASSIUT CITY

(With 4 Tables)

By

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الحالة الميكروبيولوجية لأسماك الرنجة المدخنة بمدينة أسيوط

الفونس فخرى بسطاوروس ، عبد الحكيم أبو العلا ، أشرف محمد سيد ، محمد أحمد عبد الساتر

أجريت هذه الدراسة على عدد ثلاثة وثلاثون عينة عشوائية من الأسماك المدخنة (الرنجة) المعروضة بالأسواق والمحلات ذات المستويات الصحية المختلفة بمدينة أسيوط وذلك لفحصها ظاهريا و لتقييم الحالة الميكروبيولوجية لها حيث تبين صلاحية هذه العينات ظاهريا للاستهلاك الأدمي. وأن متوسطات العدد الكلى للميكروبات الهوائية , المكور السبحي , المكور العنقودى الذهبى , الباسيللس سيريس والميكروبات العصوية المعوية ٥.٢ × ١٠^{٤} , ٤.٩ × ١٠^{٢} , ٧.٨ × ١٠^{٣} , ١.٦٥ × ١٠^{٣} , ٩.٢ × ١٠^{٢} لكل جرام على التوالي هذا وقد أمكن عزل وتصنيف العديد من الميكروبات العصوية المعوية بينما لم يستدل على وجود السالمونيلا والشيجيلا. أثبت الفحص الميكولوجى باستخدام وسطى Potato Dextrose Agar وال Czapek's agar الذى يحتوى على ١٠% كلوريد صوديوم أن متوسط العدد الكلى للفطريات كان ١٠٦٠ , ١٥٠٠ لكل جرام أما متوسط عدد الخمائر فكان ١٥٠ , ١١٠ لكل جرام بالترتيب. تم عزل وتصنيف ٨ أجناس من الفطريات بالإضافة إلى عدد من الخمائر الغير مصنفة من أنسجة الأسماك المدخنة ولقد نوقشت الأهمية الصحية للميكروبات التي تم عزلها ومدى خطورتها على الصحة العامة والطرق المقترحة الواجب إتباعها لدرء خطرها.

SUMMARY

Thirty-three random samples of smoked herring fish were collected from different markets and shops of different sanitation levels at Assiut City.

All samples were examined organoleptically, bacteriologically and mycologically. The average values of microbial counts were 5.2×10^4/g. smoked fish for aerobic plate count, 4.9×10^2/g. fish for enterococci count, 7.8×10^3 /g. fish for *Staphylococcus aureus* count, 1.65×10^3 /g. fish for *Bacillus cereus* count and 9.2×10^2 /g. smoked fish for *Enterobacteriaceae* count. The different *Enterobacteriaceae* isolates recovered from the examined samples were identified biochemically. No *Salmonella* and *Shigella* species were not isolated from any sample of smoked fish. On the other hand, the average values of filamentous fungi were 1060 and 1500 colonies/g, while the average values of total yeast count were 150 and 110 colonies/g. on Potato Dextrose Agar and 10% NaCl Czapek's agar media at 28° C respectively. At the same time a total of 8 genera in addition to some unidentified species of yeasts were isolated and identified from smoked fish samples. The public health importance and hygienic significance of the isolated organisms as well as some suggested measures for improving the quality of such product were discussed.

Key words: Microbiology, Herring

INTRODUCTION

Herring is a kind of smoked fish which may be held in brine and freshened prior to smoking. Herring is subjected to many risks of either primary or secondary contamination. Such contaminants may render the smoked fish unfit for human consumption or even harmful to consumers (Van Den Broek et al., 1984).

The smoking process retards the microbial activity of fish by showing a slight decrease in bacterial counts. The action of smoking and dehydration, however, is not sufficient to reduce the bacterial counts significantly (Deng et al., 1974).

Spoilage of smoked fishes during storage is considered an important and dangerous problem facing smoked fish producers either on the local or international level. Fungal contamination is considered the main spoilage agent of smoked fish which lead to impart musty off-flavours, sliminess, lipolysis and unpalatable taste that render the product of inferior quality, unmarkaetable or even unfit for human consumption that may constitute a public health hazard and severe economic losses (Ward and Baaji, 1988).

