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Assessment of Post-Pasteurization Contamination of Fluid Milk Products

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액상유의 살균후 오염에 관한 연구

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This study focused on the psychrotrophic post-pasteurization contamination of fluid milk products which were processed by HTST system. Pasteurized line samples and container samples of each fluid milk product (whole milk and skim milk) were taken in a large fluid milk plant. Line samples were collected through nine and five different sampling locations for whole milk and skim milk products, respectively. Each sample was subjected to preliminary incubation (PI) at 21°C for 16h followed by standard plate count (SPC) and crystal violet tetrazolium agar count (CVT). Flavor, SPC, and psychrotrophic bacteria count (PBC) were determined after 7 d at 7.2°C. In addition, ten sequential container samples (packaged in 1000 ml paperboard containers) were taken from a filler at the beginning of each product run. These samples were used for PI followed by SPC and CVT. In addition, flavor evaluations, SPC and PBC tests were conducted after 7, 10, and 14 d at 7.2°C. The mean PI-CVT values for the line samples showed differences depending on the location. There was major contamination between pasteurized storage tank and the filler. The PI-CVT counts for each container sample were negatively correlated with flavor scores at 10 and 14 d. There were good correlations among PI-CVT values of line samples and the percentage of total container samples with acceptable flavor after 10d.

In the days prior to adequate refrigeration, bacterial spoilage caused great economic losses in the dairy industry. Today, the trend towards extended storage of milk at refrigeration temperature has resulted in the problems of the growth and the metabolic activities of undesirable microorganisms which especially occur from post-pasteurization contamination (1-6). Since post-pasteurization contamination by psychrotrophic bacteria is the primary reason for reduced shelf-life in fluid milk products, controlling this type of contamination in a dairy plant is an essential part of the dairy's quality assurance program (1, 7-9). Post-pasteurization contamination can occur from several sources, including ineffective cleaning and sanitizing, cracks, scratches and pinholes in storage tanks and pipelines, malfunctioning valves, cracks in gaskets, condensation from fillers, and environmental contaminants (10-13). Identification of these possible sources of contamination is an extremely beneficial tool in a dairy's efforts to increase milk quality by eliminating post-pasteurization contamination.

Generally, gram-negative psychrotrophic bacteria are the primary cause of shelf-life deterioration (14-16). Therefore, the methods which were studied in this report were focused on the ability to measure these organisms. The preliminary incubation (PI) of milk at temperatures above 10°C has been recommended to modify various methods for microbiological evaluation (1, 12). The PI count is a stan-

Key words: Milk, contamination, preliminary incubation, shelf-life

dard plate count (SPC) after incubation of raw milk for 18 h at 12.8°C (17). In this study, the PI was applied to choose appropriate methods for predicting shelf-life of fluid milk products.

Materials and Methods

Line samples and container samples of each fluid milk products (whole milk and skim milk) were evaluated for psychrotrophic post-pasteurization contamination study twice a week over a eight week period (16 replications). These fluid milk products were pasteurized at 72°C for 15 sec by using a HTST system. Line sampling and testing of container products was performed for each replication (set).

Sample collection

Line samples were taken through sampling ports no later than 20 min after the process was started. Sampling locations for whole milk product were 'discharge of HTST #1', 'inlet to skim tank', 'outlet from skim tank', 'discharge of HTST #2', 'after blender', 'inlet to product tank', 'outlet from product tank', 'after valve manifolds', and 'inlet to the filler'. For skim milk product, 'discharge of HTST #1', 'inlet to product tank', 'outlet from product tank', 'after valve manifolds', and 'inlet to the filler' were chosen. Each line samples was used for microbial tests after PI at 21°C for 16 h and shelf-life evaluation at 7.2°C after 7 d.

At the first of each product run, ten sequential samples packaged in 1000 ml paperboard containers were taken from the densignated filler. Each container samples was used for microbial tests after PI at 21°C for 16 h and shelf-life evaluation at 7.2°C after 7, 10, and 14 d.

