SURVIVAL OF *SALMONELLA TYPHIMURIUM* IN COLD-PACK CHEESE FOOD DURING REFRIGERATED STORAGE[1]

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Abstract

Fourteen batches of cold-pack cheese food were contaminated with *Salmonella typhimurium* during manufacture. Cheese food stored at 4.4 and 12.8 C was tested at weekly intervals and salmonellae were enumerated by means of a Most Probable Number technique. A rapid decline in number of salmonellae occurred during the first week of storage regardless of temperature or composition of the product. Survival beyond this time was more closely related to both conditions. Viable salmonellae could not be recovered, after 3 weeks at 12.8 C or 5 weeks at 4.4 C, from cheese food adjusted to pH 5.0 with lactic acid and fortified with 0.24% potassium sorbate. Substituting sodium propionate for sorbate resulted in 14 and 16 weeks of survival by salmonellae when cheese food was held at 12.8 and 4.4 C, respectively. Partial or complete replacement of lactic acid by acetic acid was accompanied by somewhat longer survival of salmonellae than when only lactic acid was used. Elimination of added acid from the cheese food resulted in survival of salmonellae for 6 and 7 weeks when potassium sorbate was present, for 16 and 19 weeks when sodium propionate was used, and in excess of 27 weeks when no preservative was added.

Salmonellae have been recognized as human pathogens for nearly 100 years. Salmonellosis is primarily an animal disease; human food-borne illness is a more or less accidental consequence of animal infection. Food-borne salmonellae are most often associated with poultry, poultry products, meat, and meat products. It is estimated that each year nearly two million Americans suffer from salmonellosis *(3, 7)*. Although the disease frequently is mild, approximately 100 persons in the United States die from salmonellosis each year and the annual economic loss attributed to this problem ranges from 10 to 100 million dollars *(13)*.

Milk once was a common vehicle for the dissemination of salmonellae. Since pasteurization has become common, milk has only rarely been incriminated in outbreaks of salmonellosis. However, nonfat dry milk has recently been associated with some outbreaks of salmonellosis caused by *Salmonella newbrunswick (9)*. Other salmonellae also have been recovered from this product since the onset, in 1966, of a vigorous testing program (9). During 1969 the following serotypes were found in samples of commercial nonfat dry milk: *Salmonella cubana, Salmonella minneapolis, Salmonella newington, Salmonella albany, Salmonella anatum, Salmonella tennessee,* and *Salmonella senftenberg (16)*.

According to a recent review by Marth *(9)*, outbreaks of salmonellosis attributable to cheese were reported as early as 1923 when, in Great Britain, persons contracted paratyphoid fever from consumption of contaminated cream cheese. Since then salmonellosis, nearly always caused by *Salmonella typhimurium* or *Salmonella typhi,* has been associated with Cheddar, Colby, Camembert, Romano Dolce, Teleme, Jack, Quarg, Cream, Cottage, and some other cheeses *(9)*. Persons handling milk used for cheese making or the milk itself most often served as sources of the salmonellae. In some instances cheese was consumed before it was aged sufficiently long to insure inactivation of salmonellae. Although cheese made from raw milk must be ripened at not less than 1.67 C (35 F) for at least 60 days prior to sale, there is ample evidence that this treatment will not eliminate viable salmonellae, if present, from cheese *(1, 5, 6, 12, 15)*. In the United States approximately 35% of the ripened cheese produced is used to manufacture processed cheese products of all types including cold-pack cheese food *(8)*.

