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**PREVALENCE OF BACILLUS SPECIES
IN UHT MILK**
(With 3 Tables)

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مدى تواجد ميكروبات الباسيلس في اللبن المعقم بالطريقة السريعة

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أجري البحث على ستين عينة من اللبن المعقم بالطريقة السريعة ، جمعت من أماكن مختلفة بمحافظة الشرقية لمعرفة مدى تلوثها بميكروبات الباسيلس. وقد دلت النتائج عن تواجد ميكروبات الباسيلس في ١٨,٣% من العينات بمتوسط قدره $2,6 \times 10^2$ ميكروب لكل مليلتر من اللبن. ولقد تم عزل ميكروبات الباسيلس من نوع سيرس وساتس ونيكتيفورميس وبمبولس وكواجيولانس وبوليمكسا وميجاتيرينسب مختلفة تراوحت بين ٤,٢% إلى ٢٩,٢%. هذا وقد تمت مناقشة الأهمية الصحية والإقتصادية للمعزولات وكذا الإقتراحات الواجب إتباعها لتحسين جودة المنتج.

SUMMARY

Sixty random samples of UHT milk, collected from different localities in Sharkia Governorate, Egypt were examined bacteriologically for the presence of Bacillus species. The obtained results revealed that Bacillus species were detected in 18.3% of the examined samples with a mean value of 2.6×10^2 /ml ($20 - 1.1 \times 10^3$). Bacillus cereus, B. subtilis, B. licheniformis, B. pumilus, B. coagulans, B. polymyxa and B. megaterium could be isolated from the examined samples in variant percentages. The economic and public Health importance of the existed Bacillus species as well as the suggestive measures for improving the quality of UHT milk were discussed.

Key words: Bacillus species, UHT milk.

INTRODUCTION

UHT milk is considered as a commercially sterile product. In UHT processing, the milk is heated before it's packaging and then sealed into sterilized containers in a sterile environment. The use of higher temperatures (130-140°C) for seconds will increase the microbial death rate more than the loss of milk quality associated with thermal reactions (Adams and Moss, 1995). In recent years, the UHT processing has been widespread in Egypt as it ensures a maximum safety to the consumer and the product requires no refrigeration.

Bacillus species are Gram positive, aerobic spore forming bacteria and their endospores are heat resistant. So, they represent one of the most common spoilage organisms in heat treated milk (Westhoff and Dougherty, 1981 and Walker, 1988). Heat resistant Bacillus species can be introduced into milk supplies from water, udder teat surfaces, soil, tanks, pumps, pipelines, gaskets and processing equipment (Meer *et al.*, 1991).

Bacillus strains present in UHT milk were mesophilic or thermophilic and some of them were psychrophilic and the contamination of the UHT milk by these bacteria occurred mainly during filling process (Lee, 1984). The bacterial isolates from UHT milk consisted mainly of aerobic endospores forming organisms e.g. *Bacillus cereus*, *B. licheniformis*, *B. subtilis* and *B. pumilus* (Mostert, 1981). Several outbreaks of food poisoning caused by *Bacillus cereus* were associated with consumption of milk and its products (Iversen *et al.*, 1982).

The present investigation was planned to throw light on the prevalence of Bacillus species in market UHT milk in Sharkia Governorate, Egypt.

MATERIAL and METHODS

Sixty random samples of UHT milk were collected from different supermarkets, groceries and dairy shops in Sharkia Governorate, Egypt. The collected samples were transferred to the laboratory with a minimum of delay to be examined bacteriologically for enumeration and isolation of Bacillus species.

Preparation of samples:

The samples were incubated at 30°C for 15 days, then the containers were opened with a sterile pair of scissors under an aseptic condition.

Enumeration of Bacillus organisms:

Eleven ml of well mixed milk sample were added to 99 ml of sterile saline to make a dilution of 1/10, from which decimal serial dilutions were prepared and plated on Polymyxin Pyruvate Egg yolk Mannitol Bromothymol blue agar (PEMBA) (Holbrook and Anderson, 1980) according to the technique recommended by A.P.H.A. (1985).

Isolation and Identification:

Isolated colonies were purified and identified microscopically and biochemically according to Kreig and Holt (1984).

RESULTS

Table 1: Bacillus species count/ml of examined UHT milk samples.

No. of examined samples	Positive samples		Min.	Max.	Mean.	S.E.M.
	No.	%				
60	11	18.3	20	1.1X10 ²	2.6X10 ²	1.2X10

Table 2: Frequency distribution of examined samples based on their Bacillus species count.

Intervals	Frequency	
	No. of samples	%
≤ 10 ²	3	27.27
10 ² —10 ³	7	63.63
10 ³ —10 ⁴	1	9.10
Total	11	100.00

Table 3: Frequency distribution of Bacillus species in examined UHT milk samples.

Isolates	No. of isolates	%
Bacillus cereus	7	29.2
B. subtilis	5	20.8
B. licheniformis	4	16.7
B. pumilus	3	12.5
B. coagulans	2	8.3
B. polymyxa	2	8.3
B. megaterium	1	4.2
Total isolates	24	100.0

DISCUSSION

The results presented in Table 1 revealed that Bacillus species could be detected in 11(18.3%) out of 60 examined UHT milk samples with a mean value of $2.6 \times 10^2 \pm 1.2 \times 10^3$ (20 - 1.1×10^3). The highest frequency distribution (63.63%) lies within the range $10^2 - 10^3$ (Table 2).

Relatively similar results were reported by Saad *et al.* (1989) and El Shibiny *et al.* (1990). While higher results were reported by Amer *et al.* (1986).

Table 3 reveals that Bacillus cereus, B. subtilis, B. licheniformis, B. pumilus, B. coagulans, B. polymyxa and B. megaterium could be isolated from the examined samples in variant percentages. These bacteria could be recorded as 29.2, 20.8, 16.7, 12.5, 8.3, 8.3 and 4.2% respectively.

Lower findings were reported by Mostert *et al.* (1979), Huh and Kim (1983), Amer *et al.* (1986) and Saad *et al.* (1989).

It was clear that, Bacillus species were present in UHT milk but in relatively low numbers. The presence of Bacillus species in UHT milk may be attributed to poorly cleaned dairy processing equipment, inadequate processing temperature or post processing contamination. According to Lee (1984) the

spoilage of UHT, processed milk, is due to post UHT contamination or resistant surviving spores.

Bacillus species cause gelation, bitter flavour and sediment formation in UHT milk (Al-Ashmawy, 1990 and Kraft, 1992). In addition, Bacillus species (*B. subtilis*, *B. megaterium*, *B. coagulans* and *B. sterothermophilus*) generally affect the products which did not store at low temperatures such as UHT products and cause defects including gas production, acid coagulation, thinning, bitterness and off odours (Walker, 1988). Some strains of bacillus species especially *B. cereus* was recognized as a food poisoning pathogen and can produce diarrhoeogenic toxin (Griffiths, 1990 and Eley, 1996).

In conclusion, certain recommendation should be applied in dairy factories for the production of high quality UHT milk as regular bacteriological control of raw milk supplies, adequate and efficient cleaning of equipment and proper heat treatment processing for milk.

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