Assiut Vet. Med. J. Vol. 42 No. 83, October 1999

Dept. of Food Hyygiene
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HALOPHILIC BACTERIA IN SOME DAIRY PRODUCTS SOLD IN ASSIUT CITY

(With 6 Tables)

By

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(Received at 2/8/1999)

البكتريا المحبة للملح فى بعض منتجات الالبان المباعة فى مدينة اسيوط

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تم فحص عدد ٢٠٠ عينة عشوائية من بعض منتجات الألبان المخزنة بالتمليح وتشمل الجبن الدمياطى والجبن الكاريش المخزن بالتمليح و المش وكذلك الكشك وذلك للتعرف على مدى تلوثها بالميكروبات المحبة للملوحة وعدد هذه البكتيريا وأنواعها وخطورتها الصحية.و قد جمعت العينات بطريقة عشوائية من أسواق مدينة أسيوط المختلفة ومحلات البقالة حيث تم جمع ٥٠ عينة لكل منتج فى أوعية زجاجية معقمة وتم نقلها بدون تأخير الى المعمل حيث تم فحصها. أولا تم تقدير الرقم الهيدروجينى (pH) وكذلك نسبة الملح فى كل العينات المفحوصة ووجد أن متوسطهما كان ٤,٦٤ , ٥,١٧ , ٥,٣٢ , ٤,١١ بالنسبة للرقم الهيدروجيني و ٩,٨٨% , ١٠,٥٥% , ١١,٨١% , ٧,٣٢ % بالنسبة لكمية الملح فى عينات الجبن الدمياطي , الجبن الكاريش , المش والكشك على التوالى. أما بالنسبة للفحص البكتريولوجى فقد وجد أن جميع العينات (١٠٠%) كانت تحتوى على البكتريا المحبة للملوحة وذلك باستنباتاتها على مستنبتين مختلفين وهما Halophilic agar المحتوى على ٣% ملح , Halophilic agar المحتوى على ١٠ % ملح. بالنسبة للمستنبت الأول كان متوسط العدد الكلى للبكتريا المحبة للملوحة فى العينات المفحوصة على الترتيب كالاتى ٩٩× ١٠^{٤} , ١٧ × ١٠^{٤} , ٢٩ × ١٠^{٥} , ٩ × ١٠^{٤} / جرام بينما باستنبات العينات على المستنبت الثاني كان العدد الكلى لهذه البكتريا كالآتى ٣١ × ١٠^{٤} , ٦٢ × ١٠^{٣} , ٢٥ × ١٠^{٤} , ١٣× ١٠^{٣} لكل جرام على التوالى. وقد تم عزل وتصنيف ٧٠٠ عترة من العينات المختلفة بنسب متفاوتة وكانت كاتالى:

Staph.aureus, *Staph. epidermidis*, Miccrococci, *B. cereus*, *B. licheniformis*, *B.coagulence*, *B.subtilis*, *B.mycoids*, *E. coil* and Proteus species.

S.aureus, *Staph. epidermedis*, Micrococci, *E.coli* and بينما لم يتم عزل Proteus species. من عينات الكشك. كذلك تمت دراسة الخاصية السمية لميكروبات ال *Staph. aureus* المعزول من جميع العينات المفحوصة (٩٧ عترة) وقد وجد أن ٢٤ (٢٤,٨ %) من هذه العترات مفرزة للسموم المعوية وهى: A, B, Enterotoxins AB, AC, ABC وذلك بالاعداد التالية ٩ , ٦ , ٤ , ٣ , عترة على الترتيب. كذلك تمت دراسة الخاصية السمية لميكروب ال *B.cereus* المعزول من هذه العينات (١٠٠ عترة) وقد وجد أن ال diarrheal enterotoxin يفرز من حوالى ٣٨ عترة (٣٨%) من ال B.cereus وذلك بالاعداد التالية ١٨ , ٩ , ١٨ , ٥ من ٤٣ , ٢٢ , ١٩ , ١٦ عترة معزولة من عينات الجبن الدمياطى , الجبن الكاريش , المش والكشك على التوالى . كذلك تمت مناقشة خطوره هذه الميكروبات على الصحة العامة بالاضافة الى الشروط والاحتياطات الواجب اتخاذها نحو تقليل تلوث هذه المنتجات بهذه الانواع من البكتريا .

