A NEW METHOD FOR CONTROLLING THE PROCESS OF "FESEEKH" BY *LACTOBACILLUS PLANTARUM*

M.Z. MAHMOUD

Food Technology Research Institute , Agricultural Research Centre , Giza , Egypt.

(Manuscript received 29 December 1993)

Abstract

During the last few years feseekh is regarded as an infected food. Consequently, different treatments for mullet fish are carreid out to obtain fermented product free from pathogens. These treatments included control treatment, separation of the head and intestine as a source of pathogens, washing the fish with 5% bicarbonate to remove the slim layer, and the addition of *L.planterm* inoculum. This inoculum was grown in sterilized whey.

The main aim of the addition of the inoculum was the production of the inhibitors which prevents the proliferation of pathogens.

The different fermentations were followed through the estimation of total bacterial count, aerobic sporeformers, clostridia, lactic acid bacteria, proteolytic bacteria, *Enterobacteriaceae*, coliform group, *Salmonella sp., Staph aureus,* lipolytic microorganisms, in both flesh and curing brine. The organoleptic evaluation of the obtained feseekh had also been carried out.

It was found that the removal of the intestine, head and slime layer had contributed in the elemination of most of the pathogens, while the addition of L.planterum inoculum inhibited the remaining pathogens, thus resulting in quality product.

INTRODUCTION

Feseekh is regarded as a popular meal especialy in certain occasions in Egypt.

This salty fermented fish is now considered as a serious source of infection by different pathogens particularly during the last years. It is therefore important to modify and control such kind of fermentation. This modification aimed at removing the contaminated flora found in the intestine, gills and slime layer, as well as the use of the natural inhibitors produced by *L.plantarum* known to prohibit pathogens growth.

Musleh (1976) found that the bacterial population that exist on fresh fish is generally found in places; the outer slime, gills and the intestine of feeding fish . Fresh fish usually carries population of 10^2 -10^7 cells / cm2 of the skin surface (Spencer, 1961) and 10^3-10^9 cells/gm of gill tissue (Shewan, 1949). Bacteria are supposed to enter the gills of the fish and pass through the vascular system and thus invade the flesh or penetrate the intestinal tract and enter body cavity (Shewan, 1971). The numbers of bacteria in fresh fish flesh had been found to be as low as 104 and as high as one billion/gm (Elias, 1968). Horsley (1973) found that *pseudomonas* and *Acromobacter* repesented 60% of gills, intestine and slime isolates. *Staphylococcos aureus* and *Bacillus* were also found (Goldmintz and Hull 1970; Horsley 1973).

Liston and Matches (1976) found that *Micrococcus, Bacillus* and *Clostridium* occured variably in small numbers. Graikoski (1973) reported that fish can be contaminated with Salmonella, Shigella, *E.coli* and other coliforms, *Entrococci, Cl. perfringens* from menhaden fish samples (Goldmintz and Hull, 1970). Liston *et al.* (1971) found that *Salmonella* could be limited by the presence of NaCl. Musleh, (1976) found that 30-70% of co-agulase positive staphylococci were found in all fish parts except the gut, and were located in fish skin. *Cl.botulinum* of the types A, B, E and F is usually found in very low number, mainly in gut contents (Gangarosa *et al.,* 1971; Grakoski, 1971). Hauschild and Hilsheimer (1980) found that Cl.botulinum was relatively high by resistante to low water activity and high salt, therefore sursising curing treatments. Ferial (1986) reported that *Entrobacteriacease* and *Staphylococci* comprised a significant proportion of the population, while proteolytic, lipolytic and acid forms (Lactobacilli) were present in low numbers.

Recently it was reported that the addition of lactic acid bacteria to fermented fish was so useful in controlling fermentation. Beliard *et al.* (1989) observed the inhibition of *Pseudomonas putida* by the produced H_2O_2 by the isolates of lactic acid bacteria. Meanwhile, Lewus *et al.* (1991) found that some strains of lactic acid bacteria produced bacteriocins that were inhibitory to Staph. Sp. and *Cl.perfringens.*

Accordingly, Amechi and Thomaš (1991) suggested that low levels of *L.plantarum* BN or *L.lactic* ATCCll 454 in the presence of 3 or 4% NaCl could be formulated into minimally processed refrigerated food products for protection against possible botulism hazards. Monocytogenes, Karen *et al.* (1933) found that the inhibition of *listeria monocytogenes* by *L.bavaricus* could not be attributed to acidification but to the levels of the inoculum and glucose.

