Vet.Med.J.,Giza. Vol.46, No.4. A (1998): 375-381.

LIPOLYTIC AND PROTEOLYTIC FUNGI IN LOCALLY PRODUCED SMOKED MACKEREL

BY

AMAL M. EL-SHERIF, NADA K. MANSOUR AND NABIL A. YASSIEN

Dept. of Food Hygiene, Fac. Vet. Med., Cairo Univ.

SUMMARY

A total number of 100 samples of locally produced smoked Mackerel were collected at random from Cairo and Giza markets. Samples were examined for determination of pH, contamination with both lipolytic and proteolytic fungi, as well as, identification of the present lipolytic - and proteolytic moulds. The mean pH value of samples was 5.19 ± 0.1. Lipolytic moulds and yeasts could be recovered from 67 % and 30 % of the examined samples respectively, while 22 % and 30 % of samples were found to contain proteolytic-moulds and yeasts respectively. Identification of the isolated 527 lipolytic moulds reveal the presence of 4 different genera, namely Cladosporium, Aspergillus, Scopulariopsis and Alternaria, of which the former was the most frequent (more than 90 %). Identification of the isolated 98 proteolytic moulds revealed the presence of species of genera Cladosporium and Scopulariopsis, of which Cladosporium was the most frequent (more than 97 %). Isolated Aspergillus and Alternaria species showed no proteolytic activities. The economic and public health significance of contamination of smoked fish with such moulds was discussed.

INTRODUCTION

Smoking is a very old preservation process, and still widely used in many countries to impart flavor and/or color to some food products rather than preservation. Smoked fish is a popular type of fish products that was consumed at a large scale in Egypt, especially in picnics and some occasions as "Sham Elnaseem".

The most common types of fish used for smoking are Mackerel, Herring and Salmon. Mackerel is widely used locally for smoking, as it is one of those fish that have high protein and fat content and is considered as "fatty fish" (Herbert et al., 1971).

In foods, especially those types with low water

activity or low pH value, fungi cause more destruction of stored products than any other agents, resulting in spoilage. On the other hand, growth of fungi in food is a health hazard as they may produce mycotoxins, causing failure of liver and kidney functions, and induction of cancer (Smith and Haas, 1992 and ICMSF, 1996).

Although smoke is a complex of more than 200 compounds, including antioxidative and bactericidal ingredients, however fungi may grow on the surface of the hot smoked fish products if they are kept at room temperature for 3-4 days (FAO, 1970 and Gilbert and Knowles, 1975).

The pH value is one of the quality parameters which can be estimated to detect deterioration of smoked fish (Connell, 1990). Smoking of fresh fish results in falling in the pH due to the action of smoke rather than salting (El-Akeel, 1988 and Kansmadi and Goncharov, 1979).

Smoked and dried fish are more likely to undergo fungal spoilage through utilization of protein and lipids, however the count of lipolytic and proteolytic microorganisms are not performed on a routine basis. Food manufacturers and processors usually enumerate these organisms only when a problem occurs (Jay, 1978; Koburger & Marth, 1984 and Smith and Haas, 1992).

Due to the scanty of the available literature about the mycological quality of locally produced smoked Mackerel, this study was carried out to throw the light on the degree of contamination of such food product with lipolytic and proteolytic fungi.

MATERIAL AND METHODS

Collection of samples:

A total numbers of 100 random samples of locally produced smoked Mackerel were collected from Cairo and Giza markets, each sample was placed in a separate case before sending to the laboratory to be examined as follow:

1- Determination of pH value:

The technique recommended by AOAC (1975) was carried out using digital pH meter.

2- Preparation of samples for mycological examination:

The technique described by ICMSF (1978) was carriedout on 10 grams from the dorsal muscle of each sample, and ten fold decimal dilution was prepared.

3- Determination of lipolytic fungal count/g.:

The procedure given by Koburger and Jaeger (1987) was followed by inoculating the appropriate decimal dilutions on to the surface of Tributyrin agar plates containing Nile blue sulphate stain and supplemented with 0.05 mg. chloramphenicol "Park & Davis" per ml. Inoculated plates were incubated at 22°C for one week. Yeast or mould colonies showing lipolytic zones were counted, and the lipolytic-yeast or mould count/g. sample was determined.