Cold-pack cheese food is prepared from cheese, nonfat dry milk, dried whey, and some other ingredients. It is possible that the finished product could be contaminated with salmonellae by some of the ingredients. In addition to cheese, where extended survival of these organisms has been reported *(5, 6, 12)*, and nonfat dry milk, cheese food could be contaminated by dried whey which, on occasion has been found to contain salmonellae *(16)*. Contamination of a finished product with salmonellae from ingredients has been observed in other foods *(3)*. Additionally, cold-pack cheese food could be contaminated by personnel who are engaged in its production. Presence of salmonellae in this product

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TABLE 1. COMPOSITION OF EXPERIMENTAL BATCHES OF COLD-PACK CHEESE FOOD[1]

Ingredient	Experimental product						
	1	2	3	4	5	6	7
Cheddar cheese (g)	1698	1740	1740	1740	2112	1740	1740
NFDM (g)	100	100	100	100	125	100	100
Dried whey (g)	100	100	100	100	125	100	100
Butter (g)	76.2	76.2	76.2	76.2	95.1	76.2	76.2
Potassium sorbate (g)	5.4	—	5.4	5.4	6.8	—	—
Sodium propionate (g)	—	4.5	—	—	—	4.5	—
Lactic acid (ml)	8.5	10.5	7.0	—	—	—	—
Acetic acid (ml)	—	—	2.1	6.3	—	—	—
Water (ml)	241	261	261	261	327	261	261
Moisture (%)	41.81	43.36	43.65	43.73	43.58	43.49	43.24
Fat (%)	24.88	23.57	24.36	24.44	24.38	24.07	25.22

[1]Percentages of moisture and fat are average values obtained from tests on duplicate batches of each type of cheese food.

is of special significance because it receives no heat treatment nor is any additional aging required before it is consumed.

Since no information was available on the behavior of salmonellae in cold-pack cheese food, experiments were initiated to determine how long the organisms persist in such products when made according to different formulae and when stored at refrigeration temperatures. This paper reports results of the investigation. A preliminary report of this work has been given *(11)*.

MATERIALS AND METHODS

Culture

A 24 hr old nutrient broth culture of *S. typhimurium* (Department of Bacteriology, University of Wisconsin) was used to contaminate cold-pack cheese food during its manufacture. Sufficient culture was added to provide approximately 200 salmonellae per gram of product although this value was not attained in all experiments. The culture of *S. typhimurium* was maintained by daily transfer in nutrient broth.

Manufacture of cheese food and sampling procedure

The formulae used to prepare cheese food are given in Table 1. Two batches of cheese food were prepared according to each formula and data reported in this paper are average values obtained from tests on both similar batches of product. Cheddar cheese (6 to 10 months old) was ground using a hand-operated meat grinder after which the following ingredients were added: butter (melted in sterilized warm water prior to use), nonfat dry milk, dried whey, preservative (potassium sorbate or sodium propionate blended with dry ingredients before adding to the remainder of the product), and acid (lactic or acetic acid diluted with sterile water) when the pH of the product was adjusted. All ingredients were thoroughly mixed by hand (with sanitized rubber gloves in place) and with the aid of a potato ricer. Finally *S. typhimurium* (diluted with sterile buffered water just prior to use) was added and the mixing process just described was repeated. Preliminary trials with *Serratia marcescens* demonstrated that the mixing process was adequate to insure uniform distribution of added bacteria in the cheese food. Cheese food was then filled into 2 oz plastic containers fitted with screw caps and was stored at 4.4 and 12.8 C. The product was tested initially for moisture and fat contents, pH, and number of viable salmonellae. Stored cheese food was examined at weekly intervals for its pH value and number of viable salmonellae.

Federal standards for cold-pack cheese food require that the product contains a minimum of 23% butterfat and a maximum of 44% moisture. Additionally, use of 0.20% sorbic acid or 0.30% sodium propionate is permitted. All cheese food prepared in these trials complied with the indicated standards.

Fat, moisture, and pH determinations

The fat content of cheese food was determined by the modified Babcock procedure as described by Van Slyke and Price *(17)*. Moisture in the product was determined by drying 3 g cheese food in a 50 ml beaker at 110 C for 16 hr in a forced draft oven. The pH value was measured with a saturated calomel half-cell, gold electrode, and a Leeds and Northrup portable potentiometer.

Enumeration of salmonellae

Salmonellae in cheese food were enumerated by means of the Most Probable Number (MPN) technique. Each value for a given batch of cheese represents the average of results obtained by testing three samples. Consequently, each value reported in the figures represents the average of 6 samples, three from each of two batches of cheese food. Twenty grams of cheese food was blended (4 min in a Waring blendor) with 180 ml of a sterile 2% sodium citrate solution previously cooled to 5 C. Subsequent dilutions were made with the sodium citrate solution.

