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INCIDENCE OF BACILLUS CEREUS IN SOME SWEETENED DAIRY PRODUCTS AND DAIRY DESERTS SOLD IN ASSIUT CITY

(With 2 Tables)

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مدى تواجد ميكروب الباسيليس سيريس في بعض منتجات الألبان المحلاة والحلاوى اللبنية المباعة فى مدينة أسيوط

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تم فحص عدد ٨٠ عينة عشوائية من منتجات الألبان المحلاة وتشمل الايس كريم (٣٠ عينة) واللبن المكثف(١٠ عينات) وكذلك بعض الحلاوى اللبنية وتشمل الأرز باللبن (٢٥ عينة) والمهلبية (١٥ عينة) والتي تم جمعها من أماكن مختلفة في مدينة أسيوط منها المطاعم، محلات الألبان، والسوبر ماركت ومحلات البقالة. وقد تم فحص هذه العينات لمعرفة مدى تواجد ميكروب الباسيلس سيريس في هذه المنتجات. وباستخدام المستنبتKG ثم عزل هذا الميكروب من ٥٠% ،٩٠%، ٦٨%، ٨٠% من عينات الايس كريم واللبن المكثف والأرز باللبن والمهلبية على التوالي. بأعداد تراوحت من <١٠ - ١٧٠٠، <١٠ - ٥٠٠، <١٠٠ - ٣٠٠ و <١٠٠ - ١٠٠٠ / مل اوجرام على التوالي وقد كانت اغلب العينات وهي ٣٠% ،٥٦% ،٧٣% من عينات الايس كريم والأرز باللبن والمهلبية على التوالي تحتوي عل أعداد تراوحت من ١٠ إلى <١٠ بينما كانت ٥٠% من عينات اللبن المكثف تحتوي على عدد اقل من١٠/ جرام. وقد ناقش البحث الشروط الصحية لمنع تلوث هذه المنتجات بميكروب الباسيلس سيريس وكذلك مدى خطورته على الصحة العامة.

SUMMERY

Eighty random samples of some sweetened dairy products including ice cream (30 samples) and condensed milk (10 samples) and some dairy deserts including rice with milk (25 samples) and Mehallabeia (15 samples) were collected from different restaurants, dairy shops, supermarkets and groceries in Assuit City for enumeration and isolation of B. cereus. B. cereus was isolated from 50%, 90%, 68% and 80%. of ice cream, condensed milk, rice with milk and mehallabeia samples

respectively. The numbers ranged from < 10-1700, < 10 –500, < 100 – 300 and < 100-1000 c.f.u. / g or ml of the samples, respectively. The highest frequency distribution lies within the range of < 10-10^2 /g or ml of ice cream, rice with milk and Mehallabeia, respectively. While, 50% of condensed milk had counts < 10/g. The Public health hazard and recommended measures to prevent contamination of such products by this organism were discussed.

Key words: *B. cereus, dairy deserts , ice cream, condensed milk*

INTRODUCTION

Sweetened dairy products are most palatable nutrient, healthful dairy foods. They include sweetened condensed milk, ice cream and Traditional Egyptian dairy deserts such as Mehallabeia, rice with milk, Om Ali and others where the milk is the basic constituent and may served alone or after meal. Consumption of sweetened condensed milk and ice cream has increased dramatically all over the world. While, mehallabeia and rice with milk, the Egyptian dairy deserts are usually consumed in Egypt by a wide range of people of all ages and usually served cooled. Unfortunately, some of these products provide high favorable media for multiplication of different types of microorganisms including Bacillus cereus.

Occurrence of Bacillus cereus in milk has been reported since 1916 and this bacterium is a common contaminant of raw milk. Also, the bacterium sometimes can be found in large numbers in dairy products (Kim and Goepfert, 1971). The role of B.cereus in outbreaks of food borne illness is becoming increasingly well documented. In 1950, the first report of confirmed cases of B.cereus food poisoning appeared, which was followed by additional reports in the ensuing years confirming the role of the organism in food borne disease (Hauge, 1950; Hauge, 1955; Midura *et al.*, 1970 and Mossel *et al.*, 1967).