The aim of this investigation is to produce high quality Feseekh free from pathogens.

MATERIALS AND METHODS

Preparation of the fish

Mullet fish of similar size were purchased from one source in the market. 2kg fish + a saline solution of 10% NaCl (W/W) were added to each glass jar to cover the surfac e of the fish after the following treatments:

1- Control

The whole fish after being left to ferment partially in the sun for 1-2 days.

2- Fish without head and intestine

Before salting, the head and the intestine were separated than fermented in the sun for 1-2 days.

3- Fish without head and intestine + washing with 5% bicarbonate + washing with fresh water

Washing was carried out with 5% bicarbonate to remobe the slime layer (Musleh; 1976).

4- Fish without head & intestine + washing with 5% bicarbonate + washing with fresh water + 10ml/ jar inoculum of L.plantarum (obtained from Dairy Dept. National Res. Centre).

Inoculum was the prepared by inoculation the sterilized whey with L.platarum

and incubated at 32°C for one day to obtain young cells. The number of cells/ml was 88×10^7.

Microbiologecal estimations

The microbiological determinations were carried out on the saline or on the flesh done after the homogenization of one fish each time , then 20 gm of the homogenizate was mixed in 180 ml of 0.1% peptone water for 2 minutes. Aerobic plate count and aerobic sporeformers after pasteurization at 80°C for 15 minute were done on tryptone glucose yeat ext. agar (A.P.H.A.) modified by the addition of 0.5% NaCl as recommended by Liston and Matches (1976), and incubated at 32 and 30°C, respectively for 48 h. Closteridia count was done on sulphite iron agar (Massel *et al.,* 1966). Lactic acid bacteria was determined on Tween agar medium (Regosa *et al.,* 1951) at 30°C for 48h. Aerobic proteolytic microorganisms were counted on Gelatin agar medium at 32°C for 48h. (Smith *et al.,* 1952). *Entrobacteriaceae* was determined on violet red bile dextrose agar and *Coliform* on MacConkey's Bile Salt agar medium (Difico, 1977). *Salmonella* count was done on bacto SS agar (Difico, 1977), and *Staph. aureus* on Staphylococcos medium No. 110 (Difico, 1977). All the aformentioned pathogens were incubated at 37°C for 48 h. Lipolytic microrganisms were counted on nutrient emulsified oil agar at 32°C for 48h (Diffico 1953 and modified by Mahmoud et al. (1970).

Organoleptic evaluation

The samples of cured fish were evaluated organoleptically for taste, colour, texture, appearance and odour according to (Ferial, 1986). The samples were evaluated by pannel munbers. The scoring system was 0-05 taste, 0-15 colour, 0-15 texture, 0-10 appearance and 0-10 odour.

RESULTS AND DISCUSSION

Data tabulated in tables 1 and 2 indicated that the high bacterial counts was either in the whole fish or the curing brine of the control treatment. This treatment contained the head, intestine and the available nutrients (espeeially carbon source) in the intestine. Microorganisms in the slime layer of the fish, clostridia and patho-

Table 1. Microbiological counts in flesh during the processing of mullet by different treatmens (counts /gm x 10^2)

