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MYCOLOGICAL STATUS OF IMPORTED CANNED FISH CONSUMED IN SAUDI ARABIA WITH SPECIAL REFERENCE TO PROTEOLYTIC ACTIVITY

(With 3 Tables)

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(Received at 28/3/2002)

الحالة الفطرية للأسماك المعلبة المستوردة والمستهلكة فى المملكة العربية السعودية مع إشارة خاصة لنشاط انزيم البروتييز

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تهدف هذه الدراسة إلى التعرف على درجة تلوث الأسماك المعلبة والمستوردة شائعة الإستخدام بالمملكة العربية السعودية. أجريت هذه الدراسة على ٥٠ عينة من الأسماك المعلبة والتى تمثل ٢٥ نوعاً من التونا المعروضة بالأسواق. بإستخدام طريقة وضع اجزاء من الأسماك على الوسط الغذائي Malt extract والتحضين عند ٢٨° م، أمكن عزل وتعريف ١٧ نوعاً فطريا تنتمي إلى ٩ أجناس. من التحليل الميكروبى للعينات المختبرة لوحظ أن تلوثها بالفطريات كان قليلا نسبيا. أيضا وجد أن العينات التي تحتوى على صلصة الطماطم والماء هي الأكثر تلوثا، وأقلها تلوثا تلك التي تحتوى على الزيوت. كذلك وجد أن عينتين منها وهى جودى ألباكور وألالالى سيبجاك خالية تماما من الفطريات. كانت أكثر الأنواع الفطرية شيوعا وتعدادا تلك التي تنتمي إلى أجناس أسبيرجيللس, فيوزاريوم وبنيسيليوم . تم إختبار ٨٨ عزلة فطرية تمثل ١٠ أنواع تنتمي إلى ٥ أجناس وذلك لدراسة مقدرتها على إنتاج أنزيم البروتييز فى الوسط الصلب. وجد أن أكثر العزلات المختبرة (٦و٧٩ %) لها القدرة علىإفراز هذا الأنزيم. أيضا وجد أن ٣٦ عزلة (٢و٥١ %) من العزلات المنتجة لها إنتاجية عالية للأنزيم ، وهى تنتمي إلى أسبيرجيللس فلافس، أسبيرجيللس نيجر، فيوزاريوم أوكسيسبورم وبنيسيليوم كوريلوفيلم. بينما ٢٢ عزلة (٤و٣١ %) لها مقدرة متوسطة على إفراز الأنزيم. أما باقى العزلات (٢١ عزلة) فوجد أنها ذات إنتاجية ضعيفة للأنزيم.

SUMMARY

The mycological analysis of imported canned fish, commonly consumed in Saudi Arabia, was evaluated in 50 samples representing 25 different kinds of Tuna. Using baiting-technique on Malt extract agar medium incubated at 28°C, 17 species appertaining 9 genera could be isolated. There is low diverse fungal contamination of the samples tested. Samples containing sardines and tomato sauce in water were more polluted whereas, samples of tuna with oil were less contaminated. On the other hand, samples of Goody Albacore and Alalali Skipjack tuna were free from fungi. The genera of the highest incidence and their respective numbers of species were *Aspergillus, Fusarium* and *Penicillium*. Testing the ability of 88 isolates representing 10 species and 5 genera, to produce proteolytic enzymes indicated that most (79.6 %) of the fungal isolates tested were able to produce this enzyme. From the positive isolates 36 strains (51.4 %) could be realized as good producers and these were related to *A. flavus, A. niger, F. oxysporum* and *P. corylophilum*. Whereas, 22 isolates (31.4 %) exhibited moderate activity. The remaining positive isolates (12 isolates) were weakly producers.

