Dept. of Food Hygiene, Faculty of Vet. Med., Assiut University, Head of Dept. Prof. Dr. T.A. El-Bassiony.

## MICROBIOLOGICAL QUALITY OF EGYPTIAN ULTRA-HIGH-TEMPERATURE PROCESSED (UHT) MILK

(With 4 Tables)

By
NAGAH M. SAAD; T.A. EL-BASSIONY; A.A-H. AHMED
and M.K. MOUSTAFA
(Received at 3/8/1988)

التقييم الميكروبيولوجي للبن المعقم بالطربقة السريقة والمنتج في مصر نجاح سعد، توفيق البسيوني، أحمدعبدالحميد، مصطفى خليل

تم جمع ٢٠ عينة من اللبن المعقم بالطريقة السريعة من السوبرماركت في مدينة أسيوط، وكذلك الحلت كمية من المصنع بعدالتصنيع مباشرة ثم قسمت الى مجموعتين الأولى حفظت عند درجة حسرارة الثلاجة (٨٠ ثم) ، وتم فحص هذه العينات الثلاجة (٨٠ ثم) ، وتم فحص هذه العينات القرارنية ميكروبيولرجيا ووجد أن متوسط العدد الكلي للميكروبات ، الميكروبات المعوية ، الميكروبات القرارنية وكذلك الباسيللس سيريوس، المكتريا المحبة للبرودة ، والمكتريا التي تعيش في درجات الحسرارة العالمية كانت على التوالي ٤١٠٤/١، ١٠٤/١٠ ١/١٤/١، ١/١٤/١، ٢٥/١/مللي من العينات التي جمعت من السوبر ماركت ، كذلك تم عزل عديد من الميكروبات المختلفة المصرضة والغير مصرضة والتسسي توك الى فساد المنتج وتؤثر على صحة المستهلك ، وقد تم مناقشة الأهمية الصحية لوجود هذه الميكروبات على الصحة العامة من ناحية وعلى فساد المنتج والخسارة الاقتصادية الناتجة من ناحية أخرى، وتنسساول البحث تأثير درجة حرارة المتخزين على قيمة اللبن المعنم بالطريقة المسرعة، وقد ناقش البحسست الخطرات المحية التي يجب اتباعها عند تصنيع وتعبئة هذا المنتج حفاظا على صحة المستهلك وحفاظا على قيمة الغدائية.

#### SUMMARY

Thirty random samples of UHT-milk were collected from different supermarkets in Assiut City, as well as lot was taken from factory at the time of manufacture and stored at room and refrigerator temperatures for up to 11 weeks and examined for their microbiological quality. The obtained results revealed that the average counts of aerobic plate, enter-ococci, coliforms, B.cereus, psychrotrophic, thermophilic and thermoduric bacteria in supermarkets samples were 44x10, 50, 62.2, 13.8x10, 17x10, 73.3 and 70/ml respectively. Furthermore, E.coli, klebsiella species, Enterobacter spp., citrobacter spp., Micrococcus spp., Staph. epidermidis and Anaerobic – spore formers could be isolated from the examined samples. Also the effect of storage temperature on the quality of UHT-milk was studied. The public health and economic significance of these findings are discussed.

#### INTRODUCTION

The term ultra-high-temperature processed (UHT) milk will refer to milk that has been heated to at least 130°C for not less than 1 sec., and then aseptically packaged. Milk sterilization has been practiced and studied for more than a century. Microbiological quality control for these products is a major problem (METHA, 1980) as the enzyme produced by psychrotrophic bacteria can hydrolyze milk proteins. The protease was quite active at normal and elevated room temperature and rapidly spoiled sterile milk with the development of bitter flevor, clearing or coagulation, and the

susceptibility of sterile milk to protease enzyme increased during storage of milk (SOMUELSON and HOLM, 1966).

Inspite of the temperature attained in its preparation, UHT-milk may, at times, be responsible for transmitting some pathogenic organisms to consumers. Outbreaks of gastroenteritis traced to staphylococcal enterotoxin A have been associated with UHT-milk (GERINGER, 1983). KIM (1980) found that the viable bacterial counts of thermophilic, Psychrophilic and Coliform in packaged UHT-milk were 10-40/ml, 20/ml, 0-10/ml and Zero respectively. HUH and KIM (1983) could isolate 67 strains from UHT-milk, 41 were identified as B.subtilis, 8 as B.megatherium 5 as B.circulans, 4 each as B.lichenformis and B.sphaericum, 3 as B.cereus and 1 each of B. larvae and B.coagulans, and they mentioned that 170 of these isolates could grow at 7°C and vegetative cells of B.subtilis and B.cereus survived heating at 65°C for 40 min. Moreover LEE (1984) examined 37 samples of UHT-milk, and he isolated 40 strains, 13 were identified as Bacillus species. There were 6 B.cereus, 5 B.lichenformis, 1 B.brevis and 1 B.pumilus. The other 27 strains were identified as 5 yeast, 2 pseudomonas spp., 3 streptococcus spp., 12 lactobacillus spp., 1 shigella spp., 1 Aeromonas spp. and 3 Micrococcus spp.

