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Microbiological Qualities and Post-Pasteurization Contaminations of UHT Milk Produced in Korea

Seung Young Park, Jung Min Park[2], Jin Oh Yang[2], Hoo Kil Jung[2], Ho Nam Chun[2] and Byong Hoon Lee[1*]

Department of Food Service Industry, Cheonan Yonam College

[1]Department of Food Science and Agricultural Chemistry, McGill University, Canada

[2]R&D Center, Maeil Dairy Industry Co., Ltd.

한국에서 생산되는 UHT 우유의 살균 후 오염과 미생물학적 품질

박승용 · 박정민[2] · 양진오[2] · 정후길[2] · 전호남[2] · 이병훈[1*]

천안연암대학 외식산업과, [1]캐나다 McGill 대학교 식품과학 및 농화학과, [2]매일유업㈜ 중앙연구소

요 약

국내산 UHT-ESL우유와 UHT 처리우유 총 150 packs을 냉장온도 (7℃)와 실온 (20℃)에서 5주간 저장하면서 1주마다 5 packs씩 시료로 취하여 미생물 함유 우유 packs의 발생시기, 발생 수와 미생물 종류별 함유 수준 등을 조사하였으며, 살균 후 2차 오염미생물로서 *Salmonella* spp.와 *Staphylococcus aureus* 존재 여부를 확인하였다. 총균수는 무균포장 공정을 실시하지 않은 UHT 처리우유가 UHT-ESL 우유보다 출현시기가 빠르고, 미생물 함유수가 많아 미생물학적 품질이 약간 낮은 것으로 나타났다. 그러나 두 제품군 모두 대장균군은 출현하지 않았으며, 호기성 포자형성균은 UHT 처리우유 2 제품에서 각각 7일 및 14일 만에 포자가 발아한 우유 pack이 1 pack씩 나타났으며, 7℃에서 저장한 우유보다 20℃에서 저장한 우유에서 포자가 발아한 우유 pack의 수가 많았다. 내열성균은 2 제품군 모두 130℃ 이상의 열처리로 인하여 14일까지 출현하지 않았으나, 20℃에서 저장한 우유는 21일 후(<300~<3,000 CFU/ml), 7℃에서 저장한 우유는 28일 후(40~3,600 CFU/ml)에는 모든 pack에서 나타나기 시작하였다. 내냉성균은 UHT-ESL우유는 28일부터 측정되었으나, 한 제품의 UHT 처리우유는 7일 후부터 7℃에서는 3,900~102,000 CFU/ml까지, 20℃에서는 7일부터 28일까지 <3,000 CFU/ml 이하의 균수가 측정되었으며, 다른 제품의 UHT 처리우유는 21일에 30,000 CFU/ml 이하, 28일부터는 대부분의 pack에서 30,000 CFU/ml 이하로 발견되었다. *Salmonella* spp.와 *Staphylococcus aureus*에 의한 2차 오염은 모든 제품에서 없었던 것으로 나타났다. 이러한 결과는 UHT 우유의 열처리 방법 및 포장방법에 따라서 냉장보관 우유의 유통기한을 3주간(21일)으로 설정하는 것이 가능한 것으로 나타났다.

(Key words: UHT-ESL milk, UHT milk, microbiological quality, post-pasteurization contamination)

I. Introduction

Corresponding author : B. H. Lee, 21,111, Lakeshore Rd. Ste-Anne-de-Bellevue, Montreal, Quebec, Canada, H9X 3V9. E-mail:byong.lee@mcgill.ca

