

## Bacteriological Evaluation of Soft cheese

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### ABSTRACT

One hundred soft cheese samples (25 Feta, 25 Damiate, 25 Kareish and 25 Double Cream cheese) were collected from supermarkets in Sharkia Province. The samples were transferred directly to laboratory to be bacteriologically examined.

Our result revealed total colony count/g in examined Feta, Damiate, Double Cream and Kareish cheese samples were 64% (16), 60% (15), 72% (18) & 76% (19) respectively. Total enterococci count in Feta, Damiate, Double Cream and Kareish cheese samples were 40% (10), 48% (12), 64% (16) and 68% (17) respectively. *Staph. aureus* isolated from Feta, Damiate, Double Cream and Kareish cheese 40% (4), 41.67% (5), 62.5% (10) and 47.06% (8) respectively and *Staph. Epidermidis* 60% (6), 58.33% (7), 17.5% (6) and 52.94% (9) respectively, While *Strep faecalis*, *Faecium* and *Intermediate* could be isolated from Feta soft cheese as 16% (4), 25% (6) and 20% (5) respectively, in Damiate cheese was 12% (3), 24% (6) and 16% (4) but in Double Cream cheese was 16% (4), 8% (2) and 24% (6) and in Kareish cheese were 20% (5), 28 (7) and 16% (4).

Coliforms count in Feta, Damiate, Double Cream and Kareish cheese samples were 84% (21), 72% (18), 76% (19) and 64% (16). Identification of isolated coliforms from Feta soft cheese was detected in lactose fermentation in 90.48% (19) distributed as *E.coli* 33.33% (7), *Citrobacter freundii* 19.05% (4), *Klebsiella pneumoniae* 23.81 (5) and *Enterobacter aerogenes* 14.29 (3) beside non Lactose fermentation 9.52% (2) include *Proteus* spp, Damiate cheese was detected in Lactose fermentation in 94.44% (17) distributed as *E. coli* 22.22% (4), *Citrobacter freundii* 11.11% (2), *Klebsiella pneumo-nae* 38.89 (7) and *Enterobacter aerogenes* 22.22% (4) beside non Lactose fermentation 5.56% (1) include *Proteus* spp, Double Cream cheese was detected in Lactose fermentative in 84.21 % (16) distributed as *E.coli* 26.32 % (5), *Citrobacter freundii* 10.53% (2), *Klebsiella pneumoniae* 21.05% (4) and *Enterobacter aerogenes* 26.32(5) beside non Lactose fermentative 15.79% (3) include *Proteus* spp. and in Kareish cheese was detected in Lactose fermentative in 75% (12) distributed as *E.coli* 26.32% (5), *Citrobacter freundii* 18.75(3), *Klebsiella pneumoniae* 31.25% (5) and *Enterobacter aerogenes* 18.75(3) beside non Lactose fermentative 25%(4) include *Proteus* spp.

### INTRODUCTION

Dairy products are considered the most important food in the human diet. Cheese is an excellent food that contains wide variety of easily digested nutrients (2). Total colony count of cheese may reflect conditions as microbial content (3). Presence of *Staph*

*aureus* in cheese gives an indication about its contamination from handling (4). Cheese Contamination with coliforms gives indication of faecal contamination (5). Enterococci may have a distinct role as indicator of microorganisms of poor sanitation. (6-7). *Staph.aureus* possesses a public health hazard

(8). Coliform is enteric bacteria which live in intestinal tracts of healthy and diseased animals. It causes superficial skin lesions (9). *Staph.aureus* produces toxins cause food poisoning (10). Enterococcus species are inhabitant in intestinal tract (11). *E.coli* causing food poisoning in humans (12). *Klebsiella* produces pneumonia (13). *Citrobacter* in cheese indicates contamination (14-15). *Citrobacter* believed to be harmless to humans (16). It is a thermophilic organism (17). Enterococcus faecium and faecalis are most common in human gastrointestinal tract (18). Presences of Enterococci in food indicate indirect faecal contamination and unsanitary production of food (19). Enterococcus faecalis causes majority of human enterococcal local or systematic infections as abdominal infection.

