قسم : المراقبة الصحية على الأغذية ٠
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دراسة ميكروبيولوجية على الملوحة المصرية

حسني عبدالرحمن ، طلعت الخطيب ، رمضان رفاعي

تضمنت الدراسة فحص عدد ٥٠ عينة من الملوحة المصنفة تحت اسم كلب البحر بغرض تقيمها بكترولوجيا ومدى صحتها وملائمتها للأستهلاك الأدمي حيث تم تقدير كمية ملح الطعام وتقدير الأيون الأيدرجيني والنشاط المائي ، كما شملت الدراسة تعيين العدد الكلي لكلا من ميكروب العنقود الذهبي واللاكتوباسلاى(عصويات حمض اللكتيك)، وكذا الفطريات والخمائر المتواجدة بها ٠ كما شملت الدراسة تقدير الميكروب العنقودي الذهبي الموجب التجلط والكولستريديم بيرفرنجر مع عزل وتصنيف الميكروبات والفطريات والخمائر السابق ذكرها ٠

كما نوقشت النتائج في اطار الأهمية الصحية ولتقييم البكتريولوجي في حماية صحة المستهلك٠

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MICROBIOLOGICAL STUDIES ON THE EGYPTIAN SALTED FISH " MOLOHA "

(With 7 Tables)

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SUMMARY

"Moloha" is a quite popular type of salted fish which several months storable without refrigeration. fifty samples of "Moloha" were investigated. The salt contents were in the range from 10.8-19.6 with average 15.2%. The average of pH and the water activity (a_w) were 5.9 and 0.900 respectively. The means value of Staph. aureus, Lactobacillus, mould and yeast counts per gram were: $3X10^3$, $3X10^2$, $3X10^2$ and 7X10 respectively. Coagulase positivie Staph.aureus could be detected in 10 (20%) while Clostridium perfringens in 4(8%). The most predominant mould and yeast species could be identified. In which the significance importance of the isolates as well as the control measures are discussed.

INTRODUCTION

"Moloha" is a quite Egyptian popular salted fish which consumed during certain occasions of the year. The uneviscerated fresh water fish "Hydrocynous forskallii" is usually salted by spreading the salt among the fish layer, upon the bottom of the tin, as well as on the surface of the upper fish layer before the lid was tightly closed. The tins were stored at room temperature for at least one month before used.

Inspite of proper slating a lot of "Moloha" were spoiled and at the same times it constitute a public health hazard, YOUSSEF, 1976 and MORSHDY et al., 1982.

The purposes of this study was conducted to determine the incidence of micrococci, Staph.aureus, Lactobacillus, Clostridium perfringens, mould and yeast. Moreover some ecologicol parameters which affect the growth and multiplication of microorganisms as a_w, pH and NaCl% were determined.

MATERIAL and METHODS

Fifty random samples of "Moloha", collected from Assiut markets. Ten grams of fish muscles were placed in waring blender with 90 ml of sterile 0.1% peptone water and homogenised for 1-2 minutes to obtain the dilution of 1/10, further dilutions were made.

Isolation and identification of micrococci :

Appropriate amount of the homogenate was inoculated in sodium chloride broth, which was then incubated at 37°C for 24h. A loopfull from the incubated tubes was streaked on

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mannitol salt agar which incubated at 37°C for 24h. Fermenting and nonfermenting colonies were picked up and subjected for further identification according to FEINGOLD and MARTIN (1982).

Enumeration of Staphylococcus aureus :

Staphylococcus aureus count was made by the direct plate method using Baird-Parker's Egg Yolk Tellurite Agar plates, which were incubated for 48h. at 37°C. Colonies samples of each type, were picked and streaked on blood agar base. The isolates were tested for coagulase production by the tube method (FEINGOLD and MARTIN, 1982).

Enumeration of Lactobacilli :

Lactobacillus count was enumerated on MRS Agar which was incubated at 37°C for 48h.

Detection of Clostridium perfringens :

Clostridium perfringens was detected by using the method recommended by Beernes, et al. (1980).