Three types of milk, treated by traditional HTST, microfiltrated (MF)-ESL, and UHT-ESL, have been currently introduced to the markets around the world. A brand of milk manufactured by removing the microbial

cells and spores in raw milk by microfiltration (MF) technology and then heat treated at mild pasteurization temperature combined with aseptic packaging had been developed. Wamsler (1996) compared on the points of the processing methods of ultra-pasteurization and bacto-fugation in use to extend the shelf life of milk as potential market, evaluate the use of microfiltration, and the sensory and microbiological properties of ESL milk and cost comparison. Anon (1999) discussed the benefits of ESL milk, improved taste of 'freshness' over UHT milk, risks of re-infection and effect on shelf stability, and cost vs. benefits of ESL technology. Kelly (1999) also covered several aspects including definition, appeal of ESL milk, technical options, processing of milk to extend shelf life, increase flexibility for expansion of distribution channels and entry into distant market. The MF-ESL milk (pure filtered milk) in fact has become the most popular milk in Canada, and market sales have increased significantly due to the fresh taste, cold pasteurization, extended shelf life, and technologically innovative image. Evaluations of the milk quality in respects to microbiological, nutritional and environmental contaminations or safety, and economical respect requires the accurate detection methods (Byrne *et al.*, 1989 ; Choi *et al.*, 1999a ; Eie and Martens, 2000 ; Skladal *et al.*, 1993). Gram negative bacteria found in heat treated milks are probably the post-pasteurization contaminants during filling and sealing the milk or during from delivery to market (Choi *et al.*, 1999b ; Kang *et al.*, 1995 ; Kwon and Choi, 1998). The most frequent contaminants in pasteurized milk and UHT milk have appeared to be *Bacillus*, *Staphylococcus*, *Salmonella*, *Micrococcus* species (Kessler *et al.*, 2001 ; Silveira *et al.*, 1989 ; Zarcachenco *et al.*, 2001), and *Mycobacterium* in pasteurized milk (Grant *et al.*, 1999). Although LTLT, HTST, and UHT sterilized milks are conventionally used for decade, microfiltered (Eie and Martens, 2000 ; Skrzypek *et al.*, 2001 ; Stepaniak, 1997), bactofuged and ESL pasteurized milk have been developed (Kelly, 1999 ; Wamsler, 1996). These are currently used for the supply the fresh taste of milk to the consumers around the world. While the advantage and disadvantage of ESL milk are still on the discussion (Anon, 1999 ; Gallman *et al.*, 2001), many efforts have been made to produce high quality of fresh pasteurized milk (HQFP milk) in a small dairy farm by fast cooling and separate bulking of milk (Azzony *et al.*, 1993) or HTST shelf-stable milk (Eisner, 1995) or development of the helical tube indirect UHT system (Sahoo *et al.*, 2002). In order to enhance the keeping quality of commercialized milk, lactoperoxidase system (Barrett *et al.*, 1999), higher temperature and longer holding times of pasteurization system (Grant *et al.*, 1999) had been suggested.

UHT-pasteurized ESL milk incorporated with further special aseptic processing has been launched three years ago in Korea. Comparative studies on the microbiological quality of UHT- ESL milk and conventionally produced UHT- pasteurized milk have been performed by Jeong *et al.* (2001), Jeong *et al.* (2002), and Kim *et al.* (2003). The aim of our study was to compare the microbiological quality aspects of commercialized milks produced in spring season (May, 2003) by different processing methods in Korea.

Ⅱ. Materials and Methods

1. Enumeration of Microorganisms

Total 150 milk samples manufactured by three companies in Korea were purchased from local distributors and divided into two temperature groups. One group of milk was kept under refrigeration (7 ℃) and the other was kept at room temperature (20 ℃). Five samples of each brand were withdrawn at 7 d-intervals for 5 weeks, and subjected to microbial tests as indicated below. All pour plate methods were used by mixing 1 ml of milk into a petri-dish containing about 20 ml of molten agar and swirling. Triplicates (1 ml or 25 ml × 3 times) were separately performed for each milk sample.

2. Total Bacteria

Total bacteria in milk samples were enumerated on Standard Plate Count (SPC) agar (Difco, USA) and incubated aerobically at 30°C for 48h by the procedure of

IDF (IDF 100B, 1991). The colonies formed on the agar plate were counted as total bacteria in milk samples.

3. Psychrotrophic Bacteria

The SPC agar plates prepared by the above procedure were incubated aerobically at 4°C for 7 days (IDF 132A, 1991). The colony forming units were counted as psychrotropic bacteria in sample milks.

