PSYCHROPHILIC BACTERIA AND KEEPING QUALITY OF PASTEURIZED DAIRY PRODUCTS[1, 2]

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Summary

A study was made showing relationship between post-pasteurization contamination of milk and cream and increase in bacterial count of bottled and paper carton products during storage at 45 F for 5 days. A survey indicated extensive post-pasteurization contamination in plants not employing this type of keeping quality test. The 5-day at 45 F test was more sensitive than the coliform test in detecting post pasteurization contamination. Excessive numbers of thermoduric bacteria in the raw supply also were detected by this method when plant equipment was properly cleaned and sanitized.

Special in-line sampling techniques were developed to determine source of contamination. One procedure employed sterile disposable hypodermic syringes inserted through rubber stoppered nipples welded into lines at different locations in the system. Another technique involved removal of samples by insertion of sterile disposable hypodermic syringes through rubber or neoprene gaskets between joints in different locations in the plant.

Bottle and paper carton filler equipment offered special cleaning and sanitizing problems and suggestions were made on steps to minimize contamination from these sources.

Application of the 5-day at 45 F keeping quality test followed by careful study of contamination sources has greatly improved shelf life of pasteurized fluid milk and cream and has represented a real economic advantage to plants adopting the program.

The growth of bacteria during marketing and use of pasteurized milk and cream continues to be a serious problem for the dairy industry. The most important source of such organisms is contaminated equipment between the pasteurizer and the final container. The result of such growth is definite flavor deterioration and often excessively high bacterial counts in the finished product as it is used by the consumer. The situation is more common than generally realized; in fact it may qualify as the number one sanitation problem in the dairy industry today. Often plants are unaware of this condition until they have run bacterial counts following a keeping quality test. Another circumstance associated with this condition is that bacterial plate counts, both standard and psychrophilic, and even coliform tests on the freshly processed product provide little prediction of its subsequent bacterial condition as it is consumed.

The purpose of this discussion is to emphasize the importance of regular combined use of keeping quality tests and bacterial plate counts of pasteurized fluid milk and cream products. The idea of such a keeping quality program was originated several years ago by W. K. Moseley, Indianapolis, Indiana. Recent observations on application of this approach have indicated marked improvement in both flavor and bacterial content of milk and cream after transportation and storage with definite extension of storage life and consequent financial gain to the processor. The same principles and approach can be applied to ice cream mix and cottage cheese although equipment and handling procedures for the latter are somewhat different.

Product Defects Involved

In milk and cream the first evidence of poor keeping quality is development of off-odors and flavors like unclean, fruity, stale, rancid, bitter and cheesy. In cottage cheese such defects are followed by physical changes such as gelatinous or tapioca curd *(3, 5, 6)*. Odor and flavor defects are preceded usually by growth of bacteria which, within 5 to 7 days after processing, may number in the millions in a product stored at an average temperature of 45 F. It is common for the flavor to change from the fifth to tenth day after processing from normal or fresh to decidedly undesirable. The serious aspect of this is that the product then is in the hands of the consumer. The large number of organisms consumed in the product at this stage are not disease-producing, but their over-all effect is to depress future sales and shorten handling and market life of the product.

[1]Technical Paper No. 1773. Oregon Agricultural Experiment Station. Contribution of the Department of Microbiology.
[2]Presented at the 50th Annual Meeting of the International Association of Milk and Food Sanitarians, Inc., October 22-25, 1963, at Toronto, Canada.

SOURCES AND TYPES OF BACTERIA ASSOCIATED WITH POOR KEEPING QUALITY

Genera of bacteria most frequently involved in keeping quality problems in pasteurized milk and cream include *Pseudomonas, Achromobacter, Chromobacterium, Alcaligenes, Proteus, Escherichia* and *Aerobacter*. The gelatinous curd and flavor defects of cottage cheese produced by *Pseudomonas viscosa, Pseudomonas fragi* and *Alcaligenes metalcaligenes* are well known. In milk and cream as well as cottage cheese *P. viscosa* produces bitter, rancid and rotten flavors while that produced by *P. fragi* is the fairly common fruity defect. These organisms probably are not true psychrophiles but rather should be considered as a group of bacteria that grow well at temperatures of 45 to 90 F. An example of a true psychrophile is one isolated in our laboratories recently by R. Y. Morita and associates. This organism, a gram negative rod, grows at 30 F and is killed by brief holding at 68 F.

