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# Journal of Milk and Food Technology

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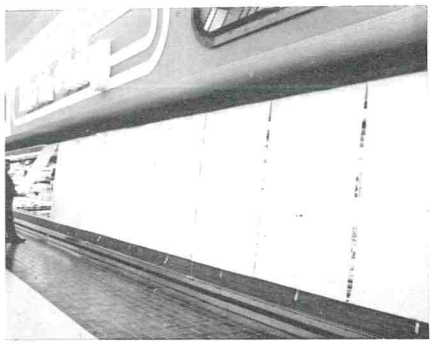

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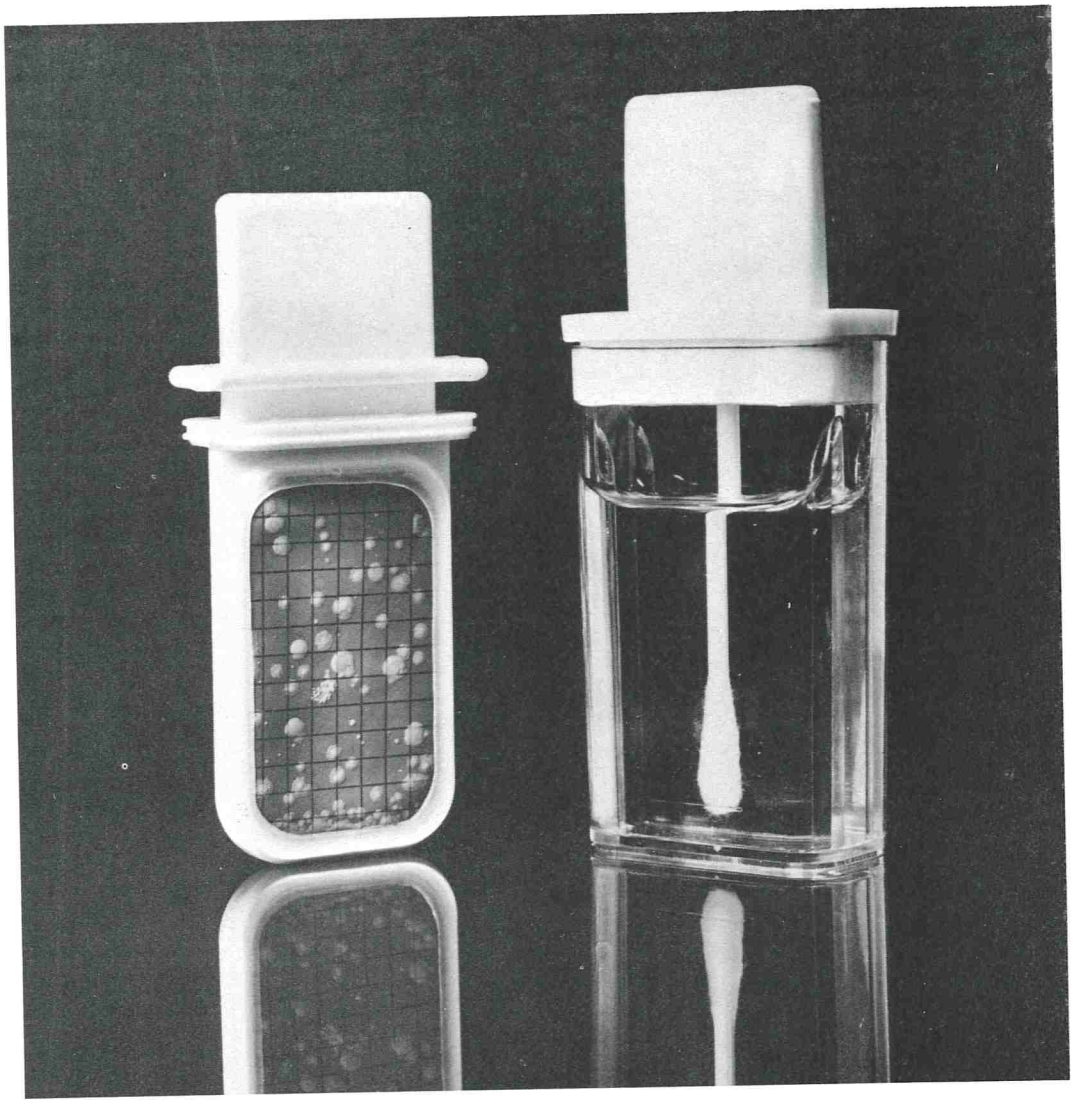

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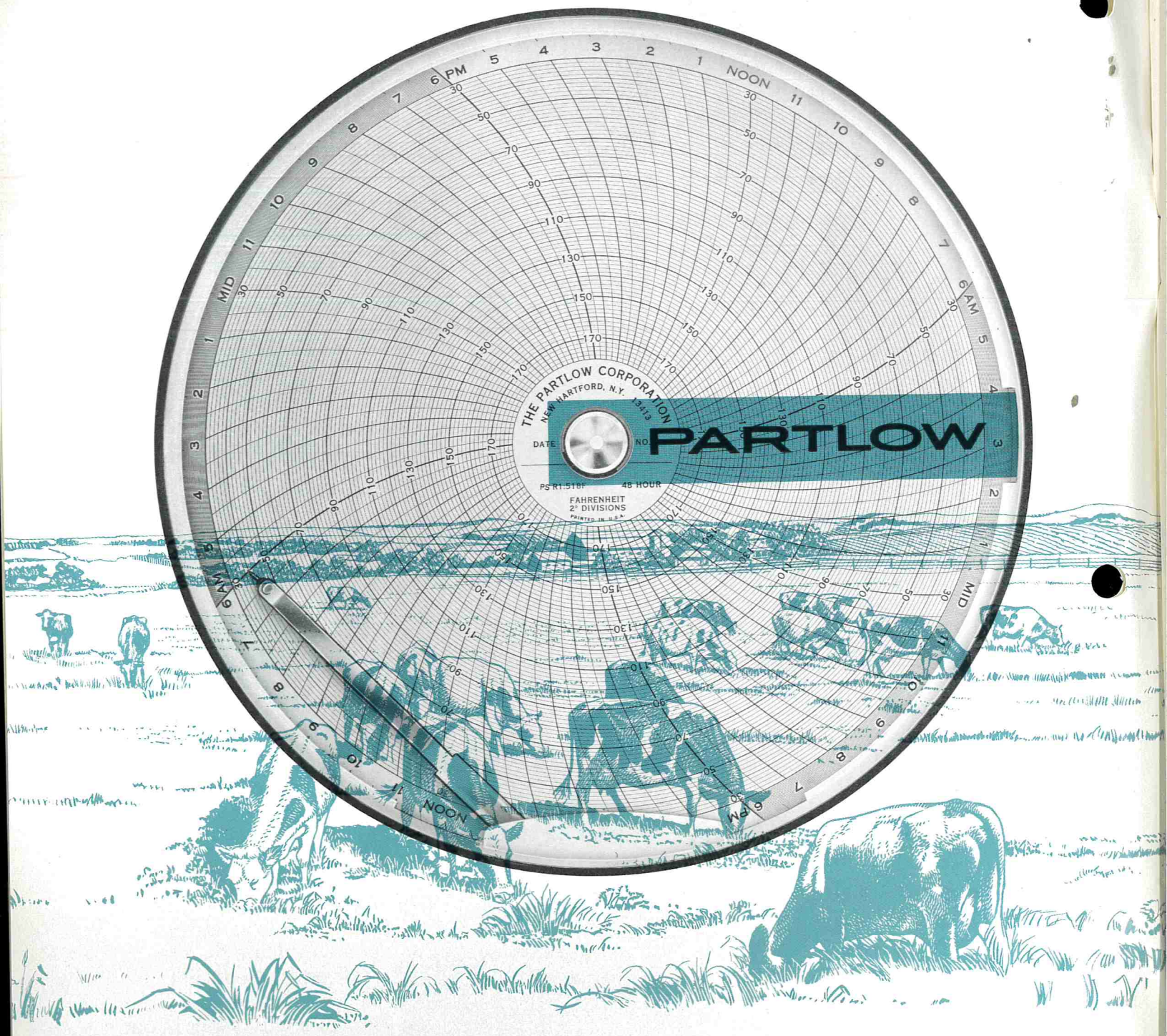


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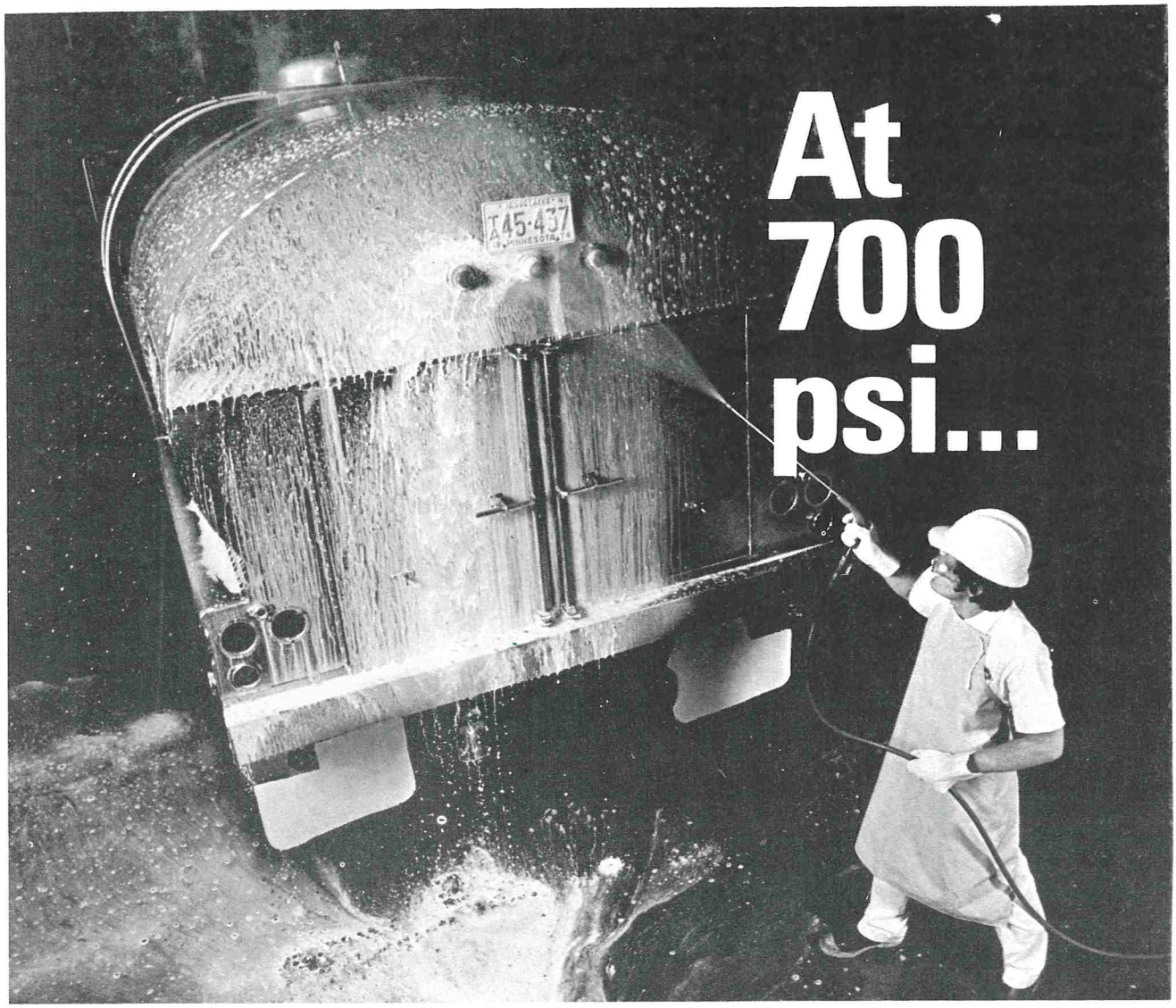


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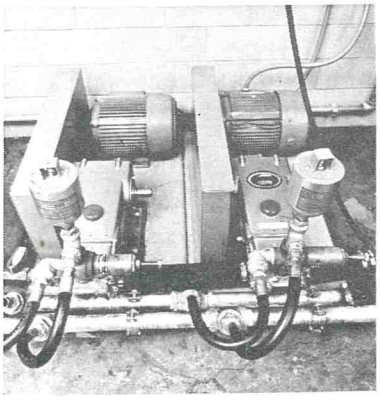

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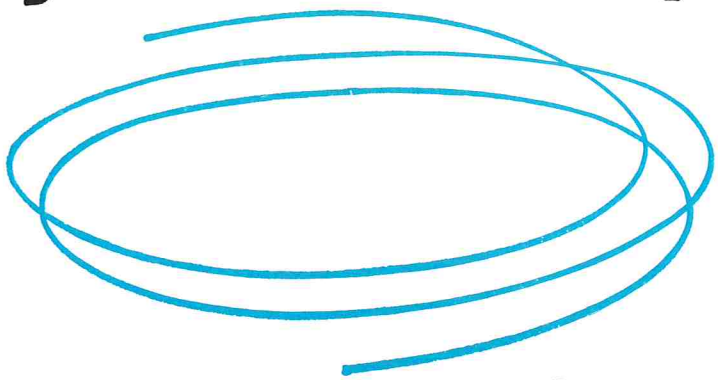

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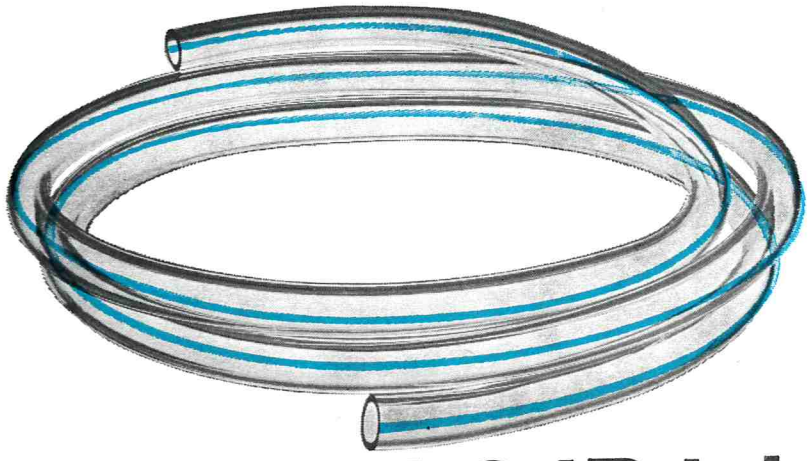

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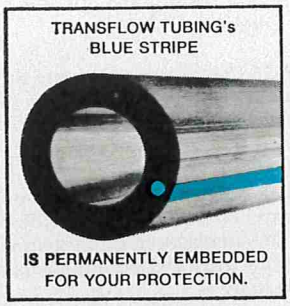


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**Editorial Offices**: Dr. Elmer H. Marth, Dept. of Food Science, University of Wisconsin, Madison, Wis. 52706. Earl O. Wright, P.O. Box 701, Ames, Ia. 50010.

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*J. Milk Food Technol. Vol. 38, No. 10, Pages 570-572 (October, 1975)*


# Use of Selective Media to Detect Enzyme Production by Microorganisms in Food Products

**SANDRA L. ANAGNOSTAKIS and LESTER HANKIN**

*Department of Genetics and Department of Biochemistry*
*The Connecticut Agricultural Experiment Station,*
*Box 1106, New Haven, Connecticut 06504*

(Received for publication February 24, 1975)

**ABSTRACT**

Media are described for use in food analysis to detect fungi and bacteria able to excrete extracellular lipases, proteases, pectinases, amylases, and acids. Antibiotics were used to suppress bacterial growth in media that detect fungi. Food products were examined to show the ability of these media to detect contaminating microorganisms able to excrete these enzymes. The possibility that keeping quality of foods could be correlated with numbers of organisms able to excrete these degradative enzymes is discussed. The test media also could be used to obtain total counts of fungal and bacterial populations.

Conventional methods for enumerating bacteria in foods involve plating dilutions of the sample on solid media usually about pH 7. For a yeast and mold count the same procedure is generally followed except that the medium is adjusted to a low pH to inhibit bacteria. An example of such a medium is potato dextrose agar acidified to pH 3.5. Several workers have proposed that the pH of media for enumeration of fungi be adjusted to 7 and antibiotics added to suppress bacterial growth (*4, 5, 7*). Such media have provided good results, and higher total counts of fungi have been obtained on these media than on acidified potato dextrose agar.

Since deterioration and keeping quality of food are related to the production of extracellular degradative enzymes by contaminating microorganisms, enumeration media which also would provide data on bacteria and fungi able to excrete degradative enzymes as well as provide a total count would be most useful. It is possible, for example, that a food sample could yield a low total microbial count and yet most of the organisms could excrete an enzyme which could degrade the food and thus lower its keeping quality.

In this paper we describe media to detect yeasts, molds and bacteria in commercially available foods. These media will differentiate organisms able to produce extracellular proteases, lipases, pectinases, amylases, and acids.

## METHODS

Food samples, purchased locally, included cottage cheese, ricotta cheese, Cheddar cheese, dehydrated potato flakes, bread without preservatives, fresh and processed cherries, walnut meats, refrigerated pastry dough, pork muscle and both ground beef and pork. Samples were chosen to represent a variety of both food substrates and manufacturing procedures.

A 45 g sample was blended for 15 s in a high speed blender with 450 ml of sterile phosphate solution, pH 7.2 (*11*). Dilutions were made in sterile 0.85% saline and 0.1 ml of the diluted sample spread on the surface of previously poured plates of each test medium.

The medium to detect protease production by fungi contained nutrient agar (Difco) to which gelatin was added as the protein source (0.4% final concn) (*10*). After incubation of inoculated plates and growth of the organisms, a saturated solution of ammonium sulfate was poured over the agar surface to enhance visibility of the zones of proteolysis (*10*). For bacterial protease production the medium described by Martley et al. (*8*) was used, except that plate count agar (Difco) was substituted for standard methods agar. In this test, protease production is seen as a precipitate of para-casein around the colonies. The medium described by Sierra (*12*) with sorbitan monolaurate (Tween 20, Fisher Scientific Co., Fairlawn, N.J.) as the lipid source was used to detect lipase production for both bacteria and fungi. A precipitate of the calcium salt of the liberated fatty acid or a clear zone around colonies indicates lipase activity. To determine pectinase production the medium described by Hankin et al. (*3*) was used. This medium (which contains mineral salts, yeast extract and pectin), when adjusted to pH 5, demonstrates polygalacturonase production and, at pH 7, pectate transeliminase production. Flooding plates after growth with a 1% aqueous solution of hexadecyltrimethylammonium bromide precipitates intact pectin, and a clear halo is seen around colonies that have produced a pectic enzyme (*3*). Excretion of amylase was detected on a medium composed of nutrient agar (Difco) to which soluble starch was added (0.2% final concn). After growth, an iodine solution (*10*) was poured over the agar surface to indicate degradation of the starch. For the detection of acid production the tomato juice medium of Wade et al. (*13*) was employed. This medium contains bromcresol purple to detect pH changes.

For bacterial growth all media were adjusted to pH 7. All test media for fungi were adjusted to pH 6 except those used to detect polygalacturonase and pectate transeliminase production, and potato dextrose agar. These were adjusted to pH 5, 7 and 3.5 respectively. It has been shown (*1*) that the media used to detect fungi can be adjusted to pH 6 without seriously interfering with enzyme production or activity, or growth.

All media for detection of fungi, except potato dextrose agar, contained both chloramphenicol (Chloromycetin, Sigma Chemical Co., St. Louis, Mo.) and neomycin sulfate (Sigma) to suppress bacterial growth. Potato dextrose agar was adjusted to pH 3.5. The aqueous chloramphenicol solution (2 mg/ml) was sterilized by autoclaving and the aqueous neomycin sulfate solution (1 mg/ml) was filter sterilized. They were added to the molten medium (48 C) just prior to pouring plates to provide a final concentration in the medium of 100 and 50 μg/ml, respectively.

We have previously shown that the antibiotics used in the test media for fungi did not inhibit growth, enzyme production by the fungi, or enzyme activity in the media (*1*).

Test plates were incubated at 22 and 30 C. Fungi were counted separately as yeasts or molds. Fungal counts and determination of enzyme production were made after 4-5 days incubation. Bacterial counts and determination of enzyme production were made after 2-3 days incubation with all media except that used for lipase production which was examined after 5 days incubation.

TABLE 1. *Analysis of foods for total numbers of bacteria and fungi on selected media and detection of organisms able to produce extracellular degradative enzymes*

| Sample number and product | Organisms | Total number per gram | | | Enzyme detected on specialized media[2] |
|---|---|---|---|---|---|
| | | Potato dextrose agar pH 3.5 | Plate count agar pH 7 | Protease detection medium | |
| 1 Cottage cheese | Molds | 1,000,000 | 940,000 | 990,000 | |
| | Yeasts | 1,200,000 | 710,000 | 7,900,000 | |
| | Bacteria | X[1] | 12,000,000 | 11,000,000 | Pr LP PT AM AC[3] |
| 2 Ricotta cheese | Molds | <1,000 | <1,000 | <1,000 | |
| | Yeasts | 61,000 | 22,000 | 238,000 | Pr |
| | Bacteria | X | 340,000,000 | >300,000,000 | Pr LP |
| 3 Cheddar cheese | Molds | 150,000 | 30,000 | >200,000 | Pr PG PT AM AC |
| | Yeasts | 16,500,000 | 24,400,000 | >200,000 | Pr |
| | Bacteria | X | 35,000,000 | 29,000,000 | Pr LP |
| 4 Dehydrated potatoes | Molds | <100 | <100 | <100 | |
| | Yeasts | 100 | <100 | 200 | LP |
| | Bacteria | X | 5,000 | 5,000 | Pr LP PT AM AC |
| 5 Bread (without Preservatives) | Molds | <100 | <100 | <100 | |
| | Yeasts | 100 | 100 | 200 | |
| | Bacteria | X | 300 | 300 | Pr LP PT AM AC |
| 6 Ground pork | Molds | 1,000 | 2,000 | 170,000 | Pr AM |
| | Yeasts | 153,000 | 160,000 | 240,000 | Pr LP PG AC |
| | Bacteria | X | 220,000,000 | 230,000,000 | Pr LP AM AC |
| 7 Fresh cherries | Molds | <100 | <100 | 5,000 | LP PG AM |
| | Yeasts | 7,600 | 5,400 | 5,400 | Pr LP PT AM |
| | Bacteria | X | 6,000 | 5,000 | LP |
| 8 Walnut meats | Molds | 69,500 | 51,000 | 24,000 | LP PG PT AM AC |
| | Yeasts | <1,000 | <1,000 | 8,000 | LP PG |
| | Bacteria | X | 3,000 | 3,000 | Pr LP PT AM AC |
| 9 Dried coconut | Molds | <100 | <100 | <100 | |
| | Yeasts | <100 | <100 | <100 | |
| | Bacteria | X | 300 | 300 | Pr LP PG AM AC |
| 10 Pastry dough (refrigerated) | Molds | 100 | 100 | 100 | |
| | Yeasts | 100 | 100 | 100 | |
| | Bacteria | X | 150,000 | 160,000 | Pr LP PT AM AC |
| 11 Processed cherries (in plastic bag—refrigerated) | Molds | 100 | <100 | <100 | |
| | Yeasts | <100 | <100 | <100 | |
| | Bacteria | X | <100 | X | |
| 12 Pork muscle | Molds | <1,000 | <1,000 | <1,000 | |
| | Yeasts | 4,000 | 1,000 | 7,000 | |
| | Bacteria | X | 2,500,000 | 3,200,000 | Pr LP PT AC |
| 13 Ground beef | Molds | <1,000 | 1,000 | 10,000 | PG |
| | Yeasts | 4,000 | 10,000 | 40,000 | Pr AC |
| | Bacteria | X | 12,800,000 | 25,000,000 | Pr LP PT AM AC |

[1]X indicates test not made.
[2]Bacteria not examined for polygalacturonase production.
[3]Pr = proteases, LP = lipases, PG =polygalacturonase, PT = pectate transeliminase, AM = amylases AC = acid.

## RESULTS AND DISCUSSION

The ability of specialized media to detect bacteria and fungi that are contaminants in foods and able to excrete specific degradative enzymes is shown in Table 1. The X's in Table 1 indicate tests that were not made. For example, though many fungi produce polygalacturonase, production by bacteria is found in only a few limited cases (*9*). Thus, such a test for bacteria would not be useful in food analysis. Also, the low pH of the potato dextrose agar generally precludes its use for enumeration of bacteria.

An example serves to illustrate our findings on the use of the media to detect enzyme excretion. Although many molds and yeasts were present in the cottage cheese (sample 1, Table 1), none were detected which were able to produce the degradative enzymes sought and none produced acid under our test conditions. Conversely, bacteria in this sample of cottage cheese were able to excrete all of the enzymes tested for.

Consumers relate keeping quality to decline in flavor, odor, and palatability. Off flavors and odors can result from breakdown of food components by degradative enzymes produced by contaminating microorganisms. The screening for a variety of extracellular degradative enzymes excreted by microorganisms is therefore useful. Thus, in a cheese sample, it would be important to know if proteolytic and lipolytic organisms are present, for such organisms could bring about a diminution in keeping quality.

Microbiol counts obtained from plates incubated at 22 C are shown in Table 1, but duplicate plates were incubated at 30 C. In most cases yeasts grew better at 22 C than at 30 C on all of the test media. Essentially the same was noted for molds. Numbers of bacteria found and relative enzyme excretion were similar at both

temperatures. Thus, in routine testing of food on these test media; 22 C is probably the preferable incubation temperature.

The antibiotics used in the fungal test media (chloramphenicol and neomycin sulfate) suppressed bacterial growth satisfactorily except with meat samples. That is, bacteria grew (especially on the media at pH 7 used to detect pectinases and proteases) but growth was suppressed for about 24 h. This suggests several possibilities: (*a*) the fungi from the meat samples were able to degrade the antibiotics and thus allow bacteria to grow, (*b*) enzymes present in the meat samples degraded the antibiotics, or (*c*) bacteria in the meat were resistant to the antibiotics used. These aspects are under investigation.

Our data indicate that most of the specialized media also could be used to obtain a total fungal count. Fungal growth on media used to detect enzyme production was compared with the growth on acidified potato dextrose agar and with plate count agar to which antibiotics were added. For comparison, only the data obtained with the protease detection media is shown in Table 1. Both potato dextrose agar and plate count agar with antibiotics gave fungal counts similar to those reported by others (*4, 5*). Media designed to detect fungal polygalacturonase activity gave the lowest total fungal counts. The medium used to detect acid production also provided somewhat lower counts than other media.

Bacterial total counts on protease detection media (as an example of enzyme detection media) were compared to those obtained on plate counts agar (Table 1). The total counts were similar on the two media and our experience has shown that the other media used to detect enzyme production would give like results. Therefore, we believe that media designed to detect enzyme production by bacteria and fungi found in foods (with exceptions noted above) would give reliable total counts as well as providing information on the degradative abilities of the microorganisms.

This paper describes selective media which could be used to evaluate foods for keeping quality based on degradative enzyme production by contaminating microorganisms. What must be done for any single food category is to correlate keeping quality with the numbers or percentages of specific organisms (bacteria, molds, yeasts) in the food able to excrete specific degradative enzymes.

In an assessment of keeping quality of foods the relationship between a total bacterial or fungal count and keeping quality is not clear. For example, it has been shown for milk that only 0.2% of the observed variability in keeping quality can be ascribed to the total bacterial count (*2*). We believe that the number or percentage of the microorganisms present in foods which are able to produce specific extracellular degradative enzymes might correlate much better with observed keeping quality.

ACKNOWLEDGMENT

We thank Margaret Staba for excellent technical assistance.

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*J. Milk Food Technol. Vol. 38, No. 10, Pages 573-580 (October, 1975)*


# The Effect of Concentration, Thermal Processing, and Storage Temperature on the Interaction Between Alpha-Ketoglutaric Acid and Tryptophan

**N. T. CHU and F. M. CLYDESDALE**

*Department of Food Science and Nutrition*
*University of Massachusetts, Amherst, Massachusetts 01002*

(Received for publication December 2, 1974)

**ABSTRACT**

Formation of colored compounds caused by the interaction of *a*-ketoglutaric acid and tryptophan was followed spectrophotmetrically at 470 and 620 nm. It was found that the rate of formation of these colored compounds was effected by the concentration of *a*-ketoglutaric acid and/or tryptophan. High concentrations of both *a*-ketoglutaric acid and tryptophan caused the greatest concentration of such colored compounds, but singly, *a*-ketoglutaric acid concentration was more effective than that of tryptophan. The amount of colored compounds formed after storage at 75 F was not altered by processing, since both processed and unprocessed samples showed the same concentration after storage. The interaction mixtures stored at 38 F proceeded at very slow rates compared with those stored at 75 F. The results also indicated that individual effects caused by processing were overcome by storage at room temperature (75 F) since all samples were similar after storage.

Formation of colored compounds by the interaction of tryptophan and *a*-ketoglutaric acid has not been noted previously in the literature. Such a reaction may be of important in food processing and therefore this study was initiated to define the effects of concentration, processing, and storage temperature.

Lin et al. (*6*) noted the presence of *a*-ketoglutaric acid in processed spinach puree and its disappearance during storage. As well, tryptophan occurs in many foods, such as beans, peas, and cereals (*2*). It is possible that formation of *a*-ketoglutaric could occur during processing and then disappear during storage in part due to its interaction with tryptophan. this would create not only color problems but also might decrease the amount of tryptophan available for nutritional needs.

Colorimetric determination of tryptophan by reaction with vanillin-HC1 was reported by Krans (*5*). Accurate and reliable methods for the estimation of tryptophan based on the glyoxylic acid reaction and spectrophotometric measurement at 520 and 540 nm have been suggested by Shaw and McFarlane (*8*). The color-forming reaction between tryptophan, p-dimethylamino benzaldehyde, and sodium nitrate was carried out satisfactorily in sulfuric acid solution and measured at wavelengths from 590 to 600 nm by Spies and Chambers (*12, 13*). The application of this method for determination of tryptophan from proteins also was described by Spies (*10, 11*). A simple, rapid spectrophotometric method for quantitative estimation of tryptophan from the interaction between tryptophan and copper was described and measured at 620 nm by Spies (*9*). A simple colorimetric method for quantitative estimation of tryptophan was described by Fischl (*3*). This method was based on the chromogen production of tryptophan when treated with acetic acid in an anhydrous medium. Oxidation was done by the use of persulfate or peroxide. Anhydrous conditions were obtained by addition of sulfuric acid. The rate of oxidation is controlled with thioglycollic acid. Absorption between 530 and 550 nm was measured. Formation of a colored compound resulting from a reaction between tryptophan, acetic acid containing iron ions, and sulfuric acid was measured at 545 nm by Opienska-Blauth et al. (*7*). The determination of tryptophan in proteins by means of its reaction with 2- or 4-nitrophenylsulfenyl chloride in aqueous acetic or formic acid was reported by Boccu et al. (*1*). In this method the nitrophenyl chromophore is covalentyly bound to the tryptophan and determined at 365 and 328 nm.

In this investigation formation of color due to the interaction between tryptophan and *a*-ketoglutaric acid was measured at 470 and 620 nm. Different concentrations of both *a*-ketoglutaric acid and tryptophan, different storage temperatures, and different processing conditions were involved in this study. The purpose of this work was not the development of a quantitative analysis for tryptophan but the investigation of production parameters and possible implications in food processing.

## MATERIALS AND METHODS

Interaction solutions were measured by an Hitachi-Perkin-Elmer 139 UV-Vis Spectrophotometer using 1-cm cuvettes. The rate of color change in the solution with different concentrations of *a*-ketoglutaric acid (Sigma Chemical Co.) and tryptophan (CalBiochem) during processing and storage was measured at wavelengths of 470 and 620 nm, respectively, where maxima occur. The decrease in transmittance after processing and storage is related to the increase in the formation of colored compounds. For the processed samples the interaction solutions were processed in TDT tubes at temperatures of 240, 270, and

300 F with an $F_0 = 4.9$ as described by Gupte and Francis (*4*). Both processed solutions and fresh solutions were stored at 75 F and 38 F and measured at intervals during storage.

The interaction solutions studied were prepared as follows:

(*a*) *a*-Ketoglutaric acid and tryptophan (67 μ mole/ml + 25 μ mole/ml) was processed at 240 F with and $F_0 = 4.9$ and measured at intervals during storage at 75 F.

(*b*) *a*-Ketoglutaric acid and tryptophan (33.5 μ mole/ml + 12.5 μ mole/ml) and *a*-Ketoglutaric acid + tryptophan (67 μ mole/ml + 25 μ mole/ml) were measured during storage at temperatures of 75 F and 38 F, respectively.

(*c*) *a*-ketoglutaric acid and tryptophan combinations of 33.5 μ mole/ml + 12.5 μ mole/ml, 33.5 μ mole/ml + 25 μ mole/ml, 67 μ mole/ml + 12.5 μ mole/ml and 67 μ mole/ml + 25 μ mole/ml were measured at intervals during storage at 75 F and 38 F, respectively.

(*d*) *a*-Ketoglutaric acid + tryptophan combinations of 16.7 μ mole/ml + 12.5 μ mole/ml, 33.5 μ mole/ml + 12.5 μ mole/ml, 50.2 μ mole/ml + 12.5 μ mole/ml were measured at intervals during storage at 75 F and 38 F, respectively.

(*e*) *a*-Ketoglutaric acid + tryptophan (33.5 μ mole/ml + 12.5 μ mole/ml) and *a*-Ketoglutaric acid + tryptophan (67 μ mole/ml + 25 μ mole/ml) were processed at temperatures of 240, 270, and 300 F, respectively, with $F_0 = 4.9$ and measured at intervals during storage at 75 F.

(*f*) *a*-Ketoglutaric acid + tryptophan combinations of 33.5 μ mole/ml + 12.5 μ mole/ml, 33.5 μ mole/ml + 25 μ mole/ml, 67 μ mole/ml + 12.5 μ mole/ml and 67 μ mole/ml + 25 μ mole/ml were processed at a temperature of 240 F with $F_0 = 4.9$ and measured at intervals during storage at 75 F.

(*g*) *a*-Ketoglutaric acid + tryptophan combination of 1.7 μ mole/ml + 12.5 μ mole/ml, 33.5 μ mole/ml + 12.5 μ mole/ml and 50.2 μ mole/ml + 12.5 μ mole/ml were processed at temperatures of 240, 270, and 300 F, respectively, with $F_0 = 4.9$ and measured at intervals during storage at 75 F.

(*h*) *a*-Ketoglutaric acid + tryptophan (50 μ mole/ml + 25 μ mole/ml) were processed at a temperature of 240 F with $F_0 = 4.9$, 9.8, and 14.7, respectively, and measured at intervals during storage at 75 $F_0$.

## RESULTS AND DISCUSSION

### *Measurement during storage*

Transmittance at 470 and 620 nm were used as an index for measurement of color formation. Decrease in transmittances at both wave-lengths indicated that colored compounds were formed. The rate of color formation during storage depended upon the concentration of *a*-ketoglutaric acid and tryptophan. To obtain a better representation of results, data were plotted on semilog paper. Figures 1a, 1b, 1c, 1d, 1e, 1f, and 1g show the curves representing the formation of colored compounds under various conditions.

Figure 1a shows the decrease in transmittances at 470 and 620 nm due to the interaction between *a*-ketoglutaric acid and tryptophan during storage at 75 F after processing at 240 F for 19.5 min. transmittance decreased slowly in the first few days from the beginning of storage, then the transmittance curves decreased rapidly in a straight line manner.

Figures 1b and 1c show the development of brown pigments due to the interaction between *a*-ketoglutaric acid and tryptophan at different concentrations during storage. The decrease in transmittance was more rapid with high concentrations of *a*-ketoglutaric acid and tryptophan in solution than in low concentration both at 75 F and at 38 F. However, the decrease in transmittance

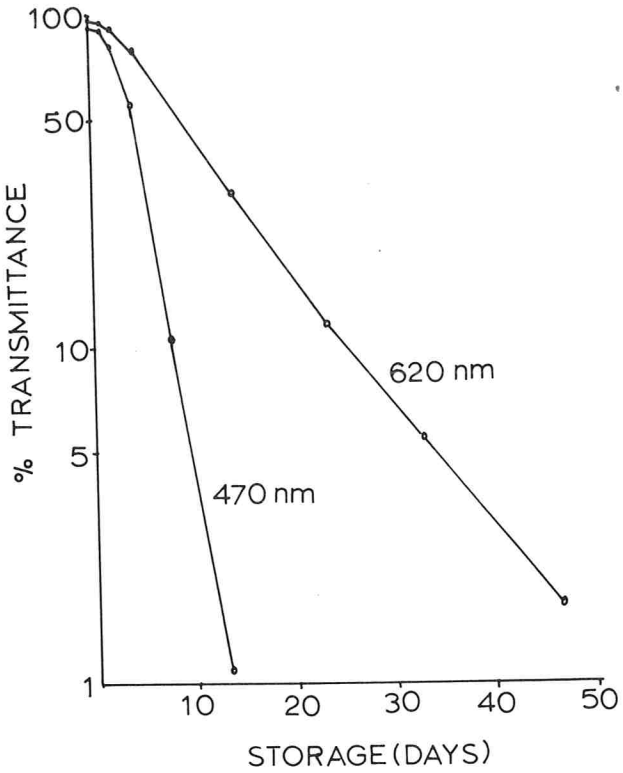


Figure 1a. *Semilogarithmic plots of transmittance at 470 nm and 620 nm versus storage for the interaction between a-ketoglutaric acid (34 μ mole/ml) and tryptophan (25 μ mole/ml) during storage at 75 F after processing at 240 F for 19.5 min.*

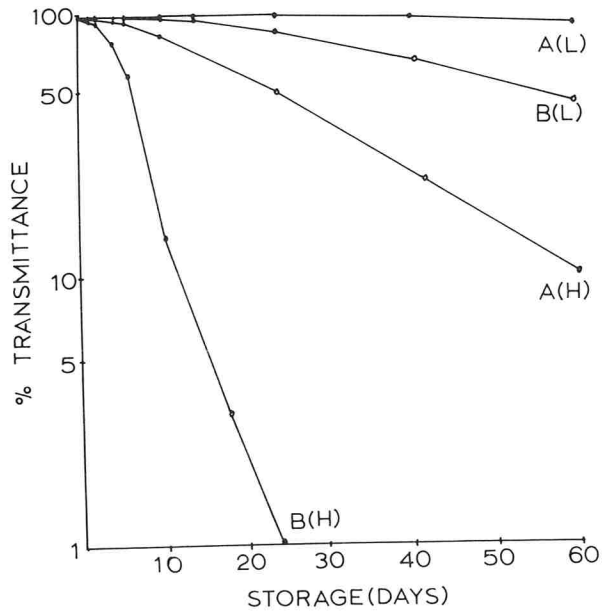


Figure 1b. *Semilogarithmic plots of the transmittance at 470 nm versus storage for the interaction between a-ketoglutaric acid and tryptophan with different concentrations during storage at 75 F and 38 F. A(h) and A(L): β-keto. + try. (47 + 12.5 μ mole/ml) at 75 F and 38 F. B(H) and B(L): a-keto. + try. (34 + 25 μ mole/ml) at 75 F and 38 F)*

for both concentrations stored at 75 F was faster than that at 38 F. This indicated that formation of colored compounds is accelerated at higher temperatures and concentrations.

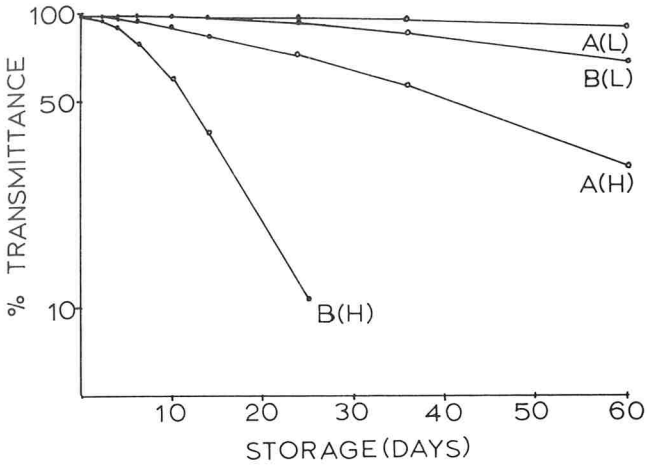


Figure 1c. *Semilogarithmic plots of the transmittance at 620 nm versus storage for the interaction between a-ketoglutaric acid and tryptophan with different concentrations during storage at 75 F and 38 F. A(H) & A(L): a-keto. + try. (17 + 12.5 μ mole/ml) at 75 F and 38 F. B(H) & B(L): a-keto. + try. (34 + 25 μ mole/ml) at 75 F and 38 F.*

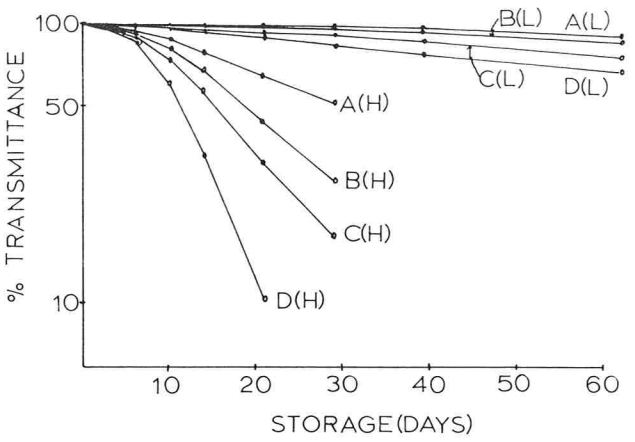


Figure 1e. *Semilogarithmic plots of the transmittance at 620 nm versus versus storage for the interactions between a-ketoglutaric acid and tryptophan with different concentrations. A(H) & A(L): (17 a-keto. + 12.5 try.) μ mole/ml at 75 F and 38 F. B(H) & B(L): (17 a-keto., + 25 try.) μ mole/ml at 75 F and 38 F. C(H) & C(L): (34 a-keto. + 12.5 try.) μ mole/ml at 75 F and 38 F. D(H) & D(L): (34 a-keto. + 25 try.) μ mole/ml at 75 F and 38 F.*

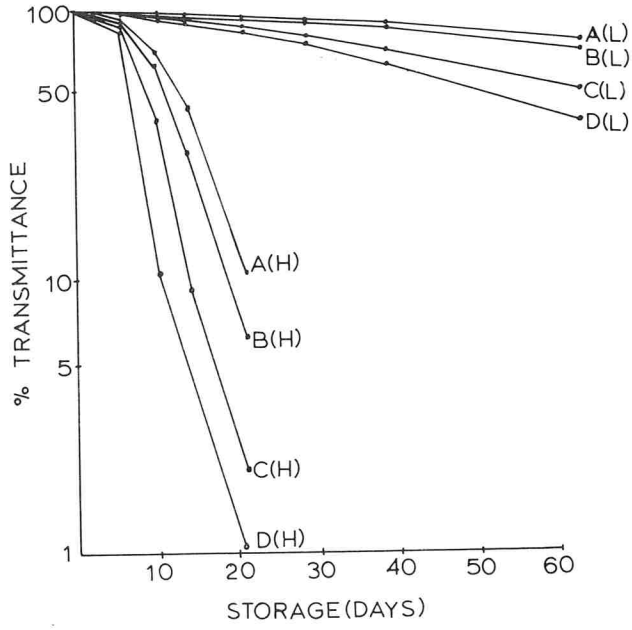


Figure 1d. *Semilogarithmic plots of the transmittance at 470 nm versus storage for the interactions between a-ketoglutaric acid and tryptophan with different concentrations during storage at 75 F and 38 F, respectively. A(H) & A(L): a-keto. + try. (17 + 12.5 μ mole/ml) at 75 F and 38 F. B(H) & B(L): a-keto. + try. (17 + 25 μ mole/ml) at 75 F and 38 F. C(H) & C(L): a-keto. + try. (34 + 12.5 μ mole/ml) at 75 F and 38 F. D(H) & D(L): a-keto. + try. (34 + 25 μ mole/ml) at 75 F and 38 F.*

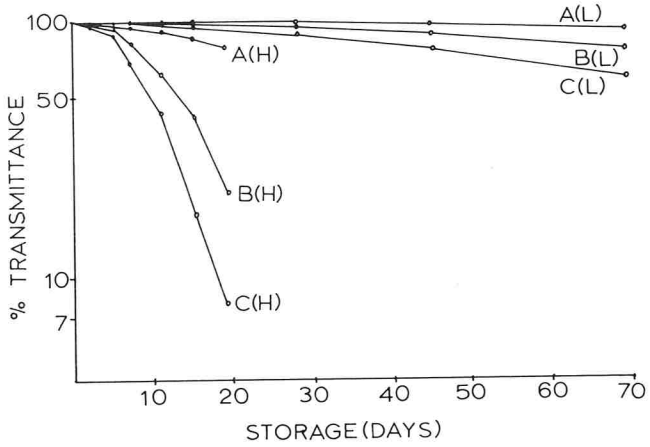


Figure 1f. *Semilogarithmic plots of the transmittances at 470 nm versus storage for the interactions of tryptophan and a-ketoglutaric acid with different concentrations during storage at 75 F and 38 F, respectively. A(H) & A(L): a-keto. + try. (8.5 + 12.5 μ mole/ml) at 75 F and at 38 F. B(H) & B(L): a-keto. + try. (17 + 12.5 μ mole/ml) at 75 F and 38 F. C(H) & C(L): a-keto. + try. (25.5 + 12.5 μ mole/ml) at 75 F and at 38 F.*

Figure 1d and 1e show the decrease in transmittance due to different concentrations of *a*-ketoglutaric acid and tryptophan during storage at 75 F and 38 F. The decrease in transmittance for all concentrations stored at 75 F was greater than for those stored at 38 F. The greatest decrease was observed with high concentrations of both *a*-ketoglutaric acid and tryptophan. The smallest was observed with low concentrations of both *a*-ketoglutaric acid and tryptophan. The combination of high *a*-ketoglutaric acid concentration with low tryptophan concentration showed a greater decrease in

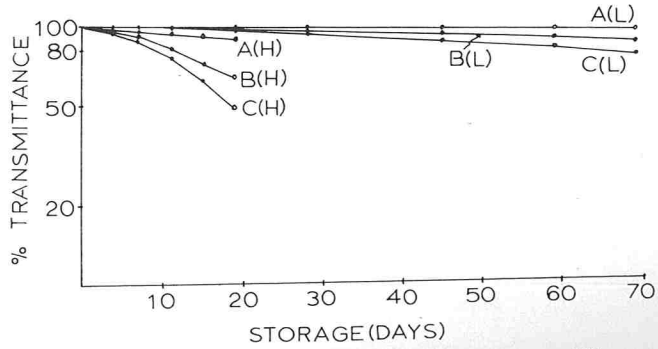


Figure 1g. *Semilogarithmic plots of the transmittances at 620 nm versus storage for theinteractions of tryptophan and a-ketoglutaric acid with different concentrations during storage at 75 F and 38 F, respectively. A(H) & A(L): a-keto. + try. (8.5 + 12.5 μ mole/ml) at 75 F and 38 F. B(H) & B(L): a-keto. + try. (17 + 12.5 μ mole/ml) at 75 F and 38 F. C(H) & C(L): a-keto. + try. (25.5 + 12.5 μ mole/ml) at 75 F and 38 F.*

transmittance than that of low *a*-ketoglutaric acid concentration with high tryptophan concentration. This may be explained by the postulation that the formation of colored compounds is catalyzed by acidity.

Figure 1f and 1g show that transmittance was decreased by utilizing different concentrations of *a*-ketoglutaric acid with one concentration of tryptophan. The transmittance decreased more rapidly in storage at 75 F than in storage at 38 F. The highest concentration of *a*-ketoglutaric acid showed the greatest decrease in transmittance, conversely, the lowest concentration of that acid showed the least decrease in transmittance. This observation along with the results obtained above clearly indicate that the formation of colored compounds probably depends upon the concentration of *a*-ketoglutaric acid. Measurements were discontinued after 19 days due to contamination.

*Measurement during storage after processing*

The samples were processed at temperatures of 240, 270, and 300 F with an $F_0 = 4.9$, and measured at weekly intervals during storage at 75 F. Results are shown in Figures 2a, 2b, 2c, 2d, 2e, and 2f.

Figure 2a shows the change in transmittance due to the interaction between tryptophan and *a*-ketoglutaric acid during storage after processing at 240 F for 19.5 min, $2 \times 19.5$ min and $3 \times 19.5$ min, respectively. All processed samples (Fig. 2a) showed the same transmittance at 470 nm after 2 weeks of storage and the same transmittance at 620 nm after 4 weeks of storage. This indicated that the color reaction was more dependent upon storage time than process time.

Figure 2b shows the interaction between *a*-ketoglutaric acid and tryptophan unprocessed and processed at 240, 270, and 300 F with an $F_0 = 4.9$ both before and after storage and at different concentrations. It may be seen that color formation at both concentrations was similar for both the unprocessed sample and those processed at 270 and 300 F before storage. However, there was increased color formation at 240 F immediately after processing. Following storage, however, all samples began to show the same degree of color formation. A difference in degree of color formation was found at both 470 and 620 nm due to concentration, since transmittance in both instances was much lower with higher concentrations of *a*-ketoglutaric acid and tryptophan.