SUMMARY

A total of 200 random samples of pickeled Damietta, pickeled kareish cheese, mish and kishk (50 each) were collected from different markets and shops in Assiut City. The average pH values of the examined samples were 4.64, 5.17, 5.32 and 4.11, while the average values of NaCl% were 9.88, 10.55, 11.81 and 7.32, of the examined samples, respectively. The samples were examined for numbers and types of halophilic bacteria. It was found that all of the examined samples contained (100%) halophilic bacteria using halophilic agar either containing 3% or 10% salt. The average numbers of halophilic bacteria recovered on halophilic agar containing 3% salt were 99×10^4, 17×10^4, 29×10^5, 9×10^4 /g of the examined pickled Damietta, kareish cheese, mish and kishk samples respectively. Crresponding counts on halophilic agar containing 10% NaCl were 31×10^4, 62×10^3, 25×10^4 and 13×10^3 /g of the examined samples, respectively. Seven hundred (700) isolates were recovered from the examined samples in different percentages. These isolates were identified biochemically as *S.aureus, S.epidermidis, Micrococci, B.cereus, B.licheniformis, B. coagulence, B. subtilis, B. mycoids, E. coli* and *Proteus species*.However, *S.aureus, Staph.epidermedis, micrococci, E. coli* and *Proteus species* failed to recover from the examined kishk samples. Enterotoxigenicity of *S.aureus* isolates reveal that 24 (24.8%)

out of 97 strains recovered from the examined samples were found to produce enterotoxins A, B, AB, BC and ABC in numbers of 9, 6, 4, 3 and 2 strains, respectively. *B.cereus* could be isolated from pickeled Damietta, pickeled kareish, mish and kishk samples in different percentages. Diarrheal enterotoxin was produced by 38% of the tested *B.cereus* strains including 18, 9, 6, 5 out of 43, 22, 19 and 16 *B.cereus* isolates recovered from the examind samples, respectively. The public health hazard of these pathogens and preventive measures were discussed.

Key words: *Halophilic, Dairy, Sold.*

INTRODUCTION

Halophilic microorganisms usually require certain minimal concentrations of sodium chloride (Na Cl) for their growth. Their requirement for salt in general, is not an exclusive need, since many halophiles require low levels of K^+, Mg^{++}, and other cations and anions in addition to NaCl (Macleod, 1965; Dundas, 1977 and Kushner, 1978). Furthermore, the apparent requirement for NaCl is not specific for some bacteria, and other salts and sugars can be substituted. The level of salt required by microorganisms varies greatly. Therefore, the types of microorganisms associated with a particular salt food depend on the concentration and type of salt, as well as, type of food.

The most practical classification of halophilic bacteria is based on the level of salt required (Eimhjellen, 1965; Gibbons, 1969 and Kushner, 1978).Slight halophilic bacteria grow optimally in media containing 0.5 to 3% salt and the moderate halophiles grow in media containing 3 to 15% salt, while extreme halophiles can grow in media containing 15-30% salt. Moreover, another group of bacteria capable of growing in salt concentration 5% and frequently higher up to 12% salt or more, as well as, in media containing no salt are called halotolerant. They are gram-positive bacteria and are species of *Micrococcaceae,* the *Bacillaceae* and some of *Coryne bacterium species.* However, the species *Bacillaceae* and Micrococcus are considered moderately halophilic and are involved in spoilage of salted food.

Some human pathogens, such as *Staph.aureus* and *Cl.perfringens* and some strains of *Cl.botulinum* can be responsible for food poisoning (Riemann, 1969 and Reimann et al. 1972). Some salted dairy products including pickeled Damietta, pickeled kareish cheese, mish and kishk are

considered among the moderately salted food (1-15% salt by weight). These products are susceptible to microbial spoilage, and also are more likely to contain viable human pathogenes. Therefore, this study was planned to secure the numbers and types of halophilic bacteria in such products as well as their public health hazard.

