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## OCCURRENCE OF SOME MICROORGANISMS IN RELATION TO PUBLIC HEALTH IN KAREISH CHEESE

(With 4 Tables)

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تواجد بعض الميكروبات ذات الصلة بالصحة العامة في الجبن القريش

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أجريت الدراسة على خمسين عينة من الجبن القريش جمعت من الأسواق المحلية بمحافظة الدقهلية (مصر) خلال ربيع ٢٠٠٥ وذلك لتحديد مدى تلوثها بالميكروبات ذات الصلة بالصحة العامة بالإضافة إلى تعيين النسبة المئوية للحموضة والرطوبة وملح الطعام. وقد أظهرت النتائج أن متوسط النسبة المئوية للحموضة والرطوبة وملح الطعام في العينات كانت ١,٥% ، ٦١,٢% ، ٥,٤% على التوالي، بينما بلغ متوسط ميكروبات الايشريشيا كولاي والمكورات العنقودية الذهبية والباسيلس سيرس والكلوستريديم بيرفرنجينز والمكورات السبجية المعوية  $1.0 \times 10^4$  ،  $3.1 \times 10^4$  ،  $1.0 \times 10^3$  ،  $1.0 \times 10^8$  ،  $1.0 \times 10^9$  ،  $1.0 \times 10^7$  لكل جرام من الجبن على التوالي. ولقد تم عزل ميكروب اليارسينيا انتيروكوليتكا من العينات بنسبة ٤% ، بينما لم يتم عزل ميكروبات السالمونيلا. كما أثبتت التحليلات الإحصائية أن هناك علاقة ارتباط بين النسبة المئوية للحموضة والرطوبة وملح الطعام وإعدادات الميكروبات المختلفة في عينات الجبن. هذا وقد تمت مناقشة النتائج من الناحية الصحية والاقتصادية واقتراح ما يجب اتبعه من إجراءات من الناحية الصحية لضمان جودة المنتج والحفاظ على صحة المستهلك.

### SUMMARY

Fifty random samples of Kareish cheese were collected from local markets in Dakahlia Province, Egypt during spring of 2005 to be examined chemically and bacteriologically. The mean acidity %, moisture % and sodium chloride % of examined kareish cheese samples were 1.5, 61.2 and 5.4 respectively. While, the mean counts/g of examined samples for *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium perfringens* and *Enterococci* were  $3.8 \times 10^3$ ,  $3.4 \times 10^4$ ,

$1.8 \times 10^4$ ,  $1.9 \times 10^2$  and  $5.7 \times 10^6$  respectively. *Yersinia enterocolitica* was isolated from 4% of examined kareish cheese samples, while *Salmonellae* failed to be detected. Significant correlation was found between acidity %, moisture % sodium chloride % and different bacterial counts in kareish cheese. The public health importance of the isolated organisms as well as sanitary measures for improving the quality of kareish cheese were discussed.

*Key words: Kareish cheese, milk products, pathogens, acidity, NaCl.*

## INTRODUCTION

Kareish cheese is a kind of soft cheese which is manufactured from raw buffaloe or cow's milk in farmer's houses. It is considered the main protein supplement to farmers and most people in Egypt. The main sources of pathogenic bacteria in cheese are contaminated raw milk, food handlers, dust, utensils and insects (Robinson, 1990).

The growth of pathogenic bacteria in dairy products is influenced by thermal treatment during processing and by conditions within the product such as pH, water activity, presence of inhibitory substances or competitive microorganisms (Ray, 1996).

Milk and its products like cheese are involved in food poisoning outbreaks. The bacteria which commonly cause food poisoning due consumption of dairy products in Canada, Britain and many other countries are *Salmonellae*, *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium perfringens* (Todd, 1985 and Trickett, 1986).

The manufacture, handling and distribution of Kareish cheese by farmers render it of public health hazards. The present study was conducted to throw light on the occurrence of some microorganisms in relation to public health importance in Kareish cheese marketed in Dakahlia Province.

## MATERIALS and METHODS

Fifty random samples of Kareish cheese (each about 50 grams) were collected from local markets in Dakahlia Province, during spring of 2005.

The collected samples were transferred directly to the laboratory in ice box to be examined chemically and bacteriologically.

### **A- Chemical examination:**

The content of water, sodium chloride and titratable acidity of kareish cheese samples were determined according to the methods



recommended by Association of Official Analytical Chemists "A.O.A.C." (1984).

**B- Bacteriological examination:**

Each sample was thoroughly mashd in a sterile mortar. Eleven grams of prepared cheese sample were aseptically homogenized with 99 ml of sterile 2% sodium citrate solution at 40C°, serial dilutions of the homogenates were prepared.

Counts of *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium perfringens* and *Enterococci/g* were determined according to A.P.H.A. (1985).