Figure 2c shows transmittance plotted versus unprocessed and processed (240 F for 19.5 min) samples of different concentrations of *a*-ketoglutaric acid and tryptophan both before and after 2 weeks of storage. Before storage a different degree of color formation was noted in the unprocessed and processed samples regardless of concentration. However, after storage a greater amount of absorption was noted in all instances at both 470 and 620 nm and the difference between unprocessed and processed samples disappeared. After storage, high concentrations of both *a*-ketoglutaric acid and tryptophan showed the greatest absorption, with a high concentration of *a*-ketoglutaric acid and a low concentration of tryptophan showing the next highest absorption. The lowest absorption was shown by low concentrations of both *a*-ketoglutaric acid and tryptophan. From these results it may be concluded that

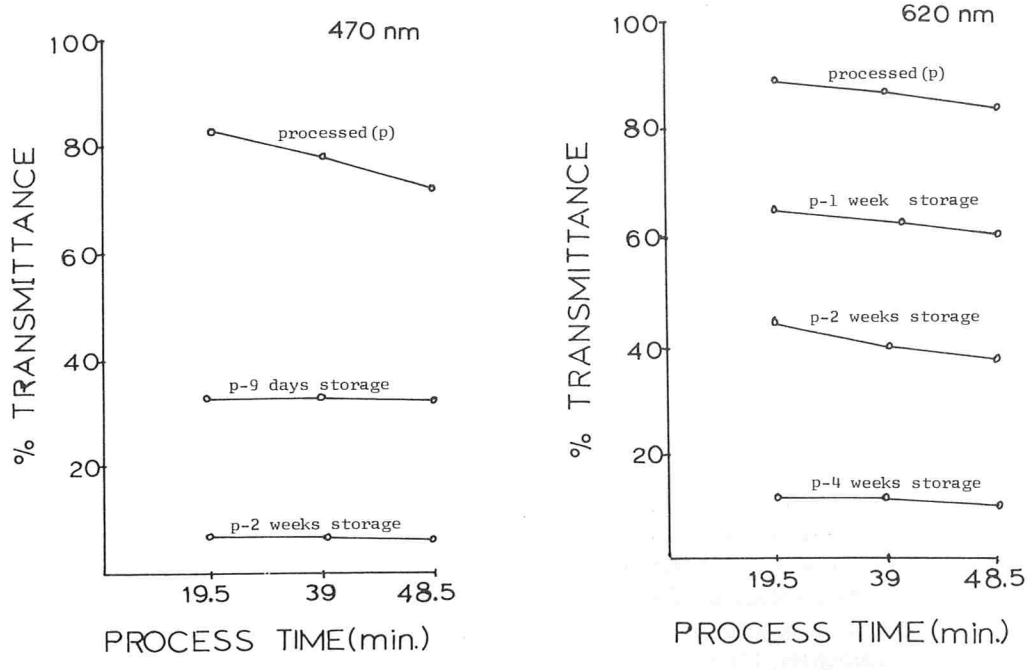


Figure 2a. *Transmittance measurements at 470 nm (left) and at 620 nm (right) versus process time for the interactions between a-ketoglutaric acid (50 μ mole/ml) and tryptophan (25 μ mole/ml) processed at 240 F during storage with an* $F_0 = 4.9$.

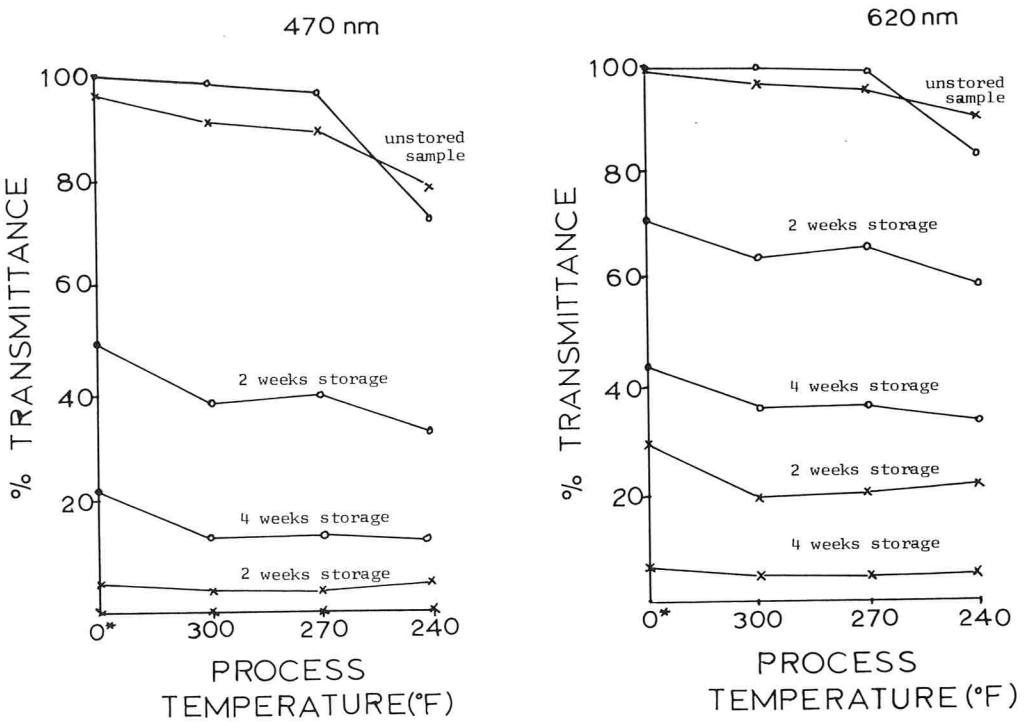


Figure 2b. *Transmittance measurements at 470 nm (left) and at 620 nm (right) versus process temperature with different concentrations of a-ketoglutaric acid and tryptophan with $F_0 = 4.9$ during storage. * unprocessed o: a-keto. + try. (17 + 12.5 μ mole/ml) x: a-keto. + try. (34 + 25 μ mole/ml)*

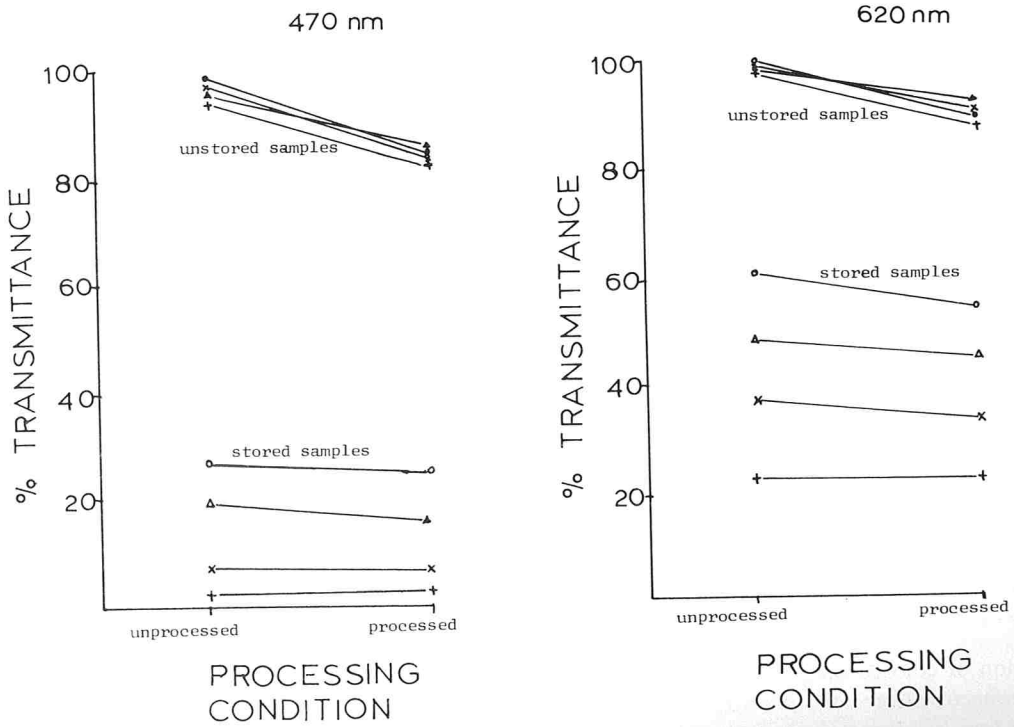


Figure 2c. *Transmittance measurements at 470 nm (left) and at 620 nm (right) versus processing conditions for different concentrations of a-ketoglutaric acid and tryptophan for both stored and unstored samples. o: a-keto. + try. (17 + 12.5 μ mole/ml) : a-keto. + try. (17 + 25 μ mole/ml) x: a-keto. + try. (34 + 12.5 μ mole/ml) + : a-keto. + try. (34 + 25 μ mole/ml).*

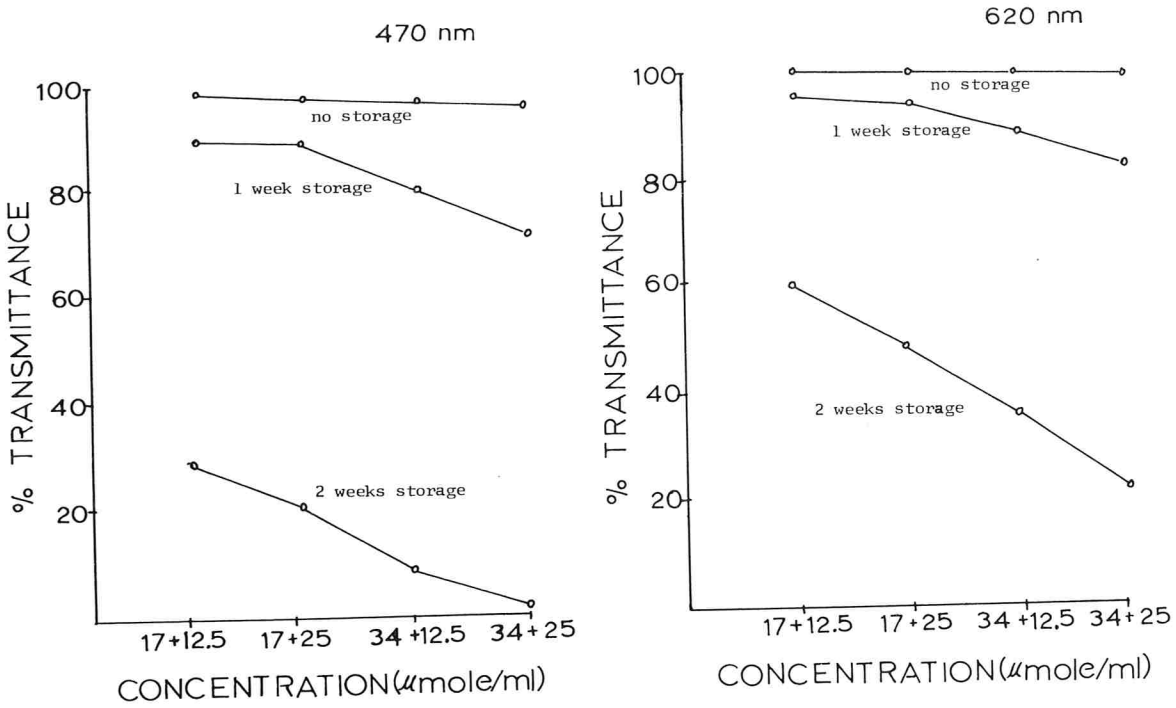


Figure 2d. *Transmittance measurements at 470 nm (left) and at 620 nm (right) versus different concentrations of a-ketoglutaric acid and tryptophan solutions. All samples were unprocessed but stored for different times.*

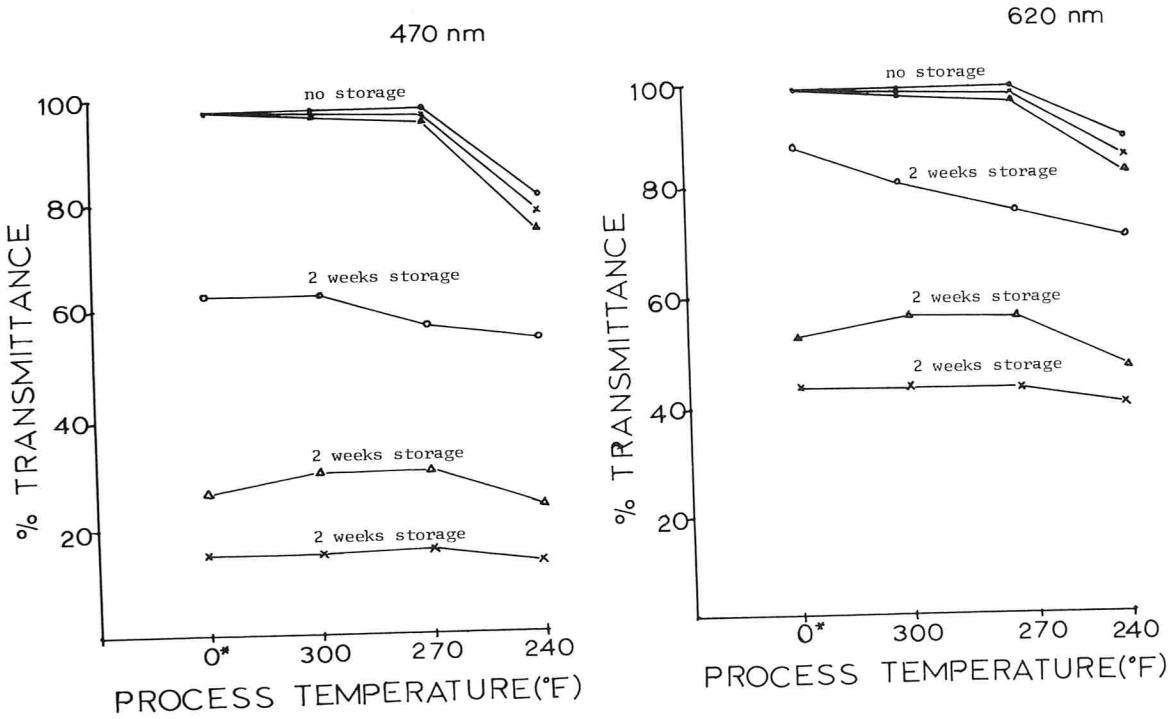


Figure 2e. *Transmittance measurements at 470 nm (left) and at 620 nm (right) versus unprocessed samples and samples processed at temperatures of 240 F and 300 F with an $F_0$ value = 4.9 containing different concentrations of a-ketoglutaric acid and a single concentration of tryptophan both before and after storage. * : unprocessed sample o: a-keto. + try. (8.5 + 12.5 μ mole/ml): a-keto. + try. (17 + 12.5 μ mole/ml) x: a-keto. + try. (25.5 + 12.5 μ mole/ml).*

the formation of colored compounds was affected most by the concentration of *a*-ketoglutaric acid and tryptophan and by storage but not by processing.

Figure 2d also shows the effect of concentration on absorption and color formation. In this figure transmittance at 470 and 620 nm is plotted versus unprocessed samples of different concentrations of *a*-ketoglutaric acid and tryptophan which were stored for different time periods. This figure indicates that color formation increased upon storage and was dependent

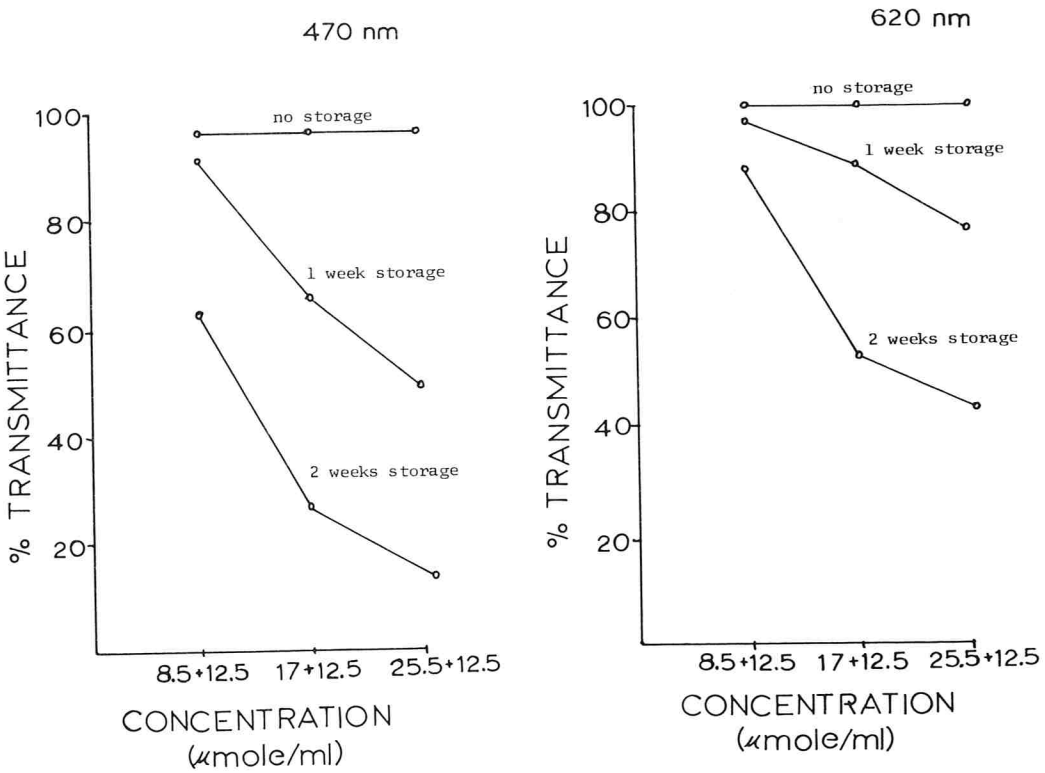


Figure 2f. *Transmittance measurements at 470 nm (left) and at 620 nm (right) versus concentration. The concentration of a-ketoglutaric acid was varied and tryptophan concentration remained constant. All samples were unprocessed but stored for different times.*

upon concentration, since the samples with the highest concentrations showed the greatest absorption.

Figure 2e shows transmittance at 470 and 620 nm plotted versus process temperature for samples containing different concentrations of *a*-ketoglutaric acid and a single concentration of tryptophan both before and after storage. It should be noted that in the samples which were not stored that the degree of absorption was greatest at 240 F. However, the amount of absorption at each single process temperature for the samples not stored was similar. After storage, concentration became important in terms of the degree of absorption and color formation since the samples with the highest concentration of *a*-ketoglutaric acid showed the greatest absorption at all processing temperatures after storage. This indicated that time in storage was an important factor in color formation because concentration effects were not evident immediately after processing.

Figure 2f shows the effect on color formation between *a*-ketoglutaric acid and tryptophan when the concentration of *a*-ketoglutaric acid was varied and tryptophan concentration remained constant. It may be seen that the degree of absorption and the color formation was increased due to increased concentrations of *a*-ketoglutaric acid but only after storage. The curve of transmittance of different acid concentrations at one week storage (Figure 2f) appears to approximate a straight line. The transmittances of low acid concentration were much higher than those of the other two acid concentrations for the two week storage period. The low absorption obtained with low concentrations of *a*-ketoglutaric acid even after storage may be due to the low acid concentration which did not lower the pH enough to catalyze polymerization of the compounds.

In conclusion these results have shown that formation of colored compounds due to the interaction of *a*-ketoglutaric acid and tryptophan depend upon concentrations of both substances and storage period. Formation of colored compounds was accelerated by long storage at 75 F while color formation was greatly slowed by storing at 38 F. Processing may be responsible for color formation, however, its effect was superseded by storage. The concentration of acid brought about significant formation compared with the concentration of tryptophan.

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# Comparison of Boil-in-Bag Plastic Pouch with Gas Pak Methods for Enumeration of Anaerobic Sporeformers in Cheese

**G. L. HONG, R. F. WILKINSON[1] and G. H. RICHARDSON**

*Department of Nutrition and Food Sciences, Utah State University, Logan, Utah 84322*

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**ABSTRACT**

Counts of anaerobic sporeformers from a cheese sample were not significantly different when conducted in individual boil-in-bag plastic pouches or on petri plates in a BBL Gas Pak system. The plastic pouch method for anaerobic culturing has significant advantages over conventional methods.

Platic pouches for boil-in-bag applications have the potential for improving culturing techniques (*2*). The pouches have been successfully used to examine Swiss cheese cultures (*5*) and proposed for general anaerobic culturing (*1*). Oxygen permeable pouches also have been suggested for aerobic culturing (*1*). "Stinker" Swiss cheese anaerobic sporeformers are successfully monitored using a commercially available boil-in-bag pouch (*3*). This study compared the anaerobic sporeformer counts of a cheese product using a boil-in-bag pouch technique to that using a conventional BBL Gas Pak method.

## MATERIALS AND METHODS

Four kilograms of "Stinker" Swiss cheese were trimmed, ground through a food chopper and thoroughly mixed by kneading. Boil-in-bag pouches (10.26 × 15.24 cm, "Scotchpak," Kapak Industries, Inc., Minneapolis, Minnesota 55431) were filled with 50 g mixed cheese, sealed and stored at − 20 C. Samples were thawed at 4 C for 12-15 h prior to assay. One gram of sample was aseptically transferred into a gas sterilized Whirl Pak (NASCO, Fort Atkinson, Wisconsin 53538) 177 ml platic pouch. The pouch was closed, the contents tempered to 25 C and mascerated by rolling against a flat table top with a round test tube (*6*). The pouch was opened and 9 ml of 2% sodium citrate was added. The pouch was again closed and the cheese sample emulsified through additional rolling. The pouch contents were heated to 80 C for 10 min in a water bath and cooled to ambient temperature. A 1 ml aliquot was transferred to 99 ml of phosphate buffer and prepared for plating using Standard Methods (*4*).

Boil-in-bag pouches were charged with 1 ml of diluted sample and 25 ml (45 C) of BACTO-Thioglycollate Medium without Dextrose, containing added 2% agar and 0.025% $FeSO_4 \cdot 7H_2O$. Each pouch was drawn between thumb and forefingers of both hands to exlude air and sealed on a "Scotchpak" sealer. Filled and sealed pouches were allowed to lie flat until the contents solidified.

One-milliliter quantities of sample dilutions were transferred to conventional 100 mm plastic petri dishes and prepared with 25 ml of thioglycollate substrate. Ten petri dishes were sealed in a BBL Gas Pak Anaerobic System 60460 using the directions of the manufacturer (BBL Div. of Bioquest, P.O. Box 175, Cockeysville, Md. 21030). Plates and pouches were incubated at 37 C for 20 h. Both $H_2S$ producing and white colonies were enumerated. The petri dish area was 63.6 $cm^2$ (90 mm ID dish) whereas the area in the pouch varied with the position of the seal. The inside width of the pouch was 8.2 cm and the inside height to the seal was approximately 10 cm (area = 82 $cm^2$).

## RESULTS AND DISCUSSION

Boil-in-bag pouches were filled with thioglycollate medium and evaluated for contaminants. Five colonies were found in six pouches. Contaminants were thus considered to have no significant effect upon the results of this study.

A single dilution of a cheese sample was plated using both the plate and pouch techniques for each trail. The results were not significantly different between the two methods (Table 1). The grouped mean for the cheese sample was 184.7 colony forming units (cfu) per plate/pouch or $1.8 \times 10^5$ anaerobic sporeformers per gram. Though the pouch method produced higher mean counts in both trials, the difference was not significant even at the 10% level.

TABLE 1. *Colony forming units per pouch/plate in replicates prepared from cheese sample dilution*

| Trial 1 | | Trial 2 | |
|---|---|---|---|
| Plate | Pouch | Plate | Pouch |
| 234 | 144 | 206 | 210 |
| 199 | 191 | 223 | 200 |
| 214 | 206 | 175 | 213 |
| 185 | 234 | 178 | 205 |
| 191 | 214 | 211 | 223 |
| 195 | 173 | 203 | 186 |
| 92 | 206 | 231 | 201 |
| 182 | 178 | 135 | 211 |
| | | 182 | 177 |
| | | 182 | 216 |
| n = 8 | 8 | 10 | 10 |
| $\bar{x}$ = 186.5 | 193.3 | 192.6 | 204.2 |
| s = 41.8 | 28.0 | 28.1 | 13.9 |
| C.V. (%) 22.4 | 14.5 | 14.6 | 6.8 |
| t = 0.38 | | 1.17 | |
| Tabular t (05) = 2.14 | | 2.10 | |

Four different dilutions of a second cheese sample were prepared and plated in the same manner (Table 2). No

[1]*Present Address: Department of Food Science, University of Missouri, Columbia, Mo. 65201*

TABLE 2. *Colony forming units per pouch/plate in replicates prepared from cheese sample dilutions (Sample number two)*

| Trial 1 | | Trial 2 | |
|---|---|---|---|
| Pouch | Plate | Pouch | Plate |
| 81 | 84 | 75 | 89 |
| 81 | 86 | 84 | 62 |
| 90 | 101 | 88 | 67 |
| 80 | 81 | 63 | 84 |
| 90 | 78 | 84 | 70 |
| 75 | 80 | 64 | 72 |
| 83 | 60 | 72 | 64 |
| 70 | 66 | 76 | 76 |
| 64 | 78 | 62 | 86 |
| 75 | 73 | 61 | 65 |
| $\bar{x}$ 78.9 | 78.7 | 72.9 | 73.5 |
| s 8.2 | 11.2 | 10.2 | 9.8 |
| **Trial 3** | | **Trial 4** | |
| Pouch | Plate | Pouch | Plate |
| 61 | 70 | 81 | 68 |
| 63 | 70 | 74 | 68 |
| 62 | 76 | 76 | 78 |
| 62 | 59 | 80 | 76 |
| 70 | 59 | 63 | 68 |
| 79 | 59 | 73 | 78 |
| 73 | 71 | 75 | 67 |
| 70 | 74 | 62 | 64 |
| 81 | 65 | 74 | 76 |
| 75 | 69 | 73 | 78 |
| $\bar{x}$ 69.6 | 67.2 | 73.1 | 72.1 |
| s 7.4 | 6.4 | 6.2 | 5.6 |

significant differences were noted.

The use of the individual plastic pouch anaerobic culture technique provided several advantages over conventional anaerobic chamber techniques. Sealed pouches were much more pleasant to work with because no odors were detected unless the pouch was ruptured. The units could be carried, with no special handling required, to milk producers to graphically show the types of organisms in their milk supply and the lack of proper sanitation in their operations. The contents did not dehydrate for several years. They therefore can be frozen for culture storage and subsequently sanitized and punctured for isolation from individual colonies. No special gas chamber was required, thus allowing application in any laboratory capable of routine SPC tests with only the addition of an economical heat sealer. Unit cost was approximately the same as with disposable plastic petri dishes.

Other researchers have used a vertical plate device to assure uniform thickness of the agar layer and help remove air from the pouch (*2, 5*). A similar device was used intially in this work. However, with the large volumes of samples routinely prepared in commercial testing, it was found to be faster and adequate to allow samples to solidify on the flat table surface. It also was easy to expel the air while holding the pouch in position for sealing. The vertical mold was therefore not used in this study and is not necessary for routine operations. Colonies could be counted adequately through the slightly concave agar medium. Edge effects were apparently insignificant.


## ACKNOWLEDGMENTS

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# Comparative and Collaborative Studies on the Preparation of Hard Cheese Samples for Microbiological Assay Using a Plastic Pouch Technique

**R. F. WILKINSON[1] and G. H. RICHARDSON**

*Dept. of Nutrition and Food Sciences, Utah State University, Logan, Utah 84322*

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**ABSTRACT**

Hard and very hard cheese samples were prepared for microbiological examination by emulsification with sodium citrate solution in a polyethylene plastic pouch. Results were comparable to those obtained using a standard blender technique.

The twelfth edition of *Standard Methods for the Examination of Dairy Products* (*4*) stated that "Sterile plastic pouches may be used for collection and preliminary preparation of cottage cheese samples before plating. The entire contents or a large portion of the carefully mixed contents of a cottage cheese carton, may be aseptically transferred to a sterile plastic pouch. After gentle mixing, weigh required amounts of the homogenous curd and creaming mixture for analysis." Mixing in the pouch was implied to provide adequate homogeniety. The thirteenth edition (*2*) omitted this technique. Samples of cottage cheese have been successfully prepared and mixed in our laboratories using plastic pouches in place of the blender technique (*2*). This study was undertaken to evaluate weighing, emulsification and dilution of hard cheese samples in a plastic pouch as an alternative to using blenders or mortars and pestles as currently recommended (*2*).

## MATERIALS AND METHODS

Four kilograms of cheese were trimmed, ground through a food chopper and thoroughly mixed. Very hard grated Parmesan samples were from commercial shaker packages. Boil-in-bag pouches (10.26 × 15.24 cm, "Scotchpak," Kapak Industries, Inc., Minneapolis, Minnesota, 55431) were filled with 50 g of well mixed cheese, heat sealed and stored at − 20 C until prepared for plating or for shipment to participating collaborators. Before testing, frozen cheese samples were tempered at 4 C overnight. Approximately 1 g ± 10 mg was rapidly weighed into a sterile 177-ml Whirl-Pak bag (NASCO, Fort Atkinson, Wisconsin, 53538). The pouch was closed, transferred to a flat laboratory table surface and the contents were mascerated by rolling them with a 15 × 125 mm test tube or similar cylindrical object. Care was taken to prevent forcing the sample into the corners of the pouch. The sample was also kept from the tied seal by holding the sealed end above the table with one hand while rolling the tube with the other. When the sample was in a fine paste the pouch was opened and 9 ml of 2% sodium citrate solution at 40 C was added. Some samples were prepared using sodium citrate at ambient temperature (25 C). The pouch was reclosed, transferred to the flat surface and the tube was again rolled over the contents to form a fine emulsion. One milliliter of emulsion was then pipetted to a 99 ml of phosphate buffer and Standard Plate Count (SPC) procedures were subsequently followed (*2*). Colony counts were adjusted to reflect any deviation of sample weights from one gram.

The Standard Method for emulsification of a cheese sample for microbiological examination involves a blender technique (*2*). This procedure was followed for the control samples.

A mild Cheddar, a sharp Cheddar, a Romano and a dry grated Parmesan cheese were prepared for storage and assay. They had 36.9, 36.8, 33.6, and 17.8% moisture respectively.

Collaborators from twelve laboratories, routinely running SPCs on various products, volunteered to evaluate the methodology. Each collaborator received samples of each cheese which had been shipped initially frozen in shipping containers with additional ice. One collaborator discarded the samples because they were at ambient temperatures upon arrival. One could find no significant microflora and another discarded the samples by mistake. Thus nine participated in the collaborative studies. Duplicate raw data were returned for statistical analysis. Laboratories were invited to comment on the workability of the system. Collaborators were not told the expected count but were asked to prepare $10^{-4}$ and $10^{-5}$ dilutions.

Statistical analyses were conducted as described by Snedecor (*3*) and Youden (*5*).

## RESULTS AND DISCUSSION

The dry grated cheese did not emulsify well in the pouch and it was concluded that very dry cheeses could not be prepared by this method. This cheese was not evaluated further.

Comparative studies were run on several different lots of Cheddar and Romano cheese (Table 1). The lowest tabular t value was 2.04, therefore, all of the comparisons in Table 1 indicate no significant differences between the blender and pouch methods for sample preparation. Standard deviations were very high in Cheddar #2 and Romano #2. Data for Cheddar samples 3 and 4 and Romano samples 3 were used as the collaborator 1 values in the collaborative study.

The use of sodium citrate solution at 40 C seemed to help in the emulsion preparation. However, no detectable difference occurred between sample preparation methods (Table 2) when sodium citrate at ambient temp-

[1]*Present address: Department of Food Science, University of Missouri, Columbia, Mo. 65201.*

TABLE 1. *Comparison of SPC on Cheddar and Romano cheese samples prepared using the blender vs. the pouch technique—each sample number (N) represents a separate dilution preparation from a frozen portion of mixed, ground cheese*

| Sample | Treatment | N | $\bar{X}$ | s | t |
|---|---|---|---|---|---|
| *Cheddar* | | | | | |
| 1 | Blender | 30 | $1.25 \pm 0.135 \times 10^5$ | | 1.735ns |
| | Pouch | 30 | $1.44 \pm 0.398 \times 10^5$ | | |
| 2 | Blender | 12 | $1.98 \pm 2.46 \times 10^5$ | | 1.528ns |
| | Pouch | 12 | $3.89 \pm 3.08 \times 10^5$ | | |
| 3[1] | Blender | 10 | $5.31 \pm 1.13 \times 10^6$ | | 1.450ns |
| | Pouch | 10 | $5.67 \pm 0.568 \times 10^6$ | | |
| 4[3] | Blender | 9 | $3.01 \pm 0.203 \times 10^7$ | | 1.22ns |
| | Pouch | 9 | $3.10 \pm 0.308 \times 10^7$ | | |
| *Romano* | | | | | |
| 1 | Blender | 26 | $1.44 \pm 0.266 \times 10^6$ | | 1.195ns |
| | Pouch | 26 | $1.49 \pm 0.392 \times 10^6$ | | |
| 2 | Blender | 11 | $2.09 \pm 2.35 \times 10^6$ | | 1.267ns |
| | Pouch | 11 | $2.99 \pm 3.94 \times 10^6$ | | |
| 3[2] | Blender | 10 | $1.92 \pm 0.621 \times 10^6$ | | 0.803ns |
| | Pouch | 9 | $2.02 \pm 0.565 \times 10^6$ | | |

[1]Sample A in collaborative study
[2]Sample B in collaborative study
[3]Sample C in collaborative study

TABLE 2. *Effect of warming sodium citrate solution on the SPC of Cheddar and Romano cheese prepared using the pouch technique*

| Sample | Pre-tempering | N | $\bar{X}$ and s | t |
|---|---|---|---|---|
| Cheddar | 40 C | 14 | $1.49 \pm 0.578 \times 10^5$ | 0.017ns |
| | 25 C | 17 | $1.50 \pm 0.426 \times 10^5$ | |
| Romano | 40 C | 15 | $1.43 \pm 0.432 \times 10^6$ | 0.235ns |
| | 25 C | 11 | $1.58 \pm 0.319 \times 10^6$ | |

erature was used. The use citrate at 40 C might be necessary where samples fail to emulsify easily. Holding some samples briefly in a 40 C bath may be helpful, such as with the Cheddar or Romano sample 2 (Table 1).

Samples arrived at the out-of-state laboratories at temperatures of 17 to 25 C though shipped frozen and iced. Samples delivered within the state remained frozen. There were no detectable differences in the data because of this temperature differential.

Upon completion of the collaborative study the raw duplicate plate data were averaged (Table 3) and ranked according to Youden (5). The collaborator scores should have been between 13 and 47 for six samples and nine collaborators in order to be free from significant bias at the 5% level. Collaborator 10 data, with a 52 score, was not within this range and data were discarded. In addition, collaborator 5 data were discarded because four of the six values were beyond two standard deviations from the mean and indicated a dilution or calculation error.

Analyses of variance were conducted on the data from the seven remaining collaborators. The F values for the blender vs. pouch treatment and for collaborator data are included in Table 4. The method of calculation is shown for sample A. There were no significant differences between treatment or among collaborators for the three cheese samples. Respective t values for these comparisons were 0.93, 0.30 and 0.81, indicating no significant differences.

TABLE 4. *Analysis of variance*

*Sample A—Mild Cheddar*

| Collaborator Number | Plate Count/g × $10^6$ AB | AP |
|---|---|---|
| 1 | 5.3 | 5.7 |
| 2 | 5.1 | 6.7 |
| 3 | 6.7 | 6.5 |
| 4 | 9.7 | 8.5 |
| 7 | 8.6 | 5.8 |
| 9 | 6.3 | 6.5 |
| 12 | 50.0 | 5.4 |

| *Source* | *df* | *ss* | *MS* | *F* | *Tabular F* |
|---|---|---|---|---|---|
| Treatment | 1 | 155.11 | 155.11 | 1.1 ns | 5.99 (0.05) |
| Collaborators | 6 | 766.31 | 127.72 | 0.91 ns | |
| Error | 6 | 845.51 | 140.92 | | |
| Total | 13 | 1766.93 | | | |

*Sample B—Romano*

| *Source* | *F* |
|---|---|
| Treatment | 1.1 ns |
| Collaborators | 1.8 ns |

*Sample C—Sharp Cheddar*

| *Source* | *F* |
|---|---|
| Treatment | 0.91 |
| Collaborators | 0.86 |

Donnelly et al. (*1*) concluded that log variances of data submitted by state laboratories conducting SPCs on split milk samples should be less than 0.05. Log variances among collaborator data on the six cheese samples in this study were 0.1184, 0.1386, 0.0827, 0.0254, 1.511, and 0.0557. Only one sample was below the suggested tolerance. The cooperating laboratories were routinely involved in testing milk and employ capable technicians. The difficulty in working with cheese samples and the fact that many are not routinely involved in testing cheese may explain the wide variance. Routine testing of these types of products would no doubt improve this

TABLE 3. *Ranking of participation collaborators*

| Collaborator number | Sample Treatment: AB[1] | BB | CB | AP[2] | BP | CP | AB | BB | CB | AP | BP | CP | Collaborator ranking score |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Plate Count/g × $10^6$ | | | | | | Ranking of Results | | | | | | |
| 1 | 5.3 | 1.9 | 30 | 5.7 | 2.0 | 31 | 7 | 3.5 | 5 | 7 | 2 | 3 | 27.5 |
| 2 | 5.1 | 0.91 | 35 | 6.7 | 0.12 | 41 | 8 | 7 | 3 | 3 | 9 | 2 | 32 |
| 3 | 6.7 | 2.0 | 34 | 5.5 | 1.9 | 32 | 5 | 3.5 | 4 | 4.5 | 3 | 4 | 24 |
| 4 | 9.7 | 1.6 | 29 | 8.5 | 1.5 | 27 | 3 | 5 | 6 | 2 | 5 | 5 | 26 |
| 5 | 200 | 0.65 | 360 | 170 | 0.53 | 340 | 1 | 8 | 1 | 1 | 7 | 1 | 19 |
| 7 | 8.6 | 1.0 | 20 | 5.8 | 1.3 | 22 | 4 | 6 | 8 | 6 | 6 | 8 | 38 |
| 9 | 6.3 | 5.1 | 24 | 6.5 | 5.2 | 24 | 6 | 1 | 7 | 4.5 | 1 | 6 | 25.5 |
| 10 | 0.52 | 0.13 | 0.22 | 0.56 | 0.15 | 0.29 | 9 | 9 | 9 | 8 | 8 | 9 | 52 |
| 12 | 50 | 9.6 | 150 | 5.4 | 1.7 | 23 | 2 | 2 | 2 | 9 | 4 | 7 | 26 |

[1]B = Blender method results
[2]P = Pouch method results

factor. However, the wide standard deviations found on samples #2 (Table 1) suggest that part of the problem might relate to the characteristics of a particular lot of cheese.

It appears that only the very hard dry grating cheese samples may not be prepared using the pouch technique. Cottage cheese and the hard and some very hard varieties exemplified by the samples used in this study can easily be prepared for microbiological evaluation.

Collaborators reported no difficulty in following or applying the methodology. There were no suggestions to improve the technique. Some advantages expressed included (*a*) elimination of sample heat up during blending, (*b*) avoiding the need to sterilize blender or mortar and pestle between samples, (*c*) the use of economical, disposable, sterile plastic pouches, and (*d*) easier emulsification than provided by a mortar and pestle.

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# Spectrophotometric Determination of Concentrations of Raw Milk in Solutions Containing Ingredients of Detergents[1]

**E. L. RUIZ[2], D. B. BROOKER[3], M. E. ANDERSON[4], J. R. FISCHER[4], and R. T. MARSHALL[5]**

*PINFST Philippine Women's University, Manila;[2] Department of Agricultural Engineering,[3] and Department of Food Science and Nutrition,[5] University of Missouri, Columbia, Missouri 65201; and U.S. Department of Agriculture, Agricultural Research Service, North Central Region, Columbia, Missouri[4] 65201*

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**ABSTRACT**

This paper reports effects of concentration of milk, concentration of individual ingredients of detergents, and temperature on transmittance of light at 527 nm through solutions of milk, water, and individual detergent ingredients. Milk in soft water caused the major portion of the variation, more than 99% of the total sum of squares, for each ingredient except sodium hydroxide and wetting agent. Milk contributed 95.7 and 17.9% to the sums of squares for the sodium hydroxide and wetting agent, respectively. Concentration of detergent ingredient and temperature were of practical significance only with wetting agent. Effects of milk on transmittance were significantly different among milk concentrations for *all* detergent ingredients, except trisodium phosphate in hard water at the lowest concentrations of milk.

Although precipitates contributed to turbidity of solutions containing trisodium phosphate in hard water, milk was responsible for 95.9% of the variance in the sum of squares. Additionally, in hard water milk accounted for 98.9, 99.1 and 99.7% of the sums of squares in analyses of effects on turbidity of sodium tripolyphosphate, sodium hexametaphosphate, and tetrasodium pyrophosphate, respectively. Turbidity increased with time of holding some phosphate solutions, however, this did not appear to pose serious problems in measuring content of milk.

The objective of this research was to determine the extent that chemical ingredients used in the formulation of commercial detergents interfere with spectrophotometric measurement of milk in aqueous solutions. The research is the initial step in investigating the possible use of the spectrophotometer as a device to measure quantities of milk residue in cleaning solutions as they pass through components of a milk processing plant or farm milking equipment.

The white color of milk is caused by the scattering of reflected light by minute fat globules, colloidal calcium caseinate, and calcium phosphate (*5*). Ashworth (*1*) observed that the turbidity per unit of concentration of total solids in milk varied with the fat content of the milk and with the efficiency of homogenization. About 75% of the total turbidity was attributed to the fat phase. Therefore, milk components in cleaning solutions, especially fat and protein, should be detectable by transmittance spectrophotometry, provided chemicals in the cleaning solutions do not interfere.

## MATERIALS AND METHODS

### *Materials*

Detergent ingredients, supplied by Economics Laboratory, Inc., St. Paul, Minnesota, consisted of four basic alkalies, three complex phosphates, two chelating agents, one wetting agent, one organic acid, and one mineral acid. Raw milk for the study was obtained from dairy farms at the University of Missouri-Columbia. Concentrations of fat and total solids in milk were determined by the Babcock test and the Mojonnier gravimetric method, respectively (*4*). Nonfat solids were determined by difference. Soft water (< 2 ppm of calcium) used in the milk-ingredient-water (MIW) solutions was softened by ion exchange. Unsoftened water contained approximately 226 ppm of hardness. Three water baths were used to temper test solutions to 20, 45, or 70 ± 2 C.

A light spectrophotometer (Bausch and Lomb, Spectronic 20), set at a wavelength of 527 nm (wavelength of maximum absorbence obtained by scanning milk-water solutions), was used in analyses of the MIW solutions. Output signals were recorded with a dual-channel strip chart recorder.

### *Methods*

The primary experiment was a 3 × 4 × 4 factorial in a randomized complete block with two replications. There were three temperatures (20, 45 and 70 C), four concentrations of detergent (they varied for each detergent ingredient), and four concentrations of raw milk (0.0, 0.01, 0.1, and 1.0%).

The same procedure was used to test each of the 12 ingredients. Various concentrations of the detergent ingredients were prepared in 300-ml BOD bottles. Ingredient concentrations were selected to cover ranges that would be used in the formulation of commercial detergents. Raw milk was added to each bottle to give the required milk concentration, and softened water was used to dilute the mixture to 300 ml.

In a second experiment each type of phosphate was prepared in hard water. The BOD bottles were shaken and placed in the appropriate water bath. When the solutions reached 20, 45 or 70 C, transmittance measurements were made. In addition, two concentrations each of four phosphates containing 0, 0.01, 0.1, or 1.0% milk were heated to and held at 70 C for 8 h per day during four days of storage. Transmittance measurements were made initially and at 24-h intervals.

Data were subjected to analyses of variance. Duncan's New Multiple Range Test (*3*) was used to determine significance of differences among means for each variable.

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## RESULTS AND DISCUSSION

### *Effects of ingredients*

Mean transmittance values (%) for four concentrations of each ingredient (indicated in parentheses) averaged over four concentrations of milk and three temperatures are shown in Table 1. Each detergent ingredient, except sodium hydroxide and the wetting agent, accounted for less than 1% of the total sum of squares.

Wetting agents lower interfacial tension between fat and water. Thus, they permit fat globules to break into smaller globules. The resulting large increase in total surface area would cause increased light absorption in the cuvette. We suggest, therefore, that increases in surface area caused the decreased transmittance we observed with increased concentrations of wetting agent ($P < 0.05$). Of the total sum of squares, 34.7% was attributable to the presence of the wetting agent. Although transmittance of the solution containing 0.3% sodium metasilicate was significantly higher ($P < 0.05$) than were those of solutions containing the other concentrations of this ingredient, the combined mean effect of this compound accounted for only 0.02% of the sum of squares. Sodium metasilicate is a relatively weaker alkali than is sodium hydroxide, and, therefore, had less effect upon turbidity. No significant differences

TABLE 1. *Mean transmittance values for four concentrations of detergent ingredients averaged over four concentrations of milk and three temperatures*

| Ingredient and concentrations (%)[a] | Concentration of detergent | | | |
|---|---|---|---|---|
| | A | B | C | D |
| | Transmittance values (%)[bc] | | | |
| Sodium hydroxide (0.0, 0.45, 0.9, 1.35) | 79.4 | 83.4 | 85.8 | 86.3 |
| Sodium metasilicate anhydrous (0.0, 0.3, 0.6, 0.9) | 76.6 | 77.9 | 77.3 | 77.0 |
| Sodium carbonate (0.0, 0.3, 0.6, 0.9) | 77.8 | 78.6 | 78.8 | 78.9 |
| Trisodium phosphate (0.0, 0.75, 1.50, 2.25) | 77.8 | 79.2 | 79.7 | 78.9 |
| (Trisodium phosphate + hard water) | 79.1 | 74.7 | 75.0 | 72.9 |
| Sodium hexametaphosphate (0.0, 0.3, 0.6, 0.9) | 75.8 | 76.7 | 76.8 | 76.6 |
| (Sodium hexametaphosphate + hard water) | 75.9 | 78.3 | 77.7 | 77.7 |
| Sodium tripolyphosphate (0.0, 0.3, 0.6, 0.9) | 78.2 | 79.5 | 79.0 | 78.2 |
| (Sodium tripolyphosphate + hard water) | 76.4 | 78.1 | 78.4 | 78.1 |
| Tetrasodium pyrophosphate (0.0, 0.3, 0.6, 0.9) | 74.5 | 77.1 | 77.0 | 76.6 |
| (Tetrasodium pyrophosphate + hard water) | 74.6 | 76.7 | 76.6 | 76.1 |
| EDTA[d] plus sodium hydroxide (0.0, 0.21, 0.42, 0.63) | 77.6 | 79.5 | 79.6 | 79.6 |
| Sodium gluconate (0.0, 0.21, 0.42, 0.63) | 77.1 | 77.9 | 78.3 | 78.6 |
| Wetting agent DC-161 (0.0, 0.06, 0.12, 0.18) | 78.6 | 38.0 | 29.4 | 26.1 |
| Gluconic acid, 50% (0.0, 0.6, 1.2, 1.8) | 77.5 | 78.6 | 79.2 | 79.1 |
| Phosphoric acid, 75% (0.0, 0.6, 1.2, 1.8) | 77.8 | 78.6 | 79.3 | 79.1 |

[a]Numbers in parentheses are concentrations of individual ingredients and correspond in order presented with transmittance values in columns A, B, C, and D.

[b]Each entry is an average of 24 readings; 12 are from each replication.

[c]Underscored values were not significantly different at the 5.0% level of probability according to Duncan's New Multiple Range Test.

[d]Ethylenediaminetetraacetic acid.

TABLE 2. *Mean transmittance values for four concentrations of milk averaged over four concentrations of detergent ingredient and three temperatures*

| Detergent ingredient | Concentration of milk (%) | | | |
|---|---|---|---|---|
| | 0.0 | 0.01 | 0.1 | 1.0 |
| | Transmittance values (%)[a,b] | | | |
| Sodium hydroxide | 99.5 | 98.5 | 91.6 | 45.3 |
| Sodium metasilicate | 99.6 | 98.0 | 86.1 | 25.0 |
| Sodium carbonate | 99.7 | 98.5 | 88.2 | 27.6 |
| Trisodium phosphate | 99.5 | 98.2 | 88.5 | 29.5 |
| (Trisodium phosphate + hard water) | 93.3 | 92.4 | 84.5 | 31.5 |
| Sodium hexametaphosphate | 98.3 | 96.8 | 85.8 | 25.0 |
| (Sodium hexametaphosphate + hard water) | 99.6 | 98.4 | 85.3 | 26.0 |
| Sodium tripolyphosphate | 99.6 | 97.6 | 86.6 | 31.0 |
| (Sodium tripolyphosphate + hard water) | 99.0 | 97.6 | 85.0 | 29.5 |
| Tetrasodium pyrophosphate | 98.8 | 96.8 | 85.6 | 24.0 |
| (Tetrasodium pyrophosphate + hard water) | 99.5 | 98.1 | 84.5 | 22.1 |
| EDTA plus sodium hydroxide | 99.3 | 98.2 | 89.2 | 29.5 |
| Sodium gluconate | 99.8 | 98.0 | 87.6 | 26.4 |
| Wetting agent | 56.0 | 52.6 | 46.0 | 17.4 |
| Gluconic acid | 99.3 | 98.1 | 88.2 | 28.7 |
| Phosphoric acid | 99.4 | 98.3 | 88.8 | 28.4 |

[a]Each entry is an average of 24 readings; 12 are from each replication.

[b]Underscored values indicate no significant difference at 5.0% level of probability according to Duncan's New Multiple Range Test.

were found among the concentrations of sodium tripolyphosphate, sodium hexametaphosphate, and gluconic acid.