MATERIAL and METHODS

Collection of samples:

A total of two hundred (200) random samples of pickeled Damietta, kariesh cheese, mish and kishk (50 each) were collected from different markets and shops in Assiut City. The samples were dispatched to the laboratory in sterile glass wide mouth bottles with a minimum of delay, where they were prepared for microbiological examination according to A.P. H.A. (1985).

1- Determination of pH value:

The pH value of the examined samples was determined using a pH meter (an Orion Model 701) equipped with standard electrode.

2- Determination of salt content:

Salt content of the examined samples was determined according to **Athertion and Nev Lander (1977).**

3- Enumeration of halophilic bacteria:

Ten fold serial ditlutions were prepared from each prepared sample using halophilic broth (Gibbons, 1969). Halophilic counts were made using halophilic agar (Gibbons, 1969) according to Baross and Matches (1984). Representative colonies of different shapes and color were picked up on agar slants for further identification.

4- Identification of the isolated bacteria:

The isolated bacteria in pure cultures were identified on the basis of Gram stain, catalse and oxidase tests, as well as other biochemical reactions according to Finegold and Martin (1982).

5- Enterotoxigenicity of the isolated Staph.aureus strains:

The identified strains of ***Staph.aureus*** were tested for production of different enterotoxins using Reserved Passive Latex Agglutination Test (Unipath/Oxoid TD 900) as described by Shingaki, et al. (1981).

6- Enterotoxigenicity of isolated strains of *B.cereus*:

The identified strains of *B.cereus* were tested for their enterotoxigenicity using Reserved Passive Latex Agglutination Test (Unipath/Oxoid TD 930) as reported by Shingaki, et al. (1981). The test

was developed for detecting diarrheal enterotoxin only using microtiter plate.

RESULTS

The obtained results are recorded in Tables, 1,2,3,4,5 and 6

DISCUSSION

The results summarized in Table 1 showed that the average values of pH and salt contents of the examined pickeled Damietta and kareish cheese, mish and kishk samples were, respectively 4.64, 5.17, 5.32 and 4.11 for pH and 9.88, 10.55, 11.81 and 7.32% for salt contents. It has been proved that pH value of these products decreased a long the days of storage, which may be due to accumulation of lactic acid produced from lactose fermentation. On the contrary, the salt contents of such pickeled products increase gradually during storage period accompanied by decrease in moisture content (water phase). This was comparable to the results obtained by Amer et al. (1979) and Ahmed et al. (1983). As kishk is a dried fermented milk product, its pH value and salt content do not change greatly during long storage period, and they are nearly similar to what they were in the finshed product after its preparation.

Evaluation of the products for slightly halophilic bacteria (Table 2), reveals that all of the examined samples contained these bacteria that recovered on a medium containing 3% NaCl. The average counts of these types were 99×10^4, 17×10^4, 29×10^5 and 9×10^4/ g of the examined Damietta and kareish cheese, mish and kishk samples, respectively. Also, the data of the Table 3 point out that using halophilic agar containing 10% salt favored the recovery of moderately halophilic bacteria in all of the examined samples in varying numbers, but comparatively lower than slight halophiles. The average counts of moderately halophilic bacteria were 31×10^4, 62×10^3, 25×10^4 and 13×10^3/ g of the examined samples, respectively.

Bacteriological identification of the different isolates recovered from the products on both types of media reveals that 97, 80 and 104 out of 700 isolates recovered from the examined products were identified as *Staph.aureus*, *Staph.epidermedis* and *Micrococcus* spp, respectively. The examined pickeled Damietta and kareish cheese and mish yielded 40, 32

and 25 strains of *Staph.aureus*; 34, 27 and 19 strains of *Staph. epidermedis* and 46, 31 and 27 strains of *Micrococcus spp.*, respectively. The kishk samples yielded no of these isolates.