After blending, 1 ml aliquots of appropriate dilutions were transferred to tubes each of which contained 9 ml of brain heart infusion broth (Difco). After overnight incubation at 37 C, 1 ml quantities were transferred to tubes containing 9 ml each of selenite-cystine broth (Difco). These cultures were incubated at 37 C for 24 hr and a sample from each tube was then streaked on SS agar (Difco) modified by adding 10 g sucrose (to screen for sucrose fermenting organisms) and 6.5 g agar (to facilitate streaking) per liter. When used as described, SS agar recovered essentially only salmonellae from cheese food. This, perhaps, is more readily understood when it is realized the 6-10 month old Cheddar cheese and the other ingredients used are not likely to contain many

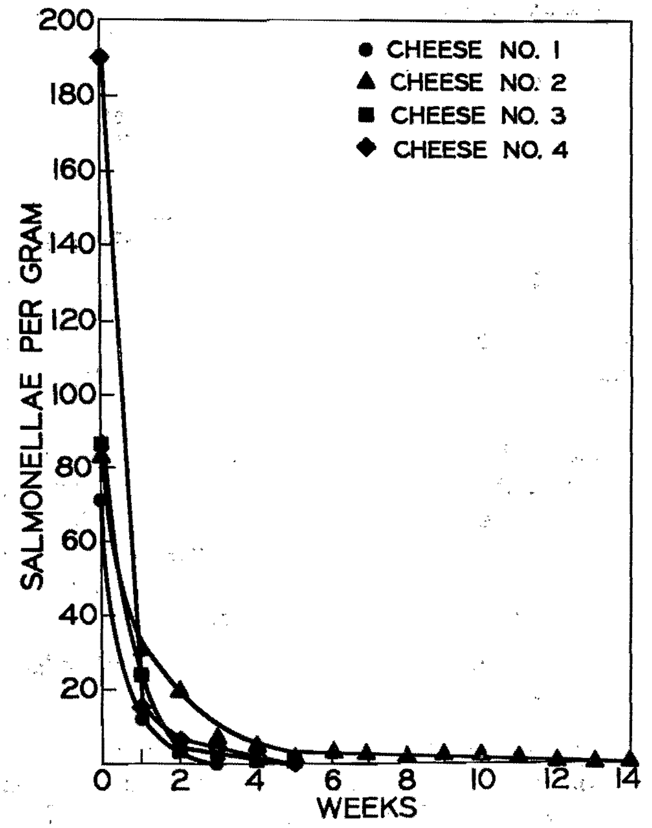

Figure 1. Survival of *Salmonella typhimurium* in cold-pack cheese food adjusted to pH 5.0, fortified with preservative, and stored at 12.8 C. Details on composition of each cheese food are in Table 1.