Because trisodium phosphate softens by precipitation (2), it was expected that transmittance values would be lower (the precipitate would deflect the light) when it was dissolved in hard water rather than soft water. Tests were conducted with hard water and each of the four phosphates. Effects of adding the phosphates were significant, but effects of concentration were not significant except in the case of the highest concentration of tetrasodium pyrophosphate (Table 1). Transmittance decreased when trisodium phosphate were added to solutions of milk and water, but transmittance increased in solutions of the other three phosphates. Precipitate was visible in solutions containing trisodium phosphate, but the other solutions were clear.

*Effects of milk*

Mean transmittance values (%) for four concentrations of milk averaged over four concentrations of detergent ingredient and three temperatures are shown in Table 2. For each increasing concentration of milk, the mean transmittance value decreased significantly except for the comparison of 0.0 to 0.01% milk in the solution of trisodium phosphate in hard water.

Solutions of trisodium phosphate in hard water allowed considerably lower transmittance than solutions of this ingredient in soft water, 93.3 vs 99.5 percent. This obviously resulted from precipitation of calcium and/or magnesium phosphates. However, concentration of milk in hard water was responsible for 95.9, 98.9, 99.1, and 99.7 percent of the sums of squares in the analyses of variance for trisodium phosphate, sodium tripolyphosphate, sodium hexametaphosphate, and tetrasodium pyrophosphate, respectively.

In solutions made from soft water, milk accounted for more than 99.0% of the total sums of squares, except when sodium hydroxide and the wetting agent were present. Milk accounted for 95.7% of the variation in data when the ingredient was sodium hydroxide and for only 17.9% when the ingredient was the wetting agent. When data related to wetting agent were omitted, mean transmittance readings ranged from a high of 99.8% to a low of 98.3% when no milk was present; a high of 98.5% to a low of 96.8% for 0.01% milk; a high of 91.6% to a low of 85.6% for 0.1% milk; and a high of 45.3% to a low of 24.0% for 1.0% milk. For milk concentrations of 0.1 and 1.0%, sodium hydroxide gave the highest transmittance readings, probably because of its solubilizing effect on colloidal calcium phosphate and calcium caseinate.

The reduction of transmittance by wetting agent was readily apparent, as milk was responsible for only 18.2% of the total sum of squares. However, even in the presence of wetting agent all mean readings averaged over all the other variables for each concentration of milk were significantly different ($P < 0.05$).

*Effects of temperature*

Mean transmittance values (%) for three temperatures averaged over four concentrations of milk and four concentrations of detergent ingredient are shown in Table 3. No significant differences were noted as a result of changes in temperature for the following ingredients in soft water: sodium hydroxide, sodium metasilicate, trisodium phosphate, sodium tripolyphosphate, and sodium gluconate. For the other ingredients, an increase in temperature caused a significant ($P < 0.05$) decrease in transmittance, except for wetting agent. In this instance the opposite effect was noted. Although effects of temperature on certain ingredients were significant, they accounted for less than 0.1% of the total sums of squares, and the results are not discussed. The effect of

TABLE 3. *Mean transmittance values for three temperatures with four concentrations of milk and four concentrations of detergent*

| Detergent ingredient | Temperature (C) | | |
|---|---|---|---|
| | 20 | 45 | 70 |
| | Transmittance values (%)[a,b] | | |
| Sodium hydroxide | 83.7 | 84.1 | 83.3 |
| Sodium metasilicate | 76.9 | 77.3 | 77.3 |
| Sodium carbonate | 77.5 | 78.8 | 79.2 |
| Trisodium phosphate | 78.4 | 79.4 | 79.0 |
| (Trisodium phosphate + hard water) | 75.1 | 77.1 | 74.0 |
| Sodium hexametaphosphate | 75.7 | 76.7 | 77.1 |
| (Sodium hexametaphosphate + hard water) | 76.1 | 78.7 | 77.4 |
| Sodium tripolyphosphate | 78.9 | 78.6 | 78.6 |
| (Sodium tripolyphosphate + hard water) | 76.9 | 78.4 | 78.4 |
| Tetrasodium pyrophosphate | 75.2 | 76.4 | 77.2 |
| (Tetrasodium pyrophosphate + hard water) | 75.9 | 76.0 | 76.0 |
| EDTA plus sodium hydroxide | 78.5 | 79.3 | 79.4 |
| Sodium gluconate | 77.9 | 77.9 | 78.1 |
| Wetting agent | 68.4 | 32.8 | 27.9 |
| Gluconic acid | 77.4 | 77.9 | 80.5 |
| Phosphoric acid | 77.8 | 79.2 | 79.2 |

[a]Each entry is an average of 32 readings; 16 are from each replication.

[b]Underscored values indicate no significant defference at 5.0% level of probability according to Duncan's New Multiple Range Test.

temperature in the experiment involving the wetting agent was more pronounced and explained 25.7% of the total variation. The wetting agent used in the study seemed to markedly emulsify the milk fat at the higher temperatures.

In hard water, solutions containing trisodium phosphate or sodium hexametaphosphate were less turbid at 45 C than at either 20 or 70 C. Solutions of sodium tripolyphosphate were less turbid at 45 C and 70 C than at 20 C, but no difference was observed among solutions at 45 and 70 C. Temperature had no effect on transmittance of solutions of tetrasodium pyrophosphate.

*Effects of time*

Because polyphosphates in solution tend to revert to simple phosphates with time (*2*), effects of holding four types of phosphate solutions at 70 C for 8 h each day for 4 days were determined. Samples contained 0, 0.01, 0.1 and 1.0% milk. No changes in transmittance with time were observed with (*a*) the lower concentration (0.75%) of trisodium phosphate, (*b*) either concentration (0.3 and 0.9%) of sodium tripolyphosphate, or (*c*) the higher concentration (0.9%) of both sodium hexametaphosphate and tetrasodium pyrophosphate. Generally, transmittance decreased with time at the higher concentration (2.25%) of trisodium phosphate and the lower concentration (0.3%) of sodium hexametaphosphate and tetrasodium pyrophosphate (Table 4). Concentration of milk appeared to effect amounts of precipitation in solutions of only sodium hexametaphosphate (0.3%) in which there was no effect of time on transmittance in water or 0.01% milk.

TABLE 4. *Effects of time on percent transmittance of solutions of phosphates and milk in hard water*[a]

| Phosphate | Days of storage | Percent milk | | | |
|---|---|---|---|---|---|
| | | 0.0 | 0.01 | 0.1 | 1.0 |
| | | Percent transmittance | | | |
| | 1 | 89.00 | 89.00 | 88.00 | 22.00 |
| Trisodium | 2 | 90.00 | 88.50 | 75.50 | 21.00 |
| (2.25%) | 3 | 77.00 | 73.75 | 63.50 | 19.40 |
| | 4 | 61.75 | 59.50 | 52.75 | 21.75 |
| Sodium | 1 | 98.75 | 97.50 | 85.00 | 23.25 |
| hexameta | 2 | 99.75 | 97.00 | 85.50 | 24.00 |
| (0.3%) | 3 | 99.50 | 96.75 | 84.50 | 22.75 |
| | 4 | 97.38 | 96.25 | 77.75 | 20.00 |
| Tetrasodium | 1 | 96.00 | 94.00 | 81.50 | 23.00 |
| pyro | 2 | 94.00 | 91.75 | 80.50 | 22.50 |
| (0.3%) | 3 | 92.00 | 87.75 | 77.25 | 23.00 |
| | 4 | 82.00 | 78.75 | 68.00 | 19.50 |

[a]Results are shown for only those ingredients and concentrations for which there was an effect of time of storage.

Although transmittance increased with time under certain conditions, effects on determining concentration of milk by transmittance measurement were minimal. Results further suggested that it would not be difficult to formulate detergents in which time would have no effect on transmittance.

## CONCLUSIONS

Major ingredients used in the formulation of commercial detergents, with the exception of the wetting agent, did not interfere with transmittance in milk-ingredient-water solutions to any practical extent. The wetting agent severly reduced transmittance. Except for the lowest concentration of milk, 0.01%, in solutions of trisodium phosphate in hard water, transmittance was lowered significantly by each stepwise increase in concentration of milk from 0.0 to 1.0%. Therefore, it appears that the light spectrophotometer may well be used to measure milk residue in cleaning solutions free from large concentrations of wetting agents like DC-161.

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# Measurement by Spectrophotometry of Concentrations of Raw Milk in Circulating Solutions of Detergents[1]

**E. L. RUIZ[2], D. B. BROOKER[3], M. E. ANDERSON[4], J. R. FISCHER[4], and R. T. MARSHALL[5]**

*PINFST Philippine Women's University, Manila[2]; Department of Agricultural Engineering,[3] and Department of Food Science and Nutrition,[5] University of Missouri; Columbia, Missouri 65201; and U.S. Department of Agriculture, Agricultural Research Service, North Central Region, Columbia, Missouri[4] 65201*

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## ABSTRACT

Studies were made with alkaline and acid detergent solutions at three temperatures (20, 45, and 70 C) and four milk concentrations (0.00, 0.01, 0.10, and 1.0%). Turbidity measurements were made continuously while the solutions were circulating through a piping system. The turbidity of milk-detergent-water solutions was found to be primarily influenced by the concentration of milk. As little as 0.01% milk caused a significant change in turbidity (1.4%), and the percentage of transmittance decreased an average of 78.6 when 1% milk was added to the water that contained the detergent. Detergent concentrations and temperature of the solutions had only minor effects on turbidity. Formation of precipitates by action of phosphates in hard water did not decrease the sensitivity of the method to added milk.

The cleaning cycle of a cleaned-in-place (CIP) dairy processing system includes four phases: rinse, alkali, acid, and postrinse. The CIP cleaning techniques now use arbitrary time limits on each phase of the cycle. Time, materials, and energy could be saved if each phase were terminated when cleaning is complete. Controls cannot be properly designed until some property of the milk-detergent-water (MDW) mixture is found that can be continuously measured or monitored under "on-line" operating conditions.

The white color of milk is caused by reflected light that is scattered by minute fat globules and colloidal calcium caseinate and calcium phosphate (*5*). These components of milk are normally found in cleaning solutions after use. We previously reported that ingredients normally used in the formulation of commercial detergents, with the exception of wetting agent, do not interfere with the turbidimetric measurement of milk in water that contains the individual ingredient (*3*).

In the present study a spectrophotometer was used to continuously monitor changes in the turbidity of MDW solutions flowing in a pipe. Relationships of turbidity to milk concentration in the MDW solution for both the alkaline and acid phases of cleaning were studied under "on-line" operating conditions.

[1]*Contribution from the University of Missouri Experiment Station. Journal Series No. 7047. Trade names and names of commercial companies are used in this publication solely to provide specific information. Such mention does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture or an endorsement by the Department over other products not mentioned.*

## MATERIALS AND METHODS

### *Materials*

Two commercial detergents, a dry alkali (Klenzade HC-41) and a liquid acid (Klenzade AC-3) were used in studies with the circulating system. The piping system and method of adding milk to the system have been reported (*1, 2*). Turbidity of solutions was determined in a

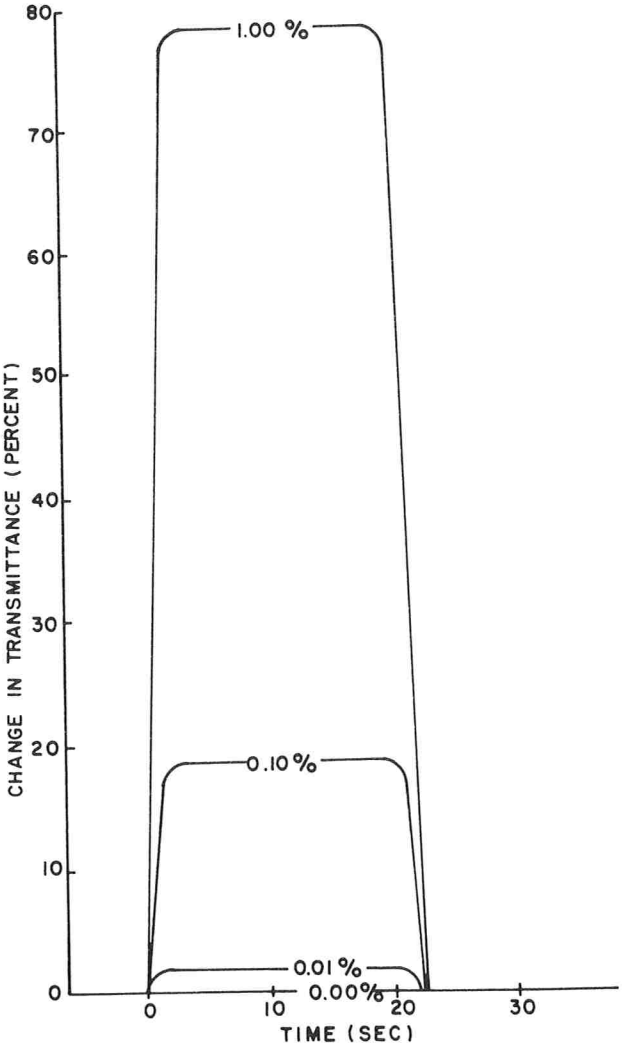


Figure 1. *Typical instrument responses for each of the four concentrations (%) of milk*

Bausch and Lomb Spectronic 20 equipped with a locally fabricated ½-inch-diameter flow-through cuvette.

Eighteen commercial detergents, sold for cleaning dairy equipment, were used to study effects of hard water (230 and 342 mg/1) on turbidity. Changes in turbidity caused by these detergents were measured in a standard cuvette.

*Methods*

The experimental design used with the circulating system was a split, split plot with two replicates. Each replicate consisted of 48 treatments. Four concentrations of detergent (0.0, 0.03, 0.3, and 3.0%), three temperatures (20, 45, and 70 C), and four concentrations of milk (0.0, 0.01, 0.1, and 1.0%) were studied in all possible combinations. The same experimental design was followed for both detergents.

Before each series of tests, the solution reservoir was filled with softened water (<5 ppm calcium), the required concentration (0.0, 0.03, 0.3, and 3.0%) of detergent was added, and the detergent-water (DW) solution was heated to the required temperature while being recirculated through the system at approximately 150 cm/sec.

A small portion of the solution flowing in the pipe was diverted continuously through a flow-through cuvette. The spectrophotometer was set at 527 nm, the wavelength of maximal absorbance of milk in water, and was adjusted to read 100% transmittance with the DW solution. Milk was then injected into the DW solution at a constant rate for 20 seconds. The instrument response was recorded on a strip chart recorder as change in the percentage of transmittance. Typical recordings for the four concentrations of milk are given in Figure 1.

The two sets of data analyzed for each experiment were: (*a*) average maximum change in instrument response and (*b*) total milligrams of milk solids measured as compared with the amount injected. Data points selected from the maximum response portion of each curve (flat portion) at 1-sec intervals were averaged to obtain the average maximum change in instrument response. Differences in average maximum changes produced by the same concentrations of milk were compared by analysis of variance (ANOVA). Totals of the three-way interactions from the ANOVA were used with the error mean square to obtain the sums of squares that were assignable to each of the 47 orthogonal components (*5*).

Response equations for each detergent were obtained by using, in a multiple regression computer program, those components that contributed significantly ($P > 0.05$) to the total sum of squares. Response equations for each detergent were used as standard curves to determine the total amount of milk detected by the spectrophotometer. The response equations and plots of the equations showing transmittance as a function of milk concentration in alkaline and acid detergents are given in Figures 2 and 3, respectively. Each measured value (change in percentage of transmittance) for each time interval (sec) was converted into its equivalent in milligrams of milk solids by using the response equations. The amounts of solids for each second were then summed over the total test time to obtain the total milligrams of solids measured.

A study was conducted to measure effects on turbidity of interactions of 18 commercial detergents with hard water (230 mg/1). Solutions were prepared in BOD bottles at concentrations recommended by the manufacturers. Milk was added at concentrations of 0.0, 0.01 and 0.1%. Samples were shaken, heated to 70 ± 2 C, and examined for turbidity. There were three replications.

In addition, tests were made to determine effects of time of holding on turbidity of solutions containing milk and 19 detergents. Milk (0.0 and 0.1%) was added to solutions containing manufacturer's recommended concentrations of detergent in hard water (342 mg/1). Hardness of the water was increased with $CaCl_2$ to provide a more informative test. Samples were shaken, heated to 70 ± 2 C and examined for turbidity. The samples were reheated daily for 8 h per day for 4 days. Turbidity measurements were taken daily.

## RESULTS AND DISCUSSION

### *Effects of main variables-alkaline detergent*

Data in Table 1 illustrate effects of the major variables for the experiments with alkaline detergent. The combined main effects of milk accounted for 99.7% of the total sum of squares attributable to regression. No significant differences ($P < 0.05$) were noted as a result of

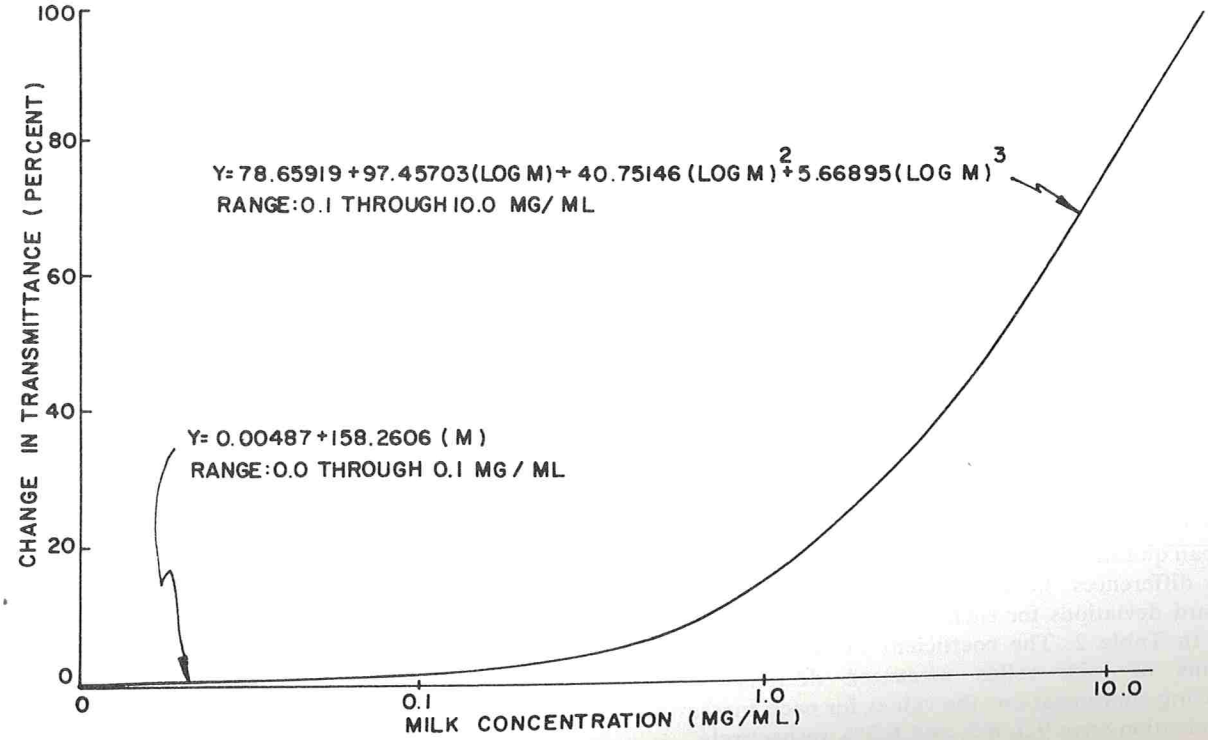


Figure 2. *Response equations and a plot of equations showing transmittance as a function of milk concentration in an alkaline detergent solution*

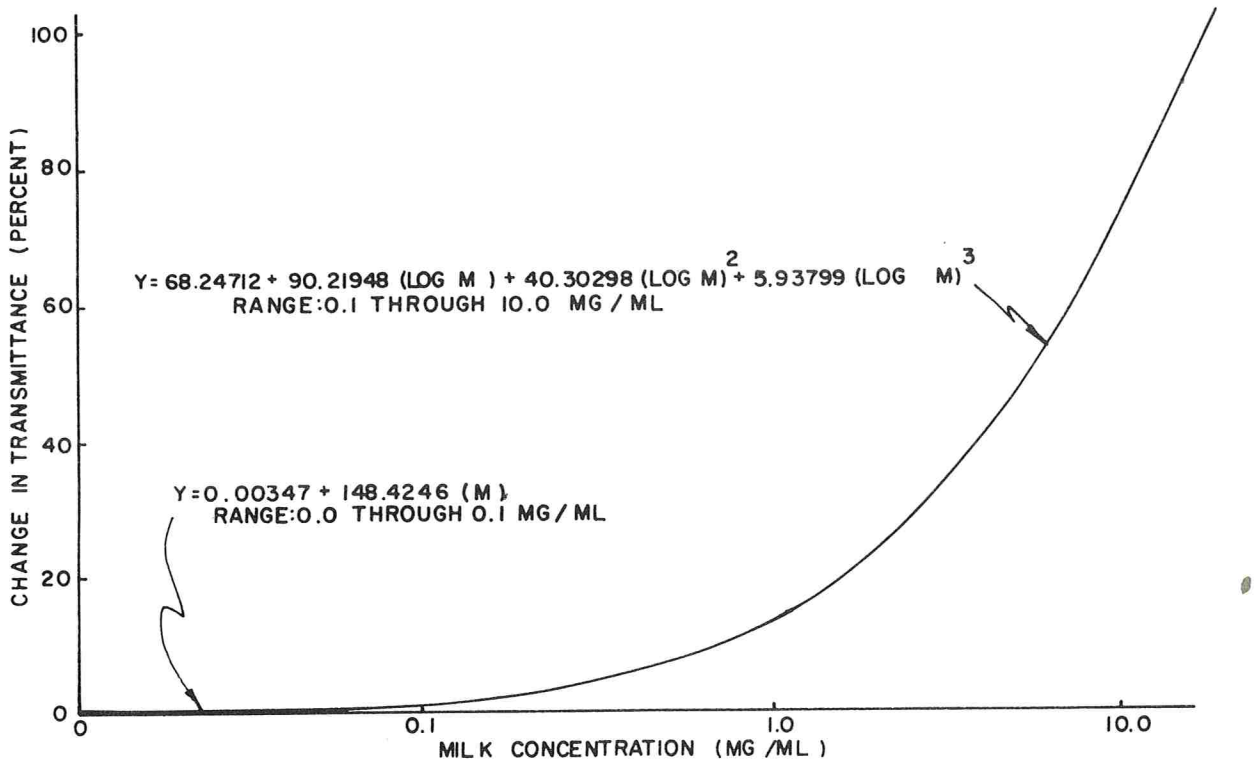


Figure 3. *Response equations and a plot of equations showing transmittance as a function of milk concentration in an acid detergent solution*

TABLE 1. *Average maximum change in percentage of transmission for four concentrations of alkaline detergent, three temperatures, and four concentrations of milk*

| | | | | |
|---|---|---|---|---|
| Alkaline detergent concentration, % | 0.00 | 0.03 | 0.30 | 3.00 |
| Mean, change in % T[a,b] | 25.2 | 24.9 | 23.2 | 23.0 |
| Temperature, C | 20 | 45 | 70 | |
| Mean, change in %T | 24.1 | 24.0 | 24.2 | |
| Milk concentration, % | 0.00 | 0.01 | 0.10 | 1.00 |
| Mean, change in % T | 0.00 | 1.40 | 16.3 | 78.6 |

[a]% T = percentage of transmission.

[b]Underscored values indicate no significant difference at the 5.0% level.

changes in concentrations of detergent or temperature. Therefore, the effects of concentration of detergent and differences in temperature were not used in the development of the response equations (Figure 2). A concentration of 0.01% milk caused a mean change of 1.4% in the percentage of transmittance. Concentrations of 0.1 and 1.0% milk caused mean changes of 16.3 and 78.6%, respectively, in the percentage of transmittance.

*Measurement of total solids injected-alkaline detergent*

Quantities of milk injected into flowing alkaline solutions were measured with acceptable accuracy (Table 2). Mean quantities of milk solids (mg) injected and measured, differences between the two, and respective standard deviations for each concentration of milk are given in Table 2. The coefficient of variation for the amounts of milk solids measured decreased with increasing concentration; the values for each increasing concentration were 9.3, 6.5, and 6.3%, respectively.

Percentage errors of -3.08, -0.50, and 2.00% were noted for the 0.01, 0.1, and 1.0% concentrations of milk, respectively. Percentage of error = (amount injected - amount measured/amount injected) × 100. A minus sign before the number indicates that the measured value was lower than the amount injected. Average percentages of error for 0.0, 0.03, 0.3, and 3.0% alkaline detergent were 4.66, 1.17, –2.80, and –4.61%, respectively. For the three temperatures the measured values, on the average, were in error by 3.10, –0.39, and –3.39%, respectively, for 20, 45, and 70 C.

TABLE 2. *Mean of quantities of milk solids (mg) injected and measured, differences between the two, and respective standard deviations for the alkaline detergent*

| Conc. of milk (%) | Amount injected (mg) | | Amount measured (mg) | | Difference (mg) | |
|---|---|---|---|---|---|---|
| | Mean | Std. dev. | Mean | Std. dev. | Mean[a] | Std. dev. |
| 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 0.01 | 409 | 43 | 394 | 37 | 15 | 28 |
| 0.10 | 4172 | 421 | 4104 | 268 | 68 | 395 |
| 1.00 | 40624 | 4160 | 40858 | 2600 | –234 | 4167 |

[a]Amount injected minus amount measured.

*Effects of main variables - acid detergent*

Milk markedly affected transmittance measurements, being responsible for 99.9% of the total sum of squares. No significant differences in instrument response were noted as a result of changes in concentration of acid detergent or temperature. These two variables were not used in formulating the response equation (Figure 3).

Comparisons among means (average maximum change in the percentage of transmittance) assigned to main effects of the variables involved in the formulated acid detergent experiment are shown in Table. 3. Only milk was a significant variable.

TABLE 3. *Average maximum change in percentage of transmission for four concentrations of acid detergent, three temperatures, and four concentrations of milk*

| | | | | |
|---|---|---|---|---|
| Acid detergent concentration, % | 0.00 | 0.03 | 0.30 | 3.00 |
| Mean, change in % T[a,b] | 20.4 | 21.1 | 20.1 | 20.5 |
| Temperature, C | 20 | 45 | 70 | |
| Mean, change in % T | 21.0 | 20.2 | 20.4 | |
| Milk concentration, % | 0.00 | 0.01 | 0.10 | 1.00 |
| Mean, change in % T | 0.00 | 1.50 | 12.4 | 68.2 |

[a]% T = percentage of transmission.
[b]Underscored values indicate no significant difference at the 5.0% level.

*Prediction of total solids injected - acid detergent*

Means of quantities of milk solids (mg) injected and measured, differences between the two, and respective standard deviations for each concentration of milk are in Table 4. The coefficients of variation for amount injected

TABLE 4. *Mean of quantities of milk solids (mg) injected and measured, differences between the two, and respective standard deviations for acid detergent*

| Conc. of Milk (%) | Amount injected (mg) | | Amount measured (mg) | | Difference (mg) | |
|---|---|---|---|---|---|---|
| | Mean | Std. dev. | Mean | Std. dev. | Mean[a] | Std. dev. |
| 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 0.01 | 410 | 30 | 396 | 36 | 14 | 27 |
| 0.10 | 4084 | 550 | 3777 | 369 | 307 | 289 |
| 1.00 | 39529 | 4010 | 37453 | 2176 | 2076 | 3481 |

[a]Amount injected minus amount measured.

for each increasing concentration of milk were 7.3, 13.5, and 10.1%, respectively. Coefficients of variation for amounts measured were 9.1, 9.7, and 5.8%, respectively. The percentages of errors for all concentrations of milk were negative, indicating that measured values were higher than the actual values. The percentages of error were –3.31, –4.56, and –6.75%, respectively, for the 0.01, 0.1, and 1.0% concentrations of milk. The average errors for 0.0, 0.03, 0.3, and 3.0% acid detergent were –5.38, –2.41, –1.51, and –5.32%, respectively. Measured quantities of milk solids detected averaged over all other variables had errors of 0.46, –3.77, and –6.73% for temperatures of 20, 45, and 70 C, respectively.

*Effects of hard water*

Precipitates which form when hard water is softened by sequestering action of phosphates could conceivably interfere with transmittance measurements. The present experiments showed, however, that this is not a significant problem. Transmittance values for solutions of detergents to which no milk was added were as follows: 12 exceeded 99%, four were greater than 98%, one was 97%, and one was 83%. Formation of precipitate did not decrease the sensitivity of the transmittance tests. Decreases in transmittance averaged over all detergents with 0.01 and 0.1% milk added were 1.4 ± 0.25 and 14.0 ± 0.53%, respectively. Solutions containing the detergent that produced the greatest turbidity in water were observed to decrease in transmittance by 2.1 and 13.8% when 0.01 and 0.1% milk were added, respectively.

*Effects of time*

Because detergent solutions may be used to clean several pieces of equipment over a period of time, the effect of time on transmission was studied. Additions of 0.1% milk to hard water (342 mg/1) caused an initial mean change in transmittance of –14.5 ± 7.; % Turbidity stabilized after 2 days so the average difference in transmittance (% T, detergent without milk *minus* % T, detergent with 0.1% milk) was –11.0 ± 7.0%. Decreases in transmittance over the first 48 h averaged 8.7 ± 7.3% and 5.1 ± 6.0% for solutions with 0.0 and 0.1% milk, respectively. Increases in transmittance with time were observed for 3 solutions containing 0.1% milk.

## CONCLUSIONS

From this study it was concluded that:

1. Changes in transmittance were mainly a function of milk concentration in solutions containing milk, detergent, and water.
2. Variations in temperature and concentration of detergent were not important factors.
3. Water hardness interacted with time of holding solutions causing variations in transmittance, but errors could be eliminated by observing transmittance prior to beginning the cleaning operation.
4. The spectrophotometer has good potential for monitoring concentrations of milk in solutions of detergents in CIP systems.

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# Induction of Prophage in Lactic Streptococci Isolated from Commercial Dairy Starter Cultures[1]

**CHANHO PARK[2] and L. L. McKAY**

*Department of Food Science and Nutrition*
*University of Minnesota, St. Paul, Minnesota, 55108*

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**ABSTRACT**

Twelve commercial dairy starter cultures were examined for presence of lysogens by treating isolated strains with ultraviolet irradiation or mitomycin C. Induction of lysis in isolates from nine of the 12 commercial cultures suggested the presence of lysogenic strains. No indicator organisms were detected, but presence of phage was confirmed by electron microscopy in two of the *Streptococcus cremoris* isolates from the commercial cultures. The results confirm that some commercial dairy cultures produced in the United States contain phage-harboring strains.

Reiter (*15*) was the first to demonstrate the occurrence of lysogenic strains of lactic streptococci in commercial dairy starter cultures. Graham et al. (*6*), however, were unsuccessful in demonstrating their presence in commercial cultures produced in the United States, although they did find that certain of these cultures carried bacteriophage. Their procedure of cross-streaking suspected lysogenic strains may not have been adequate for their detection. Recently it has been shown by using inducing agents such as ultraviolet (UV) irradiation or mitomycin C, that lysogenic strains of Group N streptococci are quite common (*8*). The origin of phage in the cheese plant is not definitely known, but as suggested by Czulak and Naylor (*4*), lysogenic strains must be considered as a possible reservoir. The objective of this investigation was to screen for lysogenic lactic streptococci in commercial dairy starter cultures produced in the United States.

## MATERIAL AND METHODS

*Growth of cultures*

All lactic streptococci used in this study were maintained by weekly transfer in sterile (121 C, 15 min) 11% reconstituted nonfat milk (RNFM) (Matrix Mother Culture Medium, Galloway-West, Fond du Lac, Wisc.) with incubation at 21 C until the milk coagulated. Between transfers, the organisms were stored at 4 C. Lactic broth (*5*) was also used to propagate the organisms. Twelve commercial starter cultures which were designated A through L to facilitate reporting, were examined as possible lysogens.

To isolate the component strains from the commercial cultures, a portion of each was inoculated into 100 ml of sterile 11% RNFM and incubated at 21 C until coagulated. Lactic agar plates were then spread with 0.1 ml of an appropriate cell dilution of the coagulated culture and incubated at 32 C for 48 h. Individual colonies were picked into 11% sterile RNFM and incubated at 21 C until coagulated. The isolates were then transferred to lactic broth and incubated at 32 C for 24 h. To distinguish between *Streptococcus lactis* and *Streptococcus cremoris* the isolates were inoculated into Niven's arginine broth, incubated at 32 C for 48 h and examined for ammonia production (*12*). To further confirm the results procedures described by Sandine et al. (*17*) were used.

*Induction with UV irradiation or mitomycin C*

Growth and UV irradiation procedures were those previously described by McKay and Baldwin (*10*). For mitomycin C (Sigma Chemical Company or Calbiochem) induction, the procedure was the same as with UV induction except centrifuged cells were suspended in broth instead of 0.85% NaCl. Each experimental tube consisted of 1 ml of the cell suspension and various concentrations (0.1, 0.5, 1.0, 2.5 or 5.0 μg per ml) of mitomycin C. Total volume of each tube was 10 ml. Tubes were incubated at 32 C and the change in absorbance was measured as for UV induction. Various strains of *S. lactis* or *S. cremoris* were examined as possible indicator strains for the phage released and induced lysates were also examined for the presence of phage using electron microscopy as previously described (*10*).

## RESULTS

The presence of bacteriophage in a mixed-strain starter culture is one of the factors which may cause a change in the balance of microorganisms. To help prevent this imbalance it should be determined if the individual strains to be incorporated into mixed cultures are lysogenic, or susceptible to phage released by lysogens. Studies were conducted to determine if

TABLE 1. *Isolation of* S. cremoris *and* S. lactis *strains from commercial dairy starter cultures*

| Commercial culture designation | No. of colonies examined | No. of *S. lactis* | No. of *S. cremoris* |
|---|---|---|---|
| A | 8 | 3(3)[a] | 5(5) |
| B | 32 | 7(1) | 25(4) |
| C | 27 | 0 | 27(6) |
| D | 22 | 0 | 22(5) |
| E | 23 | 0 | 23(4) |
| F | 16 | 2 | 14(5) |
| G | 19 | 8(3) | 11(3) |
| H | 18 | 3(2) | 15(4) |
| I | 34 | 1(1) | 33(5) |
| J | 33 | 0 | 33(6) |
| K | 35 | 0 | 35(6) |
| L | 35 | 0 | 35(6) |

[a]The number in parentheses indicates the number of strains examined for lysogeny.

[1]*Scientific Journal Series. Paper No. 9024, Minnesota Agricultural Experiment Station, St. Paul, Minnesota, 55108.*

[2]*This work was taken from a thesis submitted by Chanho Park to the faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Master of Science.*

lysogenic strains could be identified in 12 commercial lactic starter cultures used in the United States. Most of the commercial starter isolates examined appeared to contain *S. cremoris* only, or at least a high proportion of *S. cremoris* (Table 1). The purpose here was not to differentiate the strains isolated from each culture but rather to isolate at least one *S. lactis* and one *S. cremoris* from each commercial culture and examine these as possible lysogens. Data in Table 1 also indicate the number of strains examined for induction of lysis by UV irradiation or mitomycin C.

Among eight strains examined for UV induced lysis in culture A, all were lysed except for one of the *S. cremoris* strains. The seven strains were induced to lyse after a 25 sec exposure to UV irradiation. Figure 1 shows a typical

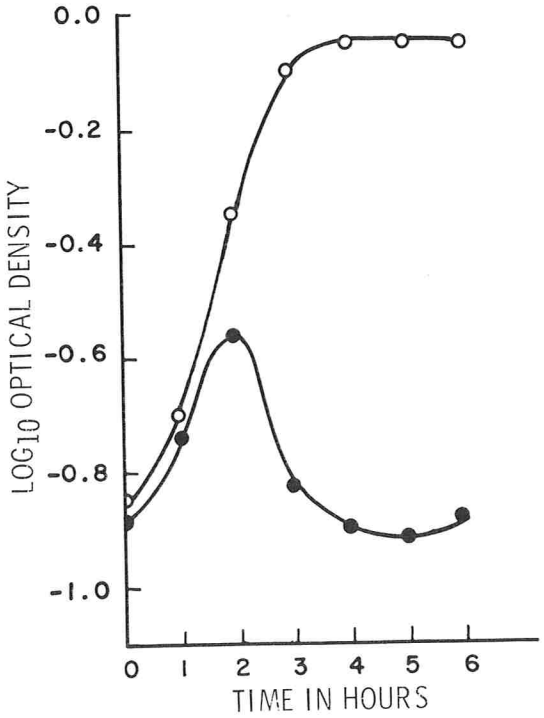



Figure 1. *Induction of lysis in* S. cremoris *A-7 by UV irradiation. An exponentially growing culture was harvested, washed, and exposed to UV irradiation for 25 sec. The irradiated suspension was then inoculated into broth and the change in absorbance was measured* ( ● ). *Unirradiated cells served as the control* ( ○ ).

lysis curve using *S. cremoris* A-7 as an example. In culture B five strains were examined and *S. lactis* B-4 and *S. cremoris* B-26 showed a similar induction response after 25 sec UV irradiation. Figure 2 shows a typical response using mitomycin C. Except for *S. cremoris* C15, no culture C strain exhibited lysis by exposure to UV irradiation. However, induction did occur in the other strains using mitomycin C. In culture D no UV induction was observed, although induction did occur using mitomycin C. In culture E, *S. cremoris* E-25 did not lyse after exposure to UV irradiation but some lysis occurred after exposure to high concentrations of mitomycin C. No induction of lysis was observed in the strains examined from culture F using different doses of

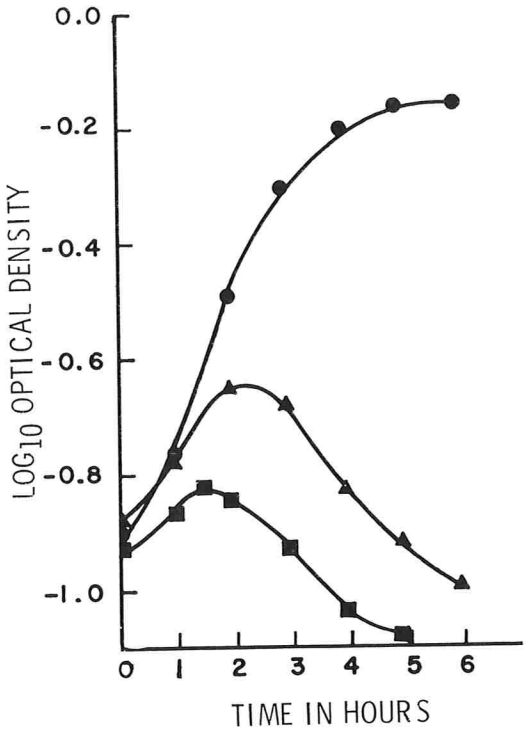
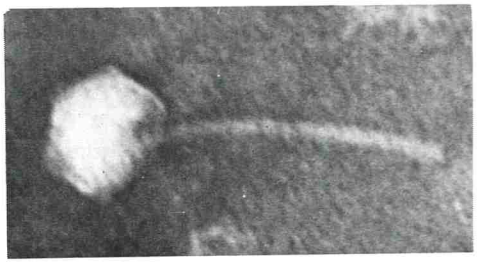



Figure 2. *Induction of lysis in* S. cremoris *B-26 by mitomycin C. An exponentially growing culture was harvested, washed, and resuspended in broth containing 0* ( ● ), *1.0 μg* ( ▲ ), *or 2.5 μg* ( ■ ) *mitomycin C per ml and the change in absorbance was measured.*



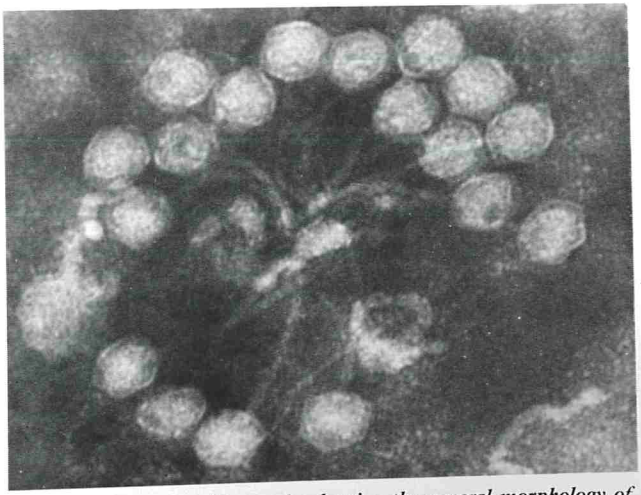


Figure 3. *Electron micrographs showing the general morphology of the latent bacteriophages induced from two commercial strains. Upper:* S. cremoris *A-7 bacteriophage* (× *125,000*). *Lower:* S. cremoris *K1 bacteriophage* (× *125,000*).

UV irradiation or mitomycin C concentrations ranging from 0.5 to 5.0 μg/ml. Similar negative results were observed for isolates from cultures G and J. With culture H, only *S. cremoris* H-18 was induced by UV irradiation. *S. lactis* H-9 showed some lysis by mitomycin C. *S. cremoris* I-25 was the only strain induced to lyse from culture I. In culture K, all the strains examined were lysed using a 25 sec irradiation period and 2.5 μg mitomycin C per ml. The strains isolated from culture L also were induced by UV irradiation or mitomycin C.

To confirm the presence of phage in the induced lysates, the spectrum of each lysate was determined to measure its absorbance at 260 nm. Only lysates showing a marked absorbance and a peak at 260 nm were treated for examination under the electron microscope. Figure 3 reveals the structure of phages induced from *S. cremoris* A-7 and *S. cremoris* K-1, respectively. Although phages were not observed in some of the other lysates examined, it is believed the concentration was not sufficient for their detection. The procedure described by Nyiendo et al. (*13*) for preparation of high-titer lactic streptococcus bacteriophages would aid in preparing lysates for EM observation. Two strains were thus confirmed to be lysogenic. This may be the first confirmed demonstration of lysogenic strains among isolates from commercial dairy cultures in the United States.

## DISCUSSION

This investigation was undertaken to examine for lysogenic lactic streptococci in commercial dairy starter cultures. To test commercial cultures, the strains were isolated from each culture and differentiated as to species. Since cultures of *S. cremoris* have been found most suitable for producing aged grade AA Cheddar cheese, it was not surprising that most of the commercial starters examined possessed a high proportion of this organism.

The phenomenon of lysogeny in Group N streptococci has recently been investigated in several laboratories. Reiter in 1949 (*15*), however, was the first to demonstrate lysogenic strains of lactic streptococci and stated that they may be common among starter cultures. Sandine et al. (*16*) demonstrated lysogeny in *S. cremoris* W and stated that temperate phages may play a role in metabolic variation among lactic streptococci. More recently, Keogh and Shimmin (*7*) demonstrated a temperate phage in *S. cremoris* C11-56 and McKay and Baldwin (*10*) induced a phage from *S. lactis* C2 which was later found to be a transducing phage (*11*). A comprehensive study of lysogeny in lactic streptococci was conducted by Kozak et al. (*8*) in 1973 in which 87 strains were tested for lysogeny. Lysogeny was found in five *S. lactis* strains and two *S. diacetilactis,* but no lysogens were found among the *S. cremoris* examined. Lowrie (*9*) however, in his recent investigation found 4 of 22 *S. cremoris* strains and 2 of 7 *S. lactis* strains to be susceptible to induction of lysis by UV irradiation or mitomycin C. Results of this study confirm the results that lysogens are quite common among the lactic streptococci. Nine of the twelve commercial dairy cultures were found to contain *S. lactis* or *S. cremoris* strains susceptible to lysis by prophage inducing agents such as UV irradiation or mitomycin C. Two of the lysates were confirmed to contain phage by electron microscopy.

Induction of prophage in lysogenic strains depends upon many factors. Since induction by UV irradiation may depend upon the dosage and environmental factors, the percentage of cells induced may be low or none at all. Kozak (*8*) reported that one strain of *S. lactis* liberated phage spontaneously but was not inducible; other workers have also shown that some phages are not inducible (*2, 3*). Kozak (*8*) also found that in one strain of *S. lactis,* only 20 to 37% of the cells in the population were induced by UV irradiation. Thus the optimal UV dosage will vary. In this report the relationship between cell lysis and duration of UV irradiation was noted. One strain lysed after 15 sec while another lysed after a 90 sec exposure to UV irradiation. The ability of UV irradiation to induce cells also depends on the nutritional environment, the depth of the sample under irradiation, and stage of growth of the bacterial culture (*1*). The effective concentration of mitomycin C was also variable and ranged from 0.5 to 5.0 μg per ml. It has been suggested that the effective concentration depends on the strain, the lysogenic state, population density, generation time, and composition of the growth medium (*3, 14*). In this report, the factors which influenced the induction of prophage from lactic streptococci were not examined other than UV dosage and mitomycin C concentration.

Since lysogeny appears to be common among Group N streptococci it may not be possible, using the present strains, to guarantee production of phage free dairy starter cultures. It may be possible, however, to isolate cured strains (strains free of phage) and if these possess no metabolic defects, to incorporate them into starter cultures. Such strains would not only guarantee phage-free cultures but also possibly reduce the phage problem.

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# Survival of Enteric Viruses on Fresh Fruit

**JACK KONOWALCHUK and JOAN I. SPEIRS**

*Bureau of Microbial Hazards, Health Protection Branch, Health and Welfare Canada, Ottawa, K1A OL2*

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**ABSTRACT**

Several enteric viruses were examined for their survival at 4 C on strawberries, cherries, and peaches. With few exceptions, recovery of virus from fruit maintained in a humid atmosphere decreased with time; recoveries from strawberries were especially low; recoveries of coxsackievirus and echovirus were greater than those of poliovirus and reovirus. Aqueous fruit infusions were antiviral and in general correlated with virus loss on the fruit surfaces. An infusion prepared from intact grapes inactivated poliovirus. Desiccation was another contributing factor in virus destruction. No naturally-occurring virus was found in nine fruit samples examined in this study.

During growth, fruit may become contaminated with enteric viruses through the use of sewage for fertilizer or polluted water for irrigation or spray. During harvesting or marketing, fruit may be contaminated by polluted rinse water or infected handlers. Since many fruits are purchased fresh or frozen and are eaten raw, their importance as a health hazard must be considered. However, we are aware of only one published report in which fruit was implicated as the cause of a virus ailment. In 1965 strawberries were suspected as the source of 19 infectious hepatitis cases in a small community in New Jersey, U.S.A. (*1*). The results of studies on the survival of several representative human enteric viruses as surface contaminants of three fruits, strawberry, cherry and peach are presented in this report.

## MATERIALS AND METHODS

### *Viruses and cells*

Coxsackievirus type B5 and poliovirus type 1 (Sabin) were grown and assayed in HEp-2 cells, echovirus type 7 in Vero cells and reovirus type 1 in primary African green monkey kidney cells. The sources of the viruses and cells have been described elsewhere (*5*). Medium 199 containing 10% fetal bovine serum was used for HEp-2 and Vero cells, medium 199 containing 2% fetal bovine serum for monkey kidney cells. Cell stocks were grown in Roux bottles. Monolayers, for assay, were prepared from stock cultures in 60 × 15 mm plastic dishes, 24 h prior to use. Virus dilutions were made in water, or if indicated, dilute feces (*5*).

### *Inoculation of fruit*

Strawberries, cherries, and peaches were purchased from a farmer's market and local grocery stores. Fruit was thoroughly rinsed in tap water and air-dried to remove surface moisture before positioning on inverted bottle tops in open glass dishes. A 0.05 ml drop of water or dilute feces containing 400 plaque-forming units (PFU) of virus was placed on each fruit. A similar inoculum was added to screw-capped bottles containing 10 ml of phosphate-buffered saline (PBS) for virus control or in an infusion of 8 ml of water and a 1 $cm^3$ piece of strawberry, cherry or peach, or a whole cherry. Samples were stored at 4 C for one to several days. Fruit samples were left uncovered to air-dry or placed in polyethylene containers with tight-fitting lids. Water in the bottom of the container provided humidity.

Virus was removed from fruit by washing with 10 ml of PBS containing 1% fetal bovine serum, pH 8.0, or if so indicated, with 8 ml of distilled water, pH 7.0 to 8.0. The wash was repipetted 10 times over the inoculum site and centrifuged at 15,000 × *g* for 30 min to remove debris and bacteria. (Comparative experiments showed that there were no significant differences in virus counts between portions of control or wash samples centrifuged and not centrifuged.) Triplicate aliquots of 2.5 ml of PBS wash or virus control were placed on appropriate monolayers for assay. Triplicate aliquots of 2.0 ml of water wash or fruit infusion (the latter were first centrifuged at 15,000 × *g*) were mixed with 0.5 ml of equal volumes of 10X medium 199, pH 7.4, and fetal bovine serum, before assay. Cultures were shaken for 2 h at room temperature (about 22 C) to allow virus to adsorb. The samples were poured off, an agar overlay applied (*3*), and cultures were incubated at 36 C in a humidified, 5% $CO_2$ atmosphere. Enterovirus plaques were counted after 3 days, and reovirus plaques after 10 days. Plaque counts were averaged and expressed as percent of the 0-time virus control (input virus). Differences greater than 25% (the maximum experimental error) were considered significant. (The percentage error of the assay system has been found to vary from 2 to 25%, with a mean of 9%.)