Furthermore, the data of Table 4 show that 100, 22, 57, 79 and 95 out of 700 isolates recovered from the examined products were identified as *B.cereus*, *B.licheniformis*, *B. coagulase*, *B. subtilis* and *B.mycoids*. The *B. cereus* could be isolated from pickeled Damietta (43 strains), pickeled kareish (22 strains), mish (19 strains) and kishk samples (16 strains). Out of 22 isolates identified as *B.licheniformis*, 12, 4, 6 and 0 strains were isolated from the examined samples, respectively. Moreover, the examined products yielded 18, 15, 13 and 11 strains of *B. coagulase*; 28, 22 19 and 10 *B. subtilis* strains and 25, 30, 22 and 18 strains of *B.mycoids*, respectively. On the other hand, out of the total isolates (700) recovered from the examined samples, 36 and 30 strains could be identified as *E. coli* and *Proteus spp.*, and they recovered from the examined samples cultured on medium containing 3% salt. *E. coli* could be isolated from pickeled Damietta (21 strains), pickeled kareish (6 strains) and mish (9 strains). Out of 30 *Proteus spp.*, 16, 8 and 6 strains could be isolated from the examined samples, respectively, while they failed to recover from kishk samples. *E.coli* and *Proteus spp.* have been isolated from Damietta and kareish cheese and other dairy products examined by Ahmed et al. (1988 a); Ahmed and Sallam (1991) and Abdel-Hady et al. (1995).

It is apparent from the previous data that kishk samples yielded only *Bacillus spp.* and failed to yield the other types of isolates, this could be attributed to the low moisture content of the product as well as the ability of Bacillus spp. to withstand the different stress factors. This was confirmed previously by Ahmed (1980). However, *Staph.aureus*, *Staph.epidermedis*, *Micrococcus spp.* and *Bacillus spp.* are considered moderately halophilic bacteria, they could be identified amomg the slight halophilic on medium containing 3% NaCl. This could be attributed to the fact that such bacteria are halotolerant and capable of growing in medium containing 5% salt and frequently higher up to 12% or more, as well as, they are able to grow on medium containing no salt.

Regarding the results recorded in Table 4, similar findings were achieved by Ahmed (1978 and 1980), who could isolate *Staph.aureus*, *Staph. epidermeidis* and *Micrococcus* from the examined Damietta and kareish cheese samples, and failed to detect them in any of the examined kishk samples. Furthermore, *S.aureus* organisms were isolated from

Damietta and kareish cheese samples examined by Ahmed et al. (1988 b) and Tawfek et al. (1988). Presence of *Staph.aureus* in such types of cheese made from unpasteurized milk is not surprizing in view of fact, that the milk itself is an endogenous source of *Staph.aureus* in dairy products. Mishandling during processing and distribution can serve as an additional sources of contamination.

Occurrence of *B.cereus* in the examined Damietta and Kareish cheese has been confirmed by El-Naway et al. (1982) and Saad (1985). The high frequency distribution of *B.cereus* among the isolates recovered from the examined products could be attributed to the contamination from surroundings, careless during processing, storage and distribution of such products, besides the ability of *Bacillus spp.* to survive pasteurization temperature as well as other stress factors.

Studying the enterotoxins production by the isolated *Staph.aureus* as recorded in Table 5, it was found that out of 97 *Staph.aureus* strains, 24 strains (24.8%) were found to be enterotoxigenic. They produce enterotoxins A (9 strains); B (6 strains); AB (4 strains); BC (3 strains) and ABC (2 strains). Out of 40 *Staph.aureus* recovered from Damietta cheese 11 strains (27.5%) were enterotoxins producers, 4 and 3 strains produced enterotoxins A and B, respectively, while one strain produced 2 enterotoxins A and B, and 2 strains produced B and C. Only one strain secreted 3 enterotoxins A, B and C. Kareish cheese samples had 7 enterotoxigenic strains (21.9%) out of 32 *Staph.aureus* recovered from the examined samples. They secreted enterotoxins A, B, AB and BC by 3, 1, 2 and 1 strain, respectively. Entertoxins A, B, AB and ABC were produced by 6 (24%) out of 10 *Staph. aureus* strains isolated from mish samples. Enterotoxins A and B were produced by 2 strains each, while one strain produced two enterotoxins, A and B. One strain secreted 3 enterotoxins AB and C. Abdel-Hakiem (1992) recorded lower occurrence of enterotoxigenic strains that 15 (18.7%) out of 80 *Staph.aureus* recovered from cheese samples produced enterotoxins. Enterotoxins A and C were produced by 2 and 10 strains, respectively. One strain produced D and 2 strains secreted AC. Different data were obtained by Ahmed (1980) who found lower findings that 8 out of 89 *Staph.aureus* strains recovered from Damietta cheese were enterotoxigenic and they produced A, B and E (one strain each), AE and BD were produced by 2 strains each, while one strain secreted 3 enerotoxins ABE. From kareish cheese one out of 13 *Staph.aureus* strains produced enterotoxin D, however, Ahmed et al. (1988 c) reported