This work was planned to determine the incidence and type of some contamination different types in soft cheese collected from supermarkets at Sharkia Province including total colony count, identification of Staphylococci, Enterococci, Enterobacteriaceae and Coliforms.

## MATERIALS AND METHODS

One hundred soft cheese samples (25 Feta, 25 Damiate, 25 Kareish and 25 Double-Cream) were collected from Supermarkets in Sharkia Province (21) and were transferred directly to the laboratory to be bacteriological examination.

Preparation of serial dilution: Each sample was mashed in a sterile mortar before being emulsified in the diluent. Eleven grams of the prepared samples were transferred to a pre-warmed sterile mortar (40°C) triturated with grams of sterile dry fine sand, to which 99 ml. of sterile 2% Sodium citrate solution as emulsifying agent warmed at 40°C were added and thoroughly mixed till completely emulsified to make solution 1:10 (22).

Total aerobic bacterial Colony Count: One ml from previously prepared dilutions was

inoculated into duplicate plates, then 10 ml. of standard plate count agar, melted and cooled at 45°C, were poured into each Petri-dish. Inoculated and control plates after being mixed and solidified were incubated aerobically at 32°C for 48 hrs. (22).

Isolation and identification of Staphylococci: from the previously prepared decimal dilutions of the examined samples 0.1ml was evenly spread on dry surface of Baird-Parker agar (25) medium plates and incubated aerobically 37°C for 48h and suspected *Staph.aureus* colonies were counted. Characterized as (black colonic surrounded by clear halo zone) was picked up and cultured on slope agar for microscopical and biochemical identification (23).

Isolation and identification of Enterococci: from each dilution 0.1 ml was spread evenly into triplicates of enterococci selective differential (E.S.D) agar medium. Inoculated plates were aerobically incubated at 37°C. Counts were determined after 17, 24 and 48hs incubation. Total enterococci counts/g of examined samples was calculated (24).

Isolation of Coliforms (MPN /Gm): One ml of previously prepared serial dilutions of the respective samples was inoculated in McConkey's broth tubes. All inoculated as well as control tubes were aerobically incubated at 37°C for 24 hr after which the tubes showing acid and gas were recorded. The most probable number of coliforms/g. was determined (22). Mac Conkey's agar plates were streaked with loopfuls from each positive liquid culture to obtain discrete colonies after the plates had been incubated for 24 hours at 37°C. Suspected colonies were picked up and isolated in a pure culture for identification (25).

## RESULTS AND DISCUSSION

The result revealed that total colony count/g. in examined Feta, Damiate, Double

Cream and Kareish cheese samples were 64% (16), 60% (15), 72% (18) and 76% (19) respectively with mean value of  $2.9 \times 10^6 \pm 2.6 \times 10^6$ ,  $3.16 \times 10^6 \pm 2.8 \times 10^5$ ,  $3.83 \times 10^6 \pm 2.71 \times 10^5$  &  $3.53 \times 10^6 \pm 2.84 \times 10^5$  respectively (Table 1). Highest frequency distribution of total colony count in examined Feta, Damiate, Double Cream and Kareish cheese were 37.5% (6), 33.33% (5), 44.44% (8) and 36.84% (7) respectively, lied within  $10^2$ - $10^4$ ,  $10^4$ - $10^6$ ,  $10^2$ - $10^4$  &  $10^2$ - $10^4$  (Table 3). These findings were agreed with (26) for Feta soft cheese (27) for Damiate cheese (28) for Kareish and Double Cream cheese. Higher total colony count was recorded (29) soft cheese. Difference of total colony count in different type of soft cheese may be due to different initial total bacteria or due to different methods of handling during or post manufacture (30-31). Total enterococci count in examined Feta, Damiate, Double Cream and Kareish cheese were 40% (10), 48% (12), 64% (16) and 68% (17) respectively with mean value  $2.7 \times 10^6 \pm 1.5 \times 10^6$ ,  $2.9 \times 10^6 \pm 1.4 \times 10^6$ ,  $3.08 \times 10^6 \pm 1.51 \times 10^6$  and  $3.064 \times 10^6 \pm 1.22 \times 10^6$  (Table 3) Highest frequency distribution of enterococci count in examined Feta, Damiate, Double Cream and Kareish cheese were 40% (4), 41.67% (5), 43.75% (7) and 41.18% (7) lies within range  $10^2$ - $10^4$ ,  $10^2$ - $10^4$ ,  $10^4$ - $10^6$  and  $10^4$ - $10^6$ . Presence of enterococci in soft cheeses may be due to insufficient sanitary condition during production and milk processing (32). Nearly enterococci range of white soft cheese was reported (33). Lower value of entero-cocci count was recorded (34) while higher levels were reported (35). Identification of *Staph. aureus* from Feta, Damiate, Double Cream and Kareish cheese were 40% (4), 41.67% (5), 62.5% (10) & 47.06% (8) respectively and *Staph. epidermidis* 60% (6), 58.33% (7), 17.5% (6) & 52.94% (9) respectively. Same *Staph. Spp.* were isolated from Feta and Double Cream cheese (36) for Damiate and Kareish cheese.