In Egypt, few surveys of microbial evaluation of smoked fish have been carried out (Hafez et al., 1992 and Edris, 1996). The current study was planned to evaluate the bacteriological and mycological quality of smoked herring fish marketed at Assiut City.

MATERIAL and METHODS

Collection of samples:

Thirty-three random samples of smoked herring fish of moderate size were collected from some markets and shops of different sanitation levels at Assiut City. The samples were collected and transferred directly to the laboratory, under aseptic conditions with a minimum of delay, where they were subjected to bacteriological and mycological examinations.

I- Organoleptic examination:

Organoleptic examination of smoked fish samples was evaluated for their skin condition, consistency, colour and odour of the fish flesh according to F.A.O. (1985).

Preparation of samples:

The samples were prepared according to the technique adopted by ICMSF (1978).

II- Microbiological examination:

A- Bacteriological examination:

1- Total colony count:

It was carried out as recommended by Dodds et al. (1992).

2- *Staphylococcus aureus* count:

It was determined using surface plating on Baird-Parker agar medium (ICMSF, 1980). The suspected colonies were subjected to coagulase test for confirmation according to Thatcher and Clark (1978).

3- Enterococci count:

This was determined using Enterococcus Selective Differential agar medium (E.S.D) as described by Efthymiou and Joseph (1974).

4- *Bacillus cereus* count:

The technique adopted is that recomended by (ICMSF, 1978) using Mannitol egg-yolk polymyxin (MYP) agar medium. The countable colonies were picked up, purified and identified according to Shinogawa (1993).

5- Total *Enterobacteriaceae* count:

The technique adopted here is that recommended by Mercuri and Cox (1979), using violet red bile glucose (VRBG) agar medium.

6- Isolation of *Enterobacteriaceae* organisms:

i) Isolation of *Salmonella* and *Shigella* spp.

Lactose broth and Selenite F. broth were used as pre-enrichment and enrichment media. While three specific solid media (Bismuth sulphite agar, MacConkey's agar and S.S. medium) were used as plating media. Suspected non-lactose fermenters colonies were screened morphologically and biochemically according to Koneman et al. (1994).

ii) Isolation and identification of other members of *Enterobacteriaceae* organisms, using MacConkey broth's as enrichment broth medium while Brilliant green phenol red and MacConkey's agar media were used as plating media. Suspected colonies were isolated in pure culture for further identification according to Koneman et al. (1994).

B- Mycological examination:

1- Total mould and yeast count: (Johnson and Curl, 1972).

Collected samples were examined for determination of total mould and yeast count/gm, using the dilution-plate method. Duplicate plates each of Potato Dextrose Agar (PDA) and 10% NaCl-Czapek agar media were used as plating media. The plates were incubated at 28°C for 10-15 days. During which the grown fungi were counted, identified and calculated per gram.

2- Identification of the isolated fungi:

The grown fungi were identified based on macro-and microscopic characteristics according to Domasch et al. (1980) and Kozakiewicz (1989).

RESULTS

The gained results of the examined samples are summarized in Tables 1, 2, 3 and 4.

DISCUSSION

I- Organoleptic examination:

The organoleptic examination showed no abnormalities and all the samples were sound as shown in (Table 1).

Table 1: Summarized results of organoleptic inspection of examined smoked fish samples (N = 33 samples)

Items	Characters	No.	%
Skin colour	Golden yellowish	33	100
	Dark brown	0	0
Consistency	Firm	33	100
	Friable	0	0
	Soft	0	0
	Dry	0	0
Flesh odour	Smoked fishy flesh	33	100
	Musty	0	0
Flesh taste	Characteristic smoked fishy delicious taste	33	100
	Salty	0	0
	Musty	0	0
	Bitter	0	0

II- Microbiological examination:

A- Bacteriological examination:

The existance of somewhat large numbers of viable counts in smoked fish where the average was $5.2x10^4$/g as shown from Table (2) indicates cross contamination from different sources such as fresh fishes, salt used, human and animal wastes, inadequately cleaned equipment and exposure to unsuitable environmental conditions (Thatcher and Clark, 1978). It shoud be mentioned that the room temperature storage of herring where the prevaling climatic condition is very hot in upper Egypt, would accelerate the growth rate of the pre-existing organisms. Therefore, storage and distribution of smoked fish at temperature ≤ 6°C was recommended (Karnop, 1980).