Suspected colonies were isolated and subjected to confirmatory tests according to Krieg and Holt (1984) and Roberts *et al.* (1995).

Isolation of salmonella spp. was carried out according to Cowan and Steel (1993).

Isolation of *Yersinia enterocolitica* was done according to the technique adopted by Walker and Gilmour (1986) and the isolates were identified according to the methods recommended by Krieg and Holt (1984). The obtained results were statistically analyzed according to Snedecor and Cochran (1989).

**RESULTS**

**Table 1:** Titratable acidity %, moisture % and sodium chloride % in examined Kareish cheese samples (n =50).

Chemical examination	Min.	Max.	Mean	± S.E.M.
Titratable acidity%	0.9	2.4	1.5	0.06
Moisture %	55.0	65.0	61.2	1.3
Sodium chloride %	2.5	9.1	5.4	0.4

**Table 2:** Bacterial counts/g in examined Kareish cheese samples (n=50).

Bacterial counts	+ ve samples		Count /g.			
	No.	%	Min.	Max.	Mean	± S.E.M.
<i>Escherichia coli</i>	25	50.0	70	7.6x10 <sup>4</sup>	4.8x10 <sup>3</sup>	1.2x10 <sup>3</sup>
<i>Staphylococcus aureus</i>	14	28.0	1.1x10 <sup>2</sup>	6.5x10 <sup>5</sup>	3.4x10 <sup>4</sup>	0.9x10 <sup>4</sup>
<i>Bacillus cereus</i>	21	42.0	4.0x10 <sup>3</sup>	2.1x10 <sup>5</sup>	1.8x10 <sup>4</sup>	0.4x10 <sup>4</sup>
<i>Clostridium perfringens</i>	10	20.0	86	3.1x10 <sup>2</sup>	1.9x10 <sup>2</sup>	0.2x10 <sup>2</sup>
<i>Enterococci</i>	50	100.0	9.2x10 <sup>3</sup>	3.5x10 <sup>7</sup>	5.7x10 <sup>6</sup>	1.6x10 <sup>6</sup>
<i>Yersinia enterocolitica</i>	2	4.0	-	-	-	-
<i>Salmonella spp.</i>	0	0.0	-	-	-	-

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

Bacterial counts	Number (%) of samples with count in range						
	10-10 <sup>2</sup>	10 <sup>2</sup> -10 <sup>3</sup>	10 <sup>3</sup> -10 <sup>4</sup>	10 <sup>4</sup> -10 <sup>5</sup>	10 <sup>5</sup> -10 <sup>6</sup>	10 <sup>6</sup> -10 <sup>7</sup>	10 <sup>7</sup> -10 <sup>8</sup>
E. coli	2 (8)	5 (20)	11(44)	7(28)			
Staph.aureus		3(21.43)	3(21.43)	5(31.71)	3(21.43)		
B. cereus			9(42.9)	8(38.1)	4(19)		
Cl. perfringens	3 (30)	7(70)					
Enterococci			9(18)	9(18)	7(14)	20(40)	5(10)

**Table 4:** Correlation between acidity %, sodium chloride % moisture % and different bacterial counts in examined Kareish cheese samples.

Bacterial counts Chemical analysis	Escherichia coli Count	Staphylococcus aureus Count	Bacillus cereus Count	Clostridium perfringens Count	Enterococci Count
Acidity%	r = -0.689*	r = -0.744**	r = -0.735**	r = -0.878**	r = -0.605**
Na Cl %	r = -0.749**	r = -0.831**	r = -0.825**	r = -0.940**	r = -0.693**
Moisture%	r = 0.673**	r = 0.743**	r = 0.743**	r = 0.886**	R = 0.591*

\* Significant (P<0.05)

\*\* Highly Significant (P<0.01)

## DISCUSSION

Average values of titratable acidity %, moisture % and sodium chloride % of examined kareish cheese samples were 1.5, 61.2 and 5.4%, respectively (Table, 1).

The results agreed to some extent with those reported by Ahmed *et al.* (1987), Moustafa (1988) and Abd-El-Haleem (1990). E. coli could be detected in 50 % of examined kareish cheese samples with a mean value of  $4.8 \times 10^3 \pm 1.2 \times 10^3$  /g ( $70- 7.6$ )  $\times 10^4$ ). Most of the samples (72 %) lay within the range of  $10^3$  to  $10^5$  (Table 2 and 3). Higher results were reported by Al-Ashmawy *et al.* (1994) and Ebrahim (1998).

The presence of E-coli in food is considered as on indicator of faecal cotamination. Moreover, particular strains are known to induce severe diarrhea in both infants and young children as well as cases of food poisoning and gastroenteritis among adult consumers (Eley, 1996).