The results explained that *Strep faecalis*, *faecium* and *intermediate* could isolated from examined samples as 16% (4), 25% (6) and 20% (5) respectively with a mean value  $2.94 \times 10^6 \pm 1.71 \times 10^6$ ,  $4.04 \times 10^6 \pm 2.32 \times 10^6$  and  $3.48$

$\times 10^6 \pm 2.06 \times 10^6$  respectively for Feta but for Damiate were 12% (3), 24% (6) and 16% (4) with a mean value of  $2.69 \times 10^6 \pm 1.68 \times 10^6$ ,  $3.97 \times 10^6 \pm 2.43 \times 10^6$  and  $3.80 \times 10^6 \pm 1.20 \times 10^6$  meanwhile for Double Cream was 16% (4), 8% (2) and 24% (6) with a mean value  $2.82 \times 10^6 \pm 1.50 \times 10^6$ ,  $4.08 \times 10^6 \pm 2.73 \times 10^6$  and  $3.94 \times 10^6 \pm 1.48 \times 10^6$  respectively and for and Karaish were 20% (5), 28 (7) and 16% (4) with a mean value of  $2.51 \times 10^6 \pm 1.43 \times 10^6$ ,  $4.42 \times 10^6 \pm 2.63 \times 10^6$  and  $3.51 \times 10^6 \pm 1.37 \times 10^6$ . (Table 2). Highest frequency distribution of *Strep. faecales*, *faecium* and *intermediate* counts in tested samples for Feta were 50% (2), 50% (3) and 60% (3) and lie between  $10^2$ - $10^4$  but for Damiate was 66.67 % (2), 50% (3) and 50% (2) lie  $10^4$ - $10^6$ , for Double Cream 50% (2), 100% (2) and 50% (3) lie  $10^2$ - $10^4$  and Kareish samples 60% (3), 42.86% (3) and 50% (2) lie  $10^4$ - $10^6$  (Table 3). Same isolates could isolate from Feta and Damiate (29), from kareish (37), from double cream (37). Higher values of *Strep. faecalis*, *faecium* and *intermediate* in soft cheese were recorded (38). Same frequency of distribution of *Strep. faecales*, *faecium* and *intermedit* were recorded (39).

Results showed that coliforms count/gm. in Feta, Damiate, Double Cream and Kareish samples were 64% (14), 44% (11), 48% (12) and 64% (16), mean value  $4.48 \times 10^6 \pm 2.06 \times 10^6$ ,  $4.85 \times 10^6 \pm 2.14 \times 10^6$ ,  $4.73 \times 10^6 \pm 2.03 \times 10^6$  and  $3.94 \times 10^6 \pm 1.084 \times 10^6$  respectively. Highest frequency of distribution of coliforms counts in tested Feta cheese samples were 33.34% (7) between  $10^4$ - $10^6$  while Damiate were 38.89 % (7) between  $10^2$ - $10^4$ , in Double Cream were 36.84% (7) lie  $10^2$ - $10^4$  and Kareish samples were 31.25% (5) lie  $10^2$ - $10^4$  as shown in table (3). Similar data were recorded (40) but high counts of coliforms in soft cheese samples were recorded (41) and lower value were reported (42). Isolated coliforms from Feta cheese samples was detected as Lactose fermentative in 90.48% (19) distributed as *E. coli* 35.75% (5), *Citrobacter freundii* 21.45% (3), *Klebsiella pneumoniae* 21.45% (3) and *Enterobacter aerogenes* 14.3% (2) beside non Lactose fermentative 7.15% (1) include *Proteus spp.*,