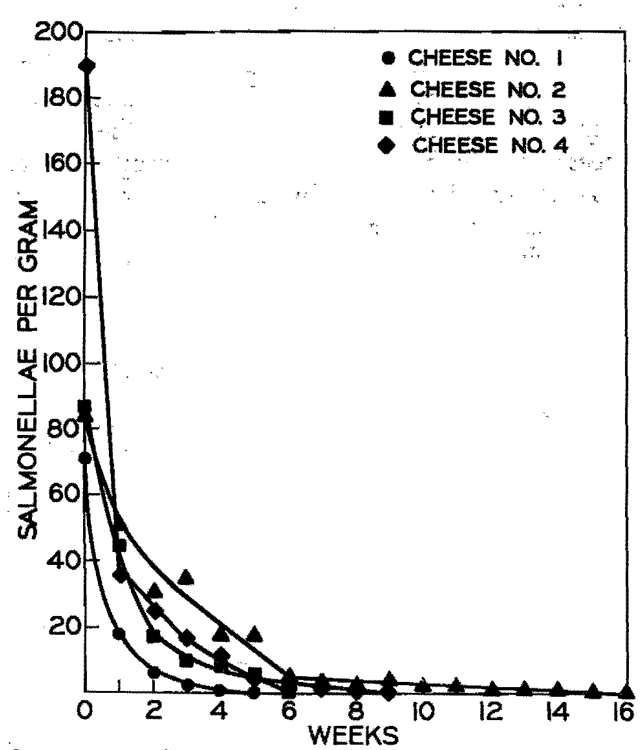

Figure 2. Survival of *Salmonella typhimurium* in cold-pack cheese food adjusted to pH 5.0, fortified with preservative, and stored at 4.4 C. Details on composition of each cheese food are in Table 1.

of the organisms which normally cause difficulty in recovery of salmonellae. Plates were examined after 24 hr at 37 C and typical *Salmonella* colonies were streaked onto and stabbed into triple sugar iron agar slants. These slants were incubated 24 hr at 37 C and checked to insure that the reaction was typical of that produced by salmonellae.

RESULTS AND DISCUSSION

Survival of salmonellae in cheese food with added acid and preservative

Figures 1 and 2 present data on the survival of salmonellae in cold-pack cheese food adjusted to pH 5.0 with lactic and/or acetic acid and fortified with either potassium sorbate or sodium propionate. Data on the pH values of these products during storage are recorded in Fig. 5 and 6.

It is evident that the number of viable salmonellae in all four types of cheese food declined most rapidly during the first week of storage at both temperatures (4.4 and 12.8 C). A less precipitous decline continued during subsequent weeks of storage. Salmonellae in cheese food containing both lactic acid and potassium sorbate declined to nondetectable levels in 3 and 5 weeks when the product was held at 12.8 and 4.4 C, respectively. In contrast, 14 and 16 weeks at the same temperatures were required before salmonellae could not be recovered from cheese food made with sodium propionate instead of potassium sorbate.

Although potassium sorbate is commonly thought to be more inhibitory to yeasts and molds than is sodium propionate *(10)*, Doell *(2)* reported that the chemical at a concentration of 0.1% and at pH values of 5.0 and 6.0 was bacteriostatic to some salmonellae. A bactericidal effect was not noted under the same conditions during a 48 hr incubation period. Doell did observe inactivation of salmonellae in 24 or 48 hr at pH 5.0 when the concentration of sorbate was increased to 1.0%. Extended storage in these experiments may have accomplished what the increase in concentration did in the tests by Doell.

Replacement of lactic acid, completely or in part, with acetic acid failed to enhance destruction of salmonellae in cheese food. In fact, the organisms persisted for 4 and 5 weeks at 12.8 C and for 6 and 9 weeks at 4.4 C. The longer periods of survival just indicated were noted when acetic acid only was used and the shorter times when a mixture of lactic and acetic acid was added. Even though acetic acid apparently had no beneficial effect over lactic acid, salmonellae in cheese foods containing acetic acid and sorbate (No. 3 and 4) remained viable for approximately one-third to one-half as long as those in cheese which contained lactic acid and sodium propionate.

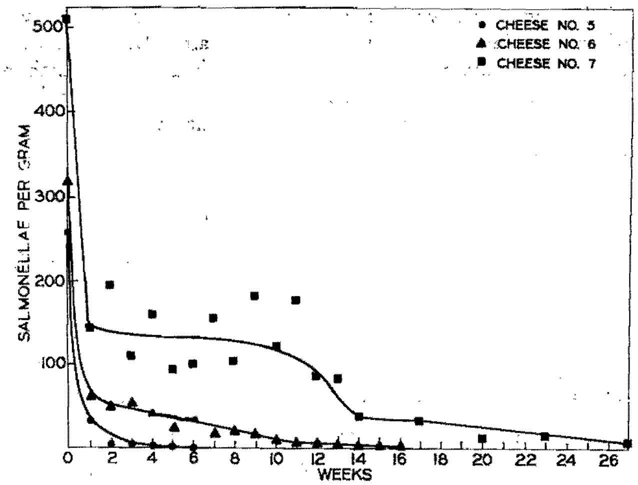

Figure 3. Survival of *Salmonella typhimurium* during storage at 12.8 C in cold-pack cheese food made without pH adjustment and with and without preservative. Details on composition of each cheese food are in Table 1.