*Staphylococcus aureus* was detected in 28% of Kareish cheese samples, with mean count of  $3.4 \times 10^4 \pm 0.9 \times 10^4$  CFU/g ( $1.1 \times 10^2$ – $6.5 \times 10^5$ ). The highest frequency percentages (31.71 %) lay within the range of  $10^4$  and  $10^5$  (Tables, 2 and 3).

Nearly similar counts were reported by Al-Ashmawy *et al.* (1994), while higher counts were reported by Kaldes (1997) and Halawa and Moawad (1999).

*Staph. aureus* is one of the most important aetiological agent of bovine and ovine mastitis and a high percentage of food handlers working in dairy industries are nasal carriers of *Staph. aureus* (Garcia *et al.*, 1986).

*Staph. aureus* has a potential importance to the public health because of its ability to produce enterotoxins, resulting in staphylococcal food poisoning (Hill, 1983).

*Bacillus cereus* could be detected in 42% of examined Kareish cheese samples, with a count ranged from  $4.0 \times 10^3$  to  $2.1 \times 10^5$  /g and an average count of  $1.8 \times 10^4 \pm 0.4 \times 10^4$ . Most of samples (81.0%) lay within the range of  $10^3$  and  $10^5$  (Tables, 2 and 3).

These results agreed with those of Khalil (1997) and Halawa and Moawad (1999). *Bacillus cereus* was recognized as a food poisoning pathogen and could produce diarrhoeal toxin (Eley, 1996). The results presented in Table (2) showed that 20% of examined Kareish cheese samples were contaminated with *Clostridium perfringens*. The maximum count /g was  $3.1 \times 10^2$ , the minimum count was 86, with a mean value of  $1.9 \times 10^2 \pm 0.2 \times 10^2$ . The highest frequency distribution (70%) lays within the range of  $10^2$  and  $10^3$  (Table 3).

Nearly similar results were reported by Amer *et al.* (1996). *Clostridium perfringens* was responsible for food poisoning among consumers duo to consumption of certain dairy products (Eley, 1996).

The presence of *Cl. Perfringens* in dairy products was indicative of faecal or soil contamination and it was associated with spoilage problems, late blowing, in cheese (Robinson, 1990).

Enterococci could be isolated from all examined Kareish cheese samples with a mean count of  $5.7 \times 10^6 \pm 1.6 \times 10^6$  ( $9.2 \times 10^3$ – $3.5 \times 10^7$ ). Most of the samples (54%) lay within the range of  $10^5$  and  $10^7$  (Tables, 2 and 3). Nearly similar results were recorded by Moustafa (1988) and El-Kholy *et al.* (1995), while higher results were reported by Kaldes (1997) and Halawa and Moawad (1999).

The presence of enterococci in milk and its products was indicative of faecal contamination and also was associated with some

cases of food poisoning outbreaks (ICMSF, 1978 and Sedova *et al.*, 1981).

*Yersinia enterocolitica* could be isolated from two samples of the examined Kareish cheese samples, while *Salmonella* failed to be detected (Table, 2).

*Y. enterocolitica* was recovered from Kareish cheese by Ebrahim (1998) and Halawa and Moawad (1999). While, El-Leboudy (1989) failed to detect the organism in Kareish cheese.

*Y. enterocolitica* has been implicated in cases of food poisoning involving milk and its products (Eley, 1996).

As presented in Table 4, there was a strong positive correlation between the bacterial counts and moisture content of Kareish cheese, while negative correlation was found between the bacterial counts and acidity and sodium chloride percentages of Kareish cheese. Higher acidity, lower moisture content and higher salt content of food are important factors for controlling the growth of contaminating microorganisms and preserving the food. On the other hand, these factors may affect the texture and flavour of cheese (Nunez *et al.*, 1985 and Forsythe and Hayes, 1998). The results of this investigation indicated that the Kareish cheese collected from local markets in Dakahlia Province was of poor sanitary quality and could pose a considerable risk to human health.

## REFERENCES

- Abd-El-Haleem, A.M. (1990):* Studies on the sanitary conditions of Kareish and Mish cheese. M.V.Sc. Thesis, Fac. Vet. Med., Zagazig Univ., Egypt.
- Ahmed, A.A.; Moustafa, M.K. and Abdel-Hakim, E.H. (1987):* Sanitary condition of Kareish cheese manufactured in Assiut City. *Assiut Vet. Med. J.* 19 (37): 76-80.
- Al-Ashmawy, A.M.; Mansour, M.A.; Amer, I.H. and Abd-El Haleem, A.M. (1994):* Studies on the microbiological quality of Egyptian Kareish and Mish cheese. *Zag. Vet. J.* 22(2): 125-132.
- Amer, I.H.; Bahout, A.A. and Hatab, M.E. (1996):* Prevalence of *Clostridium perfringens* in milk and dairy products. The proceeding of 3<sup>rd</sup> Vet. Med. Cong., 8-10 october, Fac. Vet. Med., Zagazig Univ., pp 203-208.
- American Public Health Association (A.P.H.A.) (1985):* Standard Methods for the Examination of Dairy Products. 15<sup>th</sup> ed., Washington. DC.