Common sources from which psychrophilic type bacteria enter milk and establish themselves on farm and plant equipment include soil, water, dust, and, in the case of cottage cheese, vegetables that may be incorporated with the product. The organisms grow well in milk and in milk deposits on equipment. Thorough cleaning and effective sanitizing will eliminate them from processing equipment and this is of importance for equipment following pasteurization.

Water supplies so frequently carry psychrophilic type spoilage bacteria that cottage cheese wash water should be routinely treated with additional chlorine (5 to 10 ppm), just as insurance against contamination from this source. Where growth in such water is excessive or the water is alkaline, it may be acidified before chlorine treatment in order to accelerate destructive action of the chlorine *(2, 4)*. The acidification may bring the pH down to levels such as pH 5.0 to 7.0. Treatment at this pH with 5 to 10 ppm chlorine may be quite effective in eliminating spoilage bacteria from the water on a continuous basis where retention time is no more than 15 to 60 seconds.

MOSELEY KEEPING QUALITY TEST

The procedure originally developed for this test consists of storing a container of milk or cream, preferably unopened, at 45 F for 5 days and then subjecting the product to a standard plate count with incubation of plates at 90 F *(1)* or, if desired, at room temperature (77 F). A bacterial count on the fresh product provides a useful control especially when beginning such an improvement program. Coliform counts can be run also on the fresh and stored samples although they become less useful as sanitation improves through application of the keeping quality test. Some plants have developed variations on this test but observations indicate that incubation at 45 F generally provides conditions more closely approximating those during marketing and use of the product.

The basis of the Moseley Keeping Quality Test is that practically all of the bacteria that grow well in milk in 5 days at 45 F are destroyed by pasteurization. Since these organisms gain entrance to the product from contaminated equipment, the test becomes a means of establishing sanitary condition of equipment or containers which contact the product following pasteurization. Organisms that grow well at 45 F in milk or cream almost invariably are found on poorly cleaned and sanitized equipment. A marked increase in bacterial numbers during the 5-day test period suggests a plant inspection and this usually reveals poorly cleaned and sanitized equipment. Subsequent cleanup of this equipment usually results in marked improvement of keeping quality and bacterial condition of stored samples.

Recent observations also have emphasized that unless a plant has been conscientiously engaged in such a combined keeping quality test and bacterial count program, it frequently is harboring false illusions regarding the sanitary condition of its plant and bacterial condition of the finished product.

By making a plate count on fresh samples and on a sample of the same batch of product after 5 days at 45 F, two important types of information can be obtained: First, if the bacterial numbers increase appreciably during storage, the evidence is strong that post-pasteurization contamination has occurred. If the plate count on the fresh sample is high, that suggests thermoduric bacteria in the raw product which is a situation that occurs less frequently.

SPECIAL SAMPLING PROCEDURES

To obtain specific information relating to sources of contamination it is often necessary to aseptically sample at any given point in a post-pasteurization system. These areas may be at the pasteurizer, in the network of lines or valves, at pasteurized surge tanks, at filler bowls or at the filler itself. A number of sampling procedures can be employed incorporating the use of special devices, sampling cocks or sterile pipettes and dippers. One of these special line sampling techniques involves the use of a disposable plastic hypodermic syringe. This is inserted into a pipe line and the sample removed through a previously sanitized neoprene gasket joint

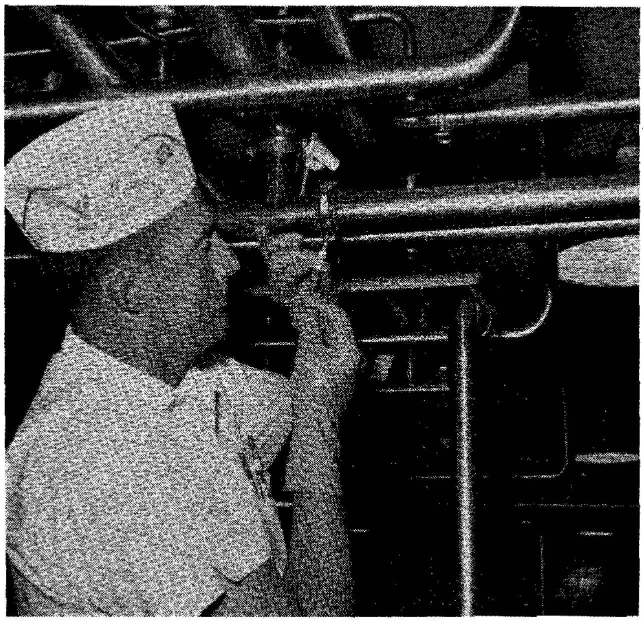

Figure 1. In-line sampling using sterile plastic syringe through sanitized neoprene gasket between pipe joints.

as shown in Figure 1.