Key words: *Microorganisms, Canned fish, Salted fish, Stored foods, Enzymes, Proteolytic enzymes.*

INTRODUCTION

Fish and fish products are considered one of the most important food stuffs as they as the cheapest source of animal protein in many countries. Fish have protein of high biological values as they contain essential amino acids and good source of minerals such as calcium, phosphorus, iron and trace elements like iodine, as well as, vitamins, in addition, the high content of polyunsaturated fatty acids (Deng *et al.* 1974, Sedik *et al.* 1989, Youssef 1998, Bastawrows *et al.* 2000). Moulds are widely distributed in nature and contaminate fish and fish products through polluted environment. Fungal contamination of fish product is considered the main cause of spoilage which lead to off flavours, sliminess and unpalatable taste that render the product of inferior quality, unmarketable or even unfit for human consumption that may constitute a public health hazard and severe economic losses

(Mossel and Shennan 1976, Thatcher and Clark 1978, Karnop 1980, Dorner 1983, Ward and Baaji 1988).

Fungi contaminated fish products are not only unpalatable but also unsafe for consumption by producing mycotoxins. Consumption of mycotoxin contaminated fish products has been associated with several cases of human poisoning or mycotoxicosis, sometimes resulting in death (Matossian 1984, Joffe 1986, Dvorackova 1990, Krogh 1992). Proteinases are a well known group of proteolytic enzymes that play an important role in the food processing industry (Ward 1983). Also, proteases are considered among the most important enzymes in the breakdown of fish materials when fungi attack their surfaces (Moharram and El-Zayat 1989).

In Saudi Arabia, no surveys on microbial evaluation of canned fish have been carried out. The current study was planned to evaluate the mycological quality of imported canned fishes commonly marketed in Saudi Arabia. Also, the capacity of the isolated fungi to produce protease enzymes in vitro was assessed.

MATERIALS and METHODS

Sampling:

Fifty samples of canned fishes, representing 25 kinds of imported products commonly consumed in Saudi Arabia were bought from different markets. The samples were transferred to the laboratory and kept in a refrigerator at 3-5°C till the mycological analysis. The types, components and producing countries were illustrated in Table (1).

Table 1: Different imported canned fishes (Tuna), their ingredients and producing countries.

Canned fish	Ingredients	Producing country
1- Diamond	Tuna in water - Tuna in water, salt	Thailand
2- Rio Mare	Light meat tuna - Tuna, olive oil, salt	Italy
3- Green Farms	Light meat tuna - Tuna, soyabean oil, salt	USA
4- Freshly	Light meat tuna - Tuna, sunflower oil, salt	Thailand
5- Alalali	Light meat tuna - Tuna, sunflower oil, salt	Thailand

Counted Table 1:

Canned fish	Ingredients	Producing country
6- Geisha	Light meat tuna - Tuna, sunflower oil, salt	Thailand
7- Nashar	White meat tuna -White meat tuna, soyabean oil, salt	Thailand
8- Goody	Light meat tuna -Cooked light meat tuna, water, salt	Indonesia
9- Botan	Light meat tuna - Tuna, sunflower oil, salt	Thialand
10- Josiane	Sardines in soya oil - Sardines, soya oil, salt	Morocco
11- Sea Belle	- Sardine in tomato sauce, hot chili	Japan
12- Manila	- Sardines in tomato sauce	Thailand
13- Baity	Light meat tuna - Tuna, sunflower oil, salt	Oman
14- California Garden	Light meat tuna - Tuna, sunflower oil, salt	Oman
15- Diamond Mackerel	Cooked mackerel, sunflower oil broth salt	Thailand
16- Oases	Sardines in tomato sauce - Sardines, tomato sauce, water, salt	Philippines
17- Milo	Sardine in vegetable oil - Sardines, soyabean oil, salt	Morocco
18- 555	Sardine in tomato sauce - Fish, tomato sauce, soya oil, sugar, salt, spices, chili	Philippines
19- Diamond	Sardine in tomato sauce -Coocked sardine,tomato paste, salt	Thailand
20- Diamond	Light meat tuna - Tuna, sunflower oil, salt	Thailand
21- Diamond	White meat tuna - Tuna, sunflower oil, salt	Thailand
22- Goody	Light meat tuna -Cooked tuna, soyabean oil, salt	Indonesia
23- Goody	Albacore white meat tuna -Cooked tuna, soyabean oil, salt	Indonesia
24- Alalali Skipjack	Light meat tuna, sunflower oil, salt	Thailand
25- California Garden	- White tuna in vegetable - Tuna, soyabean oil, salt	Oman