The presence of coliform organisms in UHT-milk provides a useful index in determining the hygienic quality of the products. The presence of coliform bacteria in UHT-milk has been reported by HAHN (1984) he could isolate Klebsiella oxytoca, Hafina alvei, Citrobacter freundii, Enterobacter cloaca and 5 strains of E.coli from contaminated UHT-milk. On other instances the contamination of UHT-milk by psychrophilic bacteria has been reported by BLANC, et al. (1984) and MOTTAR, et al. (1985). The presence of such organisms could serve in predecting the life of refrigerated foods, while ITO (1985) stated that UHT-milk may be kept for 1 day (opened carton) or 2 days (unopened carton) at 20°C and for about 10 days (opened carton) or 14 days (unopened carton) at 5°C. MOTTAR, et al. (1985) recommended that determination of psychrotrophic microorganisms is of importance for quality control of milk products in general and UHT-milk in particular as some psychrotrophs produce thermostable proteinases that limit the shelf life of UHT-milk.

As the microbial quality of UHT-milk reflects the care with which the milk was produced and the sanitary conditions prevailing during its processing and filling, therefore, this work was planned to secure information regarding the sanitary conditions of UHT-milk as well as the effect of storage temperature on its quality.

## MATERIAL and METHODS

### Part I: Microbiological quality of UHT-milk:

Collection and preparationof samples:

Thirty random samples of UHT-milk were collected from different supermarkets in Assiut City. The samples were prepared according to Standard Methods for the Examination of Dairy Products (MARTH, 1978) and were examined microbiologically for aerobic plate, enterococci, colliforms, B-cereus, psychrophilic, thermophilic and thermoduric counts.

### QUALITY OF UHT MILK

# Part II: Effect of storage temperature on the quality of UHT-milk:

A total of UHT-milk (containing 24 cartons) was taken from factory at the time of manufacture, and divided into 2 groups, the first was stored at room temperature (25°C) and the second one at refrigerator temperature (7+2°C) the stored samples were prepared according to the standard Methods for the Examination of Dairy Products (MARTH, 1978) and were examined at time zero, then weekly upto 11 weeks for their microbiological quality.

- (1) Aerobic plate, psychrophilic, thermophilic and thermoduric counts: were carried out according to the standard Methods for the Examination of Dairy products (MARTH, 1978).
- (2) Enterococcus count: Enterococcus Selective Differential (ESD) agar was used according to EFTHYMIOU, et al. (1974).
- (3) Coliform count: Violet red bile agar was used as recommended by Standard Methods for the Examination of Dairy Products (MARTH, 1978).
- (4) Detection of anaerobic sporeformers (Stormy fermentation test): The technique adopted is that recommended by CRUICHSHANK, et al. (1969).
- (5) Detection of pathogenic microorganisms: The procedure used for isolation and identification of pathogenic microorganisms were carried out according to COWAN and STEEL (1970).

#### RESULTS

All results obtained from the examined samples of UHT-milk are recorded in lables 1-4.

#### DISCUSSION

## Part I: Microbiological quality of UHT-milk:

The results in Table 1. show the min., max. and average values for aerobic plate, enterococci, coliforms, B.cereus, psychrotrophic, thermophilic and thermoduric organisms recovered from the examined samples lower counts of total bacteria, thermophilic and psychrotrophic bacteria were recorded by KIM (1980) B.cereus was previously isolated from UHT-milk by HUH and KIM (1983) and LEE (1984). While the contamination of UHT-milk with psychrotrophic bacteria was previously studied by BLANC, et al. (1984) and MOTTAR, et al. (1985).

Data in Table (2) show the frequency distribution of isolated E.coli, klebsiella species, Enterobacter species, citrobacter species, Micrococcus species, Staph. epidermidis and anaerobic spore-formers from examined samples. A higher incidence of E.coli was obtained by HAHN (1984). The findings for colliform organisms nearly go parralel with results recorded by HAHN (1984).

# Part II: Effect of storage temperature on the quality of UHT-milk:

Table 3 reveals an increase in aerobic plate count in samples stored at room temperature, while Enterococci, coliforms and B.cereus decreased in population with increasing time of storage and not detectable after 5, 8 and 9 weeks respectively.