Another arrangement that has proven useful is a stainless nipple, fitted with a sterile rubber serum cap, welded onto a standard line fitting, which can be clamped in or permanently located at any area desired (see Figure 2). Repeated samplings using sterile plastic hypodermic syringes can be taken at these locations by inserting the needle through the serum cap and withdrawing a sample. Samples can be transferred to sterile tubes or left in the disposable hypodermic syringe for immediate use or incubation.

In Europe another method of line sampling has been observed. This involves drilling a pin hole in any particular line at any given location and using this as a sampling port. The drilled hole is conveniently covered with a small rubber or plastic disc which is held in place with a spring clamp.

More conventional methods of sampling also may be employed. Sampling through appropriately sanitized sampling cocks or using sterile dippers or pipettes all may be satisfactory. If condensate droplets are to be collected, sterile swabs, sterile bacteriological loops, sterile syringes or medicine droppers can be used.

RESULTS WITH KEEPING QUALITY TEST

Table 1 illustrates typical results obtained by a plant that considered its sanitation program satisfactory. One coliform count suggested the possibility of a sanitation problem but not to the degree brought out by the plate count on the stored sample. Many of the samples tested in this series exhibited serious off-flavor by the fifth or sixth day of storage. Numerous customers must have experienced off-flavor development in these products.

This type of situation also emphasizes a paradox in our supervision of milk supplies. Consider the great effort to provide a raw milk that frequently stays under a 25,000 plate count entering the processing plant; yet, after processing, the consumer may drink a pasteurized product that runs into millions per ml. This condition is not uncommon except where plants are using a test such as this one to evaluate suitability of plant sanitation procedures.

Table 2 illustrates a different situation where contamination is apparently not as excessive. Note that the coliform test is negative in every sample. This emphasizes an important point, namely, that the keeping quality test with plate counts after 5 days at 45 F is a more sensitive and reliable indicator of plant contamination than the coliform test. Also, the standard plate count on freshly processed samples understandably provides no indication whatsoever of the keeping quality of the milk. There might be so few psychrophilic types of bacteria picked up from such equipment that even though plates of the

TABLE 1. PLATE COUNTS ON FRESH AND STORED SAMPLES WITH PLANT ON REGULAR SANITATION PROCEDURES

	Fresh		Stored
Product	Plate count	Coli-form	5 days at 45 F
Skim	<3,000	0	25,600,000
Homo	<3,000	0	24,800,000
M.V.[a]	<3,000	1	26,400,000
H.H.[b]	14,100	0	2,700,000
W.C.[c]	<3,000	0	7,500,000

[a]Multivitamin; [b]half and half; [c]whipping cream.

TABLE 2. PLATE COUNTS ON FRESH AND STORED SAMPLES WITH PLANT ON IMPROVED SANITATION PROCEDURES

	Fresh		Stored
Product	Plate count	Coli-form	5 days at 45 F
Homo	400	0	2,100,000
M.V.[a]	600	0	9,100,000
H.H.[b]	900	0	3,000,000
W.C.[c]	100	0	17,000,000
Skim	700	0	9,200,000

[a]Multivitamin; [b]half and half; [c]whipping cream.

TABLE 3. PLATE COUNTS ON FRESH AND STORED SAMPLES WITH PLANT ON IMPROVED SANITATION PROCEDURES

	Fresh		Stored
Product	Plate count	Coli-form	5 days at 45 F
Homo	400	0	700
M.V.[a]	700	0	3,300
H.H.[b]	300	0	300
W.C.[c]	500	0	100
Skim	800	0	6,000

[a]Multivitamin; [b]half and half; [c]whipping cream.

TABLE 4. PLATE COUNTS OF STORED SAMPLES SHOWING INADEQUATE POST-PASTEURIZATION CLEANING AND SANITIZING

	Plate counts after storage 5 days at 45 F			
Product	At HTST	At filler bowl bowl	In carton	Empty carton
Homo	22,000	26,000	30,000	0
Skim	<3,000	10,000	60,000	0
M.V.[a]	<3,000	520,000	1,300,000	0
2%	<3,000	8,000	1,300,000	0

[a]Multivitamin.