4. Coliform Bacteria

Violet Red Bile Lactose agar (Difco, USA) embedded 1 ml of milk samples were incubated aerobically at 30°C for 24 and 48h. Dark red colonies appeared on VRBA were counted as coliforms.

5. Thermoduric Bacteria

Thermoduric bacteria in milk was counted on SPC agar (Difco, USA) and incubated aerobically at 55°C for 16~24h.

6. Aerobic Spore

Milk samples were treated at 65°C water bath for 20 min to eliminate live cells and subsequently pour plated on SPC agar. The colonies appeared after aerobic incubation at 30°C for 48h were counted as aerobic spores.

7. *Salmonella* Species

Salmonella spp. was detected by the method of ISO 6785 (2001) ; 25 ml of milk was pipetted into a flask containing 225 ml of pre-enrichment medium, 2% (w/v) peptone water (Difco, USA), mixed, and incubated for 16~24h at 37°C. Samples (10 ml) were inoculated into 90 ml of Rappaport-Vassiliadis Soya Peptone (RVS, Oxoid, England) and incubated for 24h at 42°C. Samples (1 ml) were then plated into 20 ml of molten selective Brilliant Green Modified agar (BGM, Difco, USA), and incubated for 18~24h at 37°C.

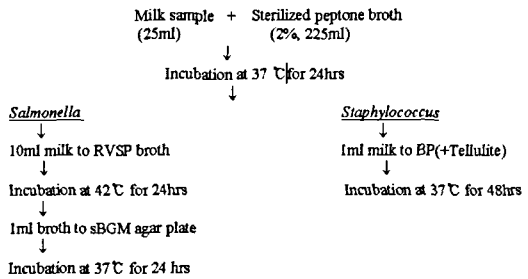

Fig. 1. Scheme for detection of post-pasteurization contaminants.

8. *Staphylococcus aureus*

Staphylococcus aureus was detected by the method of ISO (ISO 6888-1, 1999) ; a pre-enrichment step was not necessary. After 25 ml of milk was mixed in a flask containing 225 ml of 2% (w/v) peptone water (Difco, USA), portions (1 ml) were plated into 20 ml of molten Baird-Parker agar (Difco, USA) and incubated for 48h at 35~37°C. The suspected colony was confirmed by coagulase tube test using Brain Heart Infusion agar (Difco, USA).

Ⅲ. Results

1. Comparative Microbiological Qualities of UHT Milk

1) Total Bacteria in UHT Milk

On the first week from the day of the milk purchase, 2 out of 5 UHT-1 and UHT-2 milk samples stored at 7°C and 20°C, respectively showed the viable counts of less than 300 CFU/ml, whereas none of the 5 UHT-ESL milk samples revealed any colony up to 21 days. After 14 days of refrigerated storage, the total count in 1 pack of UHT-2 milk was more than 10,000 CFU/ml that was near to legal limits, and another 1 pack reached 300,000 CFU/ml more than the limits even stored at refrigerated temperature. For the milk samples stored at 20°C, 4 samples of UHT milk showed less than 300 CFU/ml, except 1 pack (168,000 CFU/ml). On the other hand, only

one UHT-ESL milk sample contained over 3,000 CFU/ml on 14 days stored at 20°C. On the second week of storage, a total of six UHT milk samples contained more than 3,000 CFU/ml, and one of these samples stored at 20°C has surpassed the regulation limit of 20,000 CFU/ml. On the third week of refrigerated storage, one UHT-ESL sample started to show less than 300 CFU/ml, whereas all UHT milk samples maintained less than 300 CFU/ml. However, one UHT-1 milk sample kept at 20°C was completely spoiled, with putrid odor and fungi on the surface, thus sample was discarded at this stage. In the remaining UHT-1 milk samples, the counts were increased dramatically (30,000~3,000,000 CFU/ml) between the fourth and fifth week at 20°C. On the other hand, only two ESL milk samples showed over 30,000 CFU/ml after 5 weeks at 20°C, whereas the entire UHT-ESL samples kept at refrigerated storage for 5 weeks contained less than 300 CFU/ml.