Assint Vet. Med. L. Vol. 21, No. 42, 1989.

### NAGAH SAAD, et al.

Psychrotrophic, thermophilic and thermoduric bacteria increased in number by the end of the 11th week. LEE (1984) suggested that Bacillus strains present in UHT-milk were mesophilic or thermophilic and some of them were also psychrophilic, and contamination by them occured mainly during filling.

From the results in Table 4 it is evident that aerobic plate count in samples stored in refrigerator was initially 2x103/ml, then decrease gradually till reach 9x102/ml by the end of storage time. While Enterococci, coliforms and B.cereus were not detectable after 3, 4 and 4 weeks respectively. Psychrotrophic bacteria decreased in population at first, then increased till reach 8x102/ml by the end of the 11th week. ADAMS, et al. (1975) stated that a milk sample that had a high population of psychrotrophic bacteria could have a reduced shelf-life. Concerning thermophilic and thermoduric counts it is clear that they decreased in numbers by the end of the 11th week of storage at refrigerator temperature. KIM (1980) mentioned that recontamination with psychrotrophic and thermophilic bacteria was experienced during packaging, 110 (1985) found that keeping UHT-milk in refrigerator increases its keeping quality than storage at room temperature.

Overall results indicated that improvements in the UHT-milk processing and filling operations are required, and storage of UHT-milk at refrigerator temperature will increase its keeping quality than storage at room temperature.

## REFERENCES

- Adams, D.M.; Barach, J.T. and Speck, M.L. (1975): Heat resistant protease produced in milk by psychrotrophic bacteria of dairy origin. J. Dairy Sci. 58: 828.
- Blanc, B.; Fluckiger, E.; Ruegg, M. and Steiger, G. (1984): Effect of cold storage and enrichment with psychrotrophic bacteria on the quality of UHT-milk. Alimenta 23 (3): 71-79.
- Cowan, S.I. and Steel, K.J. (1970): Manual for identification of medical bacteria, Cambridge Univ. Press, London.
- Cruickshank, R.; Duguid, J.P. and Swain, R.H.A. (1969): Medical microbiology 11th Ed., E and S. Livingestone Limited Edinburgh and London.
- Efthymio, C.J.; Baccash, P.; Laborardi, V.J. and Epstein, D.S. (1974): Improved isolation and differentiation of Enterécocci in cheese. Appl. Microbiol. 28, 417.
- Geringer, M. (1983): Food poisoning from enterotoxin-producing Staphylococcus aureus strain in UHT-milk and UHT flavord milk. Tieraztliche Umschau, 38: 98-100,
- Hahn, G. and Tolle, A. (1984): Growth at 30 and 44.5°C for differentiation of coliforms isolated from soft cheese. Lahn, German Federal Republic 77-80.
- Huh, C.S. and Kim, H.U. (1983): A study on the mesophilic Bacillus species occuring in Korean market milk treated by UHT. J. Dairy Sci. 1: 29-36.
- Ito, Y. (1985): Studies on the keeping quality of UHT treated milk. Proceeding of the Faculty of Agriculture, Kyushu Tokai University No. 4: 111-116.
- Kim, J.W. (1980): Studies on the sources of bacterial contamination of UHT processed milk. Research Reports of Agricultural Science and Technology 7 (2): 103-108,
- Lee, C.M. (1984): Spoilage microorganisms encountered in ultra-high-temperature plocassed milk. Chinese J. of Microbiology and Immunology 17 (2): 86-91.

#### QUALITY OF UHT MILK

Marth, E.H. (1978): Editor of Standard Methods for the Examination of Dairy Products, 14th Ed. A.P.H.A., Washington, D.C.

Mehta, R.S.(1980): Milk processed at ultra-high temperatures. A review. J. Food Prot. 43:212-225.

Mottar, J.; Renterghem, R. Yan and Vilder, J. DF. (1985): Evaluation of the raw material for UHT milk by determining the degree of protein break down through HPLC. Milchwissenschaft. 40: 717-721.

Samuelsson, E.G. and S. Holm (1966): Technological principles for utra-high heat treatment of milk. XVII. Int. Dairy Congr., Munchen, B. 1: 57.