TABLE 5. PLATE COUNTS OF STORED SAMPLES SHOWING FILLING AREA AS SOURCE OF CONTAMINATION

	Plate counts after storage 5 days at 45 F			
Product	At HTST	At filler bowl bowl	In carton	Empty carton
Homo	<3,000	<3,000	150,000	0
Skim	<3,000	<3,000	3,600,000	0
H.H.[a]	<3,000	<3,000	29,000	0
Whip	<3,000	<3,000	130,000	0
M.V.[b]	<3,000	<3,000	52,000	0

[a]half and half; [b]multivitamin.

TABLE 6. PLATE COUNTS OF STORED SAMPLES OF GLASS PRODUCTS SHOWING BOTTLE FILLER AS SOURCE OF CONTAMINATION

	Plate counts after storage 5 days at 45 F			
Product	At HTST	Above filler	Bottled product	Empty bottle
Homo	<3,000	<3,000	460,000	0
Stand[a]	<3,000	5,000	840,000	0
M.V.[b]	<3,000	<3,000	59,000	0
Skim	<3,000	<3,000	310,000	0

[a]standard milk; [b]multivitamin.

TABLE 7. PLATE COUNTS OF STORED SAMPLES TAKEN AFTER IMPROVED SANITATION

	Plate counts after storage 5 days at 45 F			
Product	At HTST	At filler bowl	In carton	Empty carton
Homo	<3,000	<3,000	4,000	0
Skim	<3,000	<3,000	<3,000	0
H.H.[a]	<3,000	<3,000	<3,000	0
Whip	<3,000	<3,000	<3,000	0
M.V.[b]	<3,000	<3,000	6,000	0

[a]half and half; [b]multivitamin.

TABLE 8. PLATE COUNTS SHOWING A PROBLEM WITH CREAM PRODUCTS AND THERMODURIC BACTERIA

Sample	Fresh	After storage 5 days at 45 F
Regular	22,000	23,000
Homo	12,000	16,000
Skim	6,000	6,300
H.H.[a]	5,300	12,000,000
C.C.[b]	<3,000	6,000,000
W.C.[c]	3,400	10,000,000

[a]half and half; [b]coffee cream; [c]whipping cream

fresh sample are incubated at low temperatures such as 41 F, little indication of numbers of such bacteria that develop during storage at 45 F can be obtained from this count. This emphasizes the limited sanitary significance of the standard plate count on a fresh, processed sample.

Table 3 shows results obtained in the same plant and provides an indication of what can be expected after results of the keeping quality test have been followed by a conscientious effort to eliminate all deposits and effectively sanitize the equipment. This may include equipment such as valves, pipelines, tanks (including surge tanks), and especially fillers.

Tables 4 and 5 illustrate how an in-line sampling method provides information on source of contamination. Samples were removed with a sterile hypodermic syringe through sterile rubber serum caps at different locations in the system. In one instance in Table 4, contamination in the line is indicated. In Table 5 low counts at the filler bowl and sterile cartons place the difficulty at the paper filling machine. Table 6 illustrates the same situation for the glass bottle filler. Paper machines sometimes offer special problems with such items as contaminated mandrels or other surfaces, excessive condensate that drips or is blown into clean cartons, and contaminated lubricants. Such equipment may have to be studied carefully to locate possible contamination sources.

Carton counts were obtained by rinsing cartons with 10 ml of sterile water and plating this out. As a further check on the bacteriological condition of the containers, sterile milk was added to a considerable number of both paper cartons and glass bottles and a count made of the milk after 5 days of storage at 45 F. Neither paper cartons nor glass bottles yielded organisms capable of increasing in the milk at 45 F.

Table 7 provides an example of excellent control over the sanitary condition of the entire system. This can be accomplished by careful analysis of the cleaning and sanitizing of all surfaces that come in contact with product or which might drip condensate into the product. Special precautions particularly with the glass and paper fillers are necessary to consistently produce results such as these. However, with such a product there is a vast improvement in keeping quality in the home and restaurant. Return date has been extended at least three days and freshness in flavor often several more.