### *Grape infusion*

Blue table grapes were purchased from a local grocery store and rinsed in tap water. Eight grapes, with stems attached, and without apparent skin blemishes, were placed stem side up in a pyrex dish. Twenty-five milliliters of distilled water were added, immersing the bottom half of each grape. After storage at 4 C for 24 h, the water (grape infusion) was removed and centrifuged for 30 min at 15,000 × *g*. An inoculum of 400 PFU of poliovirus was added to an 8-ml aliquot of grape infusion and to 8 ml of water for virus control. Samples were stored at 4 C for 24 h. Two ml of 5X concentrated medium were added, and samples assayed on four monolayers. Plaque counts were averaged and compared.

### *Strawberry extract*

Strawberries were mechanically blended for 1 min with distilled water, 2:1 (weight:volume), and centrifuged at 1000 × *g* for 20 min. The supernatant fluid was centrifuged at 15,000 × *g* for 30 min to remove solids and bacteria. The clear liquid (extract) was diluted 1:10 in water. Four 8-ml aliquots of the diluted extract or water were adjusted, if required, to pH 3.5 and 7.0 with HC1 or NaOH, inoculated with 400 PFU of coxsackievirus or poliovirus, and stored at 4 C for 24 h. After storage, acid samples were adjusted to pH 7.0, two ml of 5X concentrated medium added, and samples assayed on four monolayers. Plaque counts were averaged and expressed as percent of the count in the water sample, pH 7.0.

### *Test for naturally-occurring viruses in fruits*

At the time fruits were collected for the described tests, additional samples were examined for the presence of naturally-occurring viruses.

A quart of strawberries, a pound of cherries, or six peaches were shaken for several minutes in small groups, or individually in 300 ml of PBS containing 1% fetal bovine serum, pH 8.0. Washes were concentrated to 15 ml with a type PM30 ultrafiltration membrane fitted in a Diaflo Model 402 ultrafiltration cell (Amicon Corp., Lexington, Mass.). Concentrates were centrifuged at 15,000 × *g* for 30 min to remove debris and bacteria. The entire sample, in aliquots of 2.5 ml, was assayed on monolayers of HEp-2, Vero, and primary African green monkey kidney cells. Each monolayer received an agar or liquid overlay of the appropriate growth medium. A blind pass was made from each liquid sample.

## RESULTS

### *Virus recovery from fruit*

After storage at 4 C for 24 h, recovery of coxsackievirus and echovirus from strawberries was less efficient with water than with PBS containing 1% serum. However, a significant portion of input virus was not recovered (Table 1). Under the same conditions, virus recovery from cherries was complete with both water and PBS washes.

TABLE 1. *Recovery of coxsackievirus B5 and echovirus 7 from strawberries and cherries, as percent of input virus, after storage at 4 C for 1 day*

| Fruit | Coxsackievirus | | Echovirus | |
|---|---|---|---|---|
| | Water wash | PBS-serum wash | Water wash | PBS-serum wash |
| Strawberry | 7 ( 4-11)[a] | 43 (40- 46) | 2 ( 0-6 ) | 72 (67-74 ) |
| Cherry | 92 (85-99) | 93 (91-104) | 100 (95-105) | 100 (91-105) |

[a]An average of three samples, extremes in parenthesis

### *Virus survival on fruit and in fruit infusions*

Virus recoveries from strawberries, cherries, peaches and their infusions are shown in Tables 2, 3, and 4. In general, (*a*) virus recoveries from fruit samples, kept moist, decreased with time, (*b*) virus recoveries from strawberries were lower than from the other fruits, (*c*) air-dried fruit samples yielded little virus, (*d*) samples receiving a virus inoculum in feces yielded better recoveries than samples receiving no feces, and (*e*) coxsackievirus and echovirus recoveries were better than those of poliovirus and reovirus. Quantitative recoveries were obtained only with coxsackievirus and echovirus stored for 6 days on cherries and 1 day on peaches.

Virus survival in fruit infusions resembled survival on fruit surfaces. The infusion prepared with a cherry piece was more antiviral than either the whole cherry or the cherry surface.

Poliovirus survival in grape infusion, prepared from apparently intact grapes, was 57% of the virus control.

TABLE 3. *Recovery of viruses from single samples of cherries and infusions, as percent of input virus, after storage at 4 C*

| Storage condition | Coxsackievirus | | Echovirus | | Reovirus | |
|---|---|---|---|---|---|---|
| | 1 day | 6 days | 1 day | 6 days | 1 day | 6 days |
| Humid, no feces | 102 | 100 | 99 | 98 | 58 | 20 |
| Dry, no feces | 1 | <1 | 2 | <1 | 7 | <1 |
| Humid, feces | 98 | 100 | 95 | 98 | 58 | 30 |
| Dry, feces | 1 | <1 | 40 | <1 | 17 | <1 |
| Infusion of fruit piece | 48 | 17 | 27 | 2 | 4 | 3 |
| Infusion of whole fruit | 89 | 36 | 97 | 100 | 60 | 3 |
| Control in PBS | 95 | 106 | 99 | 89 | 87 | 86 |

TABLE 4. *Recovery of viruses from single samples of peaches and infusions, as percent of input virus, after storage at 4 C*

| Storage condition | Coxsackievirus | | Echovirus | | Reovirus | |
|---|---|---|---|---|---|---|
| | 1 day | 4 days | 1 day | 4 days | 1 day | 4 days |
| Humid, no feces | 100 | 14 | 100 | 74 | 30 | <1 |
| Dry, no feces | <1 | <1 | <1 | <1 | <1 | <1 |
| Humid, feces | 101 | 18 | 98 | 88 | 50 | 30 |
| Dry, feces | 1 | <1 | 1 | <1 | <1 | <1 |
| Infusion | 98 | 20 | 101 | 74 | 29 | 3 |
| Control in PBS | 102 | 100 | 97 | 105 | 93 | 89 |

TABLE 5. *Effect of pH of water and strawberry extract on percent survival of coxsackievirus B5 and poliovirus 1 after storage at 4 C for 1 day*

| Medium | pH | Coxsackievirus | Poliovirus |
|---|---|---|---|
| Water | 7.0 | 100[a] | 100[a] |
| Water | 3.5 | 31 (27-38 )[b] | 37 (32-41) |
| Extract | 7.0 | 32 (29-34 ) | 0 ( 0-0 ) |
| Extract | 3.5 | 100 (95-103) | 57 (55-58) |

[a]Virus control.
[b]An average (extremes in parenthesis) of three experiments, in which extracts were prepared from three different lots of strawberries.

### *Effect of pH on strawberry extract activity*

The pH of different fruit infusions after 24 h of storage was: strawberry piece, 3.5; whole cherry or a piece, 4.1; peach piece, 4.4; intact grape, 5.9. The pH of the strawberry extract was 3.5. To determine if the acidic pH was responsible for virus inactivation, coxsackievirus and poliovirus were stored for 24 h in strawberry extract and water at pH 3.5 and 7.0. Less virus was recovered from strawberry extract at pH 7.0 than at pH 3.5; the reverse occurred in water (Table 5). Poliovirus was more sensitive to inactivation by strawberry extract than coxsackievirus.

### *Naturally-occurring viruses on fruit*

In July and August, 1974, seven quarts of strawberries and one batch each of cherries and peaches were tested for the presence of naturally-occurring viruses. None were found.

TABLE 2. *Recovery of viruses from single samples of strawberries and infusions, as percent of input virus, after storage at 4 C*

| Storage conditions | Coxsackievirus | | Poliovirus | | Echovirus | | Reovirus | |
|---|---|---|---|---|---|---|---|---|
| | 2 days | 5 days | 2 days | 5 days | 2 days | 5 days | 2 days | 5 days |
| Humid, no feces | 17 | 13 | 4 | <1 | 58 | 3 | <1 | <1 |
| Dry, no feces | <1 | <1 | 1 | <1 | <1 | <1 | <1 | <1 |
| Humid, feces | 42 | 19 | 10 | <1 | 87 | 31 | <1 | <1 |
| Dry, feces | 1 | <1 | 7 | <1 | 8 | <1 | <1 | <1 |
| Infusion | 44 | 23 | <1 | <1 | 99 | 4 | <1 | <1 |
| Control in PBS | 98 | 105 | 89 | 94 | 88 | 100 | 81 | 93 |

## DISCUSSION

We demonstrated previously that PBS with serum was better than water for removing virus from lettuce (*4*). Quantitative recoveries of enteric viruses were attained from several kinds of vegetables (*5*). Also, PBS with serum was more effective than water for removing virus from strawberries, but recoveries always were less than the input virus. Since strawberry extract and infusion were antiviral by tissue culture tests, the antiviral substance was probably present on the soft strawberry surface. Cherry surface and whole-cherry infusion were less antiviral than strawberry, but infusion prepared from a cherry piece was strongly antiviral. It is suggested that the tough cherry skin exuded only minute amounts of antiviral substance. Peach surface and infusion were somewhat more antiviral than cherry surface, but less antiviral than strawberry. An infusion prepared from apparently intact grapes proved antiviral to poliovirus. The degree of viral inactivation on fruit depended also on the type of virus present.

Heidelbaugh and Giron (*2*) reported that poliovirus survival in cranberry sauce and orange juice was poor after storage for 48 h at 4 C, and this was attributed to the low pH (< 2.9) of these foods. Our studies indicated that two phenomena appeared to take place in strawberry extract, one at pH 7.0 and the other at pH 3.5. In the latter, virus was protected in comparison to aqueous controls, whereas at pH 7.0 inactivation occurred because of an antiviral agent which was more effective at pH 7.0 than pH 3.5. Both phenomena appear worthy of further investigation.

Virus loss on fruit surfaces was also related to evaporation of the inoculum; the presence of fecal material apparently delayed desiccation and therefore virus loss. Similar results were obtained with vegetable surfaces (*4, 5*). Solar radiation has been implicated as an important factor in the destruction of virus on food (*6*). Irradiation and desiccation, in combination with a natural antiviral substance present in many fruits, may reduce virus infectivity very quickly.

Virus was not found as a natural contaminant in nine samples of fruit examined in this study. In retrospect, this is not surprising. Peaches and cherries, when purchased, were firm and their skins were dry; strawberries were either dry or dampened by the juice of bruised berries. It would appear, therefore, that fruit occupies a place in the category of minimal health hazard.

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# An Evaluation of Methods for Detecting and Comparative Incidence of Penicillin Residues in Different Types of Raw Milk Supplies

**V. S. PACKARD, SITA TATINI and ROY E. GINN**

*Department of Food Science and Nutrition*
*University of Minnesota, St. Paul, Minnesota 55108 and*
*Dairy Quality Control Institute, Inc., St. Paul, Minn. 55108*

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**ABSTRACT**

Over a one-month period, 288 samples of raw milk (equally divided among grade A, and manufacturing grade can and bulk milk producers) were tested by the disc assay and Delvotest P methods for penicillin residues. All positive samples were also checked by the *Sarcina lutea* cylinder plate method. The disc assay procedure detected one positive sample (0.34%), the Delvotest P method seven positive samples (2.4%) and one questionable (+/–) reaction. When applied to samples found positive by the Delvotest P procedure, the cylinder plate method identified as positive two samples. The Delvotest P method was found sensitive to 0.002 unit of penicillin. Hydrolytic rancidity (to acid degree values as high as 4.75) caused no false positive reactions. When applied to delactosed whey, false positive reactions did occur, as has been noted with other methods.

The problem of antibiotic residues in milk and dairy products requires continuous monitoring on the part of industry and regulatory agencies. In March, 1975 a decision by the U.S.D.A. to sample every carlot of government-purchase nonfat dry milk stimulated renewed interest in the problem. Ideally, a test method to detect antibiotics would be simple and fast enough to assay milk on the farm before delivery to the plant. Thus far, no such test has been developed. The *Sarcina lutea* cylinder plate method (CP), being more sensitive than the disc assay (DA) procedure, has been adapted to nonfat dry milk in government monitoring programs. Still, the CP method is quite involved and requires 16-18 h of incubation, far longer incubation than desired for routine quality control work. Gist Brocades nv, a Netherlands firm, has developed and made commercially available a method (Delvotest P) purported to be as sensitive as the CP procedure and requiring only 2.5 h of incubation. The method is simple and would appear to have promise in a routine penicillin monitoring program in either raw milk or finished products.

The work reported herein was done to (*a*) determine the applicability of the Delvotest P method to routine penicillin monitoring programs, (*b*) compare the relative sensitivity of the disc assay, *S. lutea* CP, and Delvotest P methods on raw milk, and (*c*) determine the relative incidence of penicillin in three different types of raw milk supplies, i.e. grade A bulk, and manufacturing grade can and bulk milk.

## MATERIALS AND METHODS

### *Raw and finished product samples*

Raw milk samples were collected and analyzed at weekly intervals over a one-month period. Samples were divided evenly among (*a*) Grade A, bulk milk, (*b*) manufacturing grade can milk, and (*c*) manufacturing grade bulk milk. Twenty-four samples from each different supply type were taken each week from regular shipments to the dairy plants. A total (over one month) of 288 different producer samples were investigated.

At the same time a number of finished product composites of dairy products manufactured in the dairy plants to which the raw milk supplies were delivered were also analyzed by the Delvotest P method. Products analyzed were: Cheddar cheese, whey powder, whey cream, lactose, delactosed whey powder, and nonfat dry milk.

All raw milk was analyzed by the disc assay and Delvotest P methods. All positive raw milk samples were also analyzed by the *S. lutea* cylinder plate method.

### *Disc assay method*

The Disc Assay procedure was applied as outlined in *Standard Methods for the Examination of Dairy Products* (*1*).

### *Sarcina lutea cylinder plate method*

The procedure followed was the method of Carter (*2*) as referenced in *Standard Methods for the Examination of Dairy Products* (*1*) and citing the report of Kramer et al. (*3*).

### *Delvotest P method*

This procedure has been developed and test kits are being marketed by Gist-Brocades nv, P.O. Box 1, Delft, Holland. The method followed was that indicated in instructions supplied by the company with test-kit materials.

Test materials of this method consist of ampules of solid medium seeded with *Bacillus stearothermophilus* var. *calidolactis*, tablets of nutrient medium and indicator, and disposable syringes for sampling and dispensing 0.1 ml samples. In outline, procedural steps are as follows: (*a*) ampules are identified and placed in a suitable rack; (*b*) after placing identifying marks on ampules, the rack is placed in a water bath maintained at 64 ± 2 C; (*c*) to each ampule (broken at the neck) is added one nutrient/indicator tablet; (*d*) using the disposable syringe, 0.1 ml of mixed sample is added to each ampule; and (*e*) ampules thus prepared are incubated 2.5 h at 64 C (± 2 C) and read.

The dye (bromcresol purple) in this method is purple at initial pH levels. If at the end of the incubation period the purple color remains, the test is considered positive for inhibitor; a yellow color (resulting from growth of the organisms, production of acid and a lowering of pH) is considered negative, and a layered yellow/purple mixture,

questionable (±) for presence of inhibitor. It is the dual-colored end-point reaction that occurs at the limits of penicillin detectability, i.e. 0.002 unit.

*Confirming presence of penicillin*

All samples found positive for inhibitor were confirmed for presence of penicillin. Confirmation was done according to procedures designated in *Standard Methods for the Examination of Dairy Products* (*1*). In the Delvotest P method confirmation consisted of (*a*) heating samples to 80 C for 2 min, (*b*) adding 0.1 ml of penicillinase, and (*c*) incubating samples 0.5 h at 37 C. Samples of both the heated product (untreated with penicillinase) and heated-penicillinase-treated products were tested. If the heated sample proved positive, the penicillinase-treated sample negative, results were considered positive for penicillin. Quantitative estimates of penicillin were made by simple dilution and re-test.

*Finished product sample size*

The *S. lutea* cylinder plate method, as applied to nonfat dry milk, calls for reconstitution of the dry product at a rate of 10 g of product to 30 ml of water. Because some of the products analyzed in this study did not completely disperse at 1:3 dilution rate, different dilutions were made. By product, the dilution used was: lactose, whey powder, delactosed whey powder, 1 g of dry product to 10 ml water; whey cream, 0.2 ml of product to 1.8 ml of water; Cheddar cheese, 0.2 g cheese to 2 ml water. Cheese was blended with the water before sampling.

## RESULTS AND DISCUSSION

Initially, sensitivity of the Delvotest P method was checked against standardized dilutions of penicillin in 1% phosphate buffer. Negative results were obtained through concentrations of 0.001 unit/ml. At 0.002 unit/ml, a +/− reaction was noted, this being the ultimate sensitivity of the method as indicated by the manufacturer.

TABLE 1. *Incidence of penicillin residues in 288 samples of Grade A bulk and manufacturing can and bulk raw milk supplies as detected by the disc assay and Delvotest P methods*[1]

| | Numbers of positive samples in each class | | | | | |
|---|---|---|---|---|---|---|
| | Disc assay | | | Delvotest P | | |
| Week no. | Grade A | Mfg can | Mfg bulk | Grad A | Mfg can | Mfg bulk |
| 1 | 0 | 0 | 0 | 1 | 1 | 1[2] |
| 2 | 0 | 0 | 0 | 1 | 0 | 1 (+/—) |
| 3 | 0 | 1 | 0 | 0 | 2[3] | 0 |
| 4 | 0 | 0 | 0 | 1 | 0 | 0 |

[1]Twenty-four samples of each class were collected and analyzed each week, for a total of 288 samples over a month-long period. All samples showing positive by the Delvotest P method were analyzed by the cylinder plate method.
[2]This sample also was positive by the cylinder plate method. The concentration of penicillin was determined to be 0.025 unit/ml.
[3]One of these samples was positive by the cylinder plate method. The concentration of penicillin was determined to be 0.009 unit/ml.

Data in Table 1 indicate the incidence of penicillin residues in grade A bulk and manufacturing grade can and bulk raw milk supplies as detected using the disc assay and Delvotest P methods. Of a total of 288 samples analyzed, the disc assay method detected one positive sample (0.34%) over a one-month trial period. Twenty-four samples of each type of raw supply were collected and analyzed on each of four consecutive weeks. The Delvotest P method detected seven positive (2.4%) and one questionable sample from these same supplies. When the *S. lutea* cylinder plate method was applied to those samples found positive by the Delvotest P procedure, two samples showed positive results. Thus the Delvotest P method appears to be somewhat more sensitive that the other two methods, while being considerably faster and simpler to do. As applied to tank truck milk, such sensitivity would be a distinct advantage in screening raw milk supplies. In all instances, samples identified as positive were also confirmed positive for penicillin.

Considering positive samples as confirmed by the Delvotest P method, residue problems appear to be fairly equally spread among grade A (three positive samples) manufacturing can (three positive samples) and manufacturing bulk (one positive, one +/—) raw milk supplies.

Using $10^{-1}$ and $10^{-2}$ dilutions and re-analyzing positive samples by the Delvotest P method, two positive and one +/— reaction was noted at the former concentration. No positive results were observed at the $10^{-2}$ dilution. Thus it would appear that positive samples identified by this method ranged from 0.002 to about 0.02 unit of penicillin.

To determine whether hydrolytic rancidity would yield false positive reactions by the Delvotest P method, a series of milk samples were prepared ranging in acid degree value (*4*) from 1.00 through 4.75. At none of the levels checked were positive results observed. However, communications with the manufacturer indicated that false negative reactions could occur in rancid milk samples.

Analysis of Cheddar cheese, lactose, dry whey, and whey cream made from milk which included (but did not entirely make up the day's lot) supplies tested in this investigation showed no positive results. Delactosed whey was found to yield false positive results in all instances at a reconstitution dilution of 1 g of product to 10 ml of water.

## SUMMARY AND CONCLUSIONS

Three methods for detecting penicillin residues were compared and the relative incidence of residues in three different types of raw milk supplies determined. The Delvotest P method yielded satsifactory results for routine analyses, while being simpler and faster than the disc assay and cylinder plate methods. However, its sensitivity, as presently designed, is such that a considerably larger number of positive identifications would be anticipated if used in lieu of the disc assay method for monitoring raw milk. For testing tank truck supplies or storage tank milk, such sensitivity would be a distinct advantage.

### ACKNOWLEDGMENTS

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# Some Volatile and Nonvolatile Compounds Associated with Milk and Their Effects on Certain Bacteria. A Review[1]

**D. C. KULSHRESTHA and E. H. MARTH**

*Department of Food Science and the Food Research Institute*
*University of Wisconsin-Madison, Madison, Wisconsin 53706*

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**ABSTRACT**

Raw and heated milks contain numerous volatile and other compounds. This review discusses some of the available information with particular emphasis on compounds having a low boiling point and which are likely to be removed by the vacuum treatment that often is combined with commercial pasteurization of milk. Reports dealing with carbonyl compounds including fatty acids, alcohols, and ketones have been reviewed. Emphasis has been given to fatty acids having up to 10 carbon atoms. Sections dealing with the occurrence in milk of volatile sulfur compounds, nitrogenous compounds, and alcohols also are included. A brief discussion about some miscellaneous compounds concludes the first part of this review. A detailed discussion of the effects of some compounds associated with milk on certain bacteria constitutes the second part of this review. The inhibitory effect of fatty acids, aldehydes, ketones, sulfur compounds, amines, alcohols, and some other compounds on lactic streptococci, lactobacilli, *Escherichia coli, Salmonella typhimurium, Staphylococcus aureus* and some other organisms encountered in the dairy industry, is discussed.

Raw and heated milks contain numerous volatile and other compounds. Kulshrestha and Marth (*113*) listed many of the compounds that have been reported as present in milk. The present review is a detailed discussion of information pertaining to presence of such compounds in raw or heated whole or skim milk. Particular attention has been given to normal raw or heated milk and to milkfat. An attempt has been made to emphasize the compounds with lower boiling points, especially when fatty acids are discussed. Whenever applicable, quantities of fatty acids with shorter chain lengths, as reported by different workers, have been mentioned.

For convenience this review is in two parts; first, volatile and nonvolatile compounds associated with milk are discussed and second effects of volatile compounds on bacterial growth are considered.

## VOLATILE AND NONVOLATILE COMPOUNDS ASSOCIATED WITH MILK

### Carbonyl compounds

Eight components were detected by Nawar et al. (*146*) when they examined the volatile fraction of milkfat after it was heated to 120 C; when milkfat was heated for 2 h at 130-185 C, 19 components were found of which eight were carbonyl compounds (*146*). Later Wynn et al. (*230, 231*) isolated an unidentified carbonyl compound and other volatile compounds from milk. Keeney et al. (*88*) reported that milkfat contained 10-15 μM of carbonyl groups/g, of which 2% were fatty aldehydes and more than 95% were ketoacids.

For convenience this group of compounds will be dealt with under three headings: fatty acids, aldehydes, and ketones.

#### *Fatty acids*

Jensen et al. (*82*) reviewed detection of different fatty acids by gas-liquid-chromatography (GLC) and reported that up to that time (1967) 142 acids were isolated from milk, many of them only in trace amounts. They reported that saturated, unsaturated (cis and trans), branched chain, hydroxy and ketoacids, as well as cyclic acids have been discovered. It also was observed that most of the free fatty acids were distributed in fat and the fat globule membrane fraction (*96, 97*), and that milk serum free of lipid material contained less than 10% of the free fatty acids.

*Raw milk.* Various amounts of different free fatty acids have been reported as present in raw unprocessed milk (*42, 214*). Morr et al. (*141, 142*) detected small amounts of butyric, acetic, pyruvic, formic, lactic, propionic, and four unidentified acids in raw milk. The total concentration of these acids ranged from 160-400 μeq/100 g. Harper and Huber (*63*) found that raw milk contained 2.4 mg of α-ketoglutaric acid/l. Traces of oxalosuccinic, oxaloacetic, and acetoacetic acid also were found in milk. By using a modified GLC partition chromatography procedure milk was found to contain a variable but characteristic concentration of $C_1$-$C_8$ fatty acids (*60*). Fresh raw milk contained formic, acetic, propionic, butyric, valeric, caproic, and caprylic acid according to results obtained by Hankinson et al. (*61*). Trammel and Janzen (*210*) found that the free fatty acid distribution in raw milk ranged from $C_4$-$C_{14}$; and that 100 ml of good quality raw milk contained 0.0-3.5 mg of butyric and 0.0-2.75 mg of caproic acid.

El-Hagarawy (*38*) determined the free fatty acid content of fresh milk from Holstein cows and from Egyptian cows and buffaloes; he reported that different portions of milk from the same cow and stage of lactation exhibited differences in free fatty acid content. The free fatty acid content of milk from Holstein cows was

[1] *A contribution from the College of Agricultural and Life Sciences, University of Wisconsin-Madison.*

sufficient to neutralize 25.0-136.3 ml of 1 N alkali/100 g of fat. Ryoki and Kudo (*178*) noted that cow's milk contained $C_6$-$C_{20}$ saturated and $C_{14}$ and $C_{16}$ unsaturated fatty acids together with oleic, linoleic, and linolenic acid. $C_4$-$C_{18}$ even numbered saturated; $C_{18:1}$, $C_{18:2}$ unsaturated; and some other fatty acids were isolated from milk by Glass and Troolin (*49*). Hexanoic, octanoic, and decanoic acid were isolated from raw milk (*187*); and fresh milk was found to contain sufficient free fatty acids to neutralize 0.3-0.7 mg NaOH/100 g of fat (*198*). Yashchenko (*232*) reported that the volatile fatty acid content of normal milk was 11-12% (by weight) of fat.

Hand- and machine-milked milks differed in their content of free fatty acids; mean values and the range being 4.7 (3.5-7.1) and 5.8 (4.5-7.0) mg/100 ml for hand- and machine-milked samples, respectively (*3*). Ramamurthy and Narayanan (*171*) reported the presence of $C_{4:0}$-$C_{18:0}$ even numbered; $C_{15}$ and $C_{17}$ saturated; $C_{10:1}$, $C_{14:1}$, $C_{16:1}$, $C_{18:1}$, $C_{18:2}$, and $C_{18:3}$ unsaturated fatty acids in cow's and buffalo's milk. Milk from three breeds of cows (Holstein, Guernsey, and Jersey) contained $C_{4:0}$-$C_{18:}$ even numbered saturated and $C_{18:1}$ and $C_{18:2}$ unsaturated fatty acids (*181*). It also was found that after storage for 3 days at 8 C the amount of fatty acids with eight or more carbon atoms markedly increased in raw milk (*182*).

Quantitative determinations of different fatty acids were made by several workers (*62, 65, 97, 222*). Results reported by them are summarized in Table 1, which only lists some of the shorter chain length fatty acids. The table is not intended to be an exhaustive listing of fatty acids in raw milk.

*Heated milk.* As early as in 1926 Whittier and Benton (*216*) found that the titratable acidity (TA) of milk increased when it was heated. Later Gould (*53*) observed that the lactic acid content of whole and skim milk increased when they were heated in sealed cans at 100 C for 8 h or at 116 C for 2.5 h. This increase in TA was related to a corresponding decrease in the lactose content of milk samples. Heating skim milk in cans at 116 C for 1 and 2 h markedly increased the volatile acid content of milk (*54*). Formic acid constituted 80-85% of total volatile acids isolated from distillates of skim milk that was previously heated at 116 C for 2 h. Later Gould and Frantz (*56*) found that milk heated at 116 C for 1 and 2 h contained 9.9 and 21.8 mg formic acid/100 ml, respectively. It also was noted that the TA of milk increased by 49 and 57% when milk was heated at 116 C for 1 and 2 h, resepectively. Sterilized milk contained propionic, acetic, pyruvic, and butyric acid (*91*). These acids contributed to the increased TA and the relative proportion of these acids was related to the degree of heat treatment given the milk. Later Morr et al. (*141, 142*) confirmed that the fatty acid content of milk increased when it was heated in sealed cans for 30-60 min at 100 C.

Harper et al. (*65*) also reported that commercial pasteurization of milk resulted in a general increase in its content of free fatty acids but the increase was significant

TABLE 1. *The amount of some free fatty acids in raw milk*

| Fatty acid | Harper and Gould (*62*) | Harper et al. (*65*) | Kinter and Day (*97*) | Withycombe and Lindsay (*222*) |
|---|---|---|---|---|
| | (ppm) | | | |
| Formic | —[a] | 8.1-39.7 | — | — |
| Acetic | — | 3.1-24.6 | — | — |
| Propionic | — | 0.0- 2.2 | — | — |
| Butyric | 0.0- 0.05 | 0.2- 3.3 | 20.4 ± 0.44 | 12.8-24.0 |
| Caproic | 11.8-14.4 | 5.3-10.0 | 10.0 ± 0.30 | 7.9-10.0 |
| Caprylic | 40.3-51.8 | 13.8-21.3 | 7.3 ± 0.55 | 3.1- 5.7 |
| Capric | — | — | 23.9 ± 2.43 | 10.8-15.5 |
| Total free fatty acids | 0.7- 1.0 (ADV)[b] | — | 358.9 | 222.8-432.2 |

[a]Value not reported.
[b]Acid degree value.

TABLE 2. *The amount of some free fatty acids in pasteurized and pasteurized homogenized milk*

| Fatty acid | Harper et al. (*65*) | Kinter and Day (*97*) | | Withycombe and Lindsay (*222*) | |
|---|---|---|---|---|---|
| | Pasteurized milk | Pasteurized milk | Pasteurized and homogenized milk | Pasteurized milk LTLT | Pasteurized milk HTST |
| | | (ppm) | | | |
| Formic | 3.0-21.6 | —[a] | — | — | — |
| Acetic | 3.1- 9.2 | — | — | — | — |
| Propionic | 0.1- 0.9 | — | — | — | — |
| Butyric | 1.2- 2.1 | 20.5 ± 0.95 | 20.9 ± 0.37 | 15.9 | 15.1 |
| Caproic | 0.7-31.6 | 8.0 ± 0.10 | 7.7 ± 0.20 | 5.9 | 6.9 |
| Caprylic | 1.7- 4.6 | 5.5 ± 0.33 | 4.5 ± 0.27 | 3.1 | 2.9 |
| Capric | — | 14.0 ± 0.48 | 15.1 ± 0.77 | 11.0 | 9.5 |
| Total free fatty acids | — | 283.9 | 322.0 | 188.0 | 193.6 |

[a]Value not reported.

only for formic and acetic acid. Storage of pasteurized milk for 3 days at 8 C resulted in a slight increase in its content of $C_{18}$ unsaturated fatty acid. In contrast, later workers (*96, 97, 222*) found that pasteurization or similar heat treatments caused a decrease in the free fatty acid content of milk. Withycombe and Lindsay (*222*) observed that the free fatty acid content of milk was reduced by more than 13% in all commercial heating processes. Presence of butyric, caproic, and capric acid in commercially pasteurized milk was confirmed by Trammel and Janzen (*210*).

Canale and Sarra (*22*) studied the free fatty acid content of heated milks; immediately after heating milk contained 200 mg of free fatty acids/100 g of fat. Storage of milks led to an increase in their content of free fatty acids. Scanlan et al. (*187*) isolated hexanoic, octanoic, and decanoic acid from heated milk; acetic, propionic, and butyric acid were isolated from milk serum by Ledford (*123*). Forewarmed and UHT-treated milk contained less free fatty acids than did unheated milk, but more than that of milk only forewarmed (*222*). Quantities of free fatty acids in pasteurized homogenized (*97*) and pasteurized milk (*65, 97, 222*) were reported by some workers. Data on short-chain fatty acids are summarized in Table 2. Values for the fatty acid content of milk given heat treatments other than conventional pasteurization also have been reported by Kinter and Day (*97*) and Withycombe and Lindsay (*222*).

*Homogenized milk.* Appreciable amounts of free volatile fatty acids including butyric and caproic were detected in raw homogenized milk (*107*). Kosikowski (*108*) reported that within 20 min after homogenization the free volatile fatty acid content of milk was 0.35-0.83 (expressed as ml of 0.1 N NaOH required to titrate 100 g of milk); storage for 24 h at 4.4 C caused this value to increase to 2.25, and butyric, caproic, caprylic, and capric acid were reported to be present. Homogenization did not markedly affect the content of volatile compounds present in milk (*79*). Kinter and Day (*97*) and Canale and Sarra (*22*) also studied the free fatty acid content of homogenized milk. The former workers (*97*) reported that homogenization had no significant effect on the free fatty acid content of milk. The amounts of $C_4$-$C_{10}$ even numbered saturated fatty acids detected by them in pasteurized homogenized milk are given in Table 2.

*Milk with off-flavors.* Harper et al. (*65*) found formic, acetic, propionic, butyric, valeric, caproic, and caprylic acid in feed-, oxidized-, and rancid-flavored milk. Milks with feed flavor contained relatively larger amounts of formic, butyric, and caprylic acid; rancid-flavored milk contained larger amounts of butyric, caproic, caprylic, formic, and acetic acid than did milk with a satisfactory flavor (*65*). The quantitative values for some of the fatty acids present in off-flavored milks as reported by Harper and Gould (*62*) and Harper et al. (*65*) are given in Table 3.

*Milkfat.* Jack and Smith (*77*) reviewed the chemistry of

TABLE 3. *The amount of some free fatty acids in milk with off-flavors*

| | Harper and Gould (*62*) | Harper et al. (*65*) | | |
|---|---|---|---|---|
| | Rancid | Feed flavored | Oxidized | Rancid |
| | (ppm) | | | |
| Formic | —[a] | 28.8 | 34.2 | 43.2 |
| Acetic | — | 12.2 | 10.8 | 10.9 |
| Propionic | — | 0.9 | 0.3 | 0.7 |
| Butyric | 9.3-12.1 | 1.4 | 0.5 | 13.4 |
| Caproic | 21.3-25.8 | 7.0 | 6.7 | 16.0 |
| Caprylic | 54.4-57.9 | 25.6 | 15.3 | 27.6 |
| Total free fatty acids | 2.0- 2.3 (ADV)[b] | — | — | — |

[a]Value not reported.
[b]Acid degree value.

TABLE 4. *The amount of some free fatty acids in milkfat*

| | Fatty acid | | | |
|---|---|---|---|---|
| Investigator and unit | Butyric | Caproic | Caprylic | Capric |
| Jack and Smith (*77*), wt% | 8.1-10.5 | 1.2 -3.9 | 1.3 -2.7 | 1.5 -3.7 |
| Gander et al. (*45*), wt% | 3.6 | 2.3 | 1.2 | 2.8 |
| Jensen et al. (*81*), wt% | 3.57 | 2.22 | 1.17 | 2.54 |
| Khatri and Day (*93*), ppm | 98.3 | 3.5 | 21.8 | 117.8 |
| Bills et al. (*16*), ppm | 43.4 | 13.6 | 12.8 | 40.4 |
| Stull and Brown (*200*), % | —[a] | 0.82-0.99 | 0.76-0.88 | 2.17-2.91 |
| Robertson et al. (*175*), Area % | — | 3.58-5.60 | 2.16-2.17 | 4.03-4.74 |
| Mattson et al. (*130*), Mole % | 9.0 -9.9 | 2.8 -4.0 | 1.6 -2.4 | 3.2 -4.9 |
| Shehata et al. (*193*), % | 3.61-4.00 | 1.83-2.01 | 1.13-1.26 | 1.98-2.38 |

[a]Value not reported.

milkfat and stated that free fatty acids could always be demonstrated as present in milkfat. They also indicated that milkfat contained $C_4$-$C_{20}$ saturated fatty acids with an even number of carbon atoms; unsaturated $C_{10}$-$C_{20}$ fatty acids with an even number of carbon atoms; octadienoic; decadienoic; and some other fatty acids. Milkfat was reported to contain a variety of saturated or unsaturated fatty acids ranging in chain length from $C_4$-$C_{26}$ (*76*); the total content of $C_4$-$C_{10}$ even numbered saturated fatty acids in cow's milkfat was 9.3%. Kumarato and Jezeski (*122*) isolated butyric, caproic, caprylic, capric, lauric, myristic, palmitic, and stearic acid from milkfat. Besides $C_4$, $C_6$, $C_8$-$C_{18}$ n-alkanoic acids, milkfat also contained cis and trans $C_{18}$ n-alkanoic acid, and $C_{18}$ n-alkadienoic and trienoic acid (*159*). Other workers (*68, 80, 195*) confirmed the presence of $C_4$-$C_{18}$ saturated and $C_{14:1}$, $C_{15:1}$, $C_{16:1}$, $C_{17:1}$, and $C_{18:1}$-$C_{18:3}$ fatty acids in milkfat. Average acid degree values for milk ranged from 0.33 to 0.46 meq/kg of fat depending on the season of the year (*213*).

Jack and Smith (*77*), Gander et al. (*45*), Jensen et al. (*81*), Khatri and Day (*93*), Bills et al. (*16*), Stull and Brown (*200*), Robertson et al. (*175*), Mattson et al. (*130*), and Shehata et al. (*193*) determined quantitatively the major free fatty acid content of milkfat and reported the presence of a variety of saturated $C_4$-$C_{18}$ and unsaturated fatty acids of various chain lengths. The quantities reported by these workers for some of the shorter chain fatty acids are given in Table 4. Again, as with previous tables, data given are not intended to be exhaustive.

Presence of a variety of fatty acids in milkfat also was confirmed by other workers (*71, 128*). At least 60 fatty acids, including some previously not described (such as $C_{15}$-$C_{23}$ monethanoid acids) were identified (*128*). Besides a large number of fatty acids, 27 minor components were found in milkfat (*71*). Milkfat was analyzed for its fatty acid content by de Man (*129*), who found that it contained $C_{4:0}$-$C_{16:0}$, $C_{10:1}$, $C_{12:1}$, $C_{14:1}$, $C_{15:1}$, $C_{16:1}$, $C_{18:2}$, and $C_{18:3}$ fatty acids plus an unknown fatty acid. Milkfat obtained from hand- and machine-milked milks contained 92-127 (average 106) and 127-173 (average 149) mg of free fatty acid/100 g of fat (*3*). The increase in fatty acids in machine-milked samples was attributed to hydrolysis of milkfat during the milking operation.

### *Aldehydes*

Different aldehydes have been detected in raw or heated milk.

*Raw milk.* Forss et al. (*43, 44*) isolated acetaldehyde from normal milk, and traces of formaldehyde and acetaldehyde were detected in 12 and 13, respectively, of 20 samples of raw milk (*63*). Other workers also have reported the presence of acetaldehyde (*13, 78, 230, 231*) and propionaldehyde (*13, 126*) in milk. The acetaldehyde content of normal milk was reported to be 1.87 μg/l (*225, 226*). Raw milk also was shown to contain hexanal and benzaldehyde (*187*).

Skim milk with oxidized flavor contained acetaldehyde, n-hexanal, crotonaldehyde, and $C_5$-$C_{11}$ unsaturated aldehydes (*43, 44*). Later Wishner and Keeney (*220*) also conclusively identified formaldehyde and acetaldehyde as present in both normal milk and milk exposed to sunlight, whereas $C_5$-$C_{11}$ n-alkanals and $C_{10}$ and $C_{11}$ alk-2-enals were tentatively identified as present in these milk samples. Furthermore, Wishner and Keeney (*220*) also suggested that milk exposed to sunlight contained n-dodecanal, 2-butenal, and $C_6$-$C_9$ alk-2-enals. Exposure to sunlight markedly increased the amounts of n-heptanal and n-octanal in milk but lowered its content of formaldehyde, which dropped from 5.1 ppb in fresh milk to 2.0 ppb in milk exposed to sunlight (*221*). The formaldehyde content of milk, buttermilk, and yogurt was found to be 0.3-3.0 mg/kg (*139*); and cowy flavored milk contained 0.61 mg% of acetaldehyde (*151*). Alk-2-enals were absent in fresh milk but were found in milk exposed to sunlight (*221*).

*Heated and processed milk.* Furfural and acetaldehyde were reported to be the principal carbonyl compounds generated by heat treatment (121 C for 90 min) of skim milk (*140*). Phospholipid-free milk was found to contain about 50 mg of aldehydes (calculated as tetradecanal)/kg (*189*). Wong (*223*) isolated formaldehyde and acetaldehyde by low temperature distillation of milk; $C_1$-$C_6$ alkanals were reported to be in milkfat heated in the absence of oxygen (*147*). Later Wong and Patton (*224*) reported that formaldehyde (0.001 and 0.003 mg/l) and acetaldehyde (0.016 and 0.013 mg/l) were isolated from two milk samples when milk was distilled at 35-40 C and 15-20 mm Hg for 4 h.

Fresh high-temperature short-time treated (140.5 ± 1 C for approximately 4 sec) sterile milk contained ethanal, propanal, and furfural. Stored samples contained the aforementioned aldehydes plus butanal, hexanal, and heptanol (*98, 99*). Heated (forewarmed at 82 C for 30 min and then heated at 146 C for 4 sec) milk also contained hexanal and acetaldehyde (*187*) plus banzaldehyde, furfural, and phenylacetaldehyde (*185, 187*).

Hydroxymethylfurfural was detected in milk heated for 2 h at 120 C (*168*), in evaporated milk, and in milks heated at different temperatures for different periods (UHT-treated and sterilized milk) (*179, 180*). Schogt et al. (*188*) isolated n-$C_{13}$-$C_{16}$, branched chain $C_{13}$-$C_{15}$ aldehydes, and hexadecanal from phsophorus-free milkfat; $C_6$-$C_9$ alkanals were produced in milkfat heated in the absence of oxygen (*145*).

### *Ketones*

Both raw and heated milks have been found to contain ketones.

*Raw milk.* Bertoni (*15*) reported that the total ketone bodies content of milk was 24.9-90.0, 9.4-15.2, and 15.3-22.0 mg/l at 15-23, 66-74, and 125-133 days after calving, respectively. He also found that the acetone and acetoacetic acid content for the same stages of lactation were 1.1-2.8, 1.5-3.3, and 3.3-6.7 mg/l, and that open housing of cows was associated with an increase in both the acetone and acetoacetic acid content of milk.

Other workers (*43, 44, 63, 78, 230, 231*) have reported

that acetone is present in milk. Wishner and Keeney (*220*) conclusively identified acetone and butanone as present in both fresh milk and milk exposed to sunlight; $C_6$-$C_8$ methyl ketones were tentatively identified as present in both types of milk. Exposure to sunlight was reported to cause an increase in milk's content of 2-butanone, 2-pentanone, 2-heptanone, and 2-octanone. Wishner and Keeney (*121*) later reported that fresh milk contained 1.6 and 0.37 ppb of butanone and pentanone, respectively. Other workers (*13, 126*) also have demonstrated the presence of acetone, butanone, and 2-heptanone in milk; the amounts of acetone and 2-butanone found in normal milk were 95.7 and 9.8 μg/1, respectively (*225, 226*). Palo and Ilkova (*151*) stated that milk with a cowy flavor contained 79.4 mg% acetone.

Bassette et al. (*12*) found that feeding silage-grain, brome grass pasture, or hay-grain to cows led to the presence of acetone and butanone in milk. Raw milk also was found to contain other volatile compounds including acetone, butanone, 2-pentanone, 2-heptanone, and 2-nonanone (*187*). Cows on a hay-concentrate ration produced milk that contained only two ketones-acetone and butanone (*11*).

Unheated milk contained 0.003 ppm of diacetyl (*185*). Diacetyl was found in three of 34 samples of milk, but all 34 samples were positive for acetyl-methyl-carbinol (average 0.051 mg %); it was stated that diacetyl-positive samples of milk had more than 0.2% acidity as lactic acid (*23*). Later Scanlan et al. (*187*) found that raw milk contained 5 ppb of diacetyl.

Fresh raw milkfat was found to contain a series of saturated delta-lactones with an even number of carbon atoms ranging from $C_8$-$C_{18}$; their presumed precursors, hydroxy triglycerides of fatty acids, also were present (*227-229*). Honkanen et al. (*73*) isolated another lactone, 4-methyl, 5-hydroxy hexanoic acid lactone from normal milk. It also has been reported that raw milk contains delta-decalactone and delta-dodecalactone (*187, 204*).

*Heated and processed milk.* Steam distillate and unsaponifiable matter obtained from milkfat contained a homologous series of methyl ketones with odd numbers of carbon atoms ranging from $C_3$ to $C_{15}$ (*156*). It was reported that acetone, 2-pentanone, 2-heptanone, 2-nonanone, 2-undecanone, and relatively smaller quantities of 2-butanone, 2-hexanone, and 2-octanone were formed in milkfat by heating it in the absence of oxygen (*145, 147*).

Acetone, butanone, 2-pentanone, and 2-hexanone were isolated after low-temperature distillation of milk (*223*). Wong and Patton (*224*) confirmed the presence of acetone (1.152 and 0.847 mg/1), butanone (0.079 and 0.077 mg/1), 2-pentanone (0.007 and 0.026 mg/1), and 2-hexanone (0.007 and 0.11 mg/1) in two samples of milk; they also stated that tentatively 2-heptanone was present in these milks. Kirk et al. (*98, 99*) found that fresh high-temperature short-time (140.5 ± 1 C for approximately 4 sec) processed sterile milk contained acetone and 2-butanone. After storage the milk also contained 2-pentanone and 2-heptanone. Scanlan et al. (*187*) reported that heated milk contained acetone, butanone, 2-pentanone, 2-heptanone, 2-octanone, 2-nonanone, 2-decanone, 2-undecanone, and 2-tridecanone.

Besides 0.038 ppm of diacetyl, UHT-treated milk also contained $C_3$-$C_5$, $C_7$-$C_{11}$, and $C_{13}$ n-methyl ketones and $C_8$, $C_{10}$, $C_{12}$ delta-lactones (*185*). The diacetyl content of five samples of heated milk ranged from 29.0-31.5 ppb (mean 30.3 ppb) according to Scanlan and Lindsay (*186*). Forewarmed and UHT-treated milk contained 22 and 38 ppb of diacetyl, respectively (*187*).

Leemeu (*89*) noted that heating of milk products caused development of a coconut-like flavor which was related to formation of delta-decalactone and delta-dodecalactone. Occurrence of delta-decalactone and delta-dodecalactone in steam distillates obtained from pasteurized homogenized milkfat has been reported (*155*); 2 ppm of delta-caprolactone were found in milk heated at 130 C for 2.5 h (*153*). Later Dimick et al. (*36*) reported the presence of saturated aliphatic delta-lactones ($C_{10}$-$C_{16}$ even numbered) in milkfat of cows, goats, sows, ewes, and humans.

Honkanen and Karvonen (*72*) detected delta-decalactone and tetra-decalactone in the distillate obtained while distilling milkfat for higher boiling point flavor compounds. Even number $C_6$-$C_{16}$ n-alkanoic acids and $C_8$-$C_{14}$ delta-lactones were found in heated milk (*152*). Scanlan et al. (*187*) reported that heated milk contained delta-decalactone, delta-dodecalactone, and delta-octalactone.

### Sulfur compounds

Numerous volatile sulfur compounds have been associated with milk. Volatile compounds, that impart the typical flavor to boiled milk are formed from sulfhydryl (-SH) groups contained in milk proteins (*191*). Heat labile sulfides were reported to originate from either milk serum or material associated with and firmly attached to the fat globule. Whey proteins are thought to be the main source of these sulfides (*207*). More recently Pofahl and Vakaleris (*167*) noted that casein had no appreciable -SH groups and that heat treatment of casein resulted in only very limited generation of volatile sufur compounds. Stage of lactation (*209*), feed given to cows (*173*), homogenization (*101*), and storage (*95, 109*) all affect the content of volatile sulfur compounds in milk. An increase in liberated volatile sulfides was noted when milk was produced by cows late in their lactation period (*209*). Reddy et al. (*173*) found that the methylsulfide content did not increase above 11 ppb in milk of cows fed a normal ration, brome-grass pasture, or rye pasture; but feeding of freshly cut alfalfa increased the methylsulfide content to more than 30 ppb. Homogenization also caused an increase in the -SH content of milk (*101*). A decrease in the -SH content was noted when heated (80-90 C) milk was stored at 20 or 3 C (*95*). Losses were higher at 20 C and in daylight than at 3 C and in the dark. Furthermore, when fresh raw milk was stored at 37 C, the amount of $H_2S$

liberated was low for the first 45 min, then increased during the next 3-5 h, and finally decreased again for the rest of the storage period (*109*).

*Raw milk*

In 1939 Diemair et al. (*31*) determined the volatile sulfur content of milk and milk products. They found that raw milk contained 0.13-0.15 mg S/100 ml. Greenstein (*57*) confirmed the presence of -SH groups in cow's milk. Zweig and Block (*233*) stated that the -SH content in milk and milk products varies from batch to batch even though the milk comes from the same farm. They also found that two samples of milk contained 0.254 and 0.308 meq -SH groups/l (calculated as meq of cysteine/l of milk).

Sasago et al. (*183*) reported that the -SH content of raw milk varied according to the analytical method that was used. Values obtained by them were 0.17 and 0.24 mM of -SH/l when determined by the p-chloromercuribenzoate dithizone and amperometric methods, respectively. Later they (*184*) found that raw milk contained $0.16 \pm 0.02$ mM -SH/1. Narang et al. (*144*) reported that the mean values for free -SH content of cow and buffalo milk were 0.193 (0.175-0.220) and 0.098 (0.055-0.183) mM/1, respectively. Raw buffalo milk contained fewer -SH groups than were present in fresh cow's milk. Pofahl and Vakaleris (*167*) found that unheated milk-serum proteins contained an average of 61.4 $\mu$M of -SH/g of protein. Thanh et al. (*203*), on the basis of results obtained from 31 cows, indicated that the -SH content of raw milk was between 0.138 and 0.280 meq/1 in winter and 0.136 and 0.260 meq/1 in summer. Later other workers found the -SH content of raw cow's milk to be 0.14-0.28 meq/1 (*174*) and 0.156 mM/1 (*191*).

Sasago et al. (*184*) showed that raw milk contained $0.97 \pm 0.06$ mM disulfide (-SS), and Pofahl and Vakaleris (*167*) found that unheated milk-serum proteins contained an average of 301.6 $\mu$M of -SS/g of protein. Other workers found that raw milk contained $0.156 \pm 0.06$ mM -SS groups/1 (*192*), and 0.644 mM -SS/1 (*191*).