that *Staph.aureus* recovered from Damietta and kareish cheese samples failed to produce enterotoxins A or C.

The public health hazard of enterotoxigenic *Staph.aureus* has been well documented and incriminated in several cases of food poisoning out breaks due to consumption of contaminated dairy products (Casman and Bennet 1965; Zehren and Zehren, 1968; Bryant et al., 1988 and Bone et al., 1989). In addition to food poisoning, Staph.aureus has been reported to cause clinical infection in the skin and mucous membranes due to epidermolytic toxin. It causes chronic purulent inflammations of all organs, wound infections, abscesses, pyodermia, osteomyelitis and can lead to septiceamia (Johnson, 1981).

Testing the isolated *B.cereus* strains recovered from the examined samples for diarrheal enterotoxins proved that 38 (38%) out of 100 *B.cereus* strains were diarrheal enterotoxin producers (Table 6). Out of 43 *B. cereus* strains isolated from Damietta cheese samples 18 (41.9%) were enterotoxigenic while 9 (40.9%), 6 (31.6%) and 5 (31.3%) out of 22, 19, 16 *B.cereus* strains recovered from kareish, mish and kishk samples, respectively, were found to be diarrheal enterotoxins producers 9Table 6). Higher incidence of enterotoxigenic *B.cereus* was detected by Abdel-Hakeim (1992) who stated that 34% of the tested *B.cereus* recovered from cheese samples were diarrheal enterotoxin producer. Also, Granum et al. (1993) recorded a higher percentages (59%) of enterotoxigenic *B.cereus* strains. It has been well established that *B.*cereus was found to be the cause of several food poisoning outbreaks (Hauge, 1950 & 1955); Nikodemusez et al. (1962); Midura et al. (1970); Public Health Laboratory Service (1972); Taylor and Gilbert (1975); Mossel, (1982); Midura et al. (1970) and Gianella and Brasile (1979).

Considering the public health hazard of *E.coli* organisms, they have been associated with severe diarrhea in infants and young children. Also, they are involved in food poisoning gastroenteritis (Riley et al., 1983; Anon, 1987; Hitchins et al., 1992 and Anon, 1994). Also, the presence of *Proteus spp.* in the examined samples is of public health importance as they have been encountered in some cases of summer diarrhea in infants and were also incriminated in some cases of urinary tract affections (Smith and Canant, 1960 and Mackie and Maccartney, 1962).

Furthermore, it has been well reported that these bacteria (*Bacellus spp.*, *E.coli* and ***Proteus***, are capable to decarboxylate one or more amino acids with production of biogenic amines during the long

storage period of these products. These bacteria decarboxylate the amino acid tyrosine and histadine liberating tyramine and histamine (Rice et al., 1976 and Beutling, 1993). Presence of these biogenic amines in dairy products could be of public health hazard associated with their consumption. It has been reported that tyramine and histamine are responsible for cutaneous symptoms (itching and flushing), gastrointestinal symptoms, hematodynamic symptoms (hypo or hypertension), as well as, neurologic symptoms (Murray et al., 1982 and Varnam and Sutherland, 1995).