while Damiate cheese samples was detected in Lactose fermentative in 94.44% (17) distributed as *E.coli* 29.7% (3), *Citrobacter freundii* 19.8% (2), *Klebsiella pneumoniae* 39.6% (4) and *Enterobacter aerogenes* 9.9% (1) beside non Lactose fermentative 9.9% (1) include *Proteus* spp., while Double Cream cheese was detected in Lactose fermentation in 84.21%(16) distributed as *E. coli* 24.9%(3), *Citrobacter freundii* 16.6%(2), *Klebsiella pneumoniae* 16.6% (2) and *Enterobacter*

*aerogenes* 33.2 % (4) beside non Lactose fermentative 8.3%(1) include *Proteus* spp and from Karaish cheese samples was detected in Lactose fermentative in 64%(16) distributed as *E.coli* 6.25%(1), *Citrobacter freundii* 18.75(3), *Klebsiella pneumoniae* 31.25% (5) & *Enterobacter aerogenes* 18.75(3) beside non Lactose fermentative 25% (4) (*Proteus* spp.). Similar species were isolated from soft cheese (43).

Table 1. Statistical analytical results of bacterial count in examined soft cheese samples.

Bacterial type	No. of sample	Feta soft cheese		Dammite cheese		Double cream cheese		Karashish cheese		
		+ ve samples No	% %	Count/gm Mean ± S.E.	+ ve samples No	% %	Count/gm Mean ± S.E.	+ ve samples No	% %	Count/gm Mean ± S.E.
T. bacterial count	25	16	64	2.9x10 <sup>6</sup> ± 2.6x10 <sup>6</sup>	15	60	3.16x10 <sup>6</sup> ± 2.8x10 <sup>5</sup>	18	72	3.83x10 <sup>6</sup> ± 2.71x10 <sup>5</sup>
Staph. count	25	10	40	2.7x10 <sup>6</sup> ± 1.5x10 <sup>6</sup>	12	48	2.9x10 <sup>6</sup> ± 1.40x10 <sup>6</sup>	16	64	3.08x10 <sup>6</sup> ± 1.51x10 <sup>6</sup>
Faecalis	25	4	16	2.94x10 <sup>6</sup> ± 1.71x10 <sup>6</sup>	3	12	2.69x10 <sup>6</sup> ± 1.68x10 <sup>6</sup>	4	16	2.82x10 <sup>6</sup> ± 1.50x10 <sup>6</sup>
Faecium	25	6	25	4.04x10 <sup>6</sup> ± 2.32x10 <sup>6</sup>	6	24	± 3.97x10 <sup>6</sup> ± 2.43x10 <sup>6</sup>	2	8	4.08x10 <sup>6</sup> ± 2.73x10 <sup>6</sup>
Intermediat	25	5	20	3.72x10 <sup>6</sup> ± 1.16x10 <sup>6</sup>	4	16	3.80x10 <sup>6</sup> ± 1.20x10 <sup>6</sup>	6	24	3.94x10 <sup>6</sup> ± 1.48x10 <sup>6</sup>
Coliform count	25	14	64	4.48x10 <sup>6</sup> ± 2.06x10 <sup>6</sup>	11	44	4.85x10 <sup>6</sup> ± 2.14x10 <sup>6</sup>	12	48	1.48x10 <sup>6</sup> ± 4.73x10 <sup>6</sup>
								16	64	2.03x10 <sup>6</sup> ± 1.084x10 <sup>6</sup>

Table 2. Incidence of isolated enterococci and coliforms spp in the in examined soft cheese samples

