Dept. of Food Hygiene and Control,
Fac. of Vet. Med., Beni-Suef University.

BACTERIOLOGICAL STATUS OF SOME SOFT CHEESES SOLD IN BENI-SUEF CITY

(With 5 Tables)

By

A.M.S. MESHREF and G.M. HASSAN

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الحالة البكتريولوجية لبعض الجبن الطرى المباع فى مدينة بنى سويف

عرفه مشرف سليمان مشرف ، جمال محمد حسن

اجريت هذه الدراسة على ١٠٠ عينة من الجبن المصرى الطرى (٥٠ عينة جبن قريش و٥٠عينة جبن ثلاجة) جمعت عشوائيا من مدينة بنى سويف وقراها وذلك لتعيين حالتها البكتريولوجية ومعرفة مدى سلامتها واثرها على صحة المستهلك. وقد وجد ان متوسط العدد الكلى للميكروبات ، الميكروبات المحبة للبرودة ، الميكروب القولونى ، الميكروب القولونى البرازى والميكروب العصوى القولونى كما يلى على التوالى : ٤,١٩ x ١٠ ^٧ ، ١,٤٥ x ١٠ ^٧ ، ٧,٧ x ١٠ ^٦ ، ٢,١ x ١٠ ^٦ و ٢,٩٧ x ١٠ ^٤ / جرام من عينات الجبن القريش المفحوصة. بينما كان متوسط اعداد الميكروبات المحبة للبرودة ، الميكروب القولونى والميكروب القولونى البرازى كما يلى على التوالى : ٧,٧ x ١٠ ^٥ ، ٥,٣ x ١٠ ^٣ و ٦ x ١٠ ^٢ /جرام من عينات جبن الثلاجة المفحوصة. كما امكن عزل ميكروب الميكروب العصوى القولونى واليرسينيا انتيروكوليتكا من ٥٦ و ١٠ % من عينات الجبن القريش وبنسبة ٦ و ٤ % من عينات الجبن الثلاجة المفحوصة على التوالى. لم يتم عزل ميكروب الكورونوبكتر سكازاكى من اى من عينات الجبن المفحوصة. كما تم مناقشة الاهمية الصحية والاقتصادية للميكروبات وكذلك الشروط الصحية الواجب توافرها لتحسين جودة هذا المنتج.

SUMMARY

A total of 100 random samples of some Egyptian soft cheeses (50 each of kareish and tallaga cheese) were collected from different farmer's houses and shops in Beni-Suef city and villages around the city. The mean values of TBC, psychrotrophic, Total coliforms, faecal coliforms and E.coli counts were $4.19x10^7$, $1.45x10^7$, $7.7 x10^6$, $2.1x10^6$ and $2.97x10^4$ / g of the examined kareish cheese samples, respectively. The mean values of psychrotrophic, Total coliforms and faecal coliforms counts were $7.7x10^5$, $5.3x10^3$ and $6 x 10^2$ / g of the examined tallaga cheese samples, respectively. E.coli and Y.enterocolitica organisms were

detected in 56 and 10 % of the examined kareish cheese samples and in 6 and 4 % of the examined tallaga cheese samples, respectively. None of the examined cheese samples contained C.sakazakii. The public health significance and suggested control measures were discussed.

Key words: *Dairy products, cheese, coliforms, psychrotrophs, Y.enterocolitica.*

INTRODUCTION

Egypt has a long and rich tradition in cheese making based on the many traditional cheese varieties. The most popular varieties of cheese in Egypt are Kareish and Damietta cheese, which are usually made from either raw or heat treated milk.

Kareish cheese is one of the ancient Egyptian, fresh white soft cheeses. It is consumed largely in Egypt due to its low price and high nutritive value. It is made mainly at farmer's house either by acid coagulation of skim milk by natural microflora present in milk or by addition of rennet to skim milk. Such methods of manufacturing are still primitive and unhygienic, a fact that may expose the product to serious contamination (Ahemd and El-Bassiony, 1977 and Deeb *et al.*, 2004).