Microbiological counts											
Treatment	week	Total bactrial count	Sporeformer	Closteridia	Lactic acid bacteria	Proteolytic microorganism	Entrobacteriaces	Colfform group	Salmonellaspp,	Selmonella spp,	Lipolyti microorganism
Control	Fresh	72100	360.0	10.0	62000	1600	1410	310	160	2460	1960
	Swollen	635000	2400.0	100.0	215000	32000	12500	2040	1540	24000	18500
	1 st	435000	92.0	1000.0	30700	6000	111	19	13	31000	73600
	2 nd	117000	66.2	600.0	16300	76000	112	60	87	34000	40000
	3 rd	96000	41.0	321.0	3300	8200	96	86	170	46000	16000
	4 th	76000	24.1	100.0	450	9100	80	100	250	60000	9100
	5 th	58000	7.3	0.1	10	9660	76	112	325	70000	5543
Fish without head & intestins	Fresh	3100	5.00	4.00	161	309	6	31	40	176	192
	Swollen	29400	16.66	11.20	1400	2250	33	320	390	1100	1560
	1 st	6000	2.90	1.10	3075	7630	13	210	110	290	1615
	2 nd	6500	1.00	0.93	6100	8000	7	90	0	340	1210
	3 rd	5700	0.80	0.01	6000	5100	3	50	0	81	870
	4 th	4500	0.00	0.00	4100	1173	0	39	0	0	610
	5 th	4700	0.00	0.00	3000	800	0	28	0	0	500
Fish without head & intestins + 5% Bicarbonats	Fresh	1120	0.04	0.60	32	166	2.1	11.2	3.2	67.6	135
	Swollen	9770	0.66	2.03	330	1430	19.3	100.0	210.0	860.0	1200
	1 st	6000	0.41	0.02	270	3080	15.0	49.0	90.0	780.0	300
	2 nd	2000	0.26	0.02	156	3600	4.1	26.0	0.0	640.0	200
	3 rd	860	0.12	0.00	57	2250	3.6	10.0	0.0	580.0	170
	4 th	500	0.03	0.00	60	2300	3.2	0.0	0.0	600.0	197
	5 th	360	0.00	0.00	36	970	0.0	0.0	0.0	620.0	180
Fish without head & intestins + 5% Bicarbonats + inoculum	Fresh	4100	0.08	0.68	2100	900	4.0	26.0	23.0	80.0	141
	Swollen	30000	0.76	3.07	16700	11000	24.1	106.0	183.0	74.0	1350
	1 st	11000	0.52	0.10	14400	8000	0.0	33.0	0.0	62.0	743
	2 nd	7100	0.20	0.05	11000	6700	0.0	0.0	0.0	31.0	642
	3 rd	6600	0.04	0.01	9000	2400	0.0	0.0	0.0	22.0	420
	4 th	3160	0.00	0.00	5600	1670	0.0	0.0	0.0	10.4	216
	5 th	3500	0.00	0.00	4100	630	0.0	0.0	0.0	0.0	155

Table 2 . Microbiological counts in the burine during the process of mullet by different treatmens (counts /gm x 10^2)

Microbiological counts											
Treatment	week	Total bactrial count	Sporeformer	Closteridia	Lactic acid bacteria	Proteolytic microorganism	*Entrobacteriaces*	Colfform group	*Salmonellaspp,*	*Staph. aureus*	Lipolyti microorganism
Control	Fresh time	86000	60.00	2.0	50000	24000	2000	1000	240	1000	220
	1 st	134000	2.00	1.2	71000	34000	8300	410	50	2400	500
	2 nd	130000	1.00	0.1	78000	46000	4800	380	46	1600	1300
	3 rd	130000	0.70	0.0	66000	42000	1200	240	32	1300	1100
	4 th	105000	0.50	0.0	52000	28000	168	180	18	800	800
	5 th	83000	0.04	0.0	38000	13500	100	150	10	200	400
Fish without head & intestins	Fresh time	64200	10.00	1.500	2200	5700	7	107.0	196	710	150
	1 st	43700	1.75	0.07	12000	8000	10	30.0	84	480	400
	2 nd	48500	1.20	0.03	25000	11000	100	0.0	53	310	670
	3 rd	51000	0.08	0.07	21100	9940	0	0.0	0	67	250
	4 th	38000	0.00	0.02	18000	6700	0	0.0	0	0	460
	5 th	32000	0.00	0.00	11000	3300	0	0.0	0	0	183
Fish without head & intestins + 5% Bicarbonats	Fresh time	2506	0.75	1	760.0	2340	60	10	0.8	11.5	95
	1 st	3960	0.63	0	1000	3100	28	0	0.0	121.0	200
	2 nd	5300	0.20	0	1100	4000	2	0	0.0	109.0	510
	3 rd	3900	0.00	0	800	2700	0	0	0.0	87.0	440
	4 th	3050	0.00	0	757	2670	0	0	0.0	0.0	316
	5 th	2500	0.00	0	401	1150	0	0	0.0	0.0	150
Fish without head & intestins + 5% Bicarbonats + inoculum	Fresh time	58000	1.0	0.7	58000	3000	58	12	1.1	1.0	1450
	1 st	31000	1.0	0.0	76000	3200	10	0.0	0.0	0	390
	2 nd	98000	0.7	0.0	93000	3900	0	0.0	0.0	0	630
	3 rd	94000	0.0	0.0	71000	2500	0	0.0	0.0	0	500
	4 th	61000	0.0	0.0	58000	2200	0	0.0	0.0	0	440
	5 th	43000	0.0	0.0	41000	900	0	0.0	0.0	0	173

gens were still found either in the flesh or in the curing brine until the end of the fermentation period. However, they gradually decreased except for *Staph. aureus* which increased in number. This might be due to the high counts indicated at zaro time and / or due to the available nutrients in the intestine of the fish. *Staph aureus* relatively increased at the last week. This may be due to the suitability of salt concentration used (10%) to the growth of microorganisms.