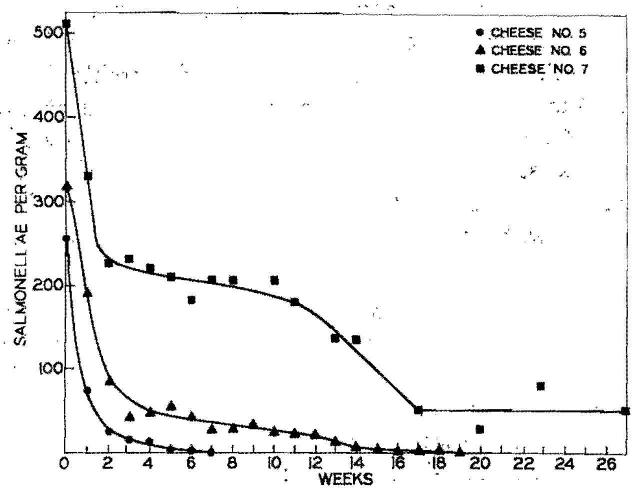

Figure. 4. Survival of *Salmonella typhimurium* during storage at 4.4 C in cold-pack cheese food made without pH adjustment and with and without preservative. Details on composition of each cheese food are in Table 1.

Several reports (*4, 9, 14, 18*) have indicated that organic acids are inhibitory to salmonellae. Propionic and acetic acids are usually thought to be more active than citric and lactic acids. Recently Subramanian and Marth (*14*) observed that citric acid retarded growth of *S. typhimurium* more than did lactic or hydrochloric acids. Wethington and Fabian (*18*) noted that *Salmonella schottmuelleri, S. typhimurium, Salmonella paratyphi, Salmonella enteritidis, Salmonella choleraesius,* and *Salmonella pullorum* survived for 144, 144, 156, 156, 156, and 132 hr, respectively, in mayonnaise made to contain 0.15% acetic acid (pH 5.0) and held at room temperature. Goepfert et al. (*5*) reported that the presence of 0.1% acetate in skimmilk at pH 4.9 enhanced inactivation of *S. typhimurium* and they suggested that accumulation of this acid during ripening of Cheddar cheese might contribute to the demise of salmonellae in cheese. In contrast to this, Hargrove et al. (*6*) claimed that acetic acid had no apparent effect greater than that of other acids on survival of salmonellae in Cheddar cheese made by direct acidification. Differences in the effect of acetic acid on salmonellae, including those reported in this paper, noted by various investigators are probably attributable to different environmental conditions which existed and which concurrent with acetic acid, affected the bacteria.

Data in Fig. 5 and 6 reveal that the pH of cheese food held at 12.8 C tended to decline during storage, whereas, at 4.4 C it remained rather constant. Undoubtedly this change in pH contributed to the more rapid disappearance of viable salmonellae from cheese food stored at the higher temperature.

Survival of salmonellae in cheese food with added preservative

Figures 3 and 4 provide data on the disappearance of viable salmonellae from cold-pack cheese food made without adjusting the pH but with added potassium sorbate (No. 5) or sodium propionate (No. 6). Detectable salmonellae persisted for 6 and 7 weeks at 12.8 and 4.4 C, respectively, when cheese food contained potassium sorbate and for 16 and 19 weeks at the same temperatures when sodium propionate was used. The pH values of cheese foods ranged between 5.20 and 5.26 when the products were prepared. During storage at 4.4 C, the pH of cheese food with potassium sorbate dropped by approximately 0.1 unit but failed to reach a pH value of 5.0 as existed in acidified products. The pH of the product with propionate declined gradually and