Large numbers of viable cells of B.cereus are required to cause illness; numbers in excess of 10^5-$16^6$1g have been encountered in food suspected as causing illness (Goepfert *et al.*, 1973). Food borne illness caused by B.cereus is considered by some investigators as food intoxication rather than a food infection, and so a significant level of growth by the organism would be required to synthesize the necessary level of extra cellular toxin. The ability of B.cereus to produce two different syndromes, has been established ,diarrhea syndrome caused by

at least two different types of enterotoxins during vegetative growth of B.cereus in food, as well as in the small intestine after consumption of contaminated food (Ombui *et al.*, 1997 and Kotiranta *et al* ., 2000). While emetic syndrome, where a quite number of vegetative cells may resporulated after heat treatment and produce the emetic toxin (Eley, 1992).

B.cereus in dairy products is not only of concern as a pbulic health hazard but also as a cause of economic losses through spoilage of contaminated products. Examples of such spoilage including bitty cream, sweet curdling of milk and off-flavors in various products (Te-Gifflel *et al.*, 1996; Pirltijarvi *et al.*, 2000 and Eneroth *et al.*, 2001). No available data concerning the occurrence of B. cereus in Mehallabeia and rice with milk could be found. However, the occurrence of the organism in ice cream and condensed milk was detected by Ahmed *et al.* (1983); Ahmed *et al.* (1988); Korashy and Sabreen (2001) and Abdel-Hameid (2004) Therefore, this work was planned to secure the occurrence of such organism in some sweetened dairy products as well as some dairy deserts sold in Assiut city markets.

MATERIALS and METHOD

Collection of samples:

Eighty (80) random samples of ice cream (30 samples), condensed milk (10 samples), rice with milk (25 samples) and Mehallabia (15 samples) were collected from different restaurants, dairy shops, supermarkets and groceries in Assiut city. Each sample was obtained in its container as it is served or sold to the public. The samples were dispatched directly to the laboratory with a minimum of delay, where they were prepared and examined.

Preparation of samples:

Ice cream samples were left to melt in a thermostatically controlled water bath at 44°c for not more than 15 min (A.P.H. A., 1992). Rice with milk and mehallabeia samples were mixed thoroughly and then 10 g were weighed in sterile stainless steel cups containing 90 ml sterile peptone water. Condensed milk cans were physically examined, cleaned aseptically opened, thoroughly mixed and then 10 g were weighed and added to 90 ml sterile peptone water.

Isolation and enumeration of B. cereus:

Ten fold serial dilutions of samples were prepared and the numbers of B. cereus were determined using surface plating technique

on KG medium (Kim and Goepfert, 1971). Furthermore, an appropriate amount of each sample was inoculated into a tube of brain heart infusion broth, which was then incubated for 24 h at 30°c .A loopful of liquid from incubated tubes was streaked onto a plate of K.G agar. Following incubation at 30°C for 24 h, KG plates were examined for typical colonies, which were dry, flat and surrounded by a wide cloudy zone. Colonies presumed to be B. cereus were transferred to nutrient agar slants, which were incubated at 30°C for 24h. The Gram stain, spore stain and motility test were done on each isolate according to Speck (1976). Also, the confirmatory tests based on carbohydrate utilization, nitrate reduction and production of acetyl methyl carbonyl were carried out as described by Speck (1976).