Table 8 illustrates a situation often occurring in smaller plants that may place their cream in cans to be later dumped into the filler for packaging cream products. Cans in a dairy plant are a common source of such bacteria because they frequently are not as well cleaned and sanitized as regular process-

TABLE 9. PLATE COUNTS SHOWING A CLEAN PLANT WITH EVIDENCE OF THERMODURIC BACTERIA IN RAW SUPPLY

Sample	Fresh	After storage 5 days at 45 F
Regular	5,300	4,400
Homo	16,000	10,000
H.H.[a]	3,100	<3,000
W.C.[b]	3,100	<3,000

[a]half and half; [b]whipping cream.

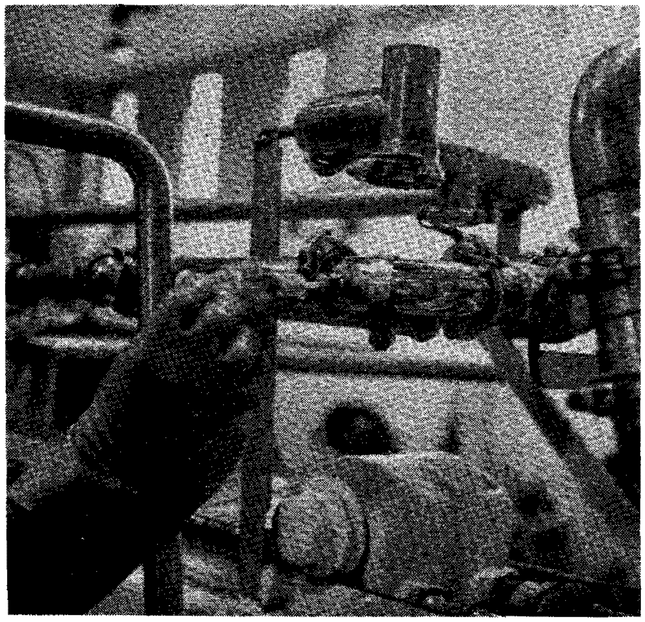

Figure 2. Sampling at HTST pasteurizer using plastic hypodermic syringe. Sample is removed through rubber serum cap inserted on nipple welded onto elbow. Metal clamp at right is placed over nipple when not sampling to maintain pressure on serum cap.

ing equipment. Any product going through such cans may thereby pick up such contamination. The first 3 products shown in Table 8 and those shown in Table 9 indicate another interesting type of information provided by the keeping quality test when counts are run on both the freshly processed product and that stored at 45 F for 5 days. The numbers in the freshly processed samples appear high, presumably due to the excessive numbers of thermoduric bacteria in the raw supply. However, the plant systems handling these products are clean as evidenced by the lack of increase in numbers during the keeping quality test.

APPLICATION OF METHOD TO ICE CREAM MIX

Long transportation and extended storage periods now employed for ice cream mix pose a real problem in bacterial control. Widespread use of counter freezers under a variety of conditions provides ample opportunity for bacteria to develop in the mix before freezing. This emphasizes importance of keeping quality tests on pasteurized mix as well. Table 10 shows results on various mix samples in an attempt to pin point sources of contamination. Careful attention to various cleaning and sanitizing steps has demonstrated that mix can be improved in keeping quality also as shown by the 12% product in Table 10.

APPLICATION OF METHOD TO COTTAGE CHEESE

Considerable information on sanitary condition of cottage cheese equipment or wash water can be obtained by applying the same type of test to this product. Excessive bacterial counts in cottage cheese after 5 days of storage at 45 F usually indicate unsanitary plant conditions assuming culture organisms are not added to the dressing.

SUGGESTIONS FOR IMPROVING SANITARY CONDITION OF POST-PASTEURIZATION EQUIPMENT

Following are some of the fine points which will make the difference between a successful or unsuccessful keeping quality program:

1. Insure an adequate cleaning system regardless of whether it is manual or CIP. The index of cleanliness is best determined by visual inspection using a strong flash light supplemented by the use of the black light. A program for periodic checking of valves and gaskets is strongly recommended in automated systems or disassembly systems. Often-times automated systems need to be checked thoroughly by local or factory representatives.