Likewise, white soft cold stored low salt Damietta "Tallaga cheese" cheese is one most popular type of cheeses in Egypt (Abd El-Shaheed, 2004). It is characterized by low salt content, slightly acid flavour and smooth creamy body texture. Its manufacturing includes heating of one third of the standardized milk to 80 °C and salt (5-6% in winter, 6-7% in spring and autumn and 7-8 % in summer) is added to the reminder milk. The two portion of milk are then mixed and renneted, and starter is added. Finally the coagulum is ladled out into moulds and consumed fresh after drainage (Abou-Donia, 1996). Poor sanitation during processing and marketing of this products, not only reduce its quality but also posses serious health risks to consumer.

The bacteriological quality and safety of cheese are the major area of concern for both producers and consumers. The coliforms and total bacterial counts are used as indicators for both sanitary quality and shelf life of the cheese. However, there have been a number of serious well documented outbreaks of foodborne diseases associated with consumption of cheeses. Cheeses made from raw milk are particularly at risk since they may become contaminated with pathogens initially present in the milk. Pathogens may also enter cheese during processing, if hygiene and process controls are inadequate (Fernandes, 2008).

Yersinia enterocolitica is potentially pathogenic for man, and has been isolated from human gastro-intestinal infections in many countries of the world (Swaminathan *et al.,* 1982). It has been isolated from a wide range of foods including cheese (Moustafa, 1990; El-Kholy *et al.,* 1991; Abdel Hady *et al.,* 1996; Hassan, 1999; Bahout and Moustafa 2006; Basha *et al.,* 2008 and Mezyed *et al.,* 2008)

Enterobacter sakazakii is a member of the family Enterobacteriaceae. It was initially known as yellow pigmented E.cloacae, but was reclassified in 1980 as E.sakazakii. Recent taxonomic work has led to an alternative classification of the organism, E.sakazakii, and the proposal of a newly defined genus, Cronobacter. C.sakazakii is considered emerging opportunistic pathogen and is associated with outbreaks of infections amongst infants, in particular neonates. Its primary origin remains unknown due its ubiquitous nature. It could be isolated from a wide variety of foods including cheese (El-Sharoud *et al.* 2009).

Therefore, the present work was planned to investigate the bacteriological status of kareish and tallaga cheese in Beni-Suef governorate as well as to secure the incidence of Y.enterocolitica and C.sakazakii in both types of cheese.

MATERIALS and METHODS

I-Collection of samples:

A total of 100 random samples of some Egyptian soft cheeses (50 each of kareish and tallaga cheese) were collected from different farmer's houses and shops in Beni-Suef city and villages around the city. The collected samples were sent to the laboratory under refrigerated condition (4 °C). Analyses were started without any delay.

II- Bacteriological analyses:

Representative 11 g of each sample were aseptically homogenized in 99 ml of a sterile 2% sodium citrate solution at 45 °C, in a stomacher for 1 minute. Ten fold serial dilutions were prepared using 0.1% sterile peptone water and appropriate dilutions were used to enumerate the following as descried by APHA (1992):

A - Total bacterial count (TBC) was carried out using plate count agar (Oxoid) after incubation at 32 ± 1 °C for 48 ±3 h. was done for kareish cheese only.

B - Psychrotrophic colony count was carried out using plate count agar (Oxoid) after incubation at 7 ± 1 °C for 10 days.

C - Total coliforms, faecal coliforms and E.coli counts were estimated by a three tube most probable number (MPN) technique.

III- Isolation and identification of Y.enterocolitica (Walker and Gilmour, 1986):

Twenty five grams of each cheese sample were added to 225 ml of Trypticase Soya Broth, homogenized and incubated at 22 °C for 24h. One ml of this pre-enrichment culture was added to 9 ml of bile oxalate sorbose broth (Schiemann, 1982) and incubated at 22 °C for 5 days. After incubation, a loopful of enrichment broth was streaked directly onto a CIN agar plate (Oxoid), then incubated at 25 °C and examined after 24 and 48 h. colonies showing typical bulls eye like" deep red center with a sharp border surrounded by a clear colourless zone" were picked and identified.