2) Coliforms

Coliforms were not detected on Violet Red Bile Lactose agar in any UHT milk samples through out five-week storage at both temperatures.

3) Aerobic spores

On the days of samples were purchased, the aerobic spore counts of UHT-2 samples were less than 30 CFU/ml in UHT-2 milk, but on the first week, 300 CFU/ml were detected in UHT-1, but no spores were present in any of ESL samples up to the second week at both temperatures. Spore counts (<300 to 144,000 CFU/ml) were high in UHT-2 after 1 week of 20°C and after 4 weeks of refrigerated storage (<300 CFU/ml), respectively. On the contrary, very low level of spores (<30 CFU/ml) were detected in refrigerated UHT-1 samples after 35 days, whereas only one UHT-1 sample kept at 20°C showed about 13,100 CFU/ml. In ESL milks, all five refrigerated samples showed the count less than 30 CFU/ml, whereas the samples at 20°C contained about 300 CFU/ml ; the most high spore count in one of these samples was 580,000 CFU/ml at 35 days.

4) Thermoduric Bacteria

Table 1. Incidences and CFU of total bacteria on SPC agar in UHT milk stored at 7°C and 20°C

Storage time (days)	UHT-ESL				UHT-1				UHT-2			
	7°C		20°C		7°C		20°C		7°C		20°C	
	CFU	Incidence	CFU	Incidence	CFU	Incidence	CFU	Incidence	CFU	Incidence	CFU	Incidence
0	-	0/5	-	0/5	-	0/5	-	0/5	0	0/5	-	0/5
7	-	0/5	-	0/5	-	0/5	<300(2)	2/5	<300	1/5	<300	1/5
14	-	0/5	<3,000(2)	2/5	<300(3)	3/5	<3,000-	1/5	<300(2) 10,500	3/5	<3,000 65,000	2/5
21	<300	1/5	-	0/5	<300	1/5	-	0/5	<300(3)	3/5	-	0/5
28	-	0/5	<3,000)	1/5		0/5	620,000 47,000 3,000,000 242,000	4/5	>300,000 46,000	2/5	168,000	1/5
35	<300(2)	2/5	<30,000(2)	2/5	<30(3)	3/5	101,500 166,000 760,000 >30,000	4/5	<300(2)	2/5	-	0/5

Table 2. Incidences and CFU of coliforms on VRBA agar in UHT milk stored at 7°C and 20°C

Storage time (days)	UHT-ESL				UHT-1				UHT-2			
	7°C		20°C		7°C		20°C		7°C		20°C	
	CFU	Incidence	CFU	Incidence	CFU	Incidence	CFU	Incidence	CFU	Incidence	CFU	Incidence
0	-	0/5	-	0/5	-	0/5	-	0/5	-	0/5	-	0/5
7	-	0/5	-	0/5	-	0/5	-	0/5	-	0/5	-	0/5
14	-	0/5	-	0/5	-	0/5	-	0/5	-	0/5	-	0/5
21	-	0/5	-	0/5	-	0/5	-	0/5	-	0/5	-	0/5
28	-	0/5	-	0/5	-	0/5	-	0/5	-	0/5	-	0/5
35	-	0/5	-	0/5	-	0/5	-	0/5	-	0/5	-	0/5

Table 3. Incidences and CFU of aerobic spores on SPC agar in UHT milk stored at 7°C and 20°C