The obtained results agree, quite well, with those reported by Dodds et al. (1992) and Edris (1996) who found that the average total colony counts were less than10^5/g and 1.48×10^4/g respectively. On the other hand, Zorn et al. (1993) reported higher count ≥. 10^6/g.

The results of the present study revealed that the enterococci were detected in 33.33% of the samples with an average count of 4.9×10^2 /g . Nearly similar counts but of higher incidence (48%) were recorded by Abd El-Daym (1999). On the other hand, Edris (1996) reported high incidence (66.67%) but of lower count (2×10 to 1.2×10^2/g).

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Enterococci, are considered as potential pathogens causing disease in human while, *Enterococcus faecalis* is considered as the etiological agent of food poisoning (Hafez et al., 1992). This organism is used by most investigators as an indicator of faecal pollution. Enterococci contaminate the food through the main source of infection which is the gastrointestinal tract of man and animals (Mousa and Mahmoud, 1997).

From Table (2), it is apparent that 18 (54.52%) of the samples contained *Staphylococcus aureus* with an average count of 7.8×10^3/g. The counts are nearly similar to that obtained by Dalmacio et al. (1988)[8.2×10^3/g] and Hafez et al. (1992)[2.2×10^3/g].

The presence of *Staphylococcus aureus* in a number of examined smoked fish samples suggests the poor handling and/or the improper storage methods given to such commodities. It is also a good indicator of the poor personal hygiene of food handlers: the organism orginates from a suppurating lesion or from the nostrils of a carrier (Elwi, 1994). Smoked fish being a commonly consumed product, can be considered as a potential cause of food poisoning (Dalmacio et al., 1988).

It is worth mentioning that the presence of *Staphylococcus aureus* in herring is not surprising because this organism is a halotolerant organism, since it can grow in food which contain from 10 to 20% NaCl concentration, although the upper limit may be lower due to the presenee of other inhibitory factors (Marcy et al., 1985).

The findings recorded in Table (2) exhibit that *Bacillus cereus* was detected in 5(15.15%) samples with an average count of 1.65×10^3 /g; higher results were reported by Plahar et al. (1991). *Bacillus cereus* had been reviewed in food poisoning cases linked with packed fish (Schmitt et al., 1976). It was also implicated in two distinct forms of food poisoning, a rapid onset emetic syndrome and a delayed onset diarrhoeal syndrome (Van Netten et al., 1990). However, in the present study, *Bacillus cereus* was found in smoked herring fishes, in such counts that may be considered innocuous since the minimum level required to cause illness had been estimated to be > 10^5/g. (Hobbs, 1974).

The achieved results in Table (2) reveal that the incidence of *Enterobacteriaceae* in herring examined was 30.30% with an average count of 9.2×10^2/g. Such count is considered an indication of inadequate

sanitary conditions during preparation, handling and storage (ICMSF, 1978).

The present findings agree, to a certain extent, with that reported by Abd El-Daym (1999). Higher results were reported by Edris (1996). While, Hafez et al. (1992) reported lower counts.

Table 2: Results of values of bacteriological examination of 33 samples of smoked herring fish.

