Occurrence Of Proteolytic Bacteria In Milk And Some Dairy Products

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ABSTRACT

One hundred random samples (50 of Market raw milk and 25 each of Damietta and Kariesh cheese) collected from different localities in Sharkia Governorate, Egypt, were examined for detection of their sanitary condition. The bacteriological examination showed that the mean proteolytic count/ml or gram in examined raw milk, Damietta and Kariesh cheese samples were 5.3×10^{6}, 3.81×10^{6} and 2.84×10^{12}, respectively. Pseudomonas spp., Enterobacteriaceae, Acenitobacter spp., Gram-positive spp., Alcaligenes spp., Achromobacter spp., Aeromonas spp., Flavobacterium spp., Vibrio and Chromobacterium spp., could be detected in the examined samples at varying percentages. The proteolytic Enterobacteriaceae species isolated from the examined raw milk, Damietta and kariesh cheese samples were Enterobacter spp., citrobacter spp., serratia spp., klebsiella spp., proteus spp. and E.coli. Gram-positive proteolytic bacteria isolated from examined samples were Bacillus spp., Micrococcus spp., Streptococcus and staphylococcus spp.. All proteolytic colonial isolates tested for proteolytic activity at 7°C for 10 days on skim milk agar. The economic importance and public health significance of existing microorganisms as well as the suggested measures for improving the keeping quality as well as the sanitary condition of raw milk and its products were discussed.

INTRODUCTION

Milk has been referred as the most perfect food. Unhygienic methods of production and high ambient temperatures along with the lack of prompt cooling after milking and unsatisfactory washed milking equipment are the main reasons influencing the bacteriological quality of raw milk *(1)*. Microorganisms in milk are classified into two main categories, spoilage and pathogenic microorganisms whereas the spoilage microorganisms impart off-flavor, increased acidity and subsequent low keeping quality. Some of those are psychrotrophic microorganisms, such as Pseudomonas fluorescence, Ps. fragi, Bacillus spp., Clostridium spp., Corynebacterium spp., Arthrobacter spp., Micrococcus spp. and streptococcus spp.. The proteolytic enzymes produced by psychrotrophs in milk are more powerful in its action on milk protein than that naturally present in milk and that produced by leucocytes even if present by great amount *(2)*. Pseudomonas are the most common microorganisms causing spoilage during refrigeration storage of milk. While their growth in raw milk, they produce extracellular proteases which resist heat-treatments and can cause the development of gelatin and bitterness of UHT milk and off-flavor of pasteurized milk and may be responsible for softening of the curd and yield losses during cheese manufacture *(3)*. This work was undertaken to detect the presence of proteolytic microorganisms in raw milk, Damietta and Kariesh cheese and to determine whether this food is a potential vehicle for such organisms or not.

MATERIALS AND METHODS

One hundred random samples (50 of market raw milk "500 ml for each" and 25 each of Damietta and Kariesh cheese "50 gm for each sample") were collected from different localities in Zagazig city, Sharkia Governorate, Egypt. All samples were collected in dry, clean and sterile containers and transferred to the laboratory with a minimum of delay to be examined microbiologically. All examined raw milk samples were examined by storch's to detect heat treated samples *(4)* .

Enumeration of proteolytic microorganisms: *(5)* Using milk agar (SPC+ 10% sterile skim milk) at 44±1°C followed by incubation at 32°C for 2 days. The plates were

flooded for 1 min. with a solution of 1% HCl and the colonies surrounded by clear zone were counted and reported. All proteolytic colonies were picked up from the countable plates and streaked on tryptone agar slants then incubated at 32°C for 24 hrs and kept in refrigerator for further identification.

Identification of proteolytic microorganisms: The previously isolated proteolytic colonies were inoculated into tryptone soya broth and incubated at 32°C for 24 hrs, then purified onto tryptone soya agar slants for further identification *(6)*.

Determination of proteolytic activity of the isolated microorganisms from Raw milk and soft cheese at 7°C *(7)*.

Preparation of isolates: The isolates were subcultured onto nutrient agar plates and incubated at 32°C for 24 hours. Pure cultures were inoculated into nutrient broth and incubated over night at 32°C.

Proteolytic activity: The over night culture were spot inoculated onto pre-poured plates of the relevant medium (standard plate count agar with 10% added skim milk). The inoculated plates were incubated at 7°C for 10 days the colonies were checked by further examination as previously mentioned *(7)*.

RESULTS

Table 1. Proteolytic bacterial count/ml. or gm. in examined samples.

Type of examined samples	Min.	Max.	Mean	± S.E. M.
Raw milk	5.0×10^{3}	9.0×10^{7}	5.30×10^{6}	2.05×10^{6}
Damietta cheese	9.0×10^{4}	5.0×10^{7}	3.81×10^{6}	1.96×10^{6}
Kariesh cheese	3.5×10^{9}	4.2×10^{13}	2.84×10^{12}	1.76×10^{12}

Table 2. Frequency distribution of examined samples based on their proteolytic bacterial count/ml. or gm.