Table (1)
Counts of some microorganisms recovered from UHT-milk samples

Types	Positive samples		Count/ml		
	N/30	%	Min.	Max.	Average
Aerobic Plate count	25	83.3	50	4x10³	44×10
Enterococci	5	16.6	20	1x10 <sup>2</sup>	50.0
Coliforms	7	23.3	20	1x10 <sup>2</sup>	62.2
B.cereus	8	26.6	40	1x103	13.8x10
Psychrotrophs	12	40.0	30	1×10 <sup>2</sup>	17×10
Thermophilic	6	20.0	30	1×10 <sup>2</sup>	73.3
Thermoduric	3	10.0	30	1x10 <sup>2</sup>	70.0

Table (2)
Frequency distribution of isolates recovered from UHT-milk samples

Isolates	No. of isolates	%
E.coli	3	18.75
Klebsiella sp.	1.	6.25
Enterobacter sp.	2	12.50
Citrobacter sp.	1	6.25
Micrococcus sp.	. 3	18.75
Staph. epidermidis	2	12.50
Staph. aureus	-	-
Anaerobic-sporeformers	4	25.00

Time/week Zero Type 2x10³
2x10³
2x10³
3x10³
3x10³
3x10³
7x10³
7x10³
7x10³
6x10³
8x10³
9x10³ APC Enterccocoi 2×10<sup>2</sup>
1×10<sup>2</sup>
1×10<sup>2</sup>
1×10<sup>2</sup>
7×10
2×10 Coliforms 1×10<sup>2</sup>
2×10<sup>2</sup>
3×10<sup>2</sup>
3×10<sup>2</sup>
6×10<sup>2</sup>
5×10<sup>2</sup>
1×10<sup>2</sup>
3×10<sup>2</sup>
8×10 B.cereus 2x10 3x10 5x10 9x10 9x10 1x10 8x10 5x10 3x10 3x10 Psychrotrophic 3×10<sup>2</sup>
6×10<sup>2</sup>
5×10<sup>2</sup>
2×10<sup>2</sup>
2×10<sup>2</sup>
2×10<sup>2</sup>
7×10<sup>2</sup>
1×10<sup>2</sup>
1×10<sup>2</sup>
1×10<sup>2</sup>
8×10
7×10
5×10
5×10 Thermophilic 4x10 4x10 7x10 1x10<sup>2</sup> 1x10<sup>2</sup> 1x10<sup>2</sup> 3x10<sup>2</sup> 6x10<sup>2</sup> 6x10<sup>2</sup> 9x10<sup>2</sup> 3x10<sup>2</sup> 2x10<sup>2</sup> 2x10<sup>2</sup> Thermoduric 1×10<sup>2</sup>
1×10<sup>2</sup>
2×10<sup>2</sup>
2×10<sup>2</sup>
7×10<sup>2</sup>
7×10<sup>2</sup>
9×10<sup>2</sup>
1×10<sup>3</sup>
1×10<sup>3</sup>
1×10<sup>3</sup>
1×10<sup>3</sup>
1×10<sup>3</sup>
1×10<sup>3</sup>
8×10<sup>2</sup>
7×10<sup>2</sup>

Effect of storage at room temperature on the quality of UHT-milk

Table (3)

## QUALITY OF UHT MILK

Zero 1 1 2 2 3 4 6 6 7 7 10 11	Туре
2×10 <sup>3</sup> 8×10 <sup>2</sup> 5×10 <sup>2</sup> 2×10 <sup>2</sup> 2×10 <sup>2</sup> 3×10 <sup>2</sup> 5×10 <sup>2</sup> 5×10 <sup>2</sup> 6×10 <sup>2</sup> 5×10 <sup>2</sup> 7×10 <sup>2</sup> 8×10 <sup>2</sup>	APC
2×10² 1×10² 7×10 1×10	Enterococci
1×10² 6×10 3×10 3×10 1×10	Coliforms
2x10 2x10 2x10 2x10 1x10	B.cereus
3×10 <sup>2</sup> 3×10 <sup>2</sup> 3×10 <sup>2</sup> 3×10 <sup>2</sup> 4×10 <sup>2</sup> 7×10 <sup>2</sup> 7×10 <sup>2</sup> 6×10 <sup>2</sup> 7×10 <sup>2</sup> 7×10 <sup>2</sup> 9×10 <sup>2</sup> 1×10 <sup>3</sup> 9×10 <sup>2</sup>	Psychrotrophs
4×10 3×10 1×10 1×10 5×10 4×10 9×10 1×10 <sup>2</sup> 1×10 <sup>2</sup> 1×10 <sup>2</sup> 1×10 <sup>2</sup> 1×10 <sup>2</sup> 1×10 <sup>2</sup>	Thermophilic
1×10² 9×10 1×10² 1×10² 1×10² 8×10 2×10² 8×10 9×10 5×10	Thermoduric

Table (4)

Effect of storage at refrigerator on the quality of UHT-milk