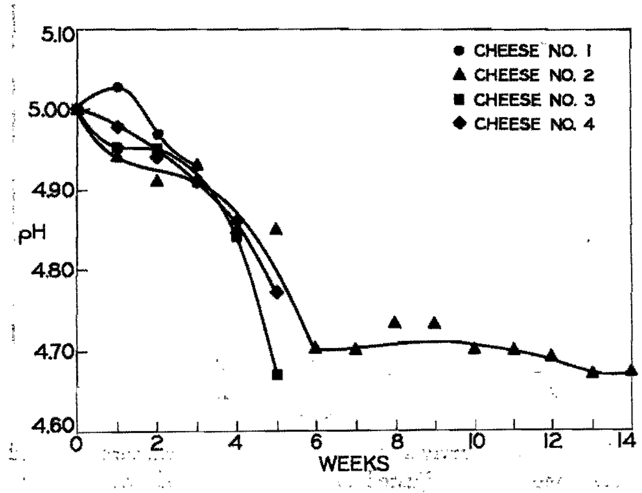

Figure 5. The pH values of acidified cold-pack cheese food containing preservatives and stored at 12.8 C.

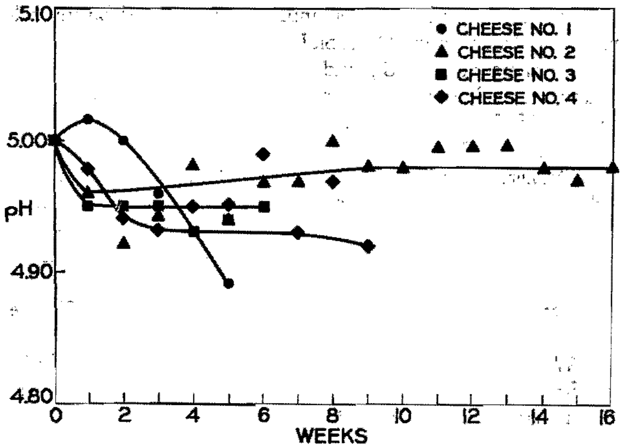

Figure 6. The pH values of acidified cold-pack cheese food containing preservatives and stored at 4.4 C.

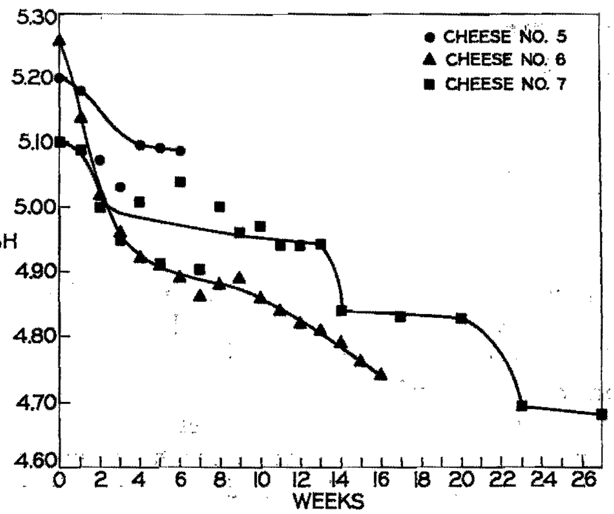

Figure 7. The pH values of cold-pack cheese food made without added acid, with or without preservatives, and stored at 12.8 C.

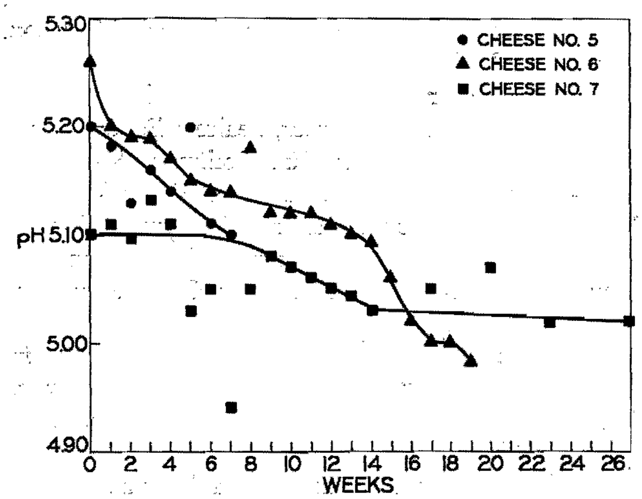

Figure 8. The pH values of cold-pack cheese food made without added acid, with or without preservative, and stored at 4.4 C.

a value of 5.0 was attained after 17 weeks of storage. Undoubtedly the higher pH of cheese food contributed to extended survival of salmonellae in the product held at 4.4 C. The decline in pH was greater in cheese food held at 12.8 C and this is probably related to more rapid d-mise of salmonellae from the product.