Intervals	Raw milk		Damietta cheese		Kariesh cheese	
	No.	%	No.	%	No.	%
10^{3}-<10^{5}	15	30.0	1	4.0	0	0.0
10^{5}-<10^{7}	29	58.0	23	92.0	0	0.0
10^{7}-<10^{9}	6	12.0	1	4.0	0	0.0
10^{9}-<10^{11}	0	0.0	0	0.0	6	24.0
10^{11}-<10^{13}	0	0.0	0	0.0	17	68.0
10^{13}-<10^{15}	0	0.0	0	0.0	2	8.0
Total	50	100.0	25	100.0	25	100.0

Table 3. Prevalence of proteolytic bacteria isolated from examined samples.

Isolates	Raw milk N=50		Damietta cheese N=25		Kariesh cheese N=25	
	No. of samples	%	No. of samples	%	No. of samples	%
Pseudomonas spp.	20	40.0	16	64.0	16	64.0
Enterobacteriaceae spp.	19	38.0	10	40.0	12	48.0
Acenitobacter spp.	12	24.0	6	24.0	5	20.0
Gram-positive spp.	12	24.0	11	44.0	9	36.0
Alcaligenes spp.	9	18.0	9	36.0	8	32.0
Achromobacter spp.	9	18.0	3	12.0	4	16.0
Aeromonas spp.	6	12.0	0	0.0	3	12.0
Flavobacterium spp.	5	10.0	1	4.0	2	8.0
Vibrio	1	2.0	0	0.0	0	0.0
Chromobacterium spp.	1	2.0	1	4.0	1	4.0

Table 4.Prevalence of proteolytic Enterobacteriaceae species isolated from examined samples.

Isolates	Raw milk N=50		Damietta cheese N=25		Kariesh cheese N=25	
	No. of samples	%	No. of samples	%	No. of samples	%
Enterobacter spp.	7	14.0	3	12.0	3	12.0
Citrobacter spp.	5	10.0	2	8.0	3	12.0
Serratia spp.	3	6.0	0	0.0	1	4.0
Klebsiella spp.	3	6.0	1	4.0	2	8.0
Proteus	0	0.0	2	8.0	1	4.0
E.coli	1	2.0	2	8.0	2	8.0

Table 5. Prevalence of proteolytic Gram-positive species isolated from examined samples.

Isolates	Raw milk N=50		Damietta cheese N=25		Kariesh cheese N=25	
	No. of samples	%	No. of samples	%	No. of samples	%
Bacillus spp.	6	12.0	2	8.0	3	12.0
Micrococcus spp.	3	6.0	0	0.0	0	0.0
Staphylococcus spp.	3	6.0	8	32.0	5	20.0
Streptococcus spp.	0	0.0	1	4.0	1	4.0

Table 6. Proteolytic properties of microorganisms isolated from the examined samples at 7°C for 10 days on skim milk agar plates.

Isolates	Raw milk N=50			Damietta cheese N=25			Kariesh cheese N=25		
	No. of tested isolates	No. of positive isolates	%	No. of tested isolates	No. of positive isolates	%	No. of tested isolates	No. of positive isolates	%
Pseudomonas spp.	26	26	100.0	18	17	94.4	17	17	100.0
Enterobacteriaceae spp.	22	18	81.8	12	9	75.0	14	11	78.5
Acenitobacter spp.	14	10	71.4	6	3	50.0	9	8	88.8
Gram-positive spp.	17	13	76.5	12	11	91.6	10	10	100.0
Alcaligenes spp.	9	5	55.5	10	4	40.0	8	2	25.0
Achromobacter spp.	9	8	88.8	5	4	80.0	8	7	87.5
Aeromonas spp.	6	2	33.3	0	0	0.0	3	1	33.3
Flavobacterium spp.	5	2	40.0	3	0	0.0	3	3	100.0
Vibrio	1	0	0.0	0	0	0.0	0	0	0.0
Chromobacterium spp.	1	0	0.0	2	0	0.0	1	1	100.0
Total	110	84	76.4	68	48	70.6	73	60	82.2

DISCUSSION

Results recorded in Table 1 proved that all examined raw milk samples were positive for proteolytic bacteria with a mean value of $5.3X10^6 \pm 2.05X10^6$. The highest frequency distribution (58%) lied within the range of 10^5-$<10^7$. Table 2 . Nearly similar findings were previously reported *(8),* while lower counts were recorded *(9).*

Inspection of data reported in Table 1 revealed that the mean proteolytic bacterial count/ gm. For the examined Damietta and kariesh cheese samples were $3.81X10^6$ and $2.84X10^{12}$, respectively. The highest frequency distribution in examined Damietta and kariesh cheese (92% and 68%) were within the range of 10^5-$<10^7$ and 10^{11}-$<10^{13}$, respectively. Table 2 Comparatively lower results were reported *(10).* The presence of proteolytic bacteria in examined raw milk samples indicated that they were produced, handled and distributed under unhygienic condition, while their presence in Damietta and kariesh cheese samples reflected that those products were manufactured, handled and stored under improper sanitation as well as absence of heat treatment.