Mycological analysis:

For estimation of moulds which polluted the tested samples, the direct-plating technique was employed (Samson *et al.* 1995). Four

pieces (about 0.5 g each) from each sample were scattered, with a sterile forceps, on the surface of previously poured and set plates containing Malt extract agar medium with the following ingredients (g/L); peptone, 20; yeast extract, 5; NaCl, 5; glucose, 10; and agar agar 20. Rose-bengal (0.003%) and chloramphenicol (0.025%) were added as bacteriostatic agents. Two plates were used for each sample, incubated at 28°C for 1-2 weeks and the developing fungi were counted, identified and calculated per 8 pieces for each sample. Identification was carried out by using the taxonomic methods of Raper and Thom (1949), Raper and Fennell (1965), Booth (1971), and Domsch *et al.* (1980).

Proteolytic activity:

Most prevalent fungi, which contaminated the canned fishes, were tested for their ability to produce protease enzymes (El-Gendy 1966). The medium of the following composition (g/L) was used: peptone 10, agar agar 20, pH 6.8. After autoclaving the medium, sterile skim milk (10% solution of powder defatted milk in water was sterilized separately) was added at rate of 5 ml per 100 ml medium before pouring in plates. After solidifying the test fungi were inoculated onto the center of plates and incubated at 28°C for 10 days. Complete degradation of milk protein was seen as clear zone around colonies. The diameter of the clear zone (mm) was recorded and the data were expressed as +, ++ and +++ for weak, moderate and strong (high) protease activity, respectively.

RESULTS and DISCUSSION

A total number of 157 colonies/200 pieces representing 17 species and 9 genera of filamentous fungi were isolated and identified from 25 different kinds of tuna commonly consumed in Saudi Arabia, on malt extract at 28°C. There is a remarkably low incidence of diverse fungal contaminate the analyzed samples. The genera of highest incidence were *Aspergillus, Fusarium* and *Penicillium*. In this respect, the microorganisms that contaminated the salted and canned fishes were analyzed. It is noticed that, large numbers of filamentous fungi and yeasts could grow on their surfaces in the presence of high concentration of salt, and lead to undesirable changes (Fugii *et al.*, 1978, Jansyn and Lahai 1992, Wheeler and Hocking 1993, Abd-Alla *et al.*, 1994, Ismail *et al.*, 1994, Edris 1996, Youssef 1998, Bastawrows *et al.*, 2000 and

Several others). Similarly, their findings indicated that *Aspergillus* and *Penicillium* species were the most common contaminants of food stored at refrigerated temperature. In the current work, samples containing sardines and tomato sauce in water were more polluted whereas, samples of tuna with oil were less contaminated. On the other hand, samples of Goody Al bacore and Alalali skipjack tuna were free from fungi. This indicate that the presence of oils in canned fish may inhibit the growth of fungi and decrease the samples contamination (Table 2).

Table 2: Total counts (TC, calculated per 4 segments in each sample) and percentage frequency (%F) of fungal genera and species recovered from imported canned tuna on Malt extract agar at 28°C.

Genera & species	TC	%F
- *Alternaria alteranta* (Fries) Keissler	3	12
- *Aspergillus*	90	89
A. flavus Link	39	80
A. fumigatus Fresenius	2	8
A. niger Van Tieghem	25	60
A. oryzae (Ahlb.) Cohn	1	4
A. sydowii (Bain. & Sart.) Thom & Church	3	8
A. tamarii Kita	18	28
A. terreus Thom	1	4
A. ustus (Bain.) Thom & Church	1	4
- *Cladosporium cladosporioides* (Fries.) De Vries	10	28
- *Cochliobolus spicifer* Nelson	1	4
- *Epicoccum purpurascens* Ehrenb. Ex Schlecht.	1	4
- *Fusarium oxysporum* Schlecht.	27	88
- *Penicillium*	21	60
P. chrysogenum Thom	4	16
P. corylophilum Dierckx	17	52
- *Phoma glomerata* (Corda) Wollonw. & Hochapfel	3	12
- *Rhizopus stolonifer* (Ehrenb.) Lindt	1	4
Total count	**157**	
Number of genera = 9		
Number of species = 17		