Storage time (days)	UHT-ESL				UHT-1				UHT-2			
	7°C		20°C		7°C		20°C		7°C		20°C	
	CFU	Incidence	CFU	Incidence	CFU	Incidence	CFU	Incidence	CFU	Incidence	CFU	Incidence
0	-	0/5	-	0/5	-	0/5	-	0/5	<30	1/5	-	0/5
7	-	0/5	-	0/5	-	0/5	<300	1/5	<30	1/5	3,800 <300(2)	3/5
14	<300	1/5	<300	1/5	<300(2)	2/5	-	0/5	-	0/5	144,000 3,700	2/5
21	<30	1/5	<300(2)	2/5	<30(4)	4/5	<300(3)	3/5	-	0/5	<3,000(2)	2/5
28	<30	1/5	<300(3)	3/5	-	0/5	13,100 <300(2)	3/5	11,100 <30(2)	3/5	<300(2)	2/5
35	<30(3)	3/5	580,000 <300	2/5	<30(3)	3/5	<300(2)	2/5	<30(2)	2/5	<300(2)	2/5

Thermoduric bacteria were not present in all UHT samples tested after two weeks of storage (7°C and 20°C), but the count started to appear on the third week at 20°C. High levels of spores as well as thermoduric bacteria were detected in UHT-2 milk samples, but interestingly, more thermoduric bacteria were present in samples kept at 7°C than at 20°C. Thermoduric bacteria counts in UHT- 2 were relatively low up to four weeks of storage, but on the five weeks of storage, the counts have increased to 3,700 ~ 49,000 CFU/ml in some samples. In UHT-1 milks, the number of thermoduric bacteria in those samples was relatively low. Two UHT-1 samples showed relatively high level of thermoduric bacteria (<3,000 CFU/ml) on the four weeks of storage, and this level was maintained even on the five weeks. Thermoduric bacteria started to appear in ESL milk samples after three weeks of storage at 20°C and four weeks of refrigerated storage, respectively, and the counts of these samples were more or less the same as the UHT milk samples.

5) Psychrotrophic Bacteria

No psychrotrophic bacteria were detected in UHT-ESL milk samples up to 28 days at 7°C and up to 14 days at

Table 4. Incidences and CFU of thermoduric bacteria on SPC agar in UHT milk stored at 7°C and 20°C

Storage time (days)	UHT-ESL				UHT-1				UHT-2			
	7°C		20°C		7°C		20°C		7°C		20°C	
	CFU	Incidence	CFU	Incidence	CFU	Incidence	CFU	Incidence	CFU	Incidence	CFU	Incidence
0	-	0/5	-	0/5	-	0/5	-	0/5	-	0/5	-	0/5
7	-	0/5	-	0/5	-	0/5	-	0/5	-	0/5	-	0/5
14	-	0/5	-	0/5	-	0/5	-	0/5	-	0/5	-	0/5
21	-	0/5	<3,000(4) 3,500	5/5	3,000 2,130	2/5	<3,000(4)	4/5	-	0/5	<300(2)	2/5
28	710 310 870 <300	4/5	<3000(3)	3/5	-	0/5	-	0/5	3,600 790 52 41	4/5	<300(3)	3/5
35	<300	1/5	<300	1/5	<3,000	1/5	<3,000	1/5	9,700 49,000 3,700 4,200	4/5	27,300	1/5

Table 5. Incidences and CFU of psychtotrophic bacteria on SPC agar in UHT milk stored at 7°C and 20°C.

Storage time (days)	UHT-ESL				UHT-1				UHT-2			
	7°C		20°C		7°C		20°C		7°C		20°C	
	CFU	Incidence	CFU	Incidence	CFU	Incidence	CFU	Incidence	CFU	Incidence	CFU	Incidence
0	-	0/5	-	0/5	-	0/5	-	0/5	-	0/5	-	0/5
7	-	0/5	-	0/5	<300-	1/5	-	0/5	3,900	1/5	<3,000	1/5
14	-	0/5	-	05	-	0/5	-	0/5	102,000	1/5-	<3,000	1/5
21	-	0/5	-	0/5	-	0/5	<30,000	1/5	-	0/5-	-	0/5
28	<300	1/5	-	0/5	-	0/5-	<30,000(4)	4/5	<3,000(2)	2/5	<3,000	1/5
35	<3,000	1/5	<30,000(3) 30,000 33,000	5/5		0/5	<30,000(4) 46,000	5/5		0/5	<3,000(4) 32,000	5/5-