4- Determination of proteolytic fungal count/g.:

The technique recommended by ICMSF (1978) was carried out by inoculating the appropriate decimal dilutions on to the surface of skim milk agar plates (APHA, 1985) supplemented with 0.05 mg. Chloramphenicol "Park & Davis" per ml. Inoculated plates were incubated at 22°C for one week. Yeast or mould colonies surrounded by clear zones of proteolysis were counted, and the proteolytic-yeast or mould count/g. sample was determined.

5- Isolation and identification of lipolytic -and proteolytic moulds:

The procedure was performed according to Raper et al., 1965; Domsch et al., 1980 and Samson et al., 1981.

6- Microscopic examination of isolated moulds:

The techniques recommended by Bailey and Scott (1985) were followed up.

RESULTS

Table (1): Statistical analytical results of the examined smoked Mackerel samples based on their pH value.

No. of samples	Minimum	Maximum	Mean	S.E.M. ±
100	4.95	5.36	5.19	0.1

Table (2): Incidence of lipolytic and proteolytic fungi in examined smoked Mackerel samples

No. of samples	Lipolytic fungi %		Proteolytic fungi %	
	Moulds %	Yeasts %	Moulds %	Yeasts %
100	67	30	67	30

Table (3): Statistical analytical results of the examined smoked Mackerel samples based on their lipolytic and proteolytic fungal count/g.

	Lipolytic fungi		Proteolytic fungi	
	Moulds	Yeasts	Moulds	Yeasts
Minimum	$< 10^2$	$< 10^2$	$< 10^2$	$< 10^2$
Maximum	$6.7x10^4$	$4x10^5$	$4.6x10^4$	$7.6x10^4$
Mean	$6.7x10^3$	$11x10^3$	$1.8x10^3$	$1.8x10^3$
S.E.M. ±	$2x10^2$	$8x10^2$	$1x10^2$	$1.5x10^2$

Table (4): Different species of isolated lipolytic and proteolytic moulds from smoked Mackerel.

Mould species	Lipolytic fungi		Proteolytic fungi	
	No. of isolates	%	No. of isolates	%
1- Cladosporium				
C.herbarum	393	74.64	52	53.06
C. sphaerospermum	72	13.72	-	-
C. macrocarpum	18	3.48	17	17.35
C. cladosporioides	1	0.19	27	27.55
2- Aspergillus				
A. fumigatus	22	4.17	-	-
A. flavus	5	0.95	-	-
A. niger	2	0.38	-	-
A. oryzae	1	0.19	-	-
A. glaucus	2	0.28	-	-
3- Scopulariopsis				
S. candida	9	1.71	2	2.04
4- Alternaria				
A. Alternata	1	0.19	-	-
Total	527	100	98	100

DISCUSSION

Results given in Table (1) revealed that the pH value of the examined smoked Mackerel ranged from 4.96 to 5.36 with a mean value of 5.19 ± 0.1. Nearly similar results were recorded by Kansmadi and Gancharov, 1979, while higher figures were reported by El-Shater (1994). The reduction in the pH value of smoked fish may be due to the formation of organic acids in the smoke contents (El-Akeel, 1988).

It is obvious from Tables (2&3) that lipolytic moulds and yeasts could be recovered from 67 % and 30 % of the examined smoked fish samples respectively, with a mean count of $6.7x10^3 \pm 2x10^2$ and $11x10^3 \pm 8x10^5$/g. respectively. On the other hand, the proteolytic moulds and yeasts could be recovered from 22 % and 38 % of the examined smoked fish samples respectively, with a mean count of and $1.8x10^3 \pm 1x10^2$ and $1.8x10^3 \pm 1.5x10^2$ /g. respectively. The high rate of contamination of the examined smoked fish samples with lipolytic fungi may be attributed to the wide distribution of fungi in the environment as airborne contaminants, or may be due to inadequate sanitation during processing and storage

(Koburger and Marth, 1984).