Microbial procedures

Standard plate count (SPC) was determined using the procedures recommended by the American Public Health Association (17). Crystal violet tetrazolium agar count (CVT) was determined by plating on crystal violet tetrazolium agar incubating at 21°C for 72 h(18). These enumerations were conducted after PI at 21°C for 16 h.

In addition, keeping quality evaluation with flavor evaluation, SPC, and psychrotrophic bacteria count (PBC) were performed for line samples at 7.2

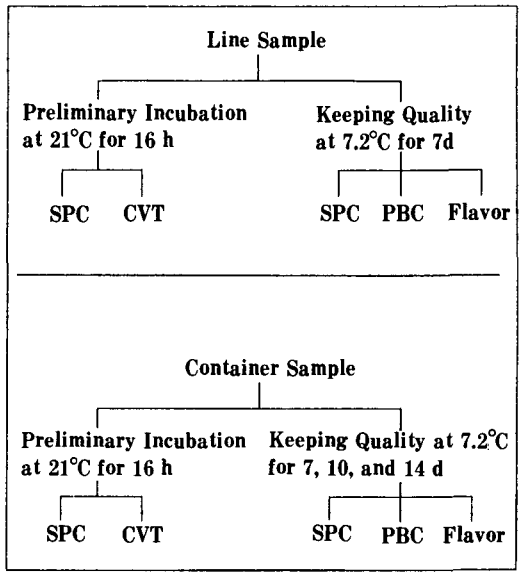

Fig. 1. Testing protocol for line samples and container samples.

°C after 7 d and for container samples at 7.2°C for 7, 10, and 14 d. Psychrotrophic bacteria count was determined using the procedures recommended by the APHA (17) (Figure 1).

Shelf-life determination

The shelf-life of each milk sample was determined by two experienced panelists. When an off-flavor was detected, the sample was considered to be unacceptable. This off-flavor would correspond to a "4" or lower on a 10 point score card for milk.

Statistical procedures

Analysis of variance and correlation coefficients were computed using the Statistical Analysis System (19). A randomized block design including blocked on each or combination of product types, replications, line sampling locations was used. The F test was used to determine if differences existed among sources of variation. Correlation coefficients were calculated among all parameters for each product.

Results and Discussion

Means of bacterial counts after PI on acceptable and unacceptable container samples

None of the line samples and container samples

Table 1. Means of bacterial counts after PI on acceptable and unacceptable container samples.

	PI-SPC		PI-CVT	
	10 d	14 d	10 d	14 d
	cfu/ml			
Whole milk				
acceptable	9,300	9,000	610	490
unacceptable	54,000	34,000	6,400	4,200
Skim milk				
acceptable	3,700	3,600	22	14
unacceptable	4,800	4,000	85	44

of both fluid milk products was considered as unacceptable after 7 d. This result revealed that whole milk and skim milk products had acceptable flavor until 7 d storage at 7.2°C.

The mean SPC values after PI (PI-SPC) for whole milk container samples which were unacceptable when they were evaluated after 10 and 14 d were 54,000 and 34,000 cfu/ml, respectively (Table 1). The mean crystal violet tetrazolium agar count values after PI (PI-CVT) were 6,400 and 4,200 cfu/ml, respectively. The counts for unacceptable samples were always higher than those for the acceptable samples. A suggested PI-CVT standard of 10,000 cfu/ml may be considered for screening whole milk container samples (Figure 2).

The PI-SPC for skim milk container samples showed mean values of 4,800 and 4,000 cfu/ml, when samples were evaluated as unacceptable after 10 and 14 d, respectively. The mean PI-CVT values for unacceptable skim milk container samples were 85 and 44 cfu/ml after 10 and 14 d, respectively. This means that there was little difference in counts for acceptable or unacceptable skim milk container samples. The PI-CVT counts would be preferable to the PI-SPC for skim milk. A suggested standard of 100 cfu/ml on the PI-CVT can be considered for skim milk container samples (Figure 3).