Enumeration and Identification of Mould and Yeast :

The enumeration of mould and yeast were carried out by using acidified malt extract agar and acidified Czapek-NaCl agar media. The inoculated plates were incubated at 25°C for 7 days and examined daily under steareomicroscope to detect the mould colonies, which were transferred to malt extract slope agar for counting and further identification. The yeast colonies were picked onto malt extract agar and kept for further identification. The identification of mould were carried out according to SAMSON et al. (1976) for genus Penicillium. SAMSON (1979) for genus Aspergillus, BARENETT and HUNTER (1972) for other gnera. While the identification of the yeast was carried out according to LODDER and KRIEGER (1952).

Determination of water activity (a_w) :

a_w was carried out by using Retronic Hygroscope DT, measuring station WA - 40.

Determination of pH :

pH was measured by using Orion research digital ionalyzer apparatus, Model 701 A. (West Germany).

Determination of NaCl% :

Sodium chloride percentage was carried out as described (in A.O.A.C., 1975).

RESULTS

Table (1), showed that the minimum, maximum and mean's values of pH and sodium chloride percentage were: (5.4, 6.2 and 5.9) and (10.8, 19.6 and 15.2) respectively, in which these findings are nearly similar to those reported by YOUSSEF, 1976. The water activity a_w- value ranged from 0.881-0.930 with average 0.900.

Table (2), showed that the minimum, maximum and mean's counts for Staph.aureus, Lactobacillus, mould and yeast were (1×10^2, 4×10^5 and 3×10^3); (10^2, 2×10^5 and 3×10^2); (1 X 10, 2×10^4 and 3×10^2) and (1 x 10, 4×10^3 and 7 X 10) per gram respectively.

Table (3) revealed that 80% from the total samples (50) had count between 10^2-10^3 and 20% between 10^4-10^5 in case of Staph.aureus, while 10%, 64% and 26% had count between 10- 10^2-10^3 and 10^4-10^5 in case of Lactobacillus respectively. The frequency distribution of the mould count were 40% between 10- 10^2, 52% between 10^2-10^3 and 8% between 10^4-10^5. On the other hand 25 samples (50%) had yeast count 10; 20 (40%) had count between 10- 10^2 while 5 (10%) had count between 10^2-10^3.

Table (4) illustrated that Clostridium perfringens could be detected in 4 (8%) out of 50 samples while in 10 (20%) in case of Staph.aureus coagulase positive. Concerning the identification of the isolated micrococcus the M.varians could be detected in 24(48%); M.roseus 19(38%) and M.Luteus 7(14%).

Eight genera from both mould and yeast could be detected ad identified in which the predominant mould genera were: Penicillium 142(38.5%); Aspergillus 97(26.3%); while the yeast genera were Debaryomyces 30(28.8%); Torulopsis 25(24.0%) Rhodotorula 18(17.3%) and Sacchromyces 15(14.4%) as recorded in Table (5).

Table (6) revealed that the most predominant identified species of Penicillium were: P.chrysogenum; P.verrucosum var verrucosum; P.corylophilum; P.citrinium and P.verrucosum var cyclopium with following numbers and percentages: 35(24.6%); 26(18.3%); 23(16.2%); 15 (10.6%) and 15(10.6%) respectively, while the predominat Aspergillus species were: A.versicolor 33(34%); A.sydowi 18(18.6%) and A.amstelodami 13(13.4%). On the other hand the most identified yeast species were: Torulopsis glabrata 16(15.4); Debaryomyces globosus 15(14.4%) Sacchromyces verona 15(14.4%) ad Rodotorula aurantiaca 14(13.5%), as recorded in Table (7).

DISCUSSION

Salting is one of the cheapest methods for food preservation but LULIPEN, 1953 reported tha the bacterial count and predominating microorganisms did not show any correlation with salt concentration, on the other hand TANNER, 1946 stated that salt did not act uniformily on all bacteria species, obligate anaerobes were found to be inhibited by 5% NaCl and above 5% facultative anaerobes and aerobic species developed, Rod were found to be easily harmed by salt, most of them were suppressed by 10% salt while most cocci seemed to be able to tolerate a salt concentration of 15% and yeast appeared to tolerate higher percentages of salt.

The a_w - value of foods influences the multiplication and metabolic activity of microorganisms, as well as the resistance and survival of spoilage and food poisoning. The value of a_w obtained lied within the range that allow growth of Lactobacillus, Staphylococci, micrococci, Rhodotorula, Pichia, Sacchromyces, Candida and Torulopsis (LEISTNER and RODEL, 1975).