RESULTS

The obtained results were summarized in Tables 1&2

Table 1: Occurrence of Bacillus cereus in some examined sweetened dairy products and dairy deserts

Samples	No	Samples containing B. cereus		B. cereus isolates/ egg yolk-positive isolates	Range of B cereus in samples (N0 /ml or g)
		No	%		
Ice cream	30	15	50	15 / 22	$<_{10}$ - 1700
Condensed milk	10	9	90	9 / 9	$<_{10}$ - 500
Rice with milk	25	17	68	17 / 24	$<_{100}$ - 300
Mehallabeia	15	12	80	12 / 14	$<_{100}$ - 1000
Total	80	53	66.25	53 / 69 (76.8 %)	

Table 2: Frequency distribution of the positive samples of some sweetened dairy products and dairy deserts based on their B. cereus count / ml or g

Count of B. cereus (No. / ml or g)	Ice cream		Condensed milk		Rice with milk		Mehallabeia	
	No /30	%	No/10	%	No/25	%	No/15	%
< 10	2	6.7	5	50	-	-	-	-
10 - < 100	9	30	-	-	14	56	11	73.3
100-< 1000	3	10	4	40	3	12	1	6.7
> 1000	1	3.3	-	-				
Total	15	50	9	90	17	68	12	80

DISCUSSION

The results summarized in Tables 1 and 2 showed that 15 out of 30 ice cream samples (50%) were contaminated with B.cereus in the range of <10 – 1700 organism/ml. The majority of positive samples (30%) contained the organism in numbers ranged from 10-10^2 / ml. About (10%) of positive samples had counts within the range of 10^2-10^3 / ml while 2 positive samples (6.7%) had counts less than 10/ ml.

The incidence of B. cereus in the examined ice cream samples was in a fair agreement with that obtained by Ahmed *et al.* (1983) as they recorded that the organism contaminated 48% of ice cream samples. The high incidence of B. cereus in ice cream samples could be attributed to contamination of raw milk or milk powder, low quality ingredients, using of polluted water supplies, lack of hygienic supervision during processing and handling besides the absence of pasteurization especially in case of small scale produced ice cream.

The summarized results in Tables 1&2 proved that 90% of condensed milk samples contained B. cereus in numbers ranging from<10 to 500/g. Most of the positive samples had numbers of < 10/g, while, the rest of the positive samples (40%) had numbers ranging from <100 to 1000/g. The incidence of B. cereus in condensed milk obtained in this study was higher than that recorded by Korashy and Sabreen (2001) who recorded that 28% of condensed milk were contaminated by B-cereus, however they counted a higher counts of organism /g (10^2 – $6x10^4$) with an average count of 7.6 $x10^3$ c.f.u. /g. However, Ahmed *et al.* (1988) could not detect the organism in the examined condensed milk samples

The data recorded in Tables 1 and 2 also postulated the B. cereus existence in 68% of examined rice with milk samples in numbers ranged from <100-300 / g. The majority of positive samples (56%) contained the organism in number ranged from 10-10^2. The recorded data (Table 1&2) point out that B. cereus could be detected in 80 % 0f mehallabeia samples in number varied from < 100-1000/ g. The highest frequency distribution of the examined mehallabeia samples (73.3%) lies between 10-10^2 /g. No available data could be compared with the obtained results. The high incidence of B. cereus in rice with milk and mehallabeia could be attributed to many factors including careless during preparation, contaminated ingredients and containers, also, contamination during cooling and storage. The organism could find the

opportunity to grow and multiply in such products during storage till serving to the public.

Inspite of the frequency with which dairy products are contaminated with B. cereus, no outbreaks of food poisoning have been occurred from consumption of milk and milk products, except for a few cases caused by cream and certain deserts. According to Goepfert et al (1972) the high numbers of B. cereus needed to elicit symptoms of food poisoning will cause visible spoilage of milk and its products and this will deter their consumption. Furthermore, Bonventre and Johnson (1970) suggested that there are unidentified nutritional factors necessary for synthesis of toxins by B. cereus, and these factors are not present in milk This was disproved by Goepfert *et al* (1973) when they demonstrated that skim milk supported synthesis of enterotoxin by B. cereus.