Several workers have reported that dimethylsulfide is present in raw milk (*12, 85, 86, 126, 158, 173, 230, 231*). Patton et al. (*158*) detected this compound in exhaust gases from an air-agitated 1000-gal cold-wall tank of raw whole milk.

Pereira (*160*) and Pereira et al. (*162*) noted small amount of $H_2S$, mercaptans, organic sulfides, and carbonyl sulfur compounds in unheated raw milk. They reported further that skim milk, cream, and rennet whey contained more free organic sulfides than did the original whole milk. Skim milk and acid whey also contained more free mercaptans than did the original milk. Dimethylsulfone (6.1-8.2 mg/1) has been isolated from raw milk (*217*).

*Heated and processed milk*

Milk pasteurized by holding or high-temperature short-time methods contained 0.11-0.14 mg S/100 ml of milk (*31*). Other workers noted that the total -SH content decreased and the free -SH content increased progressively with the severity of heating at 65-95 C for 1-30 min (*100, 176*). Instantaneous heating of whole milk to 74-76 C caused a cooked flavor to develop. This flavor also developed if whole milk was heated at 66-68 C for 30 min and corresponded with a drop in electrical potential and with formation of sulfhydryl compounds (*50*). Zweig and Block (*233*) observed that heating milk up to about 43.5 C caused a slight increase in titratable -SH groups, but if the temperature was increased further the -SH content declined.

Simultaneous determinations of volatile and nonvolatile -SH groups were made by Boyd (*20*) and Boyd and Gould (*21*). They observed that heating momentarily at 90 C resulted in a value of 21 mg/1 for nonvolatile -SH groups (expressed as thiaminedisulfide); whereas heating at 90 C for 30 min yielded a value of 433 $\mu$g/1 for volatile -SH groups (expressed as $H_2S$). On heating milk by steam injection marked sulfhydryl activity was noted which declined slowly during 2 weeks (*101*).

Sulfhydryl titers in vacuum-treated and untreated samples increased with the heat treatment given to milk (up to 115.5 C for 120 sec); with greater heat the -SH content decreased in vacuum-treated samples (*33*). Kiermeier and Hamed (*94*) studied production of -SH groups in whole milk heated at 72-150 C for different periods. They found that production of -SH was rapid during the 5-70-sec period of heating. An increase in the rate of -SH development occurred when the temperature was increased up to 95 C. Only a small rise in rate of -SH production was observed when milks were heated between 95 and 110 C; heating beyond 110 C caused a decrease in formation of -SH compounds. The combined use of both steam heating and vacuum treatment caused an appreciable denaturation of whey proteins and an increase in -SH group activity (*102*).

Preheating milk at 60-70 C for 15-60 min resulted in a decrease in heat-activated -SH groups; this was directly related to the holding time with the greatest effect apparent at 60 C (*110*). Heating of skim milk at 82.2-135 C for 3.5 sec was studied by Blankenagel and Humbert (*17*). They found that the -SH titer and cooked flavor were maximal at 128.8 C and decreased when the temperature was raised further. No -SH groups were detected in skim milk processed at 135 C and then held at room temperature, but small amounts were present in milk stored at 4.4 C for 3 weeks.

Heating skim milk to various temperatures caused liberation of a few -SH groups below 70 C. Above that temperature a marked increase in number of liberated -SH groups was noted; but some -SH groups remained masked even at 100 C and only UHT-treatment led to almost complete liberation of -SH groups in milk (*127*). Momentary heating to 75-95 C and also holding at 70-95 C for 30 min caused a faster increase in the -SH content of buffalo's milk than of cow's milk (*40*). Pofahl and Vakaleris (*167*) found that if samples of milk-serum

proteins in sealed tubes were heated at 85 C for 10 min, the -SH content was 75.4 μM/g of protein. They also observed that heating caused an increase of about 25% in the -SH content of milk proteins. Furthermore, they stated that the -SH content of two commercially pasteurized skim milk samples were 13.75 and 14.6 μM/g of protein.

Thanh et al. (*203*) studied the -SH content of heated milk obtained from 31 cows. Values were from 0.040-0.226 and 0.052-0.220 meq/1 for winter and summer milk, respectively. Later Riel and Thanh (*174*) measured the -SH content of milk from 67 cows and found that heated milk contained 0.23-0.3 meq/1. A two-fold increase in -SH content and no increase in -SH plus -SS was caused by heat denaturation of skim milk (*167*).

Generally, heat treatment results in liberation of -SS groups in milk, but some workers (*191*) found that pasteurization caused a reduction in the -SS content of milk. Townley and Gould (*206*) presented data on the amount of sulfides liberated when milk was heated either momentarily or for 30 to 60 min at temperatures between 70-95 C. The amount of sulfides liberated averaged 0.018, 0.053, 0.130, and 0.240 mg/1 when milks were momentarily heated to 76, 80, 86, and 90 C, respectively; the content of volatile sulfur compounds increased as the period of heating was extended. They also found that heating milk at 90-95 C for more than 30 min, heating in sealed cans, or heating milk above the boiling point for 30 min or less either decreased the amount or prevented liberation of sulfides (*206*).

Whole milk and skim milk contained 0.24 and 0.158 mg S/1, when they were momentarily heated to 90 C (*207*). Liberation of sulfide was greatly influenced by pH and was maximum at pH 9 (*208*). Gould and Sommer (*55*) found a close relationship between development of cooked flavor and liberation of sulfides in milk. Milk-serum proteins heated in sealed tubes at 85 C for 10 min contained 290.7 μM -SS/g of protein (*167*).

Methylsulfide present in the volatile fraction of milk was more closely related to a cowy flavor in raw skim milk than to its heat treatment (*34*). Dimethylsulfide also has been reported as present in heated milk (*85, 86, 187, 206, 230, 231*). Keenan and Lindsay (*85, 86*) stated that heating increased the dimethylsulfide content of milk; the heat labile precursor, identified as methyl methionine sulfonium salt, remained in the skim milk fraction on separation of milk.

Dill et al. (*34, 35*) determined the heat-activated volatile sulfur compounds in skim milk that were liberated as a result of direct steam heating for 2-150 sec at 87.7-144.4 C. They found that the major volatile sulfur compound formed was $H_2S$ and that a decrease in titration values occurred when the critical treatment (104.4 C for 150 sec or 126.6 C for 20 sec) was exceeded because sulfur compounds were volatilized. Dill (*32*) also found that some sulfur compounds were volatilized by vacuum treatment of milk. Hydrogen sulfide was identified as present in vapors removed by vacuum treatment of milk heated at 144.4 C for 2 sec and 126.6 and 144.4 C for 150 sec.

El-Hagarawy et al. (*39*) studied liberation of $H_2S$ after heating bulk cow's and buffalo's milk. The amount of $H_2S$ liberated from cow's milk was 26 and 170 μg/1 when samples were momentarily heated at 75 and 95 C respectively; values for buffalo's milk given similar treatments were 23 and 239 μg/1, respectively. When samples of milk were treated at 70 and 95 C for 30 min, the amount of $H_2S$ liberated was 69 and 520 μg/1 for cow's milk and 45 and 412 for buffalo's milk. When heated at 90 C for 15 and 60 min, the amount of $H_2S$ liberated from cow's and buffalo's milk was 205 and 497 μg/1 and 180 and 620 μg/1, respectively.

Hydrogen sulfide was released from proteins by heating milk at 90 C for 30-120 min; the largest amount being released from beta-lactoglobulin and the smallest from casein (*64*). Furthermore, the amount of $H_2S$ released was a function of heating time and temperature.

Other workers also have reported the presence of $H_2S$ (*10, 39, 64, 109, 160, 161, 163*) and of some other volatile sulfure compounds such as mercaptans, sulfides, disulfides, thiones, and thionals (*160, 161, 163-165*) in milk given various heat treatments. Freshly pasteurized and homogenized milk was found to contain 3.5 μg of $H_2S$/500 ml (*10*). The amount of $H_2S$ released from milk by heating at 90 C for 30 min varied between 220-490 μg/1 (*109*).

Pereira et al. (*163-165*) observed that the heat treatment given milk and other fluid milk products caused release of $H_2S$, mercaptans, organic sulfides, and sulfur-containing 2, 4, DNPH derivatives. Furthermore, they found that raising the pH of milk from 6.7 to 7.7 and 9.7 increased the amountof $H_2S$ released 1.6 and 6 times, respectively; more mercaptans and organic sulfides were released at pH 7.7 than at 9.7.

### Nitrogenous compounds

*Raw milk*

The ammonia nitrogen content of over 600 milk samples was determined by Heller and Swiechowska (*70*); they reported that the amount of ammonia nitrogen varied from 0.3-0.9 mg (average 0.64 mg)/1. They also reported that storage at different temperatures increased the ammonia content of milk samples. This was confirmed by Rafaelli (*170*) who found that after storing samples at 15-18 C for 2, 4, and 24 h the ammonia content of milk was 0.20-0.58 (average 0.37), 0.25-0.68 (average 0.46), and 0.78-3.83 (average 1.69) mg%, respectively. Furthermore Rafaelli (*170*) reported that when handled under hygienic conditions, raw and pasteurized milk contained 0.25 and 0.35 mg% of ammonia; tests on 25 samples of fresh milks from cows indicated that the average content of ammonia of such milks was 0.22 (0.10-0.38) mg%. Bagnulo (*7*) examined milk and whey samples for the presence of ammonia and reported that bulk milk and whey contained ammonia

after storage at 37 C for 5 h. Milk from individual cows when stored at room temperature or bulk milk when stored at 5 C were negative for ammonia even after 120 h at 37 C. He also reported that raw milk contained 1.10 mg % of ammonia (*8*). Cows's milk examined a few hours after milking contained 0.5-0.9 mg of ammonia nitrogen/100 ml, but bulk milk received at the dairy contained 0.8-1.2 mg of ammonia nitrogen/100 ml of milk (*9*).

Ammonia and volatile amines occur in milk in variable quantities (*59*). Cole et al. (*25*) determined the ammonia and volatile amine content of 21 samples of raw milk. The amount of ammonia in milk with a satisfactory flavor ranged from 0.3-3.0 $\mu$M/10 ml, whereas samples with a feed flavor contained 0.2-1.5 $\mu$M of ammonia/10 ml. They also reported that the content of $C_1$-$C_3$ amines in milk with a satisfactory and feed flavor was 0.5-1.7 and 0.9-2.5, $\mu$M/10 ml, respectively, whereas values for $C_4$ and higher amines were 0.5-1.7 and 0.7-2.4. Later (*215*) methyl-, ethyl-, butyl-, and dimethylamine also were tentatively identified as present in milk.

*Heated and processed milk*

The ammonia content of pasteurized milk is variable according to results of different workers; values reported were 0.35 (*170*) and 1.16 (*8*) mg% and 7 ppm (*24*) of ammonia. Bagnulo (*8*) stated that boiled milk contained 1.62 mg% of ammonia.

Heating of milk at 96 C resulted in production of ammonia. The average amounts produced were 0.96 mg%/h and 1.25 mg%/h of heating, when this was done in an open flask and in sealed cans, respectively (*199*). Browning of milk and development of a caramel-like flavor also were found to be associated with an increase in the ammonia content, but cooked flavor was not believed to be related to ammonia production (*199*).

### Alcohols

Furfuryl alcohol was isolated in fairly pure form from pasteurized (62.2 C for 30 min) milk that was then heated at 126.6 C for 90 min; the compound was not found in unheated milks (*157*). It has been suggested that furfurol may be associated with caramelization and the browning phenomenon. Patton (*154*) found that maltol was produced in skim milk by autoclaving it at 127 C for 2.5 h. He also stated that the complete lactose molecule was necessary for the reaction to occur.

Ethanol (*126, 224*), methanol, and n-propanol (*126*) were tentatively identified as present in milk; Kirk et al. (*98, 99*) reported that fresh high-temperature short-time (140.5 ± 1 C for approximately 4 sec) sterilized milk contained ethanol and butanol. Scanlan et al. (*187*) isolated ethanol from both raw and heated milk (forewarmed at 82 C for 30 min and then heated at 146 C for 4 sec), and oct-1-en-3-ol, heptanol, maltol, and 2-butoxyethanol from heated milk. Cowy flavored milk contained 3.33 mg% of ethanol, and UHT-treated milk contained 2-butoxyethanol and maltol (*151*).

### Miscellaneous compounds

Kent-Jones et al. (*90*) noted the presence of bromine in four samples of milk; later Ford et al. (*41*) reported that four samples of milk contained 1.2-2.6 ppm of bromine. Milkfat was found to contain 0.76-1.48 ppm of fluorine after milk was centrifuged (50,000 rpm) for 1 h; the amount of fluorine found in whey and in precipitated casein on a dry matter basis was 0.19-0.82 and 0.9-1.7 ppm, respectively (*135*). Samples of raw, pasteurized, homogenized, and sterilized milk also have been found to contain 310-1325 $\mu$g fluorine/1 (average 669 $\mu$g/1) (*48*); market milk contained 0.2 ppm of fluorine (*46*).

Ethyl-ether (*126, 224*), chloroform (*187, 224*), acetonitrile (*224*), and ethylenedichloride (*224*) have been reported as present in milk. It also has been noted that UHT-treated milk contained vanillin, acetophenone, benzothiozole, and benzonitrile (*185*). Scanlan et al. (*187*) isolated ethylacetate, dichlorobenzene, trichlorobenzene, and methyliodide from raw and heated milk; methyl palmitate from raw milk only; and acetonphenone, benzothiozole, toluene, naphthalene, and benzonitrile from heated milk only.

## EFFECT OF VOLATILE AND NONVOLATILE COMPOUNDS ON BACTERIAL GROWTH

It has been reported that growth of different bacteria is affected by some of the volatile compounds that occur in raw or heated milk. This review deals mainly with some of the organisms that are important to the dairy industry. Included are comments on bacteria such as those employed to manufacture fermented dairy products (*Streptococcus cremoris, Streptococcus lactis, Streptococcus thermophilus,* and *Leuconostoc citrovorum*), and undersirable kinds including one that is a common contaminant of raw milk and sometimes of dairy products (*Escherichia coli*) and several that are of importance primarily from a public health point of view (*Salmonella typhimurium* and *Staphylococcus aureus*).

### Fatty acids

It has been reported that oleic, linoleic, and linolenic acid inhibit gram-positive but not gram-negative bacteria (*104*). The influence of trace amounts of fatty acids on growth of microorganisms was reviewed by Nieman (*148*). He stated that bacterial species, fatty acid structure and concentration, presence of antagonists to neutralize inhibition, presence of detoxicants, and length of incubation all played a role in determining the effect of a fatty acid on microbial growth. Kabra et al. (*83*) studied the inhibitory effect of 30 straight chain fatty acids and their derivatives. They found that of the saturated fatty acids lauric acid was most inhibitory to gram-positive organisms. Monoenoic acid ($C_{18:1}$) was even more inhibitory than were the saturated fatty acids but was less active than dienoic acid ($C_{18:2}$).

*Lactic streptococci*

In 1923 Ayers et al. (*6*) observed that some

streptococci, including *S. lactis,* were inhibited if the surface tension of the medium was lowered. Tarassuk and Smith (*201, 202*) found that rancid milk inhibited *S. lactis.* They also noted that a reduced surface tension resulted in delayed coagulation of milk by the starter culture. Continuous growth of *S. lactis* in rancid milk caused an increase in surface tension, which was attributed to utilization of fatty acids that initially caused the surface tension to be reduced. They stated that inhibition of growth of *S. lactis* by rancid milk was observed in 1928 by Koestler (*106*).

Other investigators (*26, 28*) also have reported that rancid milk inhibited *S. lactis.* Inhibition was not caused by a slight reduction in pH or in surface tension, but a direct relationship was found between the magnitude of inhibition and the degree of rancidity in milk (*26*). *S. lactis* was inhibited by 0.1% of capric, caprylic, or lauric acid; but not by 0.1% of oleic, butyric, linoleic, and linolenic acid; 0.5% hexanoic acid; or 0.5% of stearic acid (*26, 27*). Furthermore, the inhibitory effect was greatest for decanoic acid and least for lauric acid; chain length of acid was critical for inhibition (*27*).

Strains of *S. cremoris* were found to be more active in skim milk than in whole milk and were inhibited by the cream fraction (*47*); the inhibitory activity could be prevented by constant agitation of milk to prevent creaming. Maxcy (*131*) noted that fatty acids and other surface-active agents inhibited lactic streptococci; the magnitude of inhibition was directly related to surface activity of the compound used. It also was reported that inhibition was a physical phenomenon at the bacterium: menstruum interface. Partial and complete inhibition of *S. lactis* was caused by 0.01 and 0.5% capric acid, which was the most effective of the fatty acids tested. The degree of inhibition was related to concentration of fatty acids and surface tension of the medium; butyric acid was not inhibitory (*134*). Maxcy and Dill (*132, 133*) later reported weak absorption of fatty acids at the bacterium:menstruum interface, and they believed that this absorption was critical for the inhibitory process. Fatty acids also inhibited *S. cremoris, S. lactis,* and *S. diacetilactis* (*2*) and the inhibition increased with chainlength of the acid up to $C_{10}$ or $C_{12}$; at $C_4$ or at $C_{14}$-$C_{18}$ the inhibitory effect was either less or absent.

Nilsson and Willart (*149*) observed that at concentrations of 2.5 mM or higher starter cultures were inhibited most by capric acid followed by caprylic and lauric acid; myristic, oleic, and caproic acid caused only slight inhibition; and butyric and palmitic acid were ineffective. At the 1-mM concentration only lauric, capric, and, to a limited extent, myristic acid had any inhibitory effect. Podesta and Bertoldini (*166*) found that $C_6$-$C_{12}$ fatty acids were most inhibitory to *S. lactis* when they tested butyric, caproic, caprylic, monodecanoic, lauric, tridecanoic, myristic, pentadecanoic, palmitic, stearic, oleic, linoleic, linolenic, arachidonic, or behenic acid. They also stated that growth of *S. lactis* in milk cultures was not affected as much as it was in synthetic media. *S. thermophilus, S. lactis, S. diacetilactis,* and butter starter cultures were inhibited by capric acid but not by oleic acid; the degree of inhibition was proportional to the concentration of capric acid (*169*). Milk containing an appreciable amount of free fatty acids was not suitable for growth of thermophilic lactic acid bacteria particularly streptococci (*190*).

Kulshrestha and Marth (*111, 113*), using the disc assay procedure, found that 1000 ppm of formic, butyric, hexanoic, and decanoic acid inhibited *S. cremoris. S. lactis* was only inhibited at the 1000-ppm concentration by decanoic acid, whereas 1000 ppm of both decanoic and formic acid were inhibitory to *S. diacetilactis.* Higher concentrations of all the fatty acids tested inhibited all these organisms. Later Kulshrestha and Marth (*115, 119, 121*) tested the inhibitory effect of formic, acetic, butyric, hexanoic, octanoic, and decanoic acid against *S. lactis* and *S. thermophilus* when they were grown in APT broth and confined with the chemical in an air-tight vessel. Fatty acids at 1000 ppm always inhibited *S. lactis* and *S. thermophilus.* Decanoic acid was most inhibitory to both organisms. Lower concentrations of fatty acids were inconsistently inhibitory to *S. lactis* and *S. thermophilus.*

*Leuconostoc citrovorum*

Formic, butyric, hexanoic, octanoic, and decanoic acid at the 1-100,000-ppm concentration were tested by the disc assay to determine inhibition of this organism (*111, 113*) All the fatty acids inhibited *L. citrovorum* at concentrations of 10,000 or 100,000 ppm. Only decanoic acid was inhibitory at 1000 ppm. *L. citrovorum* growing in APT broth was inhibited by lower concentrations of formic, acetic, butyric, hexanoic, octanoic, and decanoic acid (*115, 120*). Decanoic acid was most and butyric acid was least active against this organism. The inhibitory effect of fatty acids was generally insignificant when they were tested at a concentration of 1 ppm.

*Lactobacilli*

It has been reported that the ratio of *S. thermophilus* to *Lactobacillus bulgaricus* could be maintained between 1.0:0.5 and 1.0:3.0 in UHT-treated milk, but it changed quickly in favor of bacilli and stabilized at 1:20 in autoclaved milk (*18, 19*). Addition of 5 μg or more of formic acid/ml favored *L. bulgaricus.* Unsaturated fatty acids, particularly linolenic and linoleic, inhibited *Lactobacillus helveticus* when used at a concentration of 8 μg/ml (*103*). Cis forms of unsaturated fatty acids depressed acid production by *L. helveticus* and trans forms were without any effect (*105*). Spector (*197*) observed that fatty acids from maize oil and milkfat had no appreciable effect on *Lactobacillus casei* and *Lactobacillus arabinosus.* He reported that the effects caused by fatty acids, generally, were partially dependent on the concentration of a growth limiting amino acid or vitamin. Other workers (*75, 218, 219*) reported that oleic acid stimulated lactobacilli, but Norris and Lynes (*150*) observed that *L. arabinosus* was

inhibited when fats or fatty acids were present in the medium.

The effect of fatty acids on growth of certain lactobacilli was studied by still other researchers (*58, 66, 67, 205*). Hexadecanoic acid in amounts less than 300 μg/ml stimulated growth of *Lactobacillus bifidus,* but higher concentrations completely inhibited this bacterium (*66*). Fatty acids liberated by pancreatin from human or cow's milk always inhibited *L. bifidus* (*58, 205*). *L. arabinosus* and *Lactobacillus leichmanii* were inhibited by fatty acids with 8, 10, or 12 carbon atoms, and *Lactobacillus lactis* and *L. casei* were sensitive to acids with 8-14 carbon atoms. The molar concentration of acid necessary for 50% inhibition was proportional to the number of carbon atoms in the acid and was inversely proportional to its solubility (*67*). Auclair and Portman (*4, 5*) found that autoclaved milk contained formic acid which stimulated *L. lactis.* It also was reported that formic acid occurred in autoclaved milk through degradation of lactose during heating. Capric acid inhibited *L. helveticus,* but oleic acid was without any effect (*169*). *L. bulgaricus* was not inhibited in milk cultures as much as it was affected in synthetic media (*166*).

The effect of fatty acids on growth of certain lactobacilli was studied by still other researchers (*58, 66, 67, 205*). Hexadecanoic acid in amounts less than 200 μg/ml stimulated growth of *Lactobacillus bifidus,* but higher concentrations completely inhibited this bacterium (*66*). Fatty acids liberated by pancreatin from human or cow's milk always inhibited *L. bifidus* (*58, 205*). *L. arabinosus* and *Lactobacillus leichmanii* were inhibited by fatty acids with 8, 10, or 12 carbon atoms, and *Lactobacillus lactis* and *L. casei* were sensitive to acids with 8-14 carbon atoms. The molar concentration of acid necessary for 50% inhibition was proportional to the number of carbon atoms in the acid and was inversely proportional to its solubility (*67*). Auclair and Portman (*4, 5*) found that autoclaved milk contained formic acid which stimulated *L. lactis.* It also was reported that formic acid occurred in autoclaved milk through degradation of lactose during heating. Capric acid inhibited *L. helveticus,* but oleic acid was without any effect (*169*). *L. bulgaricus* was not inhibited in milk cultures as much as it was affected in synthetic media (*166*).

*Escherichia coli*

Fatty acids from maize oil and butterfat did not affect growth of five strains of *E. coli* (*197*); the concentration of growth limiting amino acid or vitamin influenced the effect of the fatty acid on growth of *E. coli.* Fatty acids from both fats inhibited *E. coli* when the concentration of growth limiting factors was high, whereas if the acids were present in small amounts growth was either not affected or stimulated. It also has been reported that growth of *E. coli* was affected less than that of *S. lactis* when the organisms were in milk which had undergone natural lipolysis; no inhibition was noted in normal milk whose surface tension or pH was adjusted to that of rancid milk (*28*). Additionally, the inhibitory effect on bacteria was proportional to the degree of lipolysis and was not the result of direct action of lipase on the bacterial cell. Inhibition of *E. coli* was greater in synthetic media than it was in milk cultures (*166*).

Kulshrestha and Marth (*113*) noted that butyric, hexanoic, and octanoic acid inhibited *E. coli* at concentrations of 10,000 or 100,000 ppm when the chemicals were tested by the disc assay procedure. Formic and decanoic acid (100,000 ppm) also inhibited this organism. Later the same workers (*116*) tested formic, acetic, butyric, hexanoic, octanoic, and decanoic acid against *E. coli* in nutrient broth and contained in an air-tight vessel. They found that all the fatty acids tested were inhibitory at 10 and sometimes at 1 ppm. Formic acid was most effective of the fatty acids involved in the study.

*Salmonellae*

Formic, acetic, propionic, and butyric acid inhibited *S. typhimurium* and the effect of the acid decreased with an increase in pH but increased with an increase in chain length of fatty acid (*51*). It also was reported that the rate of death at a particular pH value depended on chain length and concentration of the acid and on composition of the medium. Inhibition of *S. typhimurium* by fatty acids also was observed by other workers (*52, 124*). Khan and Katamay (*92*) found that volatile fatty acids with a low molecular weight were bacteriostatic or bactericidal to *Salmonella* not only in culture media but also in meat. Acetic acid, contained in culture filtrates of *L. citrovorum* was inhibitory to *S. gallinarum* (*196*).

Kulshrestha and Marth (*113*) used the disc assay method to test some fatty acids (butyric, formic, hexanoic, octanoic, and decanoic) for their inhibitory activity against *S. typhimurium.* Formic acid was most active and inhibited this bacterium at a concentration of 100 ppm. Butyric and hexanoic acid inhibited *S. typhimurium* at a concentration of 1000 ppm or more, whereas decanoic and octanoic acid were effective only at 10,000 ppm or higher concentration. Later Kulshrestha and Marth (*114, 117*) tested acetic, formic, butyric, hexanoic, octanoic, and decanoic acid against *S. typhimurium* in nutrient broth and confined in an air-tight vessel. They found that, generally all fatty acids were inhibitory at a concentration of 10 ppm, or greater; at 1 ppm none of the fatty acids tested affected growth of *S. typhimurium.* Formic acid was most inhibitory of the fatty acids tested.

*Staphylococcus aureus*

In 1944 Kodicek and Worden (*103*) reported that unsaturated fatty acids, particularly linolenic and linoleic, at a concentration of 8 μg/ml, inhibited *S. aurens.* Vadhera and Harmon (*211, 212*) found that growth of *S. aureus* in skim milk was completely inhibited by 0.05 and 0.1% of decanoic acid and 0.1% of octanoic acid; but was only partially inhibited by 0.05% of

octanoic and 0.05 and 0.1% of lauric acid. They also indicated that work by others in the same laboratory had indicated that skim milk was better than whole milk for growth of *S. aureus*. As with other organisms, *S. aureus* also was inhibited less by fatty acids in milk than in synthetic media. Acetic (*30, 136-138*), formic, and lactic acid (*30*) inhibited *S. aureus*. Daly et al. (*30*) stated that pH was inversely proportional to the magnitude of inhibition of *S. aureus*, when fatty acids were tested.

Kulshrestha and Marth (*113*) found that decanoic acid was more inhibitory to *S. aureus* than were formic, butyric, hexanoic, and octanoic acid. Decanoic acid inhibited this organism when tested at or more than 1000 ppm. Other fatty acids were inhibitory only if higher amounts (10,000 or 100,000 ppm) were tested. These results were obtained with a disc assay method. Later (*114, 118*) *S. aureus* in nutrient broth in an air-tight vessel was treated with formic, acetic, butyric, hexanoic, octanoic, and decnoic acid. When tested at 1000 ppm, butyric acid was least inhibitory, but at 10 ppm butryic, octanoic, and decanoic acid were more effective than other fatty acids.

*Other bacteria*

Some other organisms have been reported to be affected by fatty acids. $C_6$-$C_{12}$ fatty acids (*166*) and linolenic and linoleic acid (*103*) inhibited *Streptococcus agalactiae*. Again, the extent to which *S. agalactiae* was inhibited in milk was not as great as in synthetic media (*166*). *Streptococcus faecalis* (*14, 67*), *Micrococcus* (*14*), and *Bacillus cereus* (*166*) were also reported to be inhibited by fatty acids.

## Aldehydes

Ramon et al. (*172*) reported in 1945 that formaldehyde, when used with heat, lowered the number of organisms in milk more than did heat alone. However, bound formaldehyde had no antibacterial properties (*143*). In pure litmus milk cultures, *Staphylococcus* sp. and some lactic acid bacteria were sensitive to 0.004% formaldehyde, whereas *S. faecalis* was sensitive to 0.008% formaldehyde, and if present in large numbers *L. casei* and *E. coli* tolerated 0.008 and 0.02%, respectively (*1*).

Formaldehyde and acetaldehyde ($10^{-3}$ M) completely inhibited cell division by *E. coli*, but other aldehydes exerted only a moderate blocking effect and cells gradually recovered (*37*). When grown at 21 C, *L. citrovorum* 91404 rapidly utilized acetaldehyde in both acidified and nonacidified milk cultures, and 5 ppm of acetaldehyde stimulated growth of this organism (*125*). The concentration of acetaldehyde in a ripened single-strain streptococcal culture was reduced when a large inoculum of an active culture of *L. citrovorum* was added to it and incubation was continued at 21 or at 5 C (*84, 87*).

Under aerobic conditions, DL-glyceraldehyde inhibited *E. coli* probably because aldehyde bonded with cysteine, an essential amino acid for protein synthesis (*29*). No effect was observed on glucose utilization or [$^{14}$C] leucine incorporation into cell proteins by *E. coli*. Under anaerobic conditions inhibition was more intense and glycolysis and leucine incorporation also were partially affected, but there was no effect on uracil incorporation. Furthermore, inhibition of glycolysis contributed to inhibition of this organism. Rubbo et al. (*177*) confirmed that *S. aureus, E. coli,* and *Pseudomonas aeruginosa* were inhibited by glutaraldehyde.

Kulshrestha and Marth (*111, 113*) used a disc assay procedure and found that anisaldehyde, butyraldehyde, formaldehyde, and glyoxal inhibited *S. cremoris, S. lactis,* and *S. diacetilactis*. In addition to the aforementioned aldehydes *L. citrovorum* also was inhibited by isobutyraldehyde. Generally glyoxal and formaldehyde were more inhibitory to these organisms than were the other aldehydes. They (*113*) also reported that a high concentration (100,000 ppm) of anisaldehyde, butyraldehyde, isobutyraldehyde, and lower concentrations (1000 and 10,000 ppm) of glyoxal and formaldehyde, generally, were inhibitory to *E. coli, S. typhimurium,* and *S. aureus*. Acetaldehyde (100,000 ppm) only was inhibitory to *S. aureus*. Kulshrestha and Marth (*112*) later tested 1-100,000 ppm of acetaldehyde, isobutyraldehyde, and propionaldehyde against *S. cremoris* growing in autocalved skim milk. Generally aldehydes at 10-100 ppm and higher concentrations markedly inhibited both growth and acid production by the lactic streptococcus.

Subsequently, Kulshrestha and Marth (*114-121*) tested 1, 10, 100, and 1000 ppm of formaldehyde, acetaldehyde, propionaldehyde, and glyoxal against *E. coli, S. typhimurium, S. aureus, S. lactis, L. citrovorum,* and *S. thermophilus*. In this instance cultures and chemicals were confined in an air-tight vessel. Formaldehyde and glyoxal, generally, were more inhibitory to these organisms than were the other aldehydes.

## Ketones

Egyud (*37*) reported that 2-ketones caused moderate blocking of cell division by *E. coli*; subsequently this property was recovered by the cells. Hedgecock and Jones (*69*) noted that 0.05-0.25% of diacetyl inhibited numerous species of bacteria. *Lactobacillus* spp. were least sensitive, gram-negative rods were least resistant, and gram-positive cocci were intermediate in susceptibility. Later Singh et al. (*194*) reported that 1 ml of aroma producing starter distillate (containing about 30 μg diacetyl) inhibited *E. coli* and *S. agalactiae*.

Diacetyl inhibited *S. cremoris, S. lactis, S. diacetilactis, L. citrovorum, E. coli, S. aureus,* and *S. typhimurium,* whereas acetone, 2-butanone, and 2-pentanone were without effect when a disc assay procedure was used (*111, 113*). Only 100,000 ppm of diacetyl were inhibitory to *L. citrovorum,* but 10,000 and 1,000 ppm also were inhibitory to other organisms. Kulshrestha and Marth (*112*) also tested acetone,

2-butanone, and 2-pentanone against *S. cremoris* in milk. They found that three ketones inhibited *S. cremoris* but more pronounced reduction in acid development than in growth was noted. Acetone, 2-butanone, and diacetyl were also tested against *E. coli* (*116*), *S. typhimurium* (*114, 117*), *S. aureus* (*114, 118*), *S. lactis* (*115, 119*), *L. citrovorum* (*115, 120*), and *S. thermophilus* (*121*), when they were in broth and treated with the chemicals in an air-tight vessel. It was found that of the ketones tested diacetyl was most inhibitory to all the organisms, and that generally the higher (100 and 1,000 ppm) concentrations of the three ketones inhibited these organisms.

### Sulfur compounds

Ethanethiol, 1-propanethiol, 2-propanethiol, and methylsulfide inhibited *S. cremoris* during growth in milk (*112*), but the reduction in growth was not always accompanied by a reduction in acid production. Kulshrestha and Marth (*116-121*) reported that methylsulfide, methylsulfone, methanethiol, and ethanethiol caused various degrees of inhibition of *E. coli, S. typhimurium, S. aureus, S. lactis, L. citrovorum,* and *S. thermophilus.* Except when tested against *S. thermophilus,* ethanethiol was more inhibitory than methanethiol; generally thiols were more active than were methylsulfide and methylsulfone.

### Amines

Kulshrestha and Marth (*111, 113*) reported that propyl- and hexylamine inhibited *S. lactis, S. cremoris, S. diacetilactis, L. citrovorum, E. coli, S. typhimurium,* and *S. aureus* when the chemicals were tested at 10,000 and 100,000 ppm by a disc assay procedure. Propyl- and hexylamine at 10 ppm or higher concentrations caused various degrees of inhibition when tested against *E. coli* (*116*), *S. typhimurium* (*114, 117*), *S. aureus* (*114, 118*), *S. lactis,* (*115, 119*), *L. citrovorum* (*115, 120*) and *S. thermophilus* (*121*). Generally 10 ppm or higher concentrations of amines were significantly inhibitory to these organisms. Hexylamine was more detrimental to *E. coli, S. typhimurium,* and *S. lactis;* whereas propylamine was more active against the other three organisms. These tests were done with the organisms and chemicals in an air-tight vessel.

### Miscellaneous compounds

The antibacterial activity of chloroform, diethylether, and some other anaesthetic vapors against *E. coli* was demonstrated by Horton et al. (*74*). They reported that these chemicals reduced survival of organisms, and the activity was proportional to the concentration of the compound. Chloroform and ether did not cause a marked reduction in growth but considerably reduced acid production when 10 ppm or more of these compounds were tested against *S. cremoris* growing in milk (*112*). Acetonitrile (10 ppm or more) and ethylenedichloride (100 ppm or more) also inhibited growth of *S. cremoris* in milk. Furfurol, methanol, acetonitrile, chloroform, ether, and ethylenedichloride inhibited *E. coli* (*116*), *S. typhimurium* (*114, 117*), *S. aureus* (*114, 118*), *S. lactis* (*115, 119*), *L. citrovorum* (*115, 120*), and *S. thermphilus* (*121*) when organisms were in an air-tight vessel together with chemicals at concentrations of 10 ppm or more.

## IN CONCLUSION

Raw milk is richly endowed with a variety of compounds. Heat treatment can alter the concentration of some of the compounds and can cause appearance of others not indigenous to raw milk. Other processing procedures and storage of processed milk can further modify the profile of compounds that are present. These changes are fairly well documented. Effects of production conditions (kind and amount of feed, breed of cow, stage of lactation, season of the year, etc.) on the kind and amount of volatile and nonvolatile compounds in milk are less well understood.

The kind and amount of volatile and nonvolatile compounds in milk partially determines the suitability of milk as a substrate for a variety of microorganisms including the lactic acid bacteria of commercial significance. How variation in kind and amount of these compounds affects the behavior of bacteria in milk has not received much research attention and appears to be worthy of investigation.

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# Surveillance of Milk Products for Penicillin as Done by the Dairy Division of the U.S. Department of Agriculture

**HAROLD E. MEISTER**

*Dairy Division, Agricultural Marketing Service*
*U.S. Department of Agriculture, Washington, D.C. 20250*

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**ABSTRACT**

In 1974 a State regulatory agency found penicillin in an interstate shipment of nonfat dry milk (NDM). Food and Drug Administration checks in the source State showed that industry and regulatory follow up on offending producers was not always adequate to assure segregation of adulterated milk from use either in fluid or manufactured products. Other States reported the USDA their findings of penicillin-positive NDM. Dairy Division in March, 1975 inaugurated a penicillin surveillance program on NDM offered for official grading. Official grading is required for NDM purchased under the government price support program and by several commercial buyers. There was prompt action by industry to cooperate with State regulatory agencies in educational work with farmers and veterinarians to keep penicillin-positive milk from the market. Industry stepped up its testing of fluid and dry milk products. The results to date show much has been accomplished toward eliminating penicillin and other antibiotics from raw milk supplies and finished dairy products.

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Dairy Division is located in the Agricultural Marketing Service of the USDA. Our sister Divisions include Fruit and Vegetable, Poultry, Cotton, Tobacco, Grain and Livestock, the Livestock Division provides the meat grading service with which many readers are familiar.

Our authority is in the Agricultural Marketing Act of 1946 that says among other things that the Secretary of Agriculture shall provide quality standards, inspection and grading services, and market news to promote the orderly marketing of agricultural products and enhance returns to farmers. Agricultural Marketing Service (AMS) inspection services are for voluntary use and fees are charged to cover our costs.

Milk and dairy products are perishable. With this in mind it has been our policy over the years to pay attention to quality of raw material, condition of plant facilities, and equipment processing procedures and sanitary practices, all this to the end that the quality of finished products will be consistent with quality of raw material used, the products will be wholesome, and they will have good keeping quality to carry them through normal channels of distribution and give good satisfaction to consumers.

## INSPECTION

The Dairy Division inspection regulations provide that for products to be eligible for USDA grading or inspection for compliance with contract specifications the production plant shall be inspected and approved by Dairy Division. Inspections of milk drying plants are made four times per year, and the inspections include *Salmonella* surveillance. Butter and cheese plants are inpected twice a year. About 1,100 plants representing an estimated 80-85% of the U.S. production of butter, dry milk products, and cheese are inspected. Their names are listed in the booklet we issue quarterly—*Dairy Plants Inspected and Approved for USDA Inspection and Grading Service.* The booklet is available without charge. Many persons use the book to learn about the sources of products—and of course it is useful as a check to satisfy the Animal and Plant Health Inspection Service (APHIS) requirement that certain milk products used in processed meat products were produced in an approved plant.

## SURVEILLANCE FOR PENICILLIN

On March 3rd, of this year, the Dairy Division of USDA inaugurated a penicillin surveillance program on dry milk, evaporated milk, and dry whey. The purpose of the program is to eliminate marketing of adulterated manufactured dairy products under official gradings and to help assure the public of wholesome dairy products. This includes, of course, Government purchases of nonfat dry milk under the Federal Dairy Price Support Program. In this connection USDA is simply exercising the prudence it should to avoid purchasing an adulterated product.

Adulterated milk is not new to the dairy industry. Before the 1950's, mastitis was treated with various udder balms, ointments, and sulfa drugs. Then came news of wonder-drugs such as penicillin and the prospect that mastitis at last would be conquered. But as so often happens when man overcomes one major problem, he finds that in his victory he has created other problems. In this instance, the problem is the fact that penicillin-treated cows harbor antibiotic residues for several days depending upon the amount of penicillin injected into the udder and these residues are secreted with the milk. Also, penicillin is water soluble and follows the water phase in various dairy products. The usual pasteurization temperatures for milk apparently have no effect on the activity of penicillin.

The problem was brought to light last summer when a State Regulatory Agency discovered the presence of penicillin in an interstate shipment of nonfat dry milk.

Checks by the Food and Drug Administration on testing activities of the source State showed that regulatory and industry follow-up on offending milk producers was not always adequate to assure segregation of adulterated milk from use either in fluid or manufactured products. Such findings prompted an FDA survey last fall (1974) of dry milk production throughout the country.

We in the Dairy Division received reports from States on their findings of penicillin-positive lots. Our own checks on government holdings of dry milk revealed that some lots of officially graded nonfat dry milk contained measurable amounts of penicillin.

The source of the problem is on the farm and that is where the problem should be taken care of. More control is needed at the point of milk production so that surveillance on finished products can be dispensed with or at least minimized. We were obligated to initiate our own penicillin testing regimen on manufactured dairy products offered for official grade. We hope that in so doing we have given assistance and motivation to the dairy industry in eliminating milk adulterated with penicillin and other antibiotics.

In preparation for our penicillin surveillance program we sent letters to the managers of dry milk, dry whey, and evaporated milk plants; trade associations; and USDA Extension Service and State Regulatory officials explaining the purpose of our testing program and how we planned to implement it.

We also provided training for our resident graders in the techniques of performing the FDA approved *Sarcina lutea* Cylinder Plate Method. Some readers, I am sure, realize from experience that this test method is very sophisticated and requires a great deal of practice and skill.

## RESPONSE TO THE PROGRAM

Industry's response to our program was very gratifying. Dairy firms and plants immediately informed their patrons of the USDA proposed testing plan and of the importance for each dairy farmer to keep milk from treated cows separated from the normal milk supply. Meetings were held between industry representatives and State Regulatory authorities and with veterinarians to coordinate the surveillance work on producer milk. By March 3rd, most interested parties had acquired the necessary testing material, had trained company personnel in the techniques of testing, and had established a testing surveillance program of their own.

I want to now give you a brief resume of the results of our tests on dry milk since we started our surveillance on the 3rd of March.

| | |
|---|---|
| No. of tests | 2265 |
| No. of positives | 63 |
| No. of plants having positive tests | 17 |
| No. of states with positives | 8 |

I believe this low level of official positive tests is the result of the industry's own testing program. It is obvious that the dairy plants—both grade A and manufacturing milk have been doing a lot of hard work to get on top of this problem. We think this is fine and we applaud their efforts. It is not our purpose to try to test all of the product manufactured, but rather to assure ourselves and users that the dairy industry is taking corrective action to eliminate adulterated milk from the milk supply and from manufactured products. If we have inspired the industry in this direction, we have accomplished our goal.

## RELATIONSHIP WITH FDA

I think that is is important at this point to explain Dairy Division's relationship with the FDA; specifically as it has to do with sharing information on positive test results.

At the outset we envisioned a situation with the FDA similar to that involving our *Salmonella* Surveillance Program. In that program, we test a number of dry milk samples in connection with the quarterly plant inspections and report all positive *Salmonella* tests to the FDA. So long as the industry cooperates with us by segregating and reprocessing contaminated product, the FDA in general has not imposed itself into the problem. This works quite well and has virtually eliminated duplicate *Salmonella* testing on the part of the FDA. With our testing for penicillin, however, we soon learned that the FDA did not look upon this surveillance as being comparable to that which we have on *Salmonella.* You see, our current testing for penicillin is only on lots of milk powder offered for official grade or compliance with buyers' specifications. It does not provide for surveillance of the plant's production as does our *Salmonella* program. Therefore, when the FDA receives our notice of a positive penicillin test, it raises a red flag on the rest of the plant's production. We quickly realized that under this kind of a situation the FDA was obligated to follow-up on every positive report. We also realized that this action has put both the plant and USDA in an untenable position.

Accordingly we notified FDA that we would not report any positive test results to them so long as the plant in question satisfactorily segregated and disposed of its adulterated milk powder for non-food use. Based upon the cooperation shown us by industry under our *Salmonella* surveillance program, we look for an equally successful relationship concerning penicillin-adulterated product.

Buyers of milk products are concerned that they are getting penicillin-negative products. They can test the product themselves or use a commercial laboratory. Another way to get this assurance is to require USDA testing and certification as a part of purchase specifications.

## IN SUMMARY

In summary, I think we can all agree that the root of the penicillin problem is back at the farm. It is there that adulterated milk can be segregated more effectively and certainly with less trouble than when testing dairy manufactured products or even tankers or commingled milk. Such testing of milk and milk products is similar to attacking a forest fire after it has burned through several acres of trees. Such efforts would be unnecessary if proper attention had been paid at the campsite.

I will close by reiterating what I said earlier. Our penicillin testing program is designed to protect the integrity of the USDA inspection and grading services; and to make reasonably certain that no officially graded product harbors penicillin residue.

We are deeply concerned about the need for minimizing duplication of inspections by USDA, FDA, and State regulatory agencies. Industry and government cooperation should help us meet this challenge. We hope that USDA Dairy Division by our actions will be able to help the dairy industry in its efforts to eliminate penicillin and other antibiotics from the raw milk supplies and from manufactured dairy products.

The dairy industry has faced other quality and wholesomeness problems in the past. It has cooperated with government agencies to surmount those problems. The result has been quality improvement and assurance of wholesome products. Quality and wholesomeness surveillance is a never ending job but it pays off in dependable dairy products food ingredients that will help to make products that will give good satisfaction to consumers.

### ACKNOWLEDGMENTS

This paper was presented at the Annual Meeting of the Food Research Institute, University of Wisconsin-Madison, Madison, Wisconsin, May 22-23, 1975.

*J. Milk Food Technol. Vol. 38, No. 10, Pages 624-629 (October, 1975)*


# Proceedings of the Fifteenth National Conference on Interstate Milk Shipments

St. Louis, Missouri, May 11-15, 1975

**J. C. McCAFFREY**

*National Conference on Interstate Milk Shipments*
*3306 Glouster Street-Kensington Park, Sarasota, Florida 33580*

(Received for publication August 4, 1975)

## EXECUTIVE BOARD MEETING, MAY 11, 1975

The meeting, held in the Venetian Room, Chase-Park Plaza Hotel, St. Louis, was called to order by Chairman Vaux at 4:40 p.m. Voting Board members present: Causey, Gay, Harvey, Heinemann, Meister, Rich, Rowley, Russell, Schilling, Seaman, Thompson, Van Patten, Vaux; absent: Arledge, Johnson, Weckel. Non-voting Board members present: Boosinger, Conner, Gadd, Speer; guest: Eugene McGarrahan.

Minutes of the February 13, 1975 meeting were accepted as mailed. The Treasurer's report, showing a balance of $3,315.48 as of May 10, 1975, was accepted as read.

The following Council changes were proposed and accepted by the Board: (*a*) *Council I*—Chas. Morrow for Glenn Shelor; Wm. B. Hastings for James B. Honan; John D. Kaczor for Lloyd Fox. (*b*) *Council II*—Richard Dennler for Clyde Helsper; Lee Roland for Bill Semerod; Otto Hampton for Dean Stambaugh. (*c*) *Council III*—Archie Smith for Neil McBeath; Edwin Ruppert for Ford Brendle; James Carroll for Deland Morrow; Glen E. Huskey for Steve Conerly; Willis Roberts for C. Jones.

Chairman Vaux made the following committee appointments, all being approved by the Board. Resolutions Committee: James I. Kennedy, Frank L. Kelley, and Don Race, chairman. Credentials Committee: C. K. Luchterhand, Henry Atherton, and Orlowe Osten, chairman. Nominating Committee: George W. Fouse, Richard C. Dennler, Earl Helmreich, John Baghott, Archie Hurst, and Sam Noles, chairman.

Chairman Vaux discussed the proposed method of operations during the conference. The Board voted to authorize the Chairman to act for the Board in determining the order of business. Gene McGarrahan was asked to present an "overview" of FDA's plan to implement the Pure Milk Ordinance (P.M.O.). The meeting was adjourned at 5:50 p.m.

## FIRST GENERAL SESSION

The first general session of the Conference was called to order at 10:05 a.m. on Monday, May 12, 1975, by Chairman Vaux. The invocation was by Sam Noles, Florida Department of Health. The address of welcome was by Dr. Helen L. Bruse, Health Commissioner, City of St. Louis. The keynote address was by James L. Reeves, Mid-America Dairymen, Inc., Springfield, Missouri.

Chairman Vaux named the members of the Credentials, Nominating, and Resolutions committees, and gave the respective charges. Secretary McCaffrey presented the Treasurer's report and moved the acceptance of both the report and the minutes of the 1973 Conference as mailed to the participants. Motion was approved. Chairman Vaux called for reports of the standing committees: (*a*) Abnormal Milk, (*b*) Constitutional Revision, (*c*) Laboratory, and (*d*) Single Service Containers and Closures. The Conference accepted the reports and referred them to the appropriate councils.

Chairman Vaux discussed procedures under which the Conference would operate. He then assigned the various meeting rooms for the respective Councils, and instructed chairmen to make their preliminary reports to the membership on Tuesday afternoon.

## SECOND GENERAL SESSION

The second general session was convened by Chairman Vaux at 1:35 p.m. on Tuesday, May 13, 1975. The first roll call of states and delegates authorized to vote on Conference agreements was taken by Secretary McCaffrey. The chairman then called on each Council chairman to present the preliminary report of their deliberations. Dudley Conner reported for Council I, Jay Boosinger for Council II, and Erwin Gadd for Council III.

## THIRD GENERAL SESSION

Chairman Vaux convened the third general session at 1:10 p.m. on Wednesday, May 14, 1975. Each Council chairman presented his complete council report, listing all problems and proposed solutions to be voted on at the final session. Each report included both changes in and additions to existing "Procedures." Complete council

reports were given to each participant as he/she entered the meeting room for this session.