Enterococci Spp.	Species	Feta soft cheese		Dammite cheese		Double Cream cheese		Karashish cheese	
		No.	%	No.	%	No.	%	No.	%
Staphylococcus epidermidis	Staphy. aureus	4	40	5	41.67	10	62.5	8	47.06
		6	60	7	58.33	5	17.5	9	52.94
Coliforms Spp	Lactose fermentation	5	35.75	3	29.7	3	24.9	1	6.25
		3	21.45	2	19.8	2	16.6	3	18.75
		3	21.45	4	39.6	2	16.6	5	31.25
		3	21.45	4	39.6	2	16.6	5	31.25
Non lactose Fermentation	P spp	2	14.30	1	9.9	4	33.2	3	18.75
		1	7.15	1	9.9	1	8.3	4	25

CF= Citrobacter freundii

KP= Klebsiella pneumoniae

EA= Enterobacter aerogenes

P spp= Proteus sp



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## ملخص العربي

## التقييم البكتريولوجي للجبن الأبيض الطري

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أجريت الدراسة على ١٠٠ عينة من الجبن الطري موزعه كالاتي (٢٥ عينة من الجبن الأبيض- ٢٥ عينة من الجبن الدمياطي- ٢٥ عينة من الجبن القريش- ٢٥ عينة من الجبن الدوبل كريم) تم جمعها من السوبرماركت المختلفة بمحافظة الشرقية وتم فحصها بكتريولوجيا لعزل الملوثات البكتيرية.

وقد وجد ان العدد الكلي للبكتيريا في الجبن الأبيض، الدمياطي، دبل كريم والقريش ١٦ عينة (٦٤%)، ١٥ عينة (٦٠%)، ١٨ عينة (٧٢%) و ١٩ عينة (٧٦%) على التوالي بمتوسط عدد كلي البكتيري  $1.0 \times 2,9 \pm 1.0 \times 2,6$ ،  $1.0 \times 3,16 \pm 1.0 \times 2,8$ ،  $1.0 \times 3,83 \pm 1.0 \times 2,71$  و  $1.0 \times 3,53 \pm 1.0 \times 2,84$  على التوالي والمكورات العنقودية في الجبن الأبيض، الدمياطي، دبل كريم والقريش ١٠ عينة (٤٠%)، ١٢ عينة (٤٨%)، ١٦ عينة (٦٤%) و ١٧ عينة (٦٨%)، بمتوسط العدد الكلي البكتيري  $1.0 \times 2,7 \pm 1.0 \times 1,5$ ،  $1.0 \times 1,5 \pm 1.0 \times 1,5$ ،  $1.0 \times 3,08 \pm 1.0 \times 1,51$  و  $1.0 \times 3,064 \pm 1.0 \times 1,22$ . وتم تصنيف المكورات العنقودية الى المكور العنقودي الذهبي في الجبن الأبيض، الدمياطي، دبل كريم والجريش في عدد ٤ عينة (٤٠%)، ٥ عينة (٤١,٦٧%)، ١٠ عينة (٦٢,٥%) و ٨ عينة (٤٧,٠٦%) على التوالي، بينما كان المكور ابيديرمس في ٦ (٦٠%) عينة (٥٨,٣٣%)، ٦ عينة (١٧,٥%) و ٩ عينة (٥٢,٩٤%) على التوالي، ووجدت ميكروبات المكور السبحي فيكالس والمكور السبحي فيشيوم والمكور السبحي انترميديات في الجبن الأبيض عدد ٤ عينة (١٦%)، ٦ (٢٥%) و ٥ (٢٠%) على التوالي بمتوسط  $1.0 \times 2,94 \pm 1.0 \times 1,7$ ،  $1.0 \times 1,7 \pm 1.0 \times 1,7$ ،  $1.0 \times 4,04 \pm 1.0 \times 2,32$  و  $1.0 \times 3,72 \pm 1.0 \times 2,06$  على التوالي. ووجدت ميكروبات المكور السبحي فيكالس والمكور السبحي فيشيوم والمكور السبحي انترميديات في الجبن الدمياطي عدد ٣ عينة (١٢%)، ٦ (٢٤%) و ٤ (١٦%) على التوالي بمتوسط  $1.0 \times 1,68 \pm 1.0 \times 1,68$ ،  $1.0 \times 2,43 \pm 1.0 \times 3,97$  و  $1.0 \times 1,20 \pm 1.0 \times 1,20$  على التوالي. وفي الجبن الدوبل كريم وجد ميكروبات المكور السبحي فيكالس والمكور السبحي فيشيوم والمكور السبحي انترميديات في الجبن الأبيض بمتوسط  $1.0 \times 2,82 \pm 1.0 \times 1,50$ ،  $1.0 \times 4,08 \pm 1.0 \times 2,73$  و  $1.0 \times 3,90 \pm 1.0 \times 1,20$  على التوالي بينما وجدت تلك البكتيريا في الجبن القريش ٥ عينة (٢٠%)، ٧ (٢٨%) و ٤ (١٦%) على التوالي بمتوسط  $1.0 \times 1,43 \pm 1.0 \times 4,42$ ،  $1.0 \times 2,63 \pm 1.0 \times 3,51$  و  $1.0 \times 1,37 \pm 1.0 \times 2,51$  على التوالي