IV- Isolation and identification of Cronobacter sakazakii

Ten g of each cheese sample were added to 90 ml of buffered peptone water (Oxoid), homogenized and incubated at 37 °C for 16-18h. A loopful of the broth was streaked onto Violet Red Bile Glucose agar (VRBG; Oxoid) and incubated at 37 °C for 24h. Five colonies of the red or purple colonies surrounded by purple halo were transferred to Tryptic Soya agar (Oxoid) and examined after 24 h at 37 °C for yellow pigment production (Kandhai *et al.*, 2004). The suspected colonies were identified and confirmed by API 20E strips. On the same time a loopful of broth culture was streaked onto DFI chromogenic medium (Oxoid), incubated at 37 °C for 24h. Presumptive positive isolates producing blue green colonies were identified and confirmed by API 20E strips.

RESULTS

Table 1: Bacterial profiles of examined Kareish cheese samples (cfu/g)

Organisms	Min	Max	Mean	±SE
TBC	8.9×10^4	4×10^8	4.19×10^7	1×10^7
Psychrotrophic	1×10^4	2.5×10^8	1.45×10^7	5.2×10^6
Total coliforms	1.5×10^3	1.5×10^8	7.7×10^6	3.7×10^6
Faecal coliforms	<3	4.3×10^7	2.1×10^6	9.7×10^5
E.coli	<3	2.3×10^5	2.97×10^4	9.7×10^3

Table 2: Bacterial profiles of examined Tallaga cheese samples (cfu/g)

Organisms	Min	Max	Mean	±SE
Psychrotrophic	8×10^2	3.6×10^6	7.7×10^5	1.3×10^5
Total coliforms	<3	1.5×10^5	5.3×10^3	3.03×10^3
Faecal coliforms	<3	2.3×10^4	6×10^2	4.6×10^2
E.coli	<3	< 100	-	-

Table 3: Frequency distribution of the examined Kareish cheese samples based on their different bacterial counts/g.

Intervals	TBC		Psychrotrophic		Total coliforms		Faecal coliforms		E.coli	
	No	%	No	%	No	%	No	%	No	%
<3	-	-	-	-	-	-	2	4	22	44
3- $<10^1$	-	-	-	-	-	-	0	0	0	0
10^1- $<10^2$	-	-	-	-	-	-	0	0	2	4
10^2- $<10^3$	-	-	-	-	-	-	3	6	2	4
10^3- $<10^4$	-	-	-	-	10	20	8	16	9	18
10^4- $<10^5$	1	2	2	4	6	12	6	12	9	18
10^5- $<10^6$	6	12	11	22	18	36	20	40	6	12
10^6- $<10^7$	13	26	18	36	9	18	9	18	0	0
10^7- $<10^8$	24	48	18	36	6	12	2	4	0	0
10^8- $<10^9$	6	12	1	2	1	2	0	0	0	0
Total	50	100	50	100	50	100	50	100	50	100

Table 4: Frequency distribution of the examined tallaga cheese samples based on their different bacterial counts/g.

Intervals	Psychrotrophic		Total coliforms		Faecal coliforms		E.coli	
	No	%	No	%	No	%	No	%
<3	-	-	11	22	25	50	47	94
3- $<10^1$	-	-	6	12	10	20	2	4
10^1- $<10^2$	-	-	7	14	7	14	1	2
10^2- $<10^3$	1	2	4	8	4	8	0	0
10^3- $<10^4$	4	8	18	36	3	6	0	0
10^4- $<10^5$	11	22	3	6	1	2	0	0
10^5- $<10^6$	19	38	1	2	0	0	0	0
10^6- $<10^7$	15	30	0	0	0	0	0	0
Total	50	100	50	100	50	100	50	100

Table 5: Incidence of Y.enterocolitica and C.sakazakii in examined cheese samples

Organisms	No. of examined samples	Kareish cheese		No. of examined samples	Tallaga cheese	
		No	%		No	%
Y.enterocolitica	50	5	10	50	2	4
C.sakazakii		0	0		0	0

DISCUSSION

The mean values of total bacteria and psychrotrophic counts were $4.19 \times 10^7 \pm 1 \times 10^7$ and $1.45 \times 10^7 \pm 5.2 \times 10^6$ cfu/ g of examined Kareish cheese samples, with the highest frequency distribution lies within the range of $10^7 - < 10^8$ (48%) and $10^6 - < 10^8$ (72%), respectively (Tables 1&3).