## FINAL GENERAL SESSION

The final general session was called to order by Chairman Vaux at 8:35 a.m. on Thursday, May 15, 1975. The final roll call of states and delegates authorized to vote on Conference agreements was taken by Secretary McCaffrey. The roll call showed that 42 states were represented, 12 by both agriculture and health, 14 by agriculture only, and 16 by health only. The health department of the District of Columbia was also represented. Chairman Vaux presented the Past-Chairman award to John C. Schilling.

## RESOLUTIONS

Chairman Don Race presented six resolutions. Number 4 was tabled until a later time, while all others were accepted. One additional resolution was presented from the floor by Leland Lockhart of California. It was accepted by the delegates. The resolutions are listed in the order of their presentation.

(1) *Whereas* the success of a Conference such as the one which is now drawing to a close is dependent upon the untiring efforts of dedicated individuals who have given their time in making arrangements, providing facilities, and making every possible effort which adds to the comfort of all those in attendance be it therefore resolved that the Conference go on record as giving a special vote of thanks and appreciation to: (*a*) the Chairman of the Local Arrangements Committee, John C. Schilling, St. Louis Health Division, and his committee, Vernon R. Cupps, James I. Kennedy, Raymond Lange, George L. Stemmler; and to the Chairperson of the Ladies Hospitality, Mrs. Dorothy Schilling, who made possible the fine facilities and whose outstanding hospitality will leave fond memories of the 1975 St. Louis Conference; (*b*) John Speer, and the Program Committee for setting up the challenges made to stimulate and guide the Conference in its efforts to reach the goals established by the Conference; (*c*) the Chairman of the 1975 Conference on Interstate Milk Shipments, Herb Vaux, Indiana State Board of Health, whose organizational abilities and keen understanding brought the Conference to a high plane of professional pride and accomplishment; (*d*) the Chairman and Co-Chairman and members of the three governing Councils who through their patience, understanding, and tolerance guided us to the decisions which will be our guideposts for the next two years, and for the professional way in which Council deliberations were conducted at times of seeming stress and difficulties; (*e*) the members of the Executive Board who have worked dilligently in the past two years to establish policies which made the Conference a strong, viable organization; (*f*) the Chairman and members of the Single-Service Containers Committee, the Abnormal Milk Committee, and the Laboratory Committee for their diligent work in providing the Conference with direction in their various important fields of activities; (*g*) the Hotel management for providing the fine facilities, the courtesies, and the services which added to the comfort of those in attendance; (*h*) and a special note of appreciation to J. C. McCaffrey, Executive Secretary-Treasurer of the National Conference on Interstate Milk Shipments, whose keen understanding of the Conference organization, goals, and objectives and his ever-present ability to guide the Conference whenever questions arise; and (*i*) to all those in attendance whose statesmanship and intelligent deliberations continues to make the goal of the best possible milk for all the people a reality.

(2) *Whereas:* On November 29, 1974, President Ford issued an Executive Order #11821 directing all departments of the executive branch which promulgate rules and regulations to prepare an economic impact study in connection with any new proposed rules and regulations and, *Whereas:* Senators Humphrey, Dominick, and Bensten have introduced S.B. 4195 which directs all future legislative proposals to take into consideration the economic impact of such legislation and, *Whereas:* There is serious and widespread concern regarding the detrimental effect that many laws and regulations have on consumer prices, on state and local governments and on business vitality and productivity and, *Whereas:* the Food and Drug Administration on May 2, 1975, issued a pre-proposal draft of Proposed Regulations for Grade A Milk and Milk Products and, *Whereas:* these proposed regulations depart substantially from the historical role played by the U.S. Public Health Service in assisting the states in administering meaningful and uniform Grade A Milk regulatory programs through involvement in the Interstate Milk Shippers Conference, and, *Whereas:* the states which participate in the Interstate Milk Shippers Conference have historically met together with each other, the affected industry and the Public Health Service to promote better milk for all people through reaching voluntary agreement on Procedures to be followed and, *Whereas:* the voluntary agreements could be changed by the delegates to these meetings and, *Whereas:* the Proposed Regulations for Grade A Milk and Milk Products when finalized will have the full effect of law: *Therefore be it resolved* that the Interstate Milk Shippers Conference requests the Commissioner of the Food and Drug Administration prepare an economic impact study which will consider the economic impact that the form of the present proposed Grade A Milk and Milk Products Regulations will have on consumers, state and local regulatory programs, and the dairy industry, *Be it further resolved that* this economic impact study consider the economic impact on consumers, state and local governments, and the dairy industry with regard to the content of the proposed Grade A Milk and Milk Products Regulations, *Be it further resolved that* the Chairman of the Interstate Milk Shippers Conference furnish a copy of this resolution to all members of the

Appropriations Committee in the U.S. House of Representatives and the U.S. Senate.

(3) *Be it resolved that* the Board of Directors and the Chairman of the NCIMS be and are hereby authorized and directed to take, within their powers as directors and officers of the NCIMS, all action or actions that they deem necessary and appropriate at local, state, and federal levels to perpetuate and protect the traditional principles, procedures, and philosophies of the NCIMS.

(4) *Resolved that* the voting delegates of the 1975 National Conference on Interstate Milk Shipments meeting in St. Louis, Missouri, May 11-15, 1975, officially register opposition to the format, structure, direction, intent and policy embodied in the proposed Federal Regulations by USPHS-FDA and the official delegates to the NCIMS shall, at the Conference of State Officials charged with the implementation of the proposed Federal Regulations, request the USPHS-FDA delay, for a period of not less than one year, the publication of the proposed Federal Regulations in the *Federal Register* to provide sufficient opportunity for all state regulatory agencies to fully evaluate and understand the impact of these proposed regulations. If necessary, another conference should be convened to officially deliberate and offer comments to the USPHS-FDA on the proposed Federal Regulations for Grade A Milk and Milk Products.

This resolution was tabled, and later withdrawn, to be replaced by the combined policy statement of the three Councils, which was read to the delegates by Dudley Conner, and appears in the report of Council I.

(5) *Whereas* the Interstate Milk Shippers Conference was organized to permit the free flow of milk across state lines through uniformity of inspection, by creating a confident rapport between states and creating an attitude of integrity of one state with another and

*Whereas* it has been reported that conditions exist where surveys have not been reported, reports have been delayed or surveys been terminated because of unsatisfactory results, and inasmuch as this action is contrary to the intent of the IMS Conference let it be

*Resolved that* all sanitation ratings shall be conducted in an orderly and organized manner as outlined in the conference proceedings with the intent and goal to obtain as accurate a rating as possible of the true and actual condition of the milkshed, and that they be completed to fruition and reported promptly as outlined, and be it further

*Resolved that* the Regional Milk Consultants monitor the activities of the state survey programs as closely as practical to identify deviations from the outlined procedures.

(6) *Be it resolved* that all registrants of this conference forget the Delightful Duo, the Triumphant Trio, the Fearsome Foursome, and the Dirty Dozen in order that they may focus their attention and appreciation on the Unlucky Thirteen—those thirteen companies whose generosity made it possible that all of us enjoyed the relaxation and fellowship with appropriate libation on Monday evening after the stress and rigors of the initial general session and Council deliberations. The unlucky thirteen to whom we express our appreciation are: Beatrice Foods, Borden Inc., Chapman Ice Cream Co., Kroger Dairy, Mid-America Dairymen, Packet Dairy, Prairie Farms, Pevely Dairy, Raskas Dairy, Sealtest Foods, Southern Products, T. C. Jacoby Co., and Velvet Foods.

(7) In 1973 a pre-publication draft of a revision of the Public Health Service Publication #1465, *Fabrication of Single Service Containers and Closures for Milk and Milk Products, Guide for Sanitation Standards* was prepared. This document was reviewed by the Single Service Committee of the NCIMS and a few minor changes were recommended. Since it will be at least two years before a revised Pasteurized Milk Ordinance can be adopted as a regular, or recommended ordinance for adoption by states, and since the Public Health Service Publication 1465 has been out of print for several years and since it is no longer available as a guide for the manufacture of single service containers and closures, it is recommended that the U.S. Food and Drug Administration publish the recommended prepublication draft on an interim basis until the Single Service Container and Closure Guide is published with the proposed Pasteurized Milk Ordinance as a regular or a recommended ordinance.

## COUNCIL I

Chairman Vaux called on Dudley Conner, Chairman, to give the Council I report. The Council was assigned 19 problems, and voted "no action" on 15.

*Problem 3* dealt with the testing of milk and milk products for inhibitory substances. The Council proposed that the National Conference on Interstate Milk Shipments should recommend to the U.S. Food and Drug Administration that when the F.D.A. and state regulatory agencies test milk and milk products for inhibitory substances such as antibiotics, the testing methods employed and dilution factor for the final product be a procedure with sensitivity equal to the method used for testing raw milk. The delegates voted to amend the Council proposal by adding, after the words "raw milk," a comma, and then complete the sentence with the phrase "employing the disc assay method, using *Bacillus subtilis.*" The amended proposal was approved.

*Problem 9* stated: "The present standard of 1,500,000 per milliliter somatic cell count is an effective and reasonable level of enforcement for the abnormal milk control program and should be maintained." The Council recommended approval of this action. Delegates concurred.

*Problem 17* dealt with a possible change in the stability of penicillin. The Council recommended that this problem be referred to the Laboratory Committee for further study. Delegates agreed.

*Problem 19* dealt with proposed revisions of

procedures governing the cooperative state-PHS program for the certification of interstate milk shippers. The Council recommended that (*a*) to section I.A.1., add the following sentence: "In making sanitation compliance ratings of interstate milk shippers of dried sweet whey from Cheddar cheese or other cheese, the basic sanitation standards shall be the Interim Criteria and Sanitation Standards for use of sweet whey in Grade A Milk and Milk Products, Transmittal 75-1-M-A-54, February 3, 1975, dealing with acid whey from cottage cheese and, 75-2-M-A-55, issued on March 4, 1975, dealing with sweet whey by the Dairy and Lipid Products Branch, Bureau of Foods, Food and Drug Administration, United States Public Health Service," (*b*) revise Section II, 1 to read as follows: "1. Supervision of the milk supply, dry milk powder, and dried sweet whey from Cheddar or other cheese to be rated for interstate certification shall be based on the criteria and procedures for Grade A standards set forth in Section I, A, or regulations pertaining to supervision substantially equivalent thereto," and (*c*) revise section VIII to read as follows: "Agreements adopted by the National Conference on Interstate Milk Shipments shall apply to raw milk for pasteurization and to pasteurized milk, pasteurized milk products, Grade A non-fat dry milk, and dried sweet whey from Cheddar or other cheese." Delegates concurred.

Conner then listed charges that the Laboratory Committee reported. (*a*) Should goat's milk meet the same somatic cell count requirements as cow's milk? The Committee recommended that this problem be referred to the National Mastitis Council for additional study. Delegates accepted the recommendation. (*b*) Changes in procedures for the determination of the bacterial counts in both Grade A raw and pasteurized milk. The Committee recommended that this charge be continued and a report be made at the next conference. Delegates agreed. The Laboratory Committee asked for clarification as to whether problems 44B, 44C, and 44D from the 1973 deliberations of Council I should be considered by the Committee.

Chairman Conner read the report of the Committee on Abnormal Milk Control as follows: "The IMS Committee on Abnormal Milk Control reaffirms the recommendation of the Committee at the May, 1973 IMS Conference that the action level for abnormal milk control should remain at 1,500,000 somatic cells per milliliter. This recommendation is in agreement with a resolution of 6 February, 1974, by the Board of Directors of the National Mastitis Council.

The IMS Committee on Abnormal Milk urges NCIMS to forward to FDA the recommendation of the Committee that was approved by the NCIMS delegates in May 1973 as follows: that FDA encourage all laboratories to make it possible for milk control agencies to report the best estimate of somatic cell content of samples of bulk tank raw milk whenever screening tests are done. To this end, each laboratory should develop its own appropriate standard curve and conversion chart. This would require rewording of *Guidelines for the Control of Abnormal Milk* and directions in PHS Publication 1306 *Screening and Confirmarory Tests for the Detection of Abnormal Milk* when they are revised.

The IMS Committee on Abnormal Milk further recommends the following: When the 13th edition of APHA *Standard Methods for the Examination of Dairy Products* is revised, each screening test description should include the recommendation that laboratories develop and maintain their own updated appropriate standard curve and conversion charts.

State milk control agencies should use such appropriate conversions and report the best estimates of somatic cell counts of bulk tank raw milk samples to the producers.

Approved industry and commercial laboratories, to furnish valid estimates of somatic cell numbers, should use recommended protocols and develop their own standard curves and conversion charts.

The IMS Committee on Abnormal Milk Control is prepared to make specific recommendations on rewording of *Guidelines for the Control of Abnormal Milk* if requested to do so by NCIMS or FDA.

It is recommended that the IMS Committee on Abnormal Milk be continued. Gadd moved, seconded by Gay, that the Committee report be tabled and referred back to the Laboratory Committee for further study. Delegates concurred.

Conner then read the proposed combined Policy Statement of Councils I, II, and III. The amended policy statement as finally approved by the delegates, follows.

The delegates at the 1975 NCIMS hereby direct the Conference Chairman and the Executive Board to make an appropriate presentation to the Commissioner of F.D.A. that:

(*a*) The delegates reaffirm the 1973 Conference resolution which opposes the publication of the Pasteurized Milk Ordinance as a federal regulation in the *Federal Register,* and that if it is published in the *Federal Register* it be published only as a model ordinance and code for adoption by state and local governments on a voluntary basis.

(*b*) The delegates strongly recommend that the pre-proposal draft of the *Grade A Milk Sanitation Regulations* now before the Conference not be published in the *Federal Register* as a Federal Regulation.

(*c*) The Conference requests that the period for filing comments on the pre-proposal draft of the Pasteurized Milk Ordinance, the notice of which was published in the *Federal Register* dated May 5, 1975, be extended for 12 months to August 4, 1976.

(*d*) The Conference requests that a draft of the proposal, as a model ordinance for adoption by state and local regulatory agencies, be made available at least 90 days before the next operating session of the NCIMS, the date of such session to be established by the Executive Board, for further review and comment, and

that it not be printed in the *Federal Register* for any purpose until after that time.

## COUNCIL II

Chairman Vaux called on Jay Boosinger, Chairman, to give the Council report. Council II received nine problems, two of which were voted "no action."

*Problem 3* dealt with the training for milk sanitation rating officers and regional milk and food consultants. The Council recommended that the Conference request the U.S. Food and Drug Administration to provide funds for training of state milk sanitation rating officers and regional milk and food consultants in the inspection of single service milk container and closure plants. The delegates concurred.

*Problem 4* dealt with the certification of milk and food consultants. The Council recommended that Section VI, A.1 be changed to read: "The Public Health Service shall standardize at least every 3 years the rating procedures of: (*a*) Public Health Service regional personnel, and (*b*) State milk sanitation rating officers," and Section VI, A.2 be changed to read: "The Public Health Service shall publish a list of Public Health Service milk consultants and State milk sanitation rating officers whose rating methods and interpretations of the Public Health Service recommended 'Milk Ordinance' have been evaluated and approved by the Service." The delegates concurred.

*Problem 5* dealt with the procedures for deciding how many producer dairies will be evaluated during a Food and Drug Administration check rating. The Council recommended the following addition to Section VI, D.2: "One-half the number of farms needed to make an official survey will initially be visited. If the rating indicates the necessity for procedural action, the accompanying State milk sanitation rating officer shall be asked to concur with the FDA consultants as to the lack of maintenance of the sanitary status of the milk-shed. If, in the opinion of the State milk sanitation rating officer, the sample of farms selected does not appear to be sufficient to warrant procedural action, such additional producer dairies, selected at random, shall be inspected so that agreement can be reached as to the need for procedural action. The total number of dairies inspected shall not equal the number necessary for a complete survey." The delegates concurred.

*Problem 6* dealt with check ratings. The Council recommended that the following statement be added to Section III, I. 2. "And in the case of ratings 90% or over, they shall be reported to the U.S. Food and Drug Administration as 'at least 90%.' All sanitation ratings which are reported to the U.S. Food and Drug Administration as 'at least 90%' shall be listed as 'at least 90%.' After extended discussion the delegates voted *not to accept* the Council recommendation. The motion was made, seconded, and passed, that this problem be referred back to Council II for further study and a future report.

*Problem 7* dealt with the distribution of Form PHS-842 and FD 2359i. The Council recommended that the following statement be added to Section V, A.1: "The State milk sanitation and certifying agency should immediately send a completed copy of Form PHS-842 and Form FD 2359i to the State regulatory agency upon completion of any sanitation rating." The delegates concurred.

*Problem 8* dealt with check rating results. The Council recommended that the phrase "and the State regulatory agency" be added to Section VI, D.7. Delegates concurred. *Problem 9* was covered by Resolution No. 5.

Chairman Boosinger then read into the record the report of the Single Service Container and Closure Committee. "Many members of the Single Service Container and Closure Committee had questions concerning the 'proposed' Grade A Milk Sanitation Regulations. One distressing point brought out was the 'watering down' and partial elimination of Sections A, B, and C of the prepublication draft of the revised Public Health Service Publication #1465 that this committee has been working on for several years.

The deleted material included definitions and terms used by the single service container industry and cannot be found elsewhere, except in the regulations and score card, where the terms would be useless unless the document were preceded by definitions. We have objections to the listing of SSC&C plants by a point system recommended 90% or above.

We discussed the importance of single service containers and closures from the Canadian sources. It appears these fabricators are not being certified by anyone from the United States. Several questions were raised; are these sources going to meet an equivalency?, and how would we know if they were even acceptable in Canada?

The Committee agreed to accumulate their comments on the proposed Grade A Milk Sanitation Regulations through myself (Lockhart) and submit them through channels by August 4.

We had a demonstration of a plastic coated corrugated container with a plastic film liner that would be used between plastic blow molding plants and milk plants to deliver light weight plastic bottles. Due to the increasing cost of plastic resin, these fabricators are being forced to use lighter weight bottles. To deliver these bottles a more rigid container is needed. The plastic film liner would only be used once but the coated corrugated bow would be returned to the fabricator for several trips. The committee will undertake a study of the practicability and safety of this proposal. Also the recycling of chip-board liners is being studied at the same time.

The committee plans to meet again this summer in connection with the IAMFES meeting in Toronto. The delegates accepted the report.

## COUNCIL III

Chairman Vaux called on Erwin Gadd, Vice-chairman, to give the Council report. Council III received

seven problems, two of which were voted "no action." Problems 1, 2, and 4 dealt with reciprocity so were handled together. The Council recommended that Section VI, B.4 be deleted from the "Procedures." The delegates voted *not to accept* this recommendation. The Council recommended that Section I, A.2 and 3 remain as they are in the "Procedures." The delegates concurred.

*Problem 3* dealt with procedures to be followed in removing the asterisk from a listed state. The Council recommended that "Those states not practicing complete reciprocity as defined in Section I, A.2 and Section I, A.3 will continue to be identified until the problem or problems have been resolved. The Chairman of Council III shall notify the Conference Chairman when he has received sufficient information from the complaining state or states, or is otherwise satisfied that the problem leading to said identification has been corrected. The Conference Chairman shall so advise the publisher of the quarterly publication of *Sanitation Compliance and Enforcement Ratings of Interstate Milk Shippers.* The delegates concurred.

*Problem 7* dealt with the "90 day" requirement for resurvey. The Council recommended that the phrase "but not more often than 90 days from the date of the last rating" be removed from Section III, F.1 and G. The delegates voted *not to accept* this recommendation.

After the completion of the Council reports Chairman Vaux discussed the pros and cons of a non-terminal adjournment of the Conference. He explained that, if FDA agrees to a 12-month extension of the comment period, an adjournment would be in order. However, on new problems would be presented. Conner of Kentucky moved for a non-terminal adjournment, seconded by Jefferson of Virginia, to a date to be determined by the Executive Board and Chairman. The delegates were in unanimous agreement.

The non-terminal adjournment of the fifteenth National Conference on Interstate Milk Shipments occurred at 12:14 p.m. on Thursday, May 15, 1975.

## EXECUTIVE BOARD MEETING, MAY 15, 1975

The meeting was called to order by Chairman Vaux at 1:32 p.m.

Voting Board members present: Arledge, Causey, Gay, Harvey, Heinemann, Meister, Rowley, Russell, Schilling, Seaman, Thompson, Van Patten, Vaux. Absent: Johnson, Rich, Weckel. Non-voting Board members present: Boosinger, Conner, Gadd, Speer.

Chairman Vaux accepted, with regret, the resignation of Harold Thompson, Jr. from the Board. He was replaced by Eugene T. McGarrahan, F.D.A., Bureau of Foods. The Board authorized the Executive Secretary to record a unanimous vote of appreciation and thanks to "Tommie" for a job well done.

Jay Boosinger was appointed to the Board to fill the unexpired term of Wendell Carr. The Board went on record as voting that there is no objection to a Board member serving as a member of a Council.

Chairman Vaux reminded the Board that the 1975 meeting of the Conference had been *non-terminally* adjourned to a future date to be determined by the Board chairman and the Board, the reconvening of the meeting to depend on whether F.D.A. acceded to the Conference request for an additional 12-month period to study the pre-proposal draft. After considerable discussion the Board voted to reconvene the Conference within a year, if the delay is approved by F.D.A., at the Chase-Park Plaza Hotel, with the Executive Secretary being empowered to arrange for suitable dates.

Erwin Gadd, acting Chairman of Council III, reported that California and New York have now qualified for reciprocity. Russell moved, seconded by Van Patten, that the asterisk be removed from California; Russell moved, seconded by Harvey, that the asterisk be removed from New York. The Board approved both motions and instructed the Chairman to so notify F.D.A.

The Board instructed the Executive Secretary to p' an item in the next Newsletter to the effect that any .e may be changed from non-reciprocity to reciproc. or vice versa at any time, and that a complaining state shall notify the Conference Chairman or the Chairman of Council III, of a problem. Council III will then investigate the situation and report its findings, with recommendations, to the Board.

Van Patten indicated that the Dairy Division of NASDA is willing to help the Conference in Developing an economic impact statement for F.D.A. Chairman Vaux was authorized to appoint a committee to study the problem.

The Executive Secretary presented a list of hotels and cities that had submitted proposals to host the 1979 Conference. The Board voted to hold the 1979 Conference at Stouffer's Louisville Inn, the exact dates to be selected at a later time. The meeting adjourned at 2:50 p.m.

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# Penicillin in Milk and Milk Products: Some Regulatory and Public Health Considerations

**J. C. OLSON, JR. and A. C. SANDERS**

*Division of Microbiology, Bureau of Foods*
*Food and Drug Administration, Washington, D.C. 20204*

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**ABSTRACT**

Milk and milk products containing penicillin may present a health hazard to individuals who are hypersensitized to penicillin. The following aspects of this problem are discussed: (*a*) the condition of human hypersensitivity to penicillin, (*b*) the mechanism by which milk may become adulterated with penicillin, (*c*) tolerances for residues of penicillin in foods, (*d*) methods of analysis and criteria of acceptance, (*e*) use of products containing penicillin, and (*f*) surveillance of products offered at import.

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In addition to the fact that milk and milk products containing penicillin are considered adulterated under the Food, Drug, and Cosmetic Act, such products may present a significant hazard to the health of individuals who are hypersensitized to penicillin.

Since milk and milk products are consumed directly and also are used as ingredients in various food formulations, I shall attempt to bring into focus several facets of the problem presented by milk supplies containing penicillin. First of these is the condition of human hypersensitivity to penicillin which is the underlying reason for our concern; second, the mechanism through which penicillin finds its way into milk supplies; third, the matter of tolerances; fourth, methods of analysis and criteria of acceptance; fifth, the question of what can be done with products when penicillin is found in them; and finally, assurance that imports do not add to the problem.

## HUMAN HYPERSENSITIVITY TO PENICILLIN

Among the more common hypersensitivity drug reactions (allergic) in man are those caused by penicillin. Most drugs such as penicillin which cause allergic reactions contain reactive contaminants or reactive metabolic derivatives formed in vivo which are solely (or additionally) responsible for formation of hypersensitizing antigens. Mechanisms involved in drug allergies are, in general, well illustrated by the penicillin models.

One of the least toxic drugs in therapeutic use is penicillin. Even so, approximately 10% of all individuals who receive repeated doses of the antibiotic can become so highly sensitized that a single and very small amount may elicit hypersensitive reactions manifested by several physical signs and symptoms. The empirical clinical use of this antibiotic during the past 30 years has resulted in a sensitized populationof unknown proportions. Stewart (*7*) reviewed the pertinent literature in 1965 and again in 1970 and concluded that it is not possible to determine a true figure of incidence but that it lies between 1% and 10%. In 1973 he (*8*) further stated that nothing had been found in the intervening period to contradict that estimate. In 1959 Welch (*10*) had estimated the hypersensitive population in the U.S. to be 17 to 20 million individuals (based on a population of over 175 million at that time).

Penicillin hypersensitivity can be induced in two ways: (*a*) the immediate type involving reactions of humoral tissue with a specific antigen such as the penicilloyl-protein conjugates formed in tissues following intramuscular injection and the oral administration of the antibiotic, and possibly the ingestion of certain foods such as milk and milk products, and (*b*) the delayed type which is a form of immunologic response that is mediated by sensitized lymphoid cells rather than by humoral tissue. This type of sensitization can be the consequence of long exposure to and contact with penicillin not therapeutically administered (*3*), e.g., penicillin production plant workers, nurses, and pharmacists. Similarly, the dermatophyte *Trichophyton,* an etiologic agent of cutaneous mycosis, and other ubiquitous fungi produce penicillin-like molecules which may also sensitize an individual who never received penicillin therapeutically (*3*). It is conceivable that long term ingestion of milk containing low levels of penicillin could also sensitize in this way. However, regardless of the kind of exposure, reactions are varied: mild skin rashes, often urticarial, to severe generalized urticaria, edema, anaphylactic reactions, and sudden death. The most severe and critical reactions are caused by the parenteral application of this antibiotic in therapeutic use.

Anaphylactic reactions as a result of oral administration were at one time considered to be less severe and never fatal. Recently, however, it has been observed that serious reactions following the ingestion of penicillin can lead to serum sickness, the Arthus syndrome (humoral antigen-antibody complexes resulting in vascular injury), and death. In 1971 Spark (*6*) reported four fatal anaphylactic reactions following the therapeutic administration of oral penicillin. Three of the individuals had received prior penicillin therapy;

similar information was not available for the fourth victim. The interval between ingestion and death in each case was 30 min to 1.5 h. All fatalities were the consequence of a single oral dose of between 400,000 and 1 million units.

Literature is sparse regarding reactions attributed to milk and milk products and those reported have been of the urticarial type. Generally these reactions occur in individuals who have been sensitized by therapeutic applications. Vickers et al. (*9*) reported one such case from England involving a woman who drank a liter of milk each day. Since the milk produced at her farm contained 4 units/ml, she may have ingested up to 4000 units of penicillin daily; she was sensitive to 4 units of penicillin G intramuscularly. Therefore, the injection of 4 units would yield about 0.000013 unit/ml of blood. Some hypersensitized individuals have been reported to react severely to an intradermal injection of 0.000003 unit of the drug (*2*). Similarly, Zimmerman (*11*) reported on 4 cases of chronic urticaria associated with the ingestion of dairy products. In each case the reactions cleared rapidly after the intramuscular injection of 800,000 units of neutrapen (penicillinase) and remained urticaria-free when dairy products were eliminated from the diet. However, the patients could include these products in their diets without subsequent allergic reactions when neutrapen was administered prophylactically before ingestion of the foods.

Later Rosanove (*5*), with reference to Zimmerman's study, reported his observations of a patient at the Mayo Clinic who had a persistent oral and cutaneous blister-like eruption that clinically and histopathologically resembled pemphigus vulgaris (lesions of the mucocutaneous surfaces). This condition disappeared without specific treatment when milk and other dairy products were eliminated from the diet.

While it is clear that consumption of milk containing penicillin will elicit allergic reactions in the hypersensitive individual, there is no documented evidence that consumption of milk or milk products containing penicillin can alone induce the hypersensitive state.

The outstanding clinical feature of penicillin hypersensitivity is its unpredictability. Paradoxically, it is not dose-related: large doses may be well tolerated while small skin test doses may cause severe local or generalized reactions. No less paradoxical is the fact that one of the least toxic of all antibiotics has proven to be the most highly allergenic. It is suspected that individuals become hypersensitive to penicillenic acid derived from the 6-aminopenicillenic acid nucleus. Though not yet proved, this could well indicate that sensitization by one pencillin can and does result in hypersensitivity to other penicillins derived either fermentatively or biosynthetically.

## INTRAMAMMARY INFUSION OF PENICILLIN

Presence of penicillin in milk and milk products occurs almost exclusively through the therapeutic treatment of mastitis in lactating or dry cows with penicillin. The principal method of administering the drug is by intramammary infusion.

There are numerous intramammary infusion products containing certifiable antibiotics intended for use in treating mastitis in milk producing animals. With few exceptions, penicillin is one of the antibiotic components of such preparations. These products are available as single entity antibiotics or antibiotics in combination with other drugs. Furthermore, some are over-the-counter and others are prescription-type drugs. In any event, for each there is specified the appropriate dosage, condition of use (i.e., for lactating or dry cow therapy or both) and the withholding time (i.e., the period of time after last administration of the drug that must elapse before either the milk or the meat may be used). As a practical matter, relative to minimizing the presence of penicillin in milk, it is most important to strictly adhere to the withholding time indicated for each drug. For lactating animals this ranges from 36 to 96 h depending on the drug used. To date, these withholding periods have been based on residue studies that indicate the period necessary to assure the absence of detectable residues in the milk. It is conceivable that with development of penicillin detection methods of greater sensitivity, withholding times currently designated may be modified. This matter is only one of several presently receiving attention by the FDA in the interest of more effective regulation of drugs intended for use in treating mastitis. In the final analysis, with lactating animals, it is the person who does the milking who determines, in fact, whether or not the appropriate withholding time is observed. Accordingly, the mechanism of enforcing adherence to a specified withholding time resolves itself largely to a combination of (*a*) "friendly persuasion" of the person who controls the milking operation, and (*b*) use of an analytical method for assaying the presence of the drug in milk offered for sale.

## THE MATTER OF TOLERANCES

In the course of FDA's recodification program for Chapter I of Title 21 of the Code of Federal Regulations, the recodified document for Subchapter E—Animal Drugs, Feeds, and Related Products was published recently in the *Federal Register* (V. 40, No. 60, pp. 13802-13993, Thurs., March 27, 1975). Section 556 of this document establishes the tolerances for residues of new animal drugs in edible products of food producing animals treated with the respective drugs. Section 556.510 specifically covers penicillin and salts of penicillin in food as follows:

a. Uncooked edible tissues of cattle: 0.05 ppm (negligible residue).
b. Uncooked edible tissues of chickens, pheasants, quail, and swine; in eggs; and in milk or in any processed food in which such milk has been used: Zero.

c. Uncooked edible tissues of turkeys: 0.01 ppm.

One of the several conditions that are taken into account in consideration of an appropriate tolerance for a drug (CFR, Title 21, Section 556.1) is that, "Any tolerance required will, . . . be conditioned on the availability of a practical analytical method to determine the quantity of the residue." This partly explains the differences in tolerance levels given in Section 556.510; however, FDA recognizes the inconsistency and currently has the matter under review. The sensitivity of the present FDA method for penicillin in animal tissues is approximately 0.05 unit per gram.

Thus, the tolerance for penicillin in milk indicated above is ZERO. In enforcing the regulation, FDA has taken the position that through use of a specified method of analysis, the finding of no detectable amount of penicillin in milk and milk products and in any milk-containing processed food will be considered as in compliance with the specified zero tolerance for the respective product. Two methods of analysis have been approved for use; however, specific conditions have been specified governing the use of each method.

## METHODS OF ANALYSIS AND CRITERIA OF ACCEPTANCE BASED ON ANALYTICAL FINDINGS

The first of these is the Disk Assay Method-A as described in *Standard Methods* (*1*); second is the *Sarcina lutea* cylinder cup method (*4*). Table 1 lists several dairy

TABLE 1. *Acceptance criteria for penicillin residues in dairy products*

| Product | Sample dil. | Dil. factor | Method | Accept. criteria[a] (Unit/ml or g of product) |
|---|---|---|---|---|
| Raw milk (Ind. producer) | None | 1 | DA[b] | <0.05 |
| Raw milk (Commingled) | None | 1 | CC[c] | <0.01 |
| Pasteurized milk | None | 1 | CC | <0.01 |
| Buttermilk | None | 1 | CC | <0.01 |
| Cond., conc., evap. | 1 + 1 | 2 | CC | <0.02 |
| Cheese, butter, ice cream | 1 + 1 | 5 | CC | <0.05 |
| Dried milks | 1 + 3 | 4 | CC | <0.04 |

[a]See text;
[b]Disc assay;
[c]Cylinder cup

products with indicated detection levels likely to be acceptable as based upon the sensitivity of the analytical method specified.

Studies have shown that the sensitivity in terms of units of penicillin per milliliter of suspension used in testing is approximately 0.01 unit for the cylinder cup method and 0.05 unit for the disc assay method. The sensitivities will vary slightly under normal usage of the methods. Accordingly, when analyses are made to establish or support a legal action, the sensitivity of the method is established in each instance of use of the test method. Thus, for example, the acceptance criterion for a particular lot of dried milk could conceivably be less than a value somewhat below or above 0.04 unit per gram of the product.

## DISPOSAL OF MILK AND MILK PRODUCTS CONTAINING PENICILLIN

FDA has taken the position that any manufactured product, if found to conform to the acceptance criterion as specified in Table 1, would be considered to be non-actionable, regardless of whether or not the raw material from which it was manufactured contained penicillin. We expect this policy to apply to all readily forseeable situations except for blending if utilized to achieve non-detectable levels of penicillin. Any product of such blending would be considered adulterated. For example, milk containing penicillin could be separated and the cream churned. If the butter, when tested, was below the detectable level, i.e., <0.05 unit per gram, the butter would be considered non-actionable. Also, dry milk containing penicillin may be used as an ingredient in medicated animal feeds containing penicillin provided the quantity of dry milk used in the feed does not result in raising the level of penicillin in the feed above the level approved for the feed. In general, any product resulting from further processing of milk or milk product containing penicillin and when tested and found to be below the detectable level of penicillin would be considered non-actionable; provided, that the further processing would in fact serve to remove or destroy the penicillin originally present (in contrast to merely diluting it) to the extent that the processed product when tested would be below the detectable level.

## DRIED MILK PRODUCTS OFFERED FOR IMPORT

Users of dried milk products may be concerned over the adequacy of our surveillance of imported products. Perhaps the following will serve to alleviate such concern.

The obligation of maintaining an acceptable level of product quality rests with the producer and/or the exporting country. FDA is charged with the responsibility of examining dried milk products offered for import into the United States.

In the interest of fulfilling this responsibility with minimum expense, we have undertaken the development of dried milk certification agreements with several exporting countries. Currently, agreements have been made with Belgium, Denmark, France, and The Netherlands. These agreements specify processing and quality control procedures including sampling plans and testing methods.

The sampling plan is designed to give minimum coverage to dried milk entries from those countries with low violation rates and maximum coverage to dried milk product entries originating from countries with a high violation rate. The sampling will only give audit coverage to those dried milk product entries originating from countries participating in the certification agreements. Since the initiation of this program in December 1974,

we have yet to find the first dried milk product entering with a detectable level of penicillin.

## CONCLUSION

In conclusion, there is no question that penicillin in milk supplies is a serious problem for the dairy industry. It is the responsibility of the dairy industry to control it. It is a problem that originates at the dairy farm. Control measures must be applied at that point.

Since the problem arises from bovine mastitis treatment with penicillin, the only practical way of preventing penicillin from entering milk supplies is by rigid adherence to the appropriate withdrawal period specified for the penicillin preparation used. Testing programs to detect failure to observe appropriate withholding periods must be applied at the producer level by routine testing of individual producer supplies or from bulk tank truck supplies, or both. Penalty clauses of most ordinances and regulations that govern milk production and processing practices are applicable to producers whose milk supplies are found to contain penicillin. Experience has shown that enforcement of appropriate provisions of ordinances or regulations is an effective deterrent to the recalcitrant producer who fails to prevent penicillin from being present in his milk shipment.

The problem appears to be of such magnitude that would seem to require the coordinated effort of all producer cooperative organizations to bring the problem under control—and the sooner the better for all concerned.

### ACKNOWLEDGMENTS

Presented at the Annual Meeting of the Food Research Institute, University of Wisconsin, Madison, May 22-23, 1975.

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# Abstract of Papers Presented at the Sixty-Second Annual Meeting of IAMFES

Toronto, Ontario, Canada, August 10-13, 1975

The program of the 62nd Annual Meeting of IAMFES included 18 contributed research papers and 20 invited papers. Abstracts of all the research papers and most of the invited papers appear below. The complete text of many of these papers will appear in future issues of the *Journal of Milk and Food Technology.*

## CONTRIBUTED RESEARCH PAPERS

**Correlation of the Milk Quality Gauge (MQG) With the Wisconsin Mastitis Test (WMT).** R. Kevin Chumney and Dick H. Kleyn. *Department of Food Science, Rutgers-The State University, New Brunswick, NJ 08903.*

The MQG is a relatively new screening test to detect abnormal milk, particularly at cowside. This test is based on observing the rate of flow of warm milk under vacuum through a dense, test disk. Resistance to filtration is considered evidence of abnormal milk. The objective of this research was to evaluate the MQG as applied to individual cow and herd milk samples, a filtration time of 15 sec or more being considered a positive test. The WMT was used as a basis for comparison. Of 364 herd samples tested, 97 were positive to MQG and 9 to WMT, 8 of these latter 9 samples being positive for both tests. Of 181 individual cow samples tested, 75 were positive to MQG and 22 to WMT, all of these latter 22 samples being positive for both tests. Of the 31 samples positive for both tests, the presence of a heavy yellow sediment on the test disk was evident in 25 cases. Thus, the MQG, based on time of filtration and observation of the color of sediment, appears to be a very acceptable test for detection of abnormal milk.

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**Survival of Foot-and-Mouth Disease Virus in Casein Produced from Infected Cow's Milk.** H. R. Cunliffe and J. H. Blackwell. *Plum Island Animal Disease Center, ARS, USDA, Post Office Box 848, Greenport, New York 11944.*

Milk-borne transmission of foot-and-mouth disease virus (FMDV) was implicated most notably during the 1967-68 epizootic in England. Consequently, this study was designed to examine survival of FMDV in milk and dairy products, including casein. Six batches of casein was prepared from cow's milk at various times after infection with FMDV. Casein was obtained by acidulation of milk with HCl to pH 4.6. Samples for infectivity assays were made as 10% suspensions in HBSS, or as sodium caseinate in MEM before and after extraction in trifluoro-trichloroethane. Samples assayed in cell cultures were negative for FMDV. However, after normal cattle were inoculated with these samples, it was evident that FMDV survived casein production in one of two trials from raw skim milk and in two of four trials from pasteurized (72 C/15 sec) skim milk. These data suggest that a portion of the FMDV infectivity in infected cow's milk is heat and acid stable or this unique resistance is provided by one or more milk components.

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**Heat Inactivation of Conidia Produced by Toxigenic Aspergilli.** M. P. Doyle and E. H. Marth. *Department of Food Science, University of Wisconsin, Madison, Wisconsin 53706.*

Conidiospores from six strains of *Aspergillus flavus* and *Aspergillus parasiticus* were inactivated with moist heat at 45, 50, 55, and 60 C. Results indicate that heat resistance of conidia was strain-dependent. Furthermore, strains of aspergilli that produced greatest amounts of aflatoxin also were most heat resistant. Various amounts of sodium chloride, sucrose, and glucose were individually added to the heating menstruum to determine their effect on heat resistance of conidia. At equivalent water activity values above 0.93, sucrose was most protective followed by glucose and then sodium chloride. At equivalent water activity values below 0.93, sodium chloride was most protective followed by sucrose and then glucose. Other environmental conditions that influenced heat resistance of conidia included the pH value produced by various buffers in the heating menstruum, age of conidia, and substrate used to produce the conidia.

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**Influence of Location and Substrate on Levels of Trace and Heavy Metals in Mushrooms and Their Health Significance.** Amer El-Ahraf and Rudi Mattoni. *Department of Health Science and Human Ecology, California State College, San Bernardino, 5500 State College Parkway, San Bernardino, California.*

Several California mushroom farms are located in urban areas in close proximity to major traffic arteries which permit exposure to heavy and trace metal contamination. Growth substrate used include compost of horse bedding straw and manure or chicken manure, which contain heavy metals of toxic nature. The study involved mushrooms as a model agricultural crop in terms of trace and heavy metals in the edible portion (sporophore) by translocation from their growth substrate and in relation to farm location. The metals studied included mercury, arsenic, lead, cadmium, chromium, nickel, zinc, manganese, copper, and iron. Four farms, two in rural and two in urban areas were surveyed. Analyses were made of (*a*) sporophore, (*b*) compost, and (*c*) casing soil. All analyses of mushrooms and compost were performed on a dry weight basis. Casing soils were extracted with 0.1 N HCl and analyzed directly. Mercury was analyzed by flameless AA. Arsenic was analyzed by Gutzeit method following digestion. All other metals were analyzed by atomic absorption spectrophotometry following dry ash digestion. There was no association of increased lead or cadmium levels with urban farms. Comparison of metal concentrations in sporophores with substrate concentration indicates specific metals are not absorbed and translocated at the same rates, with arsenic as a striking example. In no instance was any toxic metal found at levels approaching FDA tolerance where standards exist. (Research supported by a grant from the University of California, Irvine)

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**Potential for Aflatoxin Production on Smoked Mullet.** B. Y. Farhat and J. A. Koburger. *University of Florida, Food Science Department, Gainesville, Florida 32611.*

Production of aflatoxins on smoked mullet by *Aspergillus flavus* and *Aspergillus parasiticus* was studied in relation to salt concentration and temperature of storage. Within the temperature range investigated (5 to 25 C) the greatest toxin production occurred at 25 C. Only small amounts of toxin were produced at 5 and 15 C. More Aflatoxin $B_1$ and $B_2$ were produced at 25 C; at lower temperatures of storage $B_2$ was not detected. Water phase salt within the range of 1.3 to 4.1% had no effect on the quantities or distribution of the aflatoxins produced.

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**Microbiology of the Wild Rice Fermentation.** J. F. Frank, E. H. Marth, R. C. Lindsay, and D. B. Lund. *University of Wisconsin, Department of Food Science, Madison, Wisconsin 53706.*

The predominant microorganisms in fresh and fermenting wild rice were isolated, characterized, and then used as inoculum so their effects on processing and flavor characteristics of wild rice could be determined. During storage of wild rice at 4 C for 14 weeks, *Achromobacter* spp., *Flavobacterium* spp., coryneforms, coliforms, and *Achromobacter* spp. predominated in succession. In rice stored at 21 C for 2 weeks *Pseudomonas* spp. predominated. Microorganisms isolated from fresh wild rice included pseudomonads, micrococci, coliforms, *Achromobacter* spp., and *Flavobacterium* spp. Wild rice inoculated with isolates developed a variety of odors including fecal-putrid, earthy, and rotting vegetation types at 30 C, and the previous odors plus ammonia, fatty acid, and sweet-aromatic types at 7 C. Upon inoculation of wild rice with selected isolates, a reduction in hulling efficiency was noted, but otherwise processing characteristics were not affected. Intensities of both tealike and earthy flavors in wild rice were increased by inoculation of rice with *Achromobacter, Pseudomonas, Flavobacterium,* and *Micrococcus* isolates.

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**Sensitivity of Guinea Pigs and Fetuses to Diets Containing Added Methyl Mercury and Selenium.** A. G. Hugunin, R. L. Bradley, Jr., and W. E. Ribelin. *Departments of Food Science and Veterinary Science, University of Wisconsin-Madison, Madison, Wisconsin 53706.*

Since the fetus is about four times more sensitive to methyl mercury than postnatal animals, it suggests an important toxic mechanism. This study involved seven groups of guinea pigs with five 700-g females in each group exposed to dietary levels of methyl mercury and selenium: control (<0.01 ppm Hg), 0.10, 0.50, or 2.5 ppm Hg; 2.5 ppm Hg + 2.5 ppm Se; 12.5 ppm Hg; and 12.5 ppm Hg + 2.5 ppm Se. Females were fed these rations for 30 days before mating while males were fed the control ration. For 46 days one male was caged with each group of females and these were rotated daily between cages. This allowed exposure of the females to the males during two estrus cycles. No females fed 12.5 ppm Hg had pups or lived past 76 days. Gross pathology of brains indicated a marked atrophy of the cerebrum only in pups from guinea pigs fed 12.5 ppm methyl mercury and 2.5 ppm selenium. These smaller, darker brains had a mean weight of 1.55 g while brains from pups sacrificed at a similar period were 2.87, 2.58, 2.84, and 2.70 g, and represented those from guinea pigs fed control, 0.1, 0.5, and 2.5 ppm methyl mercury. Neutron activation was used to confirm enzyme and blood studies.

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**Persistence of Enteric Organisms in Soil and on Vegetables Spray Irrigated with Effluent and Sludge.** Joseph Lovett, Brenda Boutin, and Jan M. Bisha. *Food and Drug Administration, 1090 Tusculum Avenue, Cincinnati, Ohio 45226.*

We determined survival, in soil and on vegetables, of enteric bacteria applied to vegetable plots in four weekly spray applications of secondary treatment plant effluent (STPE) or sludge. Soil and vegetables were assayed for total coliforms, fecal coliforms, *Salmonella,* and fecal streptococci before the initial spraying and following the last spraying. *Salmonella* was added to sprays to achieve a concentration of $10^9$/100 ml; all other organisms were natural flora. Bacterial levels except *Salmonella* declined to background concentrations in 40-50 days. *Salmonella* persisted throughout the sampling period in STPE-sprayed soil but were occasionally undetectable after 40 days in sludge-sprayed soil. The highest concentration achieved in soils was approximately $10^9$/100 g immediately following the last spraying. *Salmonella* on lettuce varied from $7 \times 10^7$/100 g immediately after spraying to 35/100 g after 33 days. *Salmonella* in soil persisted for >97 days and *Salmonella* on vegetables under field conditions for >30 days. Total coliforms in soil and on vegetables cannot be used as an indicator of pathogen contamination since their presence and persistence patterns are unrelated to that of *Salmonella.* Persistence of fecal streptococci and fecal coliforms follows the same pattern as *Salmonella* in soil but not on vegetables.

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**The Normal Flora of Rock Shrimp:** *Sicyonia brevirostris.* A. R. Norden and J. A. Koburger. *University of Florida, Food Science Department, Gainesville, Florida 32611.*

Rock shrimp, a deep water, nocturnal species, is presently underutilized and little is known concerning its microbiology. To obtain zero time data on the normal flora, samples were obtained and analyzed within 10 min of removal from the Atlantic Ocean. Samples were also iced and held for various storage periods, and surviving species of bacteria were determined.

The aerobic isolates were predominantly gram negative, pigmented, gelatinase positive, oxidase positive, and motile. Genera found on the fresh rock shrimp in decending order of frequency were: *Plesiomonas, Flavobacterium, Vibrio, Pseudomonas, Cytophaga, Planococcus, Micrococcus, Aeromonas, Achromobacter,* and *Beijerinckia.* In contrast, those organisms surviving iced storage were predominately gram-positive, belonging to the genus *Planococcus.*

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**Efficacy of Chemical Cleaners and Sanitizers.** J. K. Roh, R. L. Bradley, Jr., and D. B. Lund. *Department of Food Science, University of Wisconsin-Madison, Madison, Wisconsin 53706.*

The stability of sanitizing solutions and their effects were determined on several milk contacting surfaces. Results showed that iodophor solutions (25 ppm available iodine) were greatly affected by temperature, oxygen availability, presence of catalyst, and time. The half-life of these solutions were 40 min at 63 C and 7 h at 22 C. Hypochlorite solutions (200 ppm available chlorine) were stable under all conditions for 24 h. The most deleterious effect with sanitizers was with PVC tubing using iodophors and with stainless steel using hypochlorite solutions. A model clean-in-place system with a flat-flow channel was designed to monitor the cleaning process and to evaluate the efficacy of cleaning and sanitizing chemicals. Assays for calcium and protein showed that a 30-sec rinse with warm water removed over 85% of milk solids from air-dried, milk films contaminated with *Escherichia coli.* Circulating solutions of cleaning chamicals at 25-60 C for up to 2 min and a flow velocity of 1.5-5 ft/sec resulted in no significant differences in the residual on the plates.

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**Behavior of *Enterobacter* Species in Skimmilk During Fermentation by Lactic Acid Bacteria.** J. L. Rutzinski and E. H. Marth. *Department of Food Science, University of Wisconsin, Madison, Wisconsin 53706.*

Lactic acid bacteria were tested for their ability to inhibit growth of selected *Enterobacter* species in skimmilk. Skimmilk was inoculated with *Streptococcus cremoris, Streptococcus lactis,* or mixtures of *S. cremoris* and *S. lactis* plus the *Enterobacter* species (approximately $10^3$/ml). Inocula of lactic acid bacteria ranged from 0.25 to 2.0% and incubation temperatures included 21 and 32 C. *S. cremoris* and *S. lactis* and mixtures of the two repressed growth but did not always inactivate the *Enterobacter* species during 15 h of incubation at 32 C when the lactic inoculum was 0.25, 1.0, or 2.0%. An inoculum of 1.0 or 2.0% occasionally resulted in total inactivation of certain *Enterobacter* species at 32 C by some of the lactic cultures. Both *S. cremoris* and *S. lactis* usually were less inhibitory to the *Enterobacter* species than were the commercial lactic starters. Growth of most *Enterobacter* species was only slightly repressed when a lactic inoculum of 0.25 or 1.0% was

used and incubation was at 21 C for 15 h. An increase in inoculum to 2.0% resulted in greater repression of growth of the *Enterobacter* species at 21 C.