It is evident from the results given in Table 3 that members of genera Pseudomonas, Enterobacteriaceae, Acenitobacter, Aeromonas, Flavobacterium, Vibrio and Chromobacterioum were isolated from the examined raw milk samples at varying percentages ranging from 2% to 40%. These results are differ from that previously reported *(7).* The results presented in Table 3 indicated that 64%, 44%, 40%, 36%, 24%, 12%, 4% and 4% of the examined Damietta cheese samples were contaminated with Pseudomonas spp., Gram-positive spp., Enterobacteriaceae spp., Alcaligenes spp., Acenitobacter spp., Achromobacter spp., Flavobacterium spp. and Chromobacterium spp., respectively. While Pseudomonas spp., Enterobacteriaceae spp., Gram-positive spp., Achromobacter spp., Aeromonas spp., Flavobacterium spp. and Chromobacterium spp. could be isolated in 64%,48%,36%, 32%, 20%, 16%, 12%, 8%, and 4% of examined kariesh cheese samples, respectively. Moreover, the data recorded in the previously mentione table indicated that Vibrio and Aeromonas spp. could not be isolated from the examined Damietta cheese samples, while Vibrio spp. failed to be detected in the examined kariesh cheese samples.

The prevalence of proteolytic species of Enterobacteriaceae family isolated from examined samples recorded in Table 4 indicated that Enterobacter spp. was the predominated species 14% and 12% among the previously mentioned family in the examined raw milk and Damietta cheese samples, respectively. While, Enterobacter and Citrobacter species were predominating other members of such family in the examined kariesh cheese samples (12% each) but the presented data showed that Proteus and Serratia species failed to be detected in examined raw milk and Damietta cheese samples, respectively.

It is evident from the results given in Table 5 that out of the examined 50 raw milk samples, 12 % were found to be contaminated with Bacillus spp. followed by Micrococcus spp. and Staphylococcus spp. (6% each). While the incidence of proteolytic Gram-positive bacteria isolated from the examined Damietta and kariesh cheese samples proved that Staphylococcus spp. was predominating other Gram-positive bacteria followed by Bacillus spp. and Streptococcus spp.. Moreover, Streptococcus and Micrococcus spp. failed to be detected in raw milk, Damietta and kariesh cheese samples, respectively. These findings are nearly similar to that previously cited *(7).*

The proteolytic properties of the isolates incubated on skim milk agar plates at 7°C for 10 days showed that most of the tested isolates of the examined samples demonstrated the proteolytic activity, while non of vibrio

spp. tested had proteolytic activity, but Chromobacterium spp. had proteolytic activity in the examined kariesh cheese samples only"100" Table 6. Nearly similar findings were reported *(7)*. When cheese was made from heavy contaminated (10^6-10^7 psychrotrophic cell/ml.), cold stored (5°C/6days) milk, the activation of protease enzymes (proteolysis) rescued the protein yield in the range of 3-4.5%, in addition to poor cheese texture due to their effect on rennet and starter used *(11)*. All these defects in milk and its products could limit the storage life and lowering the nutritive value of the products.

Gram negative bacteria as pseudomonas which have been involved in proteolytic spoilage of cheese is due to presence of heat stable enzymes. Also, produced a large number of extracellular toxins which include phytotoxic factor, pigments, hydrocyanic acid, proteolytic enzymes, phospholipase, enterotoxin and slime. Exotoxins were the most responsible factors for Pseudomonas spp. pathogenicity because it could produce leucopoenia, circulatory collapse, liver necrosis, pulmonary odema, Heamorrhage and kidney tubular necrosis. The enterotoxin produced is responsible for diarrheal symptoms *(12)*.

Enterobacteriaceae in milk products are of greater importance with regard to the quality and shelf-life of dairy products. The species and the numbers of Enterobacteriaceae present determine the potential quality impairment of the products mainly due to extracellular proteolytic and lipolytic enzymes. These enzymes induce undesirable changes in milk proteins and fats giving rise to a rancid and off-flavor *(13)*. Presence of high incidence of coliforms in the raw milk and cheese samples may give an indication of the unsanitary practices during processing and storage or using of contaminated utensils and equipment. Some members of coliforms (Enterobacter, Citrobacter and Klebsiella species) were incriminated in acute and chronic diarrheal diseases *(14)*. The presence of E.coli is an indicative of faecal pollution *(15)*. Enterotoxigenic strains of E.coli can produce illness to both man and animals. It produce 2 toxins, heat-labile enterotoxins (LT, destroyed by heat) or heat-stable toxins (ST, not destroyed by heating up to 100°C for 5 minutes) while, some strains could produce both toxins. These toxins were associated with infantile diarrhea. But the invasive strains of E.coli could produce dysentery like disease similar to that of shigella *(16)*.