From the results obtained in this study it is acheived that salted fish "Moloha" is highly contaminated with various types of microorganisms. In spite of high salt concentration Clostridium perfringens could be detected. GOUGH and ALFORD (1965) reported that various strains of Clostridium perfringens consistently survived the normal curing, they also pointed out that the organism not only survive but also might actually grow if the suspending medium and temperature were suitable. Some strains of micrococcus are very salt-tolerant while some are pigmented and discolor the surface of foods on which they grow, M.luteus is yellow and M.roseus is pink FRAZIER and WESTHOOF (1978). The Staphylococci are ubiquitous organisms that are impossible to eleminate from our enviroment, RIEMANN and BRYAN (1979) reported that at least 50% of individuals carry these organisms in their nasal passages, throat and

on their hand, consequently the pH and a_w - value reported in this investigation were considered suitable factors for growth and enterotoxin production, LEISTNER and RODEL, 1975. On the other hand JAY, 1978 mentioned that Lactobacilli were responsible for spoilage and discoloration of foods.

Mould and yeast comprise a large group of microorganisms which are ubiquitous in nature and contaminated foods as a result of unsatisfactory hygienic measures during processing or through food additives ABDEL-RAHAMN et al., 1984. Salt used for curing play an important source of contamination with osmophilic Aspergillus and Penicillium species QUINTA, 1968. The highf incidence of Penicillium species obtained in this study were attributed to their minimal a_w requirments which ranged between 0.80 and 0.90.

Most of the isolated mould and yeast species were discussed by various investigators as spoilage microorganisms. Moreover they play a dangerous role in human mycosis and mycotoxicosis MOSSEL (1977).

REFERENCES

Abde El-Rahman, H.; Youssef, H. and Hefnewey, Y. (1984): Mycological quality of meat products in Egypt. Assiut Vet. Med. 12. 24.

A.O.A.C. (1975): Association of official analyltical chemists. official methods of analysis. 12th Ed. Box 540, Benjamin Franklin Station, Washington.

Barnett, H.L. and Hunter, B.B. (1972): Illustrated genera of imperfect fungi. 2nd Ed. Burgess Publ.

Beernes, H.; Remond, C.H.; Lepage, C. and Criquelian, J. (1980): A direct method for enumeration of C.perfringens in food and Feaces. World Congress of Foodborne Infection and Intoxication. Berlin West (BRD).

Feingold, S.M. and Martin, W.J. (1982): Bailly and Scott Diagnostic Microbiology. 6th Ed. Mosby Co. St. Louis, Toronto, London.

Frazier, W.C. and Westhoff, D.C. (1978): Food Microbiology. 3rd Ed. McGraw-Hill Book Company, New York, London.

Cough, B.J. and Alford, J.A. (1965): J. food Sci. 30. 1025 cited in bryan et al. (1979). Food. borne infection and intoxication 2nd edn. Acadmic press. London.

Jay, J.M. (1978): Modern food microbiology. 2nd Ed. D. Van Nostrand Co.

Leistner, L. and Rodel, W. (1975): The significance of water activity for microorganisms in meats. In: Duckworth, R.B. (Ed): Water relations of foods. Academic press. Page 309-323.

Lodder, J. and Krieger, R.J. (1952): The Yeast. Taxonomy study. North Holland Pub. Co. Amsterdam.

Liston, J. (1957): The occurrance and distribution of bacterial types on flat fish. J. Gen. Microbiol.16: 205.

Lulipan, A.F. (1953): Changes in the Ducn salted herring during storage and spoilage. Symposium on cured and frozen fish. Technology No. 100, Chapter 4.

Morshdy, E.A.; Sedik, F.M. and Zeidan, A.M. (1982): Bacteriological evaluation of salted fishes marked in Sharkia province. Assiut Vet. Med. Journal 9, 17.

Mossel, A.A.D. (1977): Microbiology of foods. Univ. of Utricht Faculty of vet. Med. Netherland.

Petrowa, E.K. (1933): Microbiology of salt. Arch. Microbiol. 4: 326-347.

Quinta, M.L. (1968): Natural destruction of the mycological flora of the sea salt. Food Technology, 22: 1599-1601.

