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58TH ANNUAL MEETING

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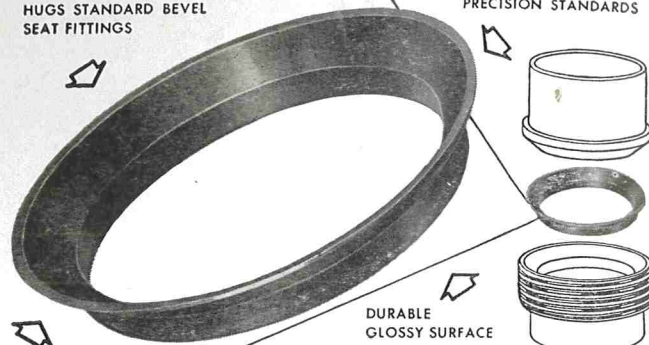
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COMPARISON OF EXTRACT-RELEASE VOLUME, PH, AND AGAR PLATE COUNT OF SHRIMP^{1, 2}

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(Received for publication October 6, 1970)

ABSTRACT

During refrigerated storage of shrimp, an initial increase in Extract-Release-Volume (ERV) with little or no change in bacterial count was followed by a rapid increase in bacterial count and decrease in ERV. The pH of shrimp increased gradually during storage. In commercial samples of stored iced shrimp little correlation existed between ERV and bacterial count, ERV and pH, and pH and bacterial count.

Loss of quality and subsequent spoilage of shrimp is caused primarily by tissue enzymes and microbial activities. At present, quality of stored iced shrimp is determined mainly by organoleptic tests. These sensory evaluations frequently lack uniformity and contribute little information about the potential shelf life of the product. The shrimp processing industry is in need of a simple rapid test to determine the sanitary quality and expected storage life of the raw product. When used with an organoleptic evaluation, it would enable processors to determine with some degree of uniformity the suitability of the product for processing. Various tests have been proposed for this purpose. Included are levels of glycogen-sugar content, lactic acid, acid-soluble orthophosphate, trimethylamine nitrogen, amino nitrogen, hydration of water-insoluble protein, pH, and bacterial count (3). Most of these tests require investment in laboratory equipment and trained personnel. Except for pH determinations, these tests are also time-consuming. A few plants now use bacterial counts as an index of sanitary quality. High bacterial counts are unacceptable but do not always indicate the extent of loss of quality or spoilage. This is caused by differences in biochemical activities of the individual bacterial species, particularly on the proteins and lipids of shrimp.

Jay (6), Jay and Kontou (7), and Kontou et al. (8) reported that changes in the hydration capacity of meat protein, expressed as Extract-Release Volume (ERV), can be used to evaluate the degree of fresh-

ness or spoilage of meat. The water holding capacity gradually increased (ERV decreased) as spoilage occurred. Recently, Adamcic and Clark (1) applied the same principle to evaluate the microbial quality of whole poultry. The object of our study was to compare changes in ERV, bacterial count, and pH during refrigerated storage of shrimp.

MATERIALS AND METHODS

Unless indicated otherwise, live white shrimp (*Penaeus setiferus*) from Galveston Bay were used. They were packed in ice and transported to the laboratory. Total time of transportation did not exceed 4 hr and tests were carried out immediately upon arrival in the laboratory. Samples for storage studies were held in sterile glass containers at 5 C. To determine ERV (5), 25 g of iced shrimp were blended for 1 min with 100 ml of distilled water (25 C) in a Waring blender. This mixture was filtered through Whatman No. 1 filter paper at 25 C. The filtrate which collected during a 20-min filtration period was measured in milliliters and expressed as ERV. Bacterial counts were made by the agar plate method (2). The pH was measured electrometrically (Corning model 12) on the homogenates. Buffers used were citrate for pH 4 and 5; phosphate for pH 6, 7, and 8; and glycine-NaOH for pH 9 and 10 (4).

RESULTS AND DISCUSSION

Data on the influence of time of blending, temperature, pH of extractant, and collection time on ERV are presented in Fig. 1 through 4. With an increase in blending time, the ERV decreased. Jay (5) observed a similar effect in ground beef up to 2 min of blending. A subsequent increase in ERV of beef after 4 min of blending may have resulted from an increase in temperature of the homogenate. With ground beef, the ERV increased somewhat with increases in temperature of the extractant (5). With shrimp, however, small decreases in ERV were noted at the higher temperatures. The decrease in ERV on continued blending (3-5 min) of shrimp also may have been caused by heat buildup in the homogenate. Subsequent experiments showed that the temperature of homogenate, initially at 25 C, rose to 30.5, 34.7, 40.0, and 44.9 C after 1, 2, 3, and 5 min of blending.

A marked decrease in ERV took place as the pH of the buffered extractant was increased from 4 to

¹Technical article no. 8752 of the Texas Agricultural Experiment Station, College Station.

²This work was funded in part by the NSF Sea Grant Program, Institutional Grant GH-59 made to Texas A&M University.

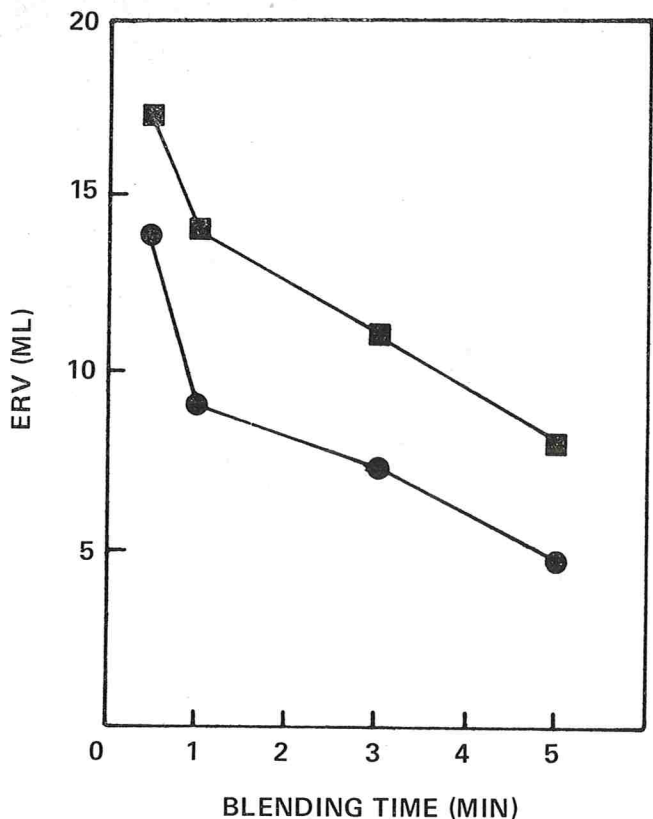


Figure 1. The effect of time of blending on ERV of 2 samples of fresh shrimp (samples were blended with dist. water at 25 C).

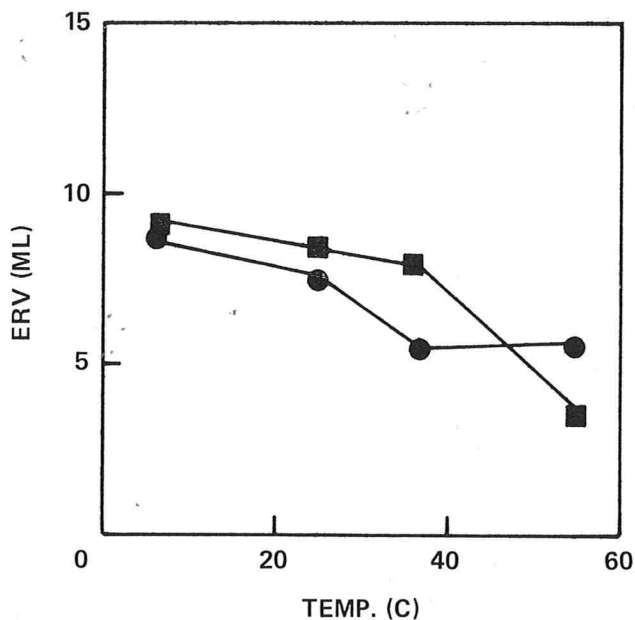


Figure 2. Effect of temperature on ERV of fresh shrimp (samples were blended with dist. water for 1 min).

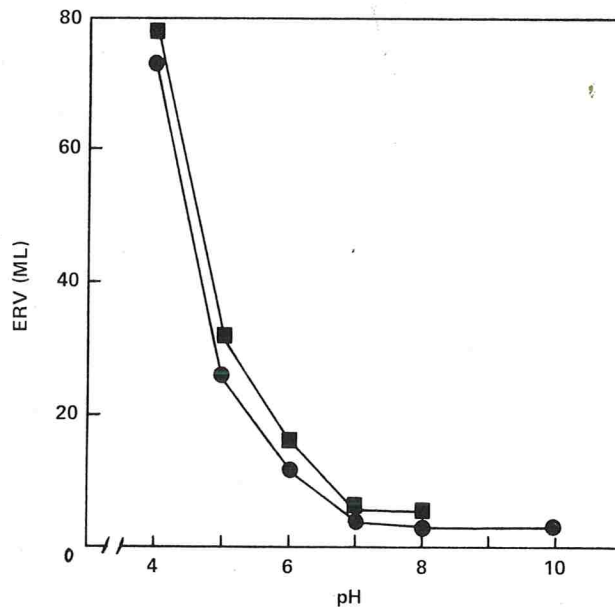


Figure 3. The effect of pH of blending diluent on ERV of 2 samples of fresh shrimp (samples were blended for 1 min at 25 C).

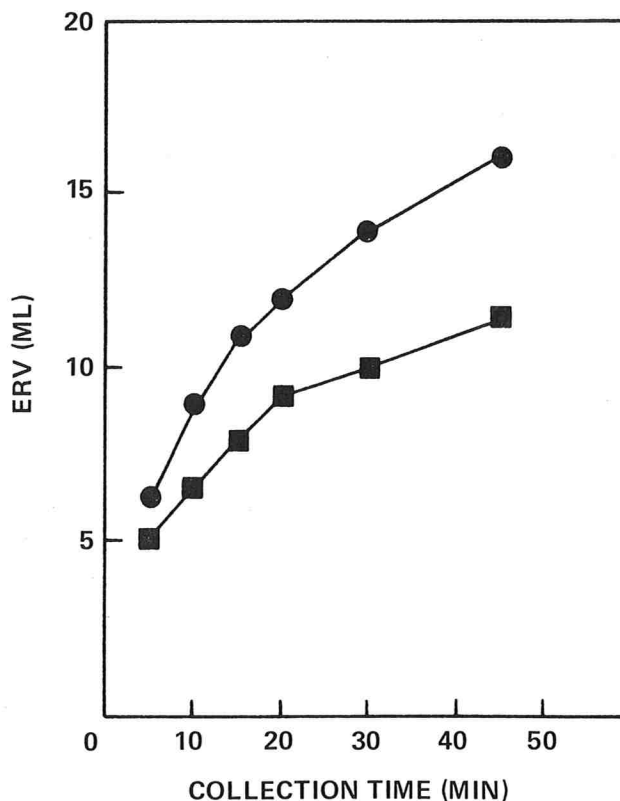


Figure 4. ERV of distilled water homogenates of 2 samples of fresh shrimp with different collection times at 25 C (samples were blended with dist. water for 1 min at 25 C).

7. With ground beef, Jay (5) observed a decrease in ERV as the pH of the extractant was raised from 5 to 7. With shrimp, highest ERV was observed at pH 4.0, with beef at pH 5.0. At pH 4.0 the ERV of beef

was zero. These differences in ERV of beef and shrimp at pH 4 and 5 most likely are caused by differences in the physical-chemical characteristics of their proteins. According to Jay (5), the high ERV

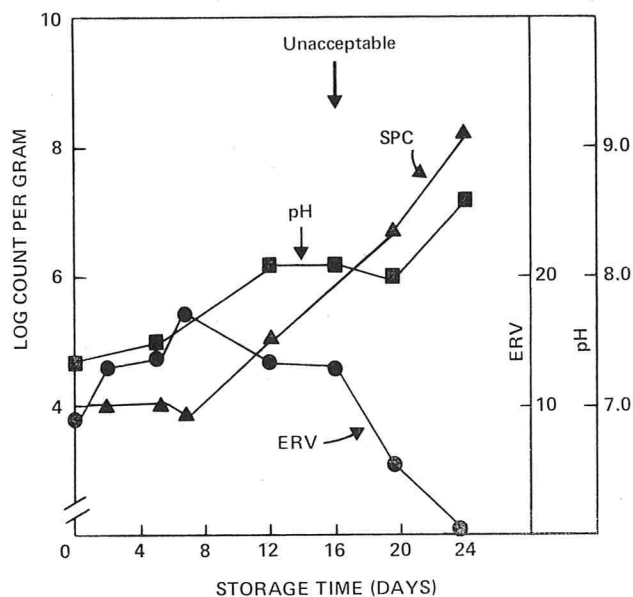


Figure 5. ERV, pH, and agar plate count of shrimp stored for 24 days at 5 C.

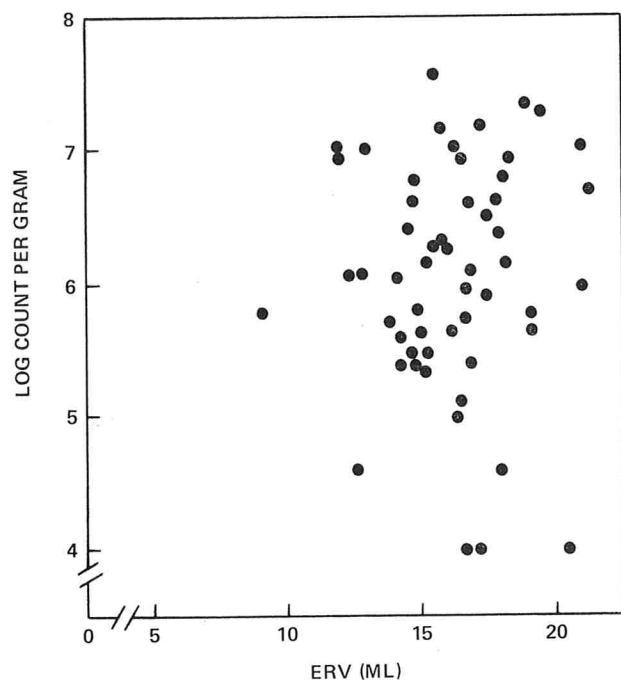


Figure 6. ERV and agar plate count of 55 samples of shrimp.

of beef at pH 5.0 probably was related to the closeness of this reaction to the isoelectric point of beef protein. The ERV of shrimp increased with increased collection times (Fig. 4). Similar results were reported for ground beef (5).

Changes in ERV, pH, and standard plate count (SPC) of shrimp during storage for 24 days at 5 C are presented in Fig. 5. During the first 7 days, the ERV increased gradually from 9 to 17 with little or

no change either in agar plate count or pH. After 7 days of storage, a marked increase in bacterial count took place. At the same time, the ERV decreased and reached zero on the 24th day. The pH increased gradually from an initial value of 7.4 to 8.6. The shrimp were considered organoleptically unacceptable after 16 days. In another sample, the ERV increased from 9 to 15 during the first 7 days at 5 C. Only minor changes in pH and agar plate count were noted during this period. From the 7th to the 14th day, the ERV decreased from 15 to 2. In the same period the agar plate count increased from 10^4 to 1.3×10^9 per gram. The pH increased gradually from 7.4 to 8.2. This shrimp was considered organoleptically unacceptable after 10 days at 5 C, when the count had increased to 2×10^7 per gram. Differences in the spoilage pattern of these samples with similar initial bacterial counts probably were caused by differences in bacterial species. However, both samples showed similar patterns in changes of ERV, bacterial counts, and pH during storage. An initial increase in ERV with little or no change in bacterial count was followed by a rapid increase in bacterial count and decrease in ERV. The pH of shrimp increased gradually during the storage period. Bailey et al. (3) showed that the water content of water-insoluble proteins of shrimp increased on iced storage. They reported that free sulfhydryl groups in shrimp proteins increased during storage providing additional sites for water binding.

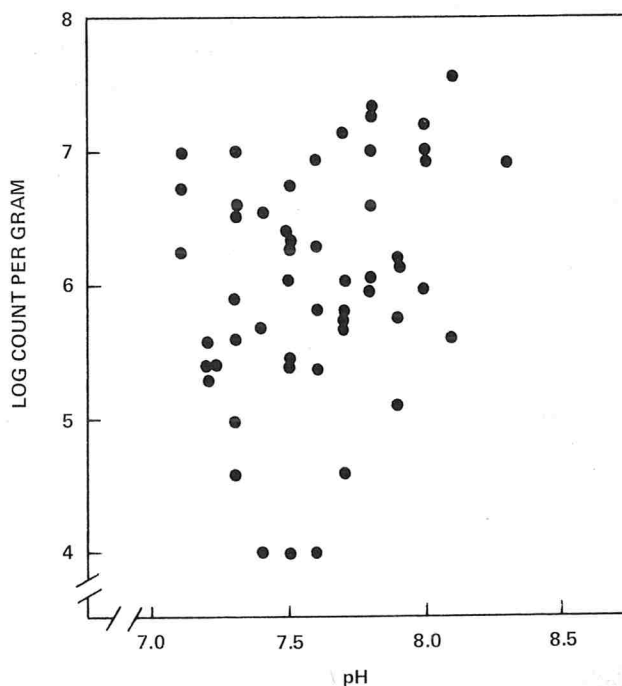


Figure 7. Agar plate count and pH of 53 samples of shrimp.

Increased waterholding capacity (decreased ERV) of beef was observed by Jay (5) and Shelef and Jay (9) when beef was treated with proteolytic enzymes or urea. Subsequent studies (10) indicated that the amino sugar content may be related to the increased hydration of spoiled meat.

Jay (5) reported that the ERV of the *longissimus dorsi* and *semitendinosus* increased to its maximum value in two days post-mortem. This was followed by a gradual decrease in ERV upon refrigerated storage. With beef the initial rise in ERV probably is caused by changes in the hydration capacity of meat proteins during rigor mortis. Too little is known about shrimp proteins to speculate that the rise in ERV during the first 7 days of storage was caused by similar processes.

At first it would appear that the gradual decrease in ERV of shrimp after 7 days possibly could be used as an index of sanitary quality similar to that with beef. However, this usage of ERV in shrimp is affected by the gradual increase in ERV during early storage (0-7 days). For example, the ERV of spoiled shrimp after 16 days at 5 C (Fig. 5) was similar to that after 2 days. This lack of relation between ERV and bacterial count is also demonstrated in Fig. 6. These samples were from boats as they arrived at processing plants in Brownsville, Texas. Correlation between ERV and bacterial count was 0.07. Bailey et al. (3) reported that pH can be used effectively as a quality index of iced stored shrimp. Below pH 7.7 they were judged of prime quality, between pH 7.7 and 7.95 acceptable but poor, and at pH 7.95 or above spoiled or on borderline of spoilage. Our observations with the laboratory stored shrimp (Fig. 5) also indicated that the samples were spoiled or near spoilage at pH 8.

Correlation between pH and bacterial count (Fig. 7) was low (-0.23), that between pH and ERV was 0.02. The pH values of shrimp with high bacterial counts (10^7 or above) ranged from 7.1 to 8.1. This observation again demonstrates that the level of viable bacterial population *per se* does not indicate the extent of biochemical changes and hence loss of fresh-

ness or spoilage of a food. Different microorganisms affect the organoleptic qualities of food in different ways. In addition, some of the high bacterial counts may reflect growth on shrimp, whereas others resulted because of contact with heavily contaminated surfaces. The latter would not be expected to cause a concomitant rise in pH. The results of the present study indicate that the ERV test, as used for beef and chicken, could not be used to evaluate the freshness or spoilage of stored iced shrimp. The data support the view (3) that the pH of shrimp perhaps could be used as a simple screening test to evaluate degree of freshness.

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A PROCEDURE FOR RAPID RECOVERY OF AFLATOXINS FROM CHEESE AND OTHER FOODS¹

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(Received for publication November 10, 1970)

ABSTRACT

A rapid and efficient method has been developed to recover aflatoxin from cheese and other foods. The procedure involves: (a) blending the sample with a mixture of chloroform, methanol, and water (solvents are used in such proportions that a miscible (monophasic) system is formed, (b) adding more chloroform and water so the mixture becomes biphasic, (c) filtering to remove the food residue, (d) separating the lower chloroform layer which contains virtually all of the aflatoxin, and (e) purification, if necessary, of the material in step (d) after it has been concentrated. Purification is achieved by sequential addition of methanol, water, and hexane; recovery of the methanol-water fraction; and extraction of aflatoxins from it with chloroform. Purification can be eliminated if the substrate contains little or no lipid or pigment which, if present, interfere with thin-layer chromatographic analysis. Extraction can be done in approximately 35 min and purification in approximately 20 min.

When aflatoxins were added to various substrates, the method recovered 92-98% B₁ and 96-100% G₁ from rice; 95-96% B₁ and 90-95% G₁ from peanut butter; 93-94% B₁ and 92-98% G₁ from Cheddar cheese; 100% B₁ and G₁ from corn meal; 91-100% B₁, 91-100% B₂, 96-96% G₁, and 92-100% G₂ from brick cheese; and 97-100% B₁, 95-100% B₂, 92-100% G₁, and 98-100% G₂ from a liquid culture medium.

Discovery of aflatoxin in 1961 has prompted much research interest in and public concern with this mycotoxin (8, 12). Since 1961 there have been numerous studies on analytical methods and consequently different procedures for recovery and measurement of aflatoxin have been suggested. In spite of this effort, no single procedure is satisfactory for use with all raw or processed agricultural commodities (15, 17). The substrate often determines the extraction method to be used (5). Many existing methods recover aflatoxin satisfactorily but are unsuitable for testing large numbers of samples because procedures are too lengthy (2, 19).

While studying formation of aflatoxin on cheese in our laboratory, it was noted that analysis of this food by the procedure of the Aflatoxin Methodology Working Group (1) was too laborious and time consuming. Consequently a method was devised which rapidly recovers a high percentage of aflatoxins from cheese and other foods. The method involves: (a)

extraction of aflatoxins with a mixture of chloroform, methanol, and water and (b) purification of the extract with methanol, water, and hexane. Results obtained from tests on cheese and several other foods indicate that the method rapidly recovers >90% of added aflatoxins. Details of the procedure are given in this paper.

MATERIALS AND METHODS

Principle

The method to be described is based on earlier findings that: (a) aflatoxins can be efficiently extracted from toxin-containing substrates by chloroform (4, 11, 16) and methanol (4, 9, 16) and (b) hexane can be used to remove interfering lipids and pigments from crude extracts of aflatoxins without significantly affecting the amount of aflatoxin which remains in the preparations (9, 13, 14). These reports and the phase diagram of chloroform, methanol, and water, (Fig. 1) led to preparation of mixtures of solvents that satisfactorily recovered aflatoxins from cheese and other foods. Toxins were purified by extraction with hexane to remove interfering lipids and pigments. These extraction and clean-up procedures reduced the time required for aflatoxin analyses.

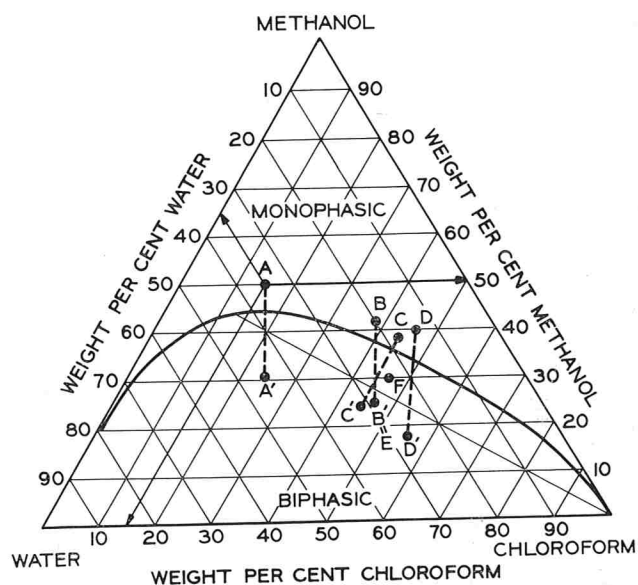


Figure 1. Chloroform-methanol-water phase diagram; per cent by weight at 20 C. Straight line immediately below heavy curved line indicates maximum chloroform tie-line at 0 C. Letters indicate solvent mixtures listed in Table 1. Dotted lines indicate addition of solvents to render monophasic mixtures biphasic.

¹Published with the approval of the Director of the Research Division of the College of Agricultural and Life Sciences, University of Wisconsin.

Extraction of aflatoxin

Solvents (chloroform, technical grade; methanol, ACS grade; and water, including water in the product) were mixed in proportions so that ternary systems were formed which had compositions designated by points A to F on the phase diagram in Fig. 1 (3, 7). See Table 1 for the actual mixtures which were used. Samples (100 g) of minced brick cheese were blended with the appropriate initial solvent mixtures (Table 1) in a Waring blender for several minutes to permit uniform contact between sample particles and the solvent mixture. Additional chloroform was then added (Table 1), the mixture was blended for 1 min, more water (Table 1) was added, and the mixture was again blended for 1 min. This served to render the monophasic extracts (A, B, C, and D in Fig. 1) biphasic (A', B', C', and D' in Fig. 1). For extracts E and F (Fig. 1 and Table 1), all solvents were added at the beginning so the system was not converted from the mono- to the biphasic condition. The actual final composition of these ternary mixtures also is given in Table 1. After filtration (vacuum from water aspirator, Whatman No. 1 filter paper in a Buchner funnel, Celite added as filter aid) to remove the cheese (or other food) residue, the filtrate was transferred to a separatory funnel and the chloroform layer (which contained the aflatoxins) was separated. Another portion of chloroform equal in volume to that added in both steps of the extraction was used to rinse the blender jar and then was added to the residue to recover residual traces of aflatoxins. The mixture was filtered to remove the residue, and the chloroform was then used to extract the original methanol-water fraction. After this extraction, the chloroform was combined with chloroform from the initial extraction.

Approximately 5 g of Na_2SO_4 was added to the combined chloroform extracts to remove traces of water. The Na_2SO_4 was recovered, washed with chloroform, and the washings added to the chloroform extract.

If the product contained little or no lipid or pigment, the chloroform extract was concentrated to 2-3 ml. The concentrate was prepared for thin-layer chromatography (TLC) by dissolving it in sufficient chloroform to yield 10 ml. If the chloroform extract contained enough lipid or pigment to interfere with TLC, then purification, as described below, was necessary. Procedures for extraction were completed in approximately 35 min.

TABLE 1. MIXTURES OF CHLOROFORM, METHANOL, AND WATER USED TO EXTRACT AFLATOXINS FROM 100 GRAMS OF CHEESE.

| Point in Fig. 1 | Initial extraction mixture | | | Dilution solvent | |
|-----------------|----------------------------|----------|--------------------|------------------|-------|
| | Chloroform | Methanol | Water ¹ | Chloroform | Water |
| | (ml) | | | | |
| A ² | 23 | 144 | 80 | 40 | 87 |
| B | 100 | 200 | 80 | 100 | 100 |
| C | 120 | 200 | 80 | 60 | 120 |
| D | 162 | 270 | 80 | 285 | 228 |
| E | 200 | 200 | 180 | — | — |
| F | 97 | 121 | 80 | — | — |

¹Includes water in the product.

²Point A indicates the solvent mixture consists, by weight, of 15% chloroform, 35% water, and 50% methanol. Specific gravity values are: chloroform, 1.498; methanol, 0.79; and water, 1.00. Hence 34.5 g (15% of the mixture) chloroform divided by the specific gravity (1.498) equals 23 ml. Quantities of other components of point A were calculated the same way as were quantities of solvents in other mixtures.

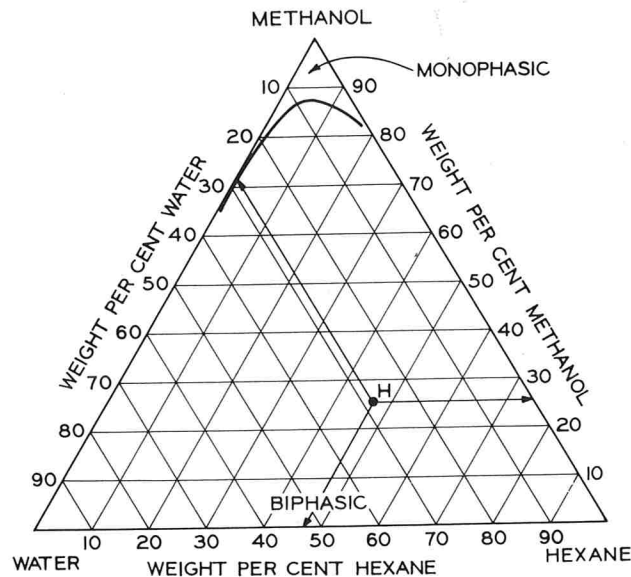


Figure 2. Hexane-methanol-water phase diagram; per cent by weight. Letter indicates mixture of solvents used for purification of the extract.

Purification of the extract

The combined chloroform extracts were evaporated (steam bath and reduced pressure) until no further reduction in volume occurred. The concentrate was then mixed with the following, each added separately and shaken in the order given: methanol, water, and hexane (commercial Skellysolve B) (50:45:120 v/v/v) (Fig. 2). Addition of solvents as indicated minimizes loss of aflatoxin.

The resulting biphasic mixture with the composition indicated by H in Fig. 2 was transferred to a separatory funnel and the lower (methanol-water) layer was removed and extracted three times with chloroform (each portion of chloroform equal in volume to that of methanol added for purification) to recover aflatoxin. The hexane layer was extracted once with methanol-water (50:45 v/v) and the methanol-water mixture was extracted three times with chloroform to recover traces of aflatoxin. Chloroform extracts were combined, evaporated to 2-3 ml, dissolved in sufficient chloroform to yield 10 ml, and used for TLC analysis. The purification procedure was completed in approximately 20-30 min.

The maximum chloroform tie-line shown in Fig. 1 is of particular significance in this extraction-purification procedure. Lower layers of ternary systems with a composition on or below the tie-line consist almost entirely of chloroform, whereas those with a composition above this line are made up of chloroform, methanol, and water. Consequently, to obtain

TABLE 2. RECOVERY OF AFLATOXINS FROM CHEESE BY CHLOROFORM-METHANOL-WATER MIXTURES.

| Extraction mixture, Table 1 | Aflatoxin B ₁ | | | Aflatoxin G ₁ | | |
|-----------------------------|--------------------------|--------------------|------|--------------------------|--------------------|------|
| | Added | Per cent recovered | | Added | Per cent recovered | |
| | | (μg) | (μg) | | (μg) | (μg) |
| A | 74.4 | 55.3 | 74.3 | 99.2 | 71.5 | 72.3 |
| B | 74.4 | 71.0 | 95.5 | 99.2 | 97.0 | 98.0 |
| C | 74.4 | 65.7 | 88.5 | 99.2 | 83.7 | 84.4 |
| D | 74.4 | 66.5 | 89.5 | 99.2 | 88.5 | 89.3 |
| E | 74.4 | 56.4 | 75.8 | 99.2 | 76.0 | 76.5 |
| F | 74.4 | 55.0 | 74.0 | 99.2 | 74.5 | 75.3 |

TABLE 3. RECOVERY OF AFLATOXINS FROM SEVERAL FOODS BY A BINARY SOLVENT (METHANOL-WATER, 50:45 v/v).

| Product | Aflatoxin B ₁ | | | B ₂ | Aflatoxin G ₁ | | | G ₂ |
|---------------------------|--------------------------|-------------------|--------------------|--------------------|--------------------------|-------------------|--------------------|--------------------|
| | Added | Recovered | Per cent recovered | Per cent recovered | Added | Recovered | Per cent recovered | Per cent recovered |
| | (μg) | (μg) | | | (μg) | (μg) | | |
| Peanut butter | 38.75 | 29.75 | 76.8 | — | 59.6 | 44.0 | 73.8 | — |
| Cheddar cheese | | | | | | | | |
| Trial 1 | 16.70 | 10.00 | 59.9 | — | 22.0 | 12.4 | 56.5 | — |
| Trial 2 | 74.40 | 52.40 | 70.5 | — | 99.2 | 68.5 | 69.0 | — |
| Brick cheese ¹ | — | — | 70.7 | 78.1 | — | — | 74.0 | 78.3 |

¹Data on per cent recovered based on comparison of peak areas.

TABLE 4. RECOVERY OF AFLATOXINS FROM VARIOUS FOODS BY THE PROPOSED TERNARY SOLVENT PROCEDURE.

| Food | Trial | Aflatoxin B ₁ | | | Aflatoxin B ₂ | Aflatoxin G ₁ | | | Aflatoxin G ₂ |
|----------------------------|-------|--------------------------|-------------------|--------------------|--------------------------|--------------------------|-------------------|--------------------|--------------------------|
| | | Added | Recovered | Per cent recovered | Per cent recovered | Added | Recovered | Per cent recovered | Per cent recovered |
| | | (μg) | (μg) | | | (μg) | (μg) | | |
| Rice | 1 | 38.75 | 35.50 | 91.8 | — ¹ | 59.60 | 60.00 | ≥ 100 | — |
| | 2 | 38.75 | 36.00 | 92.9 | — | 59.60 | 57.25 | 96.2 | — |
| | 3 | 38.75 | 37.2 | 96.0 | — | 59.60 | 59.30 | 99.5 | — |
| | 4 | 16.70 | 16.4 | 98.3 | — | 22.00 | 21.70 | 98.5 | — |
| Peanut butter ² | 1 | 37.20 | 35.30 | 95.0 | — | 53.80 | 48.50 | 90.4 | — |
| | 2 | 37.20 | 35.60 | 95.7 | — | 53.80 | 51.0 | 94.9 | — |
| Cheddar cheese | 1 | 37.20 | 35.0 | 94.1 | — | 53.80 | 49.50 | 92.1 | — |
| | 2 | 16.70 | 15.5 | 92.9 | — | 22.0 | 21.60 | 98.1 | — |
| Brick cheese ³ | 1 | — | — | ≥ 100 | ≥ 100 | — | — | 95.5 | ≥ 100 |
| | 2 | — | — | ≥ 100 | 95.0 | — | — | 90.0 | ≥ 100 |
| | 3 | — | — | 91.0 | 91.4 | — | — | 93.5 | 91.9 |
| Corn meal | | 16.7 | 17.5 | ≥ 100 | — | 22.0 | 22.5 | ≥ 100 | — |

¹— = No data

²50 g sample used

³Data on per cent recovered based on comparison of peak areas

a chloroform layer with minimal impurities, the composition of the ternary solvent system must be one located below the tie-line (Fig. 1). Reducing the quantity of impurities present at this point facilitates purification of the extract so that the relatively simple and rapid cleanup procedure just described can be used successfully.

Quantitative TLC analysis

Methods suggested by Shih and Marth (18) were used to measure aflatoxins. Thin-layer chromatographic plates were prepared with Adsorbosil-5 (Applied Science Laboratories, State College, Pa.), developed with water: methanol: chloroform (1:1:98 v/v), and scanned with a fluorometer (Model 111, G. K. Turner Associates, Palo Alto, Calif.) equipped with a thin layer plate scanner and recorder (model H, Leeds and Northrup). Some results are based on comparisons between amounts of aflatoxins added and amounts recovered and others by comparing peak areas calculated as suggested by Burriel-Marti et al. (6).

RESULTS AND DISCUSSION

Conditions for optimum extraction of aflatoxins

To establish conditions for optimum extraction of aflatoxin from cheese, experiments were conducted

in which the initial and/or final composition of solvent systems (mixtures of chloroform, methanol, and water) were selected as marked in Fig. 1 and listed in Table 1. When these systems were used to recover aflatoxins B₁ and G₁ from cheese, results shown in Table 2 were obtained. It is apparent that solvent mixtures B, C, and D provided most satisfactory recovery of aflatoxin. These results suggest that any solvent system with a composition near those of mixtures B, C, and D (see Fig. 1 and Table 1) would satisfactorily recover aflatoxin. Since mixture B provided greater recovery, use of that solvent system is preferred. The lower recovery obtained with solvent systems E and F is attributable to their biphasic rather than monophasic character when the extraction was begun.

It was concluded from these observations that optimal extraction of aflatoxin results when: (a) a solid or semi-solid food is blended with a mixture of chloroform, methanol, and water (moisture in the

sample provides some of the water for the solvent system) to form a miscible solvent mixture which efficiently extracts aflatoxin, (b) chloroform and water are added so the solvent separates into two layers with aflatoxin in the chloroform layer, and (c) the sample residue is re-extracted with a second portion of chloroform to recover traces of aflatoxin which might be present after the initial extraction.

The efficiency of extracting aflatoxin from the methanol-water layer by chloroform also was studied since this is an important step in both the extraction and purification procedures. Results indicated that total transfer of aflatoxin could be accomplished by two extractions with chloroform and are in agreement with findings of Walkling et al. (20). Although there was no detectable aflatoxin in the third chloroform extract, three extractions are recommended to preclude loss of toxin.

Recovery of aflatoxins with the ternary solvent system just discussed and with a binary solvent system made up of methanol and water was compared. The sample was extracted with a mixture of 200 ml methanol and 180 ml water and the residue was extracted a second time with 100 ml of the 50:45 (v/v) mixture of methanol:water. The two extracts were combined, were extracted with chloroform, and then were handled as were other samples when the ternary solvent was used. Results in Tables 3 and 4 show that less aflatoxin was recovered by the binary than by the ternary solvent. Chloroform appears to be an important constituent of the solvent system used for the initial extraction. When residues from samples extracted with the binary recovered. Thus the binary solvent system failed to efficiently extract aflatoxin from the products that were tested.

Extract purification

When samples contained substantial amounts of lipid and/or pigment, the initial chloroform concentrate was purified by extraction with methanol, water, and hexane (50:45:120 v/v/v) added in the sequence listed. The concentrate is likely to contain traces of water in the residual chloroform. Methanol is added first so that an excess of this chemical combined with adequate mixing yields a solvent mixture at this stage which is in the monophasic area of the phase diagram in Fig. 1. The uniform monolayer thus obtained makes possible the nearly complete transfer of aflatoxin into the methanol-water fraction after water is added so that loss of aflatoxin is avoided later when hexane is used to remove the interfering substances.

The proportion of methanol and water (50:45 v/v) used in the initial extraction was maintained in the

purification procedure. When hexane was added to the methanol-water mixture, a biphasic system resulted and the hexane layer (which contained the impurities) was easily separated. Efficiency of aflatoxin recovery in the purification step was checked and found to be 95-100%. Extraction of aflatoxin from the hexane fraction does not appear to be necessary. The purification procedure can be eliminated when the sample contains little or no lipid or pigment. Extraction and purification procedures just described can recover aflatoxins from cheese much more rapidly than the procedure described recently by Kiermeier and Groll (10).

Application of method to other substrates

The procedure just described was tested for its ability to recover aflatoxin from substrates other than cheese. Results in Table 4 indicate that satisfactory recovery of aflatoxin was obtained when rice, peanut butter, and corn meal served as substrates instead of brick and Cheddar cheese. This suggests that the procedure is suitable for recovery of aflatoxins from a variety of foods provided that chloroform, methanol, and water are kept in proportions of 25:50:20, v/v/v, before and in proportions of 50:50:45, v/v/v, after dilution.

The method also was used to recover aflatoxin from a liquid culture medium. Results in Table 5 indicate that the suggested procedure recovered 92% or more of the aflatoxins added to the liquid medium. Two other procedures, one which used the same solvent system as in the method for cheese but without passing from the monophasic to the biphasic condition and the other chloroform only, also satis-

TABLE 5. RECOVERY OF AFLATOXINS FROM A LIQUID MEDIUM¹ BY THREE DIFFERENT PROCEDURES.

| Procedure | Trial | Per cent of aflatoxins recovered | | | |
|----------------|-------|----------------------------------|----------------|----------------|----------------|
| | | B ₁ | B ₂ | G ₁ | G ₂ |
| A ² | 1 | 99.5 | 95.7 | ≥ 100 | ≥ 100 |
| | 2 | 96.7 | 95.0 | 95.9 | 98.4 |
| | 3 | ≥ 100 | ≥ 100 | ≥ 100 | ≥ 100 |
| | 4 | 96.5 | — ⁵ | 92.0 | — |
| B ³ | 1 | 96.6 | — | 95.3 | — |
| | 2 | 96.5 | — | 93.6 | — |
| C ⁴ | 1 | ≥ 100 | — | 98.1 | — |
| | 2 | 99.0 | ≥ 100 | ≥ 100 | ≥ 100 |
| | 3 | ≥ 100 | 94.5 | 93.2 | 93.0 |
| | 4 | ≥ 100 | 94.0 | 95.5 | 96.0 |
| | 5 | 93.6 | 95.9 | 92.8 | ≥ 100 |

¹Composition: 2% yeast extract, 20% sucrose, and water.

²Proposed procedure for cheese.

³Final composition of solvent A, without use of monophasic system.

⁴Extracted three times with chloroform.

⁵— = No data.

factorily recovered aflatoxins from this substrate. It is apparent that simple solvent systems can be used to recover aflatoxins from a liquid substrate which contains few if any interfering substances and in which aflatoxins are not in close association with solid particles.

ACKNOWLEDGMENT

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GROWTH AND ACTIVITY OF LACTIC-ACID BACTERIA IN SOYMILK

IV. PROTEOLYTIC ACTIVITY¹

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ABSTRACT

Three methods were examined for their suitability to measure proteolysis brought about by microbial activity in soymilk. The Anson (or Hull) method, a colorimetric measurement of tyrosine and tryptophan, was not able to measure changes detectable by other methods and hence did not appear applicable for this work. Determination of non-protein nitrogen (NPN) to follow proteolytic changes in soymilk was feasible but was time- and labor-consuming. Dye-binding methods were found most workable.

Lactobacillus delbrueckii and *Streptococcus thermophilus* exhibited proteolytic activity in soymilk but *Lactobacillus casei* did not. Two other microorganisms, *Bacillus cereus* and *Micrococcus conglomeratus*, and rennet were comparatively more proteolytic in soymilk than the lactic cultures.

In previous papers Angeles and Marth (1, 2, 3) reported that many lactic acid bacteria grew well in soymilk and that some were able to produce acid and hydrolyze soybean fat.

Proteolytic activity of lactic acid bacteria has been reported by numerous workers (10, 14, 18, 27, 29, 34, 36, 37). Such activity in milk is thought to affect curd strength (25) and speed of coagulation (41) and in cheese is associated with development of desirable (20, 32, 33) and undesirable (15, 16, 19) flavors. If soymilk is to serve as a substrate for production of cheese-like products, undoubtedly some proteolysis is needed for the cheese to be flavorful. Information on the ability of lactic acid bacteria to degrade soy protein is lacking. Consequently investigations were initiated to determine (a) which method is suitable to detect proteolysis in soy protein and (b) if certain lactic acid bacteria can degrade this substrate. Results of the studies are reported in this paper.

Mc, *Lactobacillus delbrueckii* Ld₃, and *Lactobacillus casei* 1445. As a basis for comparison and as a check for work-

ability of methods, two known proteolytic cultures, *Bacillus cereus* and *Micrococcus conglomeratus*, and rennet were tested along with the lactic cultures.

All cultures were transferred to soymilk daily for at least 3 days before being used experimentally. A 24-hr-old culture or a 1:20 dilution of commercial rennet extract (Miles Laboratories, Elkhart, Ind.) was used as inoculum.

Substrate, sampling, and duration of experiment

Sterilized soymilk prepared as described previously (1) was used as the substrate in these experiments. The medium was inoculated with 1% of the test culture or rennet solution. After thorough mixing, 1 ml of this inoculated medium was distributed into each of a sufficient number of sterile screw-cap tubes to allow for three tests (described below) with duplication. Samples were incubated at the optimum temperature for the culture; samples with rennet were incubated at 30 C. Proteolytic activity was followed over a 14-day period.

Methods for measuring proteolytic activity

Several procedures were tried and evaluated for their possible application to soymilk.

Modified Anson procedure. The procedure of Anson (4) as modified by Hull (hence, it is often referred to as the Hull method) (26), is widely used for measuring hydrolysis of milk protein. This method was further modified for use with soymilk. A concentration of 0.8 N trichloroacetic acid (TCA) was used instead of 0.3 N (4) or 0.72 N (26), in accordance with the suggestion of Becker et al. (9). The amount of sample was reduced from 5 to 1 ml to permit work within the range of the standards used.

To 1 ml of inoculated soymilk was added 5 ml water, followed by 10 ml of 0.8 N TCA. The mixture was shaken on a Vortex mixer, allowed to stand for 10 min, and then filtered through S & S No. 595 filter paper. Ten milliliters of a 15% (w/v) sodium carbonate reagent was added to 5 ml of the TCA filtrate. The mixture was thoroughly mixed before 3 ml of a 1:3 dilution of Folin-Ciocalteu reagent (Anderson Laboratories, Fort Worth, Texas) was added. The mixture was again thoroughly mixed and then allowed to stand 5 min for color development. The blue color was measured in a Bausch and Lomb Spectronic 20 at 650 m μ with the instrument set at 100% transmittance using a reagent blank.

A standard curve showing per cent light transmittance for various concentrations of tyrosine was prepared as described by Hull (26). This was used to convert sample readings into their tyrosine equivalent (μ g tyrosine per 5 ml filtrate).

Measurement of nonprotein nitrogen (NPN). The method of Becker et al. (9) for determination of NPN in soybean meal was used with slight modifications. Ten milliliters of 0.8 N (13.6% w/v) TCA was added to a 1 ml soymilk sample. The mixture was agitated for 30 min in a mechanical shaker and

MATERIALS AND METHODS

Cultures and rennet

The following lactic acid bacteria were tested for their proteolytic activity in soymilk: *Streptococcus thermophilus*

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TABLE I. CHANGES IN THE PROTEIN OF SOYMILK INOCULATED WITH MICROORGANISMS AND RENNET AS MEASURED BY THE MODIFIED ANSON (OR HULL) PROCEDURE. INCREASE IN RELEASED TYROSINE IN THE MEDIUM WITH INCUBATION TIME.

| Days | <i>S. thermophilus</i> | | <i>L. delbrueckii</i> | | <i>L. casei</i> | | <i>B. cereus</i> | | <i>M. conglomeratus</i> | | Rennet | |
|------|---------------------------------|---------|-----------------------|---------|-----------------|---------|------------------|---------|-------------------------|---------|---------|---------|
| | Trial 1 | Trial 2 | Trial 1 | Trial 2 | Trial 1 | Trial 2 | Trial 1 | Trial 2 | Trial 1 | Trial 2 | Trial 1 | Trial 2 |
| | (μg tyrosine/5 ml TCA filtrate) | | | | | | | | | | | |
| 0 | 33 | 27 | 33 | 37 | 33 | 27 | 34 | 31 | 35 | 32 | 33 | 25 |
| 1 | 33 | 27 | 33 | 27 | 33 | 27 | 63 | 67 | 35 | 32 | 33 | 25 |
| 2 | 33 | 27 | 33 | 27 | 33 | 27 | 63 | 67 | 35 | 32 | 33 | 25 |
| 3 | 33 | 27 | 33 | 27 | 33 | 27 | 63 | 67 | 35 | 32 | 33 | 25 |
| 4 | 33 | 27 | 33 | 27 | 33 | 27 | 63 | 67 | 35 | 32 | 33 | 25 |
| 5 | 33 | 27 | 33 | 27 | 33 | 27 | 63 | 67 | 35 | 32 | 33 | 25 |
| 6 | 33 | 27 | 33 | 27 | 33 | 27 | 63 | 67 | 35 | 32 | 33 | 25 |
| 7 | 33 | 27 | 33 | 27 | 33 | 27 | 63 | 67 | 43 | 40 | 33 | 25 |
| 11 | 33 | 27 | 38 | 29 | 33 | 27 | 63 | 73 | 46 | 42 | 33 | 25 |
| 14 | 33 | 27 | 38 | 29 | 33 | 27 | 70 | 73 | 46 | 42 | 50 | 44 |

then filtered through S & S No. 595 filter paper. Five milliliters of the filtrate was used for nitrogen determination by a semimicro Kjeldahl method (40).

Dye-binding methods with Orange G. Two variations of the dye-binding method were used. The first was an adaptation of the procedure of Dolby (17) for estimating protein in milk. The second was the method described by Hammond et al. (23) for determining proteolysis in milk. Both tests follow the same steps up to a certain point and hence the tests were conducted on the same samples.

Certified Orange G (National Aniline Division, Allied Chemical, N.Y.) was dried to constant weight at 110 C. A solution containing 1.00 mg of the dye per milliliter was prepared. One gram of the dye, corrected for its assay was accurately weighed, and together with 21 g citric acid and 2.5 ml of a 10% thymol solution in alcohol (used as preservative), was dissolved and made up to 1 liter with water. This dye solution had a pH value of 2.0 ± 0.2 .

Twenty milliliters of the dye solution were added to 1 ml of sample. The mixture was shaken well and allowed to stand overnight at room temperature. It was then filtered through S & S No. 595 filter paper. A blank consisting of 1 ml water and 20 ml dye solution was treated in the same manner.

The absorbance of a 1:50 dilution of the filtrates was measured in a Spectronic 20, using 0.5-inch test tube type cells.

When the Dolby method was used, the spectrophotometer was set to zero with water. The difference in absorbance between blank (representing total dye in solution) and sample (representing unbound dye left in solution) was recorded. This value is equivalent to the dye bound by proteins in the mixture. Its corresponding protein content is read from a standard curve.

The standard curve is a plot of the difference in absorbance between the blank and samples ($A_B - A_S$) containing various amounts of protein against the total protein (total N x 5.71) in the sample determined by semimicro Kjeldahl method. The curve was prepared by measuring dye bound by 1 ml of soymilk and fractions thereof made up to 1 ml with water, and plotting the values obtained for ($A_B - A_S$) against Kjeldahl protein in milligrams. The Kjeldahl protein of smaller volumes of soymilk may be calculated from one known Kjeldahl value, e.g., milligrams protein in 1 ml soymilk.

When the method of Hammond et al. (23) was used, the spectrophotometer was set to read zero with a 1:1 dilution of the dye solution (further diluted 1:50 before measuring absorbance). Absorbance values of the blank, and of fil-

trates from unproteolyzed soymilk and incubated samples were determined after proper dilution (1:50 with water). A proteolysis index was calculated from the absorbances:

$$\text{Proteolysis index} = \frac{A_S - A_0}{A_B - A_0}$$

where A_S is the absorbance of the incubated sample, A_0 that of the unproteolyzed control soymilk, and A_B that of the blank.

RESULTS AND DISCUSSION

Suitability of methods

Three methods were tried for measuring proteolytic activity in soymilk. Each is based on fractionation of the proteinaceous substrate and analysis of fractions which remain sufficiently constant in characteristics to validate conclusions from comparative results. However, sensitivities of the methods cannot be directly compared because each is based on determination of a different product.

Separation of a reaction mixture into protein and nonprotein fractions by precipitating the former with an agent such as TCA and measuring specific substances in the non-protein fraction is the basis of the first two methods used in this study. Anson's method is a colorimetric measurement for tyrosine and tryptophan and is regarded as quite sensitive for detecting small degrees of protein hydrolysis in cow's milk. The second method measures total nitrogen in the TCA filtrate. The NPN thus determined consists of free amino acids, amides, polypeptides, and possibly peptones, organic nitrogen bases, and other organic and inorganic nitrogen compounds of small molecular weight. These substances are all characterized by their ability to diffuse through semi-permeable membranes (9).

Data in Table I show that the modified Anson method is not suitable for use to study limited proteolysis in soymilk. The method did not detect small gradual changes in the medium, even when highly proteolytic cultures were used. Poor performance

of this method may be related to utilization or degradation of tyrosine or tryptophan by bacteria, before the compounds can be detected. Presence of insufficient amounts of these amino acids in soymilk can be ruled out as a problem because (a) results with *B. cereus* showed that twice as much tyrosine as was initially present was detectable after incubation; and (b) data reported by Hackler et al. (21) on

TABLE 2. CHANGES IN NONPROTEIN NITROGEN (NPN) OF SOYMILK INOCULATED WITH MICROORGANISMS AND RENNET.

| Days | <i>S. thermophilus</i> <i>L. delbrueckii</i> <i>B. cereus</i> <i>M. conglomeratus</i> Rennet | | | | |
|------|--|------|------|------|------|
| | (mg NPN/5 ml TCA filtrate) ¹ | | | | |
| 0 | 0.17 | 0.17 | 0.24 | 0.21 | 0.17 |
| 1 | 0.17 | 0.26 | 1.07 | 0.23 | ND |
| 3 | 0.18 | 0.33 | 1.36 | 0.34 | 0.19 |
| 7 | ND ² | 0.39 | 1.43 | 0.48 | 0.19 |
| 9 | 0.20 | ND | 1.52 | ND | ND |
| 11 | ND | 0.40 | ND | 0.51 | ND |
| 14 | 0.20 | 0.43 | 1.83 | 0.60 | 0.38 |

¹Values represent average of duplicate determinations from 2 trials.

²Not determined.

the amino acid content of soymilk.

The NPN procedure performed better than the first method (Table 2). It was able to detect small changes in nitrogen in the TCA filtrate. For the most part, however, this method and the first one did not give the information desired.

The third procedure and the one found most applicable for use with soymilk in terms of practicality and ability to detect proteolytic changes, was the dye-binding method. The basic principle of dye-binding methods for protein determination (5, 7, 17, 28, 38) is the formation of an insoluble complex between an acid dye and the protein in a solution at pH 2.0 ± 0.2. Enough dye must be present so that the excess can be measured by spectrophotometry. Protein content may be estimated from (a) the dye bound by the protein or (b) the concentration of unbound dye. The former is the basis for the Dolby (17) method and the latter, for the method of Hammond et al. (23).

Dye-binding methods may justifiably be used to measure proteolytic changes. The NPN of milk does not bind with Orange G, perhaps because reaction products are completely soluble and absorb light at the same wavelength as the free dye (5, 6). Furthermore, dye adsorption may be decreased not only by complete hydrolysis but also by the hydrolysis of one or more peptide bonds (23).

As shown in Table 3, the dye-binding method of Dolby was able to measure consistent gradual decreases in protein content with incubation time. The

changes measured by this method paralleled those detected by the NPN method. A comparison of the degree of proteolysis determined by the two methods is not valid since one method measures protein content and the other nitrogen content.

Dolby's method was compared to that of Hammond et al. and data are shown in Table 4. Degree of proteolysis measured by the former was consistently higher than by the latter. However, results of both methods were consistently similar and parallel, indicating that both measured the same changes.

The dye-binding method for measuring proteolysis offers many advantages over the other two methods used. Unlike the other methods which require more reagents and manual work, it is rapid, simple, and easy to carry out. Thus, the dye-binding procedure is less prone to systematic and experimental errors.

Proteolysis by lactic cultures in soymilk

The proteolytic activity of *S. thermophilus* and *L. delbrueckii* in soymilk was demonstrated using the NPN and dye-binding methods. *Lactobacillus delbrueckii* was more proteolytic than *S. thermophilus*. Both were, however, only weakly proteolytic when compared to *B. cereus* and *M. conglomeratus* (Tables 2, 3, 4).

Lactobacillus casei did not exhibit any proteolytic activity in soymilk under these test conditions. In view of the many available reports (8, 11, 12, 13, 30) regarding its proteolytic nature, this result was surprising. It is possible that the conditions, e.g., time of incubation, pH, temperature, prevailing in the experiments were unfavorable for proteolytic activity by this organism in soymilk. Some components of the proteolytic enzyme systems of *L. casei* are reportedly active at pH 5.0-6.5 (8, 35, 39). If these were operative, some activity, at least during the first 6 days of incubation when pH of the growth medium was in this range, should have been manifested. However, no such activity was observed.

The optimum pH for activity of most proteinases investigated by other workers was in the range of 6 to 8.5. Only slight or no activity was exhibited at pH 5.0. In the present study, pH was not controlled. The reaction of the substrate was well below the reported optimum for proteolysis after 24 hr incubation for *S. thermophilus* and *L. delbrueckii*. Yet, some degree of proteolytic activity was detected and it increased progressively over the 14-day-test period.

The most pronounced change produced in soymilk by *S. thermophilus* and *L. delbrueckii* is acid development. Accordingly, the possible effect of acid in decomposing protein raises a question about results obtained in this study. Hammer and Patil (22) studied

TABLE 3. CHANGES IN THE PROTEIN OF SOYMILK INOCULATED WITH MICROORGANISMS AND RENNET, AS MEASURED BY THE DYE-BINDING METHOD OF DOLBY.

| Days | <i>S. thermophilus</i> | | <i>L. delbrueckii</i> | | <i>L. casei</i> | | <i>B. cereus</i> | | <i>M. conglomeratus</i> | | Rennet | |
|------|------------------------------|---------|-----------------------|---------|-----------------|---------|------------------|---------|-------------------------|---------|---------|---------|
| | Trial 1 | Trial 2 | Trial 1 | Trial 2 | Trial 1 | Trial 2 | Trial 1 | Trial 2 | Trial 1 | Trial 2 | Trial 1 | Trial 2 |
| | (mg protein/ml) ¹ | | | | | | | | | | | |
| 0 | 35.8 | 37.0 | 36.8 | 37.0 | 35.8 | 37.0 | 35.8 | 37.0 | 35.8 | 37.0 | 35.8 | 37.0 |
| 1 | 34.8 | 35.3 | 34.8 | 35.0 | 35.8 | 37.0 | 25.0 | 25.7 | 34.8 | 33.3 | 35.8 | 37.0 |
| 3 | ND ² | 34.3 | 34.3 | 34.3 | 35.8 | 37.0 | 18.0 | 20.0 | 32.3 | 33.3 | ND | 37.0 |
| 4 | ND | ND | ND | ND | ND | ND | ND | ND | 31.8 | ND | 35.8 | ND |
| 7 | ND | 33.3 | ND | 32.3 | 35.8 | 37.0 | 15.3 | 17.0 | 29.8 | 29.5 | 33.8 | 32.3 |
| 9 | 33.8 | ND | 32.8 | ND | ND | 37.0 | ND | ND | ND | ND | 30.8 | ND |
| 10 | 32.8 | ND | ND | ND | 35.8 | ND | ND | 13.5 | ND | ND | ND | ND |
| 11 | ND | ND | 28.0 | 28.8 | ND | ND | 12.3 | ND | 28.0 | 24.7 | ND | ND |
| 14 | 31.8 | 32.5 | 28.0 | 28.8 | 35.8 | 37.0 | 7.5 | 7.5 | 25.0 | 18.0 | 28.8 | 24.7 |

¹Values were obtained by measuring the dye bound by the protein in a sample and converting this to the corresponding protein content from a standard curve.

²Not determined.

TABLE 4. COMPARISON BETWEEN THE DYE-BINDING METHODS OF DOLBY AND HAMMOND ET AL. FOR MEASURING PROTEOLYSIS IN SOYMILK. PERCENTAGE OF PROTEOLYSIS MEASURED WITH TIME.

| Days | <i>S. thermophilus</i> | | <i>L. delbrueckii</i> | | <i>B. cereus</i> | | <i>M. conglomeratus</i> | | Rennet | |
|------|------------------------|----------------|-----------------------|------|------------------|----|-------------------------|----|--------|----|
| | D ¹ | H ² | D | H | D | H | D | H | D | H |
| | (% Proteolysis) | | | | | | | | | |
| 1 | 2.6 | 0 | 5.4 | 4 | 30.2 | 25 | 2.6 | 3 | 0 | 0 |
| 3 | ND ³ | 1 | 6.8 | 6 | 49.7 | 41 | 9.8 | 4 | ND | 0 |
| 4 | ND | 2 | ND | ND | ND | ND | 11.2 | 8 | 0 | ND |
| 7 | ND | ND | ND | ND | 57.0 | 44 | 16.8 | 10 | 5.6 | 2 |
| 9 | 5.6 | 5 | 10.9 | 7 | ND | ND | ND | 14 | 14.0 | 11 |
| 10 | 8.4 | 8 | ND | ND | ND | 51 | ND | ND | ND | ND |
| 11 | ND | ND | 23.9 | ND | 66.0 | ND | 21.8 | 17 | ND | ND |
| 14 | 11.2 | 12 | 23.9 | 17.0 | 76.0 | 61 | 30.2 | 26 | 19.5 | 14 |

¹Dolby's method. Per cent proteolysis is equal to initial protein minus remaining protein after incubation divided by initial protein x 100.

²Method of Hammond et al. Proteolysis index x 100.

³ND = Not determined.

the effect of lactic acid on proteins of cow's milk. They found that up to 2% of the acid had a negligible effect on milk proteins. *Streptococcus thermophilus* produced more acid in soymilk than *L. delbrueckii* (1) yet it did not exhibit more protein breakdown. This is one indication that acid production by these cultures in soymilk is not responsible for observed proteolysis.

Hang and Jackson (24) used a strain of *S. thermophilus* for preparation of soybean cheese. In contrast to the results reported in this study, they did not observe any proteolytic activity by this organism during a ripening period of 56 days. It appeared to them that the main function of the bacterium was acid production.

Hang and Jackson (24) also observed that inclusion of rennet in soybean cheese-making improved the flavor of the product. They believed that this resulted, in part, from the action of rennet on soybean proteins. The present study has confirmed that rennet has proteolytic activity on soybean protein but

that it cannot cause coagulation of soymilk (24, 31, 41) at the concentration tested.

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MANURE HANDLING SYSTEMS AND ENVIRONMENTAL CONTROL FOR CONFINED DAIRY HOUSING¹

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ABSTRACT

There is no single best manure handling system. Odor and appropriate time of disposal are two of the most difficult waste problems facing dairymen. Systems may range from a gutter cleaner and daily hauling with a manure spreader to extended storage in concrete tanks whose contents are pumped and spread periodically. Daily hauling requires the lowest investment in equipment but has the disadvantage of possible higher labor costs and the hazard of encountering unfavorable weather, soil, or crop conditions. Slat floors in warm free-stall barns, or gutters with grated bottoms in conventional stall barns, both with under-the-building manure storage, offer suitable systems for manure handling with a minimum of labor. Carefully planned ventilation systems of high capacity must be provided for all confined units. Research shows that manure containing little bedding deposited in the end of a 30 x 90 x 8 ft external flat-bottom tank will distribute itself under its own weight. Waste heat from the dairy barn ventilation system is used to prevent freezing.

This paper will discuss the important subjects of dairy housing and manure handling systems. Both have changed markedly in the last 5 years. Unfortunately, dairymen in 1970 are governed by a U. S. Public Health Code that was last revised in 1965, before many of the current practices were in use. Most of the changes involve engineering, so it is essential that there be a mutual exchange of ideas between sanitarians and engineers in establishing uniform recommendations and realistic guidelines for the dairy producer to follow.

Handling and disposing of animal waste are two major problems facing the dairy industry. Unfortunately, there is no wholly satisfactory solution to either. Odors and appropriate time for disposal usually are two of the most difficult problems with which to deal.

Prior to World War II, removing manure from the barn was mainly a hand operation in which all the manure and bedding had to be lifted to a wheelbarrow, stoneboat, litter carrier, or manure spreader. Following the War, the gutter cleaner was developed, eliminating the need to lift manure to remove it from the stall barn. Loose housing, where cows literally were bedded on a manure pile, was revived and became popular because of its somewhat misrepresented low cost. With this system, manure generally was

removed once a year, usually with a front end loader.

All manure handling practices were based on the liberal use of bedding to absorb liquid from the waste and to help keep animals clean. The high cost of bedding, the labor required to put it in place, and the cost of getting rid of it have brought about the use of animal mats, slatted floors, and other practices to reduce bedding needs. As the amount of bedding used decreases, the consistency of the manure changes. Devoid of bedding, manure and urine must be handled in some type of water-tight container. Concrete manure storage pits are now in common use. In new systems; the manure storage pit often is placed under the barn floor, allowing the use of slatted gutters or slatted floors, depending upon the housing system. This eliminates any pre-storage labor requirements because a collection system is not necessary.

Providing reinforced concrete manure storage pits, of course, adds significantly to the cost of a waste management system. Pit costs vary widely, but observation indicates that 40 cents per ft³ of storage capacity is a practical estimate. Two cubic feet of storage per cow per day are required. A reasonable cost estimate can be obtained by multiplying the number of cows times the desired storage period by 80 cents. Thus, 150 days' storage capacity for 100 cows might be expected to cost \$12,000.

This discussion will be limited to the two general classes of housing in the temperate zone with which I am familiar: the conventional stall barn and the free-stall barn.

FREE-STALL

Free-stall barns can be either warm (completely insulated and mechanically ventilated) or cold (open uninsulated buildings where natural air movement provides ventilation and the barn temperature approximates the outside temperature). In either type of free-stall system the costs of the milking parlor, milkhouse, and feed storage and handling systems are essentially the same. Cost variation stems from the housing structure and the manure handling system (3).

The cold free-stall housing unit is mainly a shell to keep rain and snow off the ground. In severe weather, freezing temperatures will affect manure

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handling practices. In such units, manure generally is scraped daily and hauled to the field, weather permitting. Deep snow, soft fields, or other conditions may prevent daily hauling and necessitate an attempt at stacking. There are obvious sanitary and pollutional implications to such a practice when no provision is made for containment. I do not know of a cold free-stall housing unit in Minnesota that has a liquid manure storage system. The lower initial cost of the cold free-stall housing structure must be equated primarily against the problems encountered in manure handling.

Ventilation of cold free-stall units can be satisfactorily accomplished by leaving a 6 inch or wider clear opening the full length of the building at the roof peak, in combination with sufficient wall openings.

Warm free-stall barns must be heavily insulated. A general recommendation is to provide 4 inches of insulation in the walls and 6 inches over a flat ceiling at about the 8 ft level. Windows are optional, but there is no reason to provide them. They are an unnecessary expense and serve no useful purpose, since artificial light and mechanical ventilation must be provided.

Slatted alleys with storage tanks beneath offer two important advantages over solid alleys. First, manure drops directly into the storage tanks, eliminating the need for alley scraping equipment and the labor to operate it. Second, there is no opportunity for manure to build up and the cows' feet remain comparatively clean. The amount of manure tracked into the milking parlor can be substantially reduced by having the cows step in directly from the slatted alleys.

An automatic system that scrapes the alleys at regular intervals and deposits the waste into storage tanks will keep solid alleys clean. However, such systems are not in common use.

Because of the cost involved, liquid manure storage capacity is usually limited to a few months. However, many dairymen feel that greater manure storage capacity provides additional flexibility in managing the manure. With increased attention being paid to the pollution caused by animal manure, long term storage is highly important. Initially, the goal of most dairymen was to get away from having to haul manure during stormy days in winter so they wouldn't have to start the tractor every day or fight a frozen manure spreader. Significant as these two points are, they are minor ones in the total picture of waste management.

STORAGE CAPACITY

In deciding how much storage capacity to provide, a dairyman must realistically assess how he is

going to dispose of or utilize the manure. Effects of cold weather, deep snow, soft fields in the spring, and fields planted to crops must be considered. Liquid manure systems with capacities from 30 days to even 120 days or more quickly demonstrate the desirability of long term storage.

Slat floor barns are now being built with manure storage under the entire housing area. Foundation walls can be extended to also serve as the walls of the manure tanks. The floor of the barn, supported on columns and beams, can then also serve as a cover for the tanks beneath. Such buildings 40 ft wide and with a pit 8 ft deep will provide manure storage for about 9 months.

It is essential in the design of slat floor dairy barns that alleys be kept to a width no greater than about 10 ft so there is sufficient animal traffic to quickly work manure through the slotted openings. Narrow alleys also are a factor in reducing the overall cost of the building.

STALL BARNs

For new stall dairy barns, it also is practical to build manure storage tanks directly beneath the building. By using a steel grate as a gutter bottom, manure will fall through into the tank. The cost of a gutter cleaner is thus eliminated and can be written off against that of a liquid manure tank and pump. As with the free-stall barn, the floor also acts as a tank cover. Suitable openings for agitating and removing the manure are necessary. Some type of animal mats can be provided and little or no bedding need be used. This management method may conflict with some regulations, printed or implied, that prohibit milking in the same enclosure in which manure is stored. But it is my opinion that such regulations should be modified. In a number of barns producing grade A milk in Minnesota, we have observed that there is no more odor in properly ventilated barns with this system than there is in barns where manure is removed daily.

Stall barns with grated gutters or slat floor free-stall barns, both with under the building manure storage, offer a suitable system for manure handling with minimum labor (6). Adequate tank capacity should be provided so that spreading can be done when the soil can accept and use the manure.

VENTILATION

Proper ventilation of any dairy barn is essential. In barns having beneath-the-floor manure storage, it is imperative. Observation and research indicate that a number of toxic gases are produced when stored manure is agitated, and these gases may create a

dangerous condition if suitable ventilation is not provided. Dale and Ogilvie state, "Actually the problem does not seem to us to be an insurmountable one or even a difficult one. It is more one of education of individuals to make them aware of the possible effects of such gases as H_2S , NH_3 , CO_2 , and CH_4 on livestock and man if proper precautions are not taken" (4).

Except for CH_4 and NH_3 , all these gases are heavier than air. It has been my observation that with a high exhaust rate from the barn, difficulty is not encountered. We recommend a total exhaust rate of 200 ft^3/min per 1,000 lb. of animal weight (2). All this capacity should be in operation when manure is being agitated or pumped. Animals should be out of the barn if possible. About one-half the total exhaust capacity or 100 ft^3/min per 1,000 lb. of animal weight should be exhausted from the manure pits. Of this, 25 ft^3/min should be continuous, with the remainder thermostatically controlled.

For example, in a slat floor barn housing 120 animal units, the total exhaust capacity should be $120 \times 200 = 24,000$ ft^3/min . Of this total, 12,000 ft^3/min should be exhausted from the manure pits. It is suggested that the 12,000 ft^3/min total be made up of three fans of 4,000 ft^3/min each. One of these fans must operate continuously. The other two should be controlled by thermostats set at 38 F. The remaining 12,000 ft^3/min should be exhausted from the walls. This capacity can be provided by two or three conveniently located fans. Thermostats controlling wall fans should be set at least 5° higher than those controlling the pit fans.

In a slat floor barn, air exhausted by the pit fans is drawn down through the slat openings. Research in Sweden indicates that low level exhaust is an important factor in maintaining herd health compared to only high level exhaust (1, 5).

Adequate fresh air to supply the needs of the exhaust system must be provided. This can be effectively and economically done through a slot inlet system extending the full length of both long walls of a barn approximately 40 ft wide. The slot inlet is an opening 1 inch wide between the ceiling and walls. Air is drawn into the barn through it by the exhaust fans. The amount of air that enters is determined by the amount of air exhausted.

Outside air to supply the slot inlet is admitted to the attic space in winter through large louvers built in the gable in each end of the building. In the attic the air has an opportunity to be partially warmed by barn heat lost through the ceiling and by solar heat during the day.

For summer ventilation, air is allowed to enter the

slot inlet directly from the outside through continuous doors built into boxed-in eaves. When these doors are opened, outside air is drawn directly in from the shaded area under the eaves and there is little opportunity for it to be warmed in the attic (2).

In existing barns, stall or free-stall, it is not economical to provide under-the-barn manure storage, so external tanks are necessary if a liquid manure system is to be used.

MANURE STORAGE RESEARCH

In 1968, a research project was initiated at the University of Minnesota Northwest Experiment Station at Crookston to study the behavior of dairy waste stored in an external tank over an extended period. A storage tank 30 ft wide, 90 ft long, and 8 ft deep was constructed at a right angle to the end of a modern, 50 cow, tie stall barn built in 1964. The gutter cleaner elevator was lowered and extended to the center line of the tank about 5 ft from the north end. All the waste is dropped into the tank at this point. There is no mechanical distribution system.

A post and beam system to support a permanent cover or a possible slat floor was built into the tank. This has been used to support an easily removable temporary cover made by stretching woven wire across the beams and adding a polyethylene sheet topped with baled straw. A duct was built over the gutter cleaner elevator, making a tight connection between the barn wall and the top of the manure storage tank. A continuous exhaust fan was placed in this duct to exhaust air from the barn through the gutter opening and discharge it into the tank. The waste heat from the barn prevented any freezing of the elevator. A discharge opening was placed in the south end of the tank to permit air to escape.

The tank was completed in mid-December, and was filled with 3 ft of water before being used. The water quickly turned to ice. Manure was first put into the tank on December 18, 1968. The manure and the waste heat from the barn thawed the ice at the drop end of the tank and, as the winter progressed, the manure worked under the ice and lifted it up. A covering of ice remained over much of the tank surface until after the temporary cover was removed on April 15. There was no mechanical agitation of the tank until April 18, 1969. By that time, the manure had built up at the conveyor end so that it no longer would level out to allow further dumping, but there was a little usable storage space at the south end of the tank. A tractor-powered manure pump was placed in the pit along one side and, after 2 hr of agitation, the contents of the tank was level. Observations indicated that complete agitation could be accomplished satisfactorily. The agitation leveled

the contents sufficiently to permit continued storage until May 1.

On May 1, the tank was agitated for 4 hr at the mid-point and emptying was begun. Sixty-four tank loads of 1,400 gal each were removed. During removal, the pump was moved toward the drop end, where the manure was packed solidly. About 3,000 gal of water were added and the packed manure was liquified with little difficulty. The tank was then about half empty. Since the tank cover had been removed, there was ample opportunity for observation, and the mixture in the tank appeared to be uniform.

The tank was further agitated and pumped in mid-July. There was no indication that the intermittent agitation had caused undue settling of heavy material or any difficulty in removal. The tank was completely emptied in mid-July. At this time, 1 ft of water was again added. The tank was again emptied on November 20. Then it was filled with 1 ft of water and the temporary cover was replaced for winter protection. There was no freezing in the tank during the 1969-70 winter. This probably resulted from earlier placement of the cover and installation of a second fan, thermostatically controlled, that discharged into the duct.

Before the liquid manure system was put into use, poured-in-place cow mats were installed to decrease the amount of bedding entering the manure pit. Prior to the installation of the mats, bedding was used at the rate of about 16 lb. per cow per day. Since installation of the mats, bedding has been reduced to about 2.9 lb. per day, which has resulted in a savings of about 12.3 tons per month. On a yearly basis at a cost of \$15 per ton, this amounts to \$2,214.

Savings in labor cost resulting from periodic hauling of the liquid manure compared to daily hauling are estimated to be \$300 per year.

From the experience gained to date, it is apparent that: (a) Manure stored in a large tank, approximately 150,000 gal of usable capacity, can be agitated and removed without difficulty under proper management. (b) Manure with little bedding deposited in one end of a long tank will distribute itself sufficiently under its own weight, so a conveyor is not necessary. We call this the ooze system (6). (c) A full tank of stored manure can be completely agitated, partially emptied, reagitated, and completely emptied without difficulty. (d) Waste heat from the dairy barn ventilation system is a valuable resource in preventing freezing in a covered external manure storage tank.

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FOAM TEMPERATURES DURING VAT PASTEURIZATION OF MILK PRODUCTS

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ABSTRACT

Heating rates of foam during vat pasteurization were investigated to determine whether shorter holding times and higher processing temperatures would be feasible from a public health standpoint. A rake of 19 thermocouples, spaced 0.5 inch apart, was installed vertically in a 300-gal vat pasteurizer and the rake was adjusted to measure the temperature of the heated airspace, foam, and liquid product. With 200 gal of ice cream mix (16% milk fat), an 11-inch foam was generated on the liquid surface. The minimum temperature in the ice cream mix foam was below pasteurization temperature for 27 min of the 30-min holding period despite satisfactory temperatures as indicated by the product and airspace thermometers. When a 7-inch foam was generated above 218 gal of chocolate milk, the minimum foam temperature was below pasteurization temperature for the first 14 min of the holding period. Attempts to generate foams on milk were unsuccessful.

Because of the slow heating rates of milk-product foams, holding times shorter than 30 min cannot ensure the inactivation of microbial pathogens in the foam, and, consequently, they are not recommended. Some foams should be held longer than 30 min to ensure pasteurization, and additional holding time needed may be computed from known values of foam thickness, airspace temperature, and heating rate of the liquid.

Because of a potential increase in processing capacity of vat pasteurizers, there has been some interest in holding times shorter than those currently recommended by the Public Health Service (8). Equivalent time-temperature standards can be calculated, but some uncertainty is associated with vat processes of less than 30 min because of the problem of heating foam and the subsequent lack of thermal destruction of pathogens that may be in the foam.

Milk foams are difficult to heat (9), and because of this, airspace heaters are used on vat pasteurizers. Even with airspace heating, foam can be a problem, and the problem would be more severe with reduced holding times because less time is available to heat foam.

Our objective was to obtain data on the heating rates of milk and milk product foams when heated in a commercial-scale vat pasteurizer, and to establish the feasibility of vat pasteurization processes with holding times of less than 30 min.

MATERIALS AND METHODS

Pasteurizer

A round processor (Cherry Burrell¹, Model WPB-capacity 300 gal) with airspace heating was used. Internal diameter and height were both 48 inches. The agitator shaft was mounted in a vertical position and could be rotated at 25 or 50 rpm. The vat was heated by steam and the condensate temperature controller was adjusted to 194 F.

Products

The ice cream mix was a commercial product with a milk fat content of 16% and had received no heat treatment, as indicated by a phosphatase test. The chocolate milk was made with 200 gal of raw milk (4.0% milk fat) and 18 gal of commercial chocolate syrup (Kalva Corporation, Waukegan, Ill.). The syrup was blended specifically for cold mixing prior to vat pasteurization and included sugar, cocoa, malt, stabilizer, and vanilla flavoring. The raw milk used in foam tests on whole milk had a milk fat content of 4.0%.

Test procedure

With cold product in the vat, a foam was generated on the liquid surface by pumping product from the vat, metering air into the suction side of the pump, and discharging the aerated product into the vat below the level of the liquid product. Foam thickness was determined by measuring the distance from the top rim of the vat to the top of the foam and then to the liquid product's surface. The vat lid was closed and steam turned on. Airspace steam was adjusted so that temperature of the airspace was maintained at least 5 F higher than product temperature during heating and holding. At the beginning and at the end of the holding period, the vat lid was opened for measurements of foam thickness; otherwise it was kept closed during processing. All products were processed at the pasteurization temperatures and holding times specified in the *Grade "A" Pasteurized Milk Ordinance* (8).

Analytical procedures

A rake of 19 thermocouples, spaced 0.5 inch apart, was installed in the vat and adjusted to measure temperatures of the heated airspace, foam, and liquid product. The thermocouples were fabricated from No. 20 (American Wire Gage) copper constantan wire (0.032-inch diameter) and were installed on the rake so that the temperature sensitive tip was at least 2 inches away from the supporting structure. Figure 1 depicts the rake inserted in an ice cream mix foam in the vat, with the top seven thermocouples in the

¹Mention of company or product names implies neither endorsement nor criticism by the Department of Health, Education, and Welfare.

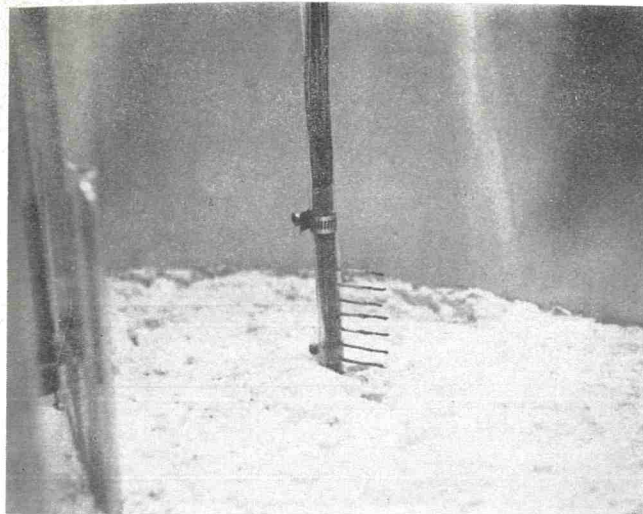


Figure 1. Rake of thermocouples positioned in an ice cream mix foam.

airspace above the foam. Temperatures were recorded with a 24-point strip chart recorder.

Foam densities were obtained from the weight of a known volume of foam. Milk fat determinations on raw milk, and phosphatase determinations on the ice cream mix were performed according to procedures prescribed in *Standard Methods for the Examination of Dairy Products* (6).

RESULTS

Not all pasteurization conditions present a foam problem. If the foam is formed after the start of holding, it consists of liquid product and air from the airspace, both of which have a temperature either at or above that required for pasteurization. Consequently, the temperature of such a foam would satisfy the temperature requirement for pasteurization. If the product is heated to pasteurization temperature in an auxiliary vat and pumped into the bottom port of the holder, any foam in the auxiliary tank would bubble up through the hot liquid, and since the foam would then be at liquid product temperature, there would be no foam heating problem. Conditions that would create a foam heating problem occur when milk is transferred from an auxiliary heater and discharged into the top port of the holder, or when the product is heated and pasteurized in the same vat and the foam is present before heating or is generated during the heating process. Our tests were based on foams generated before the start of heating.

Ice cream mix

Foams were readily produced on ice cream mix, and we selected an 11-inch foam as representative of the rarely occurring worst condition. With 200 gal of mix in the vat pasteurizer and the thermocouples in place, the lid was closed and the mix was heated to a temperature of 158 F. The slight increase in holding temperature was required to pre-

vent the temperature of liquid product from falling below 155 F during the holding period. Even though all public health requirements were met and the airspace and product thermometers indicated satisfactory temperatures, minimum foam temperature at the start of the holding period was 86 F and remained below pasteurization temperature for 27 min of the 30-min holding period (Fig. 2).

The lethal effect imposed on the foam was only 18% of a minimum pasteurization treatment of 155 F for 30 min.

When the vat lid was opened at the start of the holding period for a measurement of foam thickness, airspace temperature, as measured with a thermocouple, dropped from 170 F to 134 F. Conversely, the airspace thermometer did not respond to the lid opening; it indicated a temperature above 160 F while the lid was open.

Foam thickness and density decreased significantly during heating and holding. This resulted from drainage of liquid from the foam to the liquid product. Temperatures at successive thermocouple locations (0.5 inch apart) are shown in Fig. 3, where thermocouples 1 and 2 are in liquid product at the start of heating. At the end of the heating period (25 min later), thermocouples 1 through 5 are in liquid (as evidenced by a constant temperature), indicating a raising of liquid level caused by drainage from the foam. Moving vertically upward through the foam, isometric time lines show precipitous temperature drops to the point of minimum temperature

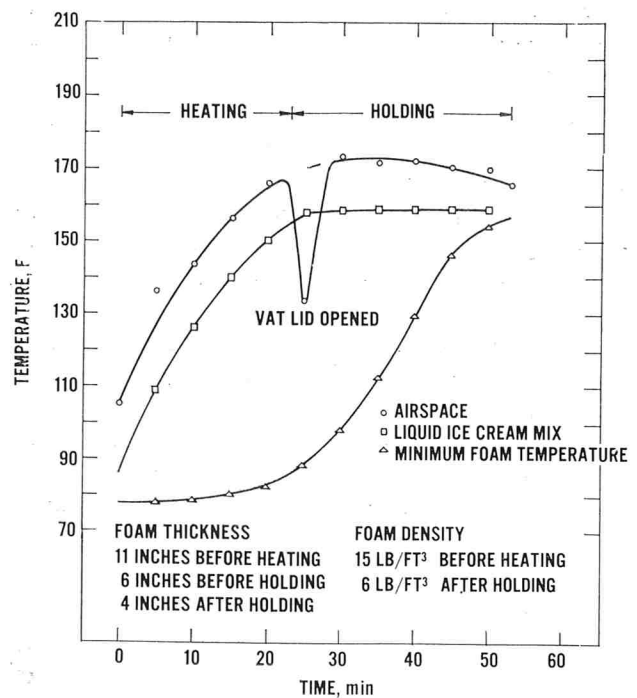


Figure 2. Minimum temperature in foam during vat pasteurization of ice cream mix—16% fat.

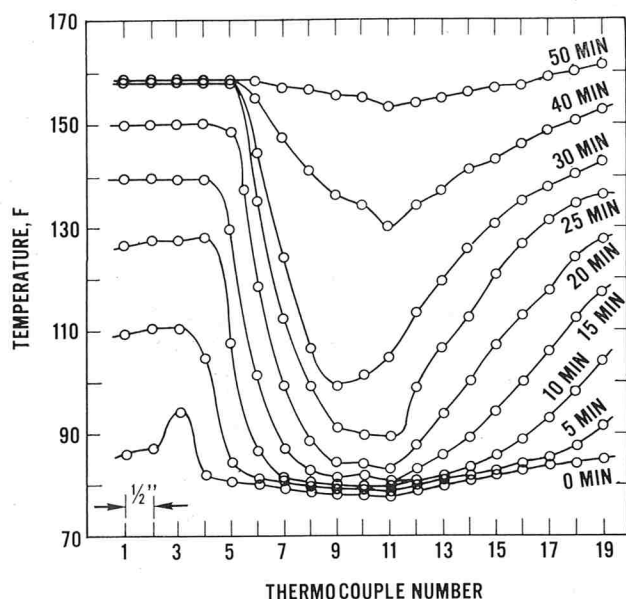


Figure 3. Temperature distributions in foam from ice cream mix-16% fat.

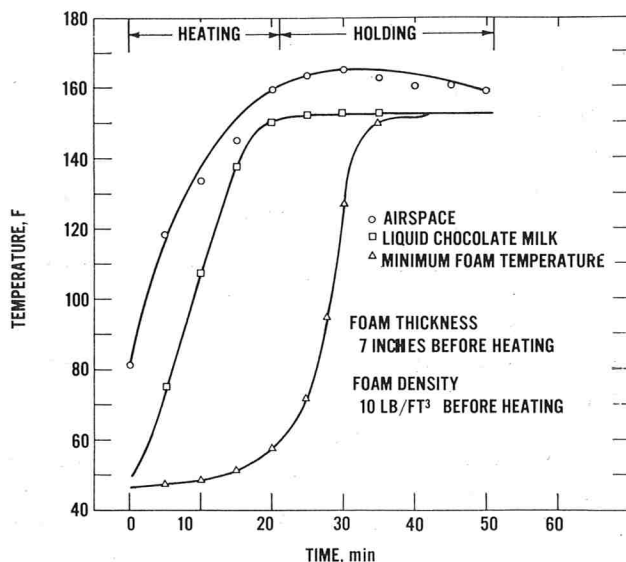


Figure 4. Minimum temperature in foam during vat pasteurization of chocolate milk.

followed by an increase to airspace temperature. The foam is heated by conduction from the hot liquid below and the hot airspace above.

Chocolate milk

Because of better mixing characteristics, chocolate syrup is usually blended with hot milk after it has been heated to pasteurization temperature; however, the worst foaming condition occurs when the syrup is blended with cold milk and is present before the start of heating. Our tests followed this procedure, and we used a commercial syrup developed specifically for cold mixing. Since the foam from chocolate

milk was neither as readily generated nor as stable as the ice cream mix foam, we selected a 7-inch foam as representative of the occasional severe condition. With 218 gal of chocolate milk in the vat, the 7-inch foam was generated on the surface of the liquid. Foam density (10 lb/ft^3) was lighter than that for ice cream mix. The product was heated to a temperature of 152 F (the pasteurization temperature is $145 \text{ F} + 5 \text{ F}$ for milk with added sweeteners) and held for 30 min. The vat lid was not opened at the start of the holding period because of the depression of airspace temperature encountered with ice cream mix.

Minimum foam temperature was below pasteurization temperature for the first 14 min of the holding period (Fig. 4). The precipitous increase in foam temperature that occurred 30 min after the start of heating suggested a loss of foam. This was verified by the absence of foam at the end of the holding period.

Whole milk

With 300 gal of whole milk in the pasteurizer, a 4-inch foam, similar to that reported by Whittaker et al. (9), was generated on the surface of the milk. The foam appeared to be not quite as stable as the chocolate milk foam, although foam density (16 lb/ft^3) was greater. For the first 9 min of heating, foam heating was delayed; minimum foam temperature was 27 F below liquid temperature after 9 min of heating. Ten minutes after the start of heating, foam temperature increased precipitously to liquid temperature, indicating a loss of foam. At the beginning of the holding period, the vat lid was opened and the loss of foam was verified.

Tests on pasteurized products

In the tests described above, steps were taken to facilitate development and retention of foam. We performed additional tests on the pasteurized ice cream mix wherein we purposely attempted to develop procedures that would be useful in reducing foam. The pasteurized product foamed just as well as the raw product, and the foams appeared to have about the same structure and stability. The previous tests on raw product were performed with an agitator speed of 25 rpm, and this yielded no noticeable wave action at the liquid surface. Conversely, an agitator speed of 50 rpm produced random waves about 2 inches high with no vortex at the agitator shaft (a vortex can lead to foam generation). Since this wave action would tend to disrupt the foam, we performed a test with an agitator speed of 50 rpm.

Using the once-pasteurized ice cream mix (200 gal), we generated a 5-inch foam over the entire surface of the vat. During heating, the higher agitator

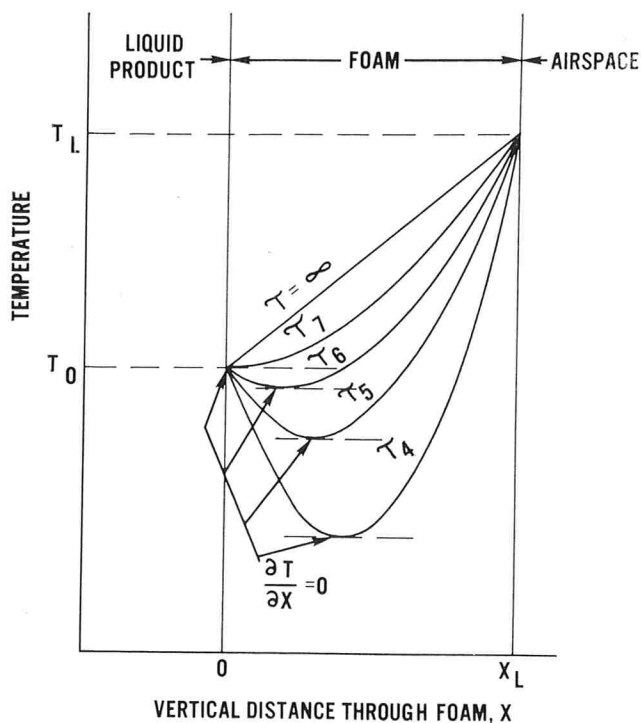


Figure 5. Theoretical temperature profiles through a foam of constant thermal diffusivity and thickness.

speed reduced foam thickness to about 2 inches. One-fourth of the liquid surface was free of foam at the end of heating. In a third test on the same 200 gal of ice cream mix and at an agitator speed of 25 rpm (slow speed), an airspace temperature of 190 F completely eliminated a 5-inch foam during heating. The airspace temperature was held at 190 F during the entire heating period.

DISCUSSION

Because of a lack of published data for ice cream mix foams, we could not make a direct comparison with previous works. Whittaker et al. (9) worked with milk foams; however, we were not able to generate a milk foam stable enough to survive the heating process with airspace heating. The work of Whittaker et al. was performed without airspace heating and was the basis for recommending airspace heaters for vat pasteurizers. They described dense foams (6.2 lb/ft³) as a "heavy soggy mass" and this is an accurate description of the foams we observed. The air was evenly distributed throughout the foam, and the air bubbles were so small that detection with the unaided eye was difficult.

Because of the slow heating rates of milk product foams, holding times of less than 30 min are not recommended. Even with a 30-min holding period, foams should be minimized by making certain that all connecting lines are tight when product is transferred to the vat. We tried several methods of gen-

erating foam, but the only successful method was that of metering air into the suction side of a transfer pump. Because of a loose fitting or a broken gasket, this metering of air is the most probable cause of foams in commercial practice.

When a foam exists prior to heating, it may be reduced in size by maintaining the airspace at a temperature of 190 F from the start of heating. Because of heating from 50 F to 190 F, the air bubbles undergo a volume increase of about 30%, and this tends to break up the foam. Agitation and baffle plates produce a wave action at the liquid surface, and this accelerates foam collapse (provided the agitation is not so severe that it produces a vortex that would pull air into the liquid and create more foam). If the foam persists into the holding period, it must be pasteurized, and this may be accomplished by holding the product longer than 30 min. The additional holding time needed depends on foam thickness, airspace temperature, and rate of heating the liquid product. Measuring the additional time required for all of these parameters is not practical. If, however, the thermal properties of the foams were available, the additional time required for pasteurization could be calculated. The thermal properties of foams were estimated, and the development of the calculations is given in the following section.

CALCULATION OF HEAT TRANSFER IN FOAMS

During pasteurization, foam density and thickness decrease by as much as two-thirds (Fig. 2), and because of these variations, a formal mathematical solution is not possible. By assuming constant thickness and constant thermal properties, however, it is possible to calculate foam temperatures, and the results will be applicable to the worst conditions that would occur in normal practice. The problem is further simplified by assuming the lateral dimension of the foam is large compared to its thickness. This establishes the geometry of an infinite slab of finite thickness and limits the calculation to one-dimensional heat transfer.

Before the temperature profiles of the holding process can be calculated, the temperature profile at the end of heating must be known. To accomplish this, the time-temperature history of the heating phase must be calculated. Consequently, an equation for temperature profile during heating must be developed, and it is then used as a boundary condition for calculating temperature profiles during the holding process. The symbols used in the following derivations are defined in the list of nomenclature.

The heating process

Taking x as the direction moving vertically upward

through the foam, the general equation for one-dimensional heat transfer is (4)

$$\frac{\partial T}{\partial \tau} = \alpha \left(\frac{\partial^2 T}{\partial x^2} \right) \quad [1]$$

The heating process of vat pasteurizers consists of a constant heat input, and this means liquid temperature will increase at a constant rate (Fig. 2, 3). After an initial transient period, foam temperatures also increase at a constant rate, therefore, $\delta\tau/\delta\tau = A$ and substituting into equation 1,

$$\frac{d^2 T}{dx^2} = \frac{A}{\alpha}$$

The solution of the above equation is

$$T = \frac{Ax^2}{2\alpha} + C_1 x + C_2 \quad [2]$$

Applying the boundary conditions, when $x = 0$, $T = T_0$, therefore $C_2 = T_0$.

When $x = L$, $T = T_L$, and substituting into equation 2

$$C_1 = \frac{T_L - T_0}{L} - \frac{AL}{2\alpha}$$

After substituting C_1 and C_2 and rearranging, equation 2 becomes

$$T = \frac{A}{2\alpha} x^2 - \frac{AL}{2\alpha} x + (T_L - T_0) \frac{x}{L} + T_0 \quad [3]$$

Equation 3 gives the temperature profile through the foam during heating where T_L and T_0 are known and $T_L - T_0$ is a constant temperature difference between airspace and liquid. At the end of heating, T_0 is the pasteurization temperature, and equation 3 is used as a boundary condition in solving for temperature profiles during holding.

The holding process

The solution to the problem of heating an infinite slab, while its surfaces are maintained at T_L and T_0 is given by Carslaw and Jaeger (1)

$$T = T_0 + (T_L - T_0) \frac{x}{L} + \sum_{n=1}^{\infty} a_n \sin \frac{n\pi x}{L} e^{-\alpha n^2 \pi^2 \tau / L^2} \quad [4]$$

where the a_n coefficients are defined by

$$a_n = \frac{2}{L} \int_0^L \left[f(x) - \left[T_0 + (T_L - T_0) \frac{x}{L} \right] \right] \sin \frac{n\pi x}{L} dx \quad [5]$$

and $f(x)$ is defined by equation 3. Substituting equations 3 and 5 into 4, integrating, and simplifying

$$T = T_0 + (T_L - T_0) \frac{x}{L} + \frac{2AL^2}{\alpha\pi^2} \sum_{n=1}^{\infty} \frac{\cos n\pi - 1}{n^3} \sin \frac{n\pi x}{L} e^{-\alpha n^2 \pi^2 \tau / L^2} \quad [6]$$

Equation 6 gives the complete temperature profile across the foam for any time after the start of the holding period. The use of equation 6 is cumbersome; it must be solved for all combinations of x and τ and each computation requires the evaluation of an infinite series. It may, however, be simplified considerably by limiting the calculation to the specific point in time when minimum foam temperature achieves pasteurization temperature, T_0 as shown in Fig. 5. Temperature profiles are shown for successive time intervals, τ_4 , τ_5 , τ_6 , etc. As the foam is heated, the location of minimum temperature (point where $\delta T/\delta x = 0$) moves closer to the liquid surface because the top surface of the foam has a higher temperature than the bottom surface. When the minimum foam temperature reaches liquid temperature, it occurs at $x = 0$, and assuming liquid temperature is pasteurization temperature, minimum foam temperature achieves pasteurization temperature when $\delta T/\delta x = 0$ at $x = 0$. Equation 6 is restricted to this specific condition as follows: Taking the partial derivative of equation 6 with respect to x , and then substituting $x = 0$

$$\left(\frac{\partial T}{\partial x} \right)_{x=0} = \frac{T_L - T_0}{L} + \frac{2AL}{\alpha\pi^2} \sum_{n=1}^{\infty} \frac{\cos n\pi - 1}{n^3} n e^{-\alpha n^2 \pi^2 \tau / L^2}$$

Applying the requirement that

$$\left(\frac{\partial T}{\partial x} \right)_{x=0} = 0$$

yields

$$\frac{\alpha\pi^2(T_L - T_0)}{2AL^2} = - \sum_{n=1}^{\infty} \frac{\cos n\pi - 1}{n^2} e^{-\alpha n^2 \pi^2 \tau / L^2} \quad [7]$$

Equation 7 predicts the specific time after the start of holding when minimum foam temperature is equal to pasteurization temperature, T_0 . Evaluating the one infinite series is somewhat time-consuming; however, it can be shown that the first term of the series predicts the value of the complete series within 10% provided $\alpha\tau/L^2 > 0.01$. This requirement is satisfied for the conditions of interest during vat pasteurization and the accuracy of 10% is good for this type of computation; therefore, equation 7 simplifies to

$$\tau = \frac{L^2}{\alpha\pi^2} \ln \left[\frac{4AL^2}{\alpha\pi^2(T_L - T_0)} \right] \quad [8]$$

provided that

$$\alpha\tau/L^2 > 0.01 \quad [9]$$

Equation 8 is used to determine heating time of the foam, which is then used in equation 9 to determine that the calculation was valid. Equation 8

shows that heating time of a foam is dependent on thermal diffusivity of the foam, temperature difference between liquid and air space, heating rate of the liquid product, and foam thickness.

Because of the original assumption that liquid temperature increases at a constant rate, equation 8 is directly applicable only to pasteurizers with a constant heat input. Some pasteurizers have a variable heat input; they operate on the principle of a constant wall temperature. A different equation could have been developed for pasteurizers with constant wall temperature (and logarithmic temperature rise of the liquid), but these pasteurizers, with automatic control of vat wall temperature, have a constant heat input during most of the heating process. Because of the small temperature difference between the liquid and the vat wall, a logarithmic temperature rise of the liquid is obtained only during the last few degrees of heating. For milk products, equation 8 may be applied to foams in both types of vat pasteurizers.

Estimating thermal diffusivity of the foam

All the terms in equation 8 can be described except thermal diffusivity of the foam, α . Thermal diffusivity may be expressed as a ratio of more commonly known terms as follows

$$\alpha = \frac{k}{\rho C_p}$$

and by estimating values of k , ρ , and C_p , α may be computed.

From the work of Whittaker et al. (9) plus the data reported here, density (ρ) of foam is known to be in the range of 10 lb/ft³.

The amount of energy required to heat the air in a foam is negligible; therefore, specific heat is dependent only on the liquid phase. By using the data of Peebles (5), the specific heat (C_p) of ice cream mix was estimated as 0.7 BTU/lb F.

Thermal conductivity of the foam is not known and, unlike specific heat of the air, thermal conductivity of the air cannot be neglected. Thermal conductivity of a condensed milk with the same fat content and total solids content as those of ice cream mix is about 0.21 BTU/hr ft F (3) and thermal conductivity of the air is 0.0165 BTU/hr ft F at 140 F (2). These data may be combined according to a method described by Tsao (7) to yield an estimate of thermal conductivity of foam. To use Tsao's method, the percentage of volume of the liquid phase must be known. Since the density of the ice cream mix is about 64 lb/ft³, the per cent (by volume) of liquid in foam is the ratio of densities, or 10/64 = 16%. By using Tsao's method, thermal conductivity (k) of the ice cream mix foam was estimated to be 0.14 BTU/hr ft F. From these data, thermal diffusivity of the foam was

TABLE 1. CALCULATED ADDITIONAL HOLDING TIME REQUIRED TO ENSURE PASTEURIZATION OF MILK PRODUCT FOAMS IN THE VAT

| Foam thickness (inch) | Heating rate of the liquid (F/min) | Temperature difference between top and bottom surfaces of foam | | | |
|--------------------------|---------------------------------------|--|------|------|-----|
| | | 5 F | 20 F | 10 F | 5 F |
| | | (Additional holding time, min) | | | |
| 1 | 4 | 0 | 1 | 3 | 4 |
| 1 | 8 | 1 | 3 | 4 | 6 |
| 2 | 4 | 8 | 16 | 21 | 27 |
| 2 | 8 | 14 | 21 | 27 | 32 |
| 3 | 4 | 33 | 49 | 62 | 75 |
| 3 | 8 | 45 | 62 | 75 | 87 |

computed as 0.048 in²/min. Subsequently, thermal diffusivity of the foam was assumed to be 0.05 in²/min, and this value was used in equations 8 and 9 for computing results for selected conditions (Table 1). These may be used to estimate additional holding time required when a foam is present as follows:

Example

A 1.75-inch foam was observed on ice cream mix before the start of the holding period. Heating rate of the liquid product, obtained from the temperature recorder, was 6 F/min. Product temperature was 156 F and airspace temperature was 177 F. What is the additional holding period required for pasteurization of the foam?

In identifying the parameters needed in Table 1, the more adverse conditions are chosen. Foam thickness is chosen as 2 inches. Heating rate is taken as 8 F/min. During pasteurization, temperature difference is 177-156, or 21 F, and 20 F is therefore selected. From Table 1, the additional holding time required for pasteurization is 21 min; therefore, the total holding period will be 51 min.

In practice, delayed heating times of foams are usually shorter than those predicted by the use of Table 1 (processing reduces foam thickness) but never greater. The calculations are based on the assumption that foam thickness is constant during processing, and, hence, Table 1 includes the most adverse condition.

Foams having a thickness greater than 3 inches at the start of holding, should be removed before pasteurization. If the foam thickness is less than 1 inch, no additional holding time is required.

NOMENCLATURE

- A = constant rate of temperature rise, F/min.
 a = the n coefficients taken one at a time.
 C = constant of integration.

- C_p = specific heat of the foam, BTU/lb F.
 k = thermal conductivity of foam, BTU/hr ft F.
 L = foam thickness, inch.
 \ln = natural logarithm.
 T = temperature, F.
 T_L = temperature of airspace, F.
 T_o = temperature of liquid product, F.
 t = time when minimum foam temperature achieves the temperature of the liquid product, min.
 x = vertical distance upward through foam, inch.
 α = thermal diffusivity, inch²/min.
 π = 3.14
 ρ = density of foam, lb/ft³.
 τ = time, min.

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AMENDMENT TO SANITARY STANDARDS FOR THERMOMETER FITTINGS AND CONNECTORS USED ON MILK AND MILK PRODUCTS EQUIPMENT

Serial #0903

Formulated by

International Association of Milk, Food and Environmental Sanitarians

United States Public Health Service

The Dairy Industry Committee

The 3-A "Sanitary Standards for Thermometer Fittings and Connections Used on Milk and Milk Products Equipment," dated March 29, 1950, Serial #0900 as amended are further amended as indicated below.

1. The title is changed to "3-A Sanitary Standards for Instrument Fittings and Connections Used on Milk and Milk Products Equipment, Serial #0900."
2. The word "instrument" shall be substituted for

"thermometer" wherever it appears in the text of the standard and on the drawings where it designates a thermometer or a fitting for a thermometer.

This amendment is effective June 1, 1971.

A Research Note

RELATIONSHIPS BETWEEN WEATHER FACTORS, SOURCE OF SAMPLE, COMPOSITION OF MILK, AND FREEZING POINT DEPRESSION

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ABSTRACT

Changes in composition of milks from individual cows did not always respond to differences in atmospheric conditions to the same extent as did herd milk. Milk from individual cows tended to have a lower chloride concentration because of conditions related to wet bulb temperature than did herd milk. Caution should be used in translating results from individual cow's milk to herd milk.

The freezing point depression of milk has been shown to be greater during the winter than during other seasons (2). These differences may, in part, result from low SNF concentration (5) and/or low chloride concentration in the milk in warm seasons (3).

The normal concentration of lactose in milk, about 4.8%, is believed to account for about one-half of the freezing point depression of milk. The purpose of this investigation was to determine the relationship of the freezing point depression of milk to certain weather variables and to the chloride, lactose, and SNF concentration of the milk. Individual cow and mixed herd samples collected over a period of several months were used.

PROCEDURE

During April and May 1968, 205 individual-cow morning milk samples from 8 Holstein cows were analyzed for chloride by titration with AgNO_3 , for lactose by the chloramine-T method (4), for SNF by drying the whole milk sample and subtracting the fat as determined by the Babcock method and for freezing point depression by use of a thermistor-type cryscope. Weather data obtained from the United States Weather Bureau station located approximately 16 km from the dairy barn were used to calculate the temperature-humidity index, $0.4(t_d + t_w) + 15$, where t_d and t_w are the dry bulb and wet bulb Fahrenheit temperatures, respectively.

During the period August, 1968 to June, 1969, 78 bulk tank samples from one herd were analyzed in a similar manner except that lactose was determined by the method of Barnett and Tawab (1).

RESULTS AND DISCUSSION

Correlation coefficients between freezing point depression, certain weather variables, and composition of milk from individual cows are presented in Table 1. Warm atmospheric temperatures (dry bulb readings) were accompanied by low freezing point

TABLE 1. CORRELATION COEFFICIENTS BETWEEN WEATHER VARIABLES, MILK COMPOSITION AND FREEZING POINT DEPRESSION OF INDIVIDUAL COWS MILK (N = 205)

| | Freezing point depression | Concentration of | |
|----------------------------|---------------------------|------------------|--------------------|
| | | Lactose | Chloride |
| Freezing point depression | | +0.01 | -0.27 ^a |
| Lactose concentration | | | -0.44 ^a |
| Dry bulb temperature | -0.20 ^a | -0.01 | -0.01 |
| Wet bulb temperature | -0.02 | -0.12 | -0.26 ^a |
| Relative humidity | +0.23 ^a | -0.06 | -0.04 |
| Temperature-humidity index | -0.03 | -0.03 | -0.02 |

^aHighly significant relationship ($P < 0.01$).

TABLE 2. CORRELATION COEFFICIENTS BETWEEN HERD MILK COMPOSITION FREEZING POINT DEPRESSIONS AND WEATHER VARIABLES, AUGUST 1968-JUNE 1969 (N = 78)

| | Freezing point depression | Concentration of | | |
|----------------------------|---------------------------|-------------------|--------------------|--------------------|
| | | Lactose | SNF | Chloride |
| Freezing point depression | | 0.12 | 0.14 | -0.34 ^b |
| Lactose concentration | | | -0.03 | -0.03 |
| SNF concentration | | | | 0.05 |
| Dry bulb temperature | -0.32 ^b | 0.27 ^a | -0.16 | 0.10 |
| Wet bulb temperature | -0.33 ^b | 0.15 | -0.14 | 0.15 |
| Relative humidity | 0.02 | -0.09 | -0.01 | -0.03 |
| Temperature-humidity index | -0.02 | 0.45 ^b | -0.22 ^a | -0.19 |

^a $P < 0.05$.

^b $P < 0.01$.

depressions of the milk and high relative humidities were accompanied by higher freezing point depressions. High chloride concentrations were accompanied by low freezing point depressions, low lactose concentrations, and low wet-bulb temperatures. No weather variable was found to be significantly related to lactose concentration.

Table 2 lists correlation coefficients between several weather variables, freezing point depression, concentrations of lactose, SNF, and chloride on 78 samples of milk collected over an 11-month period from one herd of about 100 milking cows. The herd milk had low freezing point depressions and high lactose concentrations in times of warm weather. Milk of a high

lactose concentration was produced when the temperature-humidity index was high, but the SNF concentration was low in such milk. Milks of high chloride concentration had low freezing point depressions.

Any comparison of the two sets of data (Tables 1 and 2) must be made with the recognition that the data were not collected during the same time period. Thus seasonal differences (partially reflected in weather data) and the nature of the feed might tend to alter the milk composition and thus limit the direct comparison of the data in the two tables. Recognizing this limitation, certain similarities and differences may be noted. Individual cow's milk and herd milk were alike in their relationships of freezing point depression to chloride concentration and to dry bulb reading. Milks from the two sources were not the same with respect to freezing point depression vs. relative humidity; chloride concentration vs. wet bulb reading; and chloride concentration vs. lactose concentration. These results emphasize the importance of recognizing the source of samples when consider-

ing the effect of various factors on composition of milk.

Physiologists are interested in the influence of variables on an individual cow, or even an individual quarter of the udder; but the milk processor must concern himself with factors which might influence the composition of herd milk or milk from many herds.

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REPORT OF THE COMMITTEE ON APPLIED LABORATORY METHODS, 1968-1970

During the past 2 years, the Committee on Applied Laboratory Methods (ALM) has actively provided assistance and consultation in the following areas:

- (a) Provided assistance and consultation to the Intersociety Council on *Standard Methods for the Examination of Dairy Products (SMEDP)*. This Council plans to be responsible for publication of the 13th edition of *SMEDP*. It will also conduct collaborative studies on new and old laboratory methods and will provide a mechanism for indicating to interested parties, publication of "approved" methods during the interim period between editions of *SMEDP*.
- (b) Developed a continuing program for the evaluation of methods included in the 12th edition of *SMEDP*, which should be useful to the Intersociety Council in the preparation of the next edition.
- (c) Assisted in collaborative studies concerned with established, defined, and accepted methods for the examination of milk, milk products, and other foods.
- (d) Provided assistance to the National Mastitis Council (NMC) Research Committee and the NMC Subcommittee on Screening Tests.
- (e) Continued to encourage development of criteria for certification of media, reagents, materials, and instrumentation in all laboratory disciplines concerned with protection of public health (see ALM biennial committee report 1966-1968).

Two members of the Committee died during the year—Mr. David Anderson, Past Chairman of the Subcommittee on

Laboratory Methods for the Examination of Water and Other Environmental Samples, and Dr. Herbert Hall, Past Chairman of the Subcommittee on Laboratory Methods for the Examination of Foods. Their contributions to this committee will be missed. Dr. Martin Favero has assumed chairmanship of Mr. Anderson's former Subcommittee. Dr. E. A. Zottola has resigned as Chairman of the Food Subcommittee, and Mr. Don Pusch has been appointed acting Chairman of the Food Subcommittee; we hope that the new Chairman will have accepted appointment prior to this annual meeting. Active assistance of the Applied Laboratory Methods Committee to the National Mastitis Council Subcommittee on Screening Tests was terminated in January 1969 when Dr. Brazis resigned chairmanship of that NMC subcommittee.

Mr. Charles Huhtanen replaced Dr. Elmer Marth as Chairman of the Milk and Milk Products Subcommittee when the press of additional responsibilities resulted in his resignation as Chairman of this Subcommittee.

Preparation has started on the 13th edition of *Standard Methods for the Examination of Dairy Products*, and the ALM Committee has four members who are Committee Chairmen for five chapters. One ALM committee member is also a member of the Intersociety Council on *Standard Methods for the Examination of Dairy Products*. Altogether, eight ALM committee members are actively working on preparation of the next edition of *Standard Methods*.

(Continued on Page 144)

THE FOOD ADDITIVE PETITION PROCESS¹

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ABSTRACT

Development and filing of a Food Additive Petition for a new food additive requires close liaison between the Food and Drug Administration (FDA) and the manufacturer, beginning with development of a protocol for data which will be required for the petition. Informal FDA guidelines for Food Additive Petitions require information on the identity of the additive, usage and labeling, efficacy or performance, method for determining the additive in food, proof of safety, and the proposed regulation. Pertinent literature, and biographical data on principal researchers also may be included. The clearance process, interaction of food additive regulations with food standards, time and cost for petition preparation, and possible use of the Food Additive Petition process in the future are discussed.

The concept of the Food Additive Petition process emerged from the 1958 Act wherein Congress provided that new food additives shall be subject to proof of safety. Congress did not provide any specific procedures in the law for this process, but provided the Food and Drug Administration (FDA) with the authority to establish and administer such requirements.

FDA has not incorporated guidelines for Food Additive Petitions into its formalized regulations. However, it published, in 1966, a brief outline *FDA Guidelines for Chemistry and Technology Requirements of Food Additive Petitions*, which provide a helpful summary for undertaking the petition process.

A key aspect of preparing and submitting Food Additive Petitions (FAP) is the stance under which one deals with FDA. First, it is vitally important to understand the role of FDA. FDA is basically an umpire, acting on behalf of public interest. As such, FDA is under close scrutiny, and consistent, often extreme pressure, from several directions—Congress, Industry, and, most recently, organized consumer groups. As a public agency, FDA must conduct its business in an open forum.

A good principle to follow for requesting opinions from FDA is to never make a formal request for an opinion to FDA until sufficient informal, off the record, discussions have been held to determine beyond a reasonable doubt exactly what sort of formal opinion will be given to a particular request.

REQUIRED INFORMATION

The first step in the process for FAP clearance of an additive is the development of a protocol for the various types of information required for the petition. This protocol is developed via joint discussion between various FDA divisions and branches, the manufacturer of the new additive, and other outside scientific services retained by the manufacturer, particularly in the area of toxicology. Usually, it is very useful to have a competent FDA consulting firm to assist with making appointments, seeking out firms equipped to do the specialized types of research required, particularly in toxicology, and to iron out the innumerable details which occur during development of the protocol and conduct of research, and the preparation of the petition.

The purpose of the protocol is to establish in a clear and mutually agreed upon form the type and amount of information which will be required on various aspects of the new additive. The FDA is most concerned about the safety or toxicological aspects. However, it is vital to have an understanding on all the types of information mentioned in the 1966 *Guidelines*.

The major elements or types of information required for a Food Additive Petition are grouped into seven categories.

Identity of additive

First, information is required on the identity of the additive—the name of the additive, the chemical formula if applicable, the definition or classification of the additive, and the major chemical and physical properties of the additive. Also included under identity are specifications for food grade. Here the manufacturer must list, in effect, the quality control standards—chemical, physical, and microbiological.

Usage and labeling

Second, information on the usage and labeling of the additive must be clearly set forth. What quantity of additive is required for effective use, what instructions are to be provided to the user? How is the additive to be labeled? Also required is information on levels of the additive which will be present in food. Finally, information must be provided on the fate of the additive in food—the reactions the

¹Presented at a meeting of the Wisconsin Section, Institute of Food Technologists, Oshkosh, Wisconsin, September 11, 1970.

additive undergoes, the proportion of the additive that remains in the food, and whether the additive is inert or can be considered as a metabolite.

Usefulness

The third type of information required pertains to efficacy—the usefulness of the additive. Requirements for efficacy include a description of how the additive functions, whether there is a self-limiting effect, and, most important, comprehensive data on actual performance of the additive. Data on performance must be sufficiently comprehensive to cover the entire spectrum of proposed use.

Data on food additive efficacy have received increasing emphasis from FDA in recent years. The increasing concern of consumers with “chemicals in food” will almost certainly further increase this emphasis, which will result in increasingly tougher requirements for data on efficacy.

Methods for measurement

The fourth type of information required is methodology to determine the additive in food. Analytical techniques must be provided for isolating and measuring the additive.

Toxicology

Fifth, comprehensive data on the toxicology of the additive are required. FDA requires these data to be obtained by a reputable laboratory. Although FDA cannot, of course, endorse research organizations, they can, and will indicate whether toxicological data from a particular laboratory will be accepted. Usually, toxicological data based on a minimum feeding period of 1 year for two species is required, and 2 year feeding trials are not uncommon. There are no formal requirements as to species used, length of feeding trial, or other details of toxicological work. Usually, feeding levels are sought which provide a safety factor of 100; that is, where 100 times as much additive, per unit of body weight, is fed daily than would be ingested under the conditions of actual usage.

Regulation

The sixth element of a Food Additive Petition is the proposed regulation. The petitioner must state how he would propose to have the Food Additive Regulations amended to permit the use of the additive.

References

Finally, a petition may contain literature reprints and biographical data on key individuals who conducted research on the additive. The literature used must be in English, or have a complete, accurate translation from the language in which it was originally published.

CLEARANCE PROCESS

Once all the research is done and the petition has been compiled, there remains the clearance process. The clearance process and the time table under which it operates is rather clearly spelled out in FDA Administrative Rules. The petition is submitted in triplicate to the Petitions Control Branch (PCB) of FDA. Personnel of this branch examine the petition to determine whether all the elements required have been included. The PCB has the option of returning the petition if it is deemed to be incomplete. If the petition seems to be in order, it is accepted by PCB. The examination of a petition by PCB usually requires a minimum of 15 days and may be prolonged for up to 6 months if the petition is grossly deficient and the petitioner fails to correct it.

Notice that the petition has been filed is published in the *Federal Register*. The date of publication in the *Federal Register* is the Filing Date. The *Federal Register* publication routinely calls for interested parties to comment, or request a hearing. Interested parties who either object to or are in favor of the proposed additive are free to make their own opinions known to the Petitions Control Branch. If any of the objections are sufficiently serious and well documented and are cause for concern about any aspect of the additive, the FDA can call for a hearing.

At least 90 days are required between establishment of the filing date and publication of an effective date—or, in effect, approval of the new additive for use. The 90 day period is primarily devoted to careful examination of all elements of the petition by FDA scientific personnel. If any of the data in the petition are questioned, additional data may be called for. When FDA personnel are satisfied that the data are complete and that the safety and efficacy of the new additive have been amply documented, the FDA publishes a notice in the *Federal Register* that the additive is approved for use. This publication includes description of the amended Food Additive Regulations wherein usage of the additive is provided.

RESTRICTIONS

Approval of a new additive does not automatically assure its use in all applications. If the additive is to be used in foods for which there are Food Standards, amendments to these Food Standards must be proposed and adopted. The amendment to any Food Standard proceeds in a manner similar to that just described for a Food Additive Petition. Usually a proposal to amend Food Standards evokes a great deal of interest from manufacturers and, recently, consumers.

PROBLEMS

The FAP process is lengthy and expensive. Assuming one year toxicological testing, the minimum time from decision to proceed with an FAP to completion of the Petition, ready for submission to FDA, is 18-20 months. At least four additional months are required for processing of the petition by FDA and publication of the final approval order. The absolute minimum cost for a Food Additive Petition is \$100,000.00.

Among the innumerable criticisms leveled at FDA and the food industry it is significant that there has been no specific criticism of the FAP process as being inadequate and not in the best interest of food safety. In fact, it seems probable that the FAP mechanism may well be extended, probably in a modified form, to the upcoming review of the GRAS list.

A potential precedent is to be found in FDA administration of New Drug Applications (NDA's). The *standard* NDA requires the same general types of information as the FAP—identity, efficacy, and safety. FDA also permits a *supplemental* NDA to be filed, based upon toxicological and efficacy data in a previously filed and approved standard NDA. Recently, FDA provided that an *abbreviated* NDA could be used for the clearance of certain established drugs. The abbreviated NDA, at the option of FDA, may not require comprehensive toxicological or efficacy data.

An abbreviated FAP, coupled with the recommendations and evaluations by expert panels, would seem to provide adequate and practical approach to the evaluation of certain types of substances currently on the GRAS list.

REPORT OF COMMITTEE ON APPLIED LABORATORY METHODS (Continued from Page 141)

SUBCOMMITTEE ON LABORATORY METHODS FOR THE EXAMINATION OF MILK AND MILK PRODUCTS

Introduction

The scope of the Subcommittee's activities in the last several years was limited, voluntarily, to studies of the methodology for determining standard plate counts of raw milk. There were several reasons for this. One was the feeling that some of the standard procedures specified in *Standard Methods* were rather arbitrarily derived, and their value as compared to other related methods should be confirmed or rejected. It is recognized, of course, that there must be uniformity of methods among laboratories, but this does not mean that this uniformity should be necessarily arrived at by a "consensus" rather than by rigorous study and interpretation. A second reason for concentrating on raw milk was the fact that since the producers might be penalized for high bacterial counts, the analyses of the milk samples should be as accurate and reproducible as possible. A third reason for using raw milk was the availability of this commodity to the subcommittee members and their familiarity with the problems involved.

Studies completed

Mixing methods. The first problem the Subcommittee tackled was to determine the efficiency of the "standard" method of mixing milk dilutions (25, 1-ft long, up-and-down cycles to be completed in a 7-sec period). The Subcommittee could not explore every ramification of this standard method; however, it was not even known how this mixing procedure would compare with a far less vigorous one such as simply inverting the dilutions (a procedure recommended by *Standard Methods* for mixing undiluted pasteurized milk specimens). The vigorous, standard mixing of dilution tubes causes some curdling of the cream—this could conceivably affect standard plate counts.

The first experiments undertaken by the members in the

last 2 years were therefore an evaluation of the standard milk dilution mixing procedure by comparing standard plate counts by this procedure with the counts from two inversion methods. These were: 5 inversions in a 5-sec period and 15 inversions in a 15-sec period. The experiments were summarized and explained in our Subcommittee research paper recently published in the *Journal of Milk and Food Technology* ("Effect of Dilution Bottle Mixing Methods on the Recovery of Raw Milk Bacteria"). The conclusions of the study group were: (a) the "standard" method gave higher plate counts than the gentler inversion methods, and (b) there were no significant differences ($P < 0.01$) in reproducibility among the three methods studied. The studies showed evidence of interactions between mixing methods and samples and between mixing methods and investigators. These interactions did not negate the above conclusions, but did indicate that investigations of this sort should include a variety of investigators and samples in order to avoid possible erroneous results arising from more limited studies.

Horizontal versus vertical shaking and glass versus plastic petri dishes. Some complaints have been voiced over the fact that *Standard Methods* requires that the mixing procedure for milk dilutions be a series of up-and-down cycles. This procedure is very taxing to those of limited physical endurance, and, as a matter of fact, some laboratories have routinely changed to a horizontal series of mixing strokes as a matter of convenience. A comparison of these two methods was a part of the Subcommittee's second series of experiments.

Another part of this study concerned the use of plastic and glass petri dishes in the Standard Plate Count. This investigation was prompted by the submission of comparative data for the two types of petri dishes by one of the Subcommittee members which, when statistically analyzed, showed the glass petri dishes to produce higher plate counts, and with greater reproducibility, than the plastic dishes produced. This finding was of obvious concern, and a more definitive comparative study was therefore initiated.

The results of this study were reported in another paper

(Continued on Page 149)

A SURVEY OF THE ACTUAL AND POTENTIAL BACTERIAL KEEPING QUALITY OF PASTEURIZED MILK FROM 50 PENNSYLVANIA DAIRY PLANTS¹

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ABSTRACT

The actual and potential keeping quality of milk from 50 Pennsylvania processing plants was determined by comparing Standard Plate, coliform, and psychrophilic counts on commercially processed and laboratory pasteurized portions held at 4.4 C and 7.2 C. Examinations were made on the day of processing and after 5 and 10 days storage at each temperature.

Bacterial data on fresh commercially pasteurized milk were of little or no value in predicting shelf life. As shown by previous studies, laboratory pasteurization destroys or inactivates coliform and psychrophilic organisms usually responsible for spoilage of commercially processed milk. Some laboratory pasteurized milk exhibited increases in counts when held 5 or 10 days at 7.2 C, even though freshly pasteurized portions were free of the psychrophilic or coliform organisms often found in commercially processed milk. The need for low temperature storage of pasteurized milk is clearly shown.

The most important fact illustrated by this study is that Standard Plate, coliform, or psychrophilic counts on fresh commercially processed milk are poor criteria of potential keeping quality. Even though this has been pointed out by others, official regulatory methods, as usually applied, have not recognized the need for new procedures to measure milk quality, especially at the consumer level.

Changing milk production patterns at the farm with every other day milk collection and reduced processing schedules in dairy plants have increased the age of milk, in most instances, prior to pasteurization. Milk is usually delivered to homes 3 times a week or less. More and more milk is sold through stores, often where storage conditions for peak loads are inadequate or where temperatures are too high. The consumer often purchases milk that was several days old prior to pasteurization. Perhaps a week or longer may have elapsed between pasteurization and sale. With more milk sold through stores, it seems reasonable to assume that some consumers will purchase enough to last several days. Thus, the poten-

tial keeping quality of milk must be much better than some years ago when practically all milk was produced, processed, and consumed within 2 or 3 days. In spite of these facts, quality regulations enforced by most jurisdictions have undergone little change. In an attempt to insure milk with good keeping quality, many individual dairy plants perform shelf life tests to determine the effectiveness of their cleaning, sanitizing, and processing procedures.

Unfortunately, few regulatory agencies do more than check the quality of milk at the producer or the processor level. The usual reason given is that after the processor delivers the product he no longer has any control over subsequent factors that might affect quality. In addition, many existing microbial standards and laboratory procedures, while perhaps valid quality measurements 20 years ago, have not kept pace with changes occurring in the industry. Perhaps a criticism can be made that those who promulgate standards and laboratory procedures appear to be more interested in the performance of tests according to accepted procedures and compliance with bacterial standards having dubious meaning than they are with applying methods proposed for measuring milk quality at the consumer level. It appears that little attention is given to enforcing distribution practices that would extend the time milk might be considered acceptable.

The comments of Barnum (4) on the need for a re-evaluation of the Standard Plate Count point out the inadequacy of this test, as currently applied, to yield meaningful information. Recently G6mes-de-Lass6 (10) presented data demonstrating the lack of correlation between Standard Plate, coliform, and psychrophilic counts on freshly pasteurized milk and potential keeping quality. He also emphasized the need for new tests to predict accurately and quickly the anticipated shelf life of products.

Witter's (18) review of psychrophilic bacteria showed either laboratory or commercial pasteurization effective in destroying them in the concentration usually present in raw milk.

Dabbah et al. (6) found a psychrophilic organism

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from aged pasteurized milk that appeared to be inactivated by heating at 55 C for 30 min but which subsequently grew after holding in a special medium at 20 C for 48 to 72 hr or at 4 C for 2 to 3 weeks. This culture did not survive 60 C for 30 min, a temperature and time treatment less than legal pasteurization.

Grosskopf and Harper (11) in a recent study found that fluid pasteurized milk aseptically packaged in a standard plastic coated paper container and stored at 4 C had a shelf life of 4 weeks. Subsequent loss of quality was caused by a psychrophilic sporeformer. Their initial results suggest about 25% of raw producers' supplies contain sporeformers capable of surviving UHT pasteurization.

Studies by Langlois et al. (15) have indicated significant differences among plants with respect to keeping quality of their milk, suggesting that plant sanitation procedures, storage temperatures, and time of holding were important. Barnard (3) and Witter et al. (19) reported that while the temperature of some samples of milk for sale in retail outlets was 4.4 C or below, about two-thirds of all samples were over this temperature with 17-18% over 7.2 C. Barnard (3) reported 3.6% over 10 C.

Studies by Atherton et al. (2), Blankenagel and Humbert (5), Ford and Babel (9), Huskey et al. (12), Smith (16), Elliker (8), and Witter et al. (19) have shown that as the storage temperature of pasteurized milk increases the bacterial population also increases. These studies have indicated that pasteurized milk should be held at 4.4 C or below to achieve maximum shelf life.

MATERIALS AND METHODS

In this study the actual and potential keeping quality of pasteurized milk from 50 Pennsylvania plants was determined. These 50 plants represented approximately 15% of all processors in Pennsylvania.

Commercially processed packaged samples from each plant were placed in ice water and transported to the laboratory of The Pennsylvania State University Creamery. All analyses were begun within 24 hr from the time of processing.

Twelve portions of each processed sample were aseptically transferred to sterile screw capped test tubes. To avoid airborne contamination, subdivision of samples was done in a culture transfer cabinet equipped with an ultraviolet light.

Six of the 12 tubed portions were submerged in a thermostatically controlled laboratory pasteurization bath and re-pasteurized at 62.7 C for 30 min, with a come-up time of 5 min. Immediately after the holding period these samples were cooled in ice water. Three portions of each sample were placed in a refrigerator maintained at 4.4 C and an additional three portions were held at 7.2 C. Other tubed portions of the commercially pasteurized milk were held under similar conditions.

Standard Plate, coliform, and psychrophilic counts were made immediately on the plant and laboratory pasteurized milks and on separate portions of each sample after 5 and 10

TABLE 1. SUMMARY OF STANDARD PLATE COUNT ON 50 COMMERCIALY PASTEURIZED MILK SAMPLES

| Original range ¹ | Count range ² | | | | |
|------------------------------|--------------------------|----|---|---|----|
| | 1 | 2 | 3 | 4 | 5 |
| Fresh | 28 | — | — | — | — |
| 5 days - 4.4 C | 22 | 2 | 4 | — | — |
| 10 days - 4.4 C | 16 | — | — | 3 | 9 |
| 5 days - 7.2 C | 13 | 3 | 2 | 3 | 7 |
| 10 days - 7.2 C | 4 | 1 | 1 | 5 | 17 |
| Original range ²¹ | | | | | |
| Fresh | — | 20 | — | — | — |
| 5 days - 4.4 C | 6 | 8 | 2 | 1 | 3 |
| 10 days - 4.4 C | 3 | 8 | 1 | 1 | 7 |
| 5 days - 7.2 C | 3 | 5 | 2 | 1 | 9 |
| 10 days - 7.2 C | 1 | 2 | 2 | 1 | 14 |
| Original range ³¹ | | | | | |
| Fresh | — | — | 2 | — | — |
| 5 days - 4.4 C | 2 | — | — | — | — |
| 10 days - 4.4 C | — | 1 | — | 1 | — |
| 5 days - 7.2 C | — | — | 2 | — | — |
| 10 days - 7.2 C | — | — | 2 | — | — |

| ¹ Count range | SPC per ml |
|--------------------------|-------------------|
| 1 | <1,000 |
| 2 | 1,000 - 9,999 |
| 3 | 10,000 - 99,999 |
| 4 | 100,000 - 999,999 |
| 5 | >1,000,000 |

²Number of samples within respective treatments.

days storage at 4.4 and 7.2 C. Bacterial enumeration procedures were made in accord with *Standard Methods for the Examination of Dairy Products* (1) except where very low Standard Plate Counts made it necessary to use the data from plates having less than 30 colonies. Coliform colonies per milliliter were determined on violet red bile agar. While laboratory pasteurization of the commercially pasteurized milks may have resulted in some bacterial destruction beyond that achieved with plant pasteurization, the above procedure was necessary to inactivate any organisms that might have entered post pasteurization, and to permit measuring the actual and potential keeping quality of the samples. Actually, comparison of Standard Plate Counts of the plant-pasteurized and re-pasteurized portions indicated that reheating caused little change in plate counts on the day of pasteurization.

RESULTS AND DISCUSSION

Forty-eight of the 50 commercially processed samples had original Standard Plate Counts of <10,000 /ml. Examination of data in Table 1 shows the effect of storage temperature and time on the changes in total counts of these samples. As might be expected, counts increased as a function of time and the higher storage temperature. Unfortunately, Table 1 does not show a very important fact: changes in counts as a function of time and temperature bore no relationship whatsoever to the count of the original samples. Data on two of the samples are shown in Tables 2 and 3.

TABLE 2. EXAMPLE OF MILK SHOWING EXCELLENT ACTUAL AND POTENTIAL KEEPING QUALITY

| Storage temp. | Days held | SPC per ml | Coliform colonies per ml | Psychrophilic colonies per ml |
|--|-----------|------------|--------------------------|-------------------------------|
| <i>Actual</i> (Plant pasteurized) | | | | |
| | 0 | 190 | <1 | <1 |
| 4.4 C | 5 | 150 | <1 | <1 |
| | 10 | 130 | <1 | <1 |
| 7.2 C | 5 | 130 | <1 | <1 |
| | 10 | 100 | <1 | <1 |
| <i>Potential</i> (After laboratory pasteurization) | | | | |
| | 0 | 60 | <1 | <1 |
| 4.4 C | 5 | 90 | <1 | <1 |
| | 10 | 130 | <1 | <1 |
| 7.2 C | 5 | 100 | <1 | <1 |
| | 10 | 60 | <1 | <1 |

TABLE 3. EXAMPLE OF MILK SHOWING POOR ACTUAL AND EXCELLENT POTENTIAL KEEPING QUALITY

| Storage temp. | Days held | SPC per ml | Coliform colonies per ml | Psychrophilic colonies per ml |
|--|-----------|-------------|--------------------------|-------------------------------|
| <i>Actual</i> (Plant pasteurized) | | | | |
| | 0 | 260 | <1 | <1 |
| 4.4 C | 5 | 220,000 | <1 | 170,000 |
| | 10 | 36,000,000 | <1 | 23,000,000 |
| 7.2 C | 5 | 7,800,000 | <1 | 6,100,000 |
| | 10 | 770,000,000 | <1 | 110,000,000 |
| <i>Potential</i> (After laboratory pasteurization) | | | | |
| | 0 | 200 | <1 | <1 |
| 4.4 C | 5 | 260 | <1 | <1 |
| | 10 | 230 | <1 | <1 |
| 7.2 C | 5 | 200 | <1 | <1 |
| | 10 | 140 | <1 | <1 |

Data in Table 2 are typical of those from dairy plants which were doing a good job of cleaning and sanitizing. Almost identical Standard Plate Counts were found on plant and laboratory pasteurized portions, with no increase evident during refrigerated holding. The absence of either coliforms or psychrophiles in these plant-pasteurized samples also indicated lack of post-pasteurization contamination.

Table 3 represents data from a plant which indicated the bacterial information on the freshly pasteurized product would be very misleading in terms of the shelf life of the product. The potential keeping quality data on this sample indicated that if the plant had used proper procedures and had avoided post pasteurization contamination, the product might have shown bacterial data similar to those shown in Table 2. These tables show the fallacy of relying on data on freshly commercially processed milk. Numerous examples of each of these types of data were found during this study.

With the laboratory pasteurized portions (Table 4), the Standard Plate Counts did not increase as a function of age and temperature to the degree found with the plant pasteurized portions. Counts after

laboratory pasteurization tended to remain static or decrease slightly, except for those portions held at 7.2 C for 10 days.

Perhaps the most surprising observation was the low coliform concentrations (Table 3) found in fresh samples of plant pasteurized milk and in aged portions. While a few samples of this group showed high numbers, in general, increases were minimal. This does not correlate at all with the increasing numbers of psychrophiles found in commercially processed samples as storage time progressed. Possibly growth of high concentrations of psychrophiles actually inhibited development of coliforms. It is also possible that the usual coliform determinative procedures fail to recover this group in aged samples. This phenomenon may be similar to that reported by Dack and Lupitz (7), who found the natural flora of frozen pot pies to inhibit *Escherichia coli*. Speck (17) has reviewed the subject of injury and bacterial antagonism on recovery of other organisms. Maxcy (14) recently has shown cell injury as a factor in enumeration of coliform bacteria. As might be ex-

TABLE 4. SUMMARY OF STANDARD PLATE COUNT ON LABORATORY PASTEURIZED (62.7 C - 30 MIN) PORTIONS OF 50 COMMERCIALY BOTTLED MILKS

| Original range 1 ¹ | Count range 2, 3 | | | | |
|-------------------------------------|------------------|----|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| <i>Original range 2¹</i> | | | | | |
| Fresh | 38 | — | — | — | — |
| 5 days - 4.4 C | 37 | 1 | — | — | — |
| 10 days - 4.4 C | 38 | — | — | — | — |
| 5 days - 7.2 C | 36 | 1 | 1 | — | — |
| 10 days - 7.2 C | 22 | 8 | 3 | 3 | 2 |
| <i>Original range 3¹</i> | | | | | |
| Fresh | — | 10 | — | — | — |
| 5 days - 4.4 C | 3 | 7 | — | — | — |
| 10 days - 4.4 C | 3 | 7 | — | — | — |
| 5 days - 7.2 C | 3 | 7 | — | — | — |
| 10 days - 7.2 C | 2 | 4 | 1 | 3 | — |

| Count range | SPC per ml |
|-------------|-------------------|
| 1 | <1,000 |
| 2 | 1,000 - 9,999 |
| 3 | 10,000 - 99,999 |
| 4 | 100,000 - 999,999 |
| 5 | >1,000,000 |

²Number of samples within respective treatments.

³No. samples remaining in same class or showing decrease:

- 5 days - 4.4 C - 49
- 10 days - 4.4 C - 50
- 5 days - 7.2 C - 48
- 10 days - 7.2 C - 30

TABLE 5. COLIFORM COLONIES PER MILLILITER ON 50 COMMERCIALY PASTEURIZED MILK SAMPLES¹

| | Number per milliliter | | |
|-----------------|-----------------------|------|----|
| | 1 | 1-10 | 10 |
| Fresh | 46 | 2 | 2 |
| 5 days - 4.4 C | 44 | 2 | 4 |
| 10 days - 4.4 C | 47 | 0 | 3 |
| 5 days - 7.2 C | 44 | 1 | 5 |
| 10 days - 7.2 C | 41 | 1 | 8 |

¹Coliform colonies per ml on laboratory pasteurized portions of above bottled milks: fresh, 5 days - 4.4 C, 10 days - 4.4 C, 5 days - 7.2 C, and 10 days - 7.2 C: all less than 1/ml.

TABLE 6. PSYCHROPHILIC COLONIES PER MILLILITER ON 50 BOTTLED MILK SAMPLES

| | Count range ^{1, 2} | | | | |
|-----------------|-----------------------------|---|---|---|----|
| | 1 | 2 | 3 | 4 | 5 |
| Fresh | 48 | 2 | — | — | — |
| 5 days - 4.4 C | 34 | 6 | 1 | 6 | 3 |
| 10 days - 4.4 C | 25 | 2 | 1 | 3 | 19 |
| 5 days - 7.2 C | 23 | 4 | 3 | 4 | 16 |
| 10 days - 7.2 C | 9 | 3 | 3 | 2 | 33 |

Psychrophilic colonies per ml on laboratory pasteurized portions of bottled milks

| | | | | | |
|-----------------|----|---|---|---|---|
| Fresh | 50 | — | — | — | — |
| 5 days - 4.4 C | 50 | — | — | — | — |
| 10 days - 4.4 C | 49 | 1 | — | — | — |
| 5 days - 7.2 C | 50 | — | — | — | — |
| 10 days - 7.2 C | 33 | 5 | 6 | 4 | 2 |

| ¹ Count range | SPC per ml |
|--------------------------|-------------------|
| 1 | <1,000 |
| 2 | 1,000 - 9,999 |
| 3 | 10,000 - 99,999 |
| 4 | 100,000 - 999,999 |
| 5 | >1,000,000 |

²Number of samples within respective treatments.

pected, no coliforms were found in any of the laboratory pasteurized portions, either fresh or aged.

Table 6 presents the psychrophilic counts found in this study. Many fresh commercially pasteurized samples were contaminated to some degree. The increasing counts found with some bottled products as a function of time and temperature parallel data shown in many previous studies. Data on psychrophiles in the laboratory pasteurized portions show that none were recovered in freshly pasteurized milk, or even after 5 days storage at 4.4 C. After 10 days at 7.2 C, surviving thermodurics began growing, emphasizing the need for low temperature storage of milk even though milk is completely free of post-pasteurization contamination.

Data from this study show that original bacterial counts on commercially processed milk are of little or no value in predicting bacterial keeping quality. Samples held at 4.4 or 7.2 C and examined after 5 or 10 days give a decidedly more accurate picture of

processing and packaging conditions.

The lack of correlation between psychrophilic and coliform development in commercially processed samples suggests that the coliform test is a less sensitive indicator of post-pasteurization contamination than psychrophilic counts on milk samples held at 4.4 or 7.2 C for several days.

At present various municipalities and other groups are reviving the subject of milk dating. Of more significance in terms of milk quality at the consumer level would be bacterial standards based on milk samples held under refrigerated storage equivalent to commercial conditions before examination. Surveys of milk at the retail level suggest that milk temperatures are often too high. Dating to insure good quality would seem to be a poor substitute for proper pasteurizing, freedom from contamination, and holding at temperatures low enough to inhibit the growth of those organisms present.

ACKNOWLEDGMENTS

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REPORT OF COMMITTEE ON APPLIED LABORATORY METHODS

(Continued from Page 144)

recently published in the *Journal of Milk and Food Technology* ("A Comparison of Horizontal Versus Vertical Mixing Procedures and Plastic Versus Glass Petri Dishes for Enumerating Raw Milk Bacteria"). The tests showed that there were no significant differences between types of petri dishes or the direction of the mixing stroke. It is therefore recommended that both types of petri dishes continue to be advocated and that the requirements for the standard shaking method be modified to include also the less fatiguing horizontal stroke.

Proposed work

The question of the proper incubation temperature for the Standard Plate Count continues to be in doubt since several recent papers have indicated that 32 C might be too high a temperature for some of the psychro-tolerant organisms in milk. The Subcommittee therefore will attempt to conduct studies on temperatures that will give most reproducible results and which will give the highest number of colonies. Some consideration also may be given to determining reproducibility of tests for abnormal milk.

Acknowledgments

The Subcommittee was fortunate in the last several years to retain its members and to acquire several new ones. Also, we were assisted during one of our studies by D. J. Pusch and E. Bredvold of the Minnesota State Department of Agriculture. Their help is gratefully acknowledged.

SUBCOMMITTEE ON LABORATORY METHODS FOR THE EXAMINATION OF FOODS

Activities during the first year of the biennium were nil. This resulted in part from the inactivity and unavailability of the Subcommittee Chairman. During the second year, however, attempts were made to stimulate activity of the committee. A memo to all Subcommittee members requested information on the following topics:

- (a) Evaluation and comments on methodology used for the isolation of *Staphylococcus aureus* from foods.
- (b) Comments concerning the use of indicator organisms, coliform in particular, and differentiation of fecal coliform and *Escherichia coli*.
- (c) Standardization of methodology for isolation of coliform organisms, i.e. fecal coliform.
- (d) Definition of fecal coliform.
- (e) Willingness to participate in laboratory evaluations.

Response to the request was somewhat less than overwhelming. Two committee members did not respond at all, one resigned, and the other two responded briefly. The response cannot be considered as definitive. All but one of the committee members did agree the five topics suggested for discussion were worthwhile and should be developed further. Only one respondee was willing to participate in a cooperative laboratory evaluation.

In summary, some common problems related to the methods used in the microbiological examination of foods have been defined. Solutions to these problems were not developed by this Subcommittee.

SUBCOMMITTEE ON LABORATORY METHODS FOR THE EXAMINATION OF WATER AND OTHER ENVIRONMENTAL SAMPLES

This Subcommittee has been relatively inactive during the past 2 years. This resulted, partially, from the untimely death of its past chairman, Mr. David Anderson. Dr. Martin S. Favero was appointed chairman, and Dr. John C. Hoff was appointed as a member. Mr. C. N. Huhtanen resigned. The Subcommittee is tentatively planning to meet at the American Public Health Association meeting in Houston, October 26-30, 1970.

The following projects have been suggested by the members, and attempts will be made to conduct interlaboratory tests:

- (a) Continuation of the work on slow lactose fermenters.
- (b) Mechanization and simplification of the MPN method.
- (c) Evaluation of the 7-hr fecal coliform test recently developed by Geldreich.
- (d) Consideration and discussion of special problems related to the use of fecal coliform bacteria as indicators of fecal pollution in special environments; i.e., pulp mill effluents, sugar beet waste, and hospital air, and surfaces.
- (e) Evaluation of newly developed membrane filter media.

APPLIED LABORATORY METHODS COMMITTEE

Dr. A. Richard Brazis, *Chairman*, Chief, Laboratory Development Program, Division of Microbiology, FDA, 1090 Tusculum Avenue, Cincinnati, Ohio 45226.

SUBCOMMITTEE ON LABORATORY METHODS FOR THE EXAMINATION OF MILK & MILK PRODUCTS

Mr. C. N. Huhtanen, *Chairman*, Eastern Utilization Research, and Development Division, USDA, Philadelphia, Pennsylvania.

(Continued on Page 159)

HOLDERS OF 3-A SYMBOL COUNCIL AUTHORIZATIONS ON FEBRUARY 20, 1971

"Questions or statements concerning any of the holders of authorizations listed below, or the equipment fabricated, should be addressed to Earl O. Wright, Asst. Sec'y-Treas., Dept. of Food Technology, 116 Dairy Industry Bldg., Iowa State University, Ames, Iowa 50010."

0102 Storage Tanks for Milk and Milk Products

As Amended

| | | |
|-----|--|------------|
| 116 | Jacob Brenner Company, Inc. | (10/ 8/59) |
| | 450 Arlington, Fond du Lac, Wisconsin | 54935 |
| 28 | Cherry-Burrell Corporation | (10/ 3/56) |
| | 575 E. Mill St., Little Falls, N. Y. | 13365 |
| 102 | Chester-Jensen Company, Inc. | (6/ 6/58) |
| | 5th & Tilgham Streets, Chester, Pennsylvania | 19013 |
| 1 | Chicago Stainless Equipment Corp. | (5/ 1/56) |
| | 5001 No. Elston Avenue, Chicago, Illinois | 60630 |
| 2 | CP Division, St. Regis | (5/ 1/56) |
| | 1243 W. Washington Blvd., Chicago, Illinois | 60607 |
| 117 | Dairy Craft, Inc. | (10/28/59) |
| | St. Cloud Industrial Park | |
| | St. Cloud, Minn. | 56301 |
| 76 | Damrow Company | (10/31/57) |
| | 196 Western Avenue, Fond du Lac, Wisconsin | 54935 |
| 115 | DeLaval Company, Ltd. | (9/28/59) |
| | 113 Park Street, So., Peterborough, Ont., Canada | |
| 207 | The DeLaval Separator Co. | (7/23/69) |
| | Duchess Turnpike, Poughkeepsie, N. Y. | 12602 |
| 109 | Girton Manufacturing Company | (9/30/58) |
| | Millville, Pennsylvania | 17846 |
| 21 | The J. A. Gosselin Co., Ltd. | (9/20/56) |
| | P. O. Box 280, Drummondville, Quebec, Canada | |
| 114 | C. E. Howard Corporation | (9/21/59) |
| | 9001 Rayo Avenue, South Gate, California | 90280 |
| 127 | Paul Mueller Company | (6/29/60) |
| | 1616 W. Phelps Street, Springfield, Missouri | 65801 |
| 197 | Paul Mueller (Canada), Ltd. | (9/ 9/67) |
| | 84 Wellington St., South, St. Marys, Ont. | |
| 213 | Sanitary Processing Equipment Corp. | (3/25/70) |
| | Butternut Drive E. Syracuse, N. Y. | 13057 |
| 31 | Walker Stainless Equipment Co. | (10/ 4/56) |
| | Elroy, Wisconsin | 53929 |

0204 Pumps for Milk and Milk Products

Revised, as Amended

| | | |
|------|---|------------|
| 214R | Ben H. Anderson Manufacturers | (5/20/70) |
| | Morrisonville, Wis. | 53571 |
| 212R | Babson Bros. Co. | (2/20/70) |
| | 2100 S. York Rd., Oak Brook, Ill. | 60621 |
| 29R | Cherry-Burrell Corporation | (10/ 3/56) |
| | 2400 Sixth St., S. W. Cedar Rapids, Iowa | 52406 |
| 63R | CP Division, St. Regis | (4/29/57) |
| | 1243 W. Washington Blvd., Chicago, Illinois | 60607 |
| 205R | Dairy Equipment Co. | (5/22/69) |
| | 1919 So. Stoughton Road, Madison, Wisc. | 53716 |
| 180R | The DeLaval Separator Co. | (5/ 5/66) |
| | Duchess Turnpike, Poughkeepsie, N. Y. | 12602 |
| 65R | G & H Products Corporation | (5/22/57) |
| | 5718 52nd Street, Kenosha, Wisconsin | 53140 |
| 145R | ITT Jabco, Incorporated | (11/20/63) |
| | 1485 Dale Way, Costa Mesa, Calif. | 92626 |
| 26R | Ladish Co., Tri-Clover Division | (9/29/56) |
| | 2809 60th Street, Kenosha, Wisconsin | 53140 |

| | | |
|------|--|------------|
| 148R | Robbins & Myers, Inc. | (4/22/64) |
| | Moyno Pump Division | |
| | 1345 Lagonda Ave., Springfield, Ohio | 45501 |
| 163R | Sta-Rite Products, Inc. | (5/ 5/65) |
| | 343 Wright Street, Delavan, Wisconsin | 53115 |
| 72R | L. C. Thomsen & Sons, Inc. | (8/15/57) |
| | 1303 53rd Street, Kenosha, Wisconsin | 53140 |
| 219 | Tri-Canada Limited | (2/15/71) |
| | 21 Newbridge Road, Toronto 18, Ont. | |
| 175R | Universal Milk Machine Div. | (10/26/65) |
| | National Cooperatives, Inc. | |
| | First Avenue at College, Albert Lea, Minn. | 56007 |
| 52R | Viking Pump Div.— | |
| | Houdaille Industries, Inc. | (12/31/56) |
| | 406 State Street, Cedar Falls, Iowa | 50613 |
| 5R | Waukesha Foundry Company | (7/ 6/56) |
| | Waukesha, Wisconsin | 53186 |

0402 Homogenizers and High Pressure Pumps of the Plunger Type, As Amended

| | | |
|----|--|------------|
| 87 | Cherry-Burrell Corporation | (12/20/57) |
| | 2400 Sixth Street, S. W., Cedar Rapids, Iowa | 52404 |
| 37 | CP Division, St. Regis | (10/19/56) |
| | 1243 W. Washington Blvd., Chicago, Illinois | 60607 |
| 75 | Manton, Inc. | (9/26/57) |
| | 44 Garden Street, Everett, Massachusetts | 02149 |

0506 Stainless Steel Automotive Milk Transportation Tanks for Bulk Delivery and/or Farm Pick-up Service, As Amended

| | | |
|------|--|------------|
| 131R | Almont Welding Works, Inc. | (9/ 3/60) |
| | 4091 Van Dyke Road, Almont, Michigan | 48003 |
| 98R | Beseler Steel Products, Inc. | (3/24/58) |
| | 417 East 29th, Marshfield, Wisconsin | 54449 |
| 70R | Jacob Brenner Company | (8/ 5/57) |
| | 450 Arlington, Fond du Lac, Wisconsin | 54935 |
| 40 | Butler Manufacturing Co. | (10/20/56) |
| | 600 Sixth Ave., S. E., Minneapolis, Minn. | 55114 |
| 118 | Dairy Craft, Inc. | (10/28/59) |
| | St. Cloud Industrial Park | |
| | St. Cloud, Minn. | 56301 |
| 66 | Dairy Equipment Company | (5/29/57) |
| | 1818 So. Stoughton Road, Madison, Wisconsin | 53716 |
| 123 | DeLaval Company, Ltd. | (12/31/59) |
| | 113 Park Street, South Peterborough, Ont., Canada | |
| 121 | The J. A. Gosselin Co., Ltd. | (12/ 9/59) |
| | P. O. Box 280, Drummondville, Quebec, Canada | |
| 45 | The Heil Company | (10/26/56) |
| | 3000 W. Montana Street, Milwaukee, Wisconsin | 53235 |
| 211 | Paul Krohnert Mfg., Ltd. | (4/ 1/68) |
| | West Hill, Ontario, Canada | |
| 80 | Paul Mueller (Canada), Ltd. | (11/24/57) |
| | 84 Wellington Street, So., St. Marys, Ont., Canada | |
| 85 | Polar Manufacturing Company | (12/20/57) |
| | Holdingsford, Minn. | 56340 |
| 144 | Portersville Equipment Company | (5/16/63) |
| | Portersville, Pennsylvania | 16051 |
| 71 | Progress Industries, Inc. | (8/ 8/57) |
| | 400 E. Progress Street, Arthur, Illinois | 61911 |
| 47 | Trailmobile, Div. of Pullman, Inc. | (11/ 2/56) |
| | 701 East 16th Ave., North Kansas City, Mo. | 64116 |
| 189 | A. & L. Tougas, Ltée | (10/ 3/66) |

**1400 Inlet and Outlet Leak Protector Plug Valves
for Batch Pasteurizers, As Amended**

- 122R Cherry-Burrell Corporation (12/11/59)
2400 Sixth St., S.W. Cedar Rapids, Iowa 52406
- 69 G & H Products Corporation (6/10/57)
5718 52nd Street, Kenosha, Wisconsin 53140
- 27 Ladish Co. - Tri-Clover Division (9/29/56)
2869 60th Street, Kenosha, Wisconsin 53140
- 78 L. C. Thomsen & Sons, Inc. (11/20/57)
1303 43rd Street, Kenosha, Wisconsin 53140

**1603 Evaporators and Vacuum Pans for Milk and
Milk Products, Revised**

- 132R A.P.V. Company, Inc. (10/26/60)
137 Arthur Street, Buffalo, New York 14207
- 11R Blaw-Knox Food & Chemical Equip., Inc. (2/12/59)
P. O. Box 1401
Buffalo, N. Y. 14210
- 11CR Arthur Harris & Company (11/10/58)
210-218 North Aberdeen Street, Chicago, Illinois 60607
- 164R Mora Industries, Inc. (4/25/65)
112 South Park Street, Mora, Minnesota 55051
- 107R C. E. Rogers Company (8/1/58)
8731 Witt Street, Detroit, Michigan 48209
- 186R Marriott Walker Corporation (9/6/66)
925 East Maple Road, Birmingham, Mich. 48008

**1702 Fillers and Sealers of Single Service Containers,
For Milk and Milk Products, As Amended**

- 192 Cherry-Burrell Corporation (1/3/67)
2400 Sixth St., S. W., Cedar Rapids, Iowa 52404
- 139 Exact Weight Scale Company (4/15/68)
944 West Fifth Ave., Columbus, O. 43212
- 137 Ex-Cell-O Corporation (10/17/62)
P. O. Box 386, Detroit, Michigan 48232
- 140 General Films, Inc. (4/23/63)
Covington, Ohio 55318
- 142 Polygal Company (4/15/63)
Div. of Inland Container Corp.
P. O. Box 68074, Indianapolis, Indiana 46268
- 210 Twinpak, Ltd. (2/4/70)
270 St. Joseph Blvd., Lachine, Quebec
- 211 Twinpak, Inc. (2/4/70)
1133 Avenue of the Americas, New York, N. Y. 10010

**1901 Batch and Continuous Freezers, For Ice Cream,
Ices and Similarly Frozen Dairy Foods, As Amended**

- 141 CP Division, St. Regis (4/15/63)
100 C. P. Avenue, Lake Mills, Wisconsin 53551
- 146 Cherry-Burrell Corporation (12/10/63)
2400 Sixth Street, S. W., Cedar Rapids, Iowa 52404

**2201 Silo-Type Storage Tanks for Milk and
Milk Products**

- 168 Cherry-Burrell Corporation (6/16/65)
575 E. Mill St., Little Falls N. Y. 13365
- 154 CP Division, St. Regis (2/10/65)
1243 W. Washington Blvd., Chicago, Illinois 60607
- 160 Dairy Craft, Inc. (4/5/65)
St. Cloud Industrial Park
St. Cloud, Minn. 56301
- 181 Damrow Company (5/18/66)
196 Western Ave., Fond du Lac, Wisconsin 54935

- 156 C. E. Howard Corporation (3/9/65)
9001 Rayo Avenue, South Gate, California 90280
- 155 Paul Mueller Co. (2/10/65)
1616 W. Phelps Street, Springfield, Missouri 65801
- 195 Paul Mueller (Canada), Ltd. (7/ /67)
84 Wellington St., So., St. Mary's, Ont., Canada
- 165 Walker Stainless Equipment Co. (4/26/65)
Elroy, Wisconsin 53929

**2300 Equipment for Packaging Frozen Desserts,
Cottage Cheese and Milk Products Similar to Cottage
Cheese in Single Service Containers**

- 174 Anderson Bros. Mfg. Co. (9/28/65)
1303 Samuelson Road, Rockford, Illinois 61109
- 209 Doughboy Industries, Inc. (7/23/69)
Machine Division
869 So. Main Ave., New Richmond, Wis. 54017
- 193 Triangle Package Machinery Co. (1/31/67)
6655 West Diversey Ave., Chicago, Illinois 60635

2400 Non-Coil Type Batch Pasteurizers

- 161 Cherry-Burrell Corporation (4/5/65)
575 E. Mill St., Little Falls, N. Y. 13365
- 158 CP Division, St. Regis (3/24/65)
Fort Atkinson, Wisconsin 53538
- 187 Dairy Craft, Inc. (9/26/66)
St. Cloud Industrial Park
St. Cloud, Minn. 56301
- 208 The DeLaval Separator Co., (7/23/69)
Duchess Turnpike, Poughkeepsie, N. Y. 12602
- 177 Girton Manufacturing Co. (2/18/66)
Millville, Pennsylvania 17846
- 166 Paul Mueller Co. (4/26/65)
1616 W. Phelps Street, Springfield, Missouri 65802
- 198 Paul Mueller (Canada), Ltd. (9/9/67)
84 Wellington St., So., St. Marys, Ont., Canada

**2500 Non-Coil Type Batch Processors for Milk and
Milk Products**

- 162 Cherry-Burrell Corporation (4/5/65)
575 E. Mill St., Little Falls, N. Y. 13365
- 159 CP Division, St. Regis (3/24/65)
Fort Atkinson, Wisconsin 53538
- 188 Dairy Craft, Inc. (9/26/66)
St. Cloud Industrial Park
St. Cloud, Minn. 56301
- 167 Paul Mueller Co. (4/26/65)
1616 W. Phelps Street, Springfield, Missouri 64801
- 196 Paul Mueller (Canada), Ltd. (7/6/67)
84 Wellington St., So., St. Marys, Ont., Canada
- 202 Walker Stainless Equipment Co. (9/24/68)
New Lisbon, Wis. 53950

2600 Sifters for Dry Milk and Dry Milk Products

- 171 Entoleter, Inc. (9/1/65)
Subsidiary of American Mfg. Co.
1187 Dixwell Avenue, Hamden, Connecticut 06514
- 173 Food & Chemical Equipment Div., (9/20/65)
Blaw-Knox Company
1325 S. Cicero Avenue, Chicago, Illinois 60650
- 185 The Orville-Simpson Co. (8/10/66)
1230 Knowlton St., Cincinnati, Ohio 45223
- 176 Sprout, Waldron & Co., Inc. (1/4/66)
Munsey, Pennsylvania 17756
- 172 SWECO, Inc. (9/1/65)
6111 E. Bandini Blvd., Los Angeles, California 90022

- 1 Tougas St., Iberville, Quebec, Canada
 25 Walker Stainless Equipment Co. (9/28/56)
 New Lisbon, Wisconsin 53950

**0808 Fittings Used on Milk and Milk Products
 Equipment, and Used on Sanitary Lines Conducting
 Milk and Milk Products, Revised**

- 79R Alloy Products Corporation (11/23/57)
 1045 Perkins Avenue, Waukesha, Wisconsin 53186
 138R A.P.V. (Canada) Equipment, Ltd. (12/17/62)
 103 Rivalda Rd., Weston, Ont., Canada
 82R Cherry-Burrell Corporation (12/11/57)
 2400 Sixth Street, S. W. Cedar Rapids, Iowa 52406
 124R DeLaval Company, Ltd. (2/18/60)
 113 Park Street, South, Peterborough, Ont., Canada
 184R The DeLaval Separator Co. (8/ 9/66)
 Duchess Turnpike, Poughkeepsie, N. Y. 12602
 67R G & H Products Corporation (6/10/57)
 5718 52nd Street, Kenosha, Wisconsin 53140
 199R Grayco, Inc. (12/ 8/67)
 60 Eleventh Ave., N.E., Minneapolis, Minn. 55413
 203R Grinnell Company (11/27/68)
 260 W. Exchange St., Providence, R. I. 02901
 204R Hills McCanna Company (2/10/69)
 400 Maple Ave., Carpentersville, Ill. 60110
 34R Ladish Co., Tri-Clover Division (10/15/56)
 2809 60th St., Kenosha, Wisconsin 53140
 200R Paul Mueller Co. (3/ 5/68)
 1616 Phelps St., Springfield, Mo. 65601
 149R Q Controls (5/18/64)
 Occidental, California 95465
 89R Sta-Rite Industries, Inc. (12/23/68)
 343 Wright Street, Delavan, Wis. 53115
 73R L. C. Thomsen & Sons, Inc. (8/31/57)
 1303 43rd Street, Kenosha, Wisconsin 53140
 191R Tri-Canada Fittings & Equipment, Ltd. (11/23/66)
 21 Newbridge Road, Toronto 18, Ontario
 151R Tubular Components, Inc. (11/18/64)
 Butternut Drive, East Syracuse, New York 13057
 215R Universal Milking Machine Div., (7/31/70)
 National Cooperatives, Inc.,
 First Avenue at College, Albert Lea, Minn. 56007
 86R Waukesha Specialty Company (12/20/57)
 Walworth, Wisconsin 53184
 218 Highland Equipment Corporation (2/12/71)
 175 Stockholm St. Brooklyn, N.Y. 11237

**0902 Thermometer Fittings and Connections Used
 on Milk and Milk Products Equipment and
 Supplement 1, As Amended**

- 32 Taylor Instrument Companies (10/ 4/56)
 95 Ames Street, Rochester, New York 14611
 206 The Foxboro Company (8/11/69)
 Neponset Ave., Foxboro, Mass. 02035

**1002 Milk and Milk Products Filters Using Disposable
 Filter Media, As Amended**

- 35 Ladish Co., Tri-Clover Division (10/15/56)
 2809 60th Street, Kenosha, Wisconsin 53140

**1102 Plate-Type Heat Exchangers for Milk and Milk
 Products, As Amended**

- 20 A.P.V. Company, Inc. (9/ 4/56)
 137 Arthur Street, Buffalo, New York 14207

- 30 Cherry-Burrell Corporation (10/ 1/56)
 2400 Sixth Street, S.W., Cedar Rapids, Iowa 52404
 14 Chester-Jensen Co., Inc. (8/15/56)
 5th & Tilgham Streets, Chester, Pennsylvania 19013
 38 CP Division, St. Regis (10/19/56)
 1243 W. Washington Blvd., Chicago, Illinois 60607
 120 DeLaval Company, Ltd. (12/ 3/59)
 113 Park Street, South, Peterborough, Ont., Can.
 17 The DeLaval Separator Company (8/30/56)
 Duchess Turnpike, Poughkeepsie, N. Y. 12602
 15 Kusel Dairy Equipment Company (8/15/56)
 100 W. Milwaukee Street, Watertown, Wisconsin 53094

**1202 Internal Return Tubular Heat Exchangers,
 for Milk and Milk Products, As Amended**

- 103 Chester-Jensen Company, Inc. (6/ 6/58)
 5th & Tilgham Street, Chester, Pennsylvania 19013
 96 C. E. Rogers Company (3/31/64)
 8731 Witt Street, Detroit, Michigan 48209
 152 The DeLaval Separator Co. (11/18/69)
 350 Duchess Turnpike, Poughkeepsie, N. Y. 12602
 217 Girton Manufacturing Co. (1/23/71)
 Millville, Pa. 17846

**1303 Farm Milk Cooling and Holding Tanks—
 Revised, As Amended**

- 11R CP Division, St. Regis (7/25/56)
 1243 W. Washington Street, Chicago, Illinois 60607
 119R Dairy Craft, Inc. (10/28/59)
 St. Cloud Industrial Park, St. Cloud, Minn. 56301
 4R Dairy Equipment Company (6/15/56)
 1919 S. Stoughton Road, Madison, Wisconsin 53716
 92R DeLaval Company, Ltd. (12/27/57)
 113 Park Street, South Peterborough, Ontario, Canada
 49R The DeLaval Separator Company (12/ 5/56)
 Duchess Turnpike, Poughkeepsie, N. Y. 12602
 10R Girton Manufacturing Company (7/25/56)
 Millville, Pennsylvania 17846
 95R Globe Fabricators, Inc. (3/14/58)
 7744 Madison Street, Paramount, California 90723
 179R Heavy Duty Products (Preston), Ltd. (3/ 8/66)
 635 Laurel St., Preston, Ont., Canada
 12R Paul Mueller Company (7/31/56)
 1616 W. Phelps Street, Springfield, Missouri 65801
 58R Schweitzer's Metal Fabricators, Inc. (2/25/57)
 806 No. Todd Avenue, Azusa, California 91702
 134R Universal Milking Machine Division (5/19/61)
 National Co-operatives, Inc.
 First Avenue at College, Albert Lea, Minn. 56007
 216R Valco Manufacturing Company (10/22/70)
 3470 Randolph St., Huntington Pk., Calif. 90256
 42R VanVetter, Inc. (10/22/56)
 2130 Harbor Avenue S.W., Seattle, Washington 98126
 18R Whirlpool Corporation, St. Paul Division (9/20/56)
 850 Arcade Street, St. Paul, Minnesota 55106
 55R John Wood Company (1/23/57)
 Superior Metalware Division
 509 Front Avenue, St. Paul, Minnesota 55117
 17CR The W. C. Wood Co., Ltd. (8/ 9/65)
 5 Arthur Street, South, Guelph, Ont., Canada
 16R Zero Manufacturing Company (8/27/56)
 Washington, Missouri 63090

E-3-A SANITARY STANDARDS FOR MULTIPLE-USE RUBBER AND RUBBER-LIKE MATERIALS USED AS PRODUCT CONTACT SURFACES IN EGG PROCESSING EQUIPMENT

Serial #E-1800

Formulated by
International Association of Milk, Food and Environmental Sanitarians
United States Public Health Service
United States Department of Agriculture
Institute of American Poultry Industries
Dairy and Food Industries Supply Association

It is the purpose of the IAMFES, USPHS, USDA, IAPI, and DFISA in connection with the development of the E-3-A Sanitary Standards program, to allow and encourage full freedom for inventive genius or new developments.

Multiple-Use Rubber and Rubber-Like Materials to be used as product contact surfaces in egg processing equipment heretofore or hereafter developed which so differ in specifications or otherwise as not to conform with the following standards, but which in the opinion of the manufacturer or fabricator are equivalent or better, may be submitted at any time for the consideration of IAMFES, USPHS, USDA, IAPI, and DFISA.

A. SCOPE

These sanitary standards cover the requirements of rubber and rubber-like materials for multiple use as product contact surfaces in equipment for production, processing, and handling of liquid egg and egg products. Test criteria are provided for rubber and rubber-like materials as a means of determining their acceptance as to their ability to be cleaned and to receive effective bactericidal treatment and to maintain their essential properties under repeated use conditions. In order to conform with these E-3-A Sanitary Standards multiple-use rubber and rubber-like materials shall comply with the following material, physical properties and fabrication criteria.

B. DEFINITIONS

For the purpose of these sanitary standards, the following definitions and classifications shall apply:

(1) **RUBBER AND RUBBER-LIKE MATERIALS:** Shall mean resilient (see Appendix A) compounds having natural and/or synthetic origins deriving their physical and chemical properties from chemical vulcanization.

(2) **TEMPERATURE OF EXPOSURE:** Temperatures to which material is subjected in contact with the product and/or cleaning and bactericidal treatment.

(3) **CLASSIFICATIONS:**

Class I—Temperature of exposure to product up to 300°F, and temperature of exposure to chem-

ical solution used in cleaning and bactericidal treatment up to 180°F. This classification applies when 50% or more of the entire surface area is in contact with the product.

Class II—Temperature of exposure to product up to 300°F, and temperature of exposure to chemical solution used in cleaning and bactericidal treatment up to 180°F. This classification applies when less than 50% of the entire surface area is in contact with the product.

Class III—Temperature of exposure to product up to 120°F, and temperature of exposure to chemical solution used in cleaning and bactericidal treatment up to 180°F. This classification applies when less than 50% of the entire surface area is in contact with the product.

Class IV—Temperature of exposure to product up to 100°F, and temperature of exposure to chemical solution used in cleaning and bactericidal treatment up to 180°F.

Note: See Appendix B for examples.

C. MATERIAL

Rubber and rubber-like material having any surface in contact with the product shall be non-toxic, relatively non-absorbent, relatively resistant to fat, resistant to normal cleaning and bactericidal solutions, readily cleanable, relatively insoluble, relatively stable in the environment of its intended use, and shall not adversely affect the product.

The minimum physical properties of the finished rubber and rubber-like materials, as determined by

the testing procedures specified, are the following:

I. Absorption

| | Class I | Class II | Class III | Class IV |
|--|------------|-------------|--------------|-------------|
| 1. Butteroil ¹ 22 ± 1/4 hour @158°F, (ASTM #D471) Maximum hardness change, type Shore A points | 6 | 15 | 20 | 20 |
| Maximum loss or gain— % by WT. | 5 | 25 | 25 | 60 |
| % by Vol. | 5 | 25 | 25 | 75 |
| 2. Distilled water: 22 ± 1/4 hour @ 158°F (ASTM #D471) Maximum hardness change, type Shore A points | 6 | 10 | 10 | 10 |
| Maximum loss or gain % by WT. | 5 | 15 | 15 | 20 |
| % by Vol. | 5 | 15 | 15 | 25 |

II. Stability

| | | | | |
|--|-----|-----|-----|-----|
| 1. Air Aging (ASTM #D573) Maximum hard- ness change, type Shore A points 166 ± 1/2 hour Air Oven @ 212°F ² | 20 | 20 | — | — |
| 166 ± 1/2 hour Air Oven @ 158°F ³ | — | — | 15 | 15 |
| 2. Original Tensile Strength, psi minimum (Sample prepared according to ASTM #D412) | 500 | 500 | 500 | 500 |

¹Butteroil may be prepared by melting butter at 150°F, placing in a graduate and pouring off the oil portion on top. It will be approximately 97% milk fat.

²For Class I and II only.

³For Class III and IV only.

| | Class I | Class II | Class III | Class IV |
|---|------------|-------------|--------------|-------------|
| 3. Original Elongation, % minimum (Sample prepared according to ASTM #D412) | 75 | 75 | 75 | 75 |
| 4. Original Hard- ness Range, Durometer Type Shore A points (ASTM #D676) | 50-90 | 40-90 | 35-90 | 30-90 |

D. FABRICATION

The surface of finished rubber or rubber-like fabricated parts shall be at least as cleanable as stainless steel having a 120 grit finish properly applied. Conformance with this item shall be judged by comparing the removal of standard soil from finished rubber or rubber-like fabricated parts with the removal of such soil from stainless steel having a 120 grit finish.

APPENDIX A

Hard Rubber

Hard rubber is a vulcanized rubber having a ratio of combined sulfur to rubber hydrocarbon in excess of 15% and a Shore A Durometer value in excess of 90. It is not considered a part of this standard due to its special characteristics foreign to rubber as normally used in the egg processing industry.

APPENDIX B

Examples

Rubber classes, as provided for in B. (3):

Class I—Some heat exchanger gaskets, "O" rings, C-I-P gaskets, flange gaskets, rotating seals.

Class II—Plate heat exchanger gaskets, homogenizer seals, static seals.

Class III—Cold applications such as egg and air tubing, manhole and door gaskets, seals.

Class IV—Conveyor spools, vacuum lifters.

These standards shall become effective May 7, 1971.

E-3-A ACCEPTED PRACTICES FOR SUPPLYING AIR UNDER PRESSURE IN CONTACT WITH LIQUID EGG AND EGG PRODUCTS AND PRODUCT CONTACT SURFACES

Serial #E-60400

Formulated by
International Association of Milk, Food and Environmental Sanitarians
United States Public Health Service
United States Department of Agriculture
Institute of American Poultry Industries
Dairy and Food Industries Supply Association

It is the purpose of the IAMFES, USPHS, USDA, IAPI and DFISA in connection with the development of the E-3-A Sanitary Standards program to allow and encourage full freedom for inventive genius or new developments. Practices for supplying air under pressure heretofore or hereafter developed which so differ in material, fabrication and installation or otherwise as not to conform with the following practices, but which, in the opinion of the operator, manufacturer or fabricator are equivalent or better, may be submitted for the joint consideration of IAMFES, USPHS, USDA, IAPI and DFISA, at any time.

A. SCOPE

These E-3-A Accepted Practices shall pertain to the equipment used in the supplying of air under pressure which comes in contact with liquid egg and egg products and/or any product contact surface. All equipment for supplying air as defined herein shall be considered meeting these E-3-A Accepted Practices when they comply with C. Material, D. Fabrication and Installation, and the applicable Special Requirements E., F., G., H., or I., as specified hereafter.

B. DEFINITIONS

- (1) **AIR UNDER PRESSURE:** Shall mean air, the pressure of which has been increased by mechanical means to exceed atmospheric pressure, and which is used for agitation of liquid egg and egg products, the movement of liquid egg and egg products, the automatic opening of containers, the drying of product contact surfaces, and for other purposes where specifically directed at a product contact surface.
- (2) **LOW PRESSURE AIR:** Shall mean air under pressure which does not exceed 300 p.s.i.
- (3) **HIGH PRESSURE AIR:** Shall mean air under pressure which is in excess of 300 p.s.i.
- (4) **AIR SYSTEMS:** Air systems are of two general categories:
 - (a) **Central System:** Shall mean those which furnish air to more than one piece of equipment. (See Figure No. 1). Such systems usually require the use of an air storage tank.
 - (b) **Individual System:** Shall mean those which furnish air to one piece of equipment,

and which may be an integral part of a given piece of equipment. (See Figures No. 2, No. 3 and No. 4).

- (5) **PRODUCT:** Shall mean whole egg, egg yolk, egg white, blends with or without added materials and in a liquid or frozen or dried form.
- (6) **PRODUCT CONTACT SURFACE:** Shall mean all surfaces that are exposed to the product, or from which liquid may drain, drop or be drawn into the product.
- (7) **NON-PRODUCT CONTACT SURFACE:** Shall mean all other exposed surfaces.

C. MATERIAL

(1) FILTER MEDIA

Intake and air pipeline filters shall consist of fiberglass, cotton flannel, wool flannel, spun metal, sintered metal, activated carbon, activated alumina, non-woven fabric, absorbent cotton fibre, electrostatic, or other suitable materials which, under conditions of intended use, are non-toxic and non-shedding and which do not release toxic volatiles or other contaminants to the air, or volatiles which may impart any flavor or odor to the product or interfere with organoleptical examination of the product. Chemical bonding materials contained in the media shall be non-toxic, non-volatile and insoluble under all conditions of use. Disposable media are not intended to be cleaned and re-used.

(2) FILTER PERFORMANCE

(a) **Intake Filters:** The efficiency of intake filters shall be at least 50% as measured by

the National Bureau Standards "Dust Spot Method",¹ using atmospheric dust as the test aerosol. In an aggravated atmospheric environment, e.g., industrial district, prefilters are recommended to prolong the useful life of intake filters.

(b) *Air Pipeline and Disposable Filters*: The efficiency of either air pipeline filters or disposable filters shall be at least 50% as measured by the DOP² test.

- (3) *PIPING*: Air distribution piping, fittings, and gaskets between the downstream terminal filter and the processing equipment shall conform to "3-A Sanitary Standards for Fittings Used on Milk and Milk Products Equipment and Used on Sanitary Lines Conducting Milk and Milk Products, Serial #0809".

Exceptions to this requirement are made

- (1) For compressing equipment of the fan type, blower type or high pressure type.
- (2) As provided in Section H.
- (3) For the use of transparent plastic tubing in air distribution piping in which the piping, fittings and gaskets do not actually contact the product or form a part of the product contact surfaces.

D. FABRICATION AND INSTALLATION

(1) *Air Supply Equipment*:

- (a) The air supply shall be taken from a clean space or from relatively clean outer air and shall pass through a filter upstream from the compressing equipment. This filter shall be so located and constructed that it is easily accessible for examination, and the filter media are easily removable, for cleaning or replacement. This filter shall be protected from weather, drainage, water, product spillage, and physical damage.
- (b) Relatively oil free air may be produced by one of the following known methods or its equivalent:
 - (i) Use of carbon or teflon ring piston, or diaphragm type, or water-lubricated compressors.

- (ii) Use of oil-lubricated compressors with effective provision for removal of oil.

- (iii) Water-lubricated or non-lubricated blowers.

(c) Where it is necessary to store air, an air tank(s), if used, should meet the requirements of ASME and/or National Board of Underwriters Code for unfired pressure vessels.

- (2) *MOISTURE REMOVAL EQUIPMENT*: If necessary to cool the compressed air, a liquid-cooled aftercooler shall be installed between the compressor and the air storage tank for the purpose of removing moisture from the compressed air, except that a compressor the design of which incorporates the aftercooling function does not require a separate aftercooler. (See Figure No. 1). Other moisture removal equipment may be used downstream from the compressing equipment prior to the final point of application. The resultant condensate from the aftercooler shall flow to a properly trapped outlet and shall be discharged to the atmosphere.

(3) *FILTERS AND MOISTURE TRAPS*:

- (a) Filters shall be constructed so as to assure effective passage of air through the filter media only.
- (b) The air under pressure shall pass through an oil-free filter and moisture trap for removal of solids and liquids. The filter and trap shall be located in the air pipeline downstream from the compressing equipment, and from the air tank, if one is used (See Figures No. 1 and No. 2). The filter shall be readily accessible for examination, cleaning, and for replacing the filter media. The moisture trap shall be equipped with a petcock or other means for draining accumulated water. Air pipeline filters and moisture traps downstream from compressing equipment shall not be required where the compressing equipment is of the fan or blower type (See Figures No. 3 and No. 4).
- (c) A disposable media filter shall be located in the sanitary air pipeline upstream from and as close as possible to each point of application or ultimate use of the air (See Figures No. 1 and No. 2) except that a

¹For a description of this method see: Dill, R. S. A test method for air filters. American Society Heating and Vent. Eng. Trans., Vol. 44, P. 379, 1938.

²Dioctylphthalate fog method ("DOP"). For a description of this test see: Military Standard No. 282 (MIL-STD-282, 28 May, 1956) Method 102.9.1; Naval Supply Depot, 5801 Tabor Avenue, Philadelphia, Pa. 19120.

disposable media filter shall not be required for high pressure air lines or where the compressing equipment is of the fan or blower type (See Figures No. 3 and No. 4).

- (d) Filters other than those in (b) above shall not be required on high pressure lines.
- (4) *AIR PIPING*: The requirements of D.(4)(a) which follow do not apply where the compressing equipment is of the fan or blower type.
- (a) The air piping from the compressing equipment to the filter and moisture trap described under D.(3)(b) shall be readily drainable.
- (b) A product check valve of sanitary design which complies with the criteria set forth in Section E.1 of "3-A Sanitary Standards for Fittings Used on Milk and Milk Products Equipment and Used On Sanitary Lines Conducting Milk and Milk Products, Serial #0809" shall be installed in the air piping downstream from the disposable media filter described in D. (3)(c) to prevent backflow of product into the air pipeline; except that a check valve shall not be required if the air piping enters the product zone from a point higher than the product overflow level which is open to atmosphere. These criteria do not apply to high pressure air lines. (See section I.).

E. SPECIAL REQUIREMENTS FOR AGITATION BY AIR

- (1) Tubing used to introduce air into the product and/or product zone shall be of stainless steel and shall conform to "3-A Sanitary Standards for Fittings Used on Milk and Milk Products Equipment and Used on Sanitary Lines Conducting Milk and Milk Products, Serial #0809".
- (2) No threaded fitting shall be used in the product zone.
- (3) Where drilled or perforated pipe is used, internal drilling burrs shall be removed and the orifices shall be chamfered on the outer surface of the pipe.
- (4) If the volume of the air from the compressing equipment is in excess of that required for satisfactory agitation, suitable means should be employed to eliminate the excess volume.

- (5) If the product to be agitated is in an enclosed tank, means to allow the air used for agitation to escape should be provided on the tank by a vent or a safety valve as described in F. (2).

F. SPECIAL REQUIREMENTS FOR THE MOVEMENT OF PRODUCTS BY THE AIR DISPLACEMENT METHOD.

- (1) The requirements of E.(1), E.(2) and E.(3) shall also apply to this section.
- (2) A safety (pressure relief) valve should be installed in the air line. This valve should be set to open upon reaching a pressure not greater than the maximum allowable internal working pressure specified by the manufacturer of tank from which the product is to be moved. This safety valve should have ample capacity to pass freely the entire output of the compressor.
- (3) The safe internal working pressure of the tank should be stated on a plate attached to the tank.
- (4) The check valve specified in D.(4)(b) shall be installed in the air piping wherever air is used for displacement purposes.

G. SPECIAL REQUIREMENTS FOR AIR WHICH IS TO BE INCORPORATED IN PRODUCTS

An air system in which the air is compressed by a sanitary rotary pump shall require only an intake air filter which shall be of the disposable media type. Non-sanitary air line should be pitched away from sanitary air inlet pipeline, or a transparent sump shall be provided to collect any moisture or scale that may originate from the non-sanitary air line.

H. SPECIAL REQUIREMENTS FOR MOVING CONTAINERS FROM ROTATING MANDRELS

- (1) When air under pressure is used for moving containers from rotating mandrel assemblies with integral air passages, the parts forming the air passages shall be of non-toxic, relatively non-absorbent materials.
- (2) A disposable media filter shall be located at the closest possible point upstream from the rotating mandrel assembly. (See Figure No. 5).

I. SPECIAL REQUIREMENTS FOR HIGH PRESSURE AIR WHICH IS TO BE INCORPORATED IN PRODUCTS

When high pressure air is to be incorporated in products:

(1) Stainless steel piping, tubing, and fittings in conformity with applicable ASA Standards for high pressure air should be used downstream from the filter.

(2) A high pressure stainless steel check valve

should be installed upstream from and as near as possible to the point of introduction of air to the product line.

These Practices shall become effective May 7, 1971.

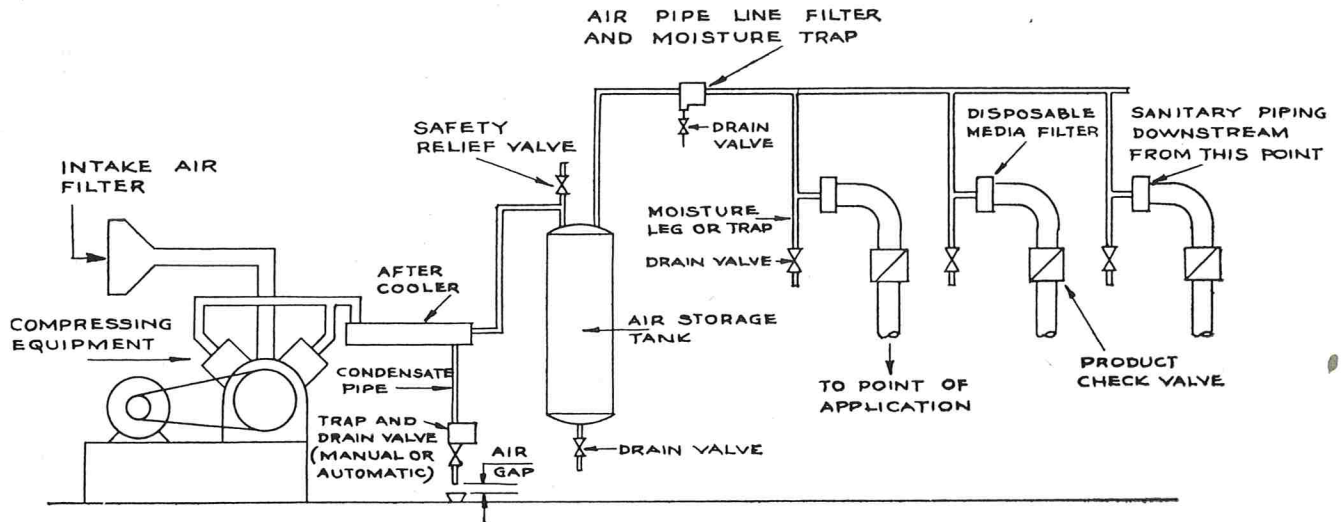


FIG. 1 CENTRAL SYSTEM

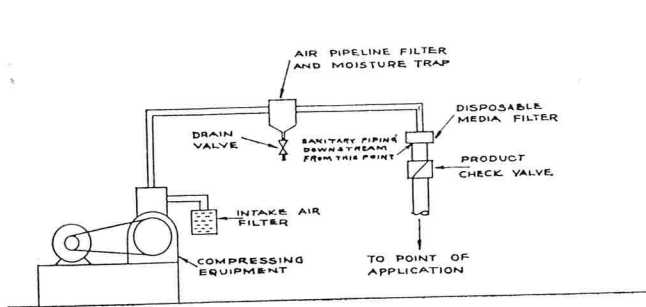


FIG. 2 INDIVIDUAL SYSTEM

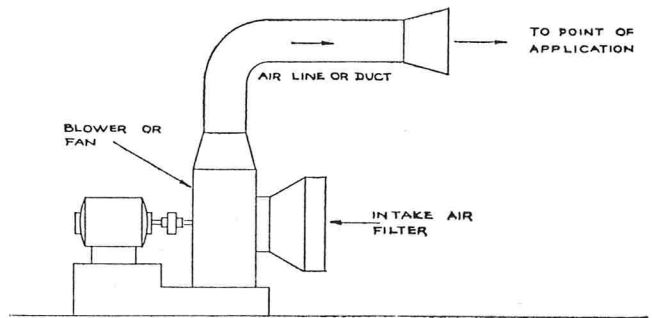


FIG. 3 INDIVIDUAL SYSTEM

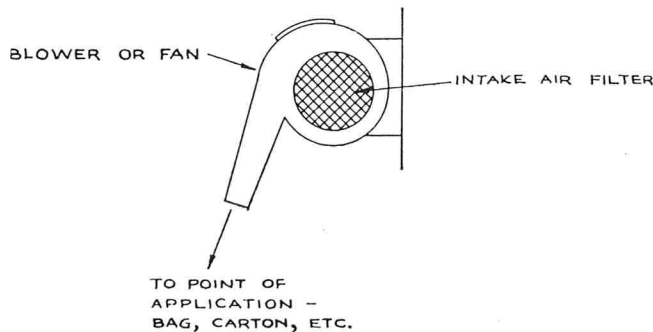


FIG. 4 INDIVIDUAL SYSTEM

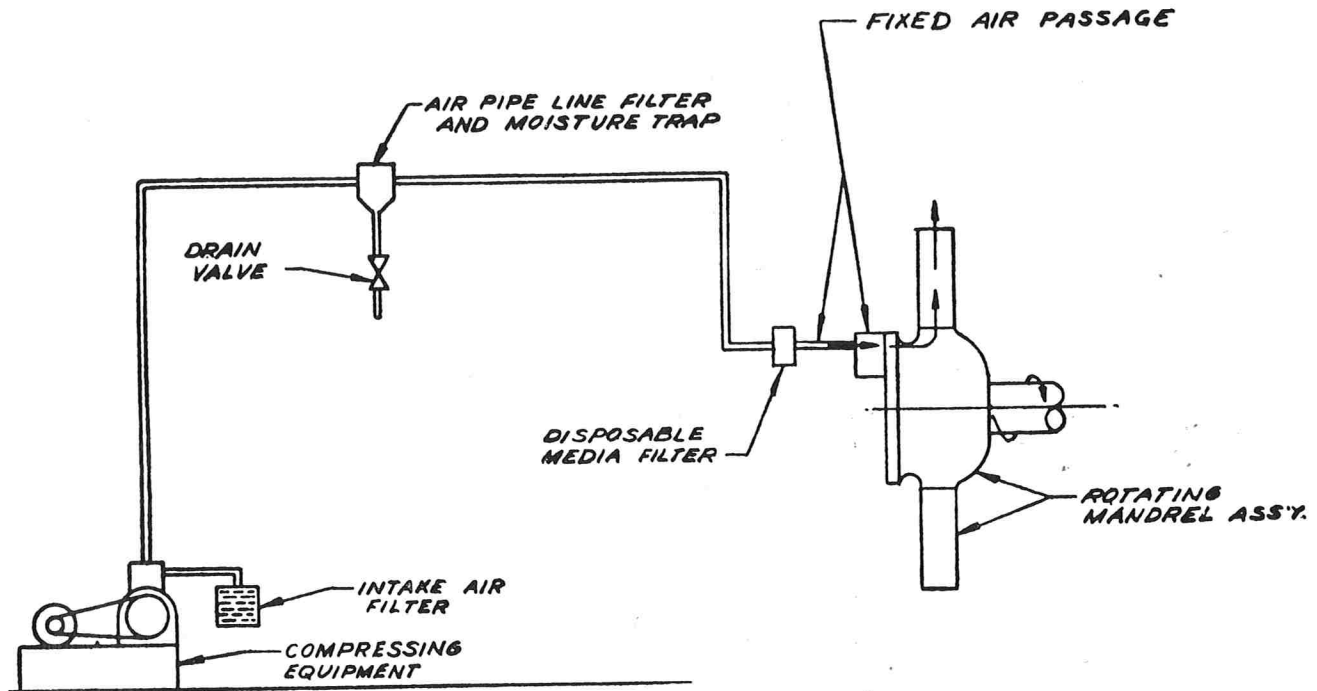


FIG. 5

ROTATING MANDREL ASSEMBLYREPORT OF COMMITTEE ON APPLIED
LABORATORY METHODS

(Continued from Page 149)

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Mr. Edmond L. Sing, Moseley Laboratories, 3862 East Washington Street, Indianapolis, Indiana 46201.

Mr. Donald I. Thompson, State Laboratory of Hygiene, State Board of Health, 437 Henry Mall, Madison, Wisconsin 53706.

SUBCOMMITTEE ON LABORATORY METHODS FOR THE
EXAMINATION OF FOODS

Dr. Edmund A. Zottola, *Chairman*, Dept. of Food Science and Industries, Institute of Agriculture, U. of Minnesota, St. Paul, Minnesota 55101.

Mr. David Q. Anderson, (deceased), Utah State Department of Health, 44 Medical Drive, Salt Lake City, Utah 84113.

Dr. Robert A. Angelotti, Deputy Director, Division of Microbiology, Food and Drug Administration, 200 "C" Street, S. W., Washington, D. C. 20204.

Dr. F. F. Busta, Department of Food Science and Industries, University of Minnesota, St. Paul, Minnesota 55101.

Dr. Herbert E. Hall (deceased), 1090 Tusculum Avenue, Cincinnati, Ohio 45226.

Dr. Laurence G. Harmon, Department of Food Science, Michigan State University, East Lansing, Michigan 48823.

Mr. Donald Pusch, Division of Laboratory Service, State Dept. of Agriculture, Room 510, State Office Building, St. Paul, Minnesota 55101.

SUBCOMMITTEE ON LABORATORY METHODS FOR THE
EXAMINATION OF WATER AND OTHER ENVIRONMENTAL SAMPLES

Dr. Martin Favero, *Chairman*, Ecological Investigations Program, Phoenix Laboratories, National Communicable Disease Center, 4402 North Seventh Street, Phoenix, Arizona 85014.

Dr. John C. Hoff, Environmental Control Administration, North Western Water Hygiene Laboratory, Route 4, Box 4129, Gig Harbor, Washington 98335.

Dr. R. L. Morris, State Hygienic Laboratory, State University of Iowa, Iowa City, Iowa 52241.

Mr. Arnold Salinger, Bureau of Laboratories, Maryland State Department of Health, Baltimore, Maryland 21218.

E-3-A ACCEPTED PRACTICES FOR PERMANENTLY INSTALLED SANITARY PRODUCT-PIPELINES AND CLEANING SYSTEMS

Serial #E-60500

Formulated by
International Association of Milk, Food and Environmental Sanitarians
United States Public Health Service
United States Department of Agriculture
Institute of American Poultry Industries
Dairy and Food Industries Supply Association

It is the purpose of the IAMFES, USPHS, USDA, IAPI, and DFISA in connection with the development of the E-3-A Sanitary Standards program, to allow and encourage full freedom for inventive genius or new developments.

Practices for permanently installed sanitary product-pipelines and cleaning systems heretofore or hereafter developed which so differ in design, material, fabrication, or otherwise as not to conform with the following practices, but which, in the fabricator's opinion, are equivalent or better may be submitted for the joint consideration of IAMFES, USPHS, USDA, IAPI, and DFISA at any time.

A. SCOPE

These E-3-A Accepted Practices provide for the installation, cleaning, and sanitizing of rigid sanitary pipelines for liquid eggs and liquid egg products in which the joints are welded or are provided with appropriate sanitary C-I-P connections. These E-3-A Accepted Practices also include provisions for rigid cleaning solution lines and cleaning systems.

B. DEFINITIONS

(1) *C-I-P Pipelines*: Shall mean rigid pipelines which have welded joints or have sanitary cleaned-in-place connections or joints of such design as to form a substantially smooth, flush interior surface.

(2) *Tungsten Shielded Arc Method*: Shall mean electric welding with a tungsten electrode shielded by an inert gas, to produce a straight butt fusion weld.

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

(4) *Solution Contact Surfaces*: Shall mean the interior surfaces of the circuit which are used exclusively for supply and re-circulation of cleaning and/or sanitizing solutions.

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

C. MATERIALS

(1) All product contact surfaces shall be of stain-

less steel of the AISI 300 series¹ or corresponding ACI² types (See Appendix, Section A), or equally corrosion resistant metal that is non-toxic and non-absorbent or heat resistant glass piping, except that rubber or rubber-like materials or plastic materials may be used for sealing applications. Paper gaskets shall not be used. Metal with free copper shall not be used.

(2) Solution contact surfaces shall be of stainless steel of the AISI 300 series¹ or corresponding ACI² types (See Appendix, Section A.), or equally corrosion resistant metal that is non-toxic and non-absorbent or of heat resistant glass piping; provided that plastic or rubber and rubber-like materials may be used for sealing applications and short flexible take-down jumpers or connectors.

(3) Plastic materials used for sealing applications and solution contact surfaces shall conform with the applicable provisions of the "3-A Sanitary Standards for Multiple-Use Plastic Materials Used as Product Contact Surfaces for Dairy Equipment, Serial #2000.", as amended.

(4) Rubber and rubber-like materials used for sealing applications and solution contact surfaces shall conform with "E-3-A Sanitary Standards for Multiple-Use Rubber and Rubber-Like Materials Used

¹The data for this series are contained in the following reference: AISI Steel Products Manual, Stainless and Heat Resisting Steels, April, 1963, Table 2-1, pp. 16-17. Available from: American Iron and Steel Institute, 633 3rd Avenue, New York, N.Y. 10017.

²Alloy Casting Institute, 300 Madison Ave., New York, N.Y. 10017.

as Product Contact Surfaces in Egg Equipment, Serial #1800-E."

(5) Lines and fittings for the application of air under pressure shall comply with the applicable provisions of "E-3-A Accepted Practices for Supplying Air Under Pressure in Contact With Egg Products, and Product Contact Surfaces, Serial #60400."

D. FABRICATION

(1) All product contact surfaces of sanitary piping utilized in C-I-P systems shall be at least as smooth as a No. 4 mill finish on stainless steel sheets. (See Appendix, Section B.).

(2) (a) All solution contact surfaces except those of castings for pumps or other appurtenances shall be at least as smooth as No. 4 mill finish on stainless steel sheets. This does not preclude the use of stainless steel sheets with No. 2B pit free finish for all solution contact surfaces.

(b) The solution contact surfaces of castings for pumps or other appurtenances shall be at least as smooth as ACI Surface Indicator Scale SIS-3, (See Appendix, Section D.) and shall be at least as cleanable as stainless steel sheet having a No. 4 mill finish. Tooled or polished areas of castings for pumps and appurtenances shall be at least as smooth as a No. 4 mill finish on stainless steel sheets. (See Appendix, Section B.).

(3) Product lines and equipment shall have C-I-P fittings or welded joints.

(4) Solution lines and equipment shall have C-I-P fittings or welded joints.

(5) Welded joints shall be smooth and free from pits, cracks, inclusions, or other defects.

(6) Removable fittings may be used with or without gaskets and shall be of such design as to form substantially flush interior joints.

(7) Appurtenances having product contact surfaces shall be cleanable, either when in an assembled position or when disassembled and shall be accessible for inspection to determine freedom from biological, chemical or physical soil contamination. Removable parts shall be readily demountable.

(8) All internal angles of 135° or less on product contact surfaces shall have minimum radii of 1/4 inch except where smaller radii are required for essential functional reasons, such as sealing ring grooves and/pumps.

(9) All internal angles of 135° or less on solution

contact surfaces shall have minimum radii of 1/4 inch except where smaller radii are required for essential functional reasons, such as for sealing ring grooves and in pumps.

(10) All solution contact surfaces shall be cleanable, either when in an assembled position or when disassembled. They shall contain no pockets or crevices that are not readily cleanable. Removable parts shall be readily demountable. Solution system appurtenances shall be accessible for inspection to determine freedom from biological, chemical, or physical soil contamination.

(11) Non-product contact surfaces shall have a smooth finish, be free of pockets and crevices, and be readily cleanable.

E. INSTALLATION

C-I-P pipeline circuits shall meet the following installation criteria:

(1) The C-I-P pipelines together with gaskets, if used, shall be supported so that they remain in alignment and position. The support system shall be designed so as to preclude electrolytic action between support(s) and pipeline(s).

(2) Each separate cleaning circuit, including product and solution lines, shall be provided with a sufficient number of access points, such as valves, fittings, or removable sections to make possible adequate inspections and examinations of the interior surfaces.

(3) Relatively horizontal lines shall be self-draining and pitched to drain points.

(4) Upon completion of welded pipeline installation and prior to use all interior line and weld areas shall be subjected to circulation of cleaning solution of 0.5 to 1.0% alkalinity at a minimum of 160°F. for 30 minutes, followed by an adequate post rinse, followed by circulation of a 0.5% minimum and 1% maximum phosphoric or nitric acid solution at 150-180°F. for 10 minutes to clean all interior surfaces of ferric impurities. This treatment shall be followed by an adequate rinse.

F. LAYOUT AND ENGINEERING REQUIREMENTS

(1) Prior to installation a drawing or equivalent plan shall be made available to the regulatory agency by the processor for each installation, or subsequent addition or modification, showing each permanent circuit to be cleaned, noting thereon the size and lengths of piping, fittings, pitch, drain points, access points, relative elevations, location and specifications of circulating unit, and other pertinent facts.

(2) The circulating unit, consisting of a motor

TABLE I
TIME, IN SECONDS, TO DELIVER 10 GALLONS AT VARIOUS VELOCITIES

Average velocity, feet per second

| Nominal Pipe Size | Inside Dimen- sion | Average velocity, feet per second | | | | | | | | | |
|-------------------------|--------------------------|-----------------------------------|-------|-------|------|------|------|------|------|------|------|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Inches | | Stainless Steel Sanitary Tubing | | | | | | | | | |
| 1 | 0.900 | 301.2 | 150.6 | 100.3 | 75.3 | 60.2 | 50.3 | 43.1 | 37.7 | 33.4 | 30.1 |
| 1-½ | 1.400 | 124.7 | 62.4 | 41.5 | 31.2 | 24.9 | 20.8 | 17.8 | 15.6 | 13.8 | 12.5 |
| 2 | 1.875 | 70.1 | 35.0 | 23.3 | 17.5 | 14.0 | 11.7 | 10.0 | 8.8 | 7.8 | 7.0 |
| 2-½ | 2.375 | 43.6 | 21.8 | 14.3 | 10.9 | 8.8 | 7.2 | 6.2 | 5.5 | 4.77 | 4.4 |
| 3 | 2.875 | 29.8 | 14.88 | 9.9 | 7.4 | 6.0 | 5.0 | 4.3 | 3.7 | 3.3 | 3.0 |
| 4 | 3.844 | 16.8 | 8.4 | 5.6 | 4.2 | 3.4 | 2.8 | 2.4 | 2.1 | 1.85 | 1.7 |
| | | Glass Pipe | | | | | | | | | |
| 1 | 1.157 | 245.1 | 122.6 | 81.6 | 61.3 | 49.0 | 40.9 | 35.0 | 30.6 | 27.2 | 24.5 |
| 1-½ | 1.672 | 108.9 | 54.5 | 36.3 | 27.2 | 21.8 | 18.2 | 15.6 | 13.6 | 12.1 | 10.9 |
| 2 | 2.172 | 61.3 | 30.6 | 20.4 | 15.3 | 12.3 | 10.2 | 8.8 | 7.7 | 6.8 | 6.1 |
| 3 | 3.203 | 27.2 | 13.6 | 9.1 | 6.8 | 5.4 | 4.5 | 3.9 | 3.4 | 3.0 | 2.7 |
| 4 | 4.265 | 15.3 | 7.6 | 5.13 | 3.83 | 3.04 | 2.6 | 2.2 | 1.92 | 1.7 | 1.52 |

driven pump and solution tank, shall provide a minimum average solution velocity at any instant of not less than five feet per second through each pipe and/or fitting in the circuit. In split flow arrangement, pressure differential must be maintained to assure the five feet per second minimum flow rate. This operation is to be checked by observation and tests. The rate of flow per second through the piping of known diameter can be determined from Table I.

(3) C-I-P systems shall be designed so that the suction intake of the primary circulating pump shall be flooded at all times during the cleaning cycle.

(4) Solution temperature shall be automatically controlled by the use of a temperature regulator with a response range of $\pm 5^{\circ}\text{F}$.

(5) The system shall be provided with a recording thermometer having a scale range of 60° to 180°F . with extension of scale on either side permitted; graduated in time scale divisions of not more than 15 minutes. Between 110° and 180°F ., the chart shall be graduated in temperature divisions of not more than 2°F ., spaced not less than 1/16 inch apart, and be accurate within 2°F . \pm . The sensor shall be protected against damage at 212°F . The sensing element of the recording thermometer shall be located in the return solution line.

(6) All connections between the solution circuit and the product circuit shall be so constructed as to positively prevent the comingling of the product and solution during processing.

G. INSTALLATION WELDING REQUIREMENTS

(1) All welding of sanitary product pipelines and solution lines shall be made by the Tungsten Shielded Arc Method or another equally satisfactory method may be used. The following precautions shall be taken.

(a) Inert back-up gas shall be used to protect and control the interior of the weld.

(b) The weld surface (interior, face and exterior) shall be cleaned and freed of all foreign matter and surface oxide before welding. Iron free abrasive shall be used when cleaning surfaces.

(c) All tube and fittings ends shall be square cut and deburred.

(d) Welding procedures shall assure uniform and complete penetration of weld at all times.

(e) All welds having pits, craters, ridges, or imbedded foreign materials shall be removed and the joints shall be properly re-welded.

(f) Internal and external grinding and/or polishing of welds is not required.

(g) An acceptable sample weld piece shall be provided at the beginning of each day and/or section welding operation or when required.

(h) A boroscope or other acceptable inspection device, to inspect representative welds, shall be made available by the processor.

H. CLEANING AND SANITIZING PROCEDURES

(1) A rinsing, cleaning, and sanitizing regimen which has been demonstrated to be effective shall be employed. Because of the possibilities of corrosion, the recommendations of the cleaning compound manufacturer shall be followed with respect to the time, temperature, and the concentration of specific acid or alkaline solutions and bactericides. To insure proper strength of solution and to avoid corrosion, the cleaning compound shall be completely dissolved or dispersed prior to circulation. One regimen found to be satisfactory is as follows:

(a) Immediately after concluding the day's operations, all connections between cleaned-in-place lines and processing equipment which are not included in the cleaning circuit shall be removed, the openings capped, by-pass connections made, and the lines rinsed thoroughly with tempered water (not to exceed 120°F., entering circuit) continuously discarding the rinse water near the downstream end of the solution return line until the discarded effluent is clear.

(b) All solution and product contact surfaces not cleanable by mechanical cleaning procedures shall be cleaned manually.

(c) Circulate an effective detergent solution for a period of time at a concentration and temperature capable of effectively removing the soil residue in the circuit.

(d) Thoroughly rinse the detergent solution from the circuit.

(e) Circulate an acid detergent, when needed, as a supplement to the routine circulation. Follow this acid detergent treatment with a thorough rinse.

(f) Sanitize all product surfaces with one or a combination of the following commonly used methods:

(1) Circulation of water at a minimum temperature of 170°F. (at the discharge end) through the circuit for five minutes and drained.

(2) Pumping of an approved chemical sanitizer solution of acceptable strength and recommended temperature through product lines and equipment for at least one minute and drained.

(3) Exposure to steam at a temperature of 170°F. (at the drainage outlet), for 15 minutes or at a temperature of 200°F. for five minutes.

(2) Prior to installation, a description of the cleaning regimen which has been demonstrated to be effective for each circuit shall be made available by the processor.

APPENDIX

A. STAINLESS STEEL MATERIALS

Stainless steel conforming to the applicable composition ranges established by AISI for wrought products, or ACI 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%. The 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 equivalent to types 303, 304, and 316 are designated CF-16F, CF-8, and CF-8M, respectively. These cast grades are covered by ASTM³ specifications A296-67 and A351-65.

B. 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 subsection D. (1) and subsections D. (2)(a) and (b).

C. TYPES OF WELDS

(1) *Automatic Weld*—A fully automatic weld is described as that made by equipment which starts and completes the weld, strikes, and controls the arc with no manual adjustment of control during the welding cycle and will consistently make repetitive welds.

(2) *Semi-automatic Weld*—A semi-automatic weld is described as that made by equipment which requires manual strike and/or control and will consistently make repetitive welds.

(3) *Hand Weld*—A weld in which the positioning of the arc is manually controlled.

D. SPECIFICATIONS FOR VISUAL INSPECTION OF CAST SURFACE FINISH

Because RMS (root mean square) values are applicable strictly to machined surfaces, it is essential to use a scale of cast surfaces in designating the general surface smoothness desired on castings. The reason for establishing a visual standard is to overcome the obvious inadequacy of any arith-

³Available from American Society for Testing and Materials, 1916 Race Street, Philadelphia, Pa. 19103.

metrical or geometrical measuring system when applied to the surface of a casting. The ACI SURFACE INDICATOR SCALE is the one to be used for the surfaces of castings for pumps or other appurtenances. The scale provides a measure of the degree of general smoothness which can be attained on alloy castings by currently available processes.

There are four surfaces shown on the scale. Copies of the SPECIFICATIONS FOR VISUAL INSPECTION OF CAST SURFACE FINISH as well as the SURFACE INDICATOR SCALE can be obtained from the Alloy Casting Institute.

These Practices shall become effective May 7, 1971.

ASSOCIATION AFFAIRS

NOMINATIONS FOR OFFICES OF IAMFES, INC.—1971-1972

FOR SECOND VICE-PRESIDENT AND SECRETARY-TREASURER



EARL O. WRIGHT

Earl O. Wright is an Associate Professor and Food Technologist in the Department of Food Technology at Iowa State University, Ames, Iowa. Raised on a dairy farm in Wisconsin, he was graduated from Wisconsin State University. Prof. Wright taught Vocational Agriculture and coached athletics in high school before entering the U. S. Army where he served for four and one-half years. He returned to University of Wisconsin where he received his M. S. in Dairy and Food Industry and Extension Education. He served as County Extension Director in Wisconsin for 3 years.

Before joining the staff at Iowa State University he served as an extension specialist and instructor in the Department of Food Science and Industry, University of Wisconsin.

He has devoted a great deal of his attention at ISU to milk quality control and dairy products processing. He initiated one of the early bulk milk drivers training school programs. He was instrumental in instigating early quality control programs with milk processors and was a member of the team that did early work with producers on abnormal milk control. Prof. Wright developed a program using the Plate Loop Count for evaluating the bacteriological quality of milk for manufacturing. This program is now being used by all milk testing laboratories in the state.

In addition, Prof. Wright is a consultant to food processing plants on quality problems. Numerous articles by him have been published in scientific and trade journals. He also publishes a monthly column in a national farm magazine on quality control and has co-authored several bulletins on milk quality.

In 1967 he was awarded the Distinguished Alumnus Award from Wisconsin State University. He is past president of the Iowa Sanitarian's Association and is presently Faculty Advisor to this organization. He is on the executive board and program chairman of Interstate Milk Shipment Conference, listed in Who's Who in the American Men of Science and past chairman of the Industry and Business Section—Resolutions Committee of the American Dairy Science Assoc. and has served actively in affairs of International Assoc. of Milk, Food and Environmental Sanitarians, Inc.

The Wright family reside at 1407 McKinley Drive in Ames. Their son, Robert, is a student at Iowa State University and their daughter, Esther, attends Junior High.



DR. C. BRONSON LANE

Dr. C. Bronson Lane is an Associate Professor of Dairy Science at the University of Florida. He is responsible for developing and implementing dairy technology extension programs for dairy processing plants and assists in obtaining high quality milk at the farm level.

The 31-year-old dairy scientist received his B. S. degree in dairy manufacturing from Pennsylvania State University, and the M. S. and Ph. D degrees in dairy science from the University of Maryland. Dr. Lane also attended Dallas Theological Seminary from 196 to 1967 for studies in Greek and Theology.

Prior to accepting the Florida position, Dr. Lane served for three years as an Assistant Extension Professor of Dairy Technology in the Department of Animal Sciences at the University of Kentucky. Lane served as an advisor to dairy processing and marketing associations during his three year stay in Kentucky, was program co-ordinator for four dairy technology societies, and planned educational meetings and conferences for dairy fieldmen and processors. In addition, he taught general Animal Sciences courses at U. K. and served as an advisor to many student groups.

The native Iowan is a member of the IAMFES Dairy Farm Methods Committee and has presented papers at two of the International's annual meetings - Louisville, Kentucky in 1969 and Cedar Rapids, Iowa in 1970. Lane is also a past president of the Kentucky Association of Milk, Food and Environmental Sanitarians and served as program co-ordinator for their 1968, 1969, and 1970 state conferences.

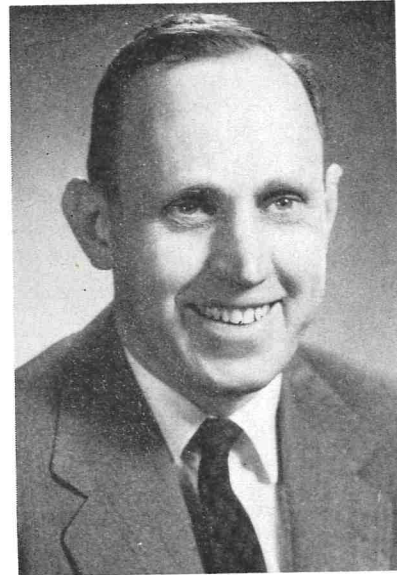
Lane is the author of numerous publications relating to the dairy industry. He has contributed frequently to the *Journal of Milk and Food Technology*, the *Dairy Fieldman*, and the *Modern Milk Hauler*.

He is also a contributing editor for *The Southern Dairy Products Journal*.

He is a member of the American Dairy Science Association, Sigma Xi, Phi Eta Sigma, Alpha Zeta, National Environmental Health Association, Florida Association of Milk, Food, and Environmental Sanitarians, and functions on committees of the Interstate Milk Shipments Conference and the National Association of Dairy Fieldmen.

Dr. and Mrs. Lane live at 927 N. W. 94th Avenue, Gainesville, Florida. They have three children - Marian 4 and 2-year-old twin sons, Charles and Clarence.

SECRETARY-TREASURER



RICHARD PELL MARCH

Richard P. March is a professor in the Department of Food Science at the New York State College of Agriculture, Cornell University, Ithaca, New York. Until 1965, he devoted 75% of his time to extension work as a specialist in milk quality and fluid milk handling and processing, and the balance of his time in research and teaching courses in fluid milk processing and quality control. At present, extension accounts for 90% of his time with 10% for research activities.

He was raised in Massachusetts, majored in dairy industry at the University of Massachusetts, receiving a B. S. degree in 1944. After a tour in the U. S. Marine Corps, he entered the Graduate School at Cornell University to major in dairy industry, receiving an M.S. degree in 1948.

Professor March taught a one-year program in dairy manufacturing until its termination in 1951, at

which time he was promoted from instructor to assistant professor. He became an associate professor in 1955, and full professor in 1965. In 1965 he also became department extension leader and is still serving in this capacity.

He is active in the New York State Association of Milk and Food Sanitarians, serving as secretary-treasurer from 1957 and executive secretary since 1967, secretary of the Dairy Industry Equipment Committee from 1952-57, secretary of the Farm Practices Committee from 1955-62, and secretary of the Council of Affiliates since 1952. He is a member of the International Association of Milk, Food, and Environmental Sanitarians, a member of their Farm

Methods Committee from 1959-65, secretary of their Council of Affiliates in 1961, and chairman in 1962 and 1963.

In both the State and International Associations he has served as chairman of a number of subcommittees including the Uniform Milkhouse Plans for the Northeast, Milk Transfer Systems, Sediment Testing, and Training Programs for Bulk Tank Truck Operators, and co-chairman of the Northeast Committee on Uniform Guidelines for Loose Housing Systems. In 1963 he was the recipient of the New York State Association's Dr. Paul B. Brooks Memorial Award for outstanding contributions to the organization.

(Notice to membership—ballots can only be mailed to paid up members as of April 15, 1971)

H. ERAUD BROWN

H. Erald Brown, 70, of 2507 Black Bridge Road, Janesville, Wisconsin, passed away January 24, 1971. "Brownie" as he was known to his many friends throughout the dairy industry came to Janesville from Chicago in 1940 and was a salesman for the Schlueter Co.

He was born Feb. 9, 1900 at Van Wert, Ohio and was married to Hazel Redueu Graves, in Chicago, November 1933. His wife and a niece, Mrs. Raymond Alm, Oaklawn, Illinois, survive.

GUESS WHO?



The photograph above was recently received by

the Journal Business Manager. Its subject is a long-term member of the Association, always active in Association affairs, the recipient of numerous Association honors, and frequently officially photographed. However, due to his informal dress when photographed, and his obvious avoidance of barbers for several months, the subject was felt to be so unrecognizable, even by intimates, that the effort to guess his identity might prove of interest. However, mechanical problems and legal restrictions to such an identity contest must first be ascertained and evaluated, furthermore, the subject of this photograph has recently been visited by several Association members, under circumstances which raised no question as to his identity.

The Journal management is of the opinion that this photograph has a potential significance to readers of the Journal of a magnitude vastly greater than that suggested. Suppose, for instance, the photograph were presented as an example of a condition to be avoided by all active members of the Association! It is widely recognized that Annual Meetings of the Association — and this is true of meetings of Affiliates — include few attendants with beards. Let's keep it that way! Members of the I.A.M.F.E.S. should not embarrass our hosts at the San Diego Annual Meeting by attending with beards, and also wearing identification badges on the streets.

This proposal constitutes a 180 degree orientation of the initial objective of the photograph submitted, which parallels the turn-around experienced by the receiving team when possession of the ball is lost by a fumble on the initial kick-off.

The subject of the photograph has indicated no objection to the proposed reoriented use of it, provided individual readers of the Journal who are so inclined may also report their identification to him

(No Prizes), because the inconveniences of continued beard culture until the San Diego Annual Meeting make it extremely unlikely that a view of his beard will be available then.

The settlement proposed by the subject of the photograph appears to present a somewhat less than positive policy of the Journal Management. But, is that not the sophisticated modern way to reach decisions, permitting both sides to claim victory?

WRIGHT APPOINTED ASSISTANT SECRETARY-TREASURER OF 3-A COUNCIL

Dr. K. G. Weckel, Chairman of the Trustees of the 3-A Sanitary Standards Administrative Council has announced the appointment of Professor Earl O. Wright as Assistant Secretary-Treasurer of the Council. Wright has been appointed to assume the duties of the Secretary-Treasurer because of the recent illness of Mr. C. A. Abele. Correspondence dealing with authorizations and use of the 3-A Symbol should be addressed to Professor E. O. Wright, 3-A Standards Administrative Council, 116 Dairy Industry Building, Iowa State University, Ames, Iowa 50010.

CUNNINGHAM LEAVES DFISA TO START SHOW MANAGEMENT FIRM

Joseph S. Cunningham, executive vice-president of the Dairy & Food Industries Supply Assn., has resigned his position effective June 30, 1971, in order to devote his efforts to activities concerned with exposition and trade show management.

Mr. Cunningham, a resident of Montgomery County, Maryland, for 30 years, joined the Washington-based organization in 1953 to assist with the Association's Exposition, the largest U. S. trade show for the food and dairy processing industries. He was appointed executive vice-president in 1960. Since that time, he has been recognized as one of the finest of exposition managers.

DFISA, without diminishing its interest in the dairy industries, has in recent years directed its activities toward the total food processing industries, including dairy. In so doing, the biennial exposition has enabled manufacturers to display their latest types of equipment and supplies for the safe manufacture of foods, treatment of waste and clean, efficient packaging of products.

FURTHER CHANGES IN THE EDITORIAL BOARD

The following persons have agreed to serve on the Editorial Board of the **Journal of Milk and Food Technology**:

G. H. RICHARDSON, Professor, Department of Food Science and Industries, Utah State University, Logan, Utah.

H. B. WARREN, Research and Development Laboratory, Fairmont Foods Company, Omaha, Nebraska.

Dr. B. J. Liska, Purdue University, has resigned from the Editorial Board. Dr. Liska recently was appointed Scientific Editor of **Food Technology** and the **Journal of Food Science** and the additional duties imposed by this appointment make it impossible for him to continue as a member of the Editorial Board.

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| Denver | 315.17 | 355.90 | 40.73 |
| Detroit | 375.64 | 447.90 | 72.26 |
| Miami | 392.32 | 495.90 | 103.58 |
| New Orleans | 344.36 | 439.90 | 95.54 |
| New York | 403.80 | 488.90 | 85.10 |
| Philadelphia | 399.63 | 482.90 | 83.27 |
| Pittsburgh | 389.20 | 461.90 | 72.70 |
| St. Louis | 338.11 | 424.90 | 86.79 |
| Washington/Balt. | 394.41 | 476.90 | 82.49 |

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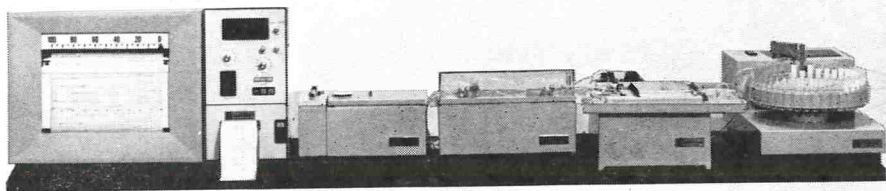
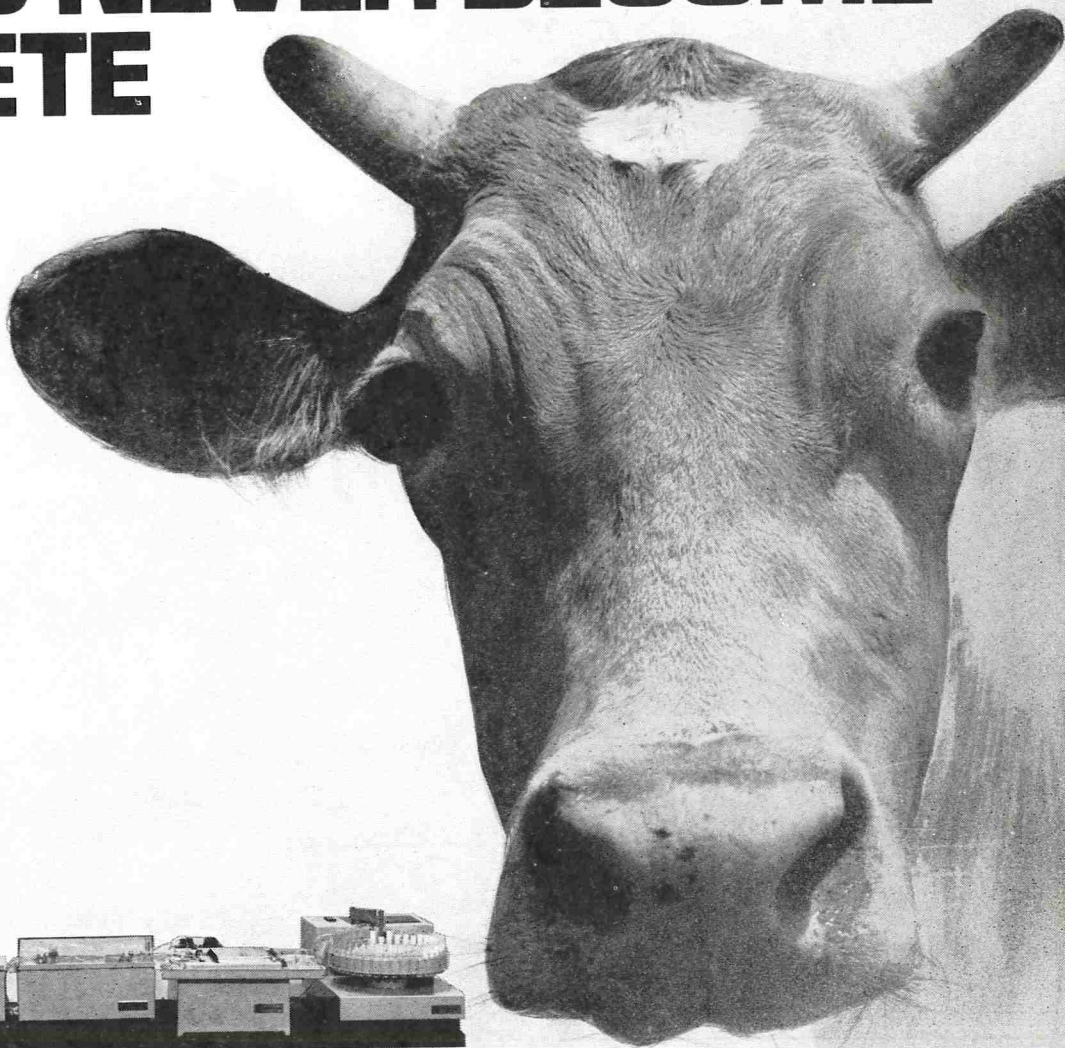
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Technicon's AutoAnalyzer is manufactured in the United States. Service including spare parts and reagents, is available through seven distribution centers strategically located throughout the country.

Should technical or operating difficulties arise, a Technicon® AutoAnalyzer specialist is as near as the telephone.

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so thoroughly trained that most problems are solved over the phone. If not, there are at least two Technicon representatives in every major city across the nation.

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We make service calls—at all hours of the day

Early in the morning, late at night or at any time in between, the Surge serviceman is on call. Because keeping dairy equipment operating efficiently is his business.

Like the doctor who gave up house calls, many suppliers have given up service. NOT SURGE. The nationwide network of Surge dealers is oriented toward regular service. They are prepared to handle every dairy service need.

Each year Surge dealers are required to submit service reports on all their customers. 1970 reports have been received on 87 percent of the Surge users in the United States and Canada

and they are still coming in. Surge dealers work hard at keeping little problems from growing into big ones.

When an emergency does arise, at any hour of the day, the Surge dealer is ready. He has the know-how to get milking systems operating.

But regular service is still the dairyman's best investment. It uncovers the little things that are costing money in reduced production, longer milking times and high mastitis treatment costs. It can also help avoid the emergency. You know sanitation, Surge knows dairying, let's work together to serve dairymen better.

SURGE...the accent is on YOU

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