In general , the other treaqtments which included the removal of the head and intesting showed a clear reduction in pathogens and clostridia found in brine or in flesh. This is in agreement with findings of Musleh (1971). These counts decreased also through the fermentation period. This could be attributed to the removal of the intestine and head as well as to mutrient deficiency resulting from the removal of the intestine.

Washing the flesh with 5% bicarbonate had resulted in to apparent decrease in total bacterial counts when compared with those obtained after the removal of the head and intestine. This evident reduction was due to the remobal of the slime layer. The high reduction of lactic acid bacteria was treated by in a culation of lactic acid bacteria. This had resulted into a complte reduction of the pathogen in the brine and flesh. An explanation for this is the inhibitor produced by the inoculum (Karen *et al.*, 1993). The addition of a low percentage of the whey through inoculation had activated both proteolytic and lactic acid bacteria, that led to high quality cured fish as revealed by the organoleptic evaluation. (Table 3).

Table 3 . Organoleptic evaluation of fermented fish using different treatments

Day of fermentation	Test	Treatment			
		Control	Fish without head & intestine	Fish without head & intestine + 5% bicarbonate	Fish without head & intestine + 5% bicarbonate + inoculum
21	Teste	21	28	25	30
	Colour	4	5	5	6
	Texture	2	3	3	3
	Apperance	3	4	4	5
	Odour	2	3	3	6
	Sum	33	43	40	50
28	Teste	22	31	29	33
	Colour	6	8	7	9
	Texture	4	6	7	8
	Apperance	5	7	7	9
	Odour	2	5	4	7
	Sum	39	57	54	66
35	Teste	20	36	34	41
	Colour	6	9	7	13
	Texture	6	8	9	9
	Apperance	7	8	7	11
	Odour	2	6	5	8
	Sum	41	67	62	82

REFERENCES

1 . Almechi, O. and J.M. Thomas 1991. Bacteriocin - mediated inhibition of *Clostridium botulinum* spores by lactic acid bacteria at refrigeration and abuse temperatures. Appl. and Environmental Microbiology. 57 (12) : 3423 - 3428.

2 . American Public Health Association (A.P.H.A.). 1971. Standard Methods for the examination of water and waste water. 13th *Ed.* - 651: 664-665.

3 . Beliard, E., B. Abgrall, and C.M. Bourgeois1989. Selection of Strains of lactic acid bacteria for inhibitory activity against Pseudomonas putida. (Application to preservation of fresh minced meat.). Seiences des Aliments 9 (4): 665-684.

4 . Difico Manual. 1953. Difico manual of dehyderated cultures media and reagent. 9th Ed : 32 Difico Laboratories incorporated, Detroit Michigan, U.S.A.

5 . Difico Manual. 1977. Difico manual of dehydrated cultures media and reagent. 9 gh Ed: Difico Laboratories incorporated, Detroit Michigan, U.S.A.

6 . Elias, A.A. 1968. Studies on the microbiology in salted Mullet Fish "Feseekh". M.Sc. Thsis. Fac. of agric., Cairo Univ.

7 . Ferial, M.R. 1986. Bacteriological and chemical Studies on Salted Mullet Fish "Feseekh-a traditional fermented fish product in Egypt. Ph.D, Dissertation, Fac. of Agri., Cairo Univ.

8 . Gangarosa, E.J., J.A. Donadio, R.W. Armestrong, K.F., Meyer, P.J. Barchman, and V.R. Dowell 1971. Botulism in the United States, 1899-1969. Am. J. Epidemiol. 93 : 93-101.

9 . Goldmintz, D. and J.C. Hull, 1970. Bacteriological aspects of fish protein. Develop. Ind. Microbial., 11:335-340.

10 . Grakoski, J.T. 1971. Microbiology of cured and fermented fish. Microbial safety of fishery products. Academic Press, Inc., New York, N.Y. pp. 97-110.

11 . Graikoski, J.T. 1973. Seafood and Botulism in "Fish Inspection and Quality Control. " (Kreuzer, R. ed.) Fishing News (Books). Limited London, England pp. 2-9-255.