- Association of Official Analytical Chemists (A.O.A.C.) (1984):* Official Methods of Analysis. 14<sup>th</sup> ed., Arlington, AV:AOAC.
- Cowan, S.T. and Steel. K.J. (1993):* Manual for the Identification of Medical Bacteria. Cambridge Univ. Press, Cambridge.
- Ebrahim, N.H. (1998):* Occurrence of enterobacteriaceae in milk and kareish cheese M.V.Sc. Thesis, Fac. Vet. Med., Zagazig Univ., Egypt.
- Eley, A.R. (1996):* Microbial Food Poisoning. 2<sup>nd</sup> ed., Chapman and Hall. London, U.K.
- El-Kholy, A.; Hafez, R. and Mahmoud, S. (1995):* Occurrence of some food poisoning bacteria in Egyptian soft cheese. Beni-Suef Vet. Med. Res., 7(1):342-355.
- El-Leboudy, A.A. (1989):* Potential food poisoning organisms in some dairy products at the consumer level. Ph.D. Thesis, Fac. Vet. Med., Alex. Univ., Egypt.
- Forsythe, S.J. and Hayes, P.R. (1998):* Food Hygiene, Microbiology and HACCP. 3<sup>rd</sup> ed., Aspen publishers, Inc., Gaithersburg, Maryland.
- Garcia, M.L.; Franciso, J.J. and Moreno, B. (1986):* Nasal carriage of Stapylococcus species by food handlers. Int. J.Food Microbiol. 3, 99-108.
- Halawa, M.A. and Moawad, A.A. (1999):* Bacteriological quality of street-vended white cheese. Alex. J. Vet.Sci. 15 (4): 855-864.
- Hill, B.M. (1983):* Enterotoxin-producing Stapylococcus aureus isolated from milk and dairy products. New Zealand J. of Dairy Sci. and Technology 18, 59-62.
- International Commission on Microbiological Specification for Food (ICMSF) (1978):* Microorganisms in food. Their significance and methods of enumeration, 2<sup>nd</sup> ed., Univ. of Toronto Press, Toronto and Buffalo's Canada.
- Kaldes, Y.T. (1997):* Microbiological examination of soft cheeses manufactured in El- Minia City. Assiut Vet. Med. J. 75, 39-47.
- Khalil, N.G. (1997):* Incidence of Bacillus cereus in some food stuffs with the special reference to its production of thrermonuclease enzyme in Assiut City. Assiut Vet. Med. J. 75, 55-63.
- Krieg, N.R. and Holt, J.N. (1984):* Bergey's Manual of Systematic Bacteriology. Vol. 1, Williams and Wilkins Co., Baltimore, USA.

- Moustafa, A.H. (1988):* Occurrence and significance of enterococci in milk and some dairy products. M.V.Sc. Thesis, Fac. Vet. Med., Zagazig Univ., Egypt .
- Nunez, M.; Gaya, P. and Medina, M. (1985):* Influence of manufacturing and ripening conditions on the survival of Enterobacteriaceae in Manchego cheese. J. Dairy Sci. 68: 794-800.
- Ray, B. (1996):* Fundamental Food Microbiology. CRC Press. Inc., Tokyo, New York.
- Roberts, D.; Hooper, W. and Greenwood, M. (1995):* Practical Food Microbiology 2<sup>nd</sup> ed., Public Health Laboratory Service, London.
- Robinson, R.K. (1990):* Dairy Microbiology 2<sup>nd</sup> ed., Chapman and Hall, London, New York.
- Sedova, N.N.; Nefed, N.P. and Skirko, B.K. (1981):* Some aspects of the pathogenicity of enterococci causing food poisoning. Voprosy Pitaniya 3, 59-61. Food Sci. and Tech. Abst. 14 (2), 1982.
- Snedecor, G.W. and Cochran, W. (1989):* Statistical Methods. 8<sup>th</sup> ed., Iowa State Univ. Press, Ames Iowa U.S.A.
- Todd, E.C.D. (1985):* Foodborne and waterborne diseases in Canada-1978. annual summary. J. Food Prot. 48(11):990-996.
- Trickett, J. (1986):* The Prevention of Food Poisoning. 2<sup>nd</sup> ed., Stanley Thornes (Publishers) Ltd., UK.
- Walker, S.J. and Gilmour, A. (1986):* The incidence of Y.enterocolitica and Y.enterocolitica-Like organisms in raw and pasteurized milk in Northern Ireland. J. Appl. Bacteriol. 61: 133-138.