20°C. However, psychrotrophic bacteria began to appear in UHT-2 milk samples after one week at both temperatures. Higher levels of psychrotrophic bacteria (3,900~102,000 at 7°C, <30,000 CFU/ml at 20°C) were present in two brands of UHT milk samples than in UHT-ESL milk samples (<300 at 7°C, <3,000 <30,000 at 20°C). In UHT-1 milk, psychrotrophic bacteria were only present in samples that kept at 20°C. After the third week of storage, the numbers were found in 4 milk packs, but the counts were less than 30,000 CFU/ml, except for one pack (46,000 CFU/ml) on the fifth week.

2. Detection of Post-Pasteurization Contaminants

1) *Salmonella* spp.

A suspected *Salmonella* species was detected in one UHT-2 sample after pre-enrichment in 2% Rappaport-Vassiliadis Soya Peptone broth. However, *Salmonella* spp. using selective Brilliant Green Modified (BGM) agar was not definitely confirmed.

Table 6. Detection of *Salmonella* spp. as a post-pasteurization contaminant in UHT milk

Detection steps	UHT-ESL				UHT-1				UHT-2			
	1st trial		2nd trial		1st trial		2nd trial		1st trial		2nd trial	
	CFU	Incidence	CFU	Incidence	CFU	Incidence	CFU	Incidence	CFU	Incidence	CFU	Incidence
Peptone broth	-	0/10	-	0/10	-	0/10	-	0/10	1	1/10	-	0/10
RVSP broth	-	0/10	-	0/10	-	0/10	-	0/10	-	0/10	-	0/10
SBG Agar	-	0/10	-	0/10	-	0/10	-	0/10	-	0/10	-	0/10

Table 7. Detection of *Staphylococcus* spp. as a post-pasteurization contaminant in UHT milk

Detection steps	UHT-ESL				UHT-1				UHT-2			
	1st trial		2nd trial		1st trial		2nd trial		1st trial		2nd trial	
	CFU	Incidence	CFU	Incidence	CFU	Incidence	CFU	Incidence	CFU	Incidence	CFU	Incidence
Peptone broth	-	0/10	-	0/10	-	0/10	-	0/10	-	0/10	-	0/10
BP agar	-	0/10	-	0/10	-	0/10	-	0/10	-	0/10	-	0/10

2) *Staphylococcus aureus*

No black colonies, the indicative of *Staphylococcus aureus* (coagulase positive) on Baird-Parker agar were detected in any of the milk samples tested.

Ⅳ. Discussion

Kwon and Choi (1998) reported that the viable counts of UHT pasteurized milk in Korea were less than 2,500 CFU/ml stored under 10°C, but increased significantly up to 10^6 CFU/ml when UHT milk stored over 10°C for three days. In our experiments we did not compare the cell number in terms of increase or decrease, but the higher cell numbers were obtained as the storage time increases. Also the cell numbers between two storage temperatures were significantly different in total bacteria and aerobic spores. Kenifel *et al.* (1991) reported the total bacterial counts ranged from 4,000 CFU/ml and 32,000 CFU/ml of pasteurized market milks in Vienna, but increased to 32,000 ~ 630,000 CFU/ml at the end of declared shelf life.

Coliforms were reduced by approximately 5 log cycles by the range of HTST heat treatment, but could recover from the heat shock (Kenifel *et al.*, 1991). They revealed that coliforms in pasteurized market milk were not detected in any sample on the first day, but 2~44% of milk samples (1,506 milks) were coliform positive at the end of shelf life. After microfiltration and pasteurization, total numbers of microorganisms were reduced by 99.88%, but coliform bacteria by approximately 5 log cycle and completed removed anaerobic spore formers (Skrypek *et al.*, 2001). A microbiological analysis of 266 pasteurized milk samples in Italy, coliforms were present in 26% of samples in the range of 3~2,155 CFU/g (Nisco *et al.*, 1989). In our experiment, no coliforms are detected in all Korean UHT milks stored at both temperatures.