Mean differences among line sampling locations for each bacterial count

Mean separation procedures were conducted on line sampling location data by Duncan's Multiple Range Test. There were no difference ($P<.05$) among mean values of PI-SPC for line samples for whole milk and skim milk. However, mean values

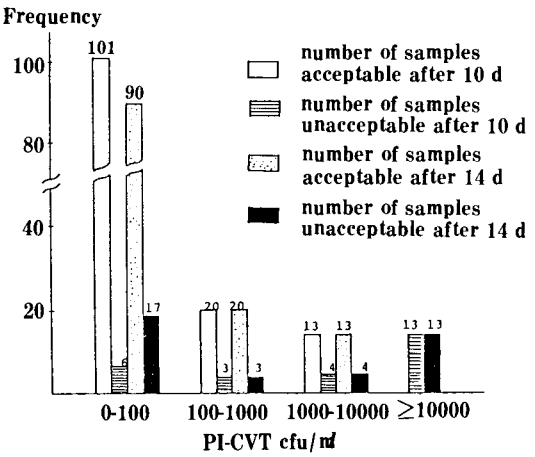

Fig. 2. Frequency of whole milk samples at different PI-CVT counts.

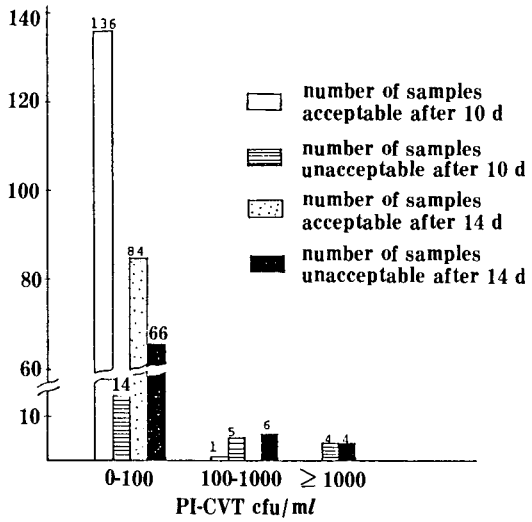

Fig. 3. Frequency of skim milk samples at different PI-CVT counts.

for PI-CVT showed differences ($P<.05$) depending on the line sampling locations (Table 2 and 3). These locations were "outlet from skim tank" for whole milk and "inlet to the filler" for skim milk. According to this results, it is quite possible that there was contamination in the skim tank for whole milk, and between pasteurized storage tank and the filler for skim milk.

Mean differences among replications for each quality factor

Table 2. Means and standard deviations of PI-CVT[a] on each line sampling location for whole milk (N = 13).

Location	Mean[b]	SD[c]
Discharge of HTST #1 (W1)	1.00[a]	.00
Inlet to skim tank (W2)	1.00[a]	.00
Outlet from skim tank (W3)	1.26[b]	.65
Discharge of HTST #2 (W4)	1.07[a]	.19
After blender (W5)	1.1[a]	.29
Inlet to product tank (W6)	1.16[a]	.46
Outlet from product tank (W7)	1.02[a]	.08
After valve manifolds (W8)	1.06[a]	.15
Inlet to the filler (W9)	1.09[a]	.23

[a]Crystal violet tetrazolium agar count (log cfu/m*l*) after PI of 21 C-16 h.
[b]Means with different letters differ (P<.05).
[c]Standard deviation.

Table 3. Means and standard deviations of PI-CVT[a] on each line sampling location for skim milk (N = 15).

Location	Mean[b]	SD[c]
Discharge of HTST #1 (S1)	1.00[a]	.00
Inlet to product tank (S2)	1.00[a]	.00
Outlet from product tank (S3)	1.00[a]	.00
After valve manifolds (S4)	1.00[a]	.00
Inlet to the filler (S5)	1.08[b]	.31

[a]Crystal violet tetrazolium agar count (log cfu/m*l*) after PI of 21 C-16 h.
[b]Means with different letters differ (P<.05).
[c]Standard deviation.

Mean separation procedures were conducted on 16 replications of samples for each product type by use of Duncan's Multiple Range Test. Overall, for whole milk container samples, 7th replication had higher (P<.05) bacterial counts and worse flavors, and 15th replication had lower (P<.05) bacterial counts and better flavors. For skim milk container samples, 2nd and 15th replications had higher (P<.05) means for quality factors. Above results indicated that the quality factors were dependent on the daily cleaning and/or processing conditions such as: cleaning and sanitizing lines, tanks, and other equipments; temperature and/or concentration of sanitizing reagent; employee's personal sanitary practice; and frequency of product change over and machine breakdown.