Microbial count	Positive samples		Microbial count/g		
	No.	%	Minimum	Maximum	Average
Total colony count	33	100	6.8×10^2	1.2×10^5	5.2×10^4
Staph. Aureus count	18	54.55	3.1×10	5.9×10^4	7.8×10^3
Enterobacteriaceae count	10	30.30	1.3×10^2	3.2×10^3	9.2×10^2
Bacillus cereus count	5	15.15	1×10^2	7.2×10^4	1.65×10^3
Enterococci count	11	33.33	9.3×10	8.1×10^3	4.9×10^2

The current study revealed the isolation of some members of family *Enterobacteriaceae* from the examined samples of smoked herring fish at varied percentages ranging from 3.03% to 39.39% (Table 3), and these organisms were identified as *Enterobacter* spp. (*Entero. aerogenes*, *Entero. cloacae*), *Proteus* spp. (*P. mirabilis*, *P. vulgaris*, *P. rettegri*), *Klebsiella* spp. (*K. pneumonia*, *K. oxytoca*) and *Escerichia coli*. These findings agree, to a certain extent, with those reported by Godayigaya and Debevere, (1990), Hafez et al. (1992) and Abd El-Daym (1999).

It should be noted that *Salmonella* and *Shigella* species were not isolated from any sample of smoked fish. This could be explained in the view that hot-smoke process was sufficient to destroy these organisms.

Our results substantiate what had been reported by Godayigaya and Debevere, (1990) and Dodds et al. (1992). On the other hand, they disagree with that reported by Heinitz and Johnson (1998) who isolated *Salmonella* species from 3.2% of 156 smoked fish samples and postulated that *Salmonella* spp. source to be post process contamination. Processing environment, processing methods and seasonal sampling may all contribute to these differences, which warrant further study.

Proteus species had been incriminated in cases of some infantile diarrhoea and urinary tract infection (Frazier, 1967). Furthermore, in Egypt Mostafa et al. (1948) reported that 30 out of 125 food borne outbreaks were due to *Proteus* organisms. Also *klebsiella* organisms are

implicated in many cases of food poisoning outbreaks in patients introduced to hospitals with symptoms of severe diarrhoea and abdominal cramps (Horvath et al. 1964).

Escherichia coli is taken as an index to indicate faecal contamination, as it is a normal inhabitant of the intestinal tract of both man and animals and always present in manure (Garrad, 1946). The enteropathogenic serotypes of *E. coli* were proved to induce severe diarrhoea in infants and young children and had been implicated in cases of food poisoning and gastroenteritis among consumers (Brayan, 1992). In this study, a good number of food borne potential pathogens was found in smoked fish at different rates of contamination. The contaminated smoked fish in this case is considered a consumer's health risk especially in certain national occasions such as feasts and Sham El-Neseam because herrings are ready-to-eat food. Therefore, this product should be routinely tested for the presence of these potential pathogens.

The Egyptian standard guidelines consider a smoked fish violative if its total bacterial count is more than 1×10^5, *Staphylococcus aureus* count is more than 1×10^3. Guidelines also consider a product violative if pathogenic organisms and coliform group are detected (Egyptian Standard, 1985).

Table 3: Incidence and frequency distribution of *Enterobacteriaceae* organisms recovered from 33 samples of smoked herring fish.

Isolates	No.	%
Enterobacter spp.		
Enterobacter aerogenes	6	18.18
Enterobacter cloacae	13	39.39
Proteus spp.		
Proteus mirabilis	8	24.24
Proteus vulgaris	5	15.15
Proteus rettegeri	3	9.09
Klebsiella spp.		
Klebsiella pneumonia	7	21.21
Klebsiella oxytoca	2	6.06
Escherichia coli (E. coli)	1	3.03
Salmonella spp.	0	0
Shigella spp.	0	0

B- Mycological examination:

A total number of 8 genera of filamentous fungi (mould) in addition to some unidentified species of yeasts were isolated and identified from 33 samples of smoked herring fish on Potato Dextrose Agar (PDA) [7 genera & 17 species] and on 10% NaCl Czapeek's agar [6 genera & 12 species] at 28°C. It was observed that the numbers of filamentous fungal genera and species recovered on PDA were higher than those recovered on 10% NaCl Czapek's agar medium (Table 4). This may be attributed to the effect of salt which inhibits the growth of some species of fungi as reported by Atapattu and Samarajeewa (1990). Some Egyptian authors could isolate the same fungi from smoked fish (Edris, 1996 and Youssef, 1998).