Survival of salmonellae in cheese food free of added acid or preservative

Data in Fig. 3 and 4 (No. 7) indicate that viable salmonellae remained in unfortified cheese food after 27 weeks of storage at either 12.8 or 4.4 C. A somewhat higher population appeared in cheese food stored at the lower temperature. The pH value of the product held at 4.4 C remained above 5.0 throughout storage, whereas cheese food at 12.8 C attained a pH value of 5.0 after two weeks of holding and then continued to decline below 4.7 after 23 weeks. In spite of this drop in pH, viable salmonellae persisted for at least 27 weeks. This suggests that in a product as complex as cheese food, pH (within the range associated with the product) is only one factor which governs survival of salmonellae.

Observations made in these experiments emphasize that cold-pack cheese food must be prepared from high quality ingredients which are free of hazardous microorganisms and the product must be handled to preclude contamination by such microorganisms during its manufacture. If present, salmonellae could survive in the product until it reaches the consumer, particularly if the pH of cheese food is not adjusted and if potassium sorbate is not used.

ACKNOWLEDGMENT

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KENTUCKY HAULERS TOLD THEY ARE KEY TO MILK QUALITY

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"Picking up milk from farms which do not have valid permits is a serious violation which occasionally plagues the bulk milk hauling industry," commented Gayle Shrader, Chief of Milk Control, Louisville and Jefferson County Department of Public Health, Louisville, Kentucky. Speaking to 150 haulers at meetings in Elizabethtown, Kentucky, Shelbyville, Kentucky and Palmyra, Indiana, Shrader urged them to eliminate partial pickups, reject milk with known added water, to rinse producers' tanks after pickups, and to take samples in the proper manner.

"Haulers are an important cog in keeping the milk industry alive," said Dudley Conner, Supervisor of the Milk Control Program, Kentucky State Health Department. Conner challenged the haulers to reject milk that does not meet state health department regulations. "An Interstate Milk Shippers rating below 90 prevents milk from being moved into another state," he reminded the hauler delegates.

A. P. Bell, Director of the Division of Environmental Services for the Louisville-Jefferson County Health Department, emphasized that there is no way to improve milk that has undergone bacterial and chemical degradation.

"You aren't doing the producer a favor by taking a partial load of milk," said featured speaker Joe Johnson, of Arlington, Texas. "After all, the producer is paying the hauling bills and deserves a fair shake," continued the AMPI representative. Johnson urged the Kentucky milk transporters to agitate the milk properly before taking samples and to carry a standard thermometer to check the bulk tank temperatures. "Too often," he said, "the tank thermometer is not functioning properly."

"Quality is our business because quality keeps us in business," stated Kentucky hauler Joe Monin of Monin Brothers Transfer. Other haulers who participated on the training programs were Cleo Mull, Mull's Milk Transfer, and Mr. Robert Hamilton, Hamilton's Milk Service.

Plant processor views on the role of the hauler were given by Mr. Ed Napier, Sealtest Foods, Louisville, Kentucky, and Mr. Jim Spillman, Dairymen, Inc. – Kyana Division, Louisville, Kentucky. Jim McDowell, field supervisor for Dairymen, Inc. – Kyana Division, spoke on hauler-fieldmen teamwork.

In summarizing the meetings, Dr. C. Bronson Lane of the University of Kentucky Animal Sciences Department said that an effective hauler is totally involved, projects a good image, and is completely informed about his role as a team leader in obtaining quality milk.

The Louisville area bulk milk hauler training programs were sponsored by the state and local health departments of Kentucky, the U. K. Cooperative Extension Service, and the dairy industry of Louisville.

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