It is evident from the results presented in Table (4) that a total of 527 lipolytic mould strains could be isolated from the examined samples. Identification of these isolates revealed that species of the genus Cladosporium were the most predominant as they constitute more than 90 % of the total isolates. Cladosporium species are known as Dematiaceous hyphomycetes (dark moulds) originated mainly from the soils and intestinal contents (Mansour et al., 1991). Cladosporium herbarum represented 74.64 % of the isolates, while C. sphaerospermum, C. macrocarpum and C. cladosporioides represented 13.72 %, 3.48 % and 0.19 % of the total isolated lipolytic moulds respectively. Aspergillus species came on the second position of the isolated lipolytic moulds, the identified Aspergillus were A. fumigatus (4.17 %), A. flavus (0.95 %), A. niger (0.38 %), A. oryzae (0.19 %) and A. glaucus (0.38 %). Scopulariopsis candida and Alternaria alternata represented 1.7 % and 0.19 % of the isolated lipolytic moulds respectively. Nearly similar moulds species were isolated by Jonsyn and Lahai, (1992) and El-Shater, (1994).

Cladosporium and Aspergillus species are often found on the surface of smoked fish which are exposed to air, when these surfaces become dehydrated during storage, bacterial growth is inhibited and mould growth becomes visible (Jay, 1978).

Although the counts of lipolytic microorganisms generally are not performed on a routine basis, the food manufacturers and processors usually enumerate lipolytic types only when a problem occurs.

Fatty fishes are susceptible to hydrolysis and oxidation, moulds and yeast are capable of causing these deteriorations. Aspergillus species are among the lipolytic moulds (Bours and Mossel, 1973). Most of isolated Aspergilli are toxin producers (Leistner and Eckardt, 1981). Aflatoxins produced by some Aspergillus species in smoked fish were detected by Farahat and Koburger, 1975 and El-Shater, 1994).

It is worth mentioning that the storage temperature of hot smoked fish in Egypt especially in summer (upper mesophile) is suitable for the rapid growth of A. flavus and aflatoxins production (Farahat and Koburger, 1975 and ICMSF, 1996).

Mackerel is one of those fish that contains comparatively high level of proteins (18.7 %) and other nitrogenous constituents (Herbert et al., 1971). Moulds and yeasts are capable of hydrolysing a wide range of proteinaceous materials (Koburger, 1972). Concerning the proteolytic moulds (Table 4), a total of 98 strains were isolated, most of them were Cladosporium species which identified as C. herbarium (53.06 %), C. cladosporioides (27 %) and C. macrocarpum (17 %), while the rest of proteolytic moulds were identified as Scopulariopsis candida (2.04 %). It is obvious from the obtained results that most of the isolated Cladosporium and Scopulariopsis

species posses both lipolytic and proteolytic activity.

In some foods the level of proteolytic microorganisms may be of value to predict refrigerated storage life and to assess processing methods (Levin, 1968). Yeasts form a significant proportion of the spoilage flora in hard-smoked products, with high heat input where yeasts are the more heat stable organisms that will be predominant (Nickelson and Finne, 1992).

From the achieved results it could be concluded that absence of visual fungal growth does not mean that moulds and yeasts are not present, their numbers may be low or their growth may be internal as smoked fish (Mackerel) are not eviscerated so the intestinal flora soon make their way through the intestinal cavity. This process is believed to be aided by the action of intestinal proteolytic enzymes. Depending on the degree of invasion, substantial economic losses may be sustained by the producer, the processor and the consumer. Improvement of the smoking process and storage condition of the products should be applied to comply with the reasonable standards to prevent the growth of moulds and their toxins production. It should be recommended that hot-smoked fish be labeled "keep refrigerated store".

REFERENCES

AOAC (1975): Official Methods of Analysis. 12th Ed. Association of Official Analytical Chemists, Washington, D.C., USA.

APHA American Public Health Association (1985): Standard methods for the examination of dairy products. 15th ed., Am. Publ. Health Assn., Washington, D.C. PP. 191.

Baily and Scott (1985): Diagnostic Microbiology. The C.V. Mosby Company, St., Louis, Toronto.

Bours, J. and Mossel, D.A.A. (1973): A comparison of methods for the determination of lipolytic properties of yeasts mainly isolated from margarine, moulds, and bacteria. Arch. Lebensmittel Hyg. 24: 197.