The most common mould genera isolated from smoked fish were *Aspergillus* and *Penicillium* which were recovered from 46% and 52%, 44% and 49% of samples on PDA and 10% NaCl Czapek's agar media respectively. They included a wide range of species representing by 7 species for *Aspergillus* and 4 species for *Penicillium* (Table, 4). During the growth of these moulds a gradual increase in amounts of volatile acid was produced. It was noticed that *Penicillium* destroys the constituents of meat more quickly than the *Aspergillus* (Butjagin, 1905). Smoked fishes can be contaminated by these mould genera mainly from contaminated smoke chamber and wood smoke as well as saw dust used in smoking of fish (Graikoski, 1973).

The filamentous fungal average count on PDA medium was 1060/g of the examined smoked fish samples, while on 10% NaCl Czapek's agar medium higher average count (1500/g) was obtained

Therefore, in the present study low mould counts ($< 10^5$) particularly on PDA medium was recorded which compel the recommended standard by Egyptian Specification (1991), and are somewhat similar to those reported in Ghana by Lu et al. (1991). In comparison moderately high counts were registered by Youssef (1998), while Hafez et al. (1992) recorded higher counts. Such variations are expected and may be attributed to the variations in the sanitary measures adopted in handling of such perishable foods.

The third predominant genus of the isolated moulds from smoked fish samples was *Eurotium*. *Eurotium amstelodami*, a well known halophilic species, was isolated only on 10% NaCl-Czapek's agar medium. It was encountered in 33% of samples examined.

Table 4: Average total counts and percentage frequency of fungal genera and species isolated from 33 samples of smoked herring on 2 types of media.

Genera & species	PDA		N.C.A	
	ATC	F%	ATC	F%
Alternaria	80	18	140	21
A. alternata	30	9	120	18
A. chlamydospora	40	12	0	0
A. tenuissima	10	3	20	6
Aspergillus	370	46	430	52
A. flavus	160	18	190	42
A. niger	170	40	200	46
A. ochraceus	0	0	30	3
A. oryzae	20	6	0	0
A. sydowii	0	0	10	3
A. Terreus Thom	10	3	0	0
A. versicolor	10	3	0	0
Cladosporium	80	15	20	6
C. cladosporioides	30	9	20	6
C. sphaerospermum	50	13	0	0
Eurotium amstelodami	0	0	180	33
Gibberella Fujikuroi	10	3	0	0
Mucor racemosus	10	3	0	0
Penicillium	390	44	670	49
P. aurantiogriseum	80	12	100	13
P. chrysogenum	50	9	0	0
P. corylophilum	160	28	350	37
P. oxalicum	100	24	220	26
Ulocladium alternariae	120	18	60	12
Average filamentous fungi (mould) count	1060		1500	
Yeasts	150	21	110	15
Total counts	1210		1610	
Number of genera = 8	7		6	
Number of species = 20	17		12	

Abbreviations:
PDA = Potato Dextrose agar medium **N.C. A. = 10% NaCl Czapek agar medium**
ATC = Average total counts. **F% = Percentage frequency.**

As regards yeasts, the average total count/gram of the examined fish samples was 150 colonies/g and 110 colonies/gram on PDA and NaCl-Czapek's agar media respectively, higher results were reported by Edris (1996). Yeasts may reach the product during processing, handling and distribution due to neglected hygienic measures.

Contamination of smoked fish with the objectionable moulds and yeasts will produce undesirable changes of fish rendering it unfit for marketing. It will also increase the risk of infection by many fungi of public health hazard which reflects the possibility of developing serious diseases to consumers (Edris, 1996). Furthermore, many strains of moulds are able to produce toxic metabolites (mycotoxins) which possess carcinogenic properties producing high levels of liver cancer, liver disease and organs, damage (Goldblatt and Stoloff, 1983).

Conclusively, strict hygienic measures must be applied during different steps between fishing and marketing and measures should be adopted to minimize fish handling. Besides, education programmes should be imposed for producers and handlers for the proper storage of smoked fish at low temperature under hygienic measures. The concerned authorities should also impose regulations and microbiological standards for marketed smoked fish. All these suggestive measures should be conducted in order to minimize the risk of bacterial and fungal contamination.

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