وتم تصنيف ميكروبات الكوليفورم المعزوله من الجبن الأبيض الطري الى كوليفورم مخمر للاكتوز في عدد ١٩ عينة (٤٨,٩٠%) موزعه كالاتي الايشرشيا كولاى في عدد ٧ عينة (٣٣,٣٣%)، سيتروباكتر فريندى في عدد ٤ عينة بنسبه ١٩,٠٥، كليسيلا نيموني في عدد ٥ عينة (٢٣,٨١) انتيروباكتر ايروجينز في عدد ٣ عينة (١٤,٢٩) وانواع كوليفورم غير مخمره للاكتوز موزعه كالاتي بروتيس في عدد ٢ عينة (٩,٥٢). بينما، وفي عينات الجبن الدمياطي تم تصنيف ميكروبات الكوليفورم المعزوله من الجبن الأبيض الطري الى كوليفورم مخمر للاكتوز في عدد ١٧ عينة (٩٤,٤٤%) موزعه كالاتي الايشرشيا كولاى في عدد ٤ عينة (٢٢,٢٢%)، سيتروباكتر فريندى في عدد ٢ عينة (١١,١١)، كليسيلا نيموني في عدد ٧ عينة (٣٨,٨٩) انتيروباكتر ايروجينز في عدد ٤ عينة (٢٢,٢٢) وانواع كوليفورم غير مخمره للاكتوز موزعه كالاتي بروتيس في عدد ١ عينة (٥,٥٦)، وفي الجبن الدبل كريم وتم تصنيف ميكروبات الكوليفورم المعزوله من الجبن الأبيض الطري الى كوليفورم مخمر للاكتوز في عدد ١٦ عينة (٨٤,٢١%) موزعه كالاتي الايشرشيا كولاى في عدد ٥ عينة (٢٦,٣٢%)، سيتروباكتر فريندى في عدد ٢ عينة (١٠,٥٣)، كليسيلا نيموني في عدد ٤ عينة (٢١,٠٥) انتيروباكتر ايروجينز في عدد ٥ عينة (٢٦,٣٢) وانواع كوليفورم غير مخمره للاكتوز موزعه كالاتي بروتيس في عدد ٣ عينة (١٥,٧٩) والجبن القريش تم تصنيف ميكروبات الكوليفورم المعزوله من الجبن الأبيض الطري الى كوليفورم مخمر للاكتوز في عدد ١٢ عينة (٧٥%) موزعه كالاتي الايشرشيا كولاى في عدد ٥ عينة (٢٦,٣٢%)، سيتروباكتر فريندى في عدد ٣ عينة (١٨,٧٥)، كليسيلا نيموني في عدد ٥ عينة (٣١,٢٥) انتيروباكتر ايروجينز في عدد ٣ عينة (١٨,٧٥) وانواع كوليفورم غير مخمره للاكتوز موزعه كالاتي بروتيس في عدد ٤ عينة (٢٥)