In previous studies on Kareish cheese low levels of TBC, 2.56×10^7 and 1.9×10^6 cfu/g were reported by Ahmed *et al.* (1987) and Abou-Ahmed (2007) respectively. On the contrary higher levels of TBC were reported by Ahmed and El-Bassiony (1977); Aman (1994); Kaldes (1997) and Amin *et al.* (2001) as they reported mean values of 2.44×10^{10}, 2.6×10^8, 2.9×10^8 and 6.74×10^7 cfu/g, respectively.

Likewise, lower levels of psychrotrophic count in Kareish cheese were reported by Saad (1983) and Ahmed *et al.* (1987) in mean values of 4.4×10^6 and 1.07×10^5cfu/g respectively. While higher levels of psychrotrophic counts were reported by El-Bassiony *et al.* (1985); Aman (1994) and Amin *et al.* (2001) in a mean values of 3.2×10^7; 5.4×10^7 and 2.04×10^7cfu/g, respectively.

Concerning Tallaga cheese, TBC was not applied since it is inoculated with starter, and in this respect, are not indicative of the hygienic quality. On the other hand the mean value of psychrotrophic count was $7.7 \times 10^5 \pm 1.3 \times 10^5$cfu /g of examined tallaga cheese samples, with the highest frequency distribution lies within the range of $10^5 - <10^6$ (38%) (Tables 2 & 4).

Psychrotrophic bacteria of major concern are Pseudomonas, Alcaligenes, Achromobacter and Flavobacterium Spp. Theses bacteria are heat labile but can produce heat resistant proteases and lipases which cause bitterness, rancidity, putrefaction of curd, slimy and mucus appearance of the curd surface and surface discolouration of cheese (Farkye and Vedamuthu, 2002).

Most foods especially dairy products should regarded as unsatisfactory when they have large population of microorganisms. Higher TBC and psychrotrophic count of Kareish and Tallaga can be originated from primary contamination of raw milk, or can be a consequence of secondary cross- contamination due to poor hygienic practice during manufacturing process and / or bad handling at a retail place.

Total coliforms and faecal coliforms were detected in 100 and 96 % of the examined Kareish cheese samples with mean values of 7.7 x $10^6 \pm 3.7x\ 10^6$ and $2.1x\ 10^6 \pm 9.7x\ 10^5$ MPN/g respectively (Tables, 1 &3). Many reports dealing with the occurrence of coliforms in Kareish cheese have been accumulated. In those studies, various rates of coliforms were reported as 87, 96, 84, 80, 80, 100, 92, 76.7, 98.8 and 70% by Ahmed *et al.* (1987); Sheliah *et al.* (1987); Ahmed *et al.* (1988); Aman (1994); Amin *et al.* (2001); El-Kosi (2001); Moawad *et al.* (2002); Hassan (2003); Deeb *et al.* (2004) and Abou-Ahmed (2007), respectively.

Concerning tallaga cheese, total coliforms and faecal coliforms were detected in 78 and 50 % of examined samples with mean values of $5.3x\ 10^3 \pm 3.03x\ 10^3$ and $6x\ 10^2 \pm 4.6x\ 10^2$ MPN/g respectively (Tables 2& 4). The results in our investigation are higher than that reported by Abd El-Shaheed (2004); Abd Elall *et al.* (2006) and Mennane *et al.* (2007), while lower than that reported by El Gamal and Abdel-Khalek (1997); Halawa and Moawad (1999) and Araujo *et al.* (2002).

All examined kareish cheese and 96% of examined tallaga cheese samples are considered having unacceptable hygienic quality, since they were above the limit "not more than 10/g of soft cheese " established for coliforms group in soft cheese by Egyptian Standards (2000).

Coliforms bacteria are able to tolerate the acid and salt conditions of most cheese, they are not inhibited by the starter bacteria, they ferment lactose readily and grow well at the temperatures used in making most varieties of cheese (Chapman and Sharpe 1990). Not less than 9.5 % Nacl should be added to milk to suppress the growth of coliforms in cheese made from raw milk (Abd El-Salam and Alichanidis 2004).

High levels of coliforms in cheese may also indicates unsanitary practices in the cheese making process and may sometimes give rise to early blowing or gassing of the product. It is characterized by large gas holes, a spongy texture of the cheese and generally occurs 1-2 days after manufacture (Bintsis, 2006).

E.coli is an indicator of faecal contamination and the possibility of the presence of enteric pathogens. E.coli was found in 28 (56%) and 3 (6%) with mean values of $2.97x10^4 \pm 9.7x\ 10^3$ and <100 MPN/g of the examined kareish and Tallaga cheese samples respectively (Tables, 1, 2, 3 &4).

According to the published data, E.coli was detected in kareish cheese by Ahmed and El-Bassiony (1977); Aboul-Khier *et al.* (1985); Ahmed *et al.* (1987); Sheliah *et al.* (1987); El-Kholy (1989); Aman (1994); Kaldes (1997); El-Shishnagni and Nazem (1999); Amin *et al.* (2001); Hassan (2003); Bahout and Moustafa (2006) and Mezyed et al. (2008) with incidence 76, 80.95, 75, 36, 23.33, 47.5, 80, 46.7, 49.33, 43.33, 50 and 43.3 % respectively.

On the other hand E.coli could be detected in tallaga cheese by El Gamal and Abdel-Khalek (1997); Halawa and Moawad (1999); Araujo *et al.* (2002) and Abd Elaal (2008) with incidence 16, 55, 97.7 and 28 % respectively. All positive samples do not comply with Egyptian standards (2000) of freedom of soft cheese from E.coli.

It is evident from Table 5 that, Y.enterocolitica was detected in 10 and 4 % of examined kareish and Tallaga cheese samples respectively. The detection rate was lower than the finding of Abdel Hady *et al.* (1996) who reported that 13.33% of those kareish cheese samples were contaminated with Y.enterocolitica. on the contrary, lower incidence rates than our result were reported by Moustafa (1990); El-Kholy *et al.* (1991); Hassan (1999); Bahout and Moustafa (2006); Basha *et al.* (2008) and Mezyed *et al.* (2008) as they found 6.7, 6.6, 2, 4, 6 and 3.3 of examined kareish cheese samples were contaminated by Y.enterocolitica respectively. Our result was not in agreement with the results of El-Kholy *et al.* (1991) and Halawa and Moawad (1999) as they found that 2 and 7.5 % of examined tallaga cheese samples were contaminated with Y.enterocolitica respectively.

C.sakazakii has been associated with outbreaks of a rare form of infant meningitis, necrotizing enterocolitis, bacteraemia and neonate deaths (Iversen and Forsythe, 2004). The organism has been isolated from a wide range of foods including cheese (Iversen and Forsythe, 2003).

In this investigation, C.sakazakii was not detected in any of the examined kareish and tallaga cheese samples. This result is in line with result obtained by El-Sharoud *et al.* (2009), who could not isolate C.sakazakii from kareish cheese. On the contrary, Iversen and Forsythe (2004) could isolate C.sakazakii from 2 out of 62 cheese samples.

There are no available data about the occurrence of C.sakazakii in cheese, so more studies are needed to determine conditions that influence the occurrence, survival and growth of C.sakazakii in cheese.

In conclusion, the current investigation has indicated that both kareish and Tallaga cheese are of inferior quality as they produced and handled under neglected sanitary measures. The presence of pathogenic bacteria may pose a risk for public health. Therefore, strict sanitary measures throughout the manufacturing steps and post-manufacture handling should be adapted.

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