Aspergillus (8 species) was the most frequently isolated genus in the tested samples (89% of the samples and 57.3% of total fungi). From the genus the predominant species were: *A. flavus, A. niger* and *A. tamarii*. They occurred in 80, 60 and 28% of the samples contributing 43.3, 27.8 and 20% of total *Aspergillus* and 24.8, 15.9 and 11.5% of total fungi, respectively. *A. fumigatus, A. oryzae, A. sydowii, A. terreus* and *A. ustus* contaminated the samples at low level (4-8% of the samples) (Table 2). The present results were greatly identical to those obtained by Ito & Abu (1985), Abdel-Rahman *et al.* (1988), Atapattu & Samarajeewa (1990), Jansyn & Lahai (1992), Singh *et al.* (1994), El-Sayed (1995) and Youssef (1998). They reported that members of *Aspergillus* were the predominant among the moulds isolated from salted fish. Also, the same previous *Aspergillus* species were the commonest in smoked herring fishes as reported by Bastawrows *et al.* (2000).

The second higher incidence rate was represented by *Fusarium oxysporum* which was identified from 88% of the samples comprising 17.8% of total fungi (Table 2). This species was also, isolated from salted fish but, with low incidence as reported by Torrey & Marth (1977), Urbanek & Yirdaw (1978), Abd-Alla *et al.* (1994), Ismail *et al.* (1994) and Bastawrows *et al.* (2000).

Penicillium came the third higher incidence in frequency of occurrence and total count. It was emerged in 60% of the samples represented 13.4% of total fungi. From the genus two species were identified of which: *P. corylophilum* was the most prevalent. It was occurred in 52% of the samples having 81.0% of total *Penicillium* and 11.2% of total fungi. *P. chrysogenum* (16% of the samples and 2.6% of total fungi) was less frequent (Table 2). Several *Penicillium* species including the above species were isolated and identified from salted fish such as: *P. citrinum, P. chrysogenum* and *P. oxalicum* (El-Sayed 1995), *P. brevicompactum* and *P. citrinum* (Abd-Alla *et al.*, 1994), *P. nigricans* and *P. chrysogenum* (Zuberi *et al.*, 1988), *P. chrysogenum* (Youssef, 1998) and *P. aurantiogriseum, P. chrysogenum, P. corylophilum* and *P. oxalicum* (Bastawrows *et al.* 2000).

The remaining following species were detected as single representatives of genera: *Alternaria alternata, Cladosporium cladosporioides, Cochliobolus spicifer, Epicoccum purpurascens, Phoma glomerata* and *Rhizopus stolonifer*. They were isolated from 4-28% of the samples matching collectively about 12.1% of total fungi. The above species and others were also isolated but, with variable

degrees from salted foods as reported by numerous workers allover the world (Dvorackova 1990, Krogh 1992, Moharram and El-Zayat 1989, Abd-Alla *et al.* 1994, Ismail *et al.* 1994, Bastawrows *et al.*, 2000 .....etc.).

Eighty- eight isolates representing 10 species were tested on plate cultures to determine protease activity. The results revealed that 79.6% of the isolates (70 isolates) could produce protease enzyme but with variable degrees (Table 3). In this respect, Moharram and El-Zayat (1989) made an extensive survey on the fungal isolates from scales of *Tilapia nilotica* fish for their ability to produce protease enzymes. They noticed that most isolates tested were able to produce this enzyme. Also, they observed that not only the species of a single genus different in the production of this enzyme but also, the different isolates within the same species.

Table 3: Screening of fungal isolates for protease production.

Species	NIT	NIP	Degree of production		
			High	Moderate	Weak
Alternaria alternata	2	1	-	1	-
Aspergillus flavus	25	22	15	3	4
A. fumigatus	2	1	-	1	-
A. niger	11	8	6	1	1
A. sydowii	3	2	-	2	-
A. tamarii	10	7	1	3	3
Cladosporium cladosporioides	8	6	-	4	2
Fusarium oxysporum	14	13	10	3	-
Penicillium chrysogenum	3	3	1	2	-
P. corylophilum	10	7	3	2	2
Total isolates	88	70	36	22	12

NIT = Number of isolates tested.
NIP = Number of isolates positive.