Certain species of genus Proteus proved to be of public health importance as they had been encountered in cases of summer diarrhea in infants as well as in urinary tract infection *(17)*. Serratia species may produce rancidity, while some species of as Serratia marcescuns may be responsible for the development of red color in milk and its product *(18)*.

Flavobacterium spp. were responsible for rancidity in milk and its products *(19)*.

Alcaligenes spp. were known to cause sliminess and slight alkalinity in the cream layer of raw milk *(20)*.

Streptococci being a normal inhabitant in the intestinal tract of both man and animals. Their presence in any food article is indicative of unsanitary production and handling *(21)*. A few numbers of Streptococci have proteolytic and lipolytic activities which lead to bitterness in milk and its products in addition to unfavorable changes. While certain species of Streptococci can grow at a wide range of temperature as well as they were implicated in cases of food poisoning *(15)*.

Bacillus species proved to induce certain objectionable changes in milk and some dairy products *(22)*. Certain Bacillus species can grow at low temperature and produce enzymes which lead to bitterness, sweet curdling, bitty cream and blood red sediment on the surface of milk. Also, they can cause carbolic fishiness

and phenol flavour. While, some strains had been implicated in cases of food poisoning.

The presence of coagualse-positive staphylococcus aureus in a food give an indication about its contamination from skin, mouth or handling the food, but inadequately cleaned utensils or equipment may also a source of contamination *(15)*. Staphylococcal food poisoning is a major form of food borne illness and appears to continue to be so as time goes on when the environmental conditions are favorable for growth and multiplication of Staph. *(23)*. The enterotoxins of staphylococcus aureus are antigenically different types include (A, B, C1, C2, D, E and TST "toxic shock toxin". All these types of enterotoxins except TST were involved in food borne diseases.

Micrococci are widely distributed in nature and can contaminated milk and its products from different sources. Certain species of these microorganisms can grow and multiply in the product to produce certain defects rendering it undesirable, unmarketable, of an inferior quality or even unfit for consumption thus causing economic losses. Some members of Micrococci are important as causative agents of mastitis in dairy animals. In addition, Micrococcus species have the ability of produce heat-stable toxin, so they are incriminated in cases of food poisoning *(21)*

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المنخص العربى

تواجد الميكروبات المحللة للبروتين في اللبن وبعض منتجاته

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**معهد بحوث صحة الحيوان – الزقازيق

أجريت هذه الدراسة علي مائة عينة (خمسون من اللبن الخام و خمسة و عشرون من كل من الجبن الدمياطي و القريش) لمعرفة مدي تلوثها بالبكتريا المحللة للبروتين حيث أن اللبن ومنتجاته مصدر هام للبروتين و بالتالي تكون أكثر أنواع الأغذية عرضة للتلوث بهذه الميكروبات و أخصب الأوساط لنموها و تكاثرها و بذلك تكون سببا أساسيا في انتشار العديد من الأمراض بالاضافة إلي تأثيرها علي جودة المنتج. كان متوسط العدد الكلي للميكروبات المحللة للبروتين في عينات اللبن الخام، الجبن الدمياطي و الجبن القريش ٣,٥×١٠^٦، ٣,٨١×١٠^٦ و ٢,٨٤×١٠^١٢ علي التوالي. كما أمكن عزل الأصناف البكتيرية الاتية: الزوائف، الميكروبات المعوية، اسينيتوباكتر، ميكروبات موجبة لصبغة الجرام، الكالجينز، اكروموباكتر، ايروموناس، فلافوباكتريم، فيبريو و كروموباكتريوم بنسب متفاوتة. من بين الميكروبات المعوية تم التعرف علي الأصناف البكتيرية الأتية: انتيروباكتر، ستروباكتر، السراتيا، كليبسيلا، المتقلبة و ايشريشيا كولاي. ومن بين الميكروبات الموجبة لصبغة الجرام تم التعرف علي الأصناف البكتيرية الأتية: العصوية، المكيرة، العنقوديات والسبحيات. تم اختبار جميع المعزولات من ناحية القدرة علي تحليل البروتين عند درجة ٥٧° م لمدة ١٠ أيام و قد أسفرت النتائج أن نسب متفاوتة من المعزولات لها القدرة علي تحليل البروتين.