Connell, J.J. (1990): Control of fish quality. Fishing New Books. Pp. 85-88.

Domsch, K.H.; Gams, T.H. and Anderson, T.H. (1980): Compendium of soil fungi. Academic Press, London.

El-Akeel, A.T. (1988): chemical, microbiological and sensory evaluation of hot smoked cat fish. Ph.D. Thesis, Fac. of Agric., Cairo Univ.

El-Shater, M.A. (1994): Quality investigation into locally produced smoked fishes. Ph.D., Thesis, Fac. Vet. Med., Cairo Univ.

FAO "Food and Agriculture Organization of the United Nations" (1970): Smoked curing of fish. Fisheries. Rep., 88, FAO, Rome, Italy.

Fatahat, B.Y. and Koburger, J.A. (1975): Potential of aflatoxin production on smoked mulled. J. Milk and Food Technol., 8, 634.

Gilbert, J. and Knowles, M.E. (1975): The chemistry of smoked foods. J. Food Technol., 10 (3): 245.

Herbert, R.A.; Hendrie, M.S.; Gibson, D.M. and Shewan, J.M. (1971): Bacteria active in the spoilage of certain sea foods. J. App. Bacteriol., 34: 41.

ICMSF (1978): Microorganisms in foods. Vol. I, Their significance and methods of enumeration. 2nd Ed., Univ. of Toronto, Press, Toronto, Canada.

ICMSF (1996): Microorganisms in foods. Microbiological specifications of food pathogens. 1st Ed. Blackie academic and Professional. London, Weinheim, New York, Tokyo, Melbourne, Madras.

Jay, J.M. (1978): Food spoilage: Spoilage of fresh and cured meats, poultry and sea foods. Chapter 7, In Modern Food Microbiology, 2nd Ed, Van Nostrand Company, New York.

Jonsyn, F.E. and Lahai, G.P. (1992): Mycotoxic flora and mycotoxins in smoke dried fish from Sierraleone. Nahrung, 36, 485.

Kansmadi, K.A. and Goncharov, A.M. (1979): Changes occurring in fish during various smoking procedures. Rybnae Knagaistu, 1:57.

Koburger, J.A. (1972): Fungi in foods. IV. Effect of plating medium pH on counts. J. Milk and Food Technol. 35: 659.

Koburger, J.A. and Marth, E.H. (1984): Yeasts and moulds. Chapter 17 In: APHA: Compendium of methods for the microbiological examination of foods, 2nd Ed. Washington, D.C. USA.

Koburger, J.A. and Jaeger, K.E. (1987): Specific and sensitive plate assay for bacterial lipases. Appl. Environ. Microbiol., 53-211.

Leistner, L. and Eckardt, C. (1981): Schimmelpilaze und Mykotoxine in Fleisch und Fleischerzeugnissen. In: Reiss. J. (ed); Mykotoxine in Lenensmittel. fischer Verlag Stuttgart.

Levin, R.E. (1968): Detection and incidence of specific species of spoilage bacteria of fish. 1. Methodology. Appl. Microbiol. 16: 1734.

Mansour, N.K.; Yassien, N.; Darwish, A. and El-Sherif, A. (1991): Psychrotrophic spoilage moulds in imported frozen beef cuts. Vet. Med. J., Giza, 39, 209.

Nickelson II, R. and Finne, G. (1992): Fish, Crustaceans and precooked sea foods. Chapter 47, In APHA: Compendium of methods for the microbiological examination of foods, 3rd Ed. Washington, D.C. USA.

Raper, K.B.; Fennel, D.I. and Austwick, A.K. (1965): The genus Aspergillus. Wiliams and Wilkins Company. Baltimore.

Samson, R.A.; Holkstia, E.S. and Van Oorschot, C.A.H. (1981): Introduction to food fungi. Central bureau voor schimmel cultures, Baarn; The Netherlands.

Smith, J.L. and Haas, M.J. (1992): Lipolytic microorganisms, chapter 11, In APHA: Compendium of methods for the microbiological examination of foods, 3rd Ed. Washington, D.C. USA.