In the current study, among the positive strains, 36 isolates (51.4 %) exhibited the highest protease production and these isolates related to *A. flavus, A. niger, F. oxysporum* and *P. corylophilum*. Twenty- two isolates (31.4 % of the positive isolates) could produce the enzyme with moderate degree, whereas, the remaining (12 isolates) positive isolates were weakly producers. Our result agree, to a great extent, with the findings reported by Gill and Modi (1981), Kimura and Tisuchiya

(1982), Patil and Shasteri (1985), Mohawed *et al.* (1993) and Wu and Hang (1998).

REFERENCES

Abd-Alla, M.H.; Omar S.A. and El-Nagdy, M.A. (1994): Protease producing microorganism inhabiting salted fish (Moloha) with special reference to protease activity of *Bacillus subtilis*. Acta Societatis Botanicorum Poloniae 63 (3/4): 303-307.

Abdel-Rahman, H.; El-Khateib, T. and Refai, R.S. (1988): Microbiological studies on the Egyptian salted fish "Moloha". Assiut Vet. Med. J. 19 (38): 91-97.

Atapattu, R.. and Samarajeewa, U. (1990): Fungi associated with dried fish in Srilank. Mycopathologia 111 (1): 55-59.

Bastawrows, A.F.; Abo-El-Alla, A.A.; Sayed, A.M. and Abdel-Sater, M.A. (2000): Microbiological quality of smoked herring fish in Assiut City. Assiut Vet. Med. J. 43 (85): 110-123.

Booth, C. (1971): The genus *Fusarium*. Commonwealth Mycological Institute, Kew, Surrey, UK.

Deng, J.; Toledo, R.T. and Lillord, D.A. (1974): Effect of smoking temperature on acceptability and storage stability of smoked Spanish mackerel. J. Food Sci. 39: 596.

Domsch, K. W;, Gams, W. and Anderson, T.H. (1980): Compendium of Soil Fungi. Academic Press, London.

Dorner, W. Joe (1983): Production of mycotoxin by *Aspergillus tamarii* Kita. Appl. Environ. Microbiol. 46 (6): 1435- 1439.

Dvorackova, I. (1990): Aflatoxins and human health. CRC Press, Inc., Boca Raton, Florida.

Edris, A.M. (1996): Microbial evaluation of some marketed smoked fish: Zag. Vet. Med. J. 24 (1): 76-81.

El-Gendy, S.M. (1966): Microbial assay of blue-veined cheese, Roqueforti type. Bull. Sci. Technol. (Univ. of Assiut, Egypt); 9: 367-384.

El-Sayed, Y.S.A. (1995): Mycological studies on locally produced smoked fish with special references to toxigenic strains. Ph.D. Thesis, Dept. Food Control, Fac. Vet. Med., Zagazig University.

Fujii, L.; Ishida, Y. and Kodota, H. (1978): Microbiological studies on salted fish stored at low temperature. Bull. of the Japanese Soc. Sci. Fisheries (Nihor Suisan Gakkaii Shi).

Gill, P.P. and Modi, V.V. (1981): Induction of extracellular proteases by egg-white in *Aspergillus nidulans*. Folia Microbiol. 26: 78-82.

Ismail, M.A.; Nassar, A.; Ahmed, A. and Youssef, H. (1994): Mycological status of ready to eat salted fish. Assiut Vet. Med. J.32 (63): 74-81.

Ito. H. and Abu, M.Y. (1985): Study of microflora in Malaysian dried fish and their decontamination by Gamma irradiation. J. Agri. Biological Chemistry 49: 1047-1051.

Jansyn, F.E. and Lahai, G.P. (1992): Mycotoxin flora and mycotoxins in smoke-dried fish from Sierraleone. Nahrung 36 (5): 485-489.