REFERENCES

A.P. H.A. (1992): Standard Method for Examination of Dairy Products .13th ed., American public health association.

Abdel- Hameid, Zeinab. (2004): Studies on Bacillus Cereus and related species in heat-treated milk and some milk products. M.v.sc. Thesis, Animal health research institute, Assiut.

Ahmed, A-H Ahmed; Moustafa, K.M. and Marth, E.H. (1983): Incidence of Bacillus cereus in milk and some milk products. Food prot. , 46(2: 126- 128.

Ahmed, A-H Ahmed; El-Basiony, T.A. and Moustafa, K.M. (1988): microbiological evaluation of condensed milk- Assiut vet Med.j. 20 (40): 98 – 102.

Bonventre, P.F. and Johnson, C.E. (1970): Bacillus cereus. P.415 .in .t.c. Montie, s. kadis, and s.j.aji (eds.), the microbial toxins. Bacterial protein toxins. Academic press, New York

Eley, A.R. (1992): Toxic bacterial food poisoning in, A.R. Eley (ed.), microbial food poisoning. 1st ed. Chapman, hall, London pp.37 – 55

Eneroth, A.; Svensson, B.; Moin, G. and Christiansson, A. (2001): contamination of pasteurized milk by Bacillus cereus in the filling machine. J. Dairy Res., 68: 189-196.

Goepfert, J.M.; Spira, W.M. and kim, H.U. (1972): Bacillus cereus food poisoning organism. A review. J. Milk food technol. 35: 213 – 227.

Goepfert, J.M.; Spira, W.M.; Glatz, B.A and Kim, H.U. (1973): Pathogenicity of Bacillus cereus .P.69.In Betly C. hobbs and J.H.B Christian (eds)., Microbiological safety of food. Academic Press, London, New York.

Hauge, S. (1950): Bacillus cereus as a cause of food poisoning. NordiskHyg. Tidskr. 31: 184–206 (cited after kim and Goeplert (1971).

Hauge, S. (1955): food poisoning caused by aerobic spore forming bacilli. J. Appl. Bacteriol. 18: 591–595

Kim, H.V. and Goepfert, J.M. (1971): Enumeration and identification of Bacillus cereus in food. Appl. Microbiol. 22: 581 – 587.

Korashy, Eman and Sabreen, M.S. (2001): Incidence of aerobic and anaerobic spore formers and thermophilic fungi in condensed milk in Assiut City. Assiut Vet. Med. J., 45 (90): 157 – 165.

Kotiranta, A.; lounatmaa, K. and Haapasalo, M. (2000): Epidemiology and pathogenesis of Bacilluse cereus infections. J. Microbes Infect., 2 (2): 189 –198.

Midura, T.M.; Wood, R. and Leonard, A.R.(1970): outbreak of food poisoning caused by Bacillus .pub. Health Rep., 85: 45-48.

Mossel, D.A.; Koopman, M.J. and Jongerius. E. (1967): Enumeration of Bacillus cereus in foods. J. Appl. Microbiol., 15: 650-653.

Ombui, j.N.; Schminger, H.; Kagiko, M.M. and Arimi, S.M. (1997): Bacillus cereus may produce tow or more diarrheal enterotoxins. FE MS Microbiol. Letl., 149 (2): 245-248.

Pirltijarvi, T.S.; Andersson, M.A. and Salkinoja –Salonen, M.S. (2000): Properties of Bacillus cereus and other bacilli contaminating biomaterial– based industrial processes. int. J. Food Microbiol., 60: 231-239.

Speck, M.L. (1976): Compendium of Methods for the Microbiological Examination of food. M.L.speck (ed.), American public Health Association Washington, D.C.

Te Gifflel, M.C.; Beumer, R.R.; leijendekkers, S. and Rombbouts, F.M. (1996): Incidence of Bacillus cereus and Bacillus subtifis in food in the Netherlands. Food Microbiol, 13: 1096 – 1100.