Kessler *et al.* (1994) reported aerobic sporeformers, specially *Bacillus* spp. that can survive at UHT heat treatment, but the numbers did not increase in our results (Table 3). When Borde-Lekona *et al.* (1995) compared the efficiency of heat treatment methods (pasteurized and UHT), no significant effects were found on the counts of the aerobic mesophilic and aerobic in milk stored at 2 °C, but the counts of sporeformers were increased in pasteurized milk and decreased in UHT milk. Kessler *et al.* (1994) insisted that *B. sphaericus* spores endured heat treatment and thus survived in UHT milk produces in

three dairies in Germany. Vyletelova *et al.* (2002) used the ribotyping of milk with a probe complementary to Coli 16s and 23s rRNA as well as by phenotyping, and found that *B. licheniformis* strains B79 passed from raw milk through UHT production process without loss of viability.

Mayr *et al.* (1999) reported that *B. circulans* was the predominant psychrotrophic aerobic sporeformers in HT-ST pasteurized milk in both winter and summer German milk, and the numbers of psychrotrophic bacilli showed as significant seasonal variations. As UHT-ESL milk was treated in aseptic packaging, the high number of thermoduric bacteria on 21 days and 28 days (Table 4) might not be originated from raw milk. Larsen and Jorgensen (1997) reported 120 milks of 257 samples found to be positive in the viable count of *B. cereus* at the range of >1,000 to <30,000 CFU/ml in pasteurized milk collected from 3 Danish dairies.

Total psychrotrophic counts in pasteurized milk in Spain ranged from <1 to 5.47 log CFU/ml and the isolates were found to be the family Enterobacteriaceae (Rodriguez *et al.*, 1990). Pure-Lac system developed by APV and Elopak A/S provides a marked reduction in thermophilic and sporeforming psychrotrophs and post-pasteurization contaminants (Stepaniak, 1997). When Pure-Lac ESL milk produced on steam-infusion equipped with aseptic filling was assessed, the results were agreed well with those of total plate counts (Eie and Martens, 2000). When *B. subtilis* and *B. stearothermophilus* were experimentally inoculated, no residual bacteria were detected in indirect type helical type UHT milk sterilizer with the length of heating section (2.28m) and cooling section (2.08m) (Sahoo *et al.*, 2002). Similarly, in our results no post-pasteurization contaminations were found in all UHT milks collected in Korea.

In conclusion, it appears that not all UHT treated milks are alike, in terms of the microbial quality. They seem to differ from one producer to the other. UHT milks that are packaged with ESL technology seem to be better in microbiological aspects than UHT milks, packaged with conventional method. In terms of the shelf life, the expiration date of ESL milks can be extended up to three weeks when refrigerated.

V. Summary

Total 150 packs of UHT milk and UHT-ESL milk, produced by different domestic milk companies, were compared at refrigerated and room temperature for 35 days in terms of microbiological qualities such as incidence date, number of incidence packs, and numbers of bacteria, and post-pasteurization contamination. Overall qualities of UHT-ESL milk were slightly better than that of UHT milk in relations to total bacteria in milk. No coliforms were detected in two groups of UHT milk samples after 35 days. Aerobic spores were more common in two brands of UHT milks stored in 20°C than those in 7°C, in which spores were broken out at 7 and 14 day. No incidences of thermoduric bacteria were founded until 14 days in two groups of UHT milks, but high level of counts (<300~<3,000 CFU/ml) after 21 days at 20°C and 40~3,600 CFU/ml at 7°C were detected, respectively. Psychrotrophic bacteria were higher in UHT milk than in UHT-ESL milk, in which began to detect at 28 days. No post-pasteurization contamination by *Salmonella* spp. and *Staphylococcus aureus* were found throughout the experimental periods. One may conclude that the shelf life of UHT milk under cold-chains system can be extended up to 21days, as long as UHT treated milk are filled in sterilized containers by aseptic packaging system.

VI. References

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