Table 4. Correlations of bacterial counts after PI with the percent of total container samples with acceptable flavor.

	Correlation	r[a]
Whole milk	PI-SPC vs flavor 10[b]	-.4057**
	vs flavor 14	-.2991**
	PI-CVT vs flavor 10	-.4156**
	vs flavor 14	-.3532**
Skim milk	PI-CVT vs flavor 10	-.2223**

[a]Correlation coefficient.
[b]The percent of total samples with acceptable flavor on 10 and 14 d.
* P<.05.
** P<.01.

Correlations of bacterial counts after PI with the percentage of total container samples with acceptable flavor

The PI-SPC and PI-CVT for whole milk had correlations (P<.01) with the percentage of total container samples with acceptable flavor on 10 (flavor 10) and 14 d (flavor 14) (Table 4). The correlation coefficients with flavor 10 were -0.4057 and -0.4156, and with flavor 14 were -0.2991 and -0.3532, respectively. The PI-CVT for skim milk was correlated (P<.01) with flavor 10 of skim milk. However, the correlation coefficient was only -0.2223. The reason of so low coefficient values may be due to the large number of observations (samples) used in the study.

Correlations of bacterial counts after PI of line samples with the percentage of total container samples with acceptable flavor

There were good correlations among PI-CVT values of line samples and flavor 10 of samples. These values demonstrated post-pasteurization contamination areas. The PI-CVT of whole milk from "outlet from skim tank" was correlated (P<.05) with flavor 10 (r = -0.6208). The PI-CVT of skim milk from "inlet to the filler" was correlated (P<.01) with flavor 10 (r = -0.8434). This was the highest correlation obtained and definitely indicated contamination at "inlet to the filler". Overall, the PI-CVT from "inlet to the filler" was correlated with flavor 10, and had higher correlation coefficients than the PI-CVT of finished products (container samples).

요 약

저온성 미생물의 살균후 오염에 관하여 HTST 살균법에 의하여 제조된 음용유를 대상으로 연구하였다. 살균후 제조공정중의 시료와 충병제품의 시료를 시유와 탈지유별로 대형 음용유 공장에서 채취하였다. 제조공정중의 시료를 시유의 경우 아홉개의 각각 다른 시료채취 장소에서 각각 채취하였다. 각각의 제조공정중의 시료는 21℃에서 16시간 동안 예비배양(PI)을 한 후 표준평판검사(SPC)와 그람음성균검사(CVT)를 실시하였다. 보존성검사를 위하여 각 시료를 7.2℃에서 7일간 보존한 후 풍미검사와 SPC 및 저온미생물검사(PBC)를 실시하였다. 제조공정중의 시료 이외에 실제품의 보존성을 검사하기 위하여 1000 ml용 종이용기에 충병된 완제품을 각 제품(시유 및 탈지유)의 생산이 시작될 때마다 일련적으로 연속하여 10개씩 충병기로부터 채취하였다. 충병제품 시료들에 대해 예비배양(PI)을 거친 후 SPC와 CVT를 실시하였다. 보존성검사를 위하여 각 충병제품 시료를 7.2℃에서 7, 10 및 14일간 보존 후 풍미검사와 SPC 및 PBC를 실시하였다. 제조공정중의 시료의 예비배양 후 CVT(PI-CVT) 평균치는 시료채취 장소에 따라 차이가 있었다. 살균유 저장탱크로부터 충병기까지의 제조공정에서 오염이 주로 발생하였다. 충병제품 시료의 PI-CVT 값은 10 및 14일간 보존 후 풍미와 밀접한 상관관계를 나타내었다. 제조공정중의 시료의 PI-CVT 값은 10일간 보존 후 양호한 풍미를 유지한 충병제품 시료의 전체 충병제품 시료에 대한 백분율과 좋은 상관관계를 보였다.

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