2. Manual valves and gaskets on take down lines are extremely critical areas. These should be individually washed or circulated and sanitized just prior to assembly. Care should be taken to sanitize

TABLE 10. PLATE COUNTS OF STORED ICE CREAM MIX SHOWING INADEQUATE POST-PASTEURIZATION CLEANING AND SANITIZING

Product	Plate counts after storage 5 days at 45 F				
	At past.	At cooler	At surge tank	At Filler	In can
12%	<3,000	<3,000	<3,000	<3,000	<3,000
4%	<3,000	15,000	18,000	9,000,000	TNC
6%	<3,000	6,000	2,000,000	3,000,000	TNC
Shake	<3,000	<3,000	4,000,000	4,000,000	4,000,000
Choc. shake	<3,000	<3,000	19,000	400,000	TNC

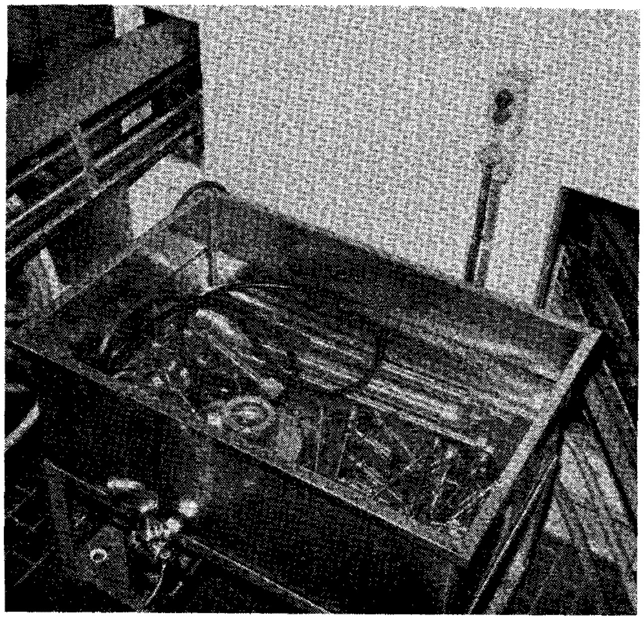

Figure 3. Immersion tank with 200 ppm chlorine solution for glass filler parts.

the contact surfaces also.

3. Sanitize with a product that will provide effective destruction of all types of bacteria. Use of proper concentrations and time intervals for bactericidal action are also important. The sanitizers most widely used due to their effectiveness and low cost are the chlorine-containing products. A 200 ppm solution of chlorine allowed to react for at least 30 sec is an adequate general sanitizer. Application of sanitizers by spraying a solution may offer better wetting action resulting in greater bactericidal efficiency than fogging.

4. Personal hygiene cannot be overemphasized. Avoid human contact, especially hands, on sanitized equipment. This particularly applies to filling machines. If it is necessary to make adjustments, be sure to wash and sanitize hands before performing an operation. In some plants operators wear rubber gloves, especially when assembling or manipulating filling machine parts.

5. Paper and bottle filling machines have many vulnerable areas that can contaminate the product before or during filling. The filling assembly is perhaps the most vulnerable. All removable parts should be thoroughly cleaned by recirculation if possible and left disassembled in an orderly fashion. Just prior to use, all parts should be sanitized individually by immersion or spraying before assembly to the previously sanitized body of the machine (Figure 3). All gaskets and "O" rings should be sanitized by immersion. Surfaces which they contact should also be sanitized. Wearing rubber gloves is a good practice to avoid contact with bare hands and also to protect hands from irritation by sanitizer solutions. The remaining body of the machine should also be sanitized by fogging or spraying with an appropriate sanitizer. Glass machines having multiple filling heads should be assembled in the same fashion described. It is also desirable to spray sanitizing solution at intervals over filler parts during assembly (Figure 4). Lubricants used should be sterile. Where detailed assembly instructions are necessary, it may be helpful to provide printed instructions listing specific operations in their order of execution.

Additional thought needs to be given to design and construction of filler assemblies to simplify and reduce contamination from other sources such as condensate.

6. Most large dairy plants will operate their fillers for long periods of time resulting in the accumulation of milk solids, fat and condensate on machine parts. These areas become ideal vehicles for contamination by psychrophilic types. Therefore, it is equally important to flush them down with sanitizing spray. A convenient time to do this is between products or while changing to a different tank. Starting with the uppermost portions such as the filler bowl and working down, spray the entire filling area (Figure 5). Condensate on some machines gathers on the carton forming mandrels. This area can also be sanitized by spraying with a sanitizer.