Riemann, H. and Bryan, L.F. (1979): Foodborne infection and intoxication, 2nd Ed. Academic Press.

Samson, R.A. (1979): A compilation of the Aspergillis described since 1965. studies in mycology, No. 18.

Samson, R.A.; Stolk, D. and Hadlok, R. (1976): Revision on the subsection fasciculata of Penicillium and some allied species. Studies in mycology, No. 15.

Tanner, R.W. (1946): The microbiology of foods. Page 861 & 889. Garrad press. Illions, U.S.A.

Youssef, H.A. (1976): Studies on the sanitary improvments of locally manufactured salted fish. pH. D. Thesis. Faculty of Vet. Med. Assiut Univ.

Table (1): Statistioal analysis of pH, sodium chloride percentage and water activity in the examined samples of "Moloha".

	pH	NaCl%	a_w
Minimum	5.4	10.8	0.881
Maximum	6.2	19.6	0.930
Mean	5.9	15.2	0.900

Table (2): Statistical analytical results of microbiological examination of examined "Moloha".

	Staph.aureus	Lactobacillus	Mould	Yeast
Minimum	$1x10^2$	$/\_10^2$	1x10	/_10
Maximum	$4x10^5$	$2x10^5$	$2x10^4$	$4x10^3$
Mean	$3x10^3$	$3x10^2$	$3x10^2$	7x10

Table (3): Frequency distribution of microbiological counts in "Moloha".

	Staph.aureus		Lacto bacillus		Mould		Yeast	
	Frequency	%	Frequency	%	Frequency	%	Frequency	%
/_10	--	-	--	-	--	-	25	50
10-/_10^2	--	-	5	10	20	40	20	40
10^2-10^3	40	80	32	64	26	52	5	10
10^4-10^5	10	20	13	26	4	8	-	-
Total	50	100	50	100	50	100	50	100

Table (4): Incidence percentages of Clostridium perfringens, Staph. aureus coagulase positive and micrococcus species in "Moloha".

No.of samples	Cl.perfringens		S.aureus		Micrococcus spp.					
					M.varians		M.roseus		M.luteus	
	F.	%	F.	%	F.	%	F.	%	F.	%
50	4	8	10	20	24	48	19	38	7	14

F. = Frequency (positive samples).

Table (5): Frequency distribution of isolated mould and yeast genera.

Moul/Yeast genera	Frequency	%
Penicillium	142	38.5
Aspergillus	97	26.3
Geotrichum	40	10.8
Cladosporium	35	9.5
Syncephalostrum	20	5.4
Fusarium	17	4.6
Mucor	10	2.7
Alternaria	8	2.2
Debaryomyces	30	28.8
Torulopsis	25	24.0
Rhodotorula	18	17.3
Saccharomyces	15	14.4
Candida	8	7.7
Endomyces	4	3.8
Pichia	2	1.9
Nematospora	2	1.9

Table (6): Frequency distribution of identified Penicillium and Aspergillus species in "Moloha".

Penicillium and Aspergillus spp.	Frequency	%
Penicillium chrysogenum	35	24.6
P.verrucosum var.verrucosum	26	18.3
P.corylophilum	23	16.2
P.citrinium	15	10.6
P.verrucosum var cyclopium	15	10.6
P.frequentans	13	9.2
P.palitans	8	5.6
P.restrictus	4	2.8
P.charlesii	3	2.1
Aspergillus versicolor	33	34.0
A.sydowi	18	18.6
A.amstelodami	13	13.4
A.terreus	8	8.2
A.niger	8	8.2
A.chevalieri	5	5.2
A.flavus link	5	5.2
A.fumigatus Fres	3	3.0
A.aureolatum	2	2.1
A.oryzae	2	2.1

Table (7): Frequency distribution of identified yeast species.

Yeast species	Frequency	%
Torulopsis glabrata	16	15.4
Debaryomyces globosus	15	14.4
Saccharomyces verona	15	14.4
Rhodotorula aurantiaca	14	13.5
T.globosa	9	8.7
D.nicotianae	8	7.7
D.vini	7	6.7
Candida tropicalis	6	5.8
Rh.glutinis	4	3.8
Endomyces capsularis	4	3.8
C.krusei	2	1.9
Pichia membranafacient	2	1.9
Nematospora coryli	2	1.9