Joffe, A.Z. (1986): In *Fusarium* species: Their biology and toxicology. P. 225-292. John Wiley & Simons, New York.

Karnop, G. (1980): Effect of storage temperature on the keeping quality of smoked habilut.Information fur die Fischwirtschaff 37 (3): 125- 128.

Kimura, T. and Tisuchiya, K. (1982): Characteristics of protease production by *Cephalosporium* sp. Appl. Environ. Microbiol. 43 (3): 654-658.

Krogh, P. (1992): Role of ochratoxin in disease causation. Food Chem. Toxicol. 30: 213- 224.

Matossian, M.K. (1984): Poisons of the past: moulds, epidemics and history. Yale University Press, New Haven, CT, USA.

Moharram, A.M. and El-Zayat, S.A. (1989): Lipase and protease production by fungi isolated from scales of *Tilapia nilotica*. Bull. Fac. Sci., Assiut Univ., 18 (1-D): 109-117.

Mohawed, S.M.; El-Sharouny, H.M. and Badran, R.A.M. (1993): Studies on the protease activity of some fungi isolated from *Tilapia* fish in River Nile water. Bull. Fac. Sci., Qena (Egypt) 1 (2): 147-171.

Mossel, A.A.D. and Shennan, J.L. (1976): Microorganisms in dried foods. Their significance, limitation and enumeration. J. Food Technol. 11: 205.

Patil, M. and Shasteri, N.V. (1985): Purification and properties of proteases produced by *Alternaria alternata*, regulation of production by fructose. J. Bio. Sci. (Bangalore) 9 (1/2): 1-12.

Raper, K.B. and Thom, C. (1949): A manual of *Penicillium*. Williams and Wilkins, Baltimore, USA.

Raper, K.B. and Fennell, D.I. (1965): The genus *Aspergillus*. Williams and Wilkins, Baltimore, USA.

Samson, R.A.; Hoekstra, E.S.; Frisvad, J.C. and Filtenborg, O. (1995): Introduction to Food- Borne Fungi. Centraalbureau voor Schimmelcultures, BAARN, The Netherlands.

Sedik, M.F.; Safwat E.E., Ibrahim, A.M. and Shaban, A.E. (1989): Studies on some species of locally produced fish. Vet. Med. J. Giza, 37(2): 197- 207.

Singh, A.; Ghosh, V.K. and Ghosh, P. (1994): Production of thermostable acid protease by *Aspergillus niger*. Letters in Applied Microbiology 18: 177-180.

Thatcher, F.S. and Clark, D.S. (1978): ICMSF. Microorganisms in foods. I- their significance and method of enumeration. Academic Press, New York.

Torrey, G.S. and Marth, E.H. (1977): Isolate and toxicity of moulds from foods stored in homes. J. Food Prot. 40 (2): 187-190.

Urbanek, H. and Yirdaw, G. (1978): Acid proteases produced by *Fusarium* species in cultures and in infected seedlings. Physiological Plant Pathology 13: 81-87.

Ward, O.P. (1983): Proteinases .In Microbial Enzymes and Biotechnology ed. Fogarty, W. M. pp. 251- 305. New York: Applied Science Publishers.

Ward, D.R. and Baaji, N.J. (1988): Factors affecting microbiological quality of seafoods. Food Technol. 42 (3): 85-89.

Wheeler, K.A. and Hocking, A.D. (1993): Interaction among xerophilic fungi associated with dried salted fish. J. Appl. Bacteriol. 74 (2): 164-169.

Wu, L.C. and Hang, Y.D. (1998): Purification and characterization of acid proteinase from *Neosartorya fischeri* var. *spinosa* IBT 4872. Letters in Applied Microbiology 27: 71-75.

Youssef, H.H.E. (1998): Mycological status of Moloha, smoked herring and frozen mackerel fish in Assiut province. Ph.D. Thesis, Dept. Food Hygiene, Fac. Vet. Med., Assiut University.

Zuberi, R.; Shamshad, S.I. and Quadri, R.B. (1988): Bacterial flora fermented fish sauce prepared from sardines in Pakistan. J. Tropical Science, 28: 239-246.