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**Effect of Medium, Dilution Fluid, and Incubation Temperature on Enumeration of Bacteria in Grade A and Manufacturing Grade Milk.** Chamras Sanghirum and B. E. Langlois. *Department of Animal Science, University of Kentucky, Lexington, Kentucky 40506.*

Total aerobic counts of manufacturing grade and Grade A raw milk samples were determined using Standard Methods (SMA), Schaedler (SA), and Eugon (EA) Agars, phosphate buffered distilled water (PB), 0.1% peptone water (PW), and Ringers' solution (RS), and incubation conditions of 28 C for 72 h and 32 C for 72 h. Comparisons of sample counts from the 18 possible combinations of medium, diluent, and incubation were made. Highest counts for both grades of milk were obtained using the combination of SMA, PB, and 28 C. Medium and diluent had a significant ($P < .01$) effect on manufacturing grade counts, while the diluents did not have an effect on Grade A counts. Results with SA were significantly different ($P < .05$) from those obtained with SMA, but did not differ from those obtained with EA. Differences between SMA and EA were not significant. Results at 28 C were significantly different from those at 32 C. Results obtained with manufacturing grade samples using SA, PB, and 32 C; SMA, PW, and 28 or 32 C; SMA, PB and 28 C; and EA, PB, or PW and 28 or 32 C were not significantly different from those obtained using *Standard Methods* (SMA, PB and 32 C). The Grade A counts for SA and all three diluents and 28 C; SMA, PW, and 32 C; and EA, PB, and 28 or 32 C, were similar to those obtained using *Standard Methods.*

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**Determining Sanitary Status of Farm Milk Pipelines Using the Rinse-Filter Procedure.** R. W. Scroggins and R. T. Marshall. *Department of Food Science and Nutrition, 203 Eckles Hall, Univ. of Missouri-Columbia, Columbia, Missouri 65201.*

Two farm milk pipelines with weigh jars and milk releasers were rinsed with water sterilized by membrane filtration. Samples of rinse water were collected and analyzed for viable bacteria and coliforms by the membrane filter method. Total aerobic counts averaged 7000/ft² of milk contact surface in a new milking system during the first month of operation. After one year of operation, when certain deficiencies of cleaning occurred, total counts averaged more than 500,000/ft². Upon correction of deficiencies, average counts dropped to 2000/ft². Counts of coliforms in clean systems averaged less than 1/ft². Gram negative bacteria constituted only 5% of the microflora in rinses from a system that had been in operation for 4 years; whereas 67% of the colonies were micrococci and 22% were streptococci. Use of the filtration system for preparation of sterile water is discussed. Contribution from the Missouri Experiment Station. Journal Series Number 7223.

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**Microbiological Evaluation of Retail Ground Beef: Centralized Versus Traditional Preparation.** J. G. Shoup, J. L. Oblinger, and J. A. Koburger. *Food Science Department, University of Florida, Gainesville, FL 32611.*

Centralized processing of meats has been shown to be economically feasible. At the same time, interest in formulating and implementing meaningful microbiological quality standards for meats presents a situation that lacks clear definition. An evaluation of the microbiological quality of retail ground beef prepared in a centralized operation as well as products prepared in the traditional manner was conducted. Forty samples of ground beef from 5 retail sources were analyzed for total aerobes (22 C and 35 C), total anaerobes (35 C), yeasts and molds, enterococci, *Clostridium perfringens, Staphylococcus aureus,* coliforms, *Escherichia coli,* and *Salmonella.* Data were subjected to the standards of microbiological quality based on proposed and/or existing standards. Standards which allowed at least 75% compliance by centralized products could not be met with equal frequency by traditionally prepared products examined except for *C. perfringens* and *S. aureus. Salmonella* screening of the samples resulted in the identification of *S. infantis* in one traditionally prepared sample. As a result of this work, it is apparent that centralized processing of ground meat can provide a product that meets many proposed and/or existing microbiological standards when good manufacturing practices are adhered to.

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**Food Poisoning Occurring in Canada During 1973.** E. Todd. *Bureau of Microbial Hazards, Health Protection Branch, Health and Welfare Canada, Ottawa, Ontario, KlA OL2, Canada.*

Data on food-poisoning outbreaks that occurred in 1973 were collected from all parts of Canada. Nearly 400 outbreaks, with about 3,000 persons ill, were recorded. Illness occurred mainly because of consumption of food containing microorganisms or their toxins. *Staphylococcus aureus* was responsible for more outbreaks (38) and more cases than any other etiological agent. Other outbreaks were caused by *Salmonella* (32), *Clostridium perfringens* (8), *Clostridium botulinum* (5), and *Bacillus cereus* (3). Yeasts and molds were suspected of causing illness in baked goods and soft drinks. Illness from chemicals occurred less frequently, but tin from canned tomato juice, cleaning and disinfecting chemicals from soft drink bottles, and toxins from plants caused a total of 36 outbreaks. Mishandling of foods in food-service facilities were responsible for about a third of the outbreaks.

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**Resumed Feeding of Apple Pomace in the Dairy Regimen.** R. H. Walter. *Department of Food Science & Technology, Cornell University, New York State Agricultural Experiment Station, Geneva, New York 14456.*

Apple pomace was analyzed for DDT over a period of 3 years, and was found to contain 0.18 ± 0.08 ppm. DDT transfer to milk, when present in dairy feed at this low concentration, is debatable. Therefore, a study was undertaken, in which a ration containing 10% of dried pomace was fed to five lactating cows for 15 weeks at a rate of 2 lb. per cow per day. No significant difference in DDT content of milkfat between the control and experimental groups was found; nor was there a significant correlation between milkfat percentage and DDT. These results lead to the conclusion that the cautious resumption in use of apple pomace in the dairy regimen would be a safe practice. The renewed feeding of apple pomace to dairy livestock will require safeguards against patulin, a toxic, heat stable, fungal metabolite that is occasionally found in apply products.

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**Removal of Bisulfite From Shrimp by Hypochlorite Rinses.** K. E. Weingartner, F. W. Knapp, and J. A. Koburger. *University of Florida, Food Science Department, Gainesville, Florida 32611.*

Sodium bisulfite is commonly used to control black spot (melanosis) on shrimp by inhibiting the enzymatic oxidation of tyrosine. The bisulfite is either applied as a dip or as a powder and at times may reach excessive levels on the raw product. Data obtained indicate that hypochlorite rinses are effective in lowering the bisulfite level on shrimp in that the bisulfite is located mainly in the shell and is therefore readily oxidized by the rinse solution. It was also observed that the bisulfite treated shrimp had lower microbial counts than the controls.

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**Recovery of Compounds responsible for Light-induced Flavor in Milk by Lyophilization.** J. D. White and A. H. Duthie. *Department of Animal Sciences, University of Vermont, Burlington, Vermont 05401.*

Samples of good and light-induced milks were dried separately and simultaneously in an all-glass lyophilization system. The dried powders and condensed waters from each sample were reconstituted to determine if light-induced compounds existed in water and/or powder. Five flavor panelists characterized six samples (two controls, four reconsituted) in five trials as good, light-induced, or other. Flavor defect intensities were scored on a scale from 0 for none to 4 for pronounced, and a mean defect intensity (MDI) was calculated for each sample. Good water (GW), good powder (GP) samples were identified as good in 95.2% of the responses with a MDI = 0.10. Light-induced water (LW), light-induced powder (LP) were characterized as light-induced in 85.7% of the responses with a MDI = 3.05. Non-lyophilized control samples of light-induced milk were always identified correctly with a MDI = 3.45. Thus, only small losses of light-induced flavor compounds occurred during lyophilization. Samples reconstituted from GW and LP were characterized as typically light-induced in 47.6% of the responses with a MDI = 1.83; samples of LW and GP were called light-induced 95.2% of the time with a MDI = 2.86. Evidence shows that most of the compounds responsible for the light-induced flavor in milk are recovered by lyophilization in the condensed water.

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## INVITED PAPERS

**An Examination of Methods for Assessing Post-Pasteurization Contamination.** G. Blankenagel. *Department of Dairy and Food Science, University of Saskatchewan, Saskatoon, Saskatchewan.*

Since post-pasteurization contamination usually shortens the shelf life of dairy products, it is of importance to have methods available for detecting the presence of contaminants. Ideally such methods should be accurate, simple, and economical, and provide results within the shortest time possible. Presently, no single test fulfills all these requirements and therefore it is necessary to compromise. In most instances the emphasis is on detecting groups of organisms which will not survive the heat treatment of pasteurization. Some of the commonly used methods are described and their advantages and disadvantages are discussed.

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**The Federal Food Service Program.** William F. Bower and A. Sidney Davis. *U.S. Food and Drug Administration, 200 C Street, SW, Washington, D.C. 20204.*

The Division of Food Service, U.S. Food and Drug Administration, is concerned with food safety when food is offered to consumers for consumption. This includes an estimated 600,000 institutional and commercial food service establishments, 300,000 retail stores, and vending machine sites numbering in the hundreds of thousands. Plans to accomplish this mission includes achievement of uniform regulations and uniform enforcement procedures nationwide based on FDA standards, providing and/or supporting training and certification programs for regulatory officials and industry, developing and supporting self-inspection/quality assurance programs, evaluation of State programs, technical assistance to State regulatory agencies, and development of a model data processing system to aid program administration at the State level.

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**In Defense of Packaging.** A. R. Chadsey. *George Weston Ltd., Toronto, Ontario M5J 1T6, Canada.*

Speaking as a packager and as a former spokesman for the packaging industry, this is an attempt to set the record straight in the face of the many often repeated and not too accurate criticisms of packages and packagers in general. The question is asked—"is packaging really a proliferate, polluting, and resource-depleting method for the fraudulent manipulation of credulous consumers?" The response points out the need to view packaging in context with the product it carries rather than as a separate entity. It stresses that *without* its package, in many cases the product would be unavailable in the form desired. The response also suggests that so-called convenience products *reduce* rather than increase solid waste, that food processing conserves energy, and explains that in many cases what is perceived as overpackaging is essential to delivering the product intact and in an acceptably wholesome condition.

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**Microbiological Standards for Cheese—A Health Protection Branch Viewpoint.** D. L. Collins-Thompson and I. E. Erdman. *Division of Evaluation, Bureau of Microbial Hazards, Health Protection Branch, Ottawa, Ontario K1A OL2.*

The Health Protection Branch, Health and Welfare Canada is considering regulations introducing microbiological standards for cheese made from pasteurized and from non-pasteurized milks. The proposed standards are based on a 2-year study carried out by the Branch. For pasteurized milk cheeses a level of 500 coliforms and/or 100 coagulase positive staphylococci appear to be readily achieved by present manufacturing practices. For non-pasteurized cheeses (i.e. made from raw or heat treated milk) a level of 5000 coliforms or 500 *Escherichia coli* or 1000 coagulase positive staphylococci is being considered. The type of standard to be used for cheeses will be of the 3-class plan suggested by the International Commission on Microbiological Specifications for Foods. Thus, a sampling plan, a defined method of analysis, and levels of acceptability are given for coliforms, *E. coli,* and staphylococci. Use of the 3-class plan in interpretation of the analytical results allows for the normal between-analytical-sample variation.

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**National Sanitation Training Program.** Richard J. Davies. *Canadian Restaurant Association, 94 Cumberland Street, Toronto, Ontario M5R 1A3.*

There are an estimated 120,000 cases of bacterial food poisoning occuring in the Canadian foodservice industry each year. The Canadian Restaurant Association in co-operation with all Provincial Departments of Health and Health and Welfare Canada, developed a sanitation code to help the food service operator understand every essential sanitation requirement. In conjunction with the code, a national sanitation training program was developed to assist in providing total education of foodservice sanitation for all personnel of the Canadian foodservice industry. To date, 3,200 personnel have successfully completed one of the national sanitation training programs and gained a more responsible level of foodservice sanitation awareness. The program as well as being accepted by all Provincial Departments of Health and Health and Welfare Canada, has officially been endorsed by the Canadian Public Health Association and the Consumers Association of Canada.

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**Fate of Animal Viruses in Effluent from Liquid Farm Wastes.** J. B. Derbyshire. *Department of Veterinary Microbiology and Immunology, University of Guelph, Guelph, Ontario, Canada.*

Various aspects of viral pollution of the environment associated with disposal of liquid farm manure on agricultural land are being investigated. Techniques have been developed for concentration and recovery of animal viruses from various field samples. Seventeen of 22 samples of liquid manure from a swine fattening house yielded enteroviruses, adenoviruses, or a coronavirus. One enterovirus was isolated from six samples of waste from a swine farrowing house, but no

virus was isolated from 18 samples of liquid cattle manure obtained from a dairy farm. A swine enterovirus was isolated from surface soil samples collected up to 8 days after liquid manure was spread on agricultural land. A swine enterovirus was also isolated from two of 26 samples of surface run-off collected from sites at which liquid pig manure was routinely spread on agricultural land. Thirty three samples of surface water and 36 samples of ground water were collected in areas in which liquid pig manure was routinely spread on farm land, and a swine enterovirus was isolated from one surface water sample. Field and laboratory experiments indicated that enteroviruses are more rapidly inactivated in aerated liquid manure than in untreated manure.

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**Bacteriological Quality of Ground Beef at the Retail Level.** C. L. Duitschaever. *Department of Food Science, University of Guelph, Guelph, Ontario, Canada.*

A total of 108 samples of various types of raw refrigerated ground beef from 13 different retail stores in Ontario (Canada) were analyzed for their bacterial content. Averages of aerobic counts (21 C for 3 days) ranged from 18 million to 82 million per gram of sample, and 76.8% of the samples had counts in excess of 10 million per gram. All samples yielded staphylococci (Baird-Parker, egg tellurite) in excess of 1000 per gram. Coagulase positive staphylococci were isolated from 46.3% of the samples and ranged from 600 to 220,000 per gram. Eighty one percent of the samples were confirmed to contain coliforms with populations ranging from 1000 to 550,000 per gram. Of these samples, 48.8% contained *Escherichia coli,* ranging from 100 to 430,000 per gram. All *E. coli* isolates were of fecal origin (EC broth, 45.5 C). Salmonellae were not isolated.

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**Recent Trends In Vending.** David E. Hartley. *National Automatic Merchandising Association, 7 S. Dearborn St., Chicago, Illinois 60603.*

The recent evolution in design and construction of food and beverage vending machines is discussed, with particular emphasis on machine features having health and safety importance. New types of equipment and a look at future innovations are explored on the basis of presently-available technology.

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**Northeast Dairy Practices Council.** Richard P. March. *Cornell University, 118 Stocking Hall, Ithaca, NY 14853.*

The Northeast Dairy Practices Council is a nonprofit organization of education, industry and regulatory personnel concerned with the dairy industry in Conn., Del., Md., Maine, Mass., N.H., N.J., N.Y., Penna., R.I., and Vt. Founded in 1970, the Council has as its objectives to "provide mutual assistance among the Northeastern States in adopting sound, uniform procedures concerning the production, processing, and distribution of milk and dairy products, especially as related to the sanitary aspects; and to eliminate the duplication of dairy industry inspection among states and municipalities in the Northeast and permit the free flow of milk and dairy products between them . . ." The work of the Council is accomplished largely by five Task Forces concerned with Buildings and Utilities, Equipment, Quality Assurance, Communication and Uniformity, and Cleaning and Sanitizing. These Task Forces have completed 15 Guidelines and are currently working on nine more. They are reviewed and approved by each state before publication. Exceptions held by any state appear as footnotes. Copies are available at a nominal cost. The Council holds an annual meeting each November. Membership is open to any interested person.

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**Microbiological Standards for Meat in Canada.** H. Pivnick, I. E. Erdman and D. Collins-Thompson. *Bureau of Microbial Hazards, Food Directorate, Health Protection Branch, Health and Welfare Canada, Ottawa.*

Microbiological standards for raw ground meat are receiving increasing attention. Consumer groups and the news media have been particularly concerned and occasionally their concerns have been justified. Standards, if required, should be uniform within a country to facilitate trade. Therefore, the Government of Canada is considering standards for this product under the Food and Drugs Act. If promulgated, standards could ensure a uniform basis for action by pertinent agencies at all levels of government. In addition, standards will enable industry to measure its own performance. The Health Protection Branch of Health and Welfare Canada has completed a Canada-wide survey of ground meat and is contemplating microbial standards based on 3-class plans as advocated by the International Commission on Microbiological Specifications for Foods. Current proposals being studied include requirements for the Aerobic Colony Count at 35 C, *Escherichia coli,* coagulase positive staphylococci, and *Salmonella* for fresh ground meat, frozen ground meat and ground meat mixed with vegetable protein.

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**A Field Study of Bulk Milk Transport Washing.** R. L. Richter, J. Bailey, and D. D. Fry. *University of Florida, Dept. of Dairy Science, Gainesville, FL 32611; Fla. Dept. of Agriculture, Tallahassee, FL 32304; and T. G. Lee Foods, Orlando, FL 32802.*

Mechanical spray devices commonly used to wash bulk milk tansport tankers in Florida were evaluated. A drop-in unit and two types of spray balls operated according to manufacturers' recommendations were used with the cleaning system supplied by the dairy. A self-contained circulating single-use system was used with one type of spray ball. When operated according to manufacturers' recommendations any of the spray devices could clean a tanker. Pressure and volume relationships below those recommended resulted in inadequate cleaning. Excessive pressure and volume damaged one spray ball which resulted in poor cleaning and required repair. A 120-day period was required to make the necessary changes in the plant cleaning system before it was possible to operate the spray devices consistently at the recommended pressure and volume. A pressure-temperature recorder in the solution supply line was used to monitor tanker washing and detect problems associated with the supply systems. It was necessary to examine routinely the tankers and spray devices to insure cleanliness of the tankers and remove trash that can collect in the spray device.

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**Intergrating Food Production and Processing into Nature's Geochemical Cycles.** J. B. Robinson. *Department of Environmental Biology, University of Guelph, Guelph, Ontario, Canada N1G 2W1.*

Elements such as nitrogen, carbon, phosphorus, and sulfur occur in the biosphere in a variety of chemical forms, each subject to biological or spontaneous conversion to other forms. In natural ecosystems such as grasslands and forests, these "cycles" operate very efficiently. The cycling of nitrogen in grassland is used to illustrate the conservative nature of natural systems. Food production, practiced on an extensive basis with minimum inputs and careful management may have minimum disruptive effects on local geochemical cycles, particularly if food is processed and consumed locally. Intensive agriculture, with its large energy inputs, and high yields, accompanied by extreme urbanization and remote food processing, induces major changes in local cycling. The localized effects of food production and processing have become a problem in industrialized countries which too often depend on food production, processing, and waste-treatment systems developed when the degree of intensification was much less than now, and when our understanding of ecosystems was only beginning. We should be using our knowledge to develop systems more compatible

with the inevitable requirements of nature. Some examples are given to indicate the direction which these developments might take.

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**Organization of Environmental Health Programs in California.** Walter F. Wilson and Amer El-Ahraf. *Los Angeles County Department of Health Services, 313 North Figueroa Street, Los Angeles, Calif. 90012.*

The organization of local and state programs for delivery of environmental health programs in California is reviewed. National and local changes in public health priorities are described. Competition for the public health dollar between "treatment medicine" and "preventive medicine" is discussed. Economic pressures bearing on the budget processes needed to fund adequate programs, "Fees for Service," "Revenue Sharing," and local property tax-based budgeting considerations are mentioned. Some experimental legislation and organization structures are being tried as solutions in California.

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**Detergents and Sanitizers as Possible Contaminants in Milk.** Robert R. Zall and David P. Brown. *Department of Food Science, Cornell University, Ithaca, New York 14853.*

Automated cleaning systems in milking parlors can and do cause situations where detergents and sanitizers are commingled with milk supplies. Studies on New York State farms to measure the effects of substituting soft water for different amounts of heat and detergent revealed design flaws in some CIP systems. Cleaning chemicals can be responsible for flavor defects and low bacterial numbers in milk. Detergents and sanitizers in concentration as low as 1.5 ppm downgrade milk taste while stone remover at slightly higher levels imparts off flavors. Bad tastes from washing compounds are intensified in raw milk after 3 days with no appreciable increase in bacterial numbers. Acid degree values are apparently not affected by these kind of contaminants. It must also follow that we do not know the potential implications for consumer health of ingestion of sanitizers and detergents in our milk supply.

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## Proposed Microbial Standards for Ground Meat in Canada

Proposed mandatory microbial standards for ground meat were made public today by the Minister of National Health and Welfare, the Honourable Marc Lalonde. Mr. Lalonde stated that the proposed standards will provide assurance to Canadians that any ground meat they purchase is safe from harmful microbial contamination. When the standards are enacted into regulations, Canada will be one of the first countries to have mandatory national standards for microbiological quality of ground meat.

The Minister underlined that the levels suggested for ground beef have deliberately been set to give a considerable margin of safety, recognizing the technical impossibility of providing ground beef or other uncooked food completely free of bacteria.

The proposed standards are based on the evaluation by staff of the Health Protection Branch of the results of the survey initiated last year to examine the microbiological content of ground beef on the Canadian market. They will be submitted shortly to other government bodies at the federal and provincial levels, as well as to consumers associations and industry, for consultation before regulations are enacted.

Microbial standards already exist for such food products as cottage cheese and ice cream, and the Health Protection Branch is developing similar standards for prepared meats, spices, chocolate and cheese.

PROPOSED MICROBIAL STANDARDS FOR GROUND BEEF
(Microbial determination at 35° C)

| | In unfrozen ground beef with or without vegetable protein | In frozen ground beef with or without vegetable protein |
|---|---|---|
| Total aerobic count/gram | 10,000,000 | 1,000,000 |
| E. coli/gram | 100 | 100 |
| Coagulase positive staphylococci/gram | 100 | 100 |
| Salmonella/25 grams | 0 | 0 |

*Technical notes*

1. The "total aerobic count" is the total number of bacteria which can grow in the presence of oxygen.
2. "E. coli" is a bacterium normally found in the intestinal tract of all warm-blooded animals in numbers of one million to one billion per gram. Small numbers of this bacterium invariably finds its way into the meat during slaughtering and processing.
3. "Coagulase positive staphylococci" are normal inhabitants of the skin and are frequently found in the nose of a wide variety of warm-blooded animals, including human beings. The presence of small numbers of staphylococci in many uncooked food is considered normal.

*J. Milk Food Technol. Vol. 38, No. 10, Pages 640-645 (October, 1975)*


# 3-A Sanitary Standards for Stainless Steel Automotive Milk and Milk Products Transportation Tanks for Bulk Delivery and/or Farm Pick-up Service

Number 05-13

*Formulated by*
*International Association of Milk, Food and Environmental Sanitarians*
*United States Public Health Service*
*The Dairy Industry Committee*

It is the purpose of the IAMFES, USPHS, and DIC in connection with the development of the 3-A Sanitary Standards program to allow and encourage full freedom for inventive genius or new developments. Milk transportation tank specifications heretofore and hereafter developed which so differ in design, material, fabrication, or otherwise as not to conform with the following standards, but which, in the fabricator's opinion are equivalent or better, may be submitted for the joint consideration of the IAMFES, USPHS, and DIC at any time.

A.

*SCOPE*

A.1

These standards cover the sanitary aspects of transportation tanks for milk and fluid milk products.

A.2

In order to conform with these 3-A Sanitary Standards, transportation tanks shall comply with the following design, material and fabrication criteria.

B.

*DEFINITIONS*

B.1

*Bulk Milk Transportation Tank:* Shall mean an over-the road truck or trailer tank used to transport milk and milk products. It may have more than one compartment.

B.2

*A Farm Pick-Up or Multiple Pick-Up and Delivery Tank:* Shall mean a bulk milk transportation tank as defined above with milk transfer attachments and facilities, including a pump and/or hose cabinet, as specified herein.

B.3

*Product:* Shall mean the milk or fluid milk product transported in the tank.

B.4

*Surfaces:*

B.4.1

*Product Contact Surfaces:* Shall mean all surfaces which are exposed to the product and surfaces from which liquids may drain, drop or be drawn into the product.

B.4.2

*Non-Product Contact Surfaces:* Shall mean all other exposed surfaces.

B.5

*Milk Outlet:* Shall mean the opening in the lining of a tank or a compartment and the outlet passage for milk to the exterior of the tank or compartment. The outlet passage starts at the opening in the lining and terminates at the connection for the outlet valve.

B.6

*Pump and/or Hose Cabinet:* Shall mean a cabinet on a farm pick-up or multiple pickup and delivery tank used to house the pump and/or transfer hose and may also house a compartment for product sample trays and samples.

B.7

*Deck Plate:* Shall mean the manhole dust cover seat or that part of the outer jacket on which the cover rests.

B.8

*Mechanical Cleaning or Mechanically Cleaning:* Shall denote cleaning, solely by circulation and/or flowing chemical detergent solutions and water rinses onto and over the surfaces to be cleaned, by mechanical means.

C.

*MATERIALS*

C.1

*Product Contact Surfaces:*

C.1.1

All product contact surfaces shall be of stainless steel of the AISI 300 series[1] or corresponding ACI[2] types (See Appendix, Section E.) or stainless steel that is non-toxic and non-absorbent and which under conditions of intended use is equally corrosion resistant as stainless Steel of the AISI 300 series[1] or corresponding ACI[2] types, except that:

[1] *The data for this series are contained in the following reference: AISI Steel Products Manual, Stainless & Heat Resisting Steels, December 1974, Table 2-1, pp. 18-19. Available from American Iron & Steel Institute, 1000-16th St. N.W., Washington, D.C. 20036.*

[2] *Alloy Casting Institute Division, Steel Founders' Society of America, 206111 Center Ridge Road, Rocky River, OH 44116.*

C.1.2

Rubber and rubber-like materials may be used for flexible product transfer tubing, gaskets, seals and parts used in similar applications. These materials shall comply with the applicable provisions of the "3-A Sanitary Standards for Multiple-Use Rubber and Rubber-Like Materials Used as Product Contact Surfaces in Dairy Equipment, Number 18-00," as amended.

C.1.3

Plastic materials may be used for flexible product transfer tubing, bearing, gaskets, seals and parts used in similar applications. These materials shall comply with the applicable provisions of the "3-A Sanitary Standards for Multiple-Use Plastic Materials Used as Product Contact Surfaces for Dairy Equipment, Number 20-00," as amended.

C.1.4

Where functional properties are required for specific applications, such as agitator bearing surfaces and rotary seals, where dissimilar materials are necessary, carbon, and/or ceramics may be used. Ceramic materials shall be inert, non-porous, non-toxic, non-absorbent, insoluble, resistant to scratching, scoring, and distortion by the temperature, chemicals, and methods to which they are normally subjected in operation, or cleaning and bactericidal treatment.

C.2

The gauge of material for the lining shall be not less than the following:

16 U.S. Standard Gauge for tanks of capacities of 1,000 gallons or less,

14 U.S. Standard Gauge for tanks of capacities of over 1,000 gallons and not exceeding 2,000 gallons,

12 U.S. Standard Gauge for tanks of over 2,000 gallons capacity,

except that lighter gauges of material shall be permitted if they are so supported that they will have equal resistance to denting, buckling and sagging, as provided by the three gauges specified above for the respective sizes of tanks.

C.3

*Non-Product Contact Surfaces:*

C.3.1

All non-product contact surfaces shall be of waterproof, corrosion-resistant material or waterproof material that is rendered corrosion-resistant. If coated, the coating used shall adhere.

C.3.2

All non-product contact surfaces shall be relatively non-absorbent, durable and cleanable. Parts removable for cleaning having both product contact and non-product contact surfaces shall not be painted.

C.4

*Pump and/or Hose Cabinet:*

C.4.1

The lining of the pump and/or hose cabinet shall be stainless steel or equally corrosion-resistant durable material.

C.4.2

Gasket material for pump and/or hose cabinet doors shall be smooth, easily cleanable, non-absorbent and without crevices in the body.

C.4.3

Sample trays and sample compartments furnished by the tank manufacturers that will be in the pump and/or hose cabinet shall be made of stainless steel, plastic or other equally corrosion-resistant material.

D.

*FABRICATION*

D.1

All product contact surfaces shall be at least as smooth as a No. 4 mill finish on stainless steel sheets. (See Appendix, Section F.)

D.2

All permanent joints in product contact surfaces shall be welded. All welded areas of product contact surfaces shall be at least as smooth as the adjoining surfaces.

D.3

All product contact surfaces shall be easily accessible for cleaning, either when in an assembled position or when removed. Removable parts shall be readily demountable.

D.4

All product contact surfaces shall be self-draining except for normal clingage. Tanks shall be so constructed that the lining will not sag, buckle or prevent complete drainage of water when the tank has a pitch of not more than 1 inch in 100 inches.

D.5

The height of the vertical axes of the lining of the tank shall not be less than the minimum heights shown in the following tables:

| *Tanks Having Uniform Vertical Axes* | Minimum Height |
|---|---|
| Up to and including 500 gallons | 36" |
| Over 500 gallons and up to and including 2,000 gallons | 40" |
| Over 2,000 gallons and up to and including 2,800 gallons | 42" |
| Over 2,800 gallons and up to and including 3,500 gallons | 44" |
| Over 3,500 gallons | 46" |

| *Tanks Having Varying Vertical Axes* | Minimum Height | |
|---|---|---|
| | Front | Rear |
| Up to and including 500 gallons | 36" | 36" |
| Over 500 gallons and up to and including 2,000 gallons | 40" | 40" |
| Over 2,000 gallons and up to and including 2,800 gallons | 41" | 51" |

| | | |
|---|---|---|
| Over 2,800 gallons and up to and including 3,500 gallons | 43" | 55" |
| Over 3,500 gallons | 43" | 57" |

D.6

The inside radii of all welded or permanent attachments shall be not less than ¼ inch. Where the head(s) and the partition wall(s) join the lining of the tank the radius shall not be less than ¾ inch.

D.7

There shall be no threads on product contact surfaces.

D.8

Sanitary pipe and fittings shall conform with "3-A Sanitary Standards for Fittings Used on Milk and Milk Products Equipment and Used on Sanitary Lines Conducting Milk and Milk Products, Number 08-09," as amended and supplemented.

D.9

The outer shell be smooth and effectively sealed except for a vent or weep hole in the outer shell of the tank. The vent or weep hole shall be located in a position that will provide drainage from the outer shell and shall be vermin proof. The outer jacket and doors of the pump and/or hose cabinet shall be smooth and effectively sealed. Outside welds need not be ground.

D.10

Non-product contact surfaces shall be free of pockets and crevices and be readily cleanable and those to be coated shall be effectively prepared for coating.

D.11

The tank and the divider between the compartments of a multi-compartment tank shall be insulated with insulating material of a nature and amount sufficient that, in an 18 hour period, when the tank is full of water, the average change in the temperature of the water will not exceed 2 F when the average difference between the temperature of the water and that of the atmosphere surrounding the tank is 30 F. Insulation material shall be installed in such a manner as to prevent shifting or settling.

D.12

*Outlet and Outlet Valve*

D.12.1

Each tank or compartment shall have a separate outlet passage. The outlet shall be of all welded construction with the exception that a rolled-on fitting may be used on the terminal end. The inside diameter of the outlet shall be at least as large as that of 2-inch 3-A sanitary tubing. The outlet(s) shall provide complete drainage of the tank(s) or compartment(s). In multi-compartment or multi-tank units, the top of the outlet passage(s) of the front compartment(s) or the front tank(s) shall be as low as the low point of the lining at the outlet at a point in the outlet passage. The horizontal distance from the opening in the lining to this point shall not be more than four times the diameter of the outlet passage. The outlet passage downstream of this point shall pitch towards the connection for the outlet valve. The terminal end shall have a bolted or a clamp-type flange or a 3-A sanitary threaded connection. The terminal end of the outlet passage shall not extend more than 6 inches beyond the inside lining of the tank or compartment(s). The outlet passage may be increased in length provided that:

D.12.1.1

The outlet passage is straight or is straight downstream of the elbow(s) or bend(s) used either to change the direction of product flow from a bottom outlet or to comply with the requirement in D.12.1 that at a specified point the top of the outlet passage shall be as low as the lowpoint of the lining at the outlet.

D.12.1.2

The outlet and outlet passage may be adequately cleaned manually or the tank or compartment with the increased outlet passage is provided with a fixed spray device(s) so that the outlet and outlet passage may be mechanically cleaned.

D.12.1.3

The outlet passage is insulated sufficiently that the temperature rise of the water in the outlet passage does not exceed the allowable average temperature rise of the tank full of water (2 F) specified in D.11.

D.12.1.4

The outlet passage is protected against damage (denting) and is braced and sloped.

D.12.2

Outlet valves shall be either close-coupled sanitary plug type or compression type tank outlet valves. The valve body shall be designed so that it can be used without a gasket or with either a single-service or multiple-use gasket.

Note: Block tin gaskets are not considered to be multiple-use gaskets

D.12.3

The tank outlet and valve bore shall be the same size and concentric or the product passage of the outlet valve (1) shall have an inside diameter no less than that of the tank outlet and (2) shall be self-draining.

D.12.4

A sanitary 3-A cap shall be furnished for the outlet opening of the outlet valve, except the outlet opening of a valve located in the pump and/or hose cabinet that is connected to the pump piping.

D.13

Unless the outlet valve is located in the pump and/or hose cabinet, it shall be provided with a dust cover which (1) encloses the entire valve assembly, (2) is dustproof and (3) has a smooth interior finish. Dust covers shall be provided with means of sealing to prevent opening or removing the cover without breaking the seal.

D.14

*Manhole Opening and Cover:*

D.14.1

The manhole(s) opening shall be not less than 16 inches by 20 inches oval or 18 inches in diameter.

D.14.2
The upper edge of a top manhole opening shall be not less than 3/8 inch higher than the surrounding area and if an exterior flange is incorporated in it, it shall slope and drain away from the opening.

D.14.3
Manholes shall be located so that the distance from either end of the tank shall not exceed 18 feet, 6 inches, except that this requirement shall not apply to tanks with a permanently installed mechanical cleaning device(s) so designed and installed that solutions are applied to all product contact surfaces except those areas requiring manual cleaning.

D.14.4
Manhole cover gaskets shall be readily removable and may have any one of the following cross-sections: flat, rectangular, square, oval, round, "L" or "Z" shape, or any other section which is equally cleanable.

D.14.5
Gasket grooves or gasket retaining grooves shall not exceed ¼ inch in depth or be less than ¼ inch wide. The minimum radius of any internal angle in a gasket groove or gasket retaining groove shall be not less than 1/16 inch.

D.14.6
A sanitary vent of sufficient free opening to prevent excess vacuum and/or internal pressure, shall be installed under the manhole dust cover. The air vent shall be designed so that parts are readily accessible, easily removable and readily cleanable. (See Appendix, Section G.)

D.15
*Manhole Dust Cover:*

D.15.1
Each manhole shall be provided with a dust cover.

D.15.2
The interior finish of the dust cover shall be smooth, readily cleanable and free from bolts and screws. Round or oval head rivets shall be deemed acceptable.

D.15.3
Welded interior attachments shall have minimum radii of 1/16 inch.

D.15.4
A suitable vent shall be provided to relieve vacuum and pressure when the dust cover is closed.

D.15.5
The dust cover when closed shall provide an effective seal to prevent entrance of dust.

D.15.6
If a rubber or rubber-like, or plastic gasket is used as a seal, it shall be smooth, either removable or firmly bonded to the dust cover to provide a smooth, easily cleanable surface without crevices.

D.15.7
Deck plate, if attached to the outer jacket, shall be effectively sealed and firmly bonded.

D.15.8
The dust cover shall be provided with means of sealing to prevent opening the dust cover without breaking the seal.

D.16
*Agitation:*

D.16.1
When specified, the tank or compartment thereof shall be provided with means for mechanical and/or air agitation that when operated 20 minutes in whole milk that has been stored 24 hours at 40 F will result in the milk fat content of the product throughout the tank or compartment being within a variation of plus or minus 0.1 per cent by an official AOAC[3] milk fat test.

D.16.2
The agitator, if not designed for mechanical cleaning, shall be located in such a manner that it shall be readily accessible for manual cleaning and inspection.

D.16.3
A mechanical agitator shall have a seal of the packless type, sanitary in design with all parts accessible for cleaning.

D.17
*Air Under Pressure and/or Mechanical Cleaning:*

D.17.1
Equipment and means for applying air under pressure or solutions for mechanical cleaning, when provided, shall conform to the applicable provisions of the "3-A Accepted Practices for Supplying Air Under Pressure in contact with Milk, Milk Products and Product Contact Surfaces," Number 604-00, as amended, except that clamp type fittings shall not be used in the product zone.

D.17.2
Tubing and related fittings within the tank shall be readily and easily removable for cleaning outside the tank or be designed to be mechanically cleaned.

D.17.3
Openings for air agitation and/or mechanical cleaning applications shall be protected against contamination by means of a removable dust cover, except where such openings are within the pump and/or hose cabinet.

D.17.4
Permanently mounted air or solution tubing shall be constructed and installed so that it will not sag, buckle, vibrate or prevent complete drainage of the tank or tubing, and shall be located so that the distance from the outside of the tubing to the lining is at least two inches, except at point of entrance. If designed for mechanical cleaning, the tubing and all related fittings shall be self-draining.

D.17.5
Means for mechanically cleaning the tank or compartment, when provided, shall clean the product contact surfaces and all non-removable appurtenances thereto

[3]*The method of making these tests will be found in the following reference: Official Methods of Analysis; Available from the Association of Official Analytical Chemists, P.O. Box 540, Benjamin Franklin Station, Washington, D.C. 20004.*

except those areas that may be manually cleaned without entering the tank. (See suggestions Appendix, Section H.)

D.18

*Baffles*

D.18.1

Baffles, when provided, shall not interfere with the free drainage of the tank or compartment.

D.18.2

The area of any one baffle plate shall not exceed 40% of the cross-sectional area of the tank and the entire baffle shall be on one side of the longitudinal center line of the tank. If more than one baffle is installed, consecutive baffles shall be installed on opposite sides of the tank and shall be at least 48 inches apart. Baffles shall be so designed that walk-through accessibility will be provided to all areas for inspection, and if the tank is not provided with means for mechanically cleaning the tank or compartment, for cleaning purposes.

D.18.3

Baffles shall be permanently attached to the tank. The radius of inside corners formed where baffles are attached to the lining shall be at least ¼ inch. There shall be no sharp edges on baffles.

D.19

*Hose Cabinets:*

D.19.1

Hose cabinets shall comply with the following as well as other applicable provisions of the Fabrication Section D:

D.19.2

The lining of cabinets, doors and fixed attachments shall be smooth.

D.19.3

All permanent joints in the lining shall be welded. All welded areas in the lining shall be at least as smooth as the adjoining surfaces.

D.19.4

The bottom shall be constructed so that it will not sag, buckle or prevent complete drainage when the truck is on a level surface.

D.19.5

All inside corners shall have minimum radii of 1/8 inch.

D.19.6

Cabinets shall be dust tight and doors shall be equipped with a compression type closing device. Gasket material for sealing cabinet doors may be installed on face of the cabinet or on the doors except along a drainage area where it shall be attached to the doors. Gasket material shall be removable or firmly bonded to provide smooth, easily cleanable surfaces without crevices.

D.19.7

A roof overhang or suitable drip molding shall be provided over the cabinet doors.

D.19.8

The cabinet and doors shall be insulated with an insulating material having an insulating value of not less than 1 inch of cork.

D.19.9

A carrier bracket shall be provided to support the flexible transfer tubing. Means shall be provided to support the loose end of the tubing above the cabinet floor.

D.19.10

Fixed attachments such as pump support brackets, tubing carrier brackets and brackets for belt and pully guards shall be easily accessible for cleaning. A pump having a base area of 1 square foot or less shall be installed so that there will be a minimum clearance of 2 inches between the base and the cabinet floor and 3 inches between the pump assembly and the cabinet walls. The minimum clearance between the base and the cabinet floor shall be increased to 3 inches if the base area of the pump exceeds 1 square foot. A pump assembly that is to be mounted on the floor of the cabinet shall have a solid base and be installed with a non-absorbent sealing gasket. It shall be installed in a position that (1) will not interfere with drainage and (2) will provide minimum clearance of 3" between the pump assembly and the cabinet walls. Except when readily removable, a side wall mounted pump assembly shall be installed with a non-absorbent sealing gasket.

D.19.11

The size and location of the cabinet shall be such that will afford easy accessibility for assembly and disassembly of removable parts and provide ample clearance around permanently installed equipment and parts. (See Appendix, Section I, Facilities for Extra Fittings.)

D.20

*Pumps:*

D.20.1

Pumps, when furnished, shall conform to the "3-A Sanitary Standards for Centrifugol and Positive Rotary Pumps for Milk and Milk Products, Number 02-06. A sanitary closure shall be furnished for the outlet opening of the pump.

D.21

*Motors for Pumps and/or Agitators:*

D.21.1

A motor when located in the pump compartment, shall be totally enclosed, non-ventilated and the electric wiring shall be water-proof and shall be conducted through the wall of pump cabinet with water-tight connections.

D.21.2

Storage space for the pump motor electrical extension cord shall be located outside the pump compartment. It is suggested that a grounded cord reel be used to store the extension cord.

D.22

*Transfer Tubing:*

D.22.1

Single lengths of transfer tubing shall not exceed 8 feet except where adequate acceptable cleaning facilities are available at the place of cleaning. The minimum inside diameter of the tubing shall be 1-3/8 inches. A sanitary closure shall be furnished for the open end(s) of the tubing.

D.22.2

If two lengths of flexible tubing are used, they shall be connected either by the use of sanitary coupling or by a piece of rigid 3-A Sanitary Tubing.

D.22.3

A piece of flexible tubing may be used for the connection from the pump to the tank.

D.22.4

Flexible tubing shall be attached to rigid 3-A Sanitary Tubing, or the tank or pump in such a manner that the flexible tubing may easily be removed without tools. If clamps are used they shall be readily removable without tools.

D.23

*Sample Trays and Sample Compartments:*

D.23.1

Sample trays and sample compartments that are furnished by the tank manufacturer that are to be in the pump and/or hose cabinet shall be of sanitary design and readily cleanable.

D.23.2

Facilities shall be provided for keeping the samples cold.

D.23.3

Permanently installed sample compartments shall (1) be attached to the cabinets by welding or with bolted connections which have non-absorbent sealing gaskets in the joints, (2) have the supporting member(s) welded if supported from the floor of the cabinet and (3) be installed so there is a minimum clearance of 6 inches between the sample compartment and the cabinet floor.

## *APPENDIX*

E.

*STAINLESS STEEL MATERIALS*

Stainless steel conforming to the applicable composition ranges established by AISI for wrought products, or by ACI for cast products, should be considered in compliance with the requirements of Section C.1.1 herein. Where welding is involved the carbon content of the stainless steel should not exceed 0.08%. The first reference cited in C.1.1 sets forth the chemical ranges and limits of acceptable stainless steels of the 300 series.

Cast grades of stainless steel corresponding to types 303, 304, and 316 are designated CF-16F, CF-8 and CF-8M, respectively. These cast grades are covered by ASTM[4] specifications A 296-67 and A 351-65.

F.

*PRODUCT CONTACT SURFACE FINISH*

Surface finish equivalent to 150 grit or better as obtained with silicon carbide, is considered in compliance with the requirements of Section D.1 herein.

G.

*AIR VENTING*

To insure adequate venting of the tank which will protect it from internal pressure or vacuum damage, the critical relationship between minimum vent size and maximum filling or emptying rates should be observed. A venting system of sufficient capacity to provide for venting during filling and emptying is not adequate during mechanical cleaning. During the cleaning cycle tanks when cleaned mechanically should be vented adequately by opening the manhole cover to prevent vacuum or pressure build-up due to sudden changes in temperature of very large volumes of air.[5] Means should be provided to prevent excess loss of cleaning solution through the manhole opening. The use of tempered water of about 95 F for both pre-rinsing and post-rinsing is recommended to reduce the effect of flash heating and cooling.

H.

*MECHANICAL CLEANING*

The mechanical cleaning system shall be so designed that solution is applied to all product contact surfaces except those areas requiring manual cleaning. When being cleaned, the tank should have sufficient pitch to accomplish draining and to have a fast flushing action across the bottom. The pitch should be at least ¼ inch per foot. Means should be provided for manual cleaning of all surfaces not cleaned satsifactorily by mechanical cleaning procedures.

NOTE: Cleaning and/or sanitizing solutions should be made up in a separate tank—not in the transportation tank.

I.

*FACILITIES FOR EXTRA FITTINGS*

If extra sanitary fittings are supplied by the manufacturer of the farm pick-up tank, facilities should be provided in the pump compartment to adequately protect such items.

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These standards are effective Nov. 25, 1975 at which time the "3-A Sanitary Standards for Stainless Steel Automotive Milk and Milk Products Transportation tanks for Bulk Delivery and/or Farm Pick-Up Service," Serial #05-11" are rescinded and become null and void.

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[4] *Available from American Society for Testing and Materials, 1916 Race Street, Philadelphia, Pa. 19103.*

[5] *For example, when a 6,000 gallon tank (with 800 cu. ft. of 135 F hot air after cleaning) is suddenly flash cooled by 50 F water sprayed at 100 gpm the following takes place:*

*Within one second, the 800 cu. ft. of hot air shrinks approximately 51 cu. ft. in volume. This is the equivalent in occupied space of approximately 382 gallons of product. This shrinkage creates a vacuum sufficient to collapse the tank unless the vent, manhole, or other openings allow air to enter the tank at approximately the same rate as it shrinks. It is obvious, therefore, that a very large air vent such as the manhole opening is required to accommodate this air flow.*

*J. Milk Food Technol. Vol. 38, No. 10, Pages 646-653 (October, 1975)*


# 3-A Sanitary Standards for Farm Milk Cooling and Holding Tanks

Number 13-06

*Formulated by*
*International Association of Milk, Food and Environmental Sanitarians*
*United States Public Health Service*
*The Dairy Industry Committee*

It is the purpose of the IAMFES, USPHS, and DIC in connection with the development of the 3-A Sanitary Standards program to allow and encourage full freedom for inventive genius or new developments. Specifications for Farm Milk Cooling and Holding Tanks heretofore or hereafter developed which so differ in design, material, fabrication, or otherwise as not to conform with the following standards, but which in the fabricator's opinion are equivalent or better, may be submitted for the joint consideration of the IAMFES, USPHS, and DIC at any time.

A.

*SCOPE*

A.1

These standards cover the sanitary aspects of tanks in which bulk milk is cooled and stored on dairy farms. They do not pertain to storage tanks nor to silo-type tanks for milk and milk products used in dairy processing plants nor do they pertain to farm milk storage tanks.

A.2

Tanks made in conformance to these standards will provide the means for cooling the milk.

A.3

In order to conform with these 3-A Sanitary Standards, farm milk cooling and holding tanks shall comply with the following design, material, fabrication and cooling criteria.

B.

*DEFINITIONS*

B.1

*Product:* Shall mean milk.

B.2

*Farm Milk Cooling and Holding Tank:* Shall mean a cylindrical, rectangular, oval or other equally satisfactorily shaped tank.

B.3

*Surfaces*

B.3.1

*Product Contact Surfaces:* Shall mean all surfaces which are exposed to the product and surfaces from which liquids may drain, drop or be drawn into the product.

B.3.2

*Non-Product Contact Surfaces:* Shall mean all other exposed surfaces.

B.4

*Lining:* Shall mean all surfaces used to contain the product, including the ends, sides, bottom and top.

B.5

*Shell:* Shall mean the material covering the exterior of the insulation.

B.6

*Breast:* Shall mean that portion of the metal used to join the lining to the shell.

B.7

*Bridge:* Shall mean a cover on an open top type tank which is open on both sides and is permanently attached to the lining on opposite sides of the tank. It may be used to support a removable or nonremovable main cover(s) and accessories.

B.8

*Outlet:* Shall mean the opening in the lining and the passage for milk to the exterior of the tank. The outlet passage starts at the opening in the lining and terminates at the connection for the outlet valve.

B.9

*Mechanical Cleaning or Mechanically Cleaning:* Shall denote cleaning, solely by circulation and/or flowing chemical detergent solutions and water rinses onto and over the surfaces to be cleaned, by mechanical means.

C.

*MATERIALS*

C.1

All product contact surfaces shall be of stainless steel of the AISI 300 series[1] or corresponding ACI[2] types (see Appendix, Section F.), or metal which under conditions of intended use is at least as corrosion resistant as stainless steel of the foregoing types and is non-toxic and non-absorbent, except that:

[1] *The data for this series are contained in the following reference: AISI Steel Products Manual, Stainless & Heat Resisting Steels, April 1963, Table 2-1, pp. 16-17. Available from: American Iron and Steel Institute, 1000-16th Street, N.W., Washington, D.C. 20036*

[2] *Alloy Casting Institute Division, Steel Founders' Society of America, 20611 Center Ridge Road, Rocky River, OH 44116*

C.1.1
Rubber and rubber-like materials may be used for slingers, drip shields, agitator seals, agitator bearings, protective caps for sanitary tubes or fittings or vents, O-Rings, seals, gaskets and parts used in similar applications. These materials shall comply with the applicable provisions of the "3-A Sanitary Standards for• Multiple-Use Rubber and Rubber-Like Materials Used as Product Contact Surfaces in Dairy Equipment, Serial #18-00."