It is clearly evident from this study that, some dairy products are preserved by salting and pickling in brine solution.However, they are still harbouring certain types of bacteria which are called halophilic or halotolerant bacteria. Some of these halopheles, including *Staph.aureus, B.cereus, E.coli* and proteus are of public health hazard through implication in several food poisoning outbreaks. Furthermore, some of these bacteria have the ability to carboxylate amino acid during the long period of storage producing biogenic amines (tyramine and hisatmine) which are of public health hazard. Pasteurization of milk is an essential step followed by strict hygiene during processing, storage and handling of such products to destroy or even minimize the existing bacteria to negligable numbers that represent no health hazard.

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Table 1. Average pH values and NaCl% of the examined samples

Product	No. of samples	average pH value	average NaCl %
Pickeled Damietta	50	4.64	9.88
Pickeled kareish	50	5.17	10.55
Mish	50	5.32	11.81
Kishk	50	4.11	7.32

Table 2. Incidence and counts of halophilic bacteria in the examined samples (using halophilic agar containing 3% NaCl)

Samples	Positive samples		Counts/g		
	No/50	%	Min.	Max.	Average
Pickeled Damietta	50	100	12×10^3	42×10^5	99×10^4
Pickeled kareish	50	100	85×10^2	90×10^4	17×10^4
Mish	50	100	29×10^3	24×10^6	29×10^5
Kishk	50	100	11×10^2	22×10^4	9×10^4

Table 3. Incidence and counts of halophilic bacteria in the examined samples (using halophilic agar containing 10% NaCl)

Samples	Positive samples		Counts/g		
	No/50	%	Min.	Max.	Average
Pickeled Damietta	50	100	4×10^2	22×10^5	31×10^4
Pickeled kareish	50	100	5×10^2	40×10^4	62×10^3
Mish	50	100	6×10^2	35×10^6	25×10^4
Kishk	50	100	6×10^2	90×10^3	13×10^3

Table 4. Frequency distrbution of the isolated halophilic bacteria recovered from the examined samples using two different halophilic agar media

Isolates	Total	Pickeled Damietta		Pickeled Kareish		Mish		Kishk	
	No.	No.	%	No.	%	No.	%	No.	%
S.aureus	97	40	41.2	32	33.0	25	25.8	0	0.0
S.epidermidis	80	34	42.5	27	33.8	19	23.7	0	0.0
Micrococci	104	46	44.2	31	29.8	27	26.0	0	0.0
B.cereus	100	43	43.0	22	22.0	19	19.0	16	16.0
B.licheniformis	22	12	54.5	4	18.2	6	27.3	0	0.0
B.coagulans	57	18	31.6	15	26.3	13	22.8	11	19.3
B.subtilis	79	28	35.4	22	27.8	19	24.1	10	12.7
B.mycoids	95	25	26.3	30	31.6	22	23.2	18	18.9
E.coli	36	21	58.3	6	16.7	9	25.0	0	0.0
Proteus spp.	30	16	53.3	8	26.7	6	20.0	0	0.0
Total	700	283	40.4	197	28.1	165	23.6	55	7.9

Table 5. Coagulase, Tnase and enterotoxins produced by the isolated *Staph.aureus* strains

The products examines	No. of strains	Coagulae +ve strains		Tnase +ve strains		Enterogenic strains		Types of enterotoxins									
								A		B		AB		BC		ABC	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Pickeled Damietta	40	33	82.5	26	65.0	11	27.5	4	36.4	3	27.3	1	9.1	2	18.2	1	9.1
Pickeled Kareish	32	24	75	18	56.3	7	21.9	3	42.9	1	14.3	2	28.6	1	14.3	0	0.0
Mish	25	10	76	13	52.0	6	24	2	33.3	2	33.3	1	16.7	0	0.0	1	16.7
Kishk	0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Total	97	76	78.4	57	58.8	24	24.8	9	9.3	6	9.3	4	4.1	3	3.1	2	2.1

Table 6. Diarrheal enterotoxin produced by the isolated *B.cereus* strains

Products examined	No. of strains tested	Toxigenic strains	
		No.	%
Pickeled Damietta	43	18	41.9
Pickeled kareish	22	9	40.9
Mish	19	6	31.6
Kishk	16	5	31.3
Total	100	38	38.0