12 . Hauschild, A.H.W., and R. Hilsheimer 1980. Incideance of *Clostridium botulinum* in commercial bacon. J. Food Protect. *43*: 564-565.

13 . Horsley, R.W. 1973. The bacterial flora of the Atlantic Salmon (Salmon suler L.) in relation to environment. J. Appl. Bact. *36* (3) : 377-386.

14 . Karen W., C. Allison, and J.M. Thomas 1993. Inhibition of *Listeria monocytogenes* by *Lactobacillus bavaricus* MN in Beef Systems at Refrigeration Temperatures. Applied & Environmental Microbiology, 59 (8) : 2552-2557.

15 . Lewus, C.R., A. Kaiser, and J.J. Mont Vitte 1991. Inhibition of food-borne bacterial pathogens by bacteriocins isolated from meat. Appl. Microbiol. 57; 1683-1688.

16 . Liston, J., J.R.N. Matches and J. Raross 1971. Survival and growth of pathogenic bacteria in foods. In Fish Inspection and Quality Control. Ed. Rudolf Kreuzer, Fishing News (books) LTD. Ludgate House - London, England pp. 246-249.

17 . Liston, J., and R. Matches, 1976. Fish crustaceans, and precooked seafoods. In compendium of Methods for Microbiological Examination of food (1976). Ed. Speck, M. American Public Health Association, pp. 507-521.

18 . Massel, D.A., S. DeBruin, H.M. Van Diepen, C.M. Venderige and G.Zaulewell. 1966. The enumeration of anaerobic bacteria and *Clostridium* species particular by in foods. J. Appl. Bact. *19*; 142-154.

19 . Musleh, R.M. 1976. Bacteriological studies on certain types of fish. M.Sc. Thesis Fac. of Agric., Cairo Univ.

20 . Regosa, M., J.A. Mitchel, and R.F. Wisman. 1951. A selective medium for the isolation of oral an dfaecal lactobacilli. J. Bact., *62*:132-133.

21 . Shewan, J.M. 1949. Some bacteriological aspects of handling, processing and distribution of fish. J. Roy. Sanit. Inst. *59*:394-397.

22 . Shewan, G.M. 1971. The microbiology of fish and fishery products. A progress report. J. Appl. Bact. 34 (20) : 299-315.

23 . Smith, N.R., R.E. Gordan, and F.E. Clark . 1952. Aerobic sporeforming bacteria. U.S. Dept. Agric. Monograph. No. 16.

24 . Spencer, R. 1961. The bacteriology of distant - water col landed. Hull. J. Appl. Bact. *24*:4.

طريقة جديدة للتحكم فى تصنيع الفسيخ بواسطة *L.plantarum*

محمود زينهم محمود

٢ – معهد بحوث تكنولوجيا الاغذية ـ مركز البحوث الزراعية ـ الجيزة .

تم عمل طريقة جديدة لتصنيع الفسيخ عن طريق ثلاثة معاملات استهدفت ازالة معظم الميكروبات المرضية وتأخير المتبقي منها بواسطة الافرازات المثبطة من ميكروب اللاكتوباسلس بلانتارم. كذلك اجريت معاملة للمقارنة لمعرفة مدي التغير الذي تحدثة المعاملات الاخري. أجريت معاملة شملت التخلص من الرأس بما فيها الخياشيم والامعاء لما تحتويه من كم كبير من الميكروبات المرضية وكذلك هذه الطبقة من ميكروبات مرضية ، ولضمان ان يكون الناتج من الفسيخ المصنع موجود تحت تأثير حافظ أضيف بادئ اللاكتوباسلس بلانتارم للسمك المنزوع الرأس والاحشاء والطبقة المخاطية ، وقد اظهرت النتائج مايلي :–

أعلى الأعداد الميكروبية الكلية وكذلك الميكروبات المرضية المختبرة كانت فى معاملة المقارنة سواء لحم أو جلد السمك او فى محلول المعالجة ، حيث ظلت اعداد هذه الميكروبات المرضية موجودة حتى نهاية فترة التخمر وان كانت فى تناقص ، بينما زادت أعداد أما نزع الرأس والأحشاء، فقد أزال عددا من الميكروبات المرضية. ولقد خفض التخلص من الطبقة المخاطية أعداد الميكروبات المرضية، وايضا إضافة البادئ قد ثبط الميكروبات المرضية لكن تأثيره كان أقل على بكتريا الاستافيلوكوكس.