7. Seeking out the source of contamination may save time and expense in the long run. By utilizing various sampling techniques such as those described earlier, one can trace back and obtain information relating, for instance to: the failure of automated CIP systems, build-up in tanks or crossover lines, non-functioning or dirty valves, dirty pumps, broken

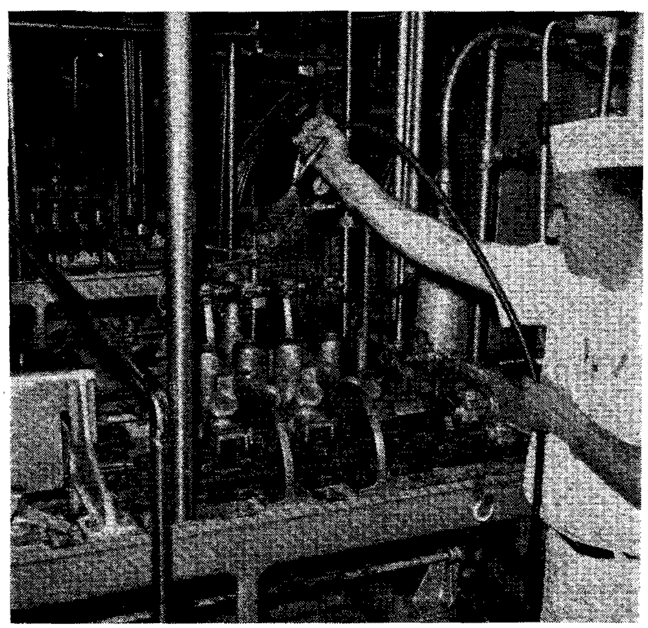

Figure 4. Spraying partially assembled filling area of paper carton machine wth 200 ppm chlorine solution.

plates of HTST and numerous other sources.

8. Pride of workmanship helps in many ways to maintain high standards and to stay on top of the keeping quality problem. Once the approach and objectives of such a program are explained to plant workers, they seem to follow it with great interest and willingness to cooperate. Management has a responsibility in recognizing such cooperation on the part of employees.

RESPONSIBILITIES FOR SANITARY CONDITION OF PLANT EQUIPMENT

The keeping quality test should be considered a useful tool that must be applied in order to provide a product that will remain in satisfactory condition through the longer marketing and consumption period encountered today. It should be utilized by the plant crew to eliminate important trouble spots. In some cases it may be necessary to call in paper carton machine representatives or sanitation automation representatives to analyze their installations for possible faulty construction or operation.

One fact graphically emphasized by application of this yardstick is that ultra-high pasteurization processes and similar improvements are not practical until the keeping quality problem is worked out. A careful study of the problem of post-pasteurization contamination has demonstrated that a satisfactory degree of cleanliness can be accomplished with very little extra effort and no more expense on the part of the plant crew. Results have further demonstrated that the improved keeping quality attained pays dividends in a longer marketing period, fewer returns and fewer customer complaints. Milk as used in restaurants, schools and the home is greatly improved; return date has been increased from 5 or 6 days to 9 or more days with product still comparatively fresh. Plants on this program are enthusiastic about it.

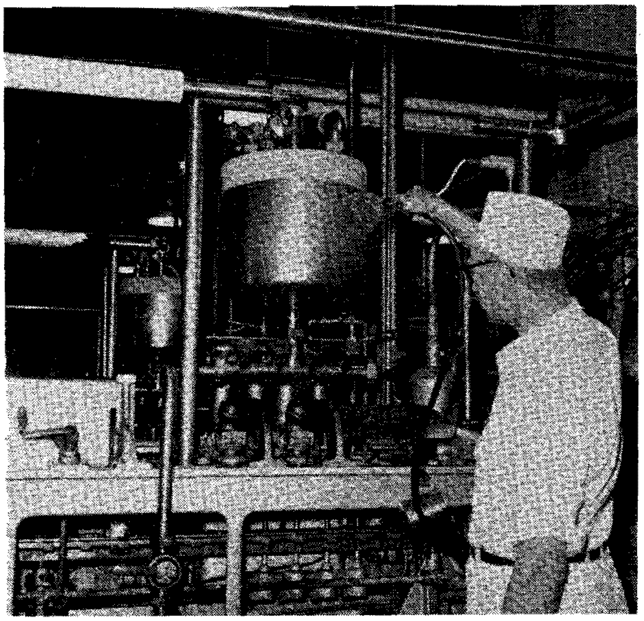

Figure 5. Flushing off filling area of paper carton machine between products with 200 ppm chlorine solution. Purpose is to remove and sanitize condensate and flush off accumulated milk solids.

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