C.1.2
Plastic materials may be used for slingers, drip shields, agitator seals, agitator bearings, protective caps for sanitary tubes or fittings or vents, O-Rings, seals, gaskets, direct reading gauge tubes, moisture traps on vacuum lines, in sight and/or light openings and parts used in similar applications. These materials shall comply with the applicable provisions of the "3-A Sanitary Standards for Multiple-Use Plastic Materials Used as Product Contact Surfaces for Dairy Equipment, Serial #20-00," as amended.

C.1.3
Except for the protective caps provided for in C.1.1 and C.1.2, sanitary fittings shall be made of materials provided for in the "3-A Sanitary Standards for Fittings Used on Milk and Milk Products Equipment and Used on Sanitary Lines Conducting Milk and Milk Products, Revised, Number 08-09," as amended and supplements thereto.

C.1.4
Glass of a clear heat resistant type may be used for direct reading gauge tubes and in sight and/or light openings.

C.1.5
Where materials having certain inherent functional properties are required for specific applications, such as bearing surfaces and rotary seals, carbon, and/or ceramic materials may be used. Ceramic materials shall be inert, non-porous, non-toxic, non-absorbent, insoluble, resistant to scratching, scoring, and distortion when exposed to the conditions encountered in the environment of intended use and in cleaning and bactericidal treatment.

C.2
The materials used for the lining shall not be less than 18 U.S. standard gauge.

C.3
All non-product contact surfaces shall be of corrosion-resistant material or material that is rendered corrosion resistant. If coated, the coating used shall adhere. All non-product contact surfaces shall be relatively non-absorbent, durable and cleanable. Parts removable for cleaning having both product contact and non-product contact surfaces shall not be painted.

D.
*FABRICATION*

D.1
All product contact surfaces shall be at least as smooth as a No. 4 finish on stainless steel sheets. (See Appendix, Section G.) The measuring rod of an immersion-type measuring device may have a dull finish to facilitate reading.

D.2
All permanent joints in product contact surfaces shall be welded except that rolled on sanitary pipeline ferrules or flanges may be used on connections beyond the shell. All welded areas of product contact surfaces shall be at least as smooth as the adjoining surfaces.

D.3
All product contact surfaces shall be easily accessible for cleaning, either when in an assembled position or when removed. Removable parts shall be readily demountable. Tanks that are to be mechanically cleaned shall be designed so that all product surfaces of the tank, including the product contact surfaces of the opening for a vertical mechanical agitator, and all non-removable appurtances thereto can be mechanically cleaned.

D.4
Product contact surfaces shall be self-draining except for normal clingage. The lining shall be so constructed that it will not sag, buckle, or become distorted in normal use. Horizontal tanks shall be so constructed that they will not prevent complete drainage of water when the tank has a pitch of not more than 1 inch in 100 inches. If the tank is designed for use on a vacuum system, the construction shall be such that the lining will not be distorted when the internal pressure is 20 inches of mercury below atmospheric pressure.

D.4.1
When the tank is level or when it is in the position in which it was calibrated or when it is in position to be calibrated, the bottom shall pitch to the outlet to effect complete drainage.

D.4.2
If the tank is designed for mechanical cleaning, and has a flat bottom, the bottom shall pitch (1) at least ¼ inch per foot toward the outlet or (2) at least ¾ inch per foot toward the outlet in a vertical tank.

D.5
Gaskets shall be removable. Any gasket groove or gasket retaining groove shall not exceed ¼ inch in depth or be less than 4 inch wide except those for standard O-Rings smaller than 4 inch.

D.6
All internal angles of 135° or less on product contact surfaces shall have minimum radii of ½ inch, except that:

D.6.1
The minimum radii for accessories, appurtenances, or bridges that are welded to product contact surfaces shall be not less than ¼ inch.

D.6.2
The minimum radii in agitator shaft bottom guide bearings and in gasket grooves or gasket retaining grooves other than those for standard ¼ inch and

smaller O-Rings shall be not less than 1/8 inch.

D.6.3

The minimum radii in grooves for standard ¼ inch O-Rings shall be not less than 3/32 inch and for standard 1/8 inch O-Rings shall be not less than 1/32 inch.

D.6.4

The minimum radii of covers and agitator assemblies shall not be less than ¼ inch.

D.7

There shall be no threads on product contact surfaces.

D.8

All sanitary fittings and connections shall conform with the applicable provisions of the "3-A Sanitary Standards for Fittings Used on Milk and Milk Products Equipment and Used on Sanitary Lines Conducting Milk and Milk Products, Serial #08-09," as amended and supplements thereto except that materials conforming to C.1.1 or C.1.2 may be used for caps of sanitary design for the protection of terminal ends of sanitary tubes, fittings or vents.

D.9

The breast shall be integral with or welded to the lining and shall be sloped so that drainage is away from the lining. The junction of the breast and the shell shall be welded or effectively sealed.

D.10

*Covers*

D.10.1

Main Covers for Open Top Type Tanks.

Main covers (1) shall be sufficiently rigid to prevent buckling, (2) shall be self-draining, (3) shall be provided with an adequate, conveniently located and durable handle(s) of sanitary design, which is welded in place or formed into the cover materials, (4) unless gasketed, shall have downward flanges not less than 3/8 inch along all edges and (5) shall be close fitting. If the cover is not gasketed, the clearance between the surface of the cover and the surface of the tank it is designed to contact shall not exceed 3/32 inch. Covers not exceeding 24 × 30 inches or 30 inches in diameter may be removable and shall be designed to be self-draining in the closed position.

D.10.2

Non-removable Covers for Open Top Type Tanks.

Non-removable covers (1) shall be of a type that can be opened and maintained in an open position, (2) shall be designed to be selfdraining when in the closed position, (3) shall be designed so that when the covers are in any open position liquid from the exterior surface will not drain onto the lining and (4) shall be designed so that when in their fully opened position, drops of condensation on the underside will not drain into the tank. Covers of openings that will be held in place by gravity or vacuum may be of the lift off type and may be provided with a clamp(s) or other device to maintain them in position.

D.10.3

Bridges and Fixed Covers for Open Top Type Tanks.

Bridges and fixed covers shall pitch to the outside edge(s) of the tank from complete drainage, and shall have a raised flange not less than 3/8 inch in height where the edge(s) meets the main cover(s). Bridges and fixed covers shall be integral or welded to the lining shall be installed so the underside is accessible for cleaning and inspection without completely entering the tank. Bridges shall not exceed 24 inches in width. Generally horizontal fixed covers, located at ends or sides of an open top type tank (or segments of cylindrical open top type tanks) with generally vertical side walls, shall not extend more than 12 inches over the surface of the product.

D.10.4

Manhole Covers for Closed Type Tanks.

Covers for manholes in side walls and/or ends shall be either of the inside or outside swing type. If the cover swings inside, it shall also swing outside, away from the opening. Threads or ball joints employed to attach the manhole cover(s) and its appendages shall not be located within the lining. Covers for manholes in the top of tanks shall be of the outside swing type or be of a removable type.

D.10.5

All openings in the lining or in fixed covers or in bridges, or main covers of open top type tanks, except those for agitators, openings with permanently attached sanitary pipeline fittings, and thermometers or immersion type measuring devices that remain in place while the product is in the tank, shall be provided with removable covers, which are designed to make close contact with the upper edges of the opening or cover surface, and when in the main cover the removable cover(s) shall remain in position when the main cover is in an open position.

D.10.6

An umbrella or drip shield of sanitary design that can be raised or dismantled, to permit cleaning of all of its surfaces, shall be provided to protect against the entrance of dust, oil, insects and other contaminants into the tank through the space around the agitator shaft.

D.10.7

The water compartment of a tank designed for refrigerated water cooling shall have a cover. The clearance between surface of the cover and surface of the water compartment it is designed to contact shall not exceed 1/16 inch.

D.11

Openings

The edges of all openings into the lining that are upward or horizontal shall extend upward or outward at least 3/8 inch above or beyond the shell or the exterior surface or be fitted with a permanently installed sanitary pipeline fitting.

D.11.1

The main openings of tanks shall be of sufficient number, adequate in size, and so located that all product

contact surfaces are easily accessible and, except for the product contact surfaces of parts removable for cleaning, can be inspected visually without entering the tank.

D.11.2

An exception to the requirements of D.11.1 is made for closed type tanks, having product contact surfaces that cannot be manually cleaned and inspected without entering the tank.

D.11.2.1

The minimum inside height of this type of tank shall be 36 inches; and if the inside height exceeds 96 inches, means shall be provided (see Appendix, Section H.) that will facilitate manual cleaning and inspection of all product contact surfaces or means shall be provided for mechanically cleaning the product contact surfaces of the tank and all non-removable appurtenances thereto. This type of tank shall have a manhole opening(s) complying with the provisions of D.11.5.

D.11.3

An inlet sanitary pipeline connection shall be at least 1½ inches or the inlet opening shall accommodate at least 1½ inch 3-A sanitary tubing.

D.11.4

Agitator openings: Agitator shaft openings through the bridge or top enclosure shall have a minimum diameter of 1 inch on tanks which require removal of the agitator shaft for cleaning or be of a diameter that will provide a 1 inch minimum annular cleaning space between the agitator shaft and the inside surface of the flanged opening on tanks which do not require removal of the agitator for cleaning.

D.11.5

Manhole openings: A manhole opening, if provided, shall be located at the outlet end or side of the tank or the top of the tank. The inside dimensions of the manhole opening shall not be less than 15 × 20 inches oval, 12 × 27 inches elliptical, or 18 inches diameter.

D.11.6

Sight and Light Openings: Sight and light openings shall be provided when no other opening is available for viewing the surface of the milk and shall be of such design and construction that the inner surfaces drain inwardly; and if the tank is designed for mechanical cleaning, the inner surface of the glass (or plastic) shall be relatively flush with the inner surface of the lining. The inside diameter of the opening, if only one is provided, shall be at least 5¾ inches. If two openings are provided, the inside diameter of each shall be at least 3¾ inches. The external flare of the opening shall be pitched so that liquid cannot accumulate.

D.11.7

Thermometer connections: A connection(s) or opening(s) which will accommodate a temperature sensing element(s) of a thermometer(s) shall be provided. The connection(s) and/or opening(s) shall be located in the top enclosure, cover, bridge or through an end or sidewall. Thermometer wells may be used. The bulb of the temperature sensing element shall be located so as to permit registering the product temperature when the tank contains no more product than 20 percent of its capacity and shall be located so that the sensing element is not influenced by the cooling medium. Connections and/or openings shall conform to one of the following:

D.11.7.1

The applicable fittings found in the "3-A Sanitary Standards for Instrument Fittings and Connections Used on Milk and Milk Products Equipment, Serial #09-07."

D.11.7.2

Fittings for temperature sensing devices which do not pierce the tank lining, but which have temperature sensing element receptacles securely attached to the exterior of the lining.

D.11.8

The vacuum connection for a tank designed to be operated under vacuum shall be standard stainless steel tubing not less than 1½ inch in diameter and not longer than 4 inches (See Appendix, Section I.).

D.12

*Outlet:* The outlet shall provide complete drainage of the tank. The outside diameter of the outlet passage shall conform to that of 3-A sanitary tubing and shall not be less than that of 2-inch tubing. The wall thickness of the outlet passage shall be no greater than 1/8 inch. The terminal end of the outlet passage shall have a rolled-on or a welded sanitary pipeline ferrule or flange. The ferrule or flange shall not be below the bottom of the shell. The distance between the nearest point on the shell to the face of the ferrule or flange on the terminal end of a horizontal type outlet shall not be more than the smaller of (1) twice the nominal diameter of the outlet passage or (2) five inches. The outlet shall be one of the following types:

D.12.1

Horizontal type. The bottom of the outlet passage shall be at least as low as the low point of the lining at the outlet. The outlet passage shall be pitched downward toward the terminal end.

D.12.2

Vertical type. The vertical centerline of the outlet passage shall be as close as practical to a side wall of the tank. The outlet passage shall be a generally horizontal extension of an elbow which is a part of or is welded the lining. The outlet passage shall not pass through the bottom of the shell if product will be held in the passage.

D.13

Outlet valves: Valves, when provided, shall conform to D.8 or if the valve is within the lining or in the outlet passage, and the seat is an integral part of the lining or the outlet passage, a compression-type valve conforming to the applicable provisions of D.13.1 may be used. A cap conforming to D.8 shall be provided for the out-

let end of valves furnished with tanks.

D.13.1

Compression-type valve in the tank or outlet passage. This type of valve shall have a metal to metal or rubber or rubber-like material to metal seat. The rubber or rubber-like material may be either removable or bonded. The handle or valve operating rod shall extend through the bridge or the handle shall be outside the shell. If the handle or valve operating lever extends through the bridge, it shall have a permanently attached shield to protect against the entrance of contaminants into the tank through the space around the handle or valve operating lever.

D.14

Agitators: Means for mechanical and/or air agitation shall be provided that will result in a variation in milk fat content of the product in the tank of not more than plus or minus 0.1 percent as determined by an Official AOAC Milk Fat Test[3], when the tank is filled to (1) 100 percent of its capacity with product and the agitator has been in operation for five minutes if the capacity of the tank is less than 1500 gallons or (2) 100 percent of its capacity with product and the agitator has been in operation for ten minutes if the capacity of the tank is 1500 gallons or larger. Agitators, if not designed for mechanical cleaning, shall be readily accessible for manual cleaning and inspection either in an assembled position or when removed. A seal for the agitator shaft, if provided, shall be of a packless type, sanitary in design and durable with all parts readily accessible for cleaning. A sanitary seal for the agitator shaft shall be provided for (1) a horizontal agitator, (2) a vertical agitator when it is specified that the tank is to be located so that the portion of the shaft outside the tank is not in the milk house or milk room, and (3) a tank designed to be operated under vacuum. The means for agitation shall be one of the following:

D.14.1

Mechanical, top entering, non-removable type.

There shall be at least a 1-inch space between the non-removable agitator and the bottom of the lining, unless the agitator is mounted on a hinged-type cover. A bottom shaft bearing shall not be provided for a non-removable type agitator.

D.14.2

Mechanical, top entering, removable type.

This type of agitator shall be provided with an easily accessible, readily demountable coupling of either a sanitary type located within the lining or a coupling located outside of the lining provided that it is above the shield provided to protect the annular space around the shaft. All product contact surfaces of the agitator shall be visible when the agitator is removed. A bottom support or guide, if used, shall be welded to the lining, shall not interfere with drainageof the tank and the inside angles shall have minimum radii of 1/8 inch. When the agitator shaft has a bearing cavity, the diameter of the cavity shall be greater than the depth. The agitator shall be easily demountable for cleaning of the bearing and any shaft cavity.

D.14.3

Mechanical side entering type.

This type of agitator and shaft and its complete seal shall be readily demountable for manual cleaning. Non-removable parts having product contact surfaces shall be designed so that the product contact surfaces are readily cleanable from the inside of the tank.

D.14.4

Air agitation.

The means for air agitation shall comply with the applicable provisions of D.15.

D.15

Air for Agitation or Movement of Product: Means for applying air under pressure shall conform to the applicable provisions of the "3-A Accepted Practices for Supplying Air Under Pressure in Contact with Milk, Milk Products and Product Contact Surfaces, Serial #604-03," and the following:

D.15.1

Clamp type fittings shall not be used within the lining.

D.15.2

Tubing and related fittings within the lining shall be readily and easily removable for cleaning outside the tank or be designed for mechanically cleaning. If designed for mechanically cleaning, the tubing and all related fittings shall be self-draining.

D.15.3

Permanently mounted air tubing shall be constructed and installed so that it will not sag, buckle, vibrate or prevent complete drainage of the tank or tubing and shall be located so that the distance from the outside of the tubing to the lining shall be at least two inches, except at point of entrance.

D.16

Mechanical Agitator Driving Mechanism Mounting: The driving mechanism when above the lining shall be securely mounted in a position that will provide a minimum distance of 4 inches measured vertically downward from the bottom of the driving mechanism housing, excluding bearing bosses and mounting bosses, to the nearest surface of the tank; and in such a manner that all surfaces of the tank under or adjacent to the driving mechanism shall be readily accessible for cleaning and inspection.

D.17

Thermometers: Each tank shall be provided with an indicating thermometer and/or a recording thermometer complying with the applicable specifications for indicating and recording thermometers in Appendix, Section J. The thermometer or the temperature sensing element of the thermometer shall fit one of the connections or openings provided for in D.11.7.1 and D.11.7.2.

[3]*The method of making these tests will be found in the following reference: Official Methods of Analysis: Available from the Association of Official Analytical Chemists, P.O. Box 540, Benjamin Franklin Station, Washington, D.C. 20004.*

D.18

Vents: A vent(s), if provided, shall be so designed to protect against the entrance of contaminants into the tank and the vent(s) shall have sufficient free opening area to prevent back pressure during filling and to prevent vacuum during emptying of the tank. It shall be in the front head near the top of the tank or in the top of the tank or in a manhole cover for a manhole in the top of the tank. The vent(s) shall terminate in the milk house or milk room. It shall be provided with a perforated cover having openings not greater than 1/16 inch diameter, or slots not more than 1/32 inch wide. Woven wire mesh shall not be used for this purpose. It shall be so designed that parts are readily removable and readily accessible for cleaning.

D.19

Cleaning: Tanks having an inside height of more than 96 inches shall be provided with means (see Appendix, Section H.) that will facilitate manual cleaning and inspection of all product contact surfaces or means shall be provided for mechanically cleaning the product contact surfaces of the tank and all non-removable appurtenances thereto.

D.20

Sample Cock: A sample cock shall be provided when a sample cannot be readily obtained from a top opening or a sample port opening in the tank. It shall be of a type that has its sealing surface relatively flush with the product contact surface of the tank and have an inside diameter no less than that of one inch 3-A sanitary tubing.

D.21

Tank Supports: The means of supporting a tank designed to be installed wholly within the milk house or milk room or the means of supporting the portion of a tank that will be in the milk house or milk room shall be one of the following:

D.21.1

With legs: Adjustable legs shall be of sufficient number and strength and so spaced that the filled tank will be adequately supported. Legs shall be smooth with rounded ends and have no exposed threads. Legs made of hollow stock shall be sealed. Legs shall be of a length that will provide (1) the distance between lowest interior surface of the outlet connection and the floor will be not less than 4 inches and (2) a clearance of at least 6 inches between the floor and the bottom of a tank 72 inches or less in diameter or width, except in the case of a V-bottom or a rounded bottom tank of which the outer shell slopes continually upward from the outlet centerline, in which case the minimum clearance may be 4 inches if it increases to 6 inches within a horizontal distance of not more than 12 inches on each side of this centerline. On a tank more than 72 inches in diameter or width, the clearance shall be at least 8 inches. (Where Weights and Measures Codes require that a seal be placed on the legs to detect height adjustment after the tank has been leveled or calibrated, the holes for seals shall be designed and located, or sealed, to prevent entrance of moisture into the legs.)

D.21.2

Mounted on a Slab or Island: The base of the tank shall be such that it may be sealed to the mounting surface (see Appendix, Section K.)

D.22

Prevention of a Significant Product Temperature Increase:

D.22.1

The tank shall be capable of preventing, in 12 hours, a product temperature increase greater than 5°F in a tank filled to 100 percent of its capacity with product when there is a difference of 50°F between the ambient temperature and the average temperature of the product in the tank. For test purposes, water may be substituted for product.

D.22.2

Insulation material, if provided, shall be of a nature and installed in a manner that will prevent shifting or settling.

D.23

A measuring device of the immersion type or of the direct reading gauge type, if provided, shall comply with D.23.1 or D.23.2.

D.23.1

Immersion Type: An immersion measuring device shall comply with the applicable provisions of the code entitled "Farm Milk Tanks" in the National Bureau of Standards Handbook 44-Fourth Edition 1971. The measuring rod shall have graduation marks not less than .005 inch in width and not exceeding .008 inch in depth. The measuring rod consists of a graduated portion, a seat to engage the measuring rod supporting bracket or other supporting means and a handle. It does not include the supporting bracket or other supporting means. The measuring rod may be two or more parts welded together or may be one piece. The handle shall extend above the bridge or main cover, or shall be located outside of the outer shell. The tank serial number stamped or etched on the rod shall be located as high on the rod as in practicable. The opening through which the measuring rod extends shall be protected against liquids or other contaminants entering the tank from that portion of the measuring rod outside the tank.

D.23.2

Direct Reading Gauge: A direct reading gauge of the glass or plastic tube type shall be sanitary in design and construction and shall be readily accessible for cleaning or shall be designed for mechanical cleaning. If designed for mechanical cleaning, the inside diameter of the gauge parts shall be sufficiently uniform that all product contact surfaces will be cleaned. It shall be designed and constructed so that all product in the gauge will be discarded. Means to accomplish this shall be provided at the lowest point and in such a manner that product in the gauge will not enter the

tank outlet line nor re-enter the tank. The valve shall be close coupled. The distance, measured along the passage for the product in the tank to the gauge valve, from the nearest point on the shell to the ferrule or flange for the valve shall not be more than the smaller of (1) twice the nominal diameter of the passage or (2) five inches.

D.24

Non-Product Contact Surfaces: Non-product contact surfaces shall comply with the following:

D.24.1

They shall be smooth, free of pockets and crevices and be readily cleanable.

D.24.2

Surfaces to be coated shall be effectively prepared for coating.

D.24.3

The shell shall be effectively sealed against moisture and vermin at all joints and at junctions with the breast, manhole openings, outlets and other openings.

D.24.4

A vent or weep hole may be provided in the shell. If provided, it shall be located in a position that will provide drainage from the shell and shall be vermin proof.

D.24.5

Outside welds need not be ground.

E.

Cooling

E.1

Cooling Requirements.

A tank when operated with a condensing unit of the minimum capacity given on the name plate shall have enough refrigerated surface to accomplish the following when the condensing unit is in operation during the filling period:

E.1.1

First Milking.

Tanks designed for the following pick up frequency to cool the milk in the tank from 90°F to 50°F within the first hour after being filled to the corresponding volume and from 50°F to 40°F within the next hour:

E.1.1.1

Everyday pick up filled to 50 percent of its rated capacity.

E.1.1.2

Every other day pick up filled to 25 percent of its rated capacity.

E.1.2

Second or Subsequent Milkings.

Prevent the blend temperature to rise above 50°F during the addition of milk.

E.2

Cooling Information.

The tank shall have an information or data plate permanently attached to it giving the following information or the information shall appear on the name plate (see E.2.2.1):

E.2.1

The maximum rate at which milk can enter the tank and comply with the cooling requirements of E.1.1 and E.1.2.

E.2.2

The minimum condensing unit capacity required when the milk enters the tank at the maximum rate.

E.2.2.1

> Maximum rate at which milk can enter this tank and meet the cooling requirements of the 3-A Sanitary Standards for Farm Milk Cooling and Holding Tanks, Serial #13-06, is _____U.S. Gallons per hour. When milk enters the tank at the maximum rate, the minimum condensing unit capacity is * _____BTU/hr. at * _____°F saturated suction temperature.

** The BTU capacity specified is to be at the saturated suction temperature designated by the manufacturer.*

E.3

Cooling System.

E.3.1

In determining cooling capacity, the ambient temperature shall be 90°F and when water cooled condensers are used, the refrigerant condensing temperature shall be not less than 103°F.

E.3.2

The tank shall be provided with an automatic refrigeration control capable of functioning on a change in product temperature of not more than plus or minus 2°F at 37°F.

## APPENDIX

F.

*STAINLESS STEEL MATERIALS*

Stainless steel conforming to the applicable composition ranges established by AISI[1] for wrought products, or by ACI[2] for cast products, should be considered in compliance with the requirements of Section C.1 herein. Where welding is involved, the carbon content of the stainless steel should not exceed 0.08 percent. The first reference cited in C.1 sets forth the chemical ranges and limits of acceptable stainless steels of the 300 series. Cast grades of stainless steel corresponding to types 303, 304, and 316, are designated CF-16F, CF-8, and CF-8M, repectively. These cast grades are covered by ASTM[4] specifications A296-68 and A351-70.

G.

*PRODUCT CONTACT SURFACE FINISH*

Surface finish equivalent to 150 grit or better as obtained with silicon carbide, is considered in compliance with the requirements of Section D.1 herein.

H.

*MANUAL CLEANING*

If the inside height of a tank exceeds 96 inches, one

[4] *Available from American Society for Testing and Materials, 1916 Race Street, Philadelphia, PA 19103.*

means for manual cleaning is to weld a sanitary stainless steel rung on each end of the tank to support a removable platform at a height which will facilitate cleaning and inspection.

I.

*VACUUM PIPING*

When vacuum piping is provided, the piping downstream from the elbow connected to the vacuum connection on the tank (see D.11.8) should pitch downward from the tank to a moisture trap. The piping between the tank vacuum connection and the moisture trap should be stainless steel and have a pitch of not less than 1 inch in the first 12 inches.

J.

*THERMOMETERS*

J.1

Indicating Thermometers.

Scale Range.—Shall have a span not less than 50°F including normal storage temperatures plus or minus 5.0°F with extension of scale on either side permitted: graduated in not more than 2.0°F divisions.

Temperature Scale Divisions.—Spaced not less than one-sixteenth of an inch apart between 35°F and 55°F.

Accuracy.—Within 2°F plus or minus, throughout the specified scale range.

Stem Fitting.—Shall conform to the "3-A Sanitary Standards for Instrument Fittings and Connections Used on Milk and Milk Products Equipment, Serial #09-07" or shall be a stem fitting that does not pierce the lining or means shall be provided to permit securely fastening the temperature sensing element to the outer surface of the lining.

J.2

Recording Thermometers.

Case.—Moistureproof under operating conditions in a milk house or milk room.

Scale.—Shall have a scale span of not less than 50°F, including normal storage temperature plus or minus 5°F, graduated in not more than 2°F divisions with not more than 40°F per inch of scale; graduated in time scale divisions of not more than 1 hour having a chord or straight line length of not less than one-eighth of an inch at 40°F. Chart must be capable of recording temperatures up to 180°F. (Span specifications do not apply to extensions beyond 100°F.)

Temperature Accuracy.—With 2°F plus or minus, between specified range limits.

Pen-Arm Setting Device.—Easily accessible; simple to adjust.

Pen and Chart Paper.—Designed to give line not over one-fortieth of an inch thick when in proper adjustment; easy to maintain.

Temperature Sensor.—Protected against damage at 212°F.

Stem Fitting.—Shall conform to the "3-A Sanitary Standards for Instrument Fittings and Connections Used on Milk and Milk Products Equipment, Serial #09-07," or shall be a stem fitting that does not pierce the lining or means shall be provided to permit securely fastening the temperature sensing element to the outer surface of the lining.

Chart Speed.—The circular chart shall make one revolution in not more than seven days and shall be graduated for a maximum record of seven days. Strip chart shall move not less than 1 inch per hour and may be used continuously for 1 calendar month.

K.

*SLABS OR ISLANDS*

When a tank is designed to be installed on a slab or an island, the dimensions of the slab or island should be such that the tank will extend beyond the slab or island at least one inch in all horizontal directions. The slab or island should be of sufficient height so that the bottom of the outlet connection is not less than 4 inches above the floor. The surface of the slab or island should be coated with a thick layer of waterproof mastic material, which will harden without cracking. The junction of the outer shell of the tank and the slab or island should be sealed.

L.

*DETERMINATION OF COOLING CAPABILITY*

In determining the capability of a farm cooling tank to meet the cooling requirements specified in E.1.1 and E.1.2 at the maximum rate at which milk can enter the tank given on the information plate:

L.1

90°F water may be substituted for milk, and

L.2

before the addition of the second and subsequent milkings, the water or milk in the tank should be cooled to 37°F and the condensing unit should be allowed to operate and automatically shut off.

M.

*SUPPLEMENTAL DATA PLATE INFORMATION*

M.1

The data plate of the tank should also include the time the agitator was designed to be in operation (5 or 10 minutes) to obtain the homogeneity required in D.14.

M.2

Example of a data plate legend:

> The agitator of this tank is designed so that it must be in continuous operation * _____ minutes before removing a product sample.

* *i.e. whether 5 or 10*

These standards are effective December 30, 1975, at which time the "3-A Sanitary Standards for Farm Milk Cooling and Holding Tanks, Revised, Serial #13-01," and the amendments to it are rescinded and become null and void.

## News and Events

### DFISA Steps up Government Liaison

A government affairs committee has been established by Dairy and Food Industries Supply Association, announced Gordon A. Houran, President.

Houran said that considerable study was given to the idea of a formal legislative monitoring and review process, with the conclusion that the growing impact of government on suppliers' operations merited higher priority on public policy matters.

The new committee will work with the DFISA staff to maintain a close surveillance of legislative and regulatory matters, alert DFISA members to issues having an impact on their operations and make representations to appropriate government bodies.

Carl Nielsen of Dairy Craft Inc. has been named the committee's first chairman. Nielsen is a member of the DFISA board of directors. Committee members are E. G. Bellows, vice chairman, De Laval Separator Co.; R. B. Adams, Virginia Chemicals Inc.; Dean Frazeur, Eskimo Pie Corp.; Richard Gross, Popsicle Industries; Alan Kligerman, Sugar-Lo Co.; Malcolm Miller, Waukesha Specialty Co. Inc.; W. Phil Niemeyer, NASCO International Inc.; Thomas Nilles, CREPACO Inc.; Michael O'Keeffe, Foss America Inc.; Carol Raulston, International Paper Co.; Edward Rhawn, B-Bar-B Inc.; Donald Rutherford, Walker Stainless Equipment Co. Inc. and John Thielke, Klenzade Products, Economics Laboratory Inc.

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### "The Role of Fiber in the Diet"
10th Annual Symposium
November 13, 1975
Hilton Inn-On-The-Campus, Rochester, New York

Sponsored by the Western New York Section of the Institute of Food Technologists and the Department of Food Science and Technology, New York State Agricultural Experiment Station, Cornell University, Geneva, New York.

Speakers will be: L. R. Hackler, Cornell University; J. Scala, Genergal Foods Corporation; P. J. Van Soest, Cornell University; J. A. Story, Wistar Institute; L. W. Allen, Cornell University, W. E. Marshall, General Foods Corporation; A. M. Connell, University of Cincinnati College of Medicine; M. H. Floch, Norwalk General Hospital; J. M. Rivers, Cornell University.

For details write to D. L. Downing, Department of Food Science and Technology, New York State Agricultural Experiment Station, Geneva, New York 14456. Telephone: (315) 787-2273.

### N. F. Sherman Elected to NIFI Board of Trustees

N. F. Sherman

N. F. Sherman, Executive Vice President and Director of United Institutional Distributors Corporation, has been named a member of the Board of Trustees of the National Institute for the Foodservice Industry, it was announced by NIFI President Theodore J. Cooney.

In reporting Sherman's election, Cooney said, "The Institute is pleased to add to its Board a representative of the vast distributor network that serves the foodservice industry. Mr. Sherman's knowledge and experience will be an important asset to NIFI."

Sherman is also Executive Vice President and Director of North American Food Service Corporation and Executive Director of ABC Affiliated Distributors, Inc.

Previously, he was Director of Purchases for the Hilton Hotels Corporation. Known throughout the foodservice industry by his nickname, "Bud," Sherman was a Lieutenant Colonel in the U.S. Army in World War II.

A graduate of the University of Maryland, he and his wife and two children reside in Oak Brook, a suburb of Chicago.

NIFI is the not-for-profit educational foundation established by the foodservice industry to advance professional standards of management through education.

## News and Events

### NMC Annual Meeting

Everyone interested in prevention and control of bovine mastitis is cordially invited to attend the 15th Annual Meeting of the National Mastitis Council to be held February 16-18, 1976, at the Executive Inn, Louisville, Kentucky. Vice President and Program Chairman Dr. R. D. Mochrie has an outstanding program planned for the meeting. Subject matter of interest to all segments will be presented.

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### Whey is Converted into Single Cell Protein

A Swiss firm specializing in converting waste porducts into valuable food stuffs and proving the environment at the importance of yeast fermentation of whey from cheese plants.

The company is PEC, Process Engineering Co. of Mannedorf, Zurich, Switzerland.

PEC, a pioneer in technology for protein production through various processes, has recently emphasized theimportance of yeast fermentation of whey from cheese plants.

PEC process converts the by-product whey into single-cell protein and lacto-proteins for human consumption and animal nutrition, and simultaneously solves a growing waste-pollution problem.

The interest in whey has been stimulated by both the growing worldwide shortage of proteins and the pressing need to clean up the United States environment through proper disposal of waste products.

Using new processes and equipment, PEC microbiologists and engineers have demonstrated that the fermentation process produces yeast with a high concentration of proteins while using less power than energy-intensive evaporation processes.

At the same time, fermentation reduces pollution in the resultant waste waters by 95 to 97 per cent. Final discharge water is as low as 5 BOD. Thus, the process water could be released into any sewage system. The cleanliness of the water actually qualifies it for recycle and re-use in the cheese plant.

Particular strains of yeast grown on waste material such as whey contain 50 to 60 per cent protein and can be used for human and animal nutrition without reservation, Judy said. Some yeast strains have been used for human consumption for 2,000 years without any evidence of toxicity, he stated.

The total market potential for protein additives has been estimated at 3.1 billion pounds annually.

### Five Food Seminars Scheduled at Chicago

Five seminars of interest to food scientists and technologists have been scheduled this fall at Chicago.

The subjects of the seminars are specific with the subjects to be presented with research specialists scheduled to discuss the themes in each case as follows:

—Protein quality in food product development—September 24, O'Hare Inn. Protein in human food, and protein quality merry-go-round, and protein quality as a regulatory tool, one-day seminar.

—New frontiers in analytical chemistry in solving your food analysis problems—September 25, O'Hare Inn. A demonstration of how new principles, instrumentation, techniques and methodology can be used in food analysis.

—Effects of texture in food systems—October 22, Sheraton O'Hare. Up-to-date information on texture measurement, interpretation and analytical data, and the relationship between texture, microstructure and processing methods.

—Recent developments in food enzymology—October 23, Sheraton O'Hare. An update of potential problems and uses of enzymes in controlling food quality and reducing processing and storage costs.

—Regulatory status of food flavors and colors—October 17, Hyatt Regency O'Hare. The lastest regulatory and technical information will be presented on important food flavors and colors.

For detailed information on these meetings, contact Technomic Publishing Co., Inc., 265 State Street, Westport, CT 06880.

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### Conferences at Ohio State University

February 11-12, 1976-Dairy Industry Conference, Center For Tomorrow, The Ohio State University, Columbus, Ohio. For information: John Lindamood, Department of Food Science and Nutrition, 2121 Fyffe Road, The Ohio State University, Columbus, Ohio 43210.

March 22-26, 1976-Mid-West Workshop in Milk and Food Sanitation, Center For Tomorrow, The Ohio State University, Columbus, Ohio. For information: John Lindamood, Department of Food Science and Nutrition, 2121 Fyffe Road, The Ohio State University, Columbus, Ohio 43210.

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## Oregon Affiliate

The reins of administrating the dairy products program of the Oregon Department of Agriculture were recently passed to Ai Tesdal (L) by Vergil N. Simmons. A special recognition program sponsored by the Oregon Affiliate for Simmons was held in Salem, Oregon.

## Notice IAMFES Awards 1976

Each year IAMFES recognizes outstanding contributions and performance by it's members.

The success of this program is dependent not only on those organizations who so generously support the monetary aspects of these awards, but it is equally dependent on your individual help in furnishing the Awards Committee with appropriate information and names of potential award winners.

Will you please give serious thought to the following Awards, which will be considered for presentation of our 1976 IAMFES Annual Meeting.

1. *The Sanitairian's Award* of $1000 to a State or Federal sanitarian, who, during the past seven years has made outstanding contributions to the health and welfare of his community.
2. *Educator-Industry Award* of $1000 to a University or Industry employee who has made outstanding contributions to food safety and sanitation. In 1976 the award will be made to an industry worker.
3. *The Citation Award* to a member who has given outstanding service to IAMFES in fulfilling it's objectives.
4. *The Shogren Award* to the affiliate organization that has the best statewide or regional program.
5. *Honorary Life Membership* to those members who have given long and outstanding service to IAMFES.

Please contact Earl O. Wright, Chairman of the IAMFES Recognition and Awards Committee, IAMFES, P.O. Box 701, Ames, Iowa 50010.

## Affiliate News

### Notice to Membership

In accordance with IAMFES Constitution and By-Laws which requires a secretary-treasurer to be elected by mail ballot, you are hereby notified that President Harold E. Thompson, Jr. at the annual meeting in Toronto, Ontario, Canada, August 1975 appointed a nominating committee for 1976. Mr. Ben Luce is the chairman of this committee.

Nominations for the office of secretary-treasurer are now open and any member wishing to make a nomination should send a picture and biographical sketch of his nominee to the nominating committee no later than November 15, 1975. To maintain proper balance on the Executive Board the nominee should be selected from local or municipal health area.

Ben Luce
Washington Association of Milk Sanitarians
2110 -2- 56th Avenue NW
Olympia, Washington 98502

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### Robinson, Ledford Head Institute

Prof. Willard B. Robinson has been named head of the Institute of Food Science at the N.Y. State College of Agriculture and Life Sciences, Cornell University.

At the same time, Prof. Richard A. Ledford, Chairman of the College's Department of Food Science, was appointed associate head of the Institute.

Both were appointed for five-year terms, effective July 1, W. Keith Kennedy, Dean of the College, announced.

Robinson is currently chairman of the Department of Food Science and Technology at the N.Y. State Agricultural Experiment Station at Geneva.

He succeeded Prof. Robert C. Baker who has completed a term as director of the Institute. Baker now devotes full time to teaching, research and extension duties in the Department of Poultry Science.

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### Index To Advertisers



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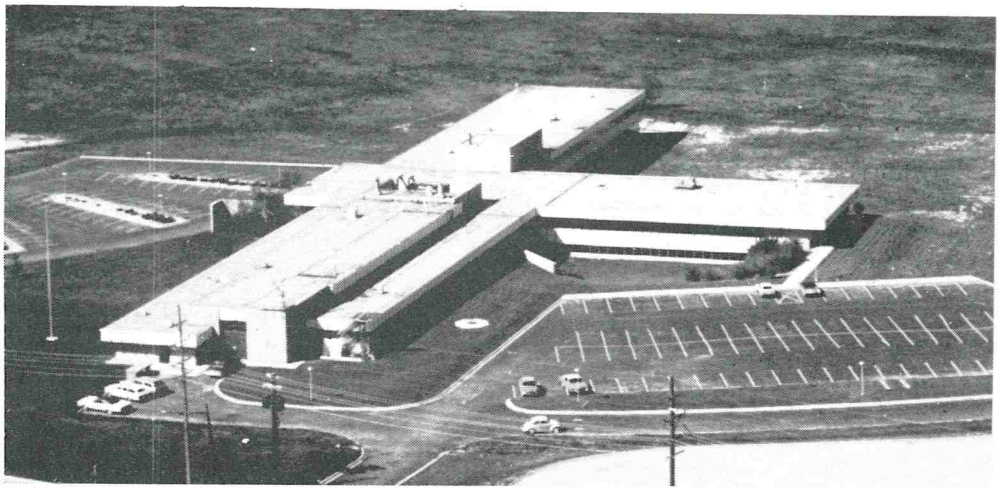
### Classified Ad

#### For Sale

Single Service milk sample tubes. For further information and a catalogue please write, Dairy Technology, Inc., P.O. Box 101, Eugene, Oregon 97401.



The NSF Building, occupying 20 acres on the northwest quadrant of Plymouth and Green Roads in Ann Arbor, contains 67,000 square feet of space for offices and laboratories.

# WHAT IS NSF?

NSF—the National Sanitation Foundation—is a non profit, non governmental organization concerned with public health and environmental safety. Our staff consists of scientists, engineers, educators and technicians working under the direction of a board of trustees and backed by a 29-member council of public health consultants.

We were established in 1944 in the School of Public Health of the University of Michigan. We are now an independent organization with regional offices throughout the United States and contractual relationships with 1,300 manufacturers of environmentally related products in North American, European and Asian countries.

We serve as a trusted neutral agency for government, industry and consumers, helping them to resolve differences and unite in finding solutions to environmental problems. Through joint NSF committees we have developed more than 50 standards and criteria since 1952. There are NSF standards for food service equipment which is used in countless thousands of eating places throughout the world. Other NSF standards relate to water treatment and purification equipment, products for swimming pool applications, plastic pipe for potable water as well as drain, waste and vent uses, plumbing components for mobile homes and recreational vehicles, laboratory furniture, hospital cabinets, polyethylene refuse bags and containers, individual aerobic wastewater treatment plants and other products related to environmental quality.

The NSF laboratories do chemical, physical, radiological and biological testing and engage in environmental research under grants from the federal government, as well as state and local agencies and foundations. NSF testing laboratory services play a key role in the development of standards and provide continuous technical support for hundreds of manufacturers who use the NSF seal to show that their products conform to NSF standards.

Surveys indicate that a substantial percentage of public health agencies at state, district, county and municipal levels require adherence to NSF standards for food service equipment, plastic pipe and other health-related products.

NSF

**National Sanitation Foundation—an independent non profit, non governmental organization dedicated to environmental quality. NSF Building, Ann Arbor, Mich. 48105. (313) 769-8010.**

Government officials and professional workers concerned with public health and environmental quality are invited to write for the NSF Publications List. It is free. It lists all NSF standards and criteria as well as listings, reports and NSF literature.

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SANITARY METHOD OF APPLYING
A U. S. P. LUBRICANT
TO DAIRY & FOOD
PROCESSING EQUIPMENT


# Haynes-Spray

## U.S.P. LIQUID PETROLATUM SPRAY

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CONTAINS NO ANIMAL OR VEGETABLE FATS. ABSOLUTELY NEUTRAL. WILL NOT TURN RANCID – CONTAMINATE OR TAINT WHEN IN CONTACT WITH FOOD PRODUCTS.

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*This Fine Mist-like*
**HAYNES-SPRAY**
*should be used to lubricate:*

SANITARY VALVES
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## *The Modern HAYNES-SPRAY Method of Lubrication Conforms with the Milk Ordinance and Code Recommended by the U. S. Public Health Service*

The Haynes-Spray eliminates the danger of contamination which is possible by old fashioned lubricating methods. Spreading lubricants by the use of the finger method may entirely destroy previous bactericidal treatment of equipment.

PACKED 6-12 oz. CANS PER CARTON  SHIPPING WEIGHT - 7 LBS

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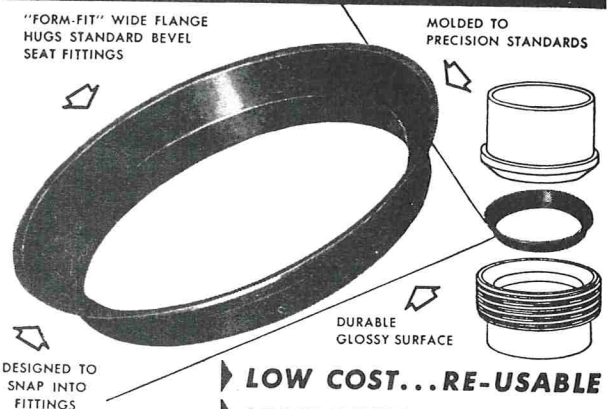


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## Specify . . . HAYNES SNAP-TITES of TEFLON

* *TEFLON ACCEPTED SAFE FOR USE ON FOOD & PROCESSING EQUIPMENT BY U. S. FOOD AND DRUG ADMINISTRATION*

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## A HEAVY DUTY SANITARY LUBRICANT



*Available in both*
**SPRAY AND TUBE**

All Lubri-Film ingredients are approved additives and can be safely utilized as a lubricant for food processing equipment when used in compliance with existing food additive regulations.

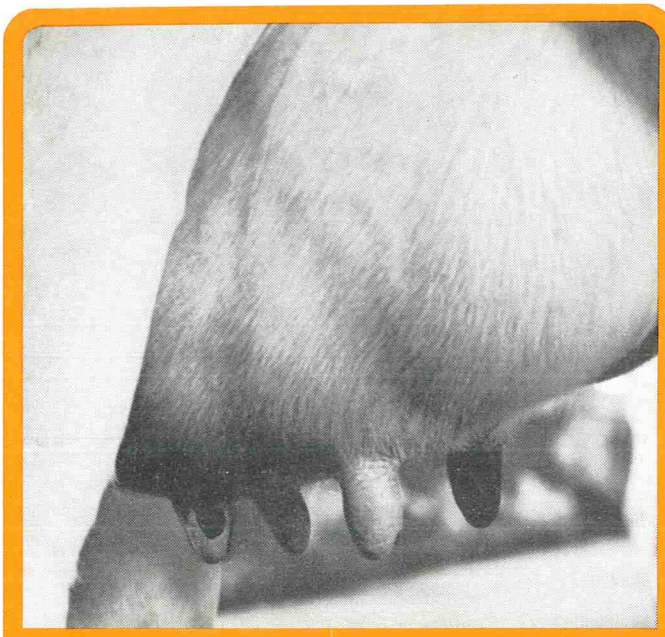
ESPECIALLY DEVELOPED FOR LUBRICATION OF FOOD PROCESSING AND PACKAGING EQUIPMENT

For Use in Dairies — Ice Cream Plants — Breweries — Beverage Plants — Bakeries — Canneries — Packing Plants

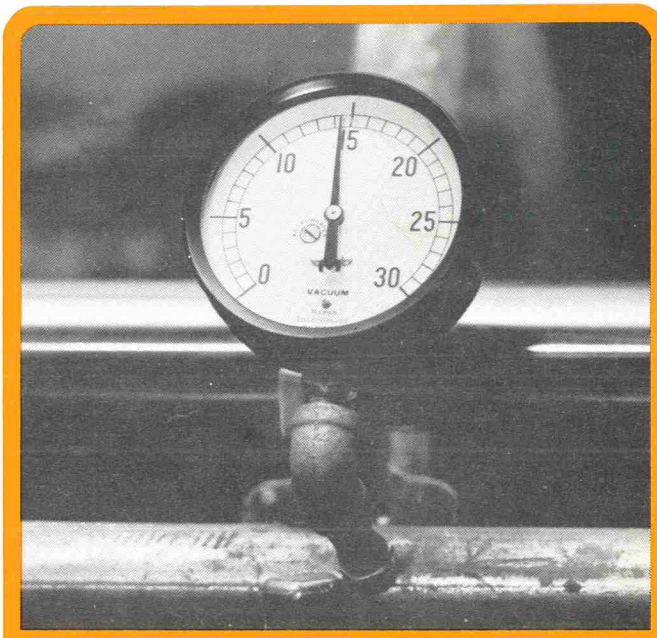
**SANITARY • NON TOXIC • ODORLESS • TASTELESS**

SPRAY — PACKED 6 — 16 OZ. CANS PER CARTON
TUBES — PACKED 12 — 4 OZ. TUBES PER CARTON

**THE HAYNES MANUFACTURING CO.**
CLEVELAND, OHIO 44113



# The place to measure vacuum pump performance is here...

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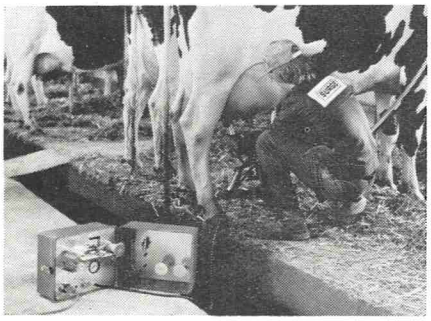


**A free levograph test from your Surge dealer evaluates your vacuum system's efficiency in minutes.**

Of all the equipment you own, none is more important than your vacuum pump, because the efficiency of your entire milking system depends on it.

You can feed the right feed; cull your cows carefully; and follow all the latest herd management practices, only to find you're being robbed by a worn or faulty vacuum pump.

Right now, your Surge dealer is offering you a valuable, money-saving opportunity. At no cost or obligation to you, he will completely test your entire vacuum system using the latest vacuum system diagnostic equipment.

Your pump may appear to be in good running order, but there's a good chance it's costing you money month-after-month. Since vacuum pumps wear out gradually, many dairymen never realize how inefficient their system has become until the installation of a new pump produces a noticeable increase in milk production.

**Surge features help produce more milk, protect herd health.**

To maintain a constant 12″-13″ of vacuum at the teat end, you need a pump that's built to last, and has a high reserve capacity. A unit like the famous Surge Alamo vacuum pump, with features like:

**1. Continuous flood-type lubrication** that assures a better seal between rotor vanes and cylinder wall, providing maximum air-moving capacity.

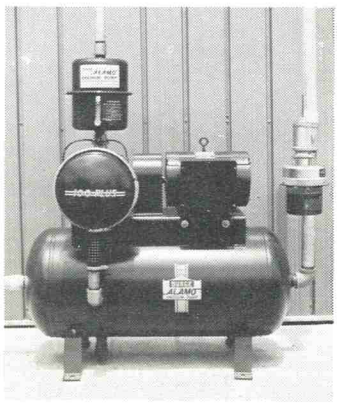
**2. An exclusive oil reclaimer system** that reclaims, filters, and recirculates up to 95% of the oil from the pump exhaust.

**3. Filtered air intake** to prevent dirt or other foreign matter from reaching the rotor.

**4. Large oil reserve** (1½ gallons) reduces the chance of pump failure or injury due to a depleted oil supply.

**5. Oil level and temperature gauges** tell you at a glance if proper oil temperatures and levels are being maintained.

Contact your Surge dealer today. He'll thoroughly test your present system at no cost, no obligation. Should you need a new pump, he'll recommend the Surge Alamo model that's right for your operation, your future.



"You're a step ahead with Surge"

SURGE

For more information see your Surge Dealer
or write to Babson Bros. Co.
2100 S. York Road, Oak Brook, Illinois 60521