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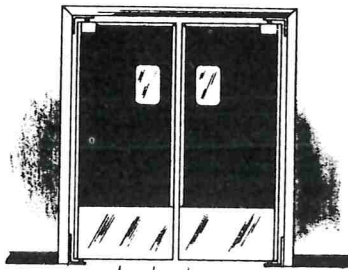


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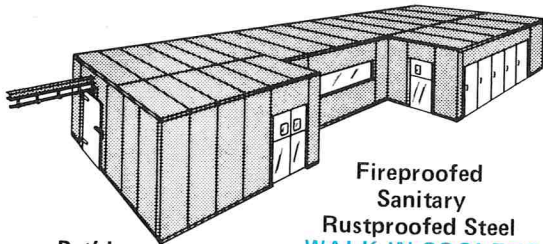
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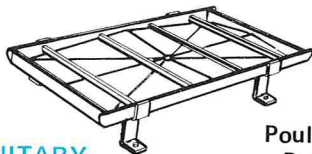
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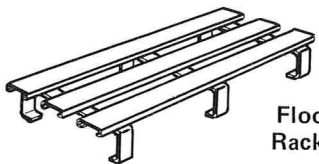
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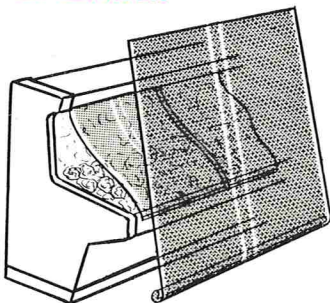
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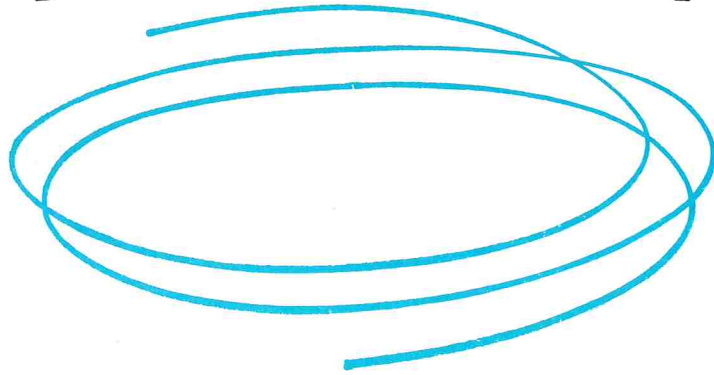
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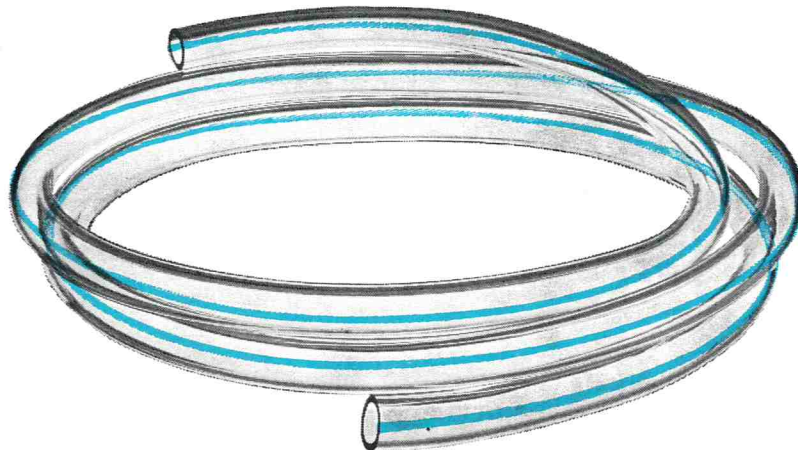
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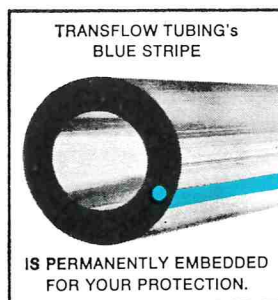


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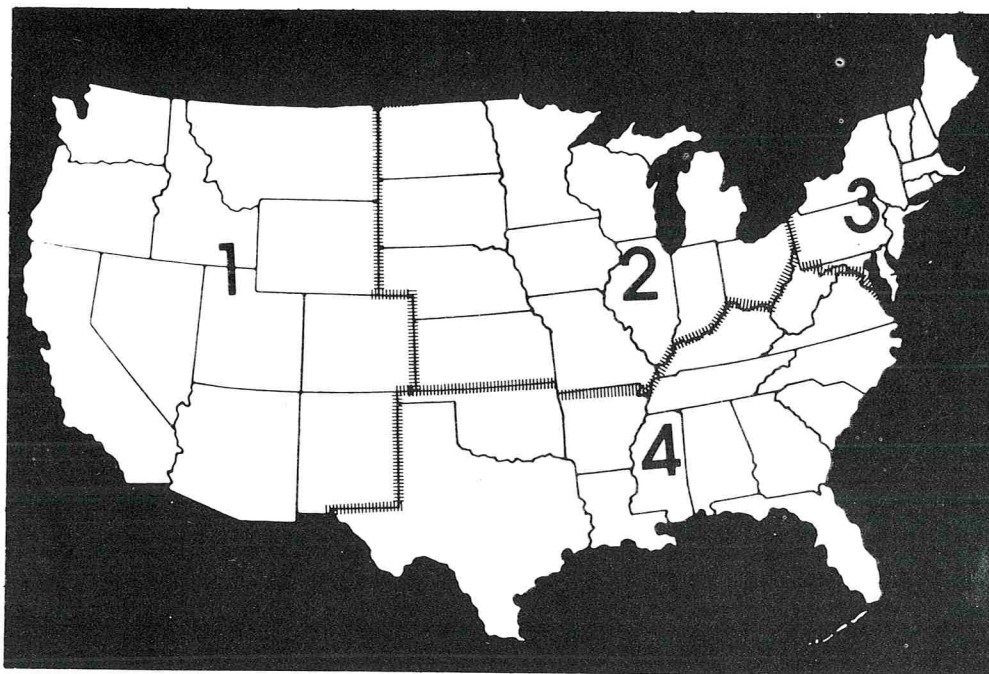
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Thermal Inactivation and Subsequent Reactivation of Staphylococcal Enterotoxin B in Selected Liquid Foods

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(Received for publication December 15, 1975)

ABSTRACT

Thermal inactivation and reactivation kinetics of staphylococcal enterotoxin B (SEB) were studied in buffer and certain liquid food systems using three serological techniques (single-gel diffusion method, radioimmunoassay, and microslide). Heating temperatures were 80-100 C and times were 0-5 min. Initial toxin concentrations employed were 0.008-5 µg/ml. Inactivation of SEB was faster in the earlier stages of heating and was much slower during continued heating. With the exception of phosphate buffered saline, reactivation of heated SEB was observed even when with small initial quantities of SEB. Reactivation (% activity) was greater when the heating time was shorter at any temperature.

Proteins including staphylococcal enterotoxins undergo denaturation when subjected to heat, chemicals, higher or low pH, or other agents. Like other proteins (1, 7, 8, 10, 13, 14), renaturation following storage or other treatment of denatured enterotoxin was noted by Fung et al. (5), Jamlang et al. (9) and Satterlee and Kraft (12). Since the initial concentration of SEB, heating system, and the detection or assay systems of SEB affect the evaluation of the safety of a thermal process, this study was conducted with small initial concentrations of SEB (0.008-5 µg/ml) utilizing very sensitive assays systems such as radio immunoassay and the microslide procedure.

MATERIALS AND METHODS

Organism, enterotoxin, and antiserum

Staphylococcus aureus S-6 was obtained from M. S. Bergdoll (Food Research Institute, University of Wisconsin). Crude staphylococcal enterotoxin B (SEB) was obtained by growing S-6 in a protein hydrolysate medium (PHP) [described by Miller and Fung (11)] at 37 C under constant shaking and supernatant fluids were collected after 24 h. Toxin levels in the supernatant fluids were measured by the capillary tube method of Fung and Wagner (6).

Staphylococcal enterotoxin B and antiserum B were obtained from Makor Chemicals Ltd., P.O. Box 6570, Jerusalem, Israel. Purified staphylococcal enterotoxin B was also obtained from another source (USA-Biological Defense Research Center).

Various concentrations of SEB were suspended in either PHP, phosphate buffered saline (PBS; 0.02 M, pH 7.4; Weirether et al., 15), or saline + 10% Brain Heart Infusion Broth (BHI; Difco Laboratories, Detroit, Michigan) depending on the final concentration desired and the assay system used for experimentation.

Serological assay procedures.

The single gel diffusion method (SGDM) of Fung and Wagner (6) was used to evaluate samples with toxin activities in the range of 3 to 100 µg/ml. The microslide double gel diffusion test of Casman et al. (2) was used to detect toxin concentration between 0.005-5 µg/ml. Quantification of enterotoxin B on the microslide was made by

matching migration band length of heated and reactivated toxins against standard curves of known concentrations of toxins using a procedure described by Fung et al. (4). The solid phase radioimmunoassay (RIA) procedure of Dickie et al. (3) was used to quantitate toxin concentration between 0.0001-0.2 µg/ml.

Iodination of enterotoxin B

To prepare radioactive enterotoxin B for the RIA, purified SEB was labeled with ¹²⁵I (Na¹²⁵I in 0.1 N NaOH; carrier-free; 5 µl of 400 mCi/ml; New England Nuclear) by the Chloramine-T method. The reaction was carried out in the rubber capped vial in which the (¹²⁵I) Iodide sample was packaged. To 5 µl of carrier-free Na¹²⁵I (2 mCi) was added to 25 µl of 0.5 M Na phosphate buffer, pH 7.5, followed by 40 µl of enterotoxin B (1 µg/ml) and fresh chloramine-T (100 µg) in 25 µl of 0.005 M phosphate buffer, pH 7.5. After each addition, made by injection through the rubber cap with a Hamilton syringe, contents of the vial were briefly mixed. Addition of 0.1 ml of sodium metabisulphite (2.4 mg/ml of 0.05 M phosphate buffer, pH 7.5) and added to a column of Sephadex G-50 Fine (10 cm × 1 cm; 1 g) to separate the labeled toxin from the reaction mixture. The reaction vial was washed twice with 0.1 ml of the carrier KI and added to the column.

Equilibration and elution from the Sephadex gel was done with 0.07 M barbiturate buffer, pH 8.6. Before the Sephadex column was used, crystalline bovine serum albumin (BSA; 20 mg in 1 ml of buffer) was passed through, followed by a 20 ml wash with the buffer. This "pre-saturation" of the Sephadex with BSA allowed a subsequent recovery of the ¹²⁵I-labeled enterotoxin from the column.

Twelve 1-ml fractions from the column were collected in tubes containing 1 ml of 1% BSA in elution buffer. The fractions were monitored for radioactivity using a standard bench top Geiger Counter (Model E-120, Eberline Instrument Corp., Santa Fe, N.M.) with the samples about 6 inches from the window. Fractions 4, 5, and 6 were pooled into a vial and stored at -20 C as undiluted stock solutions. The other fractions, a non-radioactive fraction, and a salt peak, were discarded. Due to deterioration as a result of ¹²⁵I decay, the stock solution was useful over a period of approximately 4 months or 2 half-lives of the ¹²⁵I.

The procedure to coat the plastic tubes and other aspects of the radioimmunoassay are described below and follow the procedure of Dickie et al. (3). High sensitivity of assay was favored by the use of antibody dilutions that bounded about 50% of the smallest adequate quantity of labeled toxin in the absence of unlabeled toxin. Antibody dilutions resulting in 10-50% binding of about 0.1-0.5 mg of labeled toxin proved satisfactory.

Coating of plastic tubes with antibody

Polystyrene test tubes (Packard Instrument Co., Downers Grove, Ill.) 14.5 mm × 150 mm, were used without washing or further treatment. Each tube was coated by 2.0 ml of antiserum diluted in 0.1 M carbonate-bicarbonate buffer (pH 9.6) containing 0.05% sodium azide and incubated overnight in a 30-35 C water bath.

After incubation, the coating solution was emptied from the tubes and returned to the antibody pool for further use. The same antibody pool, consisting of 100 ml of coating solution, was used for consecutive coatings until considerable reduced binding of radioactive antigen was

observed. The tubes were rinsed twice with a 0.9% NaCl solution (ca. 2 ml) and stored at room temperature in the presence of 2 ml of incubation buffer (0.05 M phosphate buffer, pH 7.4, 0.15 M NaCl, 0.05% sodium azide, 0.05% Tween 20) until use the same day.

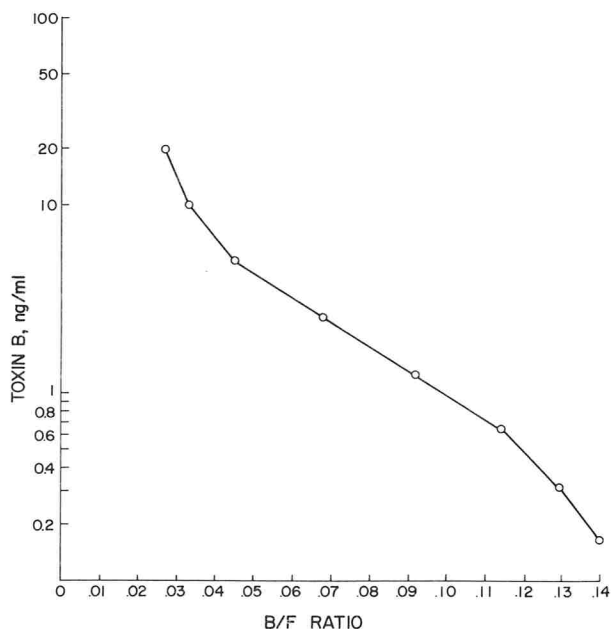


Figure 1. Standard curve of SEB determined by plotting SEB concentration against the bound/free radioactive labeled SEB ratio as measured by the RIA.

A typical standard curve of RIA is presented in Fig. 1. The linear portion of the curve (between 0.3 ng/ml to 10 ng/ml) was most reliable for use in quantification of enterotoxins in liquid foods. The background counts of this system as well as in food samples were about 20-30 cpm.

Heat inactivation and reactivation procedures

The heat inactivation and reactivation procedures according to Fung et al. (5) were followed when SEB was assayed by the SGDM and the RIA. Heated toxin samples were placed in petri dishes for 24 h before the reactivation activities were measured. For the microslide technique heated toxin samples were left in the ampules for 24 h before the reactivation activities were evaluated.

Inactivation and reactivation of SEB in liquid foods.

Initial concentrations of SEB ranging from 0.008-0.01 $\mu\text{g/ml}$ were suspended in 6 ml of PHP, PBS, BHI, whole milk, buttermilk, beef broth, tomato soup, in sterile screw capped test tubes (4.5 cm long and 0.5 cm wide). The pH values were determined before and after heating. The heat-up times to 100 C in these liquids were estimated to be 4 min for buttermilk, 5 min for BHI, 6 min for beef broth, tomato soup, PBS, and PHP, and 6.5 min for whole milk. Heating time for these liquids was 5 min at 100 C. Activity of SEB was measured after heat-up time as well as after 5 min of heating at 100 C. In both instances 4 and 25 C reactivation studies were conducted.

After heating, test tubes were removed from the oil bath and placed into ice water until cool. Two-ml samples to be used for the inactivation studies were removed and frozen in a test tube. For reactivation studies, 2-ml samples of heat treated toxin were placed into duplicate petri dishes for 4 and 25 C reactivation studies as previously described. After 24 h, all the samples were assayed by the RIA. Unheated controls were also subjected to these identical conditions.

A simple extraction was done before toxin detection on foods with particulate matters. Two ml of incubation buffer was added to the 2 ml of the buttermilk, whole milk, and the tomato soup to form a 1:2 dilution. These samples were then centrifuged at 19,000 rpm for 20 min, and the supernatant fluids used for the RIA. SEB samples in PHP, PBS, BHI and beef broth did not need any treatment. A direct 1:2 dilution was made.

RESULTS

Using the single gel diffusion test on samples with 100 $\mu\text{g/ml}$ we confirmed (data not shown) the previous published work (5) on heat inactivation and subsequent reactivation of SEB.

Heat inactivation and reactivation of 5 μg of SEB/ml

The loss and subsequent restoration of the activity of 5 μg of SEB/ml in BHI after 5, 10, and 20 min of heating at 80 C are shown in Fig. 2. Heating was done at 100 C for

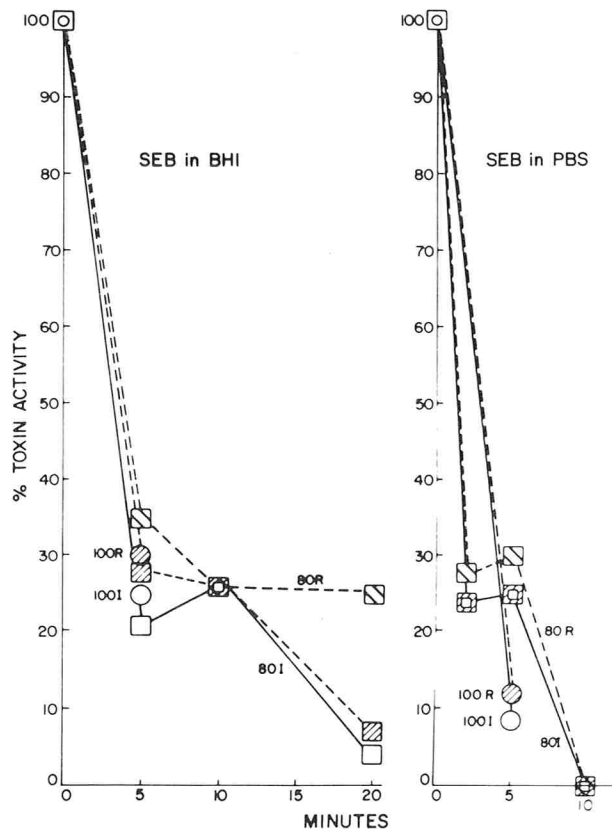


Figure 2. Heat inactivation and reactivation profiles of 5 μg of SEB/ml in BHI and PBS. Activities were measured by the microslide test. \square and \circ represent inactivation at 80 and 100 C, respectively. \boxtimes and \odot represent 24-h reactivation at 4 C of 80 and 100 C-treated SEB, respectively. \boxdot and \ominus represent 24-h reactivation at 25 C of 80 and 100 C-treated SEB, respectively. Abbreviations: I, Inactivation; R, Reactivation.

5 min only. Initially, in the first 5 min of heating at 80 C, 79% of the activity was lost, and after 20 min only 4% remained. When heated at 100 C for 5 min, 75% of the activity was lost. Although only one time period at 100 C was investigated, the data suggest that "low temperature aggregation" did occur.

Further evidence of this aggregate is exhibited by the slight increase in toxin activity between 5 and 10 min heating times at 80 C. During this prolonged heating period, the aggregates previously formed dissociated, resulting in an increase in serological activity, before the toxin decreased further. In general, reactivation of the toxin occurred after both 80 and 100 C heatings.

As a comparison to SEB in BHI, 5 μg of SEB/ml was

suspended in PBS for heat inactivation and reactivation studies. Figure 2 shows the loss and subsequent restoration of activity after 80- and 100-C treatment. Rapid decrease of toxin activity was observed in the first 5 min of heating. At 80-C heating, after 10 and 20 min no activity was detectable. At 100 C, loss of activity was rapid, with only 8% remaining after 5 min.

The slight increase in toxin activity observed at 5 min of heating at 80 C compared to 2 min of heating at 80 C suggests formation of an aggregate; toxin activity dropped to undetectable level after 10 min of heating. Reactivation was observed at 4 C incubation of 80-C treated SEB (2 and 5 min heating periods). When the toxin had decreased to undetectable level (10 and 20 min heating periods) no reactivation occurred. Slight reactivation was observed in 100-C treated SEB after 25-C incubation.

Heat inactivation and reactivation of 0.09 μg and 0.20 μg of SEB/ml

A further reduction of the quantity of SEB was made to simulate toxin concentrations which occur in natural foodborne intoxications. Figure 3 shows the loss and subsequent restoration of activity of 0.09 μg of SEB/ml in

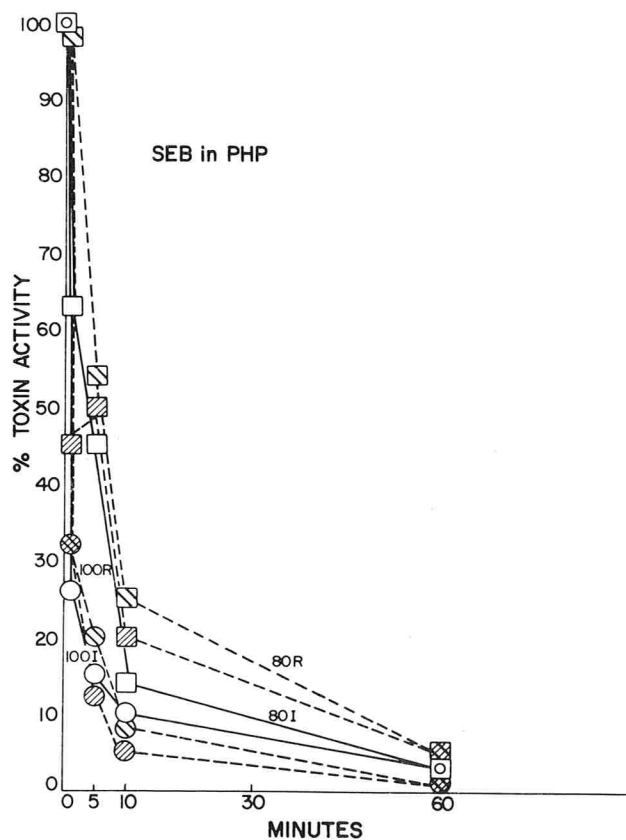


Figure 3. Heat inactivation and reactivation profiles of 0.09 μg of SEB/ml in PHP. Activities were measured by the RIA. Same legend as Figure 2.

PHP after 1 to 60 min of heating at 80 and 100 C.

In terms of reactivation, those toxin samples heated for shorter periods gained back more activity than did

those heated for longer periods. This observation further confirms previous data from this investigation (Fig. 2). Reactivation occurred after all 80-C heating periods, and after 1 and 5 min of heating at 100 C, but not after 10 and 60 min at 100 C. Also, the degree of reactivation of 80-C heat treated toxin was greater than that of SEB heated at 100 C.

It is difficult to observe the low temperature aggregate phenomenon on the time scale of Fig. 3 at this low toxin concentration. A series of experiments using 0.25 μg of SEB/ml suspended in PHP was done, heating the toxin at 80 and 100 C with samples collected at short time intervals (10 to 60 sec). Figure 4 shows that toxin

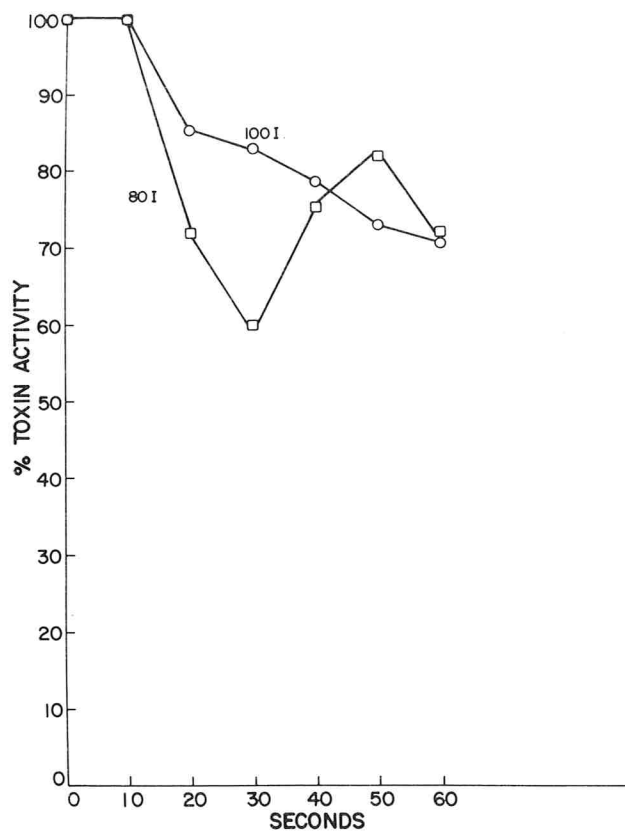


Figure 4. Heat inactivation profiles of 0.25 μg of SEB/ml in PHP After 10-60 Seconds of Heating. Activities were measured by the RIA. Open square and circles represent inactivation at 80 and 100 C, respectively.

heated at 100 C had a gradual decrease of activity with an increase in heating time, as expected. The profile of 80-C heating, however, again suggests the "low temperature aggregate" formation. Toxin activity decreased rapidly after 20 to 30 sec of heating and diminished to 72 and 60% activity, respectively. However, after 40 and 50 sec of continued heating, the toxin reactivated to 76 and 82%, respectively. Prolonged heating to 60 sec resulted in a decrease of toxin activity indicating that the aggregate phase had passed and that the aggregates were dissociated after prolonged heating.

Small concentration of SEB (0.20 $\mu\text{g}/\text{ml}$) was also suspended in PBS for thermal inactivation at 80 and

100 C. Results (data not shown) indicated that toxins were inactivated faster in PBS compared to toxins suspended in PHP. More important, no reactivation of 80- and 100-C treated toxins occurred after 24 h of incubation at either 4 or 25 C. In fact, levels of toxin after this incubation period were reduced to nondetectable levels. Thus, the type of suspending medium not only influence the heating inactivation of toxin, but the stability of the toxin as well.

A toxin stability study showed that the activity of 100 $\mu\text{g/ml}$ and 5 $\mu\text{g/ml}$ suspended in PHP, BHI, or PBS remained constant after 24 h at 4 and 25 C incubation. The activity of 0.09 μg of SEB/ml in PBS fell drastically. Activity was undetectable by the RIA after 24 h at 4 C, and only 35% remained after 24 h at 25 C.

Thermal inactivation and reactivation of 0.008-0.01 μg of SEB/ml in various liquid foods

To ascertain the practical implications of this reactivation phenomenon, it was necessary to heat inactivate and test for subsequent reactivation of SEB in various liquid foods. The RIA was used to detect toxin activity in this study. The average recovery rates of small amounts of SEB in the various foods in descending order were 91% (PBS), 88% (beef broth), 83% (buttermilk), 75% (PHP), 59% (tomato soup) and 50% (milk). The ability to recover toxin with a simple extraction procedure requiring minimal manipulation is an important advantage of the RIA system as compared to the SGDM or the microslide test in the detection of toxins in foods.

Results of the heat inactivation and reactivation study of small quantities of SEB in various liquids are in Table 1. Previous data indicated that small quantities (0.20 $\mu\text{g/ml}$) of unheated SEB were not stable in PBS. It was therefore necessary to test the stability of still smaller quantities (0.008-0.01 $\mu\text{g/ml}$) of unheated SEB in the liquid foods and laboratory media. Minute quantities of SEB showed excellent stability when stored at 4 C for 24 h. Full activity remained in all foods and laboratory media except for PBS which showed a complete spontaneous loss of all toxin activity. After 25 C incubation for 24 h, toxin in beef broth, milk, PHP, and BHI retained 100% activity while SEB in tomato soup and buttermilk retained 35 and 30% activity, respectively. SEB suspended in PBS showed no activity at all under these conditions.

Due to the large volume (6 ml) of these liquid samples compared to those of previous experiments (0.4 and 1.2 ml) the heat times to 100 C were much longer (5 to 6 min). These longer heat-up times undoubtedly inactivated some SEB since the temperature of the sample was at 80-90 C for a few minutes until the 100 C mark was reached. Columns 7, 8, and 9 of Table 1 show the percent activity of SEB after the heat-up time and the subsequent activity after exposure to reactivation conditions.

After heat-up time, beef broth, BHI, and PHP samples retained more than 50% of the original toxin activity. The low retention of SEB activity in buttermilk (26%) and tomato soup (9%) was probably due to combined effects of low pH and the heat treatment. The low SEB activity in milk (9%) was probably caused by binding of SEB to milk particles or enhancement of SEB aggregate formation due to slow heating. Interference of RIA by whole milk particles may also be a factor since recovery rates of toxin in whole milk compared with the other liquid systems was low. PBS with no protective particles had the lowest SEB activity after the heat-up time as was expected.

As far as reactivation was concerned, only beef broth and BHI provided noticeable recovery of SEB activity. PHP and milk showed no recovery of SEB activities after 24 h at 4 and 25 C incubation. The fact that a decrease of activity during reactivation time in buttermilk from 26% to 6 and 8% at 4 and 25 C incubation, respectively, reflects the instability of SEB in buttermilk during storage. Such an effect was observed in tomato soup also but with less drastic decreases. Increased acidity after heating of these foods probably also accounted for the instability of heated toxin. After heat treatment, 6% of the SEB activity remained. After 4 and 25 C reactivation conditions, however, there was still about 5% activity of SEB present, despite the fact that in the stability study no SEB activity was detectable after 24 h of storage at either 4 or 25 C incubation of unheated SEB in PBS. Heating of milk in PBS during this long heat-up time probably allowed some protein-protein aggregation to occur so that the instability exerted on native toxin by PBS was not as evident. Data on heating SEB in PBS at 80 C (Fig. 3) substantiate this protein-protein aggregation interpretation.

The toxin activity continued to decrease after heating

TABLE 1. Heat inactivation and reactivation study of 0.008-0.01 μg of SEB/ml in various liquids as measured by the RIA

Liquid sample + initial conc. of SEB ($\mu\text{g/ml}$)	pH of liquid		%SEB activity								
	Before heating	After heating	Unheated sample			Heat-up time sample			5 min at 100 C sample		
			No treatment	Reactivation at		After heating	Reactivation at		After heating	Reactivation at	
			4 C	25 C	4 C		25 C	4 C		25 C	
Beef broth (.01)	5.6	5.6	100	100	100	62	68	74	52	59	44
Buttermilk (.01)	4.4	4.0	100	100	30	26	6	8	13	9	ND ^a
Milk (.009)	6.4	6.4	100	100	100	9	9	7	11	4	9
Tomato soup (.01)	4.4	4.0	100	100	35	9	7	7	ND	3	ND
PBS (.01)	7.2	7.2	100	ND	ND	6	4	5	ND	ND	ND
PHP (.008)	6.2	6.2	100	100	100	54	54	47	23	18	16
Saline 10% BHI (.009)	6.8	6.7	100	100	100	67	75	67	54	43	38

^aND-Not detectable.

at 100 C for 5 min (Table 1, column 10). Five liquid systems retained more than 10% of the initial activity after boiling for 5 min. These data have practical significance considering the minute quantities of SEB in these samples and the severity of the treatment. More than 50% SEB activity remained in two systems (BHI, 54% and beef broth, 52%). The two dairy products, milk and buttermilk, had 12 and 11% activity remaining, respectively. It is interesting to note that the SEB activity in milk did not decrease during this prolonged 5-min heating at 100 C as compared to the activity immediately after the heat-up time. In fact, the activity of toxin increased slightly from 9% to 11%. It is possible that SEB aggregations formed with the milk protein and fat, plus the SEB-SEB interactions, were protected by the milk during this prolonged heating, and after 5 min at 100 were beginning to dissociate resulting in slight increase in activity. Toxin activity in tomato soup and PBS was reduced to a non-detectable level.

In terms of reactivation, only beef broth and tomato soup at 4 C incubation temperatures showed appreciable restoration of toxin activity after heating. All other samples had a further decrease in activity under reactivation conditions. Although the 6-ml samples were immediately placed in ice water after the heating treatment, it is possible that this volume was not cooled rapidly enough to prevent further heating effect.

Regain of toxin activity in beef broth is interesting because Chou et al. (Chou, C.H., L.G. Harmon, and K. E. Stevenson. Abs. Ann. Meet. Amer. Soc. Microbiol. 1974:14) found a dialyzable factor in beef which protected SEB during thermal inactivation. The exact nature of this substance is not known, but it may be attached to large protein molecules and is involved in the protection of SEB during heating.

Detectable amounts of toxin after 24 h of incubation at 4 C in heated tomato soup demonstrate another practical implication. The presence of SEB in heated tomato soup could be entirely overlooked due to the inability to detect its presence even by the very sensitive RIA technique. However, upon subsequent incubation, SEB regained activity and could again be detected.

DISCUSSION

Staphylococcal enterotoxin B loses 60-70% activity rapidly during the first few minutes of heating at 80 and 100 C. The remaining 30-40% is lost much less rapidly. It is proposed here that when SEB becomes heat inactivated, aggregational and / or conformational changes that occur during heating may lead to apparent loss of serological activity. Renaturation or reactivation of SEB under suitable storage conditions may lead to recovery of some of the apparently lost activity. Data presented here confirm our earlier findings (5) in that reactivation also occurred even when toxin was subjected to heat at very low initial concentrations. Data presented here also suggest that reactivation may take place during

continued heating (Fig. 4) or reheating to a higher temperature as reported by Jamlang et al. (9) might suggest that this type of regain inactivity may have occurred from disaggregation of the aggregates whereas the regain during storage of heated toxin may have occurred from refolding of unfolded molecules.

The fact that detectable activities of SEB were obtained after heat treatment of several food systems containing minute quantities of initial SEB has practical implications. Although reactivation of heat treated SEB was observed in a few food systems under certain conditions of incubation, whether or not this is a generalized phenomenon in foods remain to be investigated.

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Enzymatic Refining of Unconventional Protein for Nutritional Purposes

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ABSTRACT

Studies were made on the possibilities of refining protein from the fodder yeast, *Candida utilis*, from *Kluyveromyces fragilis* cells and from the field bean by enzymatic means using pepsin and papain, followed by the reverse synthesis of proteins from hydrolysates as treated with alpha-chymotrypsin. The yield of refined protein was about 75%, as measured by amounts of nitrogen substances precipitated in 15% trichloroacetic acid. The refined yeast proteins contained no nucleic acids, while field bean protein was free of trypsin inhibitors. The refined proteins appeared to be neutral in respect of flavor substances.

Since the shortage of proteins tends to increase, attempts have been made to develop new techniques for adaptation and refinement of unconventional nutrition sources, those of plant origin in particular (2, 15). Most plant proteins require refinement due to their unfavorable technological, nutritional, and even hygienic properties (11, 14). Refinement of plant proteins also aims at removal of accompanying protein-bound substances, such as phytic acid, saponins, pigments, and flavor substances.

An equally important problem is the endeavor to remove biologically active protein-bound substances such as trypsin inhibitors and hemagglutinating substances present, in most instances, in the seed protein of leguminous plants: soybean, field bean, string bean, pea, and lupine (1, 2, 9, 14, 19).

The principal aim of the refinement of proteins from microorganisms is removal of nucleic acids ranging from 6 to 15% present in the cells of microorganisms (13, 17).

Various procedures for refinement of plant proteins, including their modification, have been reported in the recent literature (6, 16, 18). Most of the methods suggested make it possible to obtain only a partial refinement of proteins from unconventional sources. Removal of toxicants, for example, does not always result in the elimination of color or protein-bound flavor substances (8, 11, 14, 16).

In most instances, attempts to refine proteins also lead to their modification to give them the desired physicochemical properties (4, 16). Proteins of changed isoelectric point, reduced solubility, and different electrophoretic properties are being obtained in this way (16).

Enzymatic methods of refinement and modification of proteins are believed to present better possibilities (5, 6, 16). When using proteolytic enzymes, a controlled proteolysis may be induced, as a result of which it is possible to remove foreign protein-bound substances (5, 6, 16). A considerable drawback to this method is the possibility of an unfavorable after-taste that may be produced in the course of hydrolysis due to formation of peptides bitter in taste (5).

Recently attempts have been made to find methods of protein refinement by means of a reverse enzymatic synthesis of proteins from previously prepared enzymatic protein hydrolysates (5, 6).

The aim of this study has been an attempt to refine proteins of field bean - *Vicia faba minor*, fodder yeast-*Candida utilis*, an *Kluyveromyces fragilis* using the method of reverse enzymatic synthesis with the application of alpha chymotrypsin.

MATERIALS AND METHODS

K. fragilis grown on whey medium, field bean meal, and fodder yeast were used in this experiment. Yeast cells after harvesting from the whey were washed and disintegrated mechanically. All proteins were ground, defatted, and then extracted with water. The protein solution was dialyzed using the continuous method in the "Amicon" apparatus and then was subjected to an enzymatic hydrolysis (2, 4, 8, 18).

Protein hydrolysis

A controlled protein hydrolysis was done using pepsin of an enzymatic strength 1:1500, or papain of an enzymatic strength 1:600. Enzymes were added to 2000 cm³ of 5% wt/vol protein solution at the ratio of enzyme: protein—papain 1 : 50, pepsin 1 : 100. Proteins were hydrolyzed to peptides under the following conditions: pH 2, 35 C, when pepsin was used, and pH 4.5-5.0, 65 C, when papain was used. In the course of this experiment, fodder yeast proteins and *K. fragilis* proteins were observed to be more resistant to enzymatic hydrolysis than those of field bean proteins.

After the hydrolysis had been completed, the sample was centrifuged at 3800 × g for 15 min to remove the insoluble fraction from enzymatic hydrolysis. The extent of protein hydrolysis was fixed by the amount of nitrogen compounds soluble in 15% trichloroacetic acid and in phosphotungstic acid (20).

Enzymatic protein synthesis

A definite sample of hydrolyzate containing about 10 g of dry substances was adjusted to pH 7 by adding NaOH solution, after which it was concentrated in a vacuum evaporator to the volume of 50 ml. The condensed solution was then again brought to pH 7 with the help of NaOH solution, and the alpha-chymotrypsin enzyme was added at a ratio of 1 part enzyme to 100 parts protein. This was incubated at 37 C for 24 h (5, 6). A gelatinous product was obtained which was separated

from the solution by centrifugation at $3800 \times g$ for 20 min.

Parent substances, enzymatic hydrolyzates and enzymatically synthesized proteins were examined for the contents of water, ash, total nitrogen, soluble nitrogen compounds precipitable in 15% trichloroacetic acid, and soluble nitrogen compounds precipitable in phosphotungstic acid (20). Nitrogen was determined by the Kjeldahl method (20). The content of trypsin inhibitors was determined in field bean meal and field bean proteins obtained by synthesis (10, 19). The trypsin inhibitors were determined by the Kakade method with casein substrate (10, 19). Yeast and synthesized yeast proteins were analysed for levels of nucleic acids, RNA, and DNA (7) as were the proteins of parent substances, hydrolyzates, and synthesized proteins chromatographically separated on Sephadex gel G-100. The separation of proteins was conducted in K-26/70 columns made by Pharmacia Fine Chemicals, Sweden. Blue dextran, hen egg albumen, bovine albumen, and trypsin were used to calibrate the column (3, 20). Flavor characteristics of substrates substances and synthesized proteins were determined organoleptically.

RESULTS

The total protein content in field bean meal, fodder yeast, and *fragilis* cells was 33.3%, 42.6%, and 37.1%, respectively. The content of nitrogen substances precipitated in 15% trichloroacetic acid was 4.3% in field bean meal, 4.8% in *K. fragilis* cells, and 5.2% in fodder yeast. The amounts of nitrogen substances precipitated in phosphotungstic acid were 4.9% in field bean meal, 5.4% in *fragilis* yeast, and 6.3% in fodder yeast (Table 1).

TABLE 1. Chemical composition of substrates as used for enzymatic protein hydrolysis

No.	Kind of analysis	Field bean meal	Fodder yeast	<i>K. fragilis</i> yeast
1	Dry matter content (2)	90.4	88.5	94.8
2	Total nitrogen (%)	5.3	6.8	5.9
3	Total protein ($N \times 6.25$) (%)	33.3	42.6	37.1
4	Nitrogen substances as precipitated in 15% TCA (absolute content) (%)	4.3	5.2	4.8
5	Nitrogen substances as precipitated in phosphotungstic acid (absolute content) (%)	4.9	6.3	5.4
6	Ash content of sample (%)	3.7	8.7	12.1

To determine the yield of the protein synthesis, the contents of nitrogen substances precipitated both in 15% trichloroacetic acid and phosphotungstic acid, were compared with those of total nitrogen in parent substances, enzymatic hydrolyzates, and enzyme synthesized substrates (Table 2).

^aMolecular weight of protein: blue dextran—200,000; hen egg albumen—44,000; bovine albumen—66,500; trypsin—23,800.

TABLE 2. Yield of refined proteins expressed as percent of total nitrogen

Kind of parent substance	Total nitrogen of parent substance (%)	Parent substance (substrate)		Hydrolyzates				Protein as synthesized using alpha-chymotrypsin				Yield of synthesis as percentage of parent substance protein			
		A ^a	B ^b	With pepsin		With papain		With pepsin hydrolysate		With papain hydrolysate		With pepsin hydrolysate		With papain hydrolysate	
				A	B	A	B	A	B	A	B	A	B	A	B
Field bean meal	5.3	80.1	93.0	1.9	2.0	1.7	1.9	37.0	53.0	39.0	54.0	45.8	57.0	48.0	58.1
Fodder yeast	6.8	76.4	93.1	20.0	26.0	19.0	21.0	59.1	70.5	59.5	72.0	77.7	77.4	77.4	77.4
<i>K. fragilis</i> yeast	5.9	80.6	90.7	23.9	25.0	21.0	23.0	51.0	69.0	57.0	70.0	63.3	76.1	70.8	77.2

^aA-Nitrogen as precipitated in 15% trichloroacetic solution.

^bB-Nitrogen as precipitated in phosphotungstic acid (20).

When pepsin-treated hydrolyzates were used, the yield of the protein synthesis in relation to sources was 45.8%, 77.7%, and 63.3% for field bean, fodder yeast and *K. fragilis* yeast hydrolyzates, respectively (Table 2).

The yield of protein synthesis, expressed by the number of nitrogen substances precipitable in phosphotungstic acid, was 57%, 77.4%, and 76.1% for field bean, fodder yeast, and *K. fragilis* yeast hydrolyzates, respectively.

The protein synthesis through alpha-chymotrypsin in hydrolyzates carried out by papain preparation, in relation to the total nitrogen of the parent substances, in the case of nitrogen substances precipitated by 15% trichloroacetic acid, amounted to 48.0% from field bean protein hydrolysate, 77.4% from fodder yeast, and 70.8% from *K. fragilis*. With nitrogen substances precipitable by phosphotungstic acid, the protein synthesis came to 58.1% from field bean hydrolysate and 77.4% from fodder yeast hydrolysates (Table 2).

The characteristics of proteins obtained by means of enzymatic synthesis is presented in Table 3. Preparations

TABLE 3. Characterization of enzymatically refined protein preparations

No.	Kind of analysis	Protein preparation (with papain hydrolyzate)		
		Field bean meal	Fodder yeast	<i>K. fragilis</i> yeast
1	Dry matter content (%)	24.9	19.3	21.2
2	Total nitrogen (%)	12.7	13.0	12.8
3	Total protein ($N \times 6.25$) (%)	79.8	81.4	80.1
4	Nitrogen substances as precipitated in 15% TCA (absolute content) (%)	5.5	7.8	5.2
5	Nitrogen substances as precipitated in phosphotungstic acid (absolute content) (%)	6.9	9.4	6.6
6	Ash content of sample (%)	2.9	4.7	7.2

characterized in Table 3 were obtained according to described technique but in an additional series of the experiment. The chemical contents of those preparations differed slightly from those shown in Table 2. The total protein content was 79.8% in field bean protein, 81.4% in fodder yeast protein, and 80.1% in *K. fragilis* protein. Ash contents were 2.9%, 4.7%, and 7.2%, respectively. Mean ash contents were by 36% lower than those of parent substances.

When using molecular filtration on Sephadex gel G-100, three well formed fractions were found in field bean protein. Their molecular weights were: 144.0×10^3 , 29.0×10^3 and 12×10^3 , respectively (Fig. 1).

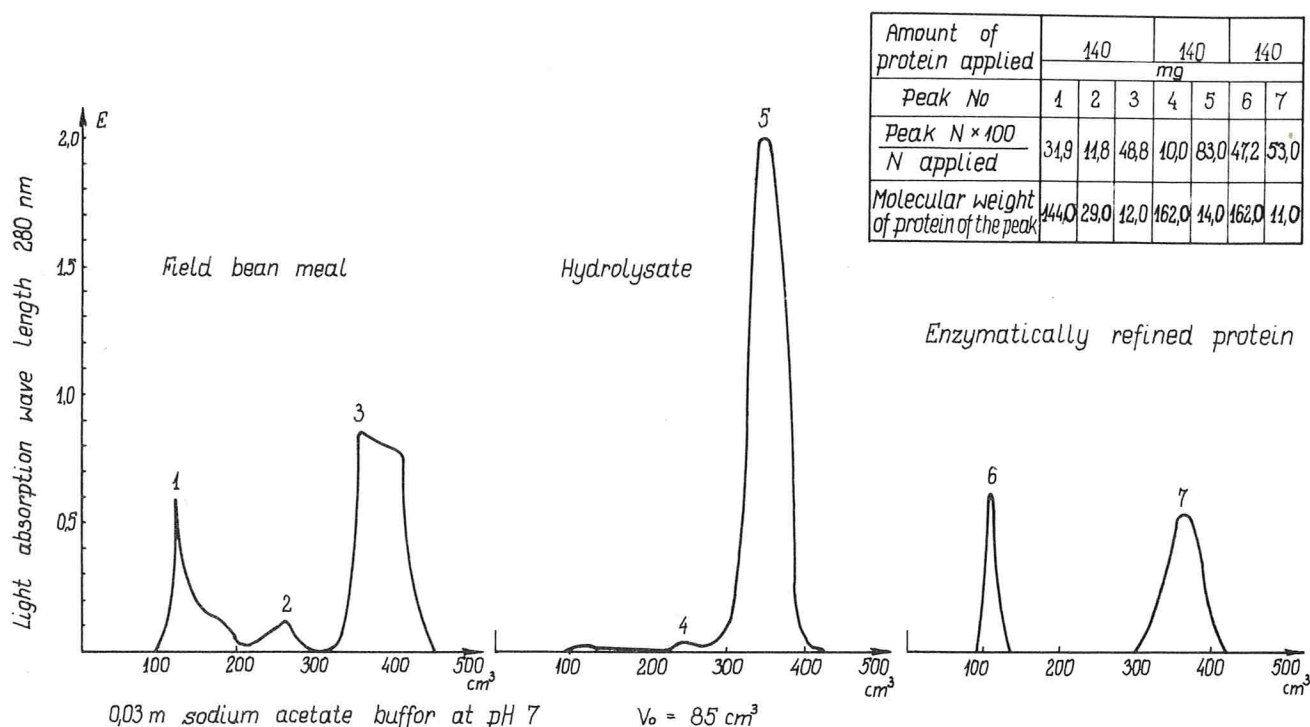


Figure 1. Chromatographic separation of field bean proteins on Sephadex gel G-100.

The enzymatic hydrolysis showed two fractions in hydrolysates of field bean protein. One of them (Peak 4) contained only 10% of nitrogen which had been applied on the column, while another (Peak 5) amounted to 83% of nitrogen, as introduced on the column. The molecular weights of the said fractions were 162.0×10^3 , and $14. \times 10^3$, respectively (Fig. 1). Two protein fractions (Peak 6 and 7) were found in field bean proteins after an enzymatic synthesis, their molecular weights being 162.0×10^3 and 11.0×10^3 .

A comparison of the patterns of fractions and their molecular weights in parent substances, enzymatic hydrolysates, and proteins after enzymatic transformations proves the existence of favorable enzymatic transformations and the possibility of synthesis of multimolecular fractions of peptides from protein hydrolysates obtained by appropriate enzymatic methods. In general, fractions of high-molecular peptides could be produced by enzymatic synthesis, the molecular weights of which approximated those of protein fractions from parent substances, viz. 144.0×10^3 and 12.0×10^3 , as compared with 162.0×10^3 and 11.0×10^3 , respectively. It has not been possible to reproduce fraction 2 of a molecular weight 29.0×10^3 which represented 11.8% of the nitrogen introduced on the column in the parent substance (field bean). However, in the field bean protein, after enzymatic synthesis, an increase of fraction 6 (Peak 6) was observed, which corresponds to fraction 1 in the parent substance, and of fraction 7 corresponding to fraction 3 of the parent substance. Upon synthesis, the percentage of these fractions was

47.2% and 53%, as compared with 31.9% and 48.8% in parent substances (Fig. 1).

Similar changes were found to occur when protein substances of fodder yeast and *K. fragilis* (Fig. 2 and 3) were separated chromatographically. In that instance, fractions which were obtained by enzymatic synthesis also approximated by their molecular weights to those of parent substances, while fraction 2 disappeared.

Results of sensory examination confirmed that synthesized proteins had a neutral flavor and gelatinous consistency in comparison with proteins of parent substances.

The enzymatic refinement of field bean protein made it possible to remove completely trypsin inhibitors from proteins, although about 14.8 T.U.I. of them were found in 1 cm^3 field bean meal extract.

The enzymatic protein synthesis of yeast hydrolysates resulted in a complete elimination of nucleic acids (RNA + DNA) present in the hydrolysed fodder yeast in amounts of 7.3% (6.7% RNA and 0.6% DNA) and *K. fragilis* in the amounts of 9.28% (8.42% RNA and 0.86% DNA).

DISCUSSION

It has been demonstrated in our experiment that hydrolysis of proteins of vascular plants and microorganisms, using pepsin or papain, makes it possible to obtain enzymatic hydrolysates suitable for resynthesis of proteins using alpha-chymotrypsin. In this way, a colorless, gelatinous protein substance was obtained resembling gelatin in appearance.

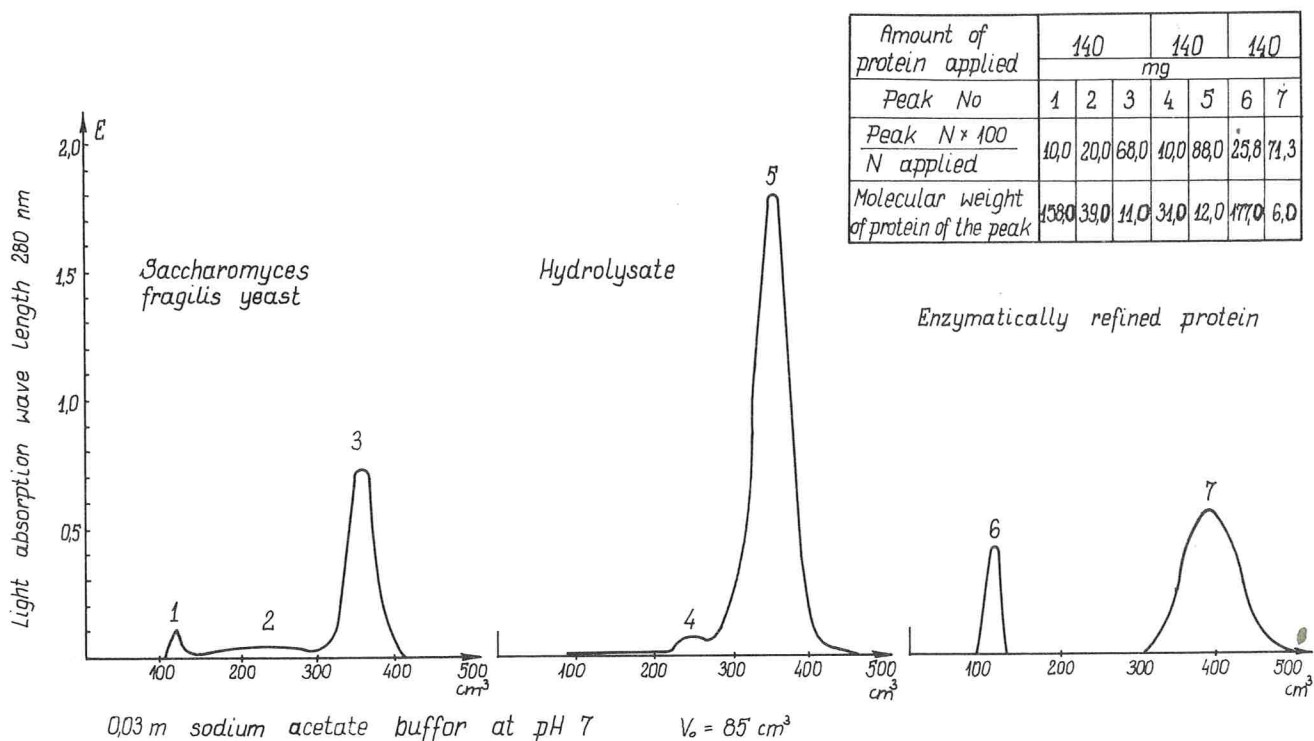


Figure 2. Chromatographic separation of Kluyveromyces (*Saccharomyces*) *fragilis* proteins on Sephadex gel G-100.

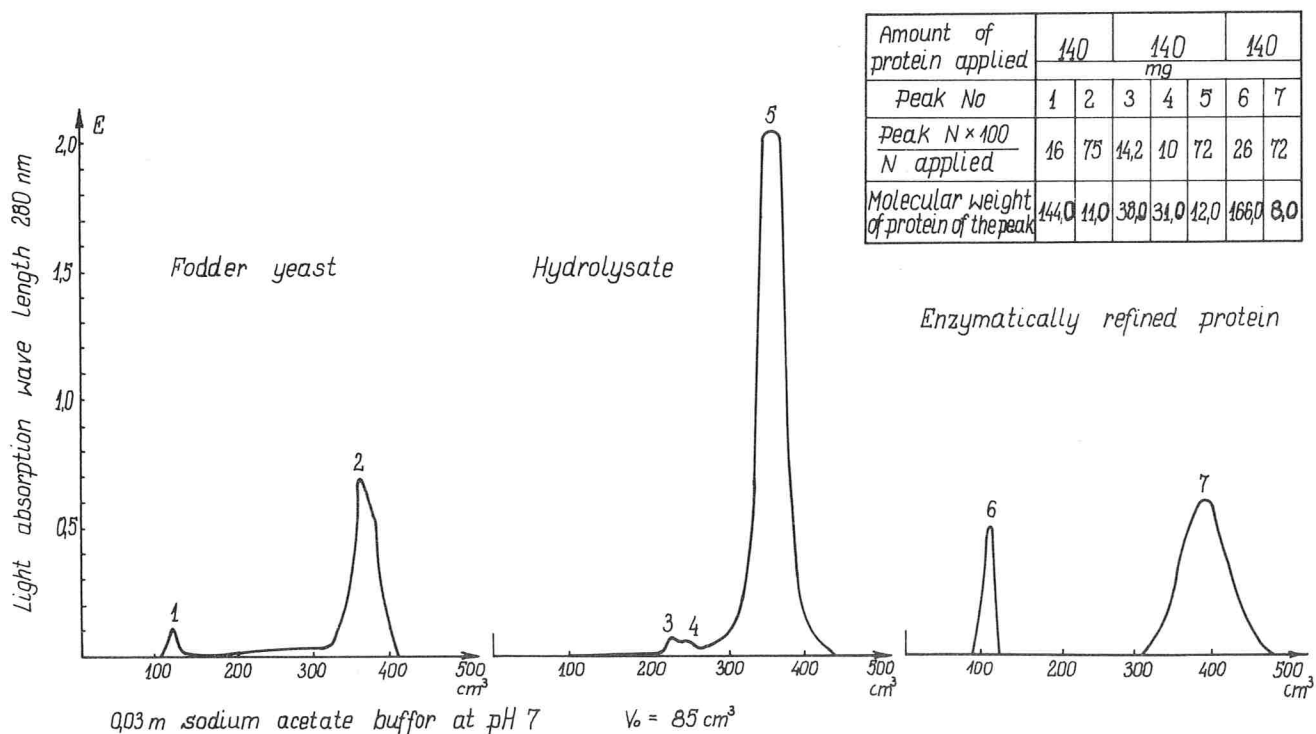


Figure 3. Chromatographic separation of *Candida utilis* proteins on Sephadex gel G-100.

Concentration of high-molecular peptides (ca. 80% of proteins in all) with simultaneous 36% decrease of ash contents, as well as elimination of the characteristic taste and flavor of leguminous plants, their concentrates, or yeasts, which occur during the resynthesis are the important observations.

Not without importance is the fact that, in yeast hydrolysates, synthesis of high-molecular peptides came up to 78% in relation to the contents of proteins in parent substances. Presumably, a further increase of the yield of high-molecular peptide synthesis from field bean protein hydrolysates will be possible. An analysis of the

amino acids composition in proteins obtained by synthesis did not show any quantitative or qualitative differences in amino acids from proteins of parent products. This, however, will be the subject of another study on this problem.

Our findings are in agreement with Japanese studies (5,6), where mainly soybean and yeast protein was refined, providing it with suitable technological features.

Moreover, the technique of protein refinement presented in this study allowed the antitryptic factor to be completely eliminated from field bean protein. It should be noted that a factor retarding the action of trypsin at the level of 45% of the original value was also found in refined protein similarly obtained by alkaline extraction and precipitation at the isoelectric point. Moreover, the described method of enzymatic protein refinement appeared to be preferable to that of alkaline extraction because neither a highly alkaline environment (pH 9-11.5) nor high temperature (80 C) are required for this process, as alkaline extraction (4,12,17).

Similarly, no nucleic acids were present in enzymatically refined yeast proteins. On the other hand, when alkaline extraction was used, the refined yeast proteins were found to contain 1.33-1.34% of nucleic acids compared with 7.3-9.3% in parent substances. The studies of Lindblom (12), Farnum (4), and Vananuvat (17) also showed that 20-85% of nucleic acids could be removed from yeast when the method of alkaline extraction was used. The above data speak in favor of the method of enzymatic protein refinement. It must be added that, in the cited studies, the extraction of nucleic acids was carried out in a highly alkaline environment, at 80 C, which must have influenced the quality of the produced proteins. The possibility of refinement of fodder yeast seems to be a matter of interest, as this is much cheaper than food yeast. It seems possible that the described method of refinement of unconventional proteins can be utilized for obtaining proteins from vegetative parts of plants to replace the acetone or alcohol extraction of pigments and accompanying substances.

Our laboratory continues its efforts for an optimum resynthesis of high-molecular peptides from enzymatic hydrolysates and examines the possibility of modification of plant and microbiological proteins to increase their

biological value and improve their physical and chemical properties vital in the technology of production of animal protein substitutes.

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Factors Related to the Occurrence of Trimethylamine in Milk¹

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ABSTRACT

The overall objectives of this research were to (a) identify the odor threshold for trimethylamine (TMA) in milk and determine how precisely different concentrations are delineated by taste, and (b) investigate the relationship of different factors to the occurrence of TMA flavor in milk of cows grazed on wheat pasture. The odor threshold for TMA in milk, defined as detection by 50% of a panel untrained in milk judging, was determined to be 1 ppm. Trained milk judges detected 5 ppm TMA in milk in every instance, but were not able to distinguish between 5 and 10 ppm TMA. There appeared to be a positive relationship between the amount of TMA administered orally to cows and its intensity in milk 2 h later. There was a linear relationship between amount of wheat forage consumed as well as stage of maturity of the forage and TMA flavor in milk. Feeding of grain or silage plus grain either before or after grazing wheat pasture had little influence on TMA milk scores. Feeding of alfalfa hay between grazing wheat pasture and milking tended to increase TMA milk scores. There was significant variation among cows in TMA milk flavor scores in all trials.

Utilization of wheat pasture by cattle is routine in wheat growing areas. However, this forage has been shown to produce an undesirable flavor, described as "fishy," in milk from dairy cows grazing this type of pasture.

In 1958, P. E. Johnson (unpublished data) found more intense fishy flavor in milk of cows that grazed on common rye and wheat than in milk from cows grazing Balbo rye, barley, or oat forage. Using mass spectrophotometry, the compound responsible for this undesirable flavor was identified in 1973 by Johnson et al. (3) as trimethylamine (TMA). In 1974, Mehta et al. (4) confirmed the relationship between the fishy flavor characteristic of milk produced on wheat pasture and concentration of trimethylamine determined by gas-liquid chromatography. Data on amounts of methylamine and trimethylamine in milk having the fishy flavor were reported, with the observation that there was no correlation between the amount of methylamine and intensity of fishy flavor.

Detection of low concentrations of TMA in milk under field conditions remains a problem in the industry.

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Bassette (2) advocated a test procedure involving addition of a base to release the amines as an aid in detection; however, this method has not gained wide acceptance in the industry. Small amounts of TMA in solution can be detected by gas chromatography (4), but this equipment is not available to milk tank truck drivers for use in the field.

The objectives of this research were to (a) define the odor threshold for TMA in milk and determine how precisely different concentrations are delineated by taste, (b) investigate the relationship of TMA dosage and wheat forage consumption to TMA concentration in milk, and (c) evaluate the effects of different feeding practices on amount of TMA in milk of cows grazed on wheat pasture.

MATERIALS AND METHODS

Odor threshold for TMA and delineation of different concentrations by taste

Three separate trials were conducted using 10 or more persons without training in milk flavor evaluation to determine the odor threshold for TMA. In each trial, each of the 10 individuals was required to indicate whether or not an undesirable odor was detected in samples of homogenized milk to which known amounts of TMA, ranging from 0 to 20 ppm, had been added. Samples were at room temperature and at normal pH. The odor threshold was defined as the lowest concentration of TMA detected by at least 50% of the test panel. This was determined by linear regression of the percentage of panel members detecting different concentrations of TMA in the three trials against the log of TMA concentration (1, 5).

Another trial was conducted to determine how precisely three trained milk judges could delineate different concentrations of TMA in milk. Samples of raw milk were prepared in duplicate with 0, 5, 10, and 20 ppm TMA. These were coded and randomized before being rated on the amount of TMA detected by taste using a scale of 1 to 5 for flavor intensity.

Factors affecting occurrence of TMA in milk

A series of trials were conducted to relate the amount of TMA administered orally and wheat forage intake by cows to flavor intensity in fresh raw milk. Cows were milked approximately 2 h after TMA administration or wheat forage consumption. Milk samples were collected, cooled immediately, and refrigerated overnight. Each sample was divided into two portions, coded, and randomized for scoring by trained milk judges, using a flavor intensity scale of 1 to 5.

The intensity of TMA flavor in milk in relation to amount of TMA administered orally was determined in two trials involving six lactating

cows. Amounts of TMA administered by capsule or stomach tube 2 h before milking were 0, .5, and 5 g per cow, with two cows receiving each dosage level in each trial.

Two trials were conducted to establish the relationship between the amount of wheat forage consumed and the intensity of TMA in milk. In one instance, 12 cows were assigned to three groups of four cows each, with the amount of time the respective groups were allowed to graze on Triumph-64 wheat forage being 0, 30, and 120 min. The cows were removed from pasture 2 h before milking. All of the cows were fed alfalfa hay after the morning milking and grain 1 h before each milking. In another trial, 12 cows were fed measured quantities of freshly clipped wheat forage to more precisely quantify the relationship between intake and TMA flavor intensity. Intakes were 0, .25, .5, and 1.0 kg of forage dry matter per 100 kg of body weight for four respective groups of cows. Alfalfa hay and grain were also fed daily in appropriate quantity to meet nutrient requirements of the cows.

The influence of feeding practices on the intensity of TMA flavor in milk of cows grazed on wheat pasture was investigated in three separate trials. These trials involved the time of feeding one or more components of the total ration in relation to the time that wheat forage was consumed. Thus, the effects of feeding alfalfa hay, grain, and silage plus grain before vs. after grazing wheat forage were determined.

RESULTS AND DISCUSSION

Odor threshold for TMA

In all three trials to determine the odor threshold of TMA in milk, at least 50% of the individuals detected TMA at a concentration of 3 ppm (Fig. 1). At lower con-

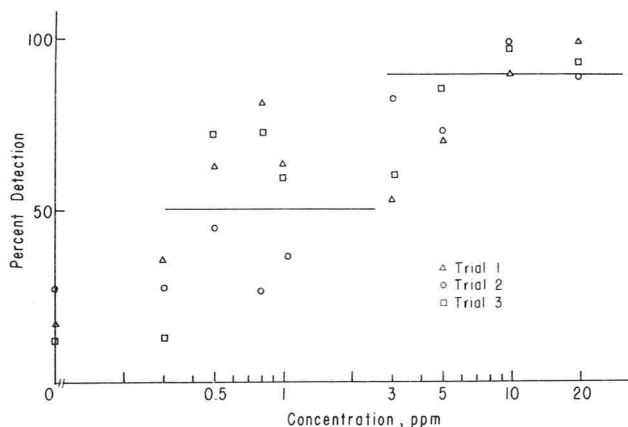


Figure 1. Detection of TMA odor in milk by persons untrained in milk flavor evaluation.

centrations, more than 50% detection was obtained in two trials, and the threshold was estimated to be near 1 ppm on the basis of regression analysis utilizing all the data. This is in general agreement with the report of Mehta et al. (4) that experienced judges detected 2 ppm by taste, more than half the time. Thus, the flavor threshold concentration for TMA as defined is quite low, and development of a reliable colorimetric test for field use does not appear likely at this time.

The merits of using people untrained in milk judging for determination of threshold levels for specific compounds or for evaluating acceptability of milk for general consumption are well illustrated in this work. A few untrained individuals did not detect as much as 20 ppm TMA in milk, whereas Mehta et al. (4) found that

all of five experienced judges detected 4 ppm TMA. This also emphasizes the need for a simple, objective, and reliable field test for TMA in raw bulk milk, since most milk truck drivers charged with the responsibility of determining acceptability of bulk tank milk on the farm are not trained milk judges. Moreover, the test procedure of Bassette (2), wherein TMA is released by adding .1 g of KOH per 20 ml of suspect milk and allowing it to stand for 5 min, has been generally unsatisfactory for field use by untrained persons, (P. E. Johnson, personal communication).

Delineation of different concentrations of TMA in milk by taste.

Experienced milk judges detected 5 ppm of TMA added to milk in every instance, but were not able to distinguish between milk having 5 and 10 ppm. Milk having 20 ppm TMA was noted to have a distinct or very strong flavor. Thus, the concentrations of TMA in milk cannot be estimated accurately in the range of 5 to 10 ppm by organoleptic evaluation. Higher concentrations can be distinguished, making this type of evaluation valid in studies where reasonably large variations in flavor intensity exist. The gas liquid chromatographic procedure developed by Mehta et al. (4) is rather time consuming, and efforts in our laboratory to develop a direct injection method for amines in milk have not been particularly productive. Meanwhile, organoleptic evaluation remains the procedure of choice when a relatively large number of samples are involved and relatively wide variation in flavor is expected.

Factors related to amount of TMA in milk.

Although there appeared to be a positive relationship between the amount of TMA orally administered to cows and its intensity in milk 2 h later, it was obvious that only a small amount of administered material was transferred to the milk (Table 1). The larger amount given two cows

TABLE 1. Detection of trimethylamine in milk following oral administration to cows

Amount administered (g/cow)	Average flavor score ^a	
	Trial 1	Trial 2
0	1.7 ^b	1.3
0.5	2.3	2.0
5.0	3.1	2.3

^aRating scale: 1 = no TMA detected, 2 = very slight, 3 = distinct, 4 = strong, 5 = very strong.

^bAverage scores by four judges tasting coded duplicate samples from each of two cows.

in each trial was calculated to be approximately 10 times the amount that would cause a very strong TMA flavor in milk of cows producing approximately 20 kg per day, if all of it were transferred to the milk. The actual metabolite from which TMA in milk is derived is not known, but it does appear that it is not merely absorbed from the rumen and transferred directly to the mammary gland.

The effect of amount of time that cows spent grazing

wheat forage on TMA flavor was different for different periods of the experiment, i.e., there was a statistically significant ($P < .05$) interaction between treatment and periods of the trial (Fig. 2). Flavor intensity in milk from

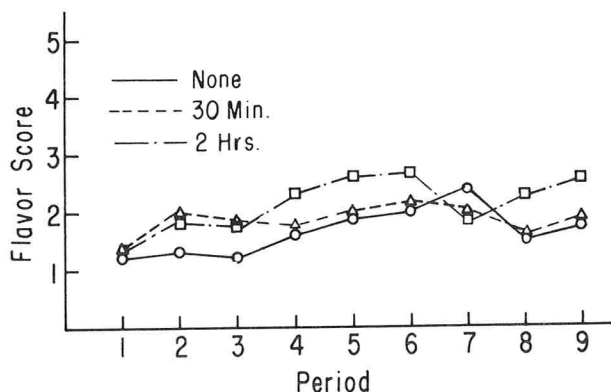


Figure 2. Average wheat flavor intensity in milk from cows grazed for different time periods on wheat pasture.

the control cows increased somewhat during periods 4 through 7, which corresponded to a period of increasingly adverse weather. During this period, unsatisfactory condition of the lots and shelters may have resulted in undesirable flavors in the milk which interfered with the ability of the judges to identify wheat pasture flavor. The irregularities occurring on the day designated Period 7 were attributed to the fact that the wheat plants were coated with ice on this particular day.

Within each group of cows grazed for a designated time, there was considerable variation among cows in flavor scores averaged over the entire trial (Table 2).

TABLE 2. Wheat flavor scores for individual cows averaged over entire grazing season

Time grazed (min)	Range of average scores for individual cows within groups ^a	Overall average
0	1.5-1.7	1.64
30	1.6-2.3	1.87
120	1.6-2.6	2.15

^aFour cows per treatment with 72 observations per cow.

During some of the periods, one or more cows in the groups grazed on wheat pasture had very little TMA flavor in the milk. Thus, differences among groups in flavor scores were statistically significant ($P < .05$) only during periods 2, 3, 6, 8, and 9 of the trial. Overall, the amount of time spent grazing accounted for only 12% of the variation in flavor scores. There was no apparent explanation for the large amount of variability among cows in TMA flavor scores.

There was a positive relationship between the amount of wheat forage consumed and intensity of TMA flavor in milk (Fig. 3). As stage of maturity of the forage advanced, intensity of TMA increased and a more definite relationship to amount of intake was evident. Thus, from 23 to 72% of the variation in flavor scores could be attributed to the linear component of variance

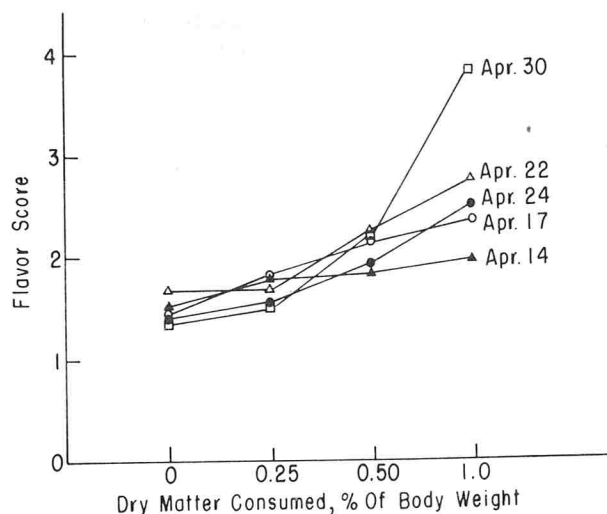


Figure 3. Average wheat flavor intensity in milk from cows fed different amounts of wheat forage dry matter.

due to amount of forage consumed. Control of amount of intake would have merit in terms of utilizing wheat forage by lactating dairy cows. However, it is obvious that factors other than the actual amount of forage consumed are responsible for part of the variability in TMA scores among cows grazed on wheat pasture for a stipulated

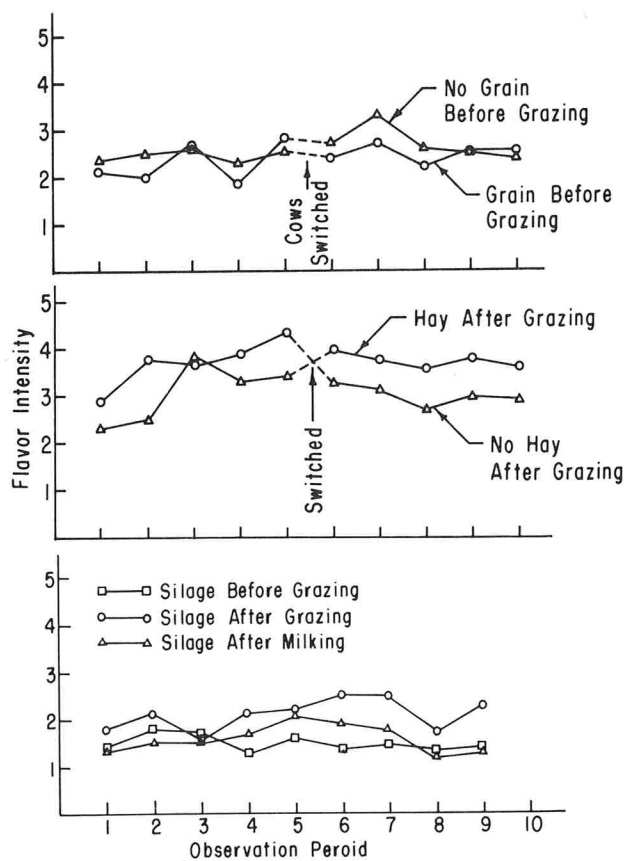


Figure 4. Average wheat flavor intensity in milk from cows fed components of the ration at different times relative to grazing wheat pasture.

amount of time. There was no relationship between daily milk yield of the cows and TMA intensity in their milk after grazing wheat forage.

Influence of feeding practices on TMA in milk.

Feeding hay, grain or grain plus silage to cows immediately before or after grazing wheat pasture has not proven particularly beneficial in reducing TMA in milk (Fig. 4). In fact, feeding of high quality alfalfa hay during the 2-h interval between grazing and milking tended to accentuate rather than reduce the problem. The trials wherein grain or silage were fed before grazing were conducted with the premise that lower rumen pH resulting from fermentation of these components might render TMA less available for absorption into the blood. Only slight reduction in TMA milk scores resulted from

this alteration in feeding regime, however, so some other approach for preventing or reducing the flavor problem is needed.

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A Microbiological Survey of Raw Ground Beef in Ohio

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ABSTRACT

A microbiological survey of raw ground beef at the retail level was conducted in Ohio. The sampling plan required that the number of samples be distributed proportionately according to the Ohio county population. Four hundred fifty-seven samples were collected from both supermarkets and independent retail outlets representing all 88 counties in Ohio. Total aerobic plate counts (TPC), oxidase-positive counts (Ox Pos), coliform counts, and pH were used as criteria to reflect poor handling, storage, and sanitation practices. After statistical treatment of the data, operational curves were developed based upon the defined critical limits derived from the survey. Proposed microbiological guidelines for reflecting proper sanitation, storage and handling were developed from this information. The proposed acceptable criteria for "fresh" raw ground beef were defined as: less than 15,000,000/g TPC and less than 300,000 Ox Pos/1,000,000 TPC/g with a pH less than 5.85 (storage and handling); and less than 3000/g coliform (sanitation).

Passage of the Federal Wholesome Meat Act of 1967 has focused considerable attention on inspection and quality assurance programs for meat products. One area of quality assurance which has attracted attention has been development of operational microbiological guidelines. One of the main objectives for establishing microbiological guidelines would be to establish a criterion that would reflect the quality of the meat at the point of slaughter, processing, and/or sale. The rationale for establishing such microbiological guidelines has been adequately discussed by Elliott and Michener (7), Thatcher and Clark (18), and Bartram (3).

Several studies (4,6,8,11,16) have been conducted on raw ground beef with total aerobic bacteria counts ranging from 250,000/g (8) to 10,000,000/g (4,6) suggested as guidelines. Perhaps reasons for this wide range of reported values were such factors as inadequate sample representation for the population surveyed, variability in plate count methodology, and existence of a variety of slaughter, processing, and product distribution conditions. With these factors in mind, the Ohio Department of Agriculture focused its microbial survey of raw ground beef on two major quality factors: (a) product

handling and storage, and (b) sanitation at the retail level.

The selected criteria used to reflect the handling and storage condition of the raw ground beef were: (a) the total aerobic plate count (TPC), (b) estimation of the psychrotrophic bacteria population by an oxidase staining technique, and (c) pH. Sanitation conditions, which would require an inspection follow-up, were identified by relating the presumptive coliform count to a percentage of the total aerobic plate count.

MATERIALS AND METHODS

A 25-g sample was weighed into a sterile pyrex glass blender jar and 225 ml of sterile phosphate buffered water (2) were added. The sample was blended for 3 min at high speed. Consecutive serial dilutions to 10^{-7} were prepared and the following analyses were done.

Total aerobic plate count (TPC)

Duplicate plates, 10^{-5} through 10^{-7} dilutions, were prepared, and thoroughly mixed with 12 to 14-ml portions of tryptone glucose yeast agar (Standard Methods Agar). The solidified plates were incubated for 48 h at 32 C. Plate count preparation, counting, and recording of results followed procedures outlined in *Standard Methods for the Examination of Dairy Products* (1).

Presumptive coliform count

Duplicate plates, 10^{-1} through 10^{-4} dilutions, were prepared and thoroughly mixed with 10 to 12-ml portions of violet red bile (VRB) agar. An overlay of 3 to 4 ml of VRB agar followed solidification of the previously poured agar. The prepared plates were incubated for 24 h at 32 C. Preparation, counting, and recording of results were in accordance with *Standard Methods for the Examination of Dairy Products* (1).

Estimation of psychrotrophic numbers (oxidase-positive count)

Hankin et al. (12, 13, 14) reported the value of the oxidase reaction in conjunction with the total aerobic plate for counting potential psychrotrophs. The estimated psychrotrophic count was done in accordance with the procedure outlined by Hankin and Dillman (12). Results were recorded as oxidase-positive count per gram.

pH Determination

The pH was determined with a Photovolt Digital read out pH meter. Approximately 30 g of raw ground beef were blended in 50 ml of distilled water before the pH determination.

Experimental design

The sample size for the survey was determined in a manner that would minimize sampling error and provide reliable estimates of the parameters being investigated.

According to the sampling formula reported by Freund et al. (10) it was determined that a minimum of 384 samples would be required to be at least 90% confident. The population to be sampled was defined as the State of Ohio. The sampling procedure was dictated by 1969

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population concentrations in each of the State's 88 counties with one sample representing, at the most, 25,000 people. The counties were also arbitrarily divided into five regions, Central, Northeast (N.E.), Northwest (N.W.), Southwest (S.W.) and Southeast (S.E.), Fig. 1. The raw ground beef samples were obtained by the Ohio Department of Agriculture Food and Drug inspectors. These inspectors were assigned the counties to be sampled and advised as to how many independent



Figure 1. Identification of the arbitrarily divided regions in Ohio.

and supermarket stores to visit. Randomly selected retail stores were visited (with a market basket-type approach) by the inspectors. Only one random sample per retail store was obtained. At least two samples were obtained from each county; one sample was purchased at a supermarket and the other from an independent retail store. In most counties, the number of samples received from supermarkets were equal to those received from independent retail stores. Samples were submitted for bacteriological analysis within 24 h after purchase and were maintained at 4 C from collection until delivery to the laboratory. A total of 457 samples of unfrozen raw ground beef were submitted to the laboratory. These samples were analyzed immediately upon receipt in the laboratory. Samples were obtained during the months of April, May, and June, 1969. The data generated from this meat survey were tabulated, statistically treated, and graphically plotted.

Statistical analyses

The variables subjected to statistical analyses were TPC, presumptive coliform count, and oxidase-positive bacteria counts. Since these are discrete data (counts) a Log_{10} transformation (15) was done before the application of statistical methods.

The statistical methods applied to the data just described were: (a) analysis of variance, (b) "t" test, and (c) correlation coefficient. The parameters examined were: (a) a comparison of samples taken from independent food stores and those taken from the five regions among independent food stores, and (b) a comparison of samples taken from the five regions among chain supermarkets. An operational curve was developed from the TPC and coliform count data.

RESULTS

The TPC data collected for the State of Ohio are graphically presented in Fig. 2. The data did not form

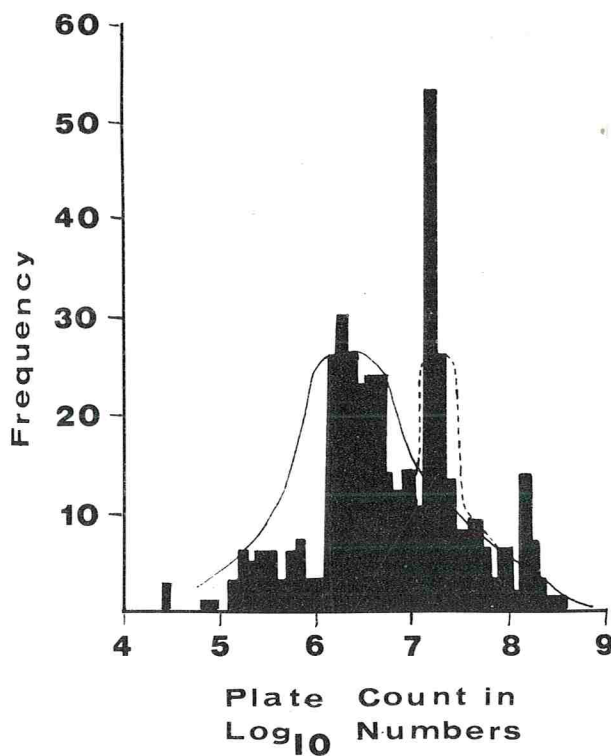


Figure 2. Frequency distribution data of total plate count (as log_{10}) for all Ohio samples analyzed.

the "normal" distribution curve which one might expect. Instead there appear to be two distribution curves partially overlapping each other. The curves seem to intersect at approximately $\text{log}_{10} = 7.18$.

The frequency distribution of the oxidase count/TPC, as percentage, is graphically presented in Fig. 3. The

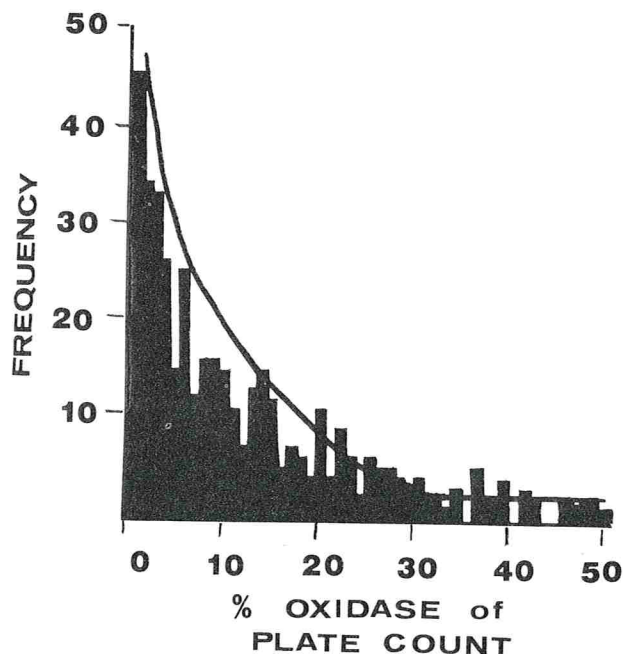


Figure 3. Frequency distribution of the oxidase positive count/total aerobic plate counts, as percentage.

data form a right tail curve whose slope markedly tapers off at 30%. This is to infer that most unfrozen Ohio raw ground beef samples analyzed exhibited 30% or less oxidase-positive colonies relative to the TPC. Observation of the initial data revealed that oxidase-positive counts begin to occur with more frequency when the TPC exceeded 5,000,000/g. The regression lines of the TPC compared with the oxidase count and with pH, respectively, are presented in Fig. 4.

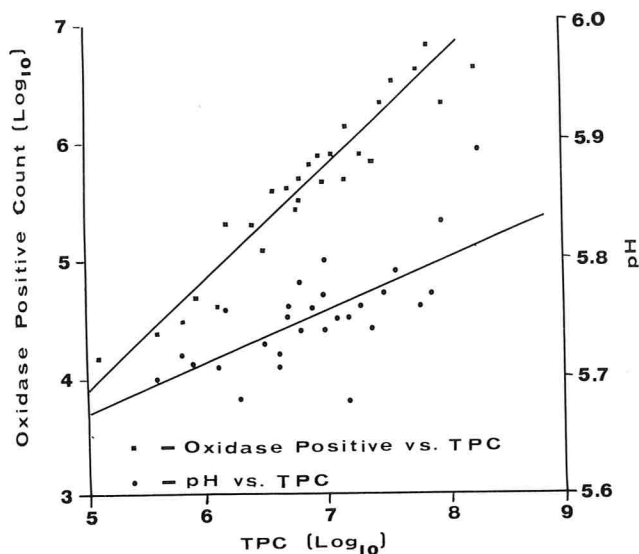


Figure 4. Correlation of the total aerobic plate counts compared to oxidase positive count and pH.

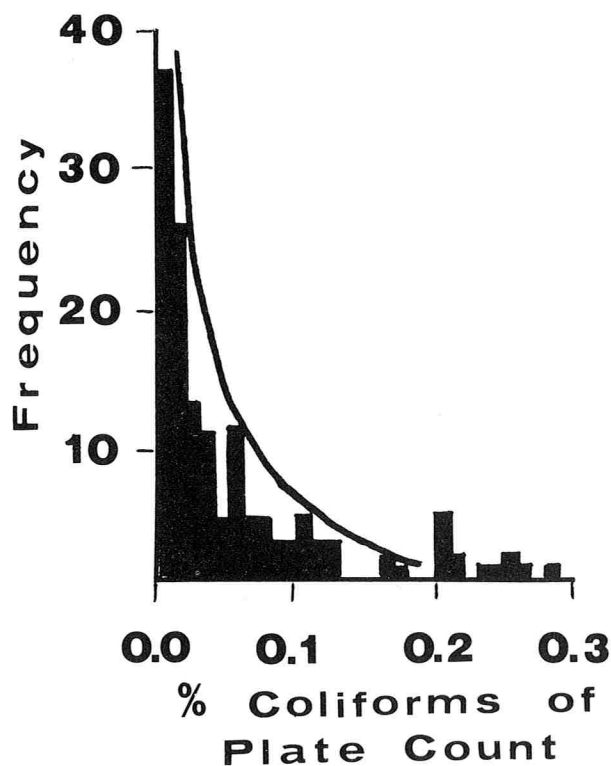


Figure 5. Frequency distribution of the coliform counts, as percentage.

A correlation coefficient of 0.95 for the oxidase positive count 0.48 for the pH values were calculated when these parameters were compared with the TPC. Thus, from the oxidase-positive count correlation one might infer that the TPC counts, demonstrated in Fig. 2, are beginning to be dominated by psychrotrophic bacteria as the count increases.

The frequency distribution for the State presumptive coliform count/TPC percentage is presented in Fig. 5. This graph shows the data are distributed as a right tail distribution curve with the slope of the curve tapering off sharply at 0.2%. This suggests that the "normal" distribution of coliform for unfrozen raw ground beef in Ohio is less than 2 coliforms per each 1,000 bacteria counted.

Table 1 reflects the descriptive statistics generated from the Ohio raw ground beef survey data. The computation took place on Ohio State University's IBM 7094 computer using a program written by the U.C.L.A. Health Sciences (19). A Statewide comparison of the TPC means of the supermarket and independent retail outlets, using a "t" test, indicates no significant difference ($P < .05$) but the standard deviation is slightly larger for the independent stores. In contrast, the presumptive coliform counts, on the average, were significantly greater ($P < .05$) for the supermarkets than for the independent food outlets. The following sets of results were obtained from regional comparisons, each utilizing a one-way analysis of variance: (a) The TPC means for independent retail outlets show no difference, on the average, when compared among the five regions. (b) The TPC means for supermarket stored show the Central Region to be significantly greater, on the average, than the S.W., S.E., N.E. and N.W. Regions ($P < .05$). No other differences were found. (c) The coliform sample means for independent retail outlets show no difference, on the average which was also the case for the coliform counts obtained for the supermarket samples. (d) The oxidase-positive means for independent retail outlets indicate the Central Region is significantly greater, on the average, than the other four regions ($P < .05$). Also, the N.E. Region is significantly greater, on the average, than the S.E. Region ($P < .05$). No other differences were found. (e) The oxidase-positive means for supermarket stores indicate the Central Region is significantly greater, on the average, than the S.E., S.W., and N.W. Regions ($P < .05$). Also, the N.E. Region is significantly greater, on the average, than the S.E. and S.W. Regions. No other differences were found.

The mean values by region, for each of these five data sets are presented in Tables 2 and 3. Comparisons among these five arbitrarily devised regions were made primarily to obtain a better insight regarding variability throughout the state

DISCUSSION

Since improper handling and storage conditions contribute to microbial contamination and/or growth, the total aerobic plate count and oxidase-positive count

TABLE 1. Descriptive statistics for the Ohio raw ground beef survey

Survey variable	Geometric mean	Means	Standard deviation	Standard error of mean	Sample
Total plate count ^a	7,357,000	6.8667	0.7811	0.0365	457
Oxidase-positive count ^a	444,600	5.6479	0.9166	0.0429	457
Coliform count ^a	537	2.7299	0.7936	0.0371	457
% Coliform		0.1107	0.4107	0.0250	270
% Oxidase-positive count		13.4443	14.3066	0.7263	388
<i>Total plate count^a</i>					
Central	15,890,000	7.2111	0.7344	0.0964	58
S.E.	5,860,000	6.7679	0.7795	0.1175	44
S.W.	5,153,000	6.7121	0.7033	0.0729	93
N.W.	6,126,000	6.7872	0.6936	0.0780	79
N.E.	8,104,000	6.9087	0.8010	0.0591	183
<i>Oxidase-positive count^a</i>					
Central	890,200	5.9495	0.8318	0.1083	58
S.E.	280,400	5.4477	0.8638	0.1302	44
S.W.	235,000	5.3710	0.8162	0.0846	93
N.W.	349,900	5.5439	0.9156	0.1039	79
N.E.	578,200	5.7621	0.9733	0.0716	183
<i>Coliform count^a</i>					
Central	944	2.9749	0.7792	0.1014	58
S.E.	529	2.7238	0.7690	0.1159	44
S.W.	464	2.6666	0.8222	0.0853	93
N.W.	439	2.6427	0.9709	0.1106	77
N.E.	564	2.7515	0.8105	0.0602	183
<i>Independent retail stores</i>					
Total plate count ^a	7,228,000	6.8590	0.8350	0.0551	232
Oxidase-positive count ^a	430,100	5.6336	0.9430	0.0622	232
Coliform count ^a	450	2.6535	0.7484	0.0493	232
% Coliform		0.1224	0.4787	0.0426	126
% Oxidase-positive count		13.3443	14.1115	1.0211	191
<i>Supermarkets</i>					
Total plate count ^a	7,869,000	6.8959	0.6883	0.0457	227
Oxidase-positive count ^a	419,600	5.6228	0.8883	0.0586	228
Coliform count ^a	632	2.8005	0.8248	0.0549	227
% Coliform		0.0928	0.3098	0.0256	146
% Oxidase-positive count		13.5364	14.1072	1.0077	196

^aValues are in logarithms

TABLE 2. Mean values for the total plate, coliform, and oxidase-positive counts (log₁₀) for independent retail outlets, by region

Count	Regions				
	Central	N.E.	N.W.	S.E.	S.W.
TPC	7.09	6.89	6.74	6.78	6.78
Coliform	2.85	2.75	2.46	2.55	2.54
Oxidase-positive	6.06**	5.75*	5.51	5.53	5.35

Note: (**) Statistically significant difference (P < .05) when compared with other four regions.

(*) Statistically significant difference (P < .05) when compared to the S.W. Region.

TABLE 3. Mean values for the total plate, coliform, and oxidase-positive counts (log₁₀) for supermarket retail outlets, by region

Count	Regions				
	Central	N.E.	N.W.	S.E.	S.W.
TPC	7.39**	6.91	6.86	6.74	6.72
Coliform	3.05	2.82	2.76	2.94	2.77
Oxidase-positive	5.89**	5.78*	5.59	5.33	5.39

Note: (**) Statistically significant difference (P < .05) when compared with the S.E., S. W., and N.W. Regions

(*) Statistically significant difference (P < .05) when compared with the S.E. and S. W. Regions.

were selected as the criteria to reflect these conditions. Use of the oxidase-positive counts as an indicator of potential psychrotrophic numbers has been documented by Hankin et al. (12,13,14) and, therefore, was selected to reflect long term storage or handling of product at elevated temperatures. The latter condition would allow the psychrotrophs to enter into the logarithmic growth

phase and reflect higher total aerobic plate counts as well as being in greater population dominance.

The criteria used to suggest poor sanitation conditions was the presumptive coliform plate count. Recognizing that members of the coliform group are ubiquitous, enumeration of this group does reflect environmental contamination due to conditions which may have existed during slaughter, processing, and/or product handling.

A review of the data presented indicated that 90% of the raw ground beef samples analyzed were below a TPC of 15,000,000/g; a presumptive coliform count of 2,000/g; and demonstrated an oxidase-positive bacteria population at less than 30% of the respective total aerobic plate counts. There was observed a wide variation in the microbial content of raw ground beef throughout the State of Ohio as reflected by data in Table 1. This tends to suggest that some inconsistency in good handling, storage, and sanitation practices existed during the survey. Therefore, an establishment of microbial guidelines seems justified to obtain a comparable measure of proper handling, storage, and/or sanitary conditions at the retail level for raw ground beef.

Considering the critical limits of raw ground beef at 15,000,000/g (log=7.2) for the total aerobic plate count and 3,000/g (log=3.47) for the presumptive coliform count, an establishment of an operation curve becomes important. Based on the protection level one desires,

several sampling plans could be developed. Therefore, the operational curves presented are only examples and are limited to the population used in this survey.

Figure 6 shows a TPC operating characteristic (O.C)

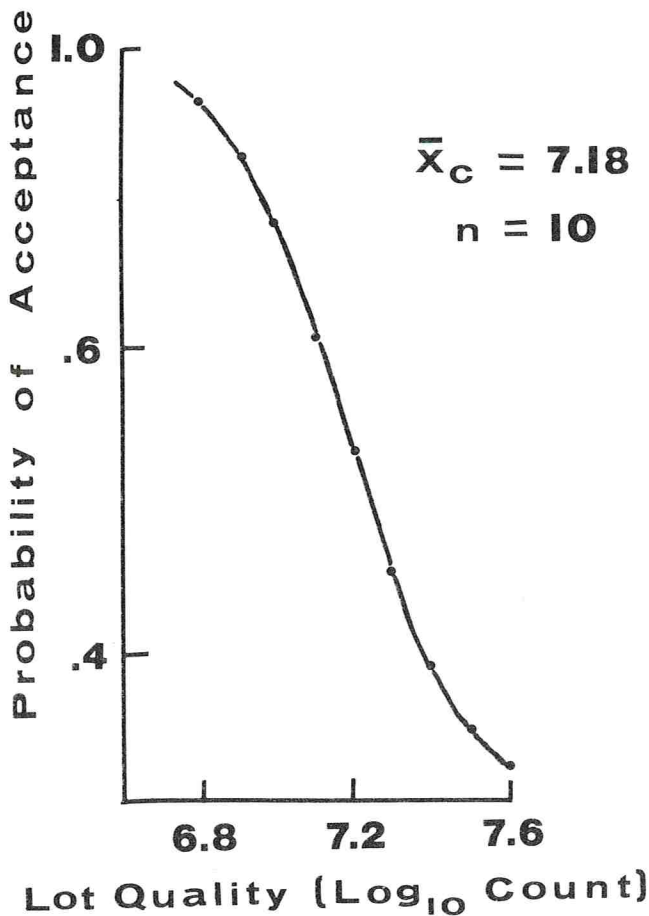


Figure 6. Total aerobic plate count operating curve and sample plan for a lot of comminuted meat.

curve for a sampling procedure using the following criteria; (a) sampling error = 0.10; (b) TPC critical limits-log 7.18 (geometric mean = 15,000,000); and (c) sample size=10. If, for example, a "true" lot quality is a TPC of 6,300,000/g (log 6.8), the probability of acceptance is 0.9357 or 936 lots out of 1,000 lots sampled. But, in contrast, this would mean that 64 lots out of 1,000 would be rejected as the probability of review (1.0-probability of acceptance). A detailed

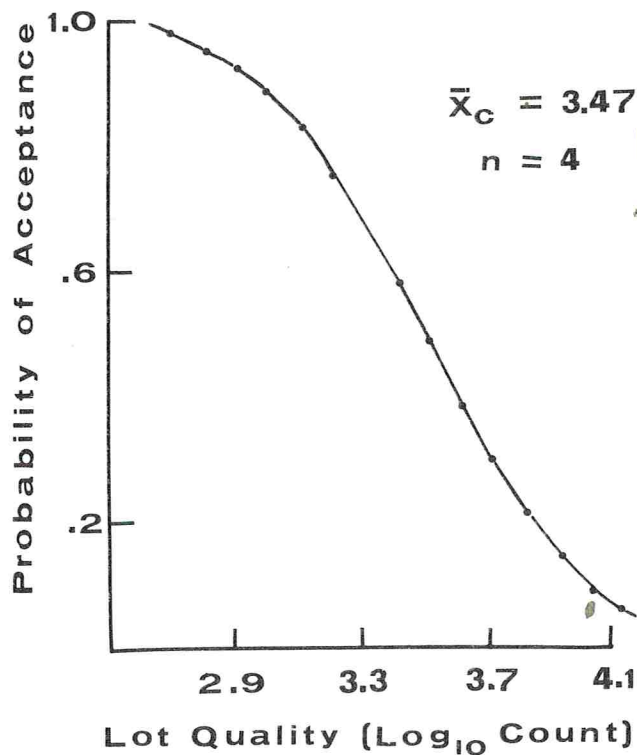


Figure 7. Coliform count operating curve and sample plan for a lot of comminuted meat.

discussion of sampling plans of O.C. curves is given by Duncan (5). Figure 7 demonstrates the coliform O.C. curve for a sampling procedure using the following criteria: (a) sampling error = 0.10; (b) presumptive coliform count critical limits = log 3.47 (geometric mean = 3,000); and (c) sample size = 4. Coliform O.C. would be used in the same manner as the example given for use of the TPC operational curve.

The use of the oxidase-positive count in conjunction with the TPC was found to be a useful tool in estimating potential psychrotrophic bacteria problems due to poor handling and/or storage practices. It was observed from the data (Fig. 2 and 4) that the possibility of two dominant flora of bacteria may exist in the raw ground beef. One dominant type appears to be the

psychrotrophic bacteria which will grow rapidly when subjected to extended storage periods at cold temperatures or when subjected to warmer temperatures (17). Rapid growing psychrotrophic bacteria (i.e. *Pseudomonas* species) have continually been documented as causing meat decomposition which lowers the quality of the meat and leads to spoilage. Warmer storage temperatures would also lead to high TPC values (9).

The investigative data generated from this survey indicate a need for some form of microbiological guidelines. It was the purpose of this survey to determine those critical limits which would indicate that raw ground beef had been subjected to probable poor sanitary and/or storage conditions and/or poor handling practices. Utilizing the TPC, presumptive coliform, and estimated psychrotrophic bacteria counts as the monitoring criteria, these undesirable conditions could be identified and corrective steps initiated. Thus, through the use of properly defined microbiological guidelines, an improvement of raw ground beef quality at the retail level should be realized.

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Variations in Organic Acid Profiles of Thermally Processed Green Beans (*Phaseolus vulgaris* L.) of Different Varietals

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ABSTRACT

Four varieties of green beans, *Phaseolus vulgaris* L. (Lake Shasta, Lake Geneva, Slimgreen, and Cascade), grown under the same conditions were analyzed for organic acid content and pH. Each variety was comminuted and processed in TDT tubes with an F_0 value of 4.9 at temperatures of 240 and 280 F. All analyses were conducted in duplicate using an Automatic Organic Acid Analyzer (Waters Associates, Milford, Mass.). Differences in the organic acid content were determined statistically at the 95% significance level for each acid. The organic acid profiles for the beans showed qualitative variations which appeared to be somewhat variety dependent and changes in the acid profile to a particular variety on processing appeared to be dependent on the variety. The effects of processing on the pH of the samples appeared to be quite similar. All varieties contained acetic, fumaric, lactic, succinic, α -ketoglutaric, malic, and citric acids both initially and after processing.

In striving to produce products for the consumer which are safe, available, and more nutritious, a great deal of research has been done on changes which occur during thermal processing. One area, in particular, which has received a great deal of attention is the change in the organic acid profiles of various fruits and vegetables.

It has been noted that changes in the organic acid content of products occurs during ripening (5) and the direction of these changes is dependent on the particular acid being observed (6, 12). Organic acid analyses have been done on many varieties of fresh fruits and vegetables (7).

Processed commodities have also received a great deal of attention in terms of the effect of the organic acids on final product quality. Weissberger et al. (16) examined cocoa beans and noted that the organic acid content was variable and could be influenced by the types of treatments the beans received during processing. It was also noted that the final organic acid content of the product influenced the product quality. Tomato products have also received careful scrutiny (3, 13). The organic acid content observed in all studies exhibited both qualitative and quantitative changes in the acids present as a result of processing of the product and later storage.

Organic acid composition and variation have been examined in spinach puree which had been processed for various times and temperatures with the same F_0 value

(10). The F_0 value has been defined by Stumbo (15) as the equivalent in minutes at 250 F, of all heat considered, with respect to its capacity to destroy spores or vegetative cells of a particular organism. Quantitative differences were found for both acetic and pyrrolidone carboxylic acid (PCA) between the processed samples. Using carrot puree, Luh et al. (11) noted differences in the organic acid content when comparing aseptic processing techniques with conventional retort processes. Work has also been done on the effects of different amounts of fertilization on the concentration of PCA found in processed table beets (9, 14). Bibeau et al. (2) developed a simple quality test for carrots based on the amount of glutamine present, the conversion of glutamine to PCA at various processing temperatures, and the taste threshold for PCA. Using this methodology, it was possible to predict the best processing parameters, based on the glutamine content, to minimize off-flavors due to PCA. As well, Bibeau and Clydesdale (2) examined the organic acid profiles of carrot puree.

In this particular study, four varieties of green beans (*Phaseolus vulgaris* L.) were processed at different temperatures and times with the same F_0 value. Organic acid profiles were examined in an attempt to determine if there were differences in the profile of the same variety when processed at different temperatures and between varieties processed at the same temperature.

MATERIALS AND METHODS

Four varieties of green beans (*Phaseolus vulgaris* L.) were planted on lima silt loam soil at different time intervals. All of the beans were fertilized using commercial rates of fertilization and were irrigated when necessary. The four varieties planted were Lake Shasta, Lake Geneva, Slimgreen, and Cascade. All seeds with the exception to the Slimgreen were from the same seed source. The beans were hand picked and chilled for 10-15 min in an ice-water mixture to decrease the product temperature. Beans were then drained, packed, and shipped from the Department of Vegetable Crops, N.Y.S. Agr. Expt. Sta., Geneva, N.Y. to our laboratory for analysis. Shipments were staggered depending on the planting data for each particular variety. Upon receipt, each sample was refrigerated and analyses were conducted within 24 h.

Each variety of beans was examined separately for organic acid content and pH. Each sample was washed and comminuted in a Fitzpatrick Mill (Fitzpatrick Mill, Model M Comminuting Machine, The W. J. Fitzpatrick Co., Chicago, Ill.) using a fine (No. 40) screen. The entrapped air, incorporated during comminuting, was removed by

placing the sample under vacuum using a vacuum dessicator attached to a water aspirator. Forty TDT tubes, which had been flushed with nitrogen, were filled with approximately 4 to 5 ml of the bean puree. A large hypodermic syringe was used to add the puree to the TDT tube. The head space was then flushed with nitrogen. The tubes were sealed using an oxygen flame. Methods developed by Gupte and Francis (4) were used in processing which was carried out in a temperature controlled glycerol bath. Each variety was processed at 240 and 280 F with an F_0 value of 4.9. Twenty tubes were used for each processing temperature. After processing the samples from each processing temperature were placed randomly in two groups of 10 tubes each. All tubes were frozen at -20 F until analyses were carried out. Samples from the initial product and from each processing temperature were analyzed to determine the pH and organic acid content.

The determination of the organic acid profile for each sample was carried out using an Automatic Organic Acid Analyzer (AOAA) (Waters Associates, Milford, Mass.). All data obtained was based on determinations made using this instrument on duplicate samples. Identification of the various organic acids present was made using both retention times and internal standards for each acid. The analyzer was calibrated using mixtures of acids determined to be present in the samples. Determinations of pH were carried out using a Beckman Expandomatic pH Meter (Beckman Instruments Inc. Fullerton, California) which had been adjusted using the appropriate buffer solution. Statistical evaluations were carried out according to Kramer and Twigg (8).

RESULTS AND DISCUSSION

Trace amounts of several organic acids were found in the different varieties of green beans examined. Propionic acid was observed in all but the Lake Shasta variety while pyruvic acid was found in trace amounts in the Cascade, the Lake Shasta, and the Lake Geneva samples (Table 1). The concentrations of organic acids presented in Table 1 are averages of the duplicate analyses conducted for the initial samples and for each processing temperature. Statistical analyses were conducted on the data using the duplicate analyses and not the averages. The lactic acid concentration was high in both the Slimgreen and Cascade varieties, but only trace amounts were found in the other samples. Pyrrolidone carboxylic acid (PCA), not observed in any of

the initial samples, was found in trace amounts in all varieties, except Slimgreen, when processed. Citric acid was found in trace amounts in all varieties, while cis-aconitic acid was found in all varieties but the Slimgreen.

The amount of each acid found in the various samples was compared to determine if there was any significant difference between varieties. Due to the sudden change in the acid profile of the Slimgreen variety and the lack of additional samples to conduct further experiments, it was not included in these analyses. It is felt that future studies should be done on this particular variety to determine if these results could be duplicated.

No significant differences in the acetic acid concentration were observed between fresh samples of the Lake Shasta and Lake Geneva varieties, while differences were found between the Lake Shasta and Cascade varieties. Significant differences were observed for both Lake Geneva and Cascade when compared to the Lake Shasta variety with the 240- and the 280-F processes. Fumaric acid concentration was significantly different at the 95% confidence level between the Lake Shasta variety and both the Lake Geneva and Cascade varieties for the fresh and processed samples. Alpha-ketoglutaric acid occurred at significantly different concentrations in all varieties processed at 280 F but not at 240 F. Malic acid exhibited no significant differences in concentration in the initial samples. Differences were found between Cascade and Shasta for the 240-F process and between all varieties for the 280-F process. No significant differences were found between green bean varieties for cis-aconitic and succinic acids.

Having examined differences due to variety it was decided to examine the effects of processing within a variety. This was done by comparing the 240-F process concentration to both the initial and 280-F process concentrations (Table 1).

TABLE 1. Average concentration of organic acids present in varieties of green bean puree. (*Phaseolus vulgaris L.*) both before and after processing at 240 F (115.6 C) and 280 F (137.8 C) with an F_0 value of 4.9

Process °F	Acid concentration (ueq/gm) dry weight of green bean puree										
	Propionic	Acetic	Pyruvic	Fumaric	Lactic	Succinic	Alpha-ketoglutaric	PCA	Cis-aconitic	Malic	Citric
<i>Lake Shasta</i>											
Initial	—	135.5*	t	45.6	t	20.4	89.5	—	13.5	231.7	t
240	—	281.6	t	57.3	t	19.6	102.5	5.45	4.5	285.7	t
280	—	222.5*	t	61.4	t	16.4	99.1	t	11.9	267.2	t
<i>Lake Geneva</i>											
Initial	t	152.5	t	35.7*+	t	20.4	97.5	—	19.0*	218.4	t
240	t	178.7+	t	42.5+	t	13.9	103.6	t	5.9	201.9	t
280	t	146.1+	t	38.1+	t	18.8	88.4+	—	14.5	174.3+	t
<i>Slimgreen</i>											
Initial	t	470.4	—	9.3	62.9	147.6	90.5	—	—	51.4	t
240	t	429.6	—	t	80.5	223.2	121.1	—	—	28.1	t
280	t	587.9	—	0.4	78.7	299.4	154.6	—	—	t	—
<i>Cascade</i>											
Initial	t	488.1+	t	18.0+	41.3*	16.7	71.0	—	t	205.9*	t
240	t	480.0+	—	20.4+	66.3	20.6	85.1	3.4	t	147.5+	t
280	t	418.3+	t	18.2+	71.1	24.1	50.0*+	t	t	110.1+	t

+ = significant difference from the Lake Shasta variety for a particular acid and process at the 95% significance level.

* = significant difference from the 240° F process for a particular acid and variety at the 95% significance level.

t = trace amount present.

Differences in acetic acid concentration due to processing were found only in the Lake Shasta variety. Differences in both fumaric and cisaconitic acid concentration were observed in the Lake Geneva variety between the initial and the 240-F processed product. With the Cascade variety, significant differences were noted for lactic and malic acids between the initial and the 240-F processed products and for alpha ketoglutaric acid between the 240- and 280-F process. No significant differences were observed with succinic acid between processes for any of the varieties.

The organic acid profiles for the green beans examined, exhibit qualitative variations which appear to be variety dependent. On processing, quantitative changes were observed in some acids within particular varieties. Between varieties, these quantitative changes varied. Changes in acid concentration for processed samples appears to be somewhat dependent on the variety of green beans being examined. These varietal differences may be of some importance in selecting beans for processing.

TABLE 2. pH of green bean puree (*Phaseolus vulgaris L.*) from different varieties both before and after processing at 240 F and 280 F with an F_0 value of 4.9

Process	pH ^a			
	Variety			
	Lake Shasta	Lake Geneva	Slimgreen	Cascade
Initial	6.16	6.39	5.81	6.08
240 F	6.01	6.22	5.70	5.78
280 F	6.12	6.29	5.20	5.91

^aAverage of duplicate readings.

The pH of each variety examined was determined in duplicate. In Table 2 it may be seen that the pH change was the greatest for the 240-F process for all varieties except the Slimgreen where the greatest change was observed with the 280-F process. The variation in the Slimgreen variety should be re-evaluated along with the organic acid profiles discussed above. The similarity of the pH readings for the other three varieties may indicate that despite the varietal differences, effects of processing on the pH of samples were quite similar.

This investigation provides some initial indications that variety of green beans may be an important factor in the quality of the thermally processed product due to the organic acid profiles and/or stability of such acids within a given variety. This could lead to further work which might indicate the usefulness of particular cultivars or the possible development of new cultivars for thermal processing, based on organic acid profiles.

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Characteristics of Yeasts Isolated From Bread Doughs of Bakeries in Shiraz, Iran

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ABSTRACT

Iranian bakers use portions of sour doughs saved from the previous day for leavening. In the present study dough samples from 43 different bakeries in Shiraz, Iran were examined for the presence of yeasts. On the basis of colonial characteristics, 92 different yeast cultures were isolated. Eighty-one of the yeast cultures were examined for their cell morphology, pellicle formation on liquid medium, ability to form ascospores on nine different sporulating media, and their ability to ferment and assimilate glucose, galactose, raffinose, sucrose, maltose, and lactose. Results showed that 15 of the yeast cultures formed pellicles, 17 cultures did not sporulate, and 33 of the sporulating cultures differed biochemically from the genus *Saccharomyces*. Based on cultural, cell morphology, and biochemical characteristics, 29 of the remaining 31 sporulating yeasts were similar to *Saccharomyces cerevisiae* and the remaining two probably belonged to the genus *Saccharomyces*. Yeast cultures were also examined for their baking strength; 11 of the cultures had baking strengths comparable to those of commercial bakers' yeast.

Use of yeasts for baking has probably been practiced since the Egyptians discovered the art of producing leavened bread about 2600 BC (2), but it is only since the 1920's that standard pure yeasts have been successfully cultivated and effectively employed for this purpose (3, 7).

In general, Iranian bakeries use portions of sour dough saved from the previous days for leavening. These uncontrolled stock cultures are used over and over again, and as far as it is known, no cultural examinations have been done on these sour doughs. There are five types of bread made in Iran; Sangak, Taftoon, Barbari, Lavash, and village bread; the ingredients, composition, and preparation of these have been described by Kouhestani et al. (4).

Except for the village bread which sometimes may be unleavened, other Iranian breads are of the leavened type. Rising of bread dough is usually due to production of carbon dioxide as a result of fermentation of flour carbohydrates by active bakers' yeast (*Saccharomyces cerevisiae*) but other types of yeasts as well as bacteria may also cause the dough to rise.

The present investigation was designed to provide some qualitative information on the presence and type of yeasts in the leavenings used for the Iranian breads. For this purpose samples of doughs from various bakeries in Shiraz and its suburbs were cultured and the yeast

strains isolated were studied culturally, morphologically, and biochemically. Ascospore-forming ability and baking strength of the cultures were studied as well.

MATERIALS AND METHODS

Samples

Dough samples were taken from 43 different bakeries, 17 of which baked sangak, 19 taftoon, and the remaining seven, barbari bread. Lavash and village breads were not tested as they were not readily available in this locality. Table 1 shows the ingredients of the five types

TABLE 1. *Ingredients of the five types of Iranian breads*

Ingredients	Types of breads				
	Sangak	Taftoon	Barbari	Lavash	Village bread
Whole wheat flour (g)	30000	1000	—	10000	—
White flour (g)	—	—	10000	—	—
Coarse wheat flour (g)	—	—	—	—	10000
Yesterday's dough (g)	1500	2500	3500	500	70
Salt (g)	330	100	130	200	70
Sodium bicarbonate ^a (g)	—	50	—	—	—
Date syrup ^a (g)	—	200	—	—	—
Water (approx.) (l)	33	7	6	6	4.5

^aDate syrup and sodium bicarbonate are not added to the doughs in Shiraz city.

of breads as reported by Kouhestani et al. (4). Depending on the types of flours used and peoples' tastes in the various localities of the country, these ingredients may vary. The samples were brought to the laboratory in clean plastic bags and a loopful from the center part of each sample was spread on a suitable medium either on its arrival at the laboratory or after overnight refrigeration. A commercial 'Red Star' baking yeast from the Universal Food Corporation, Milwaukee, Wisconsin, and a commercial baking yeast from Allinson, London, were used as standard strains for comparison throughout these experiments.

Media and conditions of growth

Difco Sabouraud dextrose agar (SDA) was used for initial examination of dough samples. Inoculated SDA plates were incubated at room temperature (approximately 24-28 C). Plates were checked every day for a maximum of 5 days and the morphological characteristics of the various colonies growing on the medium were compared. A colony from each different population was subcultured on the same medium and incubated at room temperature for a maximum of 5 days. Smears made from the subcultures were stained by the gram method and examined microscopically to check their purity. The various

populations of yeasts thus isolated were then studied and characterized independently.

Characterization

For preliminary classification and typing of the yeast-like organisms, all isolated strains were examined for their pigment formation on the SDA medium, and for cell morphology and size, after they were grown in malt extract broth for 3 days at 25 C. Cultures were then examined for their pellicle formation on the surface of the liquid medium during their growth for 1 week. Also determined were the ability of the cultures to grow at 37 C, to form ascospores on nine different sporulating media, and to ferment and assimilate, glucose, galactose, raffinose, sucrose, maltose, and lactose.

The size of the cells was measured by means of micrometer mounted on the ocular lens of a microscope. The ascospore forming ability of yeast cultures was examined according to methods described by van der Walt (6) using 5% malt extract broth as pre-sporulating medium and Gorodokowa agar, potato agar, acetate agar, corn meal agar, yeast extract agar and blocks of gypsum, potato, carrot, and cucumber as sporulating media. When active cells from the pre-sporulating media were subcultured on the sporulating media, the latter were kept at 25 C for 3 days and then at room temperature for a maximum of 6 weeks. Smears were made from the growth once a week, stained by Schaeffer's method, and studied under the microscope for formation of ascospores.

Aliquots of phenol red broth base (Difco) each containing one or another of the sugars mentioned above were used as the medium for the fermentation test. The test was done in a screw-capped test tube containing the medium and an inverted Durham tube. The medium was inoculated with the yeast strain and left at room temperature for 15 days. All tubes having acid and gas were considered as positive and were subcultured on blood agar to confirm the absence of bacterial contamination. The strains which failed to produce gas from any one of the sugars were retested in Einhorn fermentation tubes to compare the reliability of the two methods.

For the assimilation tests the method of van der Walt (6) was followed using nitrogen base agar as the medium, supplemented with 0.5% of the sugars (with raffinose, 1% solution of the sugar was used).

Baking test

The isolated yeast strains were tested for their baking strength according to the method described by White (7). In a 25-ml beaker 5 g of whole wheat flour plus 5.5 ml of a 1% solution of NaCl in water and 250 mg of wet yeast cells, (this amount of wet yeast cells were equal to about 75 mg dry weight) were mixed well by means of a clean glass rod and the resulting dough was then packed at the bottom of a beaker. The beaker was covered by a perforated plastic sheet to prevent drying and the dough was allowed to rise to 15 ml in volume. The dough was then mixed, packed down to the original volume and allowed to rise again to 15 ml in volume. The sum of the first and second rise periods

was taken as a measure of the baking strength of the yeast strains. The amount of flour, salt, water, and yeast used in this experiment was considered to be approximately similar to that used for baking sangak bread in Iran. Wet yeast cells were collected with a bacteriological loop from the yeast colonies freshly grown on the surface of the SDA medium. Conditions under which the test was done were standardized for all the yeast strains. That is, one type of flour was used for all the yeast strains, the duration of initial mixing of the doughs was fixed, and the incubation temperature was 28 C throughout this experiments.

RESULTS

Isolated microorganisms

Cultivation of the dough samples on SDA medium produced colonies which resembled yeast growth morphologically. On the basis of colonial appearance 92 different yeasts were isolated. These were kept on slants of SDA at 4 C with subculturing every 45 to 60 days. Eleven of these cultures died after several subcultivations; the remaining 81 are the subject of these experiments.

Pigmentation, cell morphology, and size

Except for one culture which produced pinkish colonies, all the isolates showed white creamy colonies. The cells from all the cultures were round, oval, and/or elongated.

The average diameter of 10 cells in each strain of yeast varied between 4 to 14 by 3 to 8 μ m. Such variation in size of the cells was seen within all 14 of the culture groups.

Ascospore formation

Only 79% of the cultures could form ascospores on the nine different sporulating media.

Fifty-seven percent of the sporulating cultures formed ascospores on Gorodokowa agar. Of the remaining 43% which did not sporulate on Gorodokowa agar 11% formed ascospores on potato blocks, 11% on corn meal agar, 7.8% on yeast extract agar, 6% on carrot blocks, and none on gypsum blocks. Also 7.2% of these cultures formed ascospores on the acetate agar, potato agar, and cucumber blocks. All the yeast cultures isolated from barbari breads were able to sporulate. Non-sporulating yeasts were isolated only from sangak and taftoon

TABLE 2. Characteristics of yeasts isolated

Groups of yeast	Number of strains	Growth at 37 C	Sporulation		Fermentation and assimilation reactions						
			+	-	Glucose	Galactose	Raffinose	Sucrose	Maltose	Lactose	
S ^a	2	+	2	—	FA ^b	FA	FA	FA	FA	FA	—
A	20	+	19	1	FA	FA	FA	FA	FA	FA	—
B	16	—	10	6	FA	FA	FA	FA	FA	FA	—
C	10	+	10	—	FA	FA	FA	FA	-A	—	—
D	17	—	11	6	FA	FA	FA	FA	-A	—	—
E	1	+	1	—	FA	-A	FA	FA	FA	FA	—
F	1	—	—	1	FA	-A	FA	FA	FA	FA	-A
G	7	—	6	1	FA	-A	FA	FA	-A	—	—
H	2	+	2	—	FA	-A	—	FA	-A	-A	-A
I	2	—	1	1	FA	— ^d	—	FA	—	—	—
J	1	+	1	—	FA	FA	-A	FA	-A	-A	-A
K	1	+	—	1	FA	-A	—	-A	-A	-A	—
L	2	+	2	—	-A ^e	-A	-A	-A	-A	-A	—
M	1	+	1	—	-A	—	-A	-A	-A	-A	—

^aS = Standard strain.

^bFA = Positive fermentation and assimilation

^c-A = Negative fermentation and positive assimilation

^d— = Negative fermentation and assimilation.

bread.

Fermentation and assimilation of carbohydrates

Results of fermentation tests done in the screw-capped test tubes containing inverted Durham tubes were the same as those done in the Einhorn tubes. Table 2 shows the results of fermentation and assimilation of sugars by the yeast cultures together with their ability to grow at 37 C and to form ascospores.

Based on these biochemical behavior and growth temperature, the cultures were divided into 13 different groups designated as A through M (Table 2). Excepting the yeasts in groups F, H, and J which assimilate lactose, none of the other cultures could ferment or assimilate this sugar. Only 39 of the yeast cultures in groups A, B, E, and I showed biochemical reactions typical of *Saccharomyces* species, but eight of these cultures did not sporulate under the present experimental conditions, and therefore may belong to the asporogenous yeasts. However, 29 of the sporulation cultures in groups A and B showed biochemical reactions similar to those of standard baker's yeast, *Saccharomyces cerevisiae*, and the remaining two cultures (groups E and I) may belong to other *Saccharomyces* species.

The biochemical reactions of 42 of the remaining cultures, in groups C, D, F, G, H, J, K, L, and M seemed to be different from those of *Saccharomyces* species. In addition, nine of the cultures in groups D, F, G, and R were unable to sporulate on the sporulating media used. Most of the non-sporulating yeasts also were unable to grow at 37 C. The yeast cultures in groups A, B, C, and D were isolated from doughs of the three types of bread examined, but all the cultures in groups E, F, G, H, J, L, and M were isolated from doughs of taftoon bread. The culture in group K was obtained from dough of sangak bread.

Temperature requirement

All the yeast cultures could grow at room temperature or at 25 C, but when they were incubated at 37 C, about 53% of them failed to grow (Table 2).

Pellicle formation

Fifteen (18.5%) of the yeast cultures formed a pellicle during their growth on the surface of malt extract broth as well as on the surface of the broth cultures used for fermentation test. Three of the cultures belonged to group A, four to group C, two to group L, and one to each of the groups B, D, H, J, K, and M. The pellicle-forming yeast of groups D and K failed to sporulate (Table 2).

Baking strength

The baking strength of the various groups of yeasts isolated is compared, both together and with those of standard strains, in Table 3. Eight of the yeasts in group A, two in group D, and one in group E had rising times comparable to those of standard bakers' yeast. Of these yeasts five were isolated from barbari breads. The yeasts in groups K, L, and M and one from group A did not show any appreciable activity, and were all isolated from doughs of taftoon bread. The remaining yeasts showed either low or reasonable baking strength and most were isolated from doughs of taftoon and sangak breads. Generally, the dough rising ability of the non-sporulating yeasts seemed to be less than that of the sporulating forms.

DISCUSSION

Results obtained from these experiments indicate that the leavenings used by Shiraz bakeries contain variable mixtures of yeast strains involving probably 13 to 23 biotypes. Some of these biotypes are not suitable for the leavening of bread (Table 3). Many dough samples contained up to four biochemically different types of yeasts. This is in agreement with the variability of taste and flavor often experienced by the local people consuming one type of bread made at different times and by different bakeries in the city of Shiraz. According to Cook (3), bakers' yeast is almost always a strain of *Saccharomyces cerevisiae*, but *Saccharomyces lactis* has been occasionally used. The present experiments showed that only 35.7% of the yeasts isolated were similar to

TABLE 3. Baking strength of various groups of yeasts isolated

Groups of yeast	Rising time of dough in minutes												
	Sporulating yeasts						Non-sporulating yeasts						
	58 80	81 100	101 120	121 200	201 300	301 560	No activity	60 80	81 100	101 120	121 200	201 540	No activity
S ^a	2	—	—	—	—	—	—	—	—	—	—	—	—
A	8	3	3	—	4	1	—	—	—	—	—	—	1
B	—	—	1	4	5	—	—	—	—	3	2	1	—
C	—	1	2	2	1	4	—	—	—	—	—	—	—
D	1	1	2	5	2	—	—	1	1	—	1	3	—
E	1	—	—	—	—	—	—	—	—	—	—	—	—
F	—	—	—	—	—	—	—	—	—	—	1	—	—
G	—	—	1	4	1	—	—	—	—	—	1	—	—
H	—	—	—	—	—	2	—	—	—	—	—	—	—
I	—	—	—	1	—	—	—	—	—	—	1	—	—
J	—	—	—	—	—	—	1	—	—	—	—	—	—
K	—	—	—	—	—	—	—	—	—	—	—	1	—
L	—	—	—	—	—	—	2	—	—	—	—	—	—
M	—	—	—	—	—	—	1	—	—	—	—	—	—

^aS = Standard strains

Saccharomyces cerevisiae; *Saccharomyces lactis* was not isolated (none of the strains were lactose fermenters), 2.5% of them may belong to other *Saccharomyces* species and the remaining 61% of the strains did not seem to fit any of the descriptions of *Saccharomyces* species, van der Walt (7). However, more biochemical examination, particularly assimilation tests, are necessary to characterize the yeast cultures completely.

Of the yeasts isolated, approximately 21% were unable to sporulate under the present experimental conditions; their ability to sporulate on the nine sporulating media has been examined three to four times. However, isolation of asporogenous yeasts from plants producing bakers' yeast and from sour doughs has already been reported by Afanaseva (1) and Sugihara et al. (5).

Dough samples of the three kinds of Iranian bread yielded yeast strains of biochemically different types with a vast range of baking strength (Tables 2 and 3). This may indicate the necessity of some kind of control over the leavenings used by Iranian bakeries.

The test for baking strength of yeasts isolated from the doughs of three types of bread showed that yeasts isolated from barbari bread had shortest rising time, but rising time for the isolates from taftoon bread could be in the various ranges of baking strengths as shown in Table 3. Generally, yeasts isolated from sangak dough had rising times between 100 and 300 min. No yeasts completely lacking rising ability were isolated from the samples of sangak dough.

Works on various other aspects of this subject are in progress at this department and will be reported in due course.

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Thermal Energy Losses and Conservation in Sauerkraut Processing Plants

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ABSTRACT

Sources and magnitudes of thermal energy losses, as well as fuels for energy, were examined in three sauerkraut processing plants in Western New York. The fuels for thermal energy accounted for 86 to 95% of the total energy purchased by the plants. With suitable energy conservation measures, present usage of thermal energy (Btu) per case containing 24 size 303 cans can be reduced by 6 to 33% depending on characteristics of the plant.

Use of energy at various stages between production and consumption of food is receiving increasing attention. Survey studies have been published on energy utilization at various stages between food production and consumption (2), and in selected food processing industries (7). Rippen (6) reviewed energy conservation procedures in the food processing industry.

The study of Unger (7) showed that about 69% of the energy used in the canned fruit and vegetable industry was obtained from coal and natural gas. In general, these fuels are used to generate thermal energy, which in turn is used for space heating and processing. For these reasons, thermal energy was the target of the present study. The aim was to determine the sources and magnitudes of thermal energy losses in three sauerkraut plants located in Western New York, and the magnitudes that can be conserved by suitable procedures. Only losses that can be minimized economically and which do not affect the safety of the product were considered. Data obtained are for the period November 1, 1973 to October 31, 1974, for which inventories of fuels and materials were kept in the plants.

Over 10 million cases, each case containing 24 size 303 cans (24/303), of sauerkraut are packed annually in the U.S., with New York State producing about 3 million. The three plants surveyed, accounted for over 1.4 million cases during 1973-74.

Because of the relatively high acid content (0.9 to 1.6%), sauerkraut can be preserved with relatively mild heat or chemical treatment. It is packed in glass jars or plastic bags after addition of 500 ppm of sodium-meta-bisulfite, and in cans after pasteurization

at about 180 F. Among the three plants surveyed, one (Plant C) packed sauerkraut in plastic and glass containers, while the other two (Plants A & B) packed in cans.

MATERIALS AND METHODS

Because the aim of the study was to determine the magnitudes of losses that can be reduced by means of economical measures, the type of data collected depended to some extent on the characteristics of each plant. For example, all the plants had ceilings made from poor insulating materials; the side walls of Plants A and B were made up of masonry walls in good condition, but those of Plant C in some sections were made up of poor insulating materials. Furthermore, Plant A had numerous windows, which were not weather stripped and which did not have storm windows.

Based on these observations, in Plants A and B, heat losses from the ceilings and windows were deemed to be more important than those from the side walls. For Plant C, however, heat losses were estimated for the ceilings as well as sections of vertical walls that were made of poor insulating materials.

Because Plant C packed sauerkraut containing sodium-meta-bisulfite, cookers were not used; in contrast, Plants A and B, employed cookers to pasteurize sauerkraut.

In each processing plant, key personnel were interviewed regarding the types of fuels used, schedule of operation, and their assessment of energy related problems in the plant. The interviews were followed by recording the inventories of fuels and products, and a preliminary inspection of the facilities with the plant manager as the guide. Each plant was inspected at least two more times.

Dimensions of cookers (Plant A and B), and uninsulated steam pipes were recorded. Areas of ceilings and side walls, that were made up of poor insulating materials, were determined with the aid of a map of each plant or by measurements when necessary. The materials of construction were noted by visual inspection or by consulting plant personnel. The flow rate and temperature of the discarded topoff hot water (water added to bring can contents to desired level) stream in Plants A and B were noted.

Data collected were fed as input to a computer program^a (4) for estimating the heat losses during winter from ceilings and side walls that were made up of poor insulating materials, as well as uninsulated heated equipment surfaces and steam pipes, and discarded hot topoff water during processing. Furthermore, magnitudes of heat losses that would occur if the various surfaces were insulated were also computed (4).

Building heat losses were estimated for the period October 1, 1973, to April 30, 1974, employing outside temperatures recorded by weather stations within a radius of about 10 miles from each plant. Indoor temperatures assumed depended on the areas of the plant being studied, and the purpose of the computations. Because the areas where the raw cabbage was cut were not used or heated during late fall and winter, a temperature of 55 F was assumed for these areas in all

^aA copy of the printout and instructions for the use of the program can be obtained by contacting the senior author.

computations. In areas such as warehouses, which are used by few people and infrequently, computations were done assuming the temperatures 70 and 55 F; the latter to determine possible energy savings. Computations were also carried out assuming the various structures were provided with 3-inch insulation to determine potential energy savings.

Infiltration losses from the numerous windows of Plant A were computed manually, employing the crack method (1). The losses were found to be about 10% of the losses through the ceilings. For Plants B and C, infiltration losses were assumed to be 5% of those through the ceilings.

Flow rates of the can cooling water (Plants A and B) could not be measured accurately for use with the computer program (4). For this reason, the energy content (Btu) of the can cooling water was estimated from the mass of product produced from November 1, 1973 to October 31, 1974, assuming the specific heat of the product to be 1.0 Btu/lb° F, and that the product is cooled from 180 to 105 F. Likewise, the heat content of the condensate from the cookers (Plant A and B) was determined from the mass of product, that it had to be heated from 65 to 180 F, and the heat lost from the cookers to the surroundings. The heat loss from the cookers was obtained using empirical natural convection equations (4).

RESULTS AND DISCUSSION

For generating thermal energy the plants employed various fuels: Plant A—coal (95.2%) and natural gas (4.8%), Plant B—natural gas (100%), and Plant C—fuel oil #2 (100%). These fuels accounted for 95.3, 86.1, and 90.1% of the total energy, expressed in Btu, purchased by Plants A, B, and C, respectively. These figures are higher than the average for the fruit and vegetable canning industry as a whole (7).

The gross thermal energy consumed per case (24/303) of product was calculated for each plant, from the magnitudes of fuels purchased, and assuming the calorific values: fuel oil #2-1.42 × 10⁵ Btu/gallon, natural gas-1.10 × 10³ Btu/ft³, and coal-2.80 × 10⁷ Btu/ton. The magnitudes of the gross thermal energy, Btu, per case (24/303) were: Plant A-9.78 × 10⁴, Plant B-2.70 × 10⁴, and Plant C-8.76 × 10⁴. The variation seen from plant to plant is due to variations in the efficiencies of boilers, thermal energy losses, and number of cases produced.

Boiler efficiencies were not measured, because of the absence of meters to indicate the quantities of steam produced, and installation of such meters was not possible. The only recourse was to assume boiler efficiencies. The coal-fired boilers of Plant A were old and based on the estimates of Lyle (3), they were assumed to have an efficiency of 60%. The natural gas-fired boilers of Plant B and the oil-fired boiler of Plant C, were assumed to have an efficiency of 70%. These magnitudes were arrived at after consulting the operators and the efficiency data of the power plant at the Geneva Experiment Station. With these boiler efficiencies the thermal energy/case consumption for each plant becomes: 5.87 × 10⁴-Plant A, 1.89 × 10⁴-Plant B, and 6.13 × 10⁴-Plant C.

The sources and magnitudes of heat losses for the three plants are given in Table 1. As seen, losses from buildings were large and represented 23.0, 28.6, and 41.1% of the net heat input for Plants A, B, and C,

TABLE 1. *Magnitudes of heat losses in the processing plants with no conservation measures*

	Plant A	Plant B	Plant C
Building heat loss area ^a , ft ²	1.15 × 10 ⁵	7.47 × 10 ⁴	5.45 × 10 ⁴
Heat lost from buildings ^b , Btu	7.38 × 10 ⁹	4.10 × 10 ⁹	2.73 × 10 ⁹
Heat lost in discarded hot water ^c , Btu	1.98 × 10 ⁹	2.75 × 10 ⁹	None
Heat lost from cookers and steam pipes, Btu	9.10 × 10 ⁷	3.80 × 10 ⁶	None

^aCeiling area for Plants A and B; includes some side wall area in Plant C.

^bFrom ceilings and windows of Plants A and B, as well as side walls in Plant C. Assumed 55 F for the cutting areas, and 70 F for the rest. Outside temperatures of Oct. 1, 1973 to Apr. 30, 1974 were used.

^cSum of condensate, can cooling and toff water losses, Plant C had no cookers.

respectively. For Plants A and B, the heat lost in discarded hot water also was large. The building heat losses reflect the large areas and the poor insulating characteristics of the surfaces. The hot water losses were higher in Plant B, because of higher production.

Several conservation measures and their effects on heat losses are discussed below, and the magnitudes are given in Table 2. Because the building heat losses were quite large, conservation measures for these are discussed first.

TABLE 2. *Thermal energy conserved with various conservation measures*

	Plant A	Plant B	Plant C
<i>Savings from buildings^a</i>			
A. Warehouses and cutting areas at 55 F, rest at 70 F, no insulation	1.42 × 10 ⁹	6.30 × 10 ⁸	3.80 × 10 ⁸
B. Warehouses and cutting areas at 55 F, rest at 70 F, 3" insulation	6.07 × 10 ⁹	3.37 × 10 ⁹	2.17 × 10 ⁹
<i>Savings from hot water^b</i>			
85% of condensate heat and 30% of heat in can cooling water recovered	5.15 × 10 ⁸	7.13 × 10 ⁸	None
<i>Savings from equipment and steam pipes</i>			
By using 1" insulation	8.76 × 10 ⁷	3.08 × 10 ⁶	None

^aAreas and outside temperatures as in Table 1.

^bNo savings in toff water were assumed.

The simple and fairly inexpensive steps, consisting of lowering the temperatures in the warehouses and the cutting room areas to 55 F, and weather stripping the windows will lower the building heat losses by about 15 to 19%. In addition to the two measures just described, if the walls and ceilings are provided with 3-inch thick insulation, building heat losses with no conservation measures can be reduced by about 80%. Obviously, installation of such insulation is expensive.

Condensate from cookers can be recycled to the boilers with little or no treatment of the water. For the purpose of illustration, we assumed that 85% of the heat in condensate can be recovered. The heat in can cooling water can also be recovered to some extent by heat exchange with boiler feed and toff water. Because of the low temperature rise of the can cooling water, it is unlikely that most of the heat energy in it can be

recovered. Also, the presence of traces of oil preclude its direct use for applications such as preheating cabbage heads. For this reason a recovery of 30% is assumed. Finally, insulating bare steam pipes and cookers can lead to additional savings in thermal energy and these are also shown in Table 2.

Implementation of the aforementioned conservation measures will reduce consumption of thermal energy per case. Lowering the temperature in the warehouses to 55 F, insulating the steam pipes and cookers, as well as recovering the heat in hot water as indicated in Table 2, shows that the consumption of thermal energy/case will be reduced by 6,10, and 6%, in Plants A,B. and C, respectively. If, in addition, buildings are provided with 3 inches of insulation, the thermal energy/case consumption can be reduced by 21, 29, and 33%, respectively.

Since the completion of this study, the processing plants enforced steps to lower temperatures in the warehouses and weatherstrip the windows in Plant A. Plans are underway for incorporation of the other conservation measures discussed in this paper.

It is of interest to note that consumption of thermal energy/case in Plants A and C, was higher than that for low acid vegetables, such as beans and beets (5). To a large extent, this was due to the much larger production of low acid vegetables per plant, and to a lesser extent due to the large building losses in Plants A and C. It appears that in some plants the large areas needed for

fermenting the cabbage and the accompanying building losses, offset any thermal energy savings in the sterilization operation.

The primary reason for the lower consumption of thermal energy/case in Plant B is due to the larger number of cases of product produced, and to some extent due to better facilities. Interplant comparisons of energy/case or energy/pound of product are favorable to the plants having the higher production capacities. For this reason, the effects of conservation measures should be assessed for each plant, relative to the energy consumption before the conservation measures were enforced.

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A Strain of *Pseudomonas aeruginosa* Resistant to a Quaternary Ammonium Compound¹

III. Electron Microscopy

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ABSTRACT

Light and electron microscopy studies were made of *Pseudomonas aeruginosa* strains which were sensitive and resistant to a quaternary ammonium compound (QAC). The colonies of the sensitive cells on Tryptone Glucose Yeast Extract Agar were granular and homogeneous in consistency. In contrast, the colonies of the resistant strain on the same medium were granular, non-homogenous, and contained numerous dense areas. Morphological observations revealed the resistant cells to be 30% smaller than sensitive cells and non-motile due to loss of polar flagella, a characteristic which was not restored when the organisms were cultured in the absence of QAC for more than 7 months. Electron-dense inclusion bodies were present in resistant cells; they ranged in size from about 0.05 to 0.2 μm in diameter. These bodies, which were not identified, were released intact from lysing cells; as many as 20 per cell were visible.

In 1945, Taft and Strandtmann (16) demonstrated that alkyldimethylbenzyl ammonium chloride, a quaternary ammonium compound (QAC), caused a marked alteration in the cellular morphology of protozoa. Two years later, electron microscope studies dealing with the effects of a surface-active agent on bacterial cells were published by Mitchell and Crowe (14). Their micrographs, although of relatively low magnification, showed an apparent cell wall disruption due to the effects of tyrocidin. The same year, the penetration of QAC into bacterial cells was reported by Dyar (8). Salton et al. (15) used electron microscopy to demonstrate a marked alteration in the cytoplasm of *Staphylococcus aureus* and *Escherichia coli* by low concentrations of cetyl trimethyl ammonium bromide; high concentrations stripped away the cell wall. Pictures published by Dawson et al. (7) indicated a similar cell wall damage by this compound to *S. aureus*. They reported an optimum range for cellular damage, with both high and low concentrations failing to produce any destructive effect on cells. The ability of EDTA to increase the activity of QAC was reported by MacGregor and Elliker (12); they suggested the presence of a

QAC-obstructing cell wall layer of lipid or lipid-protein complex bound together by multivalent inorganic cations that were chelated by EDTA, thereby increasing the permeability of the cell to germicide. This was substantiated by the recent findings of Bobo and Eagon (3) that calcium and magnesium are associated with the cell wall phospholipid of *P. aeruginosa*. MacGregor (11) also demonstrated the disruption of cells by QAC using electron microscopy and from his observations Cox (6) concluded that changes in *Bacillus megatherium* and *S. aureus*, after exposure to heterocyclic QACs, were mainly intracellular.

Each of the above cited studies has provided useful information on the mode of action of QAC on microbial cells. It was the intent of the present study to supplement these findings by comparing the effects as observed microscopically of QAC on sensitive and germicide-resistant cells.

MATERIALS AND METHODS

Quaternary ammonium compound

Accoquat 1602 used in this study was provided by Klenzade Corporation, a division of Economics Laboratory, Minneapolis, Minnesota. The chemical name for the germicide in alkyldimethylethylbenzyl ammonium chloride; the N-alkyl active ingredient (10%) was 50% C₁₂, 30% C₁₄, 17% C₁₆, and 3% C₁₈.

Cultures and media

The source of cultures, their identifying symbols and media used have been described earlier (17, 18); QRM-S identifies the sensitive strain, QRM-R the resistant, and QRM-RN the resistant strain cultured for 7 months in the absence of QAC.

Light microscopy

Cells of *P. aeruginosa* strains QRM-S, QRM-R, and QRM-RN in the late log growth phase were dried on microscope slides and fixed. The cells were stained for gram reaction and capsule using conventional methods. Motility was determined by the hanging drop technique using phase microscopy.

Colony morphology of the test organisms was compared using TGY agar slants and TGY agar plates containing various concentrations of QAC. Colonies formed on agar plates were examined under the low power objective of a light microscope.

Electron microscopy

The method of Houwink and Van Iterson (10) was used with some modifications. Sensitive and resistant strains of *P. aeruginosa* were cultivated by spotting on TGY agar and on TGY agar plus 5,000 ppm

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QAC and incubating at 32 C. A small amount of 0.01 M phosphate buffer (pH 7.0) was dripped on well-grown young cultures and the cells picked up by gently touching a formvar coated grid to the surface of the buffer. Excess moisture was drawn off with filter paper. The effect of QAC was determined by incorporating the germicide in the buffer before dripping the buffer on the culture or by adding 0.1 ml of the buffer-cell suspension to 0.1 ml of QAC (2 \times) on a wax surface. The wax caused the buffer to bead up and allowed the grid to be floated on top of it for various periods of exposure time.

Polystyrene-latex beads (Dow Chemical Company, Bioproducts Department, Midland, Michigan) were used as an internal standard for cell size determinations. The beads had an average particle diameter of 0.264 μ m. The grids were shadowed at an angle of about 30° with an alloy of platinum palladium (4:1) in a Mikros Vacuum Evaporator VE 10 (Mikros, Inc., Portland, Oregon) at a vacuum of <0.05 μ m; they were examined in an RCA EMV 2 D electron microscope.

RESULTS

Colonies of the sensitive strain on TGY agar were translucent and had a homogeneous granular texture. Streaking resistant cultures on TGY agar gave rise to colonies with dense areas that were more abundant toward the center of the colony. The peripheral regions of the colonies had a granular appearance similar to sensitive cells. Colonies of QRM-RN also exhibited a non-homogenous texture intermingled with dense areas. When sensitive cells were streaked on TGY agar containing 500 ppm QAC the resulting colonies resembled those of QRM-R. The appearance of the colonies, unaided by magnification, was much the same, except that the resistant colonies were smaller on all media.

The three strains were gram-negative and no capsule formation was observed. Phase microscopy demonstrated active motility by sensitive cells, whereas both resistant and QRM-RN cells were non-motile and appeared smaller than sensitive cells.

Electron micrographs of sensitive and resistant cells

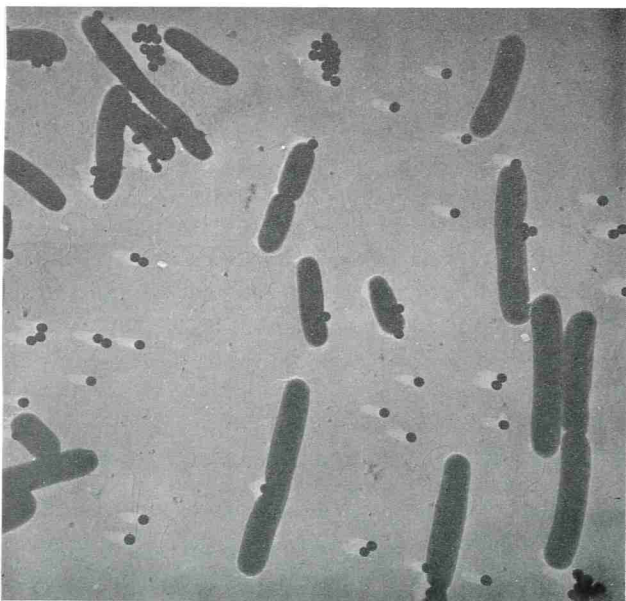


Figure 1. *P. aeruginosa* QRM-S cells showing single polar flagella. Internal standard latex beads were 0.264 μ m in diameter; Platinum: Palladium shadow cast; original magnification approximately \times 6,000.

shadowed with platinum: palladium revealed definite differences in morphology. The sensitive cells (Fig. 1) had an average size of about $3.0 \times 0.75 \mu$ m, while the resistant cells (Fig. 2) were $1.0 \times 0.35 \mu$ m. In addition, the polar flagella evident in sensitive cultures were absent in the resistant population. Micrographs of sensitive and resistant cells following treatment with QAC revealed the failure of 100 ppm QAC to disrupt the flagella of sensitive cells (Fig. 3) and the stability of resistant

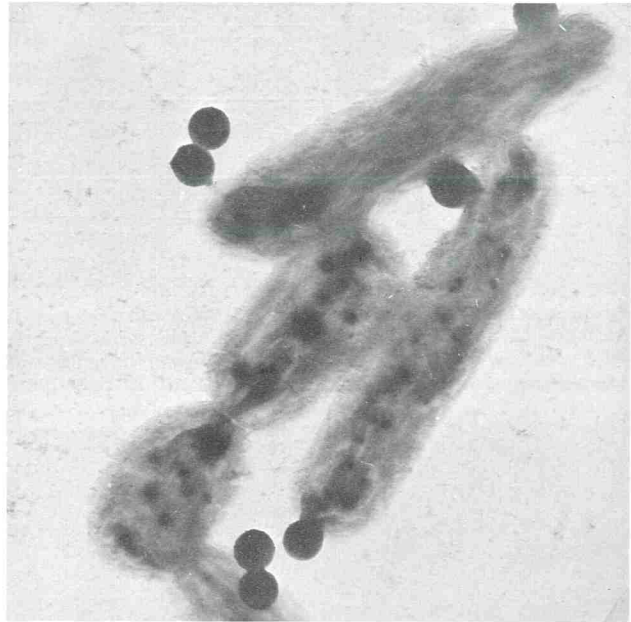


Figure 2. *P. aeruginosa* QRM-R cells shadowed with platinum: palladium. Notice the absence of flagella and the presence of dense inclusion bodies. Original magnification approximately \times 27,000.

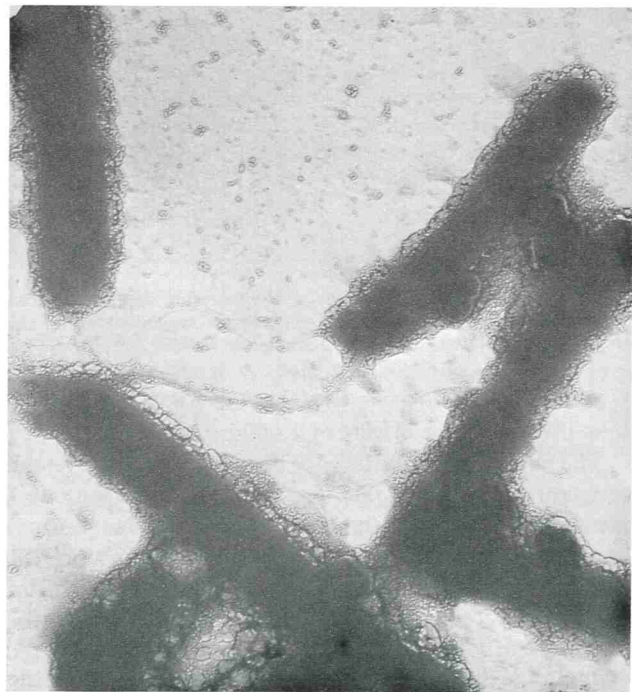


Figure 3. Cells of *P. aeruginosa* QRM-S exposed to 100 ppm QAC. The flagella appear to remain attached and no cell lysis is observable. Original magnification approximately \times 20,000.

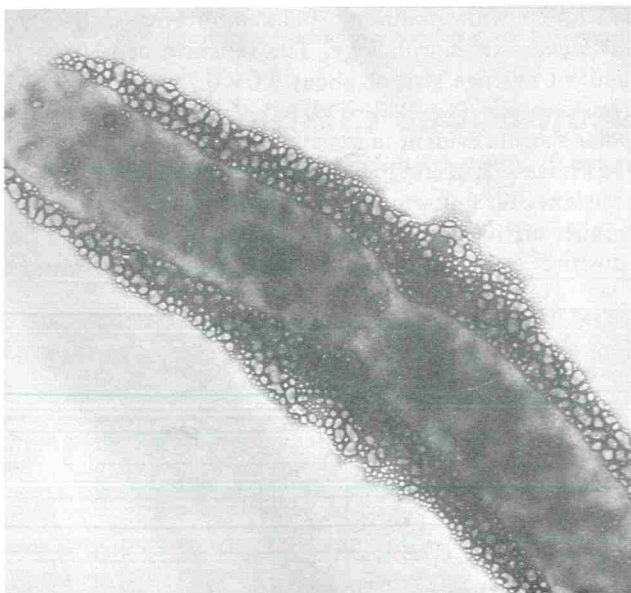


Figure 4. Dividing cells of *P. aeruginosa* QRM-R after exposure to 1,000 ppm QAC. The QAC may be seen as "bubbles" surrounding the cells. No cell wall rupture is seen. Original magnification approximately $\times 30,000$.

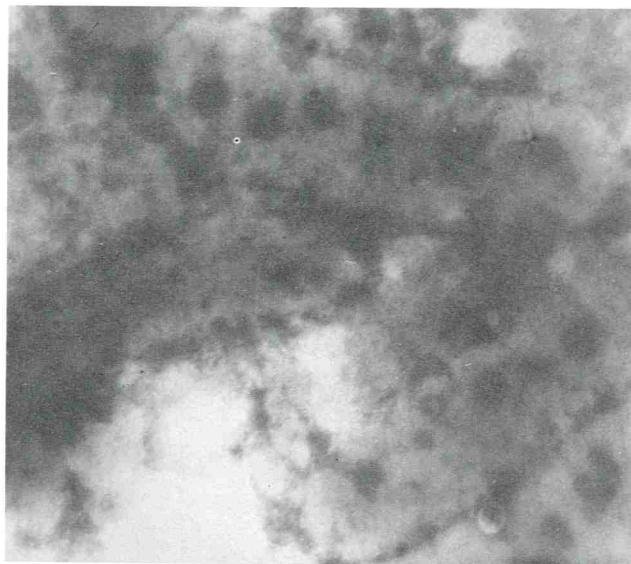


Figure 5. Unshadowed and unstained preparation of *P. aeruginosa* QRM-R. Several large electron-dense areas are prominent within the cells. Original magnification approximately $\times 50,000$.

cells (Fig. 4) to the effects of 1,000 ppm QAC.

The most striking difference between the sensitive and resistant cells was the presence of electron-dense intracellular bodies in the latter. This phenomenon may be seen in Fig. 5 through 8. The under-exposure used in Fig. 6 demonstrates the occurrence of as many as 20 of these dense areas per cell. A size determination can be made from Fig. 7 in which a latex bead ($0.264 \mu\text{m}$ diameter) is situated adjacent to and slightly below the resistant cell. The electron-dense particles range in size from a few hundredths of micrometer to about $0.2 \mu\text{m}$. It is emphasized by Fig. 8 that these bodies were discrete

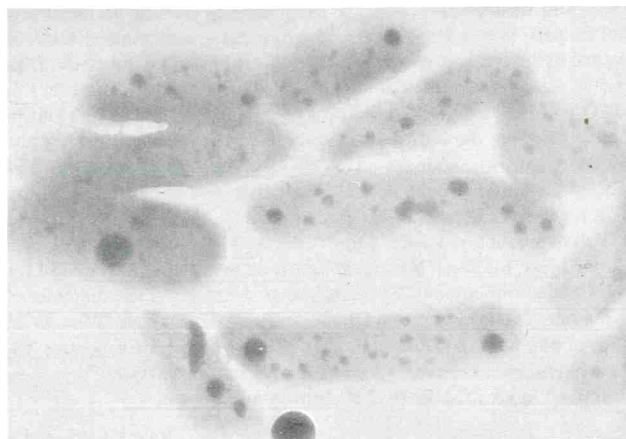


Figure 6. Underexposed micrograph of *P. aeruginosa* QRM-R showing the variation in the number and size of intracellular electron-dense bodies. Original magnification approximately $\times 12,000$.

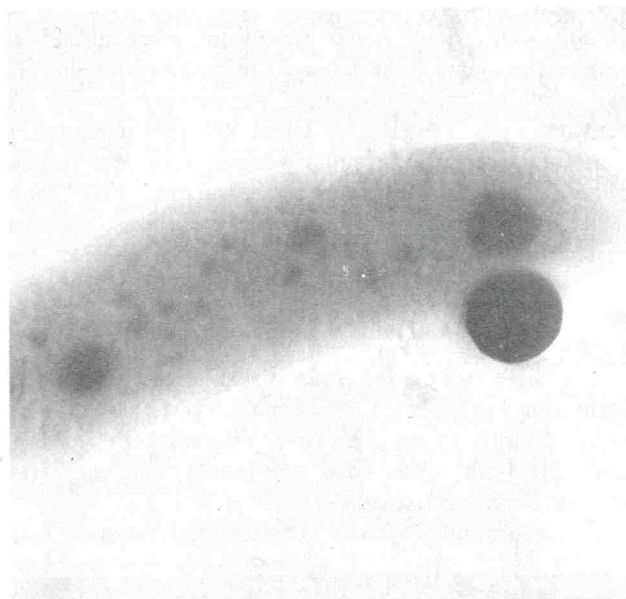


Figure 7. Micrograph of the resistant cell allowing size comparison of the internal standard latex bead ($0.264 \mu\text{m}$) and the inclusion bodies. Original magnification approximately $\times 50,000$.

and did not lose their integrity when released from the cell upon lysis.

DISCUSSION

This study was designed as a morphological comparison of three strains of *P. aeruginosa*, one, QAC sensitive, a second QAC resistant, and a third resistant to QAC, but maintained more than 7 months in the absence of germicide. The latter organism was used to establish the degree of the permanence of changes occurring in the sensitive strain during its transition to germicide resistance.

Colonies of both sensitive and resistant cells formed on TGY agar and TGY agar containing QAC revealed interesting differences. When observed microscopically, colonies of resistant cells revealed dense areas. It is possible that these dense areas were formed by cell



Figure 8. Electron micrograph of electron-dense inclusion bodies that have been released from a lysed cell of *P. aeruginosa* QRM-R. Original magnification approximately $\times 24,000$.

aggregation and cell destruction by the action of QAC. This, however seems unlikely because the resistant cells exhibited the same phenomenon in the absence of QAC. It seems more probable that the dense areas resulted from a buildup of cellular reserves. Although this may not be the sole mechanism of QAC resistance, it might well play an active role. It was also observed that the sensitive cells exhibited a similar colony morphology when exposed to a sublethal concentration of germicide.

Electron microscopy revealed that the resistant cells were non-flagellated and considerably smaller. This size difference could be a consequence of the surface activity of the QAC. This was suggested by the fact that the resistant cells when examined after transfer for only several weeks in the absence of QAC, regained a size more nearly like that of the sensitive cells, although continuous sub-culture for more than a year in the absence of QAC did not result in complete size restoration. Flagellation, so characteristic of the family *Pseudomonadaceae*, also was not reestablished over this period of stress release. This effect, therefore, appeared to be due to something other than the surface tension reducing activity of the QAC and suggested permanent genetic alterations were caused by the germicide. If so, previously published theories (1,5) that resistance is due to adaptive changes or selectivity that arises from the presence of QAC would require re-examination. These data also cast doubt on the concept (1,5) that only lipid accumulation explains QAC resistance and lends support to the findings of Bernheim (2) that a surfactant has two sites of action; one, intracellular and a second, extracellular. The permanent loss of flagella and stable diminished size of QRM-R cells observed in this study supports an intracellular activity site and the well known

surface active nature of QAC (Fig. 3) supports an extracellular site of activity. The former suggests QAC may be mutagenic and this should be studied further.

The electron photomicrographs also revealed striking differences in the cytoplasm of the sensitive and resistant cells. The resistant cells contained as many as 20 electron-dense bodies that reached $0.2 \mu\text{m}$ in diameter. Three alternatives may explain this phenomenon. First, a discontinuous cytoplasm could be envisioned from Fig. 5. However, this does not agree with our present day knowledge of the bacterial cell. Second, the areas could represent pools of cellular material trapped within a disrupted cell. This, too, seems unlikely because of the apparent discreteness of these pools of bodies (Fig. 8) when they were released from the cells. As the third alternative it could be postulated that intracellular bodies resulted from an altered metabolism giving rise to the accumulation of cytoplasmic substance that are either pooled, crystalline, or membrane enclosed. Microscopy of ultra thin sections of the sensitive cells revealed no cytoplasmic irregularities. Initial attempts to prepare thin sections of the resistant cells were unsuccessful due to difficulties not encountered with the sensitive strain. Further studies in this area as well as attempts to isolate and characterize the electron dense bodies are warranted.

From these studies, it is evident that QAC acts at the cell wall level causing lysis and may also penetrate the cell and cause effects at the genetic level. Resistant cells apparently survive because of a much smaller surface area and either an altered metabolism that averts the lethal QAC action or an intracellular reserve that tends to neutralize the QAC.

ACKNOWLEDGMENTS

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Organoleptic Evaluation of Milk in Various Containers Exposed to Fluorescent Light

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ABSTRACT

Pasteurized, homogenized milk packaged in unpigmented and variously colored paperboard and blown mold plastic containers was exposed to fluorescent light. Samples were evaluated for flavor by a trained panel after 3, 6, 12, 48, 72, 96, 120, and 144 h. All of the paperboard packages offered greater protection to light induced flavor changes than did blown mold plastic. Significant differences ($P < 0.05$) were found among various colored paperboard containers, with unpigmented, yellow, and red offering less protection than the other colors investigated. Total light transmission through the various paperboard and the plastic containers was significantly different ($P < 0.05$), with paperboard screening out more total light than plastic. Unpigmented and yellow-colored paperboard screened out significantly less total light than the other seven colors studied.

Various researchers (1, 2, 3, 4, 7) have reported on packaging materials as related to light-induced changes in milk. Paperboard cartons have been shown to offer the best protection against flavor changes, followed by pigmented plastic.

The need for protection against light-induced changes in milk has long been known. Changes in milk distribution from home delivery to store sales has resulted in long exposure to fluorescent lights (4). In 1964, 30% of all milk was sold in glass bottles, 64% in paperboard, and only 3% in blown mold plastic. By 1974 this had changed to only 3% in glass, 70% in paperboard, and 27% in blown mold plastic (9). In a recent survey (10) of 58,973 milk samples in 105 supermarkets, 58% remained in fluorescent lighted cases 24 h before sale. The average light intensity in these dairy cases was 200 ft-c. Thus, changes in distribution patterns and milk containers may present conditions where photochemically induced changes in milk are of increasing importance.

MATERIALS AND METHODS

The following report shows flavor changes occurring in milk submitted to fluorescent light exposure in blown mold plastic bottles and in unpigmented and 8 different colored paperboard packages. Following pasteurization at 74 C for 16 sec and homogenization at 2500 psig, milk (3.5% butterfat) was cooled, collected in stainless steel cans, and subsequently aseptically transferred to trial containers. Paperboard containers furnished by a commercial supplier (International Paper Co.) included unpigmented, red (Pantone 185), purple (Pantone 239), green (Pantone 354), brown (Pantone 490), black, blue, orange, and yellow. Paperboard containers were either unpigmented or solid colors

and especially prepared by the manufacturer for this study. Blown mold plastic containers (55g) were furnished by a commercial supplier (Berwick Fabrication Co., Berwick, PA.). Both paperboard and plastic containers were the half-gallon size.

The study was divided into two series of observations. In the first, milk was placed in red, blue, yellow, black, and unpigmented paperboard and blown mold plastic containers. In the second series, purple, green, orange, brown, unpigmented paperboard, and blown mold plastic containers were used. Each series also had an unexposed control from the same lot of milk held in a stainless steel can.

Trial containers of milk were held in a sliding door display case illuminated by 40-watt cool white fluorescent lamps (General Electric F40 CW), mounted parallel to the shelves at a distance of 40 cm from the containers. Illumination averaged 100 ft-c at the mid-point of the exposed vertical surface of each container. Both trial and control samples were held at 7 C. All samples were quiescent during exposure so that subsequent data reflect, at least in part, surface light induced changes.

Organoleptic examination of each series of samples was made by a panel of 12 people with extensive training in food evaluation. Preference evaluations were obtained using a 9-point hedonic scale and multiple comparison tests with the control samples as references (8). With hedonic scoring the values ranged from 1, dislike extremely, to 9, like extremely. In the multiple comparison test, the mean was based on 5 as equal to the reference. With the latter test, numbers less than 5 indicate dislike, and numbers greater than 5 indicate like better than the reference. Hedonic and multiple comparison tests were done on all samples in each series. Samples were evaluated after 3, 6, 12, 24, 48, 72, 120, and 144 h of exposure.

Observations were also made on the total light transmission through the various containers when exposed to 60, 100, 150, 200, 250, 300, 350, and 400 ft-c using a cool white fluorescent light. Illumination (ft-c) was controlled by accurately adjusting the distance between the light source and the paperboard or plastic container panel. Light readings were made inside and outside of each container using a Model 756 Weston meter.

RESULTS AND DISCUSSION

Organoleptic data from exposure of milk in the various containers to fluorescent light are shown graphically in Figures 1 and 2. These data indicate that milk in fiberboard containers changed less rapidly in favor than that in blown mold plastic bottles. Milk held in plastic containers decreased sharply in flavor score, expressed hedonically, after 12 h. These results are in agreement with observations by Dimick (5).

Data from Table 1 show that based on hedonic scores no significant differences ($P < 0.05$) were found in flavor of milk from blue, green, orange, purple, and brown containers compared to the control. With the multiple

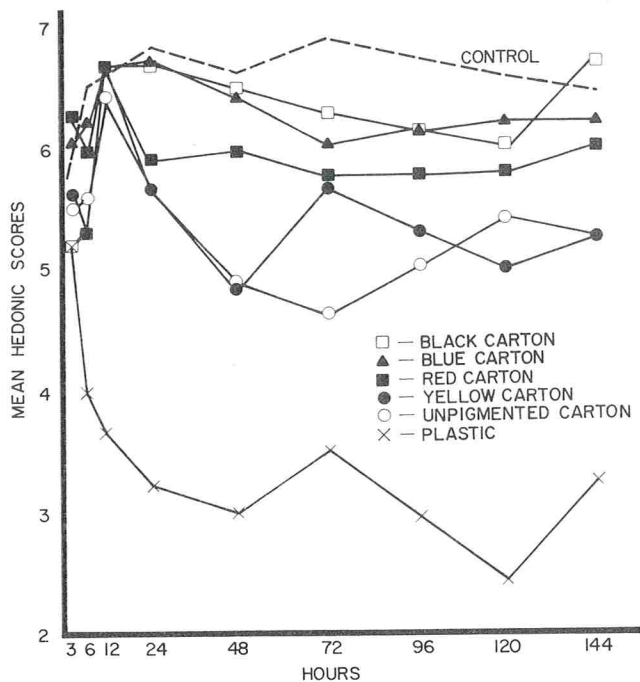


Figure 1. Trial 1: Hedonic scores of milk in black, blue, red, yellow and unpigmented paperboard, and unpigmented plastic after exposure to 100 ft-c.

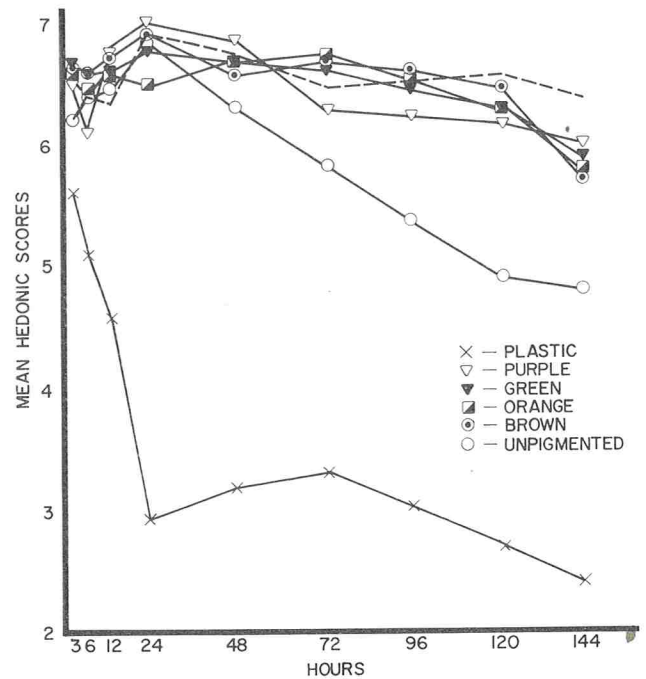


Figure 2. Trial 2: Hedonic scores of milk in purple, green, orange, brown and unpigmented paperboard and unpigmented plastic after exposure to 100 ft-c.

TABLE 1. Hedonic averages, Trials 1 and 2, Duncan's multiple range test for container differences

Trial 1			Trial 2		
Container	Mean*	Significantly different** from	Container	Mean*	Significantly different** from
1. Plastic	3.56	2,3,4,5,6,7	1. Plastic	3.77	2,3,4,5,6,7
2. Yellow	5.40	1,4,5,6,7	2. Unpigmented	5.91	1,3,4,5,6,7
3. Unpigmented	5.55	1,4,5,6,7	3. Green	6.40	1,2
4. Red	6.01	1,2,3,6,7	4. Orange	6.44	1,2
5. Black	6.15	1,2,3,7	5. Purple	6.48	1,2
6. Blue	6.33	1,2,3,4	6. Brown	6.55	1,2
7. Control	6.52	1,2,3,4,5	7. Control	6.56	1,2

*Average over trial period of 144 h.

** $(P < 0.05)$.

TABLE 2. Multiple comparison averages, Trials 1 and 2, Duncan's multiple range test for container differences

Trial 1			Trial 2		
Container	Mean*	Significantly different** from	Container	Mean*	Significantly different** from
1. Plastic	2.91	2,3,4,5,6,7	1. Plastic	3.03	2,3,4,5,6,7
2. Yellow	4.26	1,4,5,6,7	2. Unpigmented	4.36	1,3,4,5,6,7
3. Unpigmented	4.30	1,4,5,6,7	3. Orange	4.78	1,2,6,7
4. Red	4.85	1,2,3,7	4. Green	4.79	1,2,6,7
5. Black	5.00	1,2,3	5. Purple	4.89	1,2
6. Blue	5.00	1,2,3	6. Brown	4.99	1,2,3,4
7. Control	5.09	1,2,3,4	7. Control	5.00	1,2,3,4

*Average over trial period of 144 h.

** $(P < 0.05)$.

TABLE 3. Light transmission through test containers at 60, 100, 150, 200, 250, 300, 350 and 400 ft-c illumination with 40 watt cool white flourescent lamps (F40 CW)

Carton	% Light transmission	Mean	Significantly different* from
1. Unpigmented paperboard	2.5-3.0	2.8	2,4,7,8,9,10
2. Red paperboard	0.6-0.8	0.7	1,5,10
3. Green paperboard	0.9-1.1	1.0	10
4. Blue paperboard	0.6-0.9	1.8	1,5,10
5. Yellow paperboard	2.5-3.0	2.8	2,4,7,8,9,10
6. Orange paperboard	1.4-1.7	1.5	10
7. Purple paperboard	0.7-0.8	0.8	1,5,10
8. Brown paperboard	0.5-0.6	1.6	1,5,10
9. Black paperboard	0.2-0.3	0.2	1,5,10
10. Blown mold plastic	66-72	69.1	1,2,3,4,5,6,7,8,9

* $(P < 0.05)$.

comparison tests (Table 2) black, blue, purple, and brown were the only colors with which flavor scores were not significantly different from the control.

Based on light transmission (Table 3) through the paperboard container, red, blue, purple, brown, and black colors were significantly different ($P < 0.05$) from unpigmented and yellow. All paperboard containers were significantly ($P < 0.05$) better than the plastic bottles studied in screening out total light. The percentage of fluorescent light penetrating the containers varied only slightly at the intensities of light studies.

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Milk Iodides: Effects of Iodophor Teat Dipping and Udder Washing, and Dietary Iodide Supplementation

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ABSTRACT

In a small research herd, where dietary iodide intake was approximately 8 mg/head/day, and where management practices for 2½ years had included iodophor products for both udder washing and teat dipping, milk iodide values averaged 6.3 µg/100 ml, with a range of 1.0 to 24.0. The average iodide level (in µg/100 ml) in milk after udder washing and teat dipping was 3.7 with water and 3.9 with hypochlorite, with ranges of 1.6-9.6 and 1.6-9.2, respectively. Use of the iodophor udder wash under controlled conditions did not result in a detectable increase in milk iodides. Fluctuations associated with use of a commercial iodophor teat dip were essentially within the range of values obtained in the absence of iodophor use. The effect of iodide supplementation on milk iodide levels grossly overrode any contribution attributable to the use of a properly formulated iodophor teat dip product. Use of such products would seem of limited significance in the total human dietary iodide intake.

The concentration of iodide in milk has long been known to vary within wide limits with variations in the dietary intake of iodide (11). In recent years, iodophors (complexed titratable iodine products) have come into wide use in dairy herd programs for milking equipment sanitation and for the topical disinfection of udders and teats. There have been reports in the literature of increased iodide levels in milks from farms using these products (4, 10, 12). However, in the literature reports it is generally difficult to dissociate the effects of misused iodophor equipment sanitizers, udder washes, and teat dips, from the effect of dietary iodide intake on milk iodide. For this reason, the effect on milk iodide of the topical use of such products, for pre-milking udder washing and post milking teat dipping, was the subject of this study. In addition, the effect of oral iodide supplementation on the milk iodide levels associated with the topical use of the iodophor products tested was also determined in an exploratory terminal phase of this study.

Because the natural iodide content of forage is affected by geographical location as well as fertilization practices, and because of the uncertainties associated with the actual iodide content of forage grasses and feed grains and of the presence of goitrogens, fortification of animal feed has become an accepted and widespread practice (6). The total contribution of feed components (including iodized salt licks) to the daily iodide intake often goes unrecognized. In the United States, recommended iodide intakes for mature dairy cows are in the range of

0.6-0.8 ppm; this amounts to 9-12 mg for a 500-kg cow consuming 15 kg of dry matter daily (14). The British recommend that the allowance be increased to 30 mg/day when goitrogens are present in feed (7). In a study of 13 Illinois farms, some form of iodide supplementation, beyond that added to the feed grain ration, was practiced in seven of the farms; the milk iodide contents varied with the extent of total supplementation, being 1610 µg/liter where a mineral mixture with 0.055% iodide and iodized salt were used (8). Milk iodide content has been found to vary linearly with iodide intake up to about 150 mg of iodide/day, after which there is a leveling, perhaps due to limitations in the efficiency of the bovine mammary gland to concentrate additional iodide (14). In certain countries where milk is an important source of iodide for large population groups, and where for seasonal and/or geographical reasons the milk iodide contents were judged inadequate, a systematic program to increase the amount of iodide fed to cows was initiated (3, 9).

Iodide carryover into milk from iodophors used to sanitize dairy equipment is generally the result of improper attention to the label directions. The use of iodophor solutions for udder washing, at the recommended use-dilution of 25 ppm titratable iodine, has generally been premised not to result in any detectable increase in milk iodides. Painting the udder with an undiluted iodophor has been reported to have increased milk iodides only minimally (19).

MATERIALS AND METHODS

The herd utilized for this study consisted initially of 35 cows, maintained for research and teaching purposes. For at least 2½ years

TABLE 1. Approximate iodine intake of herd, based on analyses of feed components and water

Component	Total iodides (u/g)	Iodine intake mg/head/day
Mineral salt mixture ^a	23.5	3.7
Grains	0.7	1.5
Salt block	5.5	0.75
Hay and corn silage	0.4 (hay)	1.5
Water	0.3 (corn)	0.05 ^b
TOTAL	<0.002	7.5

^aApproximate intake was 84 lb/week/35 cows; use was discontinued at the start of Phase 2.

^bBased on average daily water intake of 23 kg/cow; water source was municipal supply.

before initiation of the study, the herd had a dietary iodide intake of approximately 8 mg/head/day (Table 1). During this period, udders had been washed before milking with an acidified iodophor udder wash product, diluted to 25 ppm titratable iodine, and teats had been dipped after milking with a teat dip iodophor product containing 1.0% (10,000 ppm) titratable iodine.¹

This study, divided into six phases, began in late summer and continued into winter, with temperatures reaching a low of 16 F. The 32 cows carried through the course of the investigation were in various stages of lactation: 3, very late; 19, late; 3, midway; 5, early; 2, very early. The teat dip materials to be tested (Table 2), the teat dip cups, and the cows' stalls were color coded to match. Initially, five iodine formulations (four experimental and one marketed product) and one hypochlorite product were put on the test. When it became obvious, during Phase 3, that no distinctive information was being developed for two of the experimental iodine formulations, they were discontinued. Two udder washes were used during Phases 1 to 5 (one was an acidified iodophor product², the other a hypochlorite product); one was assigned to each of the two rows of the barn. The cows within each teat dip group were distributed across the two rows, as illustrated schematically in Fig. 1. The milk samples from each cow were taken from the milk weighing can, using a 100 ml beaker and rinsing the beaker twice with approximately 50-ml aliquots, before sampling from a third aliquot into pre-coded sample bottles. Excess used iodophor was collected in a closed waste container to minimize contribution to the iodine concentration of the air in the relatively closed environment of the barn.

The study was phased as follows (see Fig. 2): (a) *Phase 1* (3 milkings): A continuation of pre-tested practices described above; samples from each milking were analyzed for iodide content (milk samples 1-3). (b) *Phase 2* (26 milkings): Mineral salt was withdrawn; water was used to wash the udders of cows on the North Row of the barn, and use of the acidified iodophor was continued on the South Row; all teats were dipped with water. Milk samples were analyzed four times (milk samples 4 to 7). (c) *Phase 3* (46 milkings): Teat dipping and udder washing with test materials were started. Udders of all cows in the barn were washed according to the row assignment, and teats were dipped by group assignment, regardless of whether all the cows in the barn were assigned to the project, to reduce the chances for error. As a result, some of the cows in each teat dip product group were washed with the acidified iodophor product, diluted to provide 25 ppm titratable iodine (South Row), and the udders of the remaining cows in each group were washed with the hypochlorite solution containing 600 ppm available chlorine (North Row). Milk samples were analyzed four times (samples 8-11). (d) *Phase 4* (25 milkings): Procedures outlined in Phase 3 were continued, after adding to the group of cows on teat dip product "F"

SOUTH ROW NORTH ROW

171	Teat Dip F (black)	
25		
104		
384 ^a		
13 ^a		26 ^a
4	Teat Dip A (red)	
11		519
509		32
8		312
		6 ^b
23		361
520		342
15	Teat Dip G (white)	32
		40
28		330
22		5 ^b
510	Teat Dip D (green)	7
29		10
20		332
27		

SOUTH ROW	NORTH ROW
Udder Wash:	Udder Wash:
Phase 1: iodophor	Phase 1: iodophor
Phase 2: iodophor	Phase 2: water
Phases 3-5: iodophor	Phases 3-5: hypochlorite
Phase 6: hypochlorite	Phase 6: hypochlorite

^atransferred, Phase 4
^badded, Phase 3

Figure 1. Schematic barn lay-out.

three cows from the teat dip products discontinued at the end of Phase 3, as noted in Fig. 1. Milk samples were analyzed twice (samples 12 and 13). (e) *Phase 5* (41 milkings): During Phase 5, the number of animals originally on the test was sharply reduced, by culling or drying off, leaving only one or two of the animals originally assigned to each teat dip product group. The Phase 5 cumulated data were therefore limited

¹"Bovadine", West Agro-Chemical, Inc., subsidiary, West Chemical Products, Inc. (used undiluted, 10,000 ppm titratable iodine); coded as Product "A".

²"Iosan", West Agro-Chemical, Inc. subsidiary, West Chemical Products, Inc. (diluted 1 part plus 640 parts water, 25 ppm titratable iodine).

TABLE 2. Teat dip products: description

	Code designation for teat dips tested			
	"A"	"D"	"F"	"G"
Titratable iodine, %	1.0	1.0	0.25	0.0
Thermodynamically free iodine, ppm	3	>50	0.3	0.0
D.C. Number ^a	140	5	420	N.A.
pH	3.9	4.5	5.0	—
Characterization	commercial product "Bovadine"	same iodine content as commercial product but no significant complexing of iodine	lower iodine content & higher degree of complexing than commercial product	NaOCl, 4.0% available chlorine, free of excess alkali (NaOH, >0.1%)
Tested in phases ^b	1,3-5	3-5	3-5	3-5

^aThe Distribution Coefficient is a measure of the degree of complexing of the thiosulfate titratable iodine, determined by partitioning the iodine between immiscible solvents (Ref. 17).

^bWater was used for teat dipping in Phase 2.

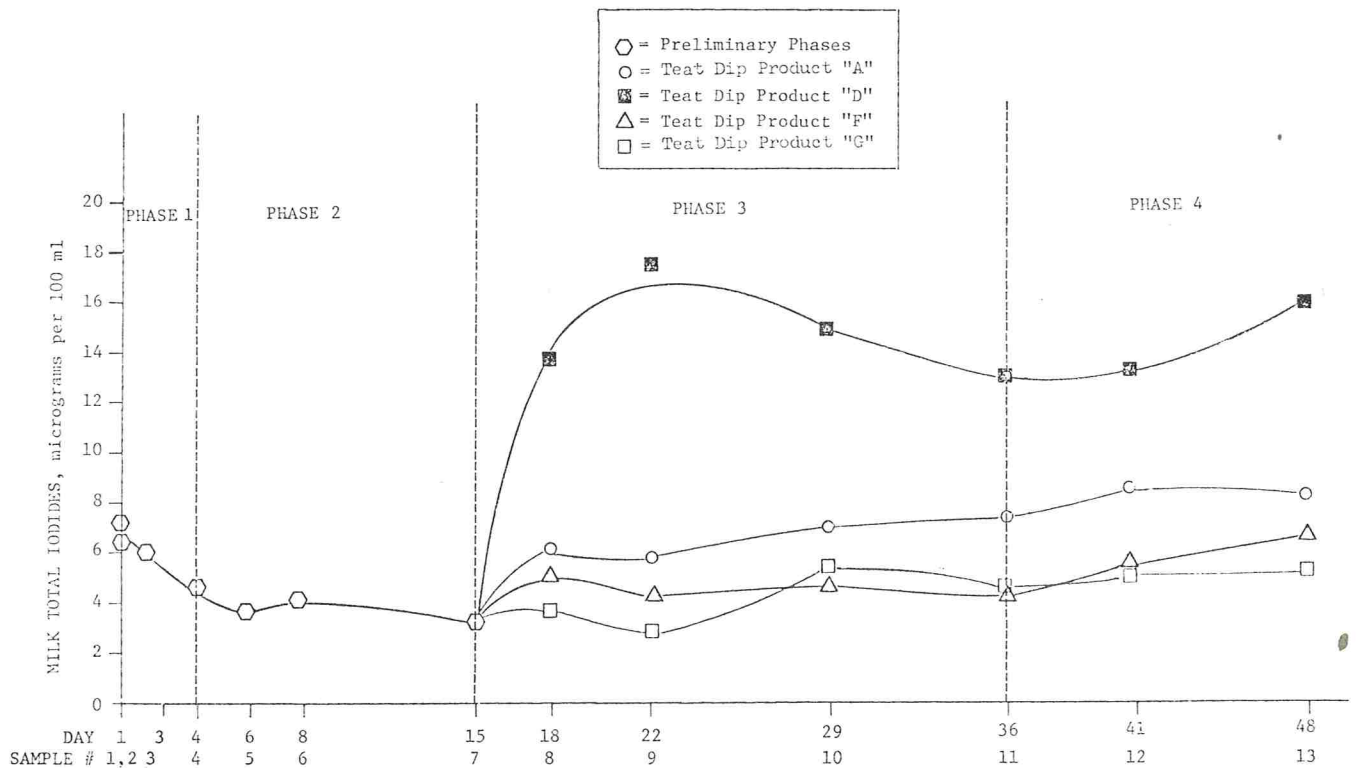


Figure 2. Milk iodide levels during Phases 1-4.

to a determination of the carryover of iodide on the teat skins from one milking to the next (Phase 5A) and to the results of iodide supplementation of the animals (Phase 5B), as follows: 5A. The teats of 6 cows were tested for residual iodine left from the milking and subsequent teat dipping 14 h previously, by extraction (repeated dipping and rinsing) into milk as the solvent. Two groups of three cows each were used; the cows in each group had been on one or the other of the two teat dip products "A" and "D." The sampling was done by sequentially dipping and rinsing the 12 unwashed teats, on each group of three cows, into one 50-ml sample of milk; the two milk samples representing the two groups of cows were then analyzed for milk iodide content. 5B. Capsules containing supplemental iodide were administered to 23 additional cows, as follows: 5 cows each, 5 mg/day for 7 days; 8 cows, 10 mg/day for 7 days; 8 cows, 15 mg/day for 14 days; 5 cows, 15 mg/day for 7 days and then 30 mg/day for an additional 7 days. All dosing was scheduled to be completed simultaneously. Phase 5 was terminated by removing from the barn all topical iodine products and iodide capsules. Milk samples were collected for analysis 5 times at 2-4 day intervals. (f) Phase 6 (26 milkings): Udders of all cows were washed with a hypochlorite solution containing 600 ppm available chlorine and teats dipped in a solution containing 4% available chlorine, until the study was terminated. Milk samples were analyzed twice at weekly intervals.

All samples (milk, water, feed) were analyzed by Boston Medical Laboratory³ by their published methods (1, 2). The lower limit of detection, based on two standard deviations, was 0.25 $\mu\text{g}/100\text{ ml}$. As will become apparent, although the cumulated data developed in this limited study were adequately supportive of the general conclusions, it was not considered sufficiently extensive to permit quantifying the statistical significance of the differences.

RESULTS AND DISCUSSION

Analytical results for feed components and water are presented in Table 1; milk iodide values through

Phases 1 and 2 are given in Table 3; milk iodide values through Phases 3 and 4 are given in Tables 4 and 5, respectively; milk iodide values for Phases 1 through 4 are graphed in Figure 2; Phase 5A results are discussed in the text below; and milk iodide values associated with Phase 5B iodide capsule dosing are given in Table 6.

In this small herd, where management practices for at least 2½ years had included udder washing with one iodine product (25 ppm titratable iodine), and teat dipping with another (10,000 ppm titratable iodine), initial milk iodide values for three consecutive milkings (Phase 1) averaged 6.3 $\mu\text{g}/100\text{ ml}$, with a range of 1.0-24 $\mu\text{g}/100\text{ ml}$.⁴ Concentrations of iodide in milk decreased slowly during Phase 2, averaging 3.5 $\mu\text{g}/100\text{ ml}$ for cows where udders were washed with the iodophor product (South Row), and 4.1 for cows where udders were washed with water (North Row). During these two weeks, all teats were dipped with water, and the only external source of iodide was the udder wash used on the South Row cows (Table 4, Phase 2). Since there was no increase in milk iodides for the South Row cows during this baseline stabilizing period, and milk iodide values for North Row and South Row cows showed no significant change relative to each other, use of the udder wash iodide product did not contribute to milk iodides.

During the approximately 3-week Phase 3 period, the changes in the baseline milk iodide concentrations established in Phase 2 were determined. Phase 3 cumulated data suggest that for the two 1.0% titratable iodine products, "A" and "D," teat dipping with the complexed iodine product "A" may have added a relatively insignificant increment (<3 $\mu\text{g}/100\text{ ml}$),

³15 Lunda Street, Waltham, Mass. 02154.

⁴One animal (cow No. 26) was the source of anomalous results, reaching 40 $\mu\text{g}/100\text{ ml}$ in one Phase 1 sample.

TABLE 3. Concentrations of iodide in milk during Phases 1 and 2

Phase	Sample/row ^a	No. of cows	Udder wash	Teat dip	Micrograms/100 ml	
					Average	Range
1	1/S&N	23	I-25 ppm ^b	"A"	6.9	1.0-24.0
1	2/S&N	23	I-25 ppm	"A"	6.3	1.2-24.0
1	3/S&N	23	I-25 ppm	"A"	5.8	1.0-17.2
1 Aver.			I-25 ppm	"A"	6.3	
2	4/S	15	I-25 ppm	Water	4.2	2.0- 8.8
2	4/N	7	Water	Water	5.0	3.2- 6.8
2	4/S&N	22	I-25 ppm/Water	Water	4.5	
2	5/S	16	I-25 ppm	Water	3.2	1.6- 7.2
2	5/N	7	Water	Water	4.2	2.4- 6.0
2	5/S&N	23	I-25 ppm/Water	Water	3.5	
2	6/S	16	I-25 ppm	Water	3.7	1.6- 4.8
2	6/N	7	Water	Water	4.3	2.4- 7.2
2	6/S&N	23	I-25 ppm/Water	Water	3.9	
2	7/S	20	I-25 ppm	Water	3.0	1.6- 4.8
2	7/N	13	Water	Water	3.1	1.6- 9.6
2	7/S&N	33	I-25 ppm/Water	Water	3.0	
2 Aver.	4-7/S		I-25 ppm	Water	3.5	
2 Aver.	4-7/N		Water	Water	4.1	
2 Aver.	4-7/S&N		I-25 ppm/Water	Water	3.7	

^aRefers to sample number (see Fig. 2) and to row of stalls in barn (see Fig. 1).

^bIodophor, 25 ppm titratable iodine.

TABLE 4. Concentrations of iodide in milk during Phase 3

Sample/row ^a	No. of cows	Udder wash Row:S/N	Teat dip	Micrograms/100 ml		Phase 3 average
				Average	Range	
8/S&N	10	I-25 ppm ^b /G ^c	"A"	5.9	3.2-10.4	6.3
9/S&N	10	I-25 ppm/G	"A"	5.6	4.4-10.4	
10/S&N	10	I-25 ppm/G	"A"	6.7	4.4-10.4	
11/S&N	11	I-25 ppm/G	"A"	7.1	3.2-11.6	
8/S&N	3	I-25 ppm/G	"D"	13.5	8.4-21.2	14.5
9/S&N	3	I-25 ppm/G	"D"	17.3	12.0-25.6	
10/S&N	3	I-25 ppm/G	"D"	14.7	10.4-20.0	
11/S&N	3	I-25 ppm/G	"D"	12.7	10.4-14.0	
8/S	3	I-25 ppm	"F"	4.8	3.6- 6.8	4.3
9/S	3	I-25 ppm	"F"	4.0	3.2- 5.6	
10/S	3	I-25 ppm	"F"	4.4	3.2- 5.2	
11/S	3	I-25 ppm	"F"	4.0	3.6- 4.4	
8/S&N	4	I-25 ppm/G	"G"	3.7	2.4- 5.2	3.9
9/S&N	5	I-25 ppm/G	"G"	2.6	1.6- 4.0	
10/S&N	5	I-25 ppm/G	"G"	5.1	2.4- 9.2	
11/S&N	5	I-25 ppm/G	"G"	4.2	3.2- 4.8	

^aRefers to sample number (see Fig. 2) and to row of stalls in barn (see Fig. 1).

^bIodophor, 25 ppm titratable iodine.

^cSodium hypochlorite, 600 ppm available chlorine.

TABLE 5. Concentrations of iodide in milk during Phase 4

Sample/row ^a	No. of cows	Udder wash Row S/N	Teat dip	Micrograms/100 ml		Phase 4 average
				Average	Range	
12/S&N	12	I-25 ppm ^b /G ^c	"A"	8.1	2.0-16.0	7.9
13/S&N	12	I-25 ppm/G	"A"	7.8	3.2-16.0	
12/S&N	7	I-25 ppm/G	"D"	12.9	7.2-19.6	14.2
13/S&N	7	I-25 ppm/G	"D"	15.6	12.0-22.4	
12/S&N	6	I-25 ppm/G	"F"	5.2	4.4- 6.4	5.7
13/S&N	6	I-25 ppm/G	"F"	6.3	4.8- 8.8	
12/S&N	7	I-25 ppm/G	"G"	4.7	2.8- 9.6	4.7
13/S&N	7	I-25 ppm/G	"G"	4.8	2.0- 6.4	

^aRefers to sample number (see Fig. 2) and to row of stalls in barn (see Fig. 1).

^bIodophor, 25 ppm titratable iodine.

^cSodium hypochlorite, 600 ppm available chlorine.

TABLE 6. Concentrations of iodide in milk during Phase 5B (iodide supplementation) and Phase 6 (termination)

Iodide dosage (mg/day) (capsule)	No. of cows	Micrograms/100 ml					
		Plateau values while on dosage ^a		1 week after cessation		2 weeks after cessation	
		Average	Range	Average	Range	Average	Range
0.0	13	12.0	5.6- 28.8	8.6	4.4-13.4	9.8	5.4-16.4
5.0	4	24.0	18.0- 48.0	14.0	8.0-27.0	12.7	8.0-20.0
10.0	5	29.2	13.6- 66.0	7.6	4.6- 9.6	9.9	5.0-12.0
15.0	8	44.2	30.2-116.0	11.1	6.4-19.4	11.3	6.0-16.4
30.0	5	75.5	36.8-116.0	11.0	4.6-16.0	11.2	5.4-17.4

^aThe range values are for samples taken on the same sampling day, toward the end of the dosing period at each of the four iodide dosages.

compared with the increment added by the uncomplexed iodine product "D" ($>10\mu\text{g}/100\text{ ml}$) (see Table 4 and Fig. 2). The reproducibility of the $2\mu\text{g}/100\text{ ml}$ difference in milk iodide between the two complexed iodine products, "A" (1.0% iodine) and "F" (0.25% iodine), would also require validation.

A comparison of the Phase 3 milk iodide values for the three iodine products ("A," 1.0% complexed iodine; "D," 1.0% iodine, not complexed; "F," 0.25% complexed iodine) suggests that for such products the concentration of the uncomplexed or "free" iodine may be of primary importance. That is, a 2-log difference in free iodine concentration (see Table 2) may be necessary to elicit significant differences in milk iodide values attributable to teat dipping with such iodine products. It is likewise apparent that there was no appreciable effect on milk iodides by teat dipping with the complexed 0.25% titratable iodine product, when compared with the hypochlorite teat dip "G".

In Phase 4, iodide concentrations in all four groups tended to increase with time of lactation, an observation previously associated with decreasing yields of milk (14). Those Phase 4 further marginal increases in milk iodide concentrations took place in all four teat dip groups, ranging from increases of 1.3 to $3.3\mu\text{g}/100\text{ ml}$. However, the cumulated Phase 4 data are also less reliable than the Phase 3 data as a result of the intervening transfer of animals between teat dip groups. Furthermore, since there was a marginal reduction in dietary iodide by withdrawal of the mineral salt at the beginning of Phase 2, the operation of the mammary gland milk iodide concentrating mechanism may have been sensitized to subsequent fluctuations in iodide input as the study progressed. An illustration of such sensitivity of the milk iodide concentrating mechanism to the baseline level of systemic iodide in the animal may be suggested by a currently published note (5). That paper reports that a very low $1\mu\text{g}/100\text{ ml}$ milk iodide level in iodide-depleted animals (at pasture, without mineral iodine supplements) rose sharply to a plateau of $27\mu\text{g}/100\text{ ml}$ within 6 days of teat dipping with a teat dip containing 0.5% available iodine. However, the same teat dipping practice on 30 herds with an initial milk iodine level of $26\mu\text{g}/100\text{ ml}$, associated with dietary iodide supplements, resulted (over 14 to 21 days) in a much more limited increase of $12\mu\text{g}/100\text{ ml}$. In eight of the latter herds, the mean iodide concentration of the milk decreased whereas in 26

it increased (12 significantly; $P<0.05$).

Reverting to our own investigation, a significant recovery of iodide was found in our Phase 5A probe to determine the residual iodide left on the unwashed teats from a 14-h prior milking and iodophor teat dipping. For the cows which had been teat dipped with product "A," the 50 ml milk sample contained $1085\mu\text{g}$ of added iodide; this amount in the 30 liters of milk for the three animals, or $3.7\mu\text{g}/100\text{ ml}$, was a calculated incremental addition to the pre-existing milk iodide level. For product "D," a likewise calculated incremental addition was almost twice that, i.e. $6.7\mu\text{g}/100\text{ ml}$. In both calculations, substantially total elution was premised for the iodine residues from the prior teat dipping. The suggested importance of adequate udder and teat washing before milking was also reported in the previously cited investigations where it was found that when teats dipped in the iodophor were washed with water before each milking a plateau of $27\mu\text{g}/100\text{ ml}$ was reached, whereas without water washing a plateau of $40\mu\text{g}/100\text{ ml}$ was reached (5). Likewise, in a radioiodine tagged teat dip study, it was found that the tagged iodine "localized mainly on the teat skin and teat canals of the udder" and that "there appears to be a distinct correlation between udder cleaning before milking and the increase in milk iodine levels" (18).

In the foregoing radioiodine study the percentage transfer of iodine into the milk, from topically (teat dip) applied iodine was also determined. Of 15.2 mg of iodide reported as found on four teats dipped with that product, 2% was present in the milk. In our investigations, we calculated that about 0.5% of the approximately 50 mg of total iodide in the film initially deposited on four teats dipped with product "A" could represent the percentage transferred to the milk, after discounting the contribution of dietary iodide. The limited difference in transfer percentages for the two products (2% as against 0.5%) may be related to the difference in total iodine concentration in the two products and to a difference in viscosity.

In Phase 5B, the effect on milk iodide levels of daily (capsule) administration of dosages as low as 5.0 mg iodide/cow overrode any contribution by topically applied iodine products, either from udder washing or from teat dipping, with the possible exception of the uncomplexed teat dip product "D." Additional increases in milk iodides varied directly with the amounts of iodide

administered, as summarized in Table 6. The return towards normal iodide levels upon withdrawal of the iodide capsule supplements was most striking from the higher iodide levels. The results are in agreement with studies reported in the literature, in which iodide intakes at "nutritional levels" are generally associated with milk iodide levels of under about 10 $\mu\text{g}/100\text{ ml}$ (11), and "prophylactic levels" are usually associated with milk iodide levels considerably in excess of 75 $\mu\text{g}/100\text{ ml}$ (6, 13, 14).

The iodide intake of the subject herd is estimated to have been about 8 mg/head/day before initiation of this study. The withdrawal of the trace mineral salt (which had been contributing approximately 3 mg iodide/head/day) at the beginning of this investigation, some 2 months before initiation of the experimental oral iodide supplementation, may have resulted in some degree of iodide deficit, and may therefore have favored its subsequent concentration by mammary glands (5).

Approximately 50% of the orally administered iodide was secreted in the milk at intake levels in the range of approximately 10-30 mg/head/day. This 50% absorption/secretion rate is in contrast to the approximately 1% topical route rate of transfer from teat dips. The ready absorption of iodide supplements and the mammary gland concentration of that systemic iodide makes such ingested iodide by far the major contributor to increased milk iodides.

There has been recent activity on the part of a few countries to set tolerance limits for iodide in milk. The Swiss Federal Health Department has imposed a limit of 50 $\mu\text{g}/100\text{ ml}$ for iodide in milk resulting from the use of iodophor teat dips. Australia (Dairy Industry Association of New South Wales), based on milk consumption of 300 ml/day, has a current limit of 50 $\mu\text{g}/100\text{ ml}$. These limits permit the safe use of properly complexed iodophor teat dip products.

In the United States, the National Research Council's Recommended Daily Allowances (RDA) for iodide for adults are in the 100-150 μg range (16); daily intakes of up to 1000 μg are considered safe (15). A recent review of dietary iodide intakes in the United States concludes that the average intake has increased in recent years and currently ranges from 380 to 450 $\mu\text{g}/\text{day}$ (6). For the 1-year old child, the RDA of 60 μg would be met by approximately 1 quart of milk containing the 6.3 $\mu\text{g}/100\text{ ml}$ of iodide found in the milk from the animals whose teats were dipped with product "A" over a 2-year period. An adult drinking 2 glasses/day of such milk would require additional dietary iodide sources.

SUMMARY AND CONCLUSIONS

1. Milk iodide values initially averaged 6.3 $\mu\text{g}/100\text{ ml}$ in a small research herd where dietary iodide intake was estimated at 8 mg/head/day and where management practices over a 2½ year period had included iodophor products for both udder washing and teat dipping.
2. The controlled use of an iodophor solution for udder

washing, at 25 ppm titratable iodine, did not result in a detectable increase in milk iodides.

3. Milk iodide values during the control period use of water for teat dipping ranged from 1.6-9.6 $\mu\text{g}/100\text{ ml}$ (average, 3.7).
4. Milk iodides, in a controlled comparison of teat dip products, ranged as follows, in $\mu\text{g}/100\text{ ml}$: (a) 1.6-9.2 (average 3.9) with hypochlorite; (b) 3.2-11.6 (average, 6.3) with a 1.0% complexed iodine commercial product; (c) 8.4-25.6 (average, 14.5) with a 1.0% uncomplexed iodine experimental formulation; and (d) 3.2-6.8 (average, 4.3) with a 0.25% complexed iodine experimental formulation.
5. The average iodide level found in milk after udder washing and teat dipping with the commercial iodophor product tested, 6.3 $\mu\text{g}/100\text{ ml}$, was well within the range of values found for the control animals udder washed and teat dipped with the hypochlorite product.
6. Failure to wash teats previously dipped in 1% iodophor, in preparation for the next milking, may cause increases of about 5 $\mu\text{g}/100\text{ ml}$ above the normally present iodide concentration.
7. Approximately 50% of orally administered iodide is secreted in the milk of cows given approximately 10-30 mg/head/day.
8. The effect of iodide supplementation on milk iodide levels overrides any contribution by properly formulated and used iodophor teat dip products. Such milk iodide fluctuations as were found associated with the use of properly formulated iodophor teat dips would seem of limited significance in the total human dietary intake.

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EXECUTIVE BOARD MEETING, JANUARY 18, 1976

Chairman Vaux convened the meeting at 1:10 p.m. Voting Board members present: Arledge, Boosinger, Causey, Gay, McGarahan, Meister, Rich, Rowley, Russell, Schilling, Van Patten, Vaux, Weckel. Absent: Harvey, Heinemann, Johnson, Seaman. Non-voting members present: Conner, Huskey, Speer.

The following Council changes were proposed and accepted by the Board: (a) Council I—Ray Morrison for Holsinger, James Carroll for Ray Ebbert, L. A. Maes for John Kaczor; (b) Council II—Robert Ryan for Dwayne Lipinski; and (c) Council III—Douglas Braatz for Archie Smith.

Chairman Vaux stated that Sam Noles, Chairman, Nominating Committee, wished a policy statement regarding eligibility of William Arledge as a representative of industry from Group I. The Board decided that the nominating committee should present another name to replace Arledge, but that Arledge would be eligible to replace Burdet Heinemann in Group II, since Heinemann had resigned from the Board due to retirement.

The Board authorized the chairman to reaffirm to members of the Conference that its position on publication of the document has not changed. Arledge presented a resolution in this regard, and the Board directed the Chairman to use this resolution as a basis for his presentation to the conference at the business session on Monday morning, January 19, 1976.

The Board directed the Chairman to charge the councils to use the seven documents and to stick to the technical aspects of the problems, not to include philosophies, and to report their recommendations as specifically as possible. A uniform format is to be used for all Council reports.

The Board directed the Chairman to present the Memorandum of Understanding to the Conference on January 19, and to assign it to Council II for action. Meeting was adjourned at 1:45 p.m.

FIRST GENERAL SESSION

Chairman Vaux convened the session at 9:15 a.m. on Monday, January 19, 1976. The invocation was by Sam Noles. Secretary McCaffrey called the role of states and delegates authorized to vote.

Chairman Vaux instructed the council chairmen, council members, and delegates on procedures to be used in deliberations. He stated that the rules would have to be waived to consider deletion of Section 7, C (special emphasis on 5) and Section 6, B, 4 from Procedures as requested by F.D.A. Dally moved, seconded by Jefferson, that the rules be waived as requested by the Chairman. Approved. Section 6, B, 4 was assigned to Council II; and Section 7, C was assigned to Council III. Gadd moved, seconded by Kennedy, that the Memorandum of Understanding be considered. Approved. It was assigned to Council II. Council I was assigned parts 962 and 963; council III was assigned parts 964, 965, and 966 as well as the Laboratory Committee report. Harold Thompson stated that F.D.A. wished the Conference to delete Section 6, G from the Procedures.

SECOND GENERAL SESSION

Chairman Vaux convened the session at 2:15 p.m. on Wednesday, January 21, 1976. Sam Noles presented the slate of the Nominating Committee for representatives from Group I, as follows: Don Race, Industry; E. Marion Causey, Jr., Health; Jay B. Boosinger, Agriculture; Sam Rich, Local Health. The name of William Arledge was presented to replace Burdet Heinemann. Members of the Committee: John Baghott, Richard Dennler, G. W. Fouse, Archie Hurst, Earl Helmreich, Sam Noles.

FINAL GENERAL SESSION

The final general session was called to order by Chairman Vaux at 8:12 a.m. on Thursday, January 22, 1976. The final roll call of states and delegates authorized to vote was taken by Secretary McCaffrey.

The roll call showed that 38 states and the District of Columbia were represented, 12 states by both Agriculture and Health, 13 by Agriculture only, and 13 by Health only. Chairman Vaux explained that he would establish priorities, indicating that proposed changes in Procedures would precede all other problems.

Jay Boosinger, chairman, Council II, recommended that the Conference delete Section VI, B, 4 of the Procedures, and that Section VII, C, 3 be amended by deleting the words "shall direct a letter to the State agency, with a copy to the shipper concerned notifying it that the shipper will be deleted from the next list of Sanitation Compliance and Enforcement Ratings of Interstate Milk Shippers" and substitute the wording "shall identify those states in the next listing of Sanitation Compliance and Enforcement Ratings of Interstate Milk Shippers as not being in compliance with Section VII, C." Approved. Glenn Huskey, Chairman, Council III, recommended that Section VII, C, 4 be amended by deleting the words "shall direct a letter to the State agency, with a copy to each listed shipper in that State, notifying that the names and ratings of all shippers will be deleted from the next list," and substituting the wording "shall identify those States in the next listing of the Sanitation Compliance and Enforcement Ratings of Interstate Milk Shippers as not being in compliance with Section VII, C" and that Section VII, C, 5 be amended by striking the words "The Service shall direct a letter to the State Milk Sanitation Rating Agency requesting such Agency to formally show cause as to why the names and ratings of all certified shippers in the State should not be deleted from the next listing," and substituting "The Public Health Service shall identify such States in the next listing of Sanitation Compliance and Enforcement Ratings of Interstate Milk Shippers as not being in compliance with Section VII, C of the IMS Procedures Manual." Delegates approved both suggestions.

Chairman Boosinger listed proposed amendments to the Memorandum of Understanding. After paragraph II-A-7 add paragraph 8, which reads: "To assist and cooperate with FDA in the standardizations and certification of State rating officers and FDA personnel." After paragraph II-B-10, add a paragraph 11, which reads "To continue to serve NCIMS in an advisory capacity in connection with the Procedures Manual in clarifying questionable areas of the current PMO, to evaluate supplies and equipment for compliance with the current PMO." Delete the wording of paragraph III-D and substitute: "The FDA and NCIMS agree that problems involving interpretations on application of any provisions of the Procedures Manual shall be subject to resolution by mutual agreement." Add, under General A, after the words "Laboratory Survey Officers," the words "State sampling surveillance officers." Delegate Kimball, Indiana, proposed an additional amendment which would become paragraph (a) under Section III, General, and would read as follows: "Future changes in

National Conference on Interstate Milk Shipments procedures and the documents basic to the NCIMS operations affecting the U.S. Food and Drug Administration shall be mutually concurred with by the National Conference on Interstate Milk Shipments and the U.S. Food and Drug Administration." Delegates approved.

REPORTS OF COUNCILS

The next order of business is the regular reports of Councils. In the summation of these deliberations your Secretary will mention only those proposed changes that were accepted or rejected by the delegates. If accepted no comment will be made; if rejected, the action will be so noted.

COUNCIL I

Chairman Vaux asked Dudley Conner, Chairman, to report on Recommended changes in the Pre-Publication draft. Conner announced that parts 962 and 963 were assigned to Council I. 962.1—Council recommended that the word "excessive" be added after the word "no" on lines 1 and 2. Council recommended that (b) be changed, deleting the words "milk rooms shall be free of insects and rodents" and substitute "insects and rodents shall be controlled." Delete "and screen doors shall open outward" Last recommendation was *rejected*. 962.3(b) insert a statement "state programs approved by E.P.A. shall be recognized." 962.4—Change (d) by deleting "free of" in the second line and insert the words "controlled for." 962.5—Council recommended that in (a) "45d." be deleted and "50d." be substituted. *Rejected*. 962.10—The only change was to delete "interstate" in the last line. 962.11—The word "interstate" was deleted. 962.12—(a) Delete "shall" and insert "should" in both the second and the fourth line. 963.13(a)—Delete "interstate" and (d) delete "interstate". 962.20(d)—The only change was the proper reference of 963.3 and 963.20, so it has to be corrected editorially. Delete (h) in its entirety and pick up the original wording for Grade A Pasteurized Milk Ordinance, 1965, as it was previously used.

962.30(g)—Delete "if ground, or chopped feed is stored in the barn." In (c) and (h) insert the word "reasonably" after "shall be kept" in the sixth line; in the eighth line insert the word "reasonably." Insert a new (i) which would make a change to have an insert and would recognize a provision for a three-wall milking parlor. 962.31(a)—Insert the letter "d" after "the product an." 962.51—The Council recommended deletion of "including a lavatory fixture" in the first and second lines. *Rejected*. 963, Subpart A, General Provisions, (b)—Change the letter "g" to "f," as a proper reference for clarification. 963.2 (b)—Delete the paragraph beginning with "Equipment and operations," (c) Change the letter "c" to the letter "b." Also, in (c) delete "the access of insects, dust, condensation, and others." In (d) change "d" to "c" to renumber the whole system. In (e) change the letter "e"

to the letter "d." In (f) change the letter "f" to the letter "e." Council recommended the deletion of "Such milk and milk products shall be protected against contamination. In no case shall pasteurized milk or milk products be standardized with unpasteurized milk unless the standardized product is subsequently pasteurized. Reconstituted or recombined milk and milk products shall be pasteurized after reconstitution or recombining of all ingredients." *Rejected*. Delete "This regulation permits standardization as a process of adjusting the butterfat of milk in a plant by the addition or removal of cream or skim milk." *Rejected*. In (g) delete the paragraph beginning with "Means shall be provided," in (h) change letter (h) to "f," change "i" to "g," "j" to "h," "k" to "l." Delete "45d." and insert "50d." *Rejected*.

963.4 (b)—Insert, after "Except" the words "those which have been ultrapasteurized and." *Rejected*. Delete "45d." and insert "50d." This recommendation was rejected but at the end of the Council report the motion was reconsidered and passed 963.5—Change in the third line "45d." to "50d." 963.13—In the fourth line change "30" to "20". 963.14 (b)—Insert the words "provided that for purposes of transferring milk from tank trucks, the room in which the transfer takes place shall be considered sufficient if overhead protection is provided for manholes and outlets." (c) Insert in the third line after "vents" the words "may also be" and after "elsewhere," the words "when they." Delete, in the third line, the word "which." In (d) change, in the second line after "required partitions," the words "except that this provision shall not apply when milk is being unloaded from bulk transport tanks, and the size of the bulk transport tank precludes the closing of entrance and or exit doors." 963.16 (d)—Delete, in the third line, "be kept free of evidence of" and insert, after "shall" the word "have," insert in the fourth line after "rodents" the word "controlled." 963.20 (a)—In the second line delete "or from which liquids may drip, drain, or be drawn into milk or milk products." (b) Insert, in the third line, after the words "shall contain no" the word "unnecessary." (d) Delete, in the second line, after "shall be," the word "rigid." 963.21 (a)—Delete, in the second line, "or from which liquids may drip, drain, or be drawn into milk or milk products." 963.21 (k)—Delete in its entirety and insert the wording regarding the standards as previously contained in the Grade A Pasteurized Milk Ordinance.

963.22 (a) — Insert, in the fourth line, after "cleaned," the words "at least once each day, if emptied." Delete, in the fourth line, "When emptied." Council recommended deletion of "Storage tanks which are used to store milk longer than 24 h and silo tanks shall be equipped with a temperature recording device complying with the specifications of 964.36 of this chapter, to verify that they are emptied, washed, and sanitized at least every 72 h." Insert "Verification that they are emptied, washed, and sanitized at least every 72 h must be provided." After the effective date of this section, newly installed storage tanks that are used to store milk longer than 24 h shall

be equipped with a temperature recording device complying with the specifications of 964.36 of this chapter to verify that they are emptied, washed, and sanitized at least every 72 h." *Rejected*. (e) Delete, in the fourth line, the word "immediately." (g) (3). Insert, after "all," the word "shipping." 963.30 — Council recommended no change. However, delegate Helmreich, Ohio, proposed to amend by adding, under line 1, at 175d.F. for 25 sec, add another line, at 185d. F. 15 sec, and then a note that if this proposed temperature and time relationship is not sufficient to provide proper pasteurization, then raise the temperature so that the proper pasteurization may be achieved at a 15-sec time.

963.31(a)—Change "966" to "964." 963.40 (k)—Insert "The equipment should be so designed that the lubricants can not fall or drip onto filler parts or the package or to any surface which in turn contacts the contact surface of the container." 963.41 (d)—Insert, after "all cans and closures" the words "for fluid products as defined in 960.1." 963.42 (a)—Delete, in the seventh line, "45d." and insert "50d." *Rejected*. 963.60—Delete the first paragraph reference to numerical ratings and add scope, definitions, and purpose to this particular document. 963.61 (a) (2).—Change "waste" to "trim" in line two. In (e) change "30" to "20" and "20" to "5" in line three. In (h) change "sanitary" to "clean" in line two; change "sanitary" to "clean" in the last line of (1). In (i) change "on pallets above" in line two of (1) to "off;" in line four of (1) delete "on pallets" and change to "off the floor;" in the last line of (2) delete "on pallets or in bins." In (j) last line of (1) add "and storage" between "fabricating" and "area." In (k) last line, change "plumbing codes" to "regulations." On (o) in line three, delete "and" and add "or" between plastic and metal. In (8) add the sentence "Air tubes used to convey resin shall have permanently attached removable covers that prevent contamination." In (q) (3) delete "of this chapter" in the last line. Under (s) (3) last line, add "and in good repair" between the words "clean" and "when."

963.70 (j) — Council recommended, in line six, to change "45d." to "50d." *Rejected*. 963.73 (c) (4) — In line three, delete "at the shipping plant before loading" and change to "after cleaning and before their next use." 963.79 (a)—Change, in line four, "30" to "20." 963.80—Recommended that it be made consistent with 963.14. 963.81—In line two change "sanitary" to "clean." 963.81(e)—Delete, in line four, "be kept free of evidence" and change to have; in the same line, add "controlled" after the word "rodents." 963-83 be kept consistent with 963.20, except for (e) and (f) parts of 963. 963.54 (1)—Insert the words "Grade A Pasteurized Milk Ordinance—1965—Recommendations of the Public Health Service." 963.85—Recommended that it be made consistent with 963.22 (a) in reference to recording thermometers.

Chairman Conner stated that Council I made two final recommendations: (1) revision of all inspection sheets

that was to be considered as consistent with the finally adopted and rewritten Ordinance to be in compliance with 962 and 963 that was under consideration; (2) the administrative procedures, where applicable, be included in the proposed recommended Ordinance in the same format as they appeared in the 1965 Pasteurized Milk Ordinance.

COUNCIL II

Jay Boosinger, Chairman, stated that Parts 960 and 961 were assigned to Council II. 960.1 (e) — Delete the words "and/or receiving stations" in the first line, and between 960.1 (e) and 960.1 (f) add the following definition: "A 'receiving station' is any place, premises, or establishment where raw milk is received, collected, handled, stored or cooled and prepared for further transportation." 960.1 (i) — add the word "processed" between the words "any" and "milk." Between 960.1 (1) and 960.1 (m) add a definition of "person" as found in the 1965 PMO.

Add a definition of "Certified Industry Personnel" after the previous definition. 960.1 (n) — Under the line "175d. F. — 25 seconds" add another line "185d.F. — 15 seconds" with a footnote if this proposed temperature and time relationship is not sufficient to provide proper pasteurization, then raise temperature so that proper pasteurization may be achieved at 15-sec time. 960.1 (q) — Add the definition for adulterated milk and milk products that appears in the 1965 PMO. Add the definition for misbranded milk and milk products from the 1965 PMO. 960.2(a)—Change the first four lines to read "No person shall cause to be delivered or shall sell, otherwise distribute or hold for sale or other distribution after shipment any milk or milk product that is not Grade A, if such milk or milk product." 960.2 (b) — Delete and replace with the following: "This requirement is not intended to include such products as sterilized milk and milk products hermetically sealed in a container and so processed, either before or after sealing, as to prevent microbial spoilage, evaporated milk, evaporated skim milk, condensed milk (sweetened or unsweetened), dietary products, infant formula, butter, ice cream and other frozen desserts, dry milk products (except as defined herein) or cheese except when they are combined with other substances to produce any pasteurized milk or milk products defined herein. These defined products may be labeled Grade A only if all milk-derived components are products which have been produced under the provisions of the Grade A Pasteurized Milk Ordinance." 960.2 (b) — Add paragraph (c) as in Council II's report to the delegates; add the paragraph regarding police jurisdiction. 960.3 — Delete (a) and (b) and add a Penalty Section as is currently in the 1965 PMO. 960.4 (a) — Delete the word "which" and add the final 960.1 of this document which---.960.4 — Council recommended deletion of paragraphs (b) and (c). Paragraphs 960.5, 6, 7, 9, 10, and 11 were considered procedural matters and should not be included in PMO for adoption.

960.12 (a) (1) — Change second sentence to read: "Permits shall not be transferable with respect to persons and/or locations except that the number may remain unchanged with permission of the regulatory agency. 960.12 (a) (2) — Add the following paragraph regarding reinstatement of permit. "Upon written application of any person whose permit has been suspended, or upon application within 48 h of any person who has been served with a notice of intention to suspend, and, in the latter case, before suspension, the health authority shall within 72 h proceed to a hearing to ascertain the facts of such violation or interference and upon evidence presented at such hearing shall affirm, modify, or rescind the suspension or intention to suspend." 960.12 (3) — After the word "sale" add the phrase "as Grade A" 960.12 (a) (i)—Delete the words "written application" and substitute the words "a request." 960.12 (a) (5) (ii) — In the first sentence delete the words "one week," and substitute the words "3 days;" delete the words "written application" and substitute the word "request." 960.12 (a) (5) (iii) — In the second sentence delete the words "1 week" and substitute the words "3 days;" delete the words "an application" and substitute the word "request." 960.12 (b) — Delete paragraph (b), (1), (2), and (3) on pages 40 and 41 and substitute the material as found on pages 7 and 8 of Council II minutes, with the amendment that in (2) in both the fourth and fifth lines the word "shall" be changed to "should." 960.12 (c) (6) — Change the word "forwarded" on line 5 to "available," and in line 8 change "annually" to "every 3 years."

960.12 (c) (7) (vi) — Change "1 year" to "3 years." 960.12 (c) (7) (vii) — On line 5 change "1 year" to "3 years" and add "except that recertification is allowed during routine inspection work" to the end of the last sentence. 960.12 (c) (7) (ix) — In the last sentence change "failing" to "failure" and delete "Part 962 of this chapter" and substitute "the Grade A farm sanitation requirements of the PMO." 960.12 (c) — Top of page 46, change to (d). 960.12 (d) (3) — Delete "961.5 of this chapter" and substitute "Section 6 of PMO." 960.12 (d) (4) — Delete "at least once every six months" and substitute "as the regulatory agency determines are necessary;" (d) (5) — Delete "should" and substitute "shall;" (d) (6) — Modify temperature to include both F and C temperature levels on page 47 and all other locations in the proposed ordinance; (d) (7) — Delete "three" and substitute "four," and add "single strip reticle method" to list of tests; (d) (8) — Delete "two" and substitute, "three" and add "optical somatic cell counter "latest edition of AOAC to list of tests; (d) — Add a new paragraph (9) as follows: "It shall be the responsibility of the milk hauler to collect a sample of milk from each bulk tank prior to his transferring milk from a farm bulk tank to a truck, tank, or other container. All samples shall be collected and held in compliance with the requirements of 960.12 (d) (6) and delivered to a milk plant, receiving station or transfer

station, or other locations approved by the regulatory agency. Change 960.12 (d) to 960.12 (e). 960.12 (e) (3) — Delete last sentence and substitute "Following any positive test, no milk shall be shipped from the milk producer source involved, unless and until it shall have been sampled and tested and thereby determined to be below actionable levels specified in Part 122 as applied to pesticides or free of antibiotic residue as specified by the methods in Part 960.12 (d) (6) (iv)."

961.1 (a) and (b) — Delete "Commissioner of the Food and Drug Administration" and substitute "Regulatory Agency." 961.2 — Delete the words "in interstate commerce." 961.3 — Add the words "at reasonable times" in line three following the word "facilities" and delete the word "tests" from the last sentence. 961.4 — Delete paragraph (a) (2). 961.4 (c) (ii) — Add the words "pasteurized for repasteurization" following pasteurized. 961.5 — Delete (a) (4); (b) delete entire paragraph 961.6 — Delete the entire paragraph and substitute the wording as reflected in Council II minutes *Rejected*. (c) — Modify the temperature standard to read "Cooled to 45d.F. within 2 hours after each milking and will not go above 50d.F. thereafter." Modify the bacterial limits to read: "Individual producer milk not to exceed 100,000 per ml prior to commingling with producer milk. Not exceeding 300,000 per ml as commingled milk prior to pasteurization." Delete all the antibiotic standard except "No detectable zone with the *Bacillus subtilis* method or equivalent." 961.6 (c) — Add the words "and those products to be repasteurized" following milk products in the items covered list. Modify the temperature standard by deleting "thereat" and adding "or stored at the appropriate temperature specified in Part 963." Modify the coliform limits by adding "Except that bulk shipments of pasteurized products for repasteurization shall not exceed 100 per ml." Add "*" following antibiotics; modify antibiotic standard to read "No detectable zone with the *Bacillus subtilis* method or equivalent."

At this time the delegates discussed extensively the temperature to be maintained in raw milk tanks. Delegate VanPatten finally made a motion as follows: "For the record it would be the intent of this Conference to have raw milk cooled to 45d.F. within 2 h after milking. The blend temperature of the milk in the farm tank must not exceed 50d.F. at any time during the addition of the second or subsequent milkings." Approved by the delegates.

The Council had several recommendations to FDA regarding surveys. 960.21 — Change to method of making survey to allow more latitude in grading with respect to enforcement rating that included two or more enforcement agencies. 960.20 — Add a new section C1: "Pasteurized milk rating is the arithmetic average of the sanitation compliance rating of raw milk for pasteurization and of the milk plants." 960.21 (2) — Add, after a survey has been completed, the method used to

randomly select the dairy farms surveyed shall be made available when requested. 960.21 (a) (4) (ii) — With respect to debiting bacterial counts, confirmed somatic cell counts or cooling temperatures, the Council made three recommendations, all of which were rejected. The following amended motion was approved: "If two of the last four samples are bad, but the last one is good, then he shall not be debited on the survey." Page 63, 960.22, replace with 960.22 (a) (iii), page 64: "The number of pounds of milk and milk products sold daily is needed for computing the rating and is entered in the appropriate place at the top of Form 2359 Milk Plant Inspection Report. When a deficiency in a plant affects the entire plant output, then it is recorded for use in computation of the plant sanitation compliance rating. Only violation of 963.22, 963.31, 963.32, 963.40 and 963.41 of this chapter are to receive partial debits. All other violations should be considered as affecting the entire product output of the plant." 960.24 — Require that the copy of the narrative report be sent to the enforcement agency.

Council recommended that FDA should be required to review Form 2359 Series to see if they could reduce the number of reports necessary; also that FDA should be requested to review the problem of several sources of milk going into one plant so that all the sources could be weighted for a combined compliance rating, and report back to the 1977 Conference.

COUNCIL III

Chairman Vaux called on Glenn Huskey, Chairman, to present the report of Council III. Chairman Huskey reported that Council III was assigned Parts 964, 965, 966, and 960 Subpart II.

964.20 — Strike all words under (a) and replace with "Source of boiler feed water. Boiler feed water shall be from a safe sanitary source approved by the regulatory agency." II Subpart D — Thermometer Specifications — It is recommended that when reference is made to the Fahrenheit (F) scale that the Centigrade (C) equivalent be listed there and should encompass the entire proposal document. 964.31 — Amend (b) to read "350d.F." rather than "300d.F." in last line. Revise 964.33 to coincide with the 1972 Memorandum MB-177 which changes spacing of thermometers in temperature scale divisions from 0.060 inch to 0.040 inch. 964.34 (g) — Amend to read "350d.F." instead of "300d.F." in last line. 964.35 (a) — Change to read "Type. Direct reading; contained in corrosion resistant case which protects against breakage and permits easy observation of the scale." All other language under (a) to be omitted. 964.36 (h) — Insert the words "not more than" between the word "in" and the figure "7" at the end of the first line. 964.37 (c) — Amend by inserting "to 180d.F." after 60d.F. and before the word "with" in the first line. 964.40 (b) — Amend by inserting the temperature "350d.F." in place of "300d.F." on page 347. A footnote should be made to

Part 964.40 (b) as follows: "The specifications for test thermometers should not be included in this document until FDA can determine availability and cost of such thermometers to satisfy demands of regulatory agencies." 964.50 (g) — A footnote should be added in reference to 964.50 (g) to request FDA to investigate with other federal agencies the requirement and acceptance of Section 964.50 (g) for test procedures up to 350d.F. 964.50 (f) change in its entirety. It is recommended that "HTST" be added after the word "Procedures" to apply to all pertinent sections, and that all reference to HTST or UHT be deleted. All reference to HTST which have been removed from (f) be included in a separate subpart approved as to format by FDA. Pending development of provisions for the operation and control of UHT systems and the inclusion of such provisions in a separate subpart approved as to format by FDA, each such system shall be recognized by FDA on a case-by-case basis. 964.56 — Change wording to read "in" rather than "on" pipeline throughout Section I. 965.2 — Insert the following words as opening sentence of first paragraph: "Water used for chemical sanitation shall be from a safe and sanitary source approved by the regulatory agency. 965.20 (A) PP 414 — Strike words "11th Ed." and insert words "current edition". 960.40, 960.41, 960.42 — Subpart D of Part 960, Evaluation of Milk Laboratories and Sampling Surveillance Programs, not be included in the proposed PMO.

The report of the Laboratory Committee was accepted in its entirety as presented to Council III. Council recommended to the Executive Board that appropriate steps be taken to determine if an asterisk should be placed after the state of New York in the Sanitation Compliance and Enforcement Ratings of Interstate Milk Shippers as provided for in Section VI, B, 3 of the Conference Procedures. Thus ended the Council reports.

There being no new business, Chairman Vaux asked Secretary McCaffrey for dates of 1977 meeting. The dates are May 22-26, 1977, at Stouffers Inn in downtown Cincinnati, Ohio.

NEW BOARD MEMBERS

Chairman Vaux asked the Nominating Committee to present their recommendations from Group I. Archie Hurst presented the report in the absence of Chairman Noles. Sam Rich was nominated to represent local health, E. Marion Causey, Jr., state health department, Jay Boosinger, state department of agriculture, and Donald Race, industry. William Arledge was nominated to complete the unexpired term of Burdet Heinemann in Group II. Chris Sykes was nominated from the floor to represent industry in Group I. In a written ballot, Don Race was declared the industry representative. The Secretary was directed to cast a unanimous ballot for each of the new Board members.

RESOLUTIONS

Chairman Vaux asked Don Race, Chairman, to present the deliberations of the Resolutions Committee.

Two resolutions were given.

(1) *Whereas*; a successful federal-state cooperative Interstate Milk Shippers Program has been in existence for more than 20 years, and

Whereas; the Federal Food and Drug Administration made available a Pre-Proposal draft of the Pasteurized Milk Ordinance for publication as a Federal Regulation, according to a notice published in the *Federal Register* dated May 5, 1975, and

Whereas; the N.C.I.M.S. was vitally concerned that such document was in fact being considered as a Federal Regulation, and

Whereas; the N.C.I.M.S., in its May, 1975 meeting in St. Louis, Missouri, issued a statement to the effect that such action is not compatible or in conformance with the IMS program, and

Whereas; representatives of NCIMS met with Dr. Sherwin Gardner, Deputy Commissioner, and other FDA officials on June 22, 1975, to convey the views and concern of the NCIMS, and

Whereas; officials of FDA have given indications that they are willing to consider alternatives other than publishing said document as a Federal Regulation,

Therefore be it resolved; that the NCIMS, meeting in St. Louis, Missouri on January 22, 1976, instruct its Board of Directors to present to the FDA for its consideration, the Memorandum of Understanding adopted at this meeting, which outlines the responsibilities of the States and FDA as they may relate to the IMS Program.

(2) *Whereas*; the Pre-Proposal draft of the "Grade A Milk Sanitation Regulations," notification of which was dated April 29, 1975, and published in the *Federal Register* on May 5, 1975, has been reviewed by the NCIMS, meeting in St. Louis, Missouri, January 18-22, 1976,

Therefore be it resolved; that the comments and recommendations are submitted to the FDA for the purpose of updating the 1965 Pasteurized Milk Ordinance and Code as a model ordinance for adoption by state and local government agencies and not as a Federal regulation.

The terminal adjournment of the non-terminally adjourned fifteenth National Conference on Interstate Milk Shipments occurred at 1:45 p.m. on Thursday, January 22, 1976.

EXECUTIVE BOARD MEETING, JANUARY 22, 1976

Chairman Vaux convened the meeting at 2:00 p.m. The first order of business was election of officers. H. H. Vaux was reelected Chairman, and Jay Boosinger was elected vice-chairman, both by unanimous ballot. J. C. McCaffrey was reappointed to the office of Executive Secretary-treasurer, at a salary of \$175.00 per month.

After considerable discussion the Board voted that Council Chairmen and Vice-Chairmen should remain in their present positions through the 1977 Cincinnati meeting. John Speer was reappointed Program

Chairman. It was pointed out that Bob Farst had been appointed Local Arrangements Chairman for the Cincinnati meeting during the Board meeting last May.

The next Board meeting will be held in conjunction with the meeting of the IAMFES at the Arlington Hilton Hotel, Arlington Heights, Illinois, at 8:30 a.m. on Monday, August 9, 1976. The Executive Secretary will make the necessary arrangements with the hotel and will so notify the Board.

Leland H. Lockhart was reappointed Chairman of the Single Service Container and Closure Committee; John C. Flake, Chairman of the Abnormal Milk Committee, and Kenneth Whaley, Chairman of the Laboratory Methods Committee.

Don Kimball replaced Raleigh Richard on Council I; Bill Trobaugh replaced Larry Christiansen on Council II.

The meeting was adjourned at 2:35 p.m.

L. L. McKay Honored with Pfizer Award in Cheese Research



Dr. Larry L. McKay, recipient of the 1976 Pfizer Inc. Award was born in 1943 in Oregon City, Oregon. He obtained the B.A. degree with honors in medical microbiology from the University of Montana in 1965. During his senior year, as a National Science Foundation Undergraduate Research Participant, McKay examined the effect of age on macromolecular components in the yeast phase of a pathogenic fungus, *Blastomyces dermatitidis*. Professor McKay received the Ph.D. degree in Microbiology from Oregon State University in 1969. His dissertation dealt with mechanisms of lactose utilization by lactic acid streptococci: enzymatic and genetic analyses. In 1971, McKay was the

senior author of a review on lactose utilization by lactic acid bacteria, thus revealing his dynamic interest and growing authority on this subject.

During 1969-70, Dr. McKay conducted post-doctoral research at Michigan State and investigated control of acetate metabolism in *Bacillus popilliae* and attempted to achieve in vitro sporulation by this bacterium.

Since August, 1970 McKay has been a staff member in the Department of Food Science and Nutrition at the University of Minnesota where he is responsible for the research program on dairy and other food starter cultures. From the beginning, he has demonstrated excellence and great originality in his research. He has utilized concepts of molecular biology in his study of lactose metabolism in streptococci. Solving the problem of decreased acid production in starter cultures have proven to be very difficult and challenging. Through selection of appropriate techniques, Dr. McKay has been able to determine the presence and function of plasmids in lactic streptococci. The ability of these organisms to grow in milk and rapidly produce acid (a vital function in fermented dairy product manufacture) was found to be dependent on presence of certain plasmid DNA. Instability of the proteinase system was also related to their presence.

This knowledge should ultimately lead to isolation of the specific plasmid so that it may be transferred to slow acid producing variants of lactic streptococci to increase the number of rapid acid producing strains needed by the dairy industry.

McKay has also conducted a thorough study of the distribution of lysogenic phages in commercial starters and worked out the parameters for batch production of concentrated starters. Results of these experiments have been reported in the *Journal of Milk and Food Technology*. His present research activities include development of methodology for selection and isolation of new strains of lactic streptococci to be used by the dairy industry, physiologic and genetic control of acid and flavor production by lactic streptococci, production and control of the proteinase enzyme system of lactics, plasmids in dairy starter cultures, and the problem of lactic bacteriophages in the dairy industry. In addition, Dr. McKay has teaching responsibilities in several microbiology courses.

Professor McKay belongs to several honorary societies and professional organizations. He has over 20 scientific publications in six different journals. In addition, he is a frequent contributor of papers or invited speaker at scientific meetings.

E-3-A Sanitary Standards for Liquid Egg Products Cooling and Holding Tanks

Number E-1300

Formulated by

*International Association of Milk, Food and Environmental Sanitarians
 United States Department of Agriculture
 Poultry and Egg Institute of America
 Dairy and Food Industries Supply Association*

It is the purpose of the IAMFES, USDA, PEIA, 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. Specifications for Liquid Egg Products Cooling and Holding Tanks heretofore or hereafter developed which so differ in design, material, fabrication, or otherwise as not to conform with the following standards, but which in the fabricator's opinion are equivalent or better, may be submitted for the joint consideration of the IAMFES, USDA, PEIA and DFISA at any time.

A.

SCOPE

A.1

These standards cover the sanitary aspects of tanks in which liquid egg products are cooled and stored. They do not pertain to non-refrigerated storage tanks used in egg processing plants.

A.2

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

B.

DEFINITIONS

B.1

Product: Shall mean liquid egg product.

B.2

Liquid Egg Products Cooling and Holding Tank: Shall mean a cylindrical, rectangular, oval or other equally satisfactorily shaped tank.

B.3

Surfaces

B.3.1

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

B.3.2

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

B.4

Lining: Shall mean all surfaces used to contain the

product, including the ends, sides, bottom and top.

B.5

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

B.6

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

B.7

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

B.8

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

B.9

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

C.

MATERIALS

C.1

Product contact surfaces shall be of stainless steel of the AISI 300 series¹ or corresponding ACI² types (See Appendix, Section F.), or metal which under conditions of intended use is at least as corrosion resistant as stainless steel of the foregoing types and is non-toxic and non-absorbent, except that:

C.1.1

Rubber and rubber-like materials may be used for slingers, drip shields, agitator seals, agitator bearings, protective caps for sanitary tubes or fittings or vents, O-Rings, seals, gaskets and parts used in similar appli-

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

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

cations. These materials shall comply with the applicable provisions of the "E-3-A Sanitary Standards for Multiple-Use Rubber and Rubber-Like Materials Used as Product Contact Surfaces in Egg Processing Equipment, Number 1800."

C.1.2

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

C.1.3

Except for protective caps provided for in C.1.1 and C.1.2, sanitary fittings shall be made of materials provided for in the "E-3-A Sanitary Standards for Fittings Used on Egg and Egg Products Equipment and Used on Sanitary Lines Conducting Egg and Egg Products, Number E-0800."

C.1.4

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

C.1.5

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

C.2

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

C.3

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

D.

FABRICATION

D.1

Product contact surfaces shall be at least as smooth as a No. 4 finish on stainless steel sheets. (See Appendix, Section G.)

D.2

Permanent joints in product contact surfaces shall be welded except that rolled on sanitary pipeline ferrules or flanges may be used on connections beyond the

shell. Welded areas of product contact surfaces shall be at least as smooth as the adjoining surfaces.

D.3

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

D.4

Product contact surfaces shall be self-draining except for normal clingage. The lining shall be so constructed that it will not sag, buckle, or become distorted in normal use. Horizontal cylindrical tanks shall be so constructed that they will permit complete drainage of water when the tank has a pitch of not more than 1 inch in 100 inches.

D.4.1

If the tank is designed for mechanical cleaning, and has a flat bottom, the bottom shall pitch (1) at least ¼ inch per foot toward the outlet in a horizontal tank or (2) at least 3/4 inch per foot toward the outlet in a vertical tank.

D.5

Gaskets shall be removable. Any gasket groove or gasket retaining groove shall not exceed 1/4 inch in depth or be less than 1/4 inch wide except those for standard O-Rings smaller than 1/4 inch.

D.6

Internal angles of 135° or less on product contact surfaces shall have minimum radii of ½ inch, except that:

D.6.1

The minimum radii for accessories, appurtenances, or bridges that are welded to product contact surfaces shall be not less than 1/4 inch.

D.6.2

The minimum radii in agitator shaft bottom guide bearings and in gasket grooves or gasket retaining grooves other than those for standard 1/4 inch and smaller O-Rings shall be not less than 1/8 inch.

D.6.3

The minimum radii in grooves for standard 1/4 inch O-Rings shall be not less than 3/32 inch and for standard 1/8 inch O-Rings shall be not less than 1/32 inch.

D.6.4

The minimum radii of covers and agitator assemblies shall not be less than 1/4 inch.

D.7

There shall be no threads on product contact surfaces.

D.8

Sanitary fittings and connections shall conform with the applicable provisions of the "E-3-A Sanitary Standards for Fittings Used on Egg and Egg Products Equipment and Used on Sanitary Lines Conducting

Egg and Egg Products, Number E-0800," except that materials conforming to C.1.1 or C.1.2 may be used for caps of sanitary design for the protection of terminal ends of sanitary tubes, fittings or vents.

D.9

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

D.10

Covers

D.10.1

Main Covers for Open Top Type Tanks.

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

D.10.2

Non-removable Covers for Open Top Type Tanks.

Non-removable covers (1) shall be of a type that can be opened and maintained in an open position, (2) shall be designed to be self-draining when in the closed position, (3) shall be designed so that when the covers are in any open position liquid from the exterior surface will not drain onto the lining and (4) shall be designed so that when in their fully opened position, drops of condensation on the underside will not drain into the tank.

D.10.3

Bridges and Fixed Covers for Open Top Type Tanks.

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

D.10.4

Manhole Covers for Closed Type Tanks.

Covers for manholes in side walls and/or ends shall be of either the inside or outside swing type. If the cover swings inside, it shall also swing outside, away from the opening. Threads or ball joints employed to attach

the manhole cover(s) and its appendages shall not be located within the lining. Covers for manholes in the top of tanks shall be of the outside swing type or be of a removable type.

D.10.5

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

D.10.6

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

D.11

Openings

The edges of all openings into the lining that are upward or horizontal shall extend upward or outward at least 3/8 inch beyond the shell or be fitted with a permanently installed sanitary pipeline fitting.

D.11.1

The main openings of tanks shall be of sufficient number, adequate in size, and so located that all product contact surfaces are easily accessible and, except for the product contact surfaces of parts removable for cleaning, can be inspected visually without entering the tank.

D.11.2

An exception to the requirements of D.11.1 is made for closed top type tanks, having product contact surfaces that cannot be manually cleaned and inspected without entering the tank.

D.11.2.1

The minimum inside height of this type tank shall be 36 inches; and if the inside height exceeds 96 inches, means shall be provided (See Appendix, Section H) that will facilitate manual cleaning and inspection of all product contact surfaces or means shall be provided for mechanically cleaning the product contact surfaces of the tank and all non-removable appurtenances thereto. This type of tank shall have a manhole opening(s) complying with the provisions of D.11.5.

D.11.3

An inlet sanitary pipeline connection shall be at least 1½ inches or the inlet opening shall accommodate at least 1½ inch E-3-A Sanitary tubing.

D.11.4

Agitator openings: Agitator shaft openings through the bridge or top enclosure shall have a minimum diameter of 1 inch on tanks which require removal of the agitator shaft for cleaning or be of a diameter that will provide a 1 inch minimum annular cleaning space be-

tween the agitator shaft and the inside surface of the flanged opening on tanks which do not require removal of the agitator for cleaning.

D.11.5

Manhole openings: A manhole opening, if provided, shall be located at the outlet end or side of the tank or the top of the tank. The inside dimensions of the manhole opening shall not be less than 15 × 20 inches oval, 12 × 27 inches elliptical, or 18 inches diameter.

D.11.6

Sight and Light Openings: Sight and light openings, when provided, shall be of such design and construction that the inner surfaces drain inwardly; and if the tank is designed for mechanical cleaning, the inner surface of the glass (or plastic) shall be relatively flush with the inner surface of the lining. The inside diameter of the opening shall be at least 3¾ inches. The external flare of the opening shall be pitched so that liquid cannot accumulate.

D.11.7

Thermometer connections: A connection(s) or opening(s) which will accommodate a temperature sensing element(s) of a thermometer(s) shall be provided. The connection(s) and/or openings shall be located in the top enclosure, cover, bridge or through an end or sidewall. Thermometer wells may be used. The bulb of the temperature sensing element shall be located so as to permit registering the product temperature when the tank contains no more product than 20 percent of its capacity and shall be located so that the sensing element is not influenced by the cooling medium. All connections and/or openings shall conform to one of the following:

D.11.7.1

The applicable fittings found in the "E-3-A Sanitary Standards for Instrument Fittings and Connections Used on Egg Products Equipment, Number E-0900," as amended and supplements thereto.

D.11.7.2

Fittings for temperature sensing devices which do not pierce the tank lining, but which have temperature sensing element receptacles securely attached to the exterior of the lining.

D.12

Outlet: The outlet shall provide complete drainage of the tank. The outside diameter of the outlet passage shall conform to that of E-3-A sanitary tubing and shall not be less than that of 2 inch tubing. The wall thickness of the outlet passage shall be no greater than 1/8 inch. The terminal end of the outlet passage shall have a rolled-on or welded sanitary pipeline ferrule or flange. The ferrule or flange shall not be below the bottom of the shell. The distance between the nearest point on the shell to the face of the ferrule or flange on the terminal end of a horizontal type outlet shall not be more than the smaller of (1) twice the nominal diameter of the outlet passage or (2) five inches. The outlet shall be one of the following types:

D.12.1

Horizontal type. The bottom of the outlet passage shall be at least as low as the low point of the lining at the outlet. The outlet passage shall be pitched downward toward the terminal end.

D.12.2

Vertical type. The vertical centerline of the outlet passage shall be as close as practical to a side wall of the tank. The outlet passage shall be a generally horizontal extension of an elbow which is a part of or is welded to the lining. The outlet passage shall not pass through the bottom of the shell if product will be held in the passage.

D.13

Outlet valves: Valves, when provided, shall conform to D.8 or if the valve is within the lining or in the outlet passage, and the seat is an integral part of the lining or the outlet passage, a compression-type valve conforming to the applicable provisions of D.13.1 may be used. A cap conforming to D.8 shall be provided for the outlet end of valves furnished with tanks.

D.13.1

Compression-type valve in the tank or outlet passage. This type of valve shall have a metal to metal or rubber or rubber-like material to metal seat. The rubber or rubber-like material may be either removable or bonded. The handle or valve operating rod shall extend through the bridge or the handle shall be outside the shell. If the handle or valve operating level extends through the bridge, it shall have a permanently attached shield to protect against the entrance of contaminants into the tank through the space around the handle or valve operating lever.

D.14

Agitators: Means for mechanical agitation of product shall be provided that when operated intermittently or continuously shall be sufficient to maintain uniformity of product throughout the tank. Agitators, if not designed for mechanical cleaning, shall be readily accessible for manual cleaning and inspection either in an assembled position or when removed. A seal for the agitator shaft, if provided, shall be of a packless type, sanitary in design and durable with all parts readily accessible for cleaning. A sanitary seal for the agitator shaft shall be provided for (1) a horizontal agitator, and (2) a vertical agitator when it is specified that the tank is to be located so that the portion of the shaft outside the tank is not in a processing area. The means for agitation shall be one of the following:

D.14.1

Mechanical, top entering, non-removable type. There shall be at least a 1-inch space between the non-removable agitator and the bottom of the lining, unless the agitator is mounted on a hinged-type cover. A bottom shaft bearing shall not be provided for a non-removable type agitator.

D.14.2

Mechanical, top entering, removable type.

This type of agitator shall be provided with an easily accessible, readily demountable coupling of either a sanitary type located within the lining or a coupling located outside of the lining provided that it is above the shield provided to protect the annular space around the shaft. All product contact surfaces of the agitator shall be visible when the agitator is removed. A bottom support or guide, if used, shall be welded to the lining, shall not interfere with drainage of the tank and the inside angles shall have minimum radii of 1/8 inch. When the agitator shaft has a bearing cavity, the diameter of the cavity shall be greater than the depth. The agitator shall be easily demountable for cleaning of the bearing and any shaft cavity.

D.14.3

Mechanical side entering type.

This type of agitator and shaft and its complete seal shall be readily demountable for manual cleaning. Nonremovable parts having product contact surfaces shall be designed so that the product contact surfaces are readily cleanable from the inside of the tank.

D.15

Air Under Pressure: Equipment and means for applying air under pressure for movement of product, when provided, shall conform to the applicable provisions of the "E-3-A Accepted Practices for Supplying Air Under Pressure in Contact with Liquid Eggs and Egg Products and Product Contact Surfaces, Number E-60400."

D.16

Mechanical Agitator Driving Mechanism Mounting: The driving mechanism when above the lining shall be securely mounted in a position that will provide a minimum distance of 4 inches measured vertically downward from the bottom of the driving mechanism housing, excluding bearing bosses and mounting bosses, to the nearest surface of the tank; and in such a manner that all surfaces of the tank under or adjacent to the driving mechanism shall be readily accessible for cleaning and inspection.

D.17

Thermometers: Each tank shall be provided with an indicating thermometer and/or a recording thermometer complying with the applicable specifications for indicating and recording thermometers in Appendix Section I. The thermometer or the temperature sensing element of the thermometer shall fit one of the connections or openings provided for in D.11.7.1 and D.11.7.2.

D.18

Vents: A vent(s), if provided, (1) shall be so designed to protect against entrance of contaminants into the tank, (2) shall be so designed that parts are readily removable and readily accessible for cleaning, (3) shall have sufficient free opening area to prevent back pressure during filling and to prevent vacuum during emptying of the tank, (4) shall be in the front head near the top of the tank or in the top of the tank or in a top

manhole cover, (5) shall terminate in a processing area, (6) shall be provided with a perforated cover having openings not greater than 1/16 inch diameter, or slots not more than 1/32 inch wide, and (7) woven wire mesh shall not be used for this purpose.

D.19

Cleaning: Tanks having an inside height of more than 96 inches shall be provided with means (see Appendix, Section H.) that will facilitate manual cleaning and inspection of all product contact surfaces or means shall be provided for mechanically cleaning the product contact surfaces of the tank and all non-removable appurtenances thereto.

D.20

Sample Cock: A sample cock, if provided, shall be of a type that has its sealing surface relatively flush with the product contact surface of the tank and have an inside diameter no less than that of 1 inch E-3-A sanitary tubing.

D.21

Tank Supports: The means of supporting a tank designed to be installed wholly within a processing area or the means of supporting the portion of a tank that will be in a processing area shall be one of the following:

D.21.1

With legs: Legs shall be of sufficient number and strength and so spaced that the filled tank will be adequately supported. Legs shall be smooth, have no exposed threads and if made of hollow stock shall be sealed. Legs that are not to be cemented into the floor shall have rounded ends. Legs shall be such that will provide (1) the minimum distance between lowest interior surface of the outlet connection and the floor will be 4 inches and (2) a minimum clearance of 6 inches between the floor and the bottom of a tank 72 inches or less in diameter or width, except in the case of a V-bottom or a rounded bottom tank of which the outer shell slopes continually upward from the outlet centerline, in which case the minimum clearance may be 4 inches if it increases to 6 inches with a horizontal distance of not more than 12 inches on each side of this centerline. On a tank more than 72 inches in diameter or width, the minimum clearance shall be 8 inches.

D.21.2

Mounted on a Slab or Island: The base of the tank shall be such that it may be sealed to the mounting surface. (See Appendix, Section J)

D.22

Prevention of a Significant Product Temperature Increase:

D.22.1

The tank shall be capable of preventing, in 12 hours, a product temperature increase greater than 5° F in a tank filled to 100 percent of its capacity with product when there is a difference of 50° F between the ambient and the average temperature of the product in the tank. For test purposes, water may be substituted

for product.

D.22.2

Insulation material, if provided, shall be of a nature and installed in a manner that will prevent shifting or settling.

D.23

A direct reading gauge of the glass or plastic tube type, if provided, shall be sanitary in design and construction and shall be readily accessible for cleaning or shall be designed for mechanical cleaning. If designed for mechanical cleaning, the inside diameter of the gauge parts shall be sufficiently uniform that all product contact surfaces will be cleaned.

It shall be designed and constructed so that all product in the gauge may be discarded. Means to accomplish this shall be provided at the lowest point and in such a manner that product in the gauge will not enter the tank outlet line nor re-enter the tank.

The valve shall be close coupled. The distance, measured along the passage for the product in the tank to the gauge valve, from the nearest point on the shell to the ferrule or flange for the valve shall not be more than the smaller of (1) twice the nominal diameter of the passage or (2) five inches.

D.24

Non-Product Contact Surfaces: Non-product contact surfaces shall comply with the following:

D.24.1

They shall be smooth, free of pockets and crevices and be readily cleanable.

D.24.2

Surfaces to be coated shall be effectively prepared for coating.

D.24.3

The shell shall be effectively sealed against moisture and vermin at all joints and at junctions with the breast, manhole openings, outlets and other openings.

D.24.4

A vent or weep hole may be provided in the shell. If provided, it shall be located in a position that will provide drainage from the shell and shall be vermin proof.

D.24.5

Outside welds need not be ground.

E.

Cooling

E.1

Cooling Requirements

A tank shall have enough refrigerated surface to cool one or more products through the temperature range within the time specified on the data plate in E.2.

E.2

Cooling information

The tank shall have an information or data plate permanently attached to it giving the following information or the information shall appear on the name plate. (See Appendix, Section K).

<p>This tank is designed to cool* _____ from ** _____° F to *** _____° F within _____ hours after the tank is filled. The minimum capacity of the refrigeration system to accomplish this is _____ BTU/hour.</p>
--

If the tank is designed to use a direct expansion condensing unit to furnish the refrigeration, the second sentence of the information required in the above data or information plate shall be replaced by the following:

The minimum capacity of the condensing unit to accomplish this is *** _____ BTU/hour

**** _____° F saturated suction temperature.

*The name of the product

**The initial product temperature

***Cooled product temperature

****The BTU capacity specified is to be at the saturated suction temperature designated by the manufacturer.

E.3

Cooling System

E.3.1

If the tank is designed to use an integral direct expansion condensing unit, in determining cooling capacity, the ambient temperature shall be 90° F and when water cooled condensers are used, the refrigerant condensing temperature shall be not less than 103° F.

E.3.2

If the tank is designed to use an integral direct expansion condensing unit, the tank shall be provided with an automatic refrigeration control capable of functioning on a change in product temperature of not more than plus or minus 2° F at 40° F.

APPENDIX

F.

STAINLESS STEEL MATERIALS

Stainless steel conforming to the applicable composition ranges established by AISI¹ for wrought products, or by ACI² for cast products, should be considered in compliance with the requirements of Section C.1 herein. Where welding is involved, the carbon content of the stainless steel should not exceed 0.08 percent. The first reference cited in C.1 sets forth the chemical ranges and limits of acceptable stainless steels of the 300 series. Cast grades of stainless steel corresponding to types 303, 304, and 316, are designated CF-16F, CF-8, and CF-8M, respectively. These cast grades are covered by ASTM³ specifications A296-68 and A351-70.

G.

PRODUCT CONTACT SURFACE FINISH

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

¹Available from American Society for Testing and Materials, 1916 Race Street, Philadelphia, PA 19103.

H.

MANUAL CLEANING

If the inside height of a tank exceeds 96 inches, one means for manual cleaning is to weld a sanitary stainless steel rung on each end of the tank to support a removable platform at a height which will facilitate cleaning and inspection.

I.

THERMOMETERS

I.1

Indicating Thermometers

Scale Range.—Shall have a span not less than 50° F including normal cooling temperatures plus or minus 5.0° F with extension of scale on either side permitted; graduated in not more than 2.0° F divisions.

Temperature Scale Divisions.—Spaced not less than one-sixteenth of an inch apart between 35° F and 75° F.

Accuracy.—Within 2° F plus or minus, throughout the specified scale range.

Stem Fitting.—Shall conform to the “E-3-A Sanitary Standards for Instrument Fittings and Connections Used on Egg Products Equipment, Number E-0900,” as amended and supplements thereto or shall be a stem fitting that does not pierce the lining or means shall be provided to permit securely fastening the temperature sensing element to the outer surface of the lining.

I.2

Recording Thermometers

Case.—Moistureproof under operating conditions in a processing area.

Scale.—Shall have a scale span of not less than 50° F, including normal cooling temperature plus or minus 5° F, graduated in not more than 2° F divisions with not more than 40° F per inch of scale; graduated in time scale divisions of not more than 1 hours having a chord or straight line length of not less than one-eighth of an inch at 40° F. Chart must be capable of

recording temperatures up to 180° F. (Span specifications do not apply to extensions beyond 100° F).

Temperature Accuracy.—Within 2° F plus or minus, between specified range limits.

Pen-Arm Setting Device.—Easily accessible; simple to adjust.

Pen and Chart Paper.—Designed to give line not over one-fortieth of an inch thick when in proper adjustment; easy to maintain.

Temperature Sensor.—Protected against damage at 212° F.

Stem Fitting.—Shall conform to the “E-3-A Sanitary Standards for Instrument Fittings and Connections Used on Egg Products Equipment, Number E-0900,” as amended and supplements thereto or shall be a stem fitting that does not pierce the lining or means shall be provided to permit securely fastening the temperature sensing element to the outer surface of the lining.

Chart Speed.—The circular chart shall make one revolution in not more than seven days and shall be graduated for a maximum record of seven days. Strip chart shall move not less than 1 inch per hour and may be used continuously for 1 calendar month.

J.

SLABS OR ISLANDS

When a tank is designed to be installed on a slab or an island, the dimensions of the slab or island should be such that the tank will extend beyond the slab or island at least one inch in all horizontal directions. The slab or island should be of sufficient height so that the bottom of the outlet connection is not less than 4 inches above the floor. The surface of the slab or island should be coated with a thick layer of waterproof mastic material, which will harden without cracking. The junction of the outer shell of the tank and the slab or island should be sealed.

K.

PRODUCT COOLING TEMPERATURES

Unpasteurized product temperature within 2 hours from time of breaking					
Product	Liquid (other than salt product) to be held 8 hours or less	Liquid (other than salt product) to be held in excess of 8 hours	Liquid salt product	Temperature within 2 hours after pasteurization	Temperature within 3 hours after stabilization
Whites (not to be stabilized).	55° F. or lower	45° F. or lower		45° F. or lower	
Whites (to be stabilized).	70° F. or lower	55° F. or lower		55° F. or lower	
All other product (except product with 10 percent or more salt added).	45° F. or lower	40° F. or lower		If to be held 8 hours or less 45° F. or lower. If to be held in excess of 8 hours, 40° F. or lower.	If to be held 8 hours or less, 45° F. or lower. If to be held in excess of 8 hours, 40° F. or lower.
Liquid egg Product with 10 percent or more salt added.			If to be held 30 hours or less, 65° F or lower. If to be held in excess of 30 hours, 45° F or lower.	65° F or lower	

K.1

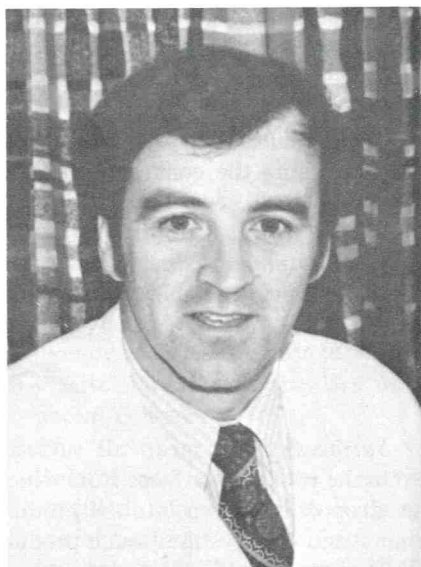
The previous table gives the minimum cooling and temperature requirements for liquid egg products found in the Regulations Governing the Inspection of Eggs and Egg Products⁴, Code of Federal Regulations⁵ Title 7, Chapter 1, Part 59, Section 59.530.

⁴Copies of the Regulation Governing the Inspection of Eggs and Egg Products are available without charge from the Standardization Branch Poultry Division, Agricultural Marketing Service, U.S. Department of Agriculture, Washington, D.C. 20250.

⁵The Code of Federal Regulations, published annually, is for sale by the Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402.

These standards are effective September 22, 1976.

Borden Award Goes to J. E. Kinsella



This year's Borden Award recipient, Dr. J. E. Kinsella, was selected from numerous outstanding nominees. His record reveals a high degree of professional versatility and diverse research interests. He is the author or co-author of more than 100 papers published in reputable scientific journals during the past 10 years. His papers are characterized by extensive literature reviews in which the implications of his own and other data are pointed out. They cover his work dealing with biochemistry of cells, tissues and organs, animal physiology, and

nutrition and technology of food systems.

Employing cell cultures, tissue homogenates and enzyme studies, Kinsella, his colleagues, and students have provided the most advanced insight yet attained regarding lipid synthesis and metabolism within the secreting mammary cell. He has done an outstanding job of interpreting and communicating the practical meaningfulness of research to consumers, processors, and producers. Among his publications are a number of excellent review articles and papers in such applied journals as the *Journal of Milk and Food Technology* covering the flavor potential of milk fat, the importance and synthesis of milk lipids, and functional chemistry of milk products in candy, chocolate manufacture, butter flavor, and cheese. Based on his research, new and improved ways of processing dairy products for use in candies, baked goods, and other food systems have been developed.

Dr. Kinsella's ability to attract financial support for his extensive research program, including a sizeable amount of grant support, have led to a large graduate training program from which 17 M.S. and Ph.D. dissertations have resulted

since 1969. Also eight post doctoral fellows have studied with him during the past 4 years.

In addition to his research activities, Kinsella has developed and teaches three courses, Fats and Oils Technology, Food Biochemistry, and Lipid Chemistry. He also participates in team teaching of Food Chemistry and Dairy Chemistry courses.

This outstanding scientist was born in Wexford, Ireland on February 22, 1938. He earned the B.S. degree at the National Institute of Ireland in 1961. He then taught Biology and Latin in Nigeria for 2 years before going to Pennsylvania State University where he earned the M.S. degree in 1965 and the Ph.D. degree in 1967. Kinsella joined the faculty of the Department of Food Science at Cornell University as assistant professor in 1967 and advanced to his present position as associate professor in 1973.

He is a member of numerous professional societies and has served on many important university, national, and international committees including the ADSA Milk Synthesis Committee and Kinsella served briefly as Associate Editor of the *Journal of Dairy Science*.

E-3-A Sanitary Standards for Fillers and Sealers of Single Service Containers for Liquid Egg Products

Number E-1700

Formulated by
International Association of Milk, Food and Environmental Sanitarians
United States Department of Agriculture
Poultry and Egg Institute of America
Dairy and Food Industries Supply Association

It is the purpose of the IAMFES, USDA, PEIA 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. Specifications for Fillers and Sealers of Single Service Containers for Liquid Egg Products heretofore and hereafter developed which so differ in design, material, fabrication, or otherwise as not to conform with the following standards, but which, in the fabricator's opinion, are equivalent or better, may be submitted for the joint consideration of the IAMFES, USDA, PEIA and DFISA at any time.

A.

SCOPE

A.1

These standards cover the sanitary aspects of equipment for performing all or a part of the following functions: mechanically opening, filling, and sealing single service containers and all parts which are essential to these functions that are furnished by the filler and sealer manufacturer. It does not pertain to other integral equipment embodied on certain machines which perform such functions as container fabricating; nor to the single service container.

A.2

In order to conform with these E-3-A Sanitary Standards, fillers and sealers of single service containers shall comply with the following design, material, and fabrication criteria that are applicable.

B.

DEFINITIONS

B.1

Product: Shall mean the liquid egg product which is filled into the container.

B.2

Container: Shall mean a single service package which is to be filled with the product.

B.3

Mechanical Opening Equipment: Shall mean the equipment for opening a container without manual

contact with any product contact surface of the container.

B.4

Mechanical Filling Equipment: Shall mean the equipment for mechanically filling the container with the product.

B.5

Mechanical Sealing Equipment: Shall mean the equipment for mechanically closing and/or sealing the filled container.

B.6

Surfaces:

B.6.1

Product Contact Surfaces: Shall mean all surfaces which are exposed to the product, surfaces from which liquids may drain, drop or be drawn into the product or into the containers, and surfaces that touch product contact surfaces of the container.

B.6.2

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

B.7

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

B.8

Engineering Plating: Shall mean plated to specific dimensions or processed to specified dimensions after plating.¹

C.

MATERIALS

C.1

Product contact surfaces shall be of stainless steel of

¹QQ-C-320a Federal Specification for Chromium Plating (Electrodeposited), July 26, 1954. Available from: General Services Administration, Seventh and D Streets NW, Room 1643, Washington, D.C.

QQ-N-290 Federal Specification for Nickel Plating (Electrodeposited), April 5, 1954, and Amendment 1, December 13, 1961. Available from: General Services Administration, Seventh and D Streets NW, Room 1643, Washington, D.C.

the AISI 300 series² or corresponding ACI³ types (See Appendix, Section E.), or metal which under conditions of intended use is at least as corrosion resistant as stainless steel of the foregoing types and is non-toxic and non-absorbent, except that:

C.1.1

Optional metal alloy may be used but only in applications requiring disassembly and manual cleaning. (See Appendix, Section F. for the composition of an acceptable optional metal alloy).

C.1.2

Those surfaces of container opening, closing and sealing devices which touch the product contact surfaces of the container or from which liquids may drain or drop into the container may be made of a non-toxic, non-absorbent metal that is corrosion resistant under conditions of intended use or may be made of metal made corrosion-resistant and wear-resistant by a covering of an engineering plating of chromium or nickel or an equally corrosion and wear-resistant non-toxic metal.

C.1.3

The valve plug of compression-type valves may be covered with rubber or rubber-like materials or plastic materials. Rubber or rubber-like materials and plastic materials used as a coating shall be of such composition as to retain their surface and conformation characteristics under conditions encountered in the environment of intended use and in cleaning and bactericidal treatment.

C.1.4

Rubber or rubber-like materials or plastic materials may be used for filling nozzles, plungers, bonded or removable gaskets, diaphragms, sealing rings, rollers, belts, drip shields, protective caps for sanitary connections, container opening and closing parts, filling valve members, seals and parts used in similar applications. Plastic materials may be used for short flexible transparent connectors.

C.1.5

Rubber and rubber-like materials when used for specified applications shall comply with the applicable provisions of the "E-3-A Sanitary Standards for Multiple-Use Rubber and Rubber-Like Materials Used as Product Contact Surfaces in Egg Processing Equipment, Number 1800."

C.1.6

Plastic materials when used for specified applications shall comply with the applicable provisions of the "3-A Sanitary Standards for Multiple-Use Plastic Materials Used as Product Contact Surfaces for Dairy Equipment, Number 20-00," as amended.

C.1.7

Silver solder material shall be non-toxic and corrosion

resistant.

C.1.8

Single service gaskets of a sanitary type may be used.

C.2

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

D.

FABRICATION

D.1

Product contact surfaces shall be at least as smooth as a No. 4 finish on stainless steel sheets. (See Appendix, Section G.)

D.2

Permanent joints in product contact surfaces shall be welded or may be silver soldered if welding is not feasible. An exception is made to the foregoing for product connections which may have rolled-on sanitary pipeline ferrules or flanges. Welded or silver soldered areas of product contact surfaces shall be at least as smooth as the adjoining surfaces.

D.3

The minimum thickness of engineering plating shall be 0.0002-inch for all product contact surfaces except that when the parts listed in C.1.2 that are to be plated are other than stainless steel, the minimum thickness of the engineering plating shall be 0.002-inch.

D.4

Product contact surfaces shall be easily accessible, visible, and readily cleanable, either when in an assembled position or when removed. Removable parts shall be readily demountable. Fillers designed to be mechanically cleaned shall be accessible for manual cleaning and inspection.

D.5

Product contact surfaces shall be self-draining or self-purging except for normal clingage. The bottom of the filler bowl shall have a minimum pitch of 1/8 inch per foot toward the plane of the outlets.

D.6

The filler bowl shall be equipped with a cover having a drop-flange which overlaps the rim of the bowl by at least 3/8 inch. The edges of all openings in the bowl cover shall extend upward at least 3/8 inch or be fitted with a permanently attached sanitary pipeline connection conforming to D.13. Openings in the bowl cover, except those fitted with a permanently installed sanitary pipeline connection, shall be provided with covers having a downward flange of not less than 1/4 inch so designed as to prevent liquid from entering the filler bowl. Covers shall be self-draining.

D.7

The filling equipment shall be so designed that adjustments necessary during the operation may be made

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

³*Alloy Casting Institute Division, Steel Founders' Society of America, 20611 Center Ridge Road, Rocky River, OH 44116.*

- without raising or removing the filler bowl cover(s).
- D.8 Rubber or rubber-like materials and plastic materials having product contact surfaces that are a covering or a gasket to be bonded shall be bonded in such a manner that the bond is continuous and mechanically sound, and so that when exposed to the conditions encountered in the environment of intended use and in cleaning and bactericidal treatment, the rubber and rubber-like material or the plastic material does not separate from the base material. The final bond shall conform to the criteria in C.1.5 or C.1.6.
- D.9 Gasket grooves or gasket retaining grooves shall not exceed 1/4 inch in depth or be less than 1/4 inch wide except those for standard O-Rings smaller than 1/4 inch.
- D.10 Internal angles of 135° or less on product contact surfaces shall have minimum radii of 1/4 inch, except that:
- D.10.1 Where smaller radii are required for essential functional reasons, such as those in filler nozzles. In no case shall such radii be less than 1/32 inch.
- D.10.2 The minimum radii in gasket grooves or gasket retaining grooves other than those for standard 1/4 inch and smaller O-Rings shall be not less than 1/8 inch.
- D.10.3 The minimum radii in grooves for standard 1/4 inch O-Rings shall be not less than 3/32 inch and for standard 1/8 inch O-Rings shall be not less than 1/32 inch.
- D.11 Shields or guards shall be provided and shall be so designed and located to prevent liquid or other contaminants from draining or dropping into the container or product, or onto product contact surfaces.
- D.12 There shall be no threads on product contact surfaces.
- D.13 Sanitary fittings and connections shall conform with the applicable provisions of the "E-3-A Sanitary Standards for Fittings Used on Egg and Egg Products Equipment and Used on Sanitary Lines Conducting Egg and Egg Products, Number E-0800," except that sanitary fittings made of optional metal alloy shall not be used if the filler is designed for mechanical cleaning.
- D.14 Coil springs having product contact surfaces shall have at least 3/32 inch openings between coils including the ends when the spring is in a free position. Coil springs shall be readily accessible for cleaning and inspection.
- D.15 The filler shall be mounted on legs or casters that will provide a clearance between the lowest fixed point on the filler and the floor of at least 4 inches when the base outlines an area in which no point is more than 12½ inches from the nearest edge, or a clearance of at least 6 inches when any point is more than 12½ inches from the nearest edge.
- D.15.1 Legs, if provided, shall be smooth with rounded ends and have no exposed threads. Legs made of hollow stock shall be sealed.
- D.15.2 Casters, if provided, shall be durable and of a size that will permit easy movement of the filler.
- D.16 Any guard(s) required by a safety standard that will not permit accessibility for cleaning and inspection, shall be designed so it (they) can be removed without tools.
- D.17 Non-product contact surfaces shall be smooth, free of pockets and crevices and be readily cleanable and those to be coated shall be effectively prepared for coating.
- D.18 A defoamer system, if provided, shall comply with the applicable parts of the following:
- D.18.1 Steam defoamer systems shall be provided with a suitable self-draining water condensation trap and strainer on the steam supply line just prior to the defoamer head. The defoamer head shall be constructed in conformance with D.4. (See Appendix, Section H. for suggested design of water condensation trap and strainer and recommendations).
- D.18.2 A vacuum system designed to return foam continuously to the filler bowl. In this type, surfaces from which foam may drain, drop or be drawn into the product shall be constructed in conformance with D.4. Surfaces of blower or vacuum lines subject to contact with foam shall be constructed in such a manner as to be readily accessible for cleaning. (See Appendix, Section I. for suggested design).
- D.18.3 A vacuum system designed not to return foam to the filler bowl. In this type, surfaces from which foam may drain, drop or be drawn into the product or the sanitary container shall conform with D.4. Surfaces of blower or vacuum lines subject to contact with foam shall be constructed in such a manner as to be readily accessible for cleaning. (See Appendix, Section J. for suggested design and recommended operation).
- D.19 Fillers shall have an information plate in juxtaposition to the name plate containing a statement that the filler *is* or *is not* designed for mechanical cleaning or the statement shall appear on the name plate.

APPENDIX

E.

STAINLESS STEEL MATERIALS

Stainless steel conforming to the applicable composition ranges established by AISI² for wrought products, or by ACI³ 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 first reference cited in C.1 sets forth the chemical ranges and limits of acceptable stainless steels of the 300 series. Cast grades of stainless steel corresponding to types 303, 304, and 316 are designated CF-16F, CF-8 and CF-8M, respectively. These cast grades are covered by ASTM⁴ specifications A 296-68 and A 351-70.

F.

OPTIONAL METAL ALLOY

⁴Available from American Society for Testing and Materials, 1916 Race Street, Philadelphia, PA 19103.

An optional metal alloy having the following minimum and maximum composition is deemed to be in compliance with C.1.1 herein.

Zinc	8% maximum
Nickel	19½% minimum
Tin	3½% minimum
Lead	5% maximum
Iron	1½% maximum
Copper	the balance

An alloy of the composition given above is properly designated "nickel silver," or according to ASTM⁴ Specification B 149-70, may be entitled, "leaded nickel bronze."

G.

PRODUCT CONTACT SURFACE FINISH

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

These standards are effective September 22, 1976.

Drawings are available in the reprint of this Standard.

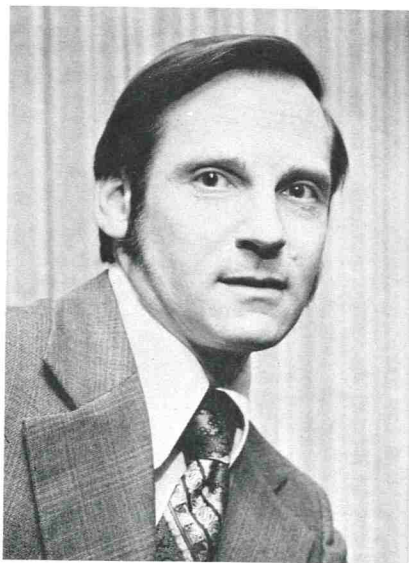
News and Events
Calendar of Events

January 12-13, 1977. DAIRY PROCESSORS CONFERENCE. Quality Inn Motel, Madison, Wisconsin. Sponsored by the Food Science Department, University of Wisconsin-Madison.

February 9-10, 1977. DAIRY INDUSTRY CONFERENCE. Center for Tomorrow, Ohio State University, Columbus, Ohio. For information contact: John Lindamood, Dept. of Food Science and Nutrition, 2121 Fyffe Road, Ohio State University, Columbus, OH 43210.

February 13-16, 1977. INTERNATIONAL EXPOSITION FOR FOOD PROCESSORS. Superdome, New Orleans, Louisiana. Sponsored by the Food Processing Machiner and Supplies Association, 7758 Wisconsin Avenue, Washington, DC 20014.

March 21-25, 1977. MID-WEST WORKSHOP IN MILK AND FOOD SANITATION. Center for Tomorrow, Ohio State University, Columbus. For information contact: John Lindamood, Dept. of Food Science and Nutrition, 2121 Fyffe Road, Ohio State University, Columbus, OH 43210.

Clark Named Executive Director

Warren S. Clark, Jr., Assistant Executive Director, has been named to succeed John T. Walsh as Executive Director of the American Dry Milk Institute and the Whey Products Institute, effective July 1, 1976.

Clark is a native of Connecticut and received his BS degree with honors and distinction in dairy

manufacturing from the University of Connecticut. Following a tour of military service, he earned MS and PhD degrees from Iowa State University in dairy microbiology and human nutrition.

Before joining the staff of the American Dry Milk Institute in 1967, Clark served on the faculty at Iowa State University and was employed in industry. He previously served both the American Dry Milk Institute and the Whey Products Institute in the capacity of Director of Research. In that capacity he was responsible for the development of product specifications and quality standards and has represented the dry milk and whey processing industries in various aspects of government relations. Additionally, he serves on the Intersociety Council of the American Public Health Association which publishes "Standard Methods for the Examination of Dairy Products" and is Vice-Chairman of the Board of Trustees, 3-A Sanitary Standards Symbol Administrative Council.

Clark is a member of many professional and honorary societies and is listed in American Men of Science and Who's Who In America.

News and Events

Technicon Instruments to Hold International Congress

Technicon Instruments Corporation, Tarrytown, New York, has announced that the 7th Technicon International Congress will be held December 13-15, 1976 at the New York Hilton in New York City.

Technicon Industrial Systems, a division of Technicon Instruments Corporation, will play a major role in the Congress with a total of 14 separate sessions over the 3-day period for which more than 50 papers are anticipated. In addition, Technicon will have on display, for the first time, a number of new systems for industrial analyses, some, if not all of which, are expected to be pertinent to the food and agriculture industries.

Four sessions over a two day period will be held under the theme, "Automated Instrumental Analysis and the Food Supply." Recognizing the growing concern over world food shortages, as well as the need for improved nutritional value in foods, two sessions will be devoted to advanced analytical techniques being employed to cope with these problems. They will be held December 14th, with the morning session covering the Automated Analysis of Soils/Fertilizers/Plants/Feeds, and the afternoon session devoted to Product Process and Quality Control.

Additionally, two sessions will be held December 13th, covering advances made in the relatively new field of Infrared Reflectance Analysis and its impact on commodity trading and processing.

The Technicon InfraAlyzer Infrared Reflectance Unit will be displayed and demonstrated together with the latest development in infrared reflectance analysis, the Technicon InfraAlyzer Plus Unit, which adds computer capability, fulfilling the need for a reliable, completely self-contained instrument

that will rapidly analyze not only basic commodities, but also mixed products, such as forages and feeds.

The last Technicon International Congress was held in New York in 1972, and attracted upwards of 3,700 persons in the fields of science and medicine.

Persons wishing to attend this congress or interested in presenting a paper, can obtain further information by contacting Technicon Industrial Systems, Tarrytown, NY 10591. (914) 631-8000.

Posters Promote Employee Safety for Food Processors

The Food Processing Section of BASF Wyandotte Corporation's Chemical Specialties Division has made available a series of color posters, stressing sanitation and safety for employees, to food processing plants.

The bright, colorful poster campaign is intended to assist BASF Wyandotte customers in improving employees' attitudes regarding cleanliness and safety. The same cartoon character is shown in situations relating to six different slogans:

Dirt Belongs in the Garden . . .

Wash Your Hands!

Stay Healthy . . . We Need You!

Keep on Truckin', But . . . Be Careful!

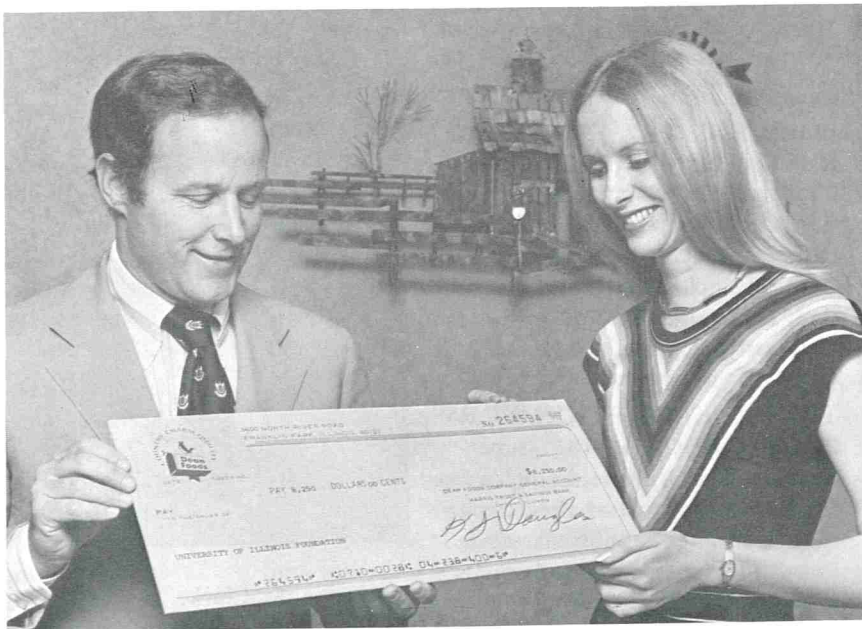
Why Take Any Chances? Dress Suitably!

Blow the Whistle on Trouble! Report Hazards!

A Place for Everything, and . . . Everything in its Place!

These posters are available, free of charge, upon request to:

BASF Wyandotte Corporation,
Chemical Specialties Division,
Food Processing Products,
Dept. LRS,
Wyandott, MI 48192.



Howard M. Dean, Jr., president of Dean Foods Company recently presented a check for \$8,250 to Susan Sundman, director of the University of Illinois Foundation at Circle Campus in Chicago. The Franklin Park-based company's contribution will be placed in the Samuel E. Dean, Jr. Memorial Award Fund, established in May of 1975 to financially assist undergraduate students in the Food Science Department at the University of Illinois in Champaign-Urbana.

Dean Foods was founded by Samuel E. Dean, Sr., in 1925 and under the direction of Sam E. Dean, Jr., Class of '30 at the University of Illinois, grew from a small dairy into a major diversified food processor serving the U.S., Canada and a number of foreign countries.

News and Events

Kraftco Teaching Award Presented to H. A. Morris



Dr. Howard A. Morris, recipient of the 1976 Kraftco Teaching Award is an outstanding educator who exemplifies the true purpose of this award. His primary activity for 28 years has been teaching and counseling students. Students are impressed with his lectures which are presented with confidence and enthusiasm and by his mastery of the subjects he teaches. Currently, he teaches courses in the Technology of Fermented Dairy Products, Understanding Cheese, International Dairy Technology, and Man's Food. In teaching these courses he makes ample use of visual aids and demonstrates how to apply research findings to practical applications. In addition to his teaching, Morris serves as advisor to about 50 students per year, coordinates the undergraduate registration within his department, serves as Secretary of his Department Curriculum Committee, and as Associate Director of Graduate Studies in Food Science. In 1975, Dr. Morris was honored by being a recipient of an All-University Morse-Amoco Award for teaching effectiveness. He has also aided in establishing new instructional programs in Universities in Turkey and Uruguay. Besides his teaching, he has been active in dairy products research and has authored or

co-authored more than 50 scientific publications of which several have appeared in the *Journal of Milk and Food Technology*.

Professor Morris was born in Draper, Utah in 1919. He obtained the B.S. degree in Dairy Manufacturing from Utah State University in 1941, and the M.S. and Ph.D. degrees from the University of Minnesota in 1949 and 1952, respectively.

He began his teaching and research career at the University of Minnesota in 1946 as a teaching assistant and was promoted through the ranks of instructor, assistant professor, associate professor and professor; attaining the latter rank in 1960. Dr. Morris has been a member of the American Dairy Science Association for 30 years and served on its Education Committee. His father received this same Award in 1963.

R. T. Marshall Receives DRINC Award



Criteria for the Dairy Research Inc. Award require that the recipient shall have demonstrated outstanding ability during the past 5 years in basic research on milk, milk components, or milk products, the results of which are applicable to solution of problems of the dairy

industry. This year's recipient, Dr. R. T. Marshall, has a distinguished record to document his research achievements in each of these areas. He has established himself as a highly competent researcher in dairy microbiology, dairy plant automation and cleaning, and mastitis control.

In the mid 1960's Marshall recognized the rapid trend to automated processing and cleaning in the dairy industry. He directed a major research effort to establish practical and reliable solutions to problems in this area. His work resulted in improved control of dairy plant processing operations through automation, improved cleaning of milk handling equipment, control of wastes, and improved efficiencies of energy and detergent utilization. Many of the papers that reported results of this work appeared in the *Journal of Milk and Food Technology*.

Professor Marshall also recognized major innovations in distribution patterns for dairy products. He and his fellow researchers undertook an intensified research effort to obtain basic knowledge on behavior of psychrotrophic microorganisms and their enzymes that were largely responsible for spoilage of refrigerated milk products. The knowledge gained from this work has been well documented in numerous publications. Marshall has made numerous other contributions. He has served on several key committees of several professional organizations. He has given presentations at numerous state and national dairy industry conferences and has received honors from several professional associations and councils. For example, he received the outstanding teaching award from the American Dairy Science Association last year. Professor Marshall also serves on the Editorial Board of the *Journal of Milk and Food Technology*. Dr. Marshall is a native of the state of Missouri and obtained all of his college education from the University of Missouri.

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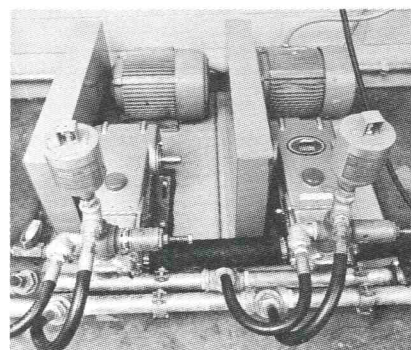
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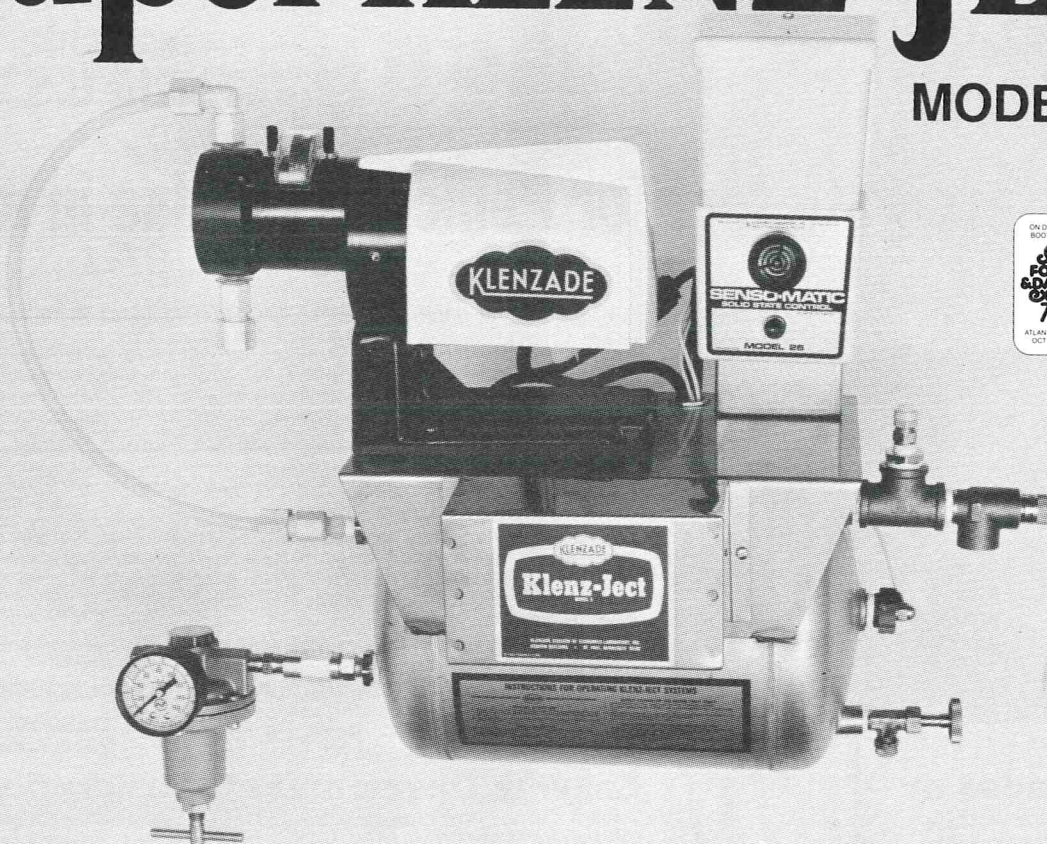
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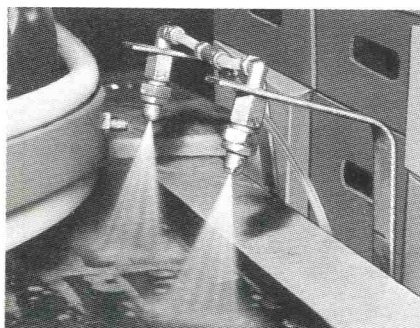
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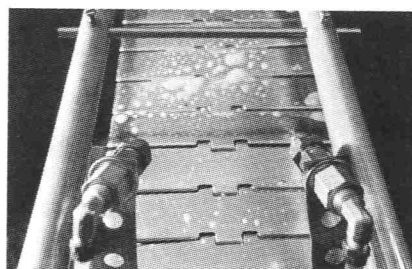
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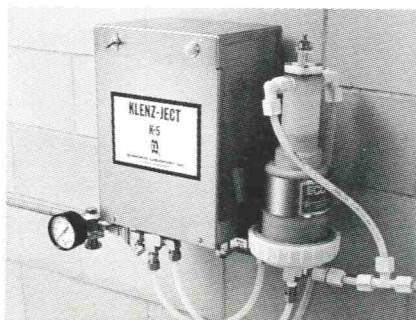
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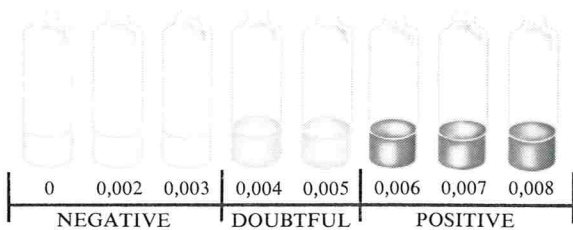
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