

MICROBIOLOGICAL EVALUATION OF CHEESES AND MILK POWDER IN KAFR EL-SHEIKH AND EL-GHARBIA GOVERNORATES

BY

I.I. AL-Hawary , Azza M.K.Sobeih and I. Aman
Fac. Vet. Med. Kafr-El-Sheikh Tanta Univ.

SUMMARY

A total of 135 samples (representing 45 samples each of soft Damietta cheese, ras cheese and milk powder) collected randomly from 6 districts in Kafr El-Sheikh and El-Gharbia Governorate Egypt (3 each) . Collected samples were examined microbiologically. Soft cheese were contaminated with *Staphylococcus aureus*, of them 11 samples agree with standards abroad ($< 10^4/g$), 30 samples contained coliforms and 13 samples contained *E.coli*, all samples agree with standards abroad ($< 10^4/g$ and $10^3/g$) and only one sample contained *Listeria monocytogenes*. 41 (91%), 22 (48.8%), 16 (35.5%), 44(97.7), 37 (84.4%) and 33 (73.3%) of Ras cheese samples exceeded the upper permissible limit for aerobic count , coliforms, *E.Coli* , *Staphylococcus aureus* , mould and yeast counts respectively : On the other hand, all milk powder samples contained aerobic bacteria, but only two samples exceed the upper allowable limit, two samples contained anaerobic bacteria (100 /g) which exceed the allowable limit of ($> 1/g$). *Salmonellae* were not detected in any examined sample.

INTRODUCTION

Milk products are universally recognized as first class foods due to their contribution of high quality animal protein, high level of calcium, phosphorous and vitamins. So they are indispensable in human feeding. They are essential not only for the healthy development of infants, but also as a good supplement for deficient diet of all ages at all times.

Cheeses are one of the preferable milk products all over the world. In Egypt, the cheese may be eaten fresh as in Kareish cheese, or ripened for short period of time as Damietta cheese. Moreover, Ras cheese is a hard variety, which ripened for 6 months. Although cheese is generally considered to be a low risk food, both

hard and soft types have been associated with significant outbreaks of food borne disease in recent years. (Vasavada, 1988; Mutlur, et. al., 1993 and Ko, & Chang, 1995). The situation is complicated by the ability of some pathogenic microorganisms including *Listeria monocytogenes*, some types of *E.coli* and *Staphylococci* to grow in some soft cheese, but not semi-hard and hard varieties. (Vasavada, 1988; Said and Fahmy, 1991)

However, defects which either reduce the value of the cheese or render it inedible can arise from the use of poor quality milk if it contains large numbers of thermophilic and /or psychrotrophic bacteria. (Norris and Pettipher, 1987).

Milk powder is consumed by infants and in sometimes used for manufacturing of dairy products, therefore the presence of organisms even in low number constitute a major public health concern for infants and economic loss and incriminated in many outbreaks of gastroenteritis and food poisoning (Luck et.al, 1980). Therefore, this work was planned to investigate the microbiological status of some types of cheeses and milk powder with various microorganisms in Kafr El-Sheikh and El- Gharbia Governorates.

MATERIALS AND METHODS

Collection of samples

A total of 135 samples (representing 45 samples each of soft Damietta cheese, ras cheese and milk Powder) were collected randomly from 3 districts in each of Kafr El-Sheikh and El-Gharbia Governorates. The samples were transferred to the laboratory with a minimum of delay to be examined microbiologically.

Preparation of samples: -

Soft and Ras cheese:

10 grams of each sample were weighted in a stomacher bag and homogenized with 90 ml of 2 % sodium citrate using the stomacher to give a 1:10 dilution (APHA, 1992).

Milk powder

Ten grams of each sample were reconstituted by soaking them in 90-ml warm sterile distilled water. The reconstituted samples were shaken at 200 rpm for 10 minutes to obtain 1: 10 dilution. All 1:10 dilutions were heated at 80 ° C for 10 minutes in a thermostatically controlled water bath, cooled in melted ice and submitted to further decimal serial dilution for counting of aerobic sporeformers.

Microbiological examination:

Soft cheese:

Samples were examined for *Staphylococcus aureus* count according to Finegold and Martin, (1982); Coliform count (MPN/g) according to ICMSF; (1982); *Escherichia coli* were counted, isolated and identified according to AOAC (1980). Isolation and identification of *Listeria monocytogenes* according to IDF (1995).

Ras Cheese :

Samples were examined for total colony count according to APHA, (1992); Coliform count (MPN/g) according to ICMSF, (1982); *Escherichia coli* were counted, isolated and identified according to AOAC, (1980); *Staphylococcus aureus* were counted as recorded by Finegold and Martin, (1982), Mould and Yeast counts according to APHA (1992).

Milk Powder :

Collected samples were examined for total colony count according to APHA, (1992); aerobic sporeformers were counted according to Harrigan, (1998); *Bacillus cereus* was counted, isolated and identified according to Holdbrook and Anderson, (1980) and Anaerobic bacteria were counted according to Cruickshank, et. al., (1969).

RESULTS AND DISCUSSIONSoft cheese :

Staphylococcus aureus count /g for the samples taken from Kafr El- Sheikh and El-Gharbia Governorates districts has been presented in Table (1). Six out of 45 samples taken from Kafr El-sheikh contained the organism in counts ranged from 4.0×10^3 to 3.0×10^6 cfu/g. Higher incidence (17 samples) and counts ranged from (90 to 8.0×10^7 cfu/g). In Samples taken from El- Gharbia districts. 11 samples contained *Staphylococcus* in counts above the M. values (10^4 /g) proposed by Canadian standards for cheese made with unpasteurized milk, (Collins- Thompson et. al., 1977). Lower results were detected by Aman & Ahmed, (1997).

Thirty samples contained coliforms, of them sixteen from Kafr El-sheikh districts and fourteen from El-Gharbia districts, with counts ranged from 100-333 and form 40 to 2.1×10^5 respectively(Table 1). Higher results were reported by Ahmed et. al., (1988); Ahmed & Sallam, (1991) and Aman & Ahmed, (1997). Among the thirty samples containing coliforms seven had coliforms above the M values (5.0×10^4 cfu/g) and two had *E. coli* over M values (1.0×10^3 /g) proposed by the Canadian standard for cheese made with raw milk (Collins-Thompson, et. al., 1977). Seven of the thirteen samples containing *E. coli*, had bacteria counts ≤ 100 ; 4 had ≤ 200 and 2 with lower than 2.0×10^3 (Table, 1).

Analysis for *Listeria monocytogenes* revealed that one samples collected from El- Riad district had the organism.

Listeria monocytogenes is a food borne pathogen which is lately of great concern not only for food industry but also for regulatory agencies in different countries (Farber et. al., 1988 and Lamont et. al., 1988). The pathogen is wide spread in the environment, it has been isolated from food processing environments, finished food products and various dairy products such as ice cream, cheese as well as raw milk (Fathi and Saad, 1992; Abdel. Hakiem and Sabreen, 1993;

Hassanein 1994 ; El-Kholy and El-Ieboudy, 1995 and Nawal Khalil and Bastawrows, 1997)

Ras cheese :

Aerobic bacteria (AB) were detected in all examined samples (100%) (Table 2a), with mean numbers of 2.0×10^6 ; 1.3×10^7 and 6.4×10^4 cfu/g for samples taken from Kafr El-Sheikh Governorate districts. Higher mean values of 5.8×10^6 , 2.1×10^7 and 5.2×10^7 cfu/g were detected for samples taken from El-Gharbia Governorate districts with the highest mean value of 5.2×10^7 for samples taken from Ketor district. Although a limit value for the AB count in cheese does not exist in international standards, the number being above 10^6 cfu/g suggest that they are not suitable for public consumption. Our results show from its point of view that 4 samples taken from Disuque and El-Mehala districts exceed these values.

All samples taken from Kafr El-Sheikh and El-Gharbia Governorates districts contained coliforms and E. coli (Table, 2 a). Kafr El-Sheikh and El-Gharbia districts showed counts from 1.1×10^2 to 1.1×10^6 / g, with the highest mean value (6.1×10^4 /g) for samples taken from Ketor districts. E.Coli, in samples taken from Disuque district contained the highest mean value (7.1×10^3 /g), compared to 1.2×10^3 and 1.7×10^2 cfu/g for the samples taken from Kafr El-Sheikh and El-Riad districts respectively (Table,2a). 15 samples and 6 samples exceed 1.5×10^3 and 5×10^2 /g total coliforms and fecal coliforms respectively for coliform E.coli as proposed by Canadian standard for cheese made with pasteurized milk (Collins-Thompson et. al.,1977)

Contamination of cheese by Coliform has been used as index of unsanitary manufacturing or handling practices. Growth of E.coli in cheese can cause defects in texture and flavor. However since the multiplication of certain strain of E.coli designated as enteropathogenic (EEC) in certain outbreaks of food-borne illness and presence of E.coli in dairy products has become a public health concern. Several investigators have surveyed dairy products including cheese for presence of E.coli (Gad El-Rab, 1983; Abbar and Mohamed, 1987 and Ahmed et. al. 1988).

Coagulase positive Staphylococcus aureus levels in Ras cheese samples taken from both Governorates are shown in Table (2 b). All samples (100%) had counts ranged from 1.0×10^2 to 5.4×10^7 cfu/g. The greater mean count 9.2×10^4 /g was found for samples taken from El-Mehala district while lower mean value (7.1×10^3 /g) was detected in samples taken from Ketor district. 44 (97.7%) of the samples examined exceed the M value (10^3 /g) proposed by Canadian standard, that suggest they are not suitable for public consumption .

Staphylococci are expected to be among the contaminant microorganisms in dairy products, and these organisms under favorable conditions can grow and causing several outbreaks of staphylococcal food poisoning worldwide (Ahmed et.al, 1983; Bakheit, et. al., 1991; El-Baradie, 1993; Mutlur, et. al., 1993; and Ko & Chang. 1995).

Mould and yeast organisms were detected in percentage of 84.4 and 73.3 respectively (table, 2 b). Mould numbers ranged from 1.0×10^2 to 4.0×10^6 cfu/g, while yeast counts ranged from 5.0×10^4 to 1.2×10^7 cfu/g. According to standards abroad, ($\leq 10^2$ /g), for mould and yeast numbers suggest that they are not suitable for public consumption. Mould in El-Riad and Ketor districts showed higher mean values (2.7×10^5 and 2.1×10^5 respectively) than other districts , while El-Raid and El-Mehala districts showed higher mean values (7.0×10^5 and 3.2×10^5 cfu /g) respectively for yeast .

Many different moulds and yeasts have been implicated in such spoilage and include species of penicillium (green discoloration). Cladosporium (green to black) and candid (black). However, the harder cheese have a wax coating or develop a rind and this minimized the problem. In more recent years film wrapped and vacuum-packed cheese have become popular and these forms of packaging should prevent fungal growth by excluding air (Hayes, 1992).

Milk Powder :

Aerobic mesophilic bacteria were detected in all milk powder samples examined (Table 3). The total numbers ranged from 1.0×10^2 to 11×10^4 cfu/g for samples taken from Kafr El-Sheikh Governorate districts while El-Gharbia Governorate districts showed count ranged from 1.0×10^2 to 5.0×10^5 cfu/g. Six samples (2 from El-Riad ; 3 from Disuque and 1 from Ketor districts) of the 45 samples exceed the limit value 5×10^4 cfu/g (Lovell,1992). Lower results were obtained by Waguida,(1986).While higher values were recorded by Moustafa et. al., (1984).

Four samples (one each of Kafr El-Sheikh ; El-Riad ; Disuque and El-Mehala districts) contained *B. cereus* organisms , with numbers ranged from 100 to 250 cfu/g. Higher counts were encountered by several authors in milk powder (Wong et. al., 1988; Shinagawa , 1993 and Aman et. al, 1998). From the public health point of view Aman et. Al., (1998) stated that sample contained 100 *B. cereus* /g constitutes a public health risk if such milk is reconstituted and remained for 8 to 12 hours at room temperature. Moreover, it plays a major role for spoilage of diary products , as well as one of the potential food poisoning and its role in outbreaks of food born illness has been well documented (Mossel, 1982, and Sallam et.al., 1991).

The presence of anaerobes in dried milk products consider as an indicative of careless method of production besides some species impair the utility of the products. As well as several outbreaks of food poisoning (ICMSF, 1982). *Bacillus* and *clostridia* have been implicated in spoilage and several defects in milk products (Roberts, 1982 and Khan & Natarajan , 1986).

Salmonellosis is the classical form of microbial food poisoning in milk powder was a potential source of infections and outbreaks. (Eley, 1992) . Fortunately , salmonellae failed to be detected in all examined samples .

From the results obtained we can conclude that strict hygienic measures and rigid regulations should be imposed for production of clean sound products to protect consumers against infection as well as save a lot of products from being deteriorated on the market .

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Table (1) Statistical analytical results of bacteriological examination of examined soft cheese samples (n = 45)

Districts	Staphylococcus aureus count					Coliform count (MPN/g)					E. coli count					Listeria monocytogenes	
	No. of Positive samples		Min.	Max.	Mean	No. of positive samples		Min.	Max.	Mean	No. positive samples		Min.	Max.	Mean	No. positive samples	
	No	%				No.	%				No.	%				No.	%
Kafr El-Sheikh	1/8	12.5				6	75	100	330	271	4	50	100	85	0	0	0
El-Rida	4/8	50	1.3 x 10 ⁴	3 x 10 ⁵	9.8 x 10 ⁵	4	50	100	330	268	1	12.5	150	150	0	0	0
Disuqe	1/6	16.7		4000		6	100	100	333	271	4	66.7	100	200	0	0	0
El-Mehala	6/8	75	90	8 x 10 ⁷	6.0 x 10 ⁵	5	80	70	2.1 x 10 ⁵	6.4 x 10 ⁵	3	37.5	4	1.1 x 10 ³	2.0 x 10 ²	0	0
Tanta	9/9	100	90	1000	8.0 x 10 ²	4	44.4	40	1.1 x 10 ⁵	2.7 x 10 ⁴	0	0	0	0	0	0	0
Ketor	2/6	33.3	12.0 x 10 ⁵	3 x 10 ⁶	21.0 x 10 ⁵	5	83.3	90	2.0 x 10 ⁴	3.0 x 10 ²	1	16.7	100	100	0	0	0
Total	23	51.1				30	66.7				13	28.9			1	2.2	2.2

Tables (2, a) Statistical analytical results of microbiological examination of examined ras cheese samples (n = 45)

Districts	Total colony count					Coliform count (MPN/g)					E.coli count				
	No. of Positive samples		Min	Max	Mean	No. of positive samples		Min	Max	Mean	No. of positive samples		Min	Max	Mean
	No	%				No	%				No	%			
Kafr El-Sheikh	10	100	3.0×10^4	5.0×10^7	2.0×10^5	10	100	2.1×10^3	1.1×10^5	3.2×10^4	10	100	1.5×10^2	1.1×10^4	1.2×10^3
El-Rida	10	100	6.0×10^4	4.6×10^8	1.3×10^7	10	100	1.1×10^3	9.0×10^4	4.1×10^3	10	100	2.1×10	3.0×10^2	1.7×10^2
Disuge	7	100	2.0×10^2	6.9×10^7	6.4×10^4	7	100	2.0×10^2	1.1×10^5	1.7×10^4	7	100	5.4×10	1.1×10^5	7.1×10^3
El-Mehala	6	100	7.0×10^4	8.6×10^7	5.8×10^6	6	100	$< 10^2$	1.1×10^6	1.3×10^4	6	100	3.9×10^2	1.3×10^6	3.1×10^3
Tanta	8	100	7.0×10^4	1.7×10^8	2.1×10^7	8	100	$< 10^2$	2.1×10^4	7.1×10^3	8	100	6.0×10	5.2×10^2	1.1×10^2
Ketor	4	100	1.0×10^7	2.4×10^8	5.2×10^7	4	100	$< 10^2$	5.0×10^5	6.1×10^4	4	100	4.5×10	6.5×10^2	3.9×10^2
Total	45	100				45	100				45	100			

Table (2, b) Statistical analytical results of microbiological examination of examined ras cheese samples (n = 45)

Districts	Staphylococcus aureus count					Mould count					Yeast count				
	No. of Positive samples		Min	Max	Mean	No. of positive samples		Min	Max	Mean	No. of positive samples		Min	Max	Mean
	No	%				No	%				No	%			
Kafr El-Sheikh	10	100	1.0×10^4	8.0×10^4	3.1×10^4	10	100	1.0×10^4	5.7×10^4	3.0×10^4	10	100	1.8×10^5	7.1×10^6	2.1×10^5
El-Rida	10	100	4.5×10^3	2.9×10^6	3.6×10^4	6	60	1.0×10^5	4.0×10^4	2.7×10^5	5	50	5.0×10^5	9.0×10^6	7.0×10^5
Disuge	7	100	1.0×10^2	3.0×10^6	1.7×10^4	6	85.7	1.0×10^2	3.1×10^5	1.9×10^4	5	71.4	1.8×10^5	7.0×10^6	1.2×10^5
El-Mehala	6	100	2.0×10^2	1.0×10^6	9.2×10^4	6	100	1.0×10^4	2.0×10^5	2.6×10^4	5	83.3	5.0×10^4	1.2×10^7	3.2×10^5
Tanta	8	100	5.9×10^3	5.4×10^7	6.8×10^4	8	100	1.0×10^4	1.6×10^6	4.3×10^4	6	75	1.0×10^5	1.2×10^7	1.3×10^5
Ketor	4	100	4.9×10^3	1.0×10^4	7.1×10^3	2	50	1.0×10^5	2.0×10^5	2.1×10^5	2	50	4.0×10^5	5.0×10^5	4.5×10^5
Total	45	100				38	84.4				33	75.3			

Table (3) Statistical analytical results of bacteriological examination of examined milk powder samples (n = 45)

Districts	Total colony count						Total aerobic spore count						Bacillus cereus count			anaerobic sporeform count	
	No. of Positive samples		Min.	Max.	Mean	No. of positive samples		Min.	Max.	Mean	No. of positive samples		Min.	Max.	Mean	No.	%
	No	%				No	%				No	%					
	No	%	No. of positive samples		Mean	No. of positive samples		Mean	No. of positive samples		Mean	No. of positive samples		No. of positive samples			
Kafr El-Sheikh	8	100	1.0×10^3	2.2×10^4	5.0×10^3	8	100	1.0×10^3	4.0×10^3	2.3×10^3	1	12.5	250		1	12.5	
El-Rida	11	100	1.0×10^3	7.0×10^4	12.4×10^3	11	100	2.0×10^3	1.0×10^4	4.9×10^3	1	9.1	100		1	9.1	
Dikuqe	8	100	2.0×10^3	11×10^4	7.5×10^3	8	100	2.0×10^3	2.0×10^4	10.5×10^3	1	12.5	200		0	0	
El-Mehala	6	100	2×10^3	1.2×10^4	6.2×10^3	6	100	1.0×10^3	2.2×10^4	5.6×10^3	1	16.7	100		0	0	
Tanta	5	100	3×10^3	1.2×10^4	7.2×10^3	5	100	2.0×10^3	3.0×10^3	2.7×10^3	0	0	0	0	0	0	
Ketor	7	100	1.0×10^3	5.0×10^5	13.0×10^3	7	100	1.0×10^3	1.4×10^4	6.7×10^3	0	0	0	0	0	0	
Total	45	100				45	100				4	8.9			2	4.4	

التقييم الميكروبيولوجي للجبن و اللبن البودرة بمحافظة كفر الشيخ و الغربية

ابراهيم ابراهيم الهوارى - عزة محمود كامل صبيح - ابراهيم محمد امان

قسم مراقبة الأغذية كلية الطب البيطرى كفرالشيخ جامعة طنطا

تم تجميع ١٣٥ عينة (٤٥ عينة جبن طرى و ٤٥ عينة جبن رومى و ٤٥ عينة البان جافة) من ٦ مراكز بمحافظة كفر الشيخ و الغربية. تم فحص هذه العينات ميكروبيولوجيا و اظهرت النتائج ان كل عينات الجبن الطرى ملوثة بالميكروب العنقود الذهبى وان ١١ عين منهم كان العدد اقل من ١٠ /جم حسب القياسات العالمية . كما اظهرت النتائج ان ٣٠ عينة ملوثة بالميكروبات العسوية القولونية و ١٣ عينة ملوثة بالميكروبات الأيشيريكية بعدد اقل من (١٠ و ١٠٠ /جم على التوالى) حسب القياسات العالمية وكانت عينة واحدة ملوثة بميكروب الليستيريا مونوسيتوجين وقد اوضحت النتائج ان الجبن الرومى ملوث بالبكتيريا الهوائية و البكتيريا العسوية القولونية و الميكروبات الأيشيريكية القولونية و الميكروب العنقود الذهبى وكذلك بالعفن والخمائر وكان العدد الكلى لهذه الميكروبات اعلى من الحد المسموح به فى ٤١ (٩١%) و ٢٢ (٤٨,٨%) و ١٦ (٣٥,٥%) و ٤٤ (٩٧,٧%) و ٣٧ (٨٤,٤%) و ٣٣ (٧٣,٣%) من العينات على التوالى و بفحص عينات الألبان المجففة اتضح ان كل العينات تحتوى على البكتيريا الهوائية و عينتان فقط اعلى من الحد المسموح به و عينتان فقط تحتوى على الميكروبات اللا هوائية (١٠٠ /جم) اى اعلى من الحد المسموح به و جميع العينات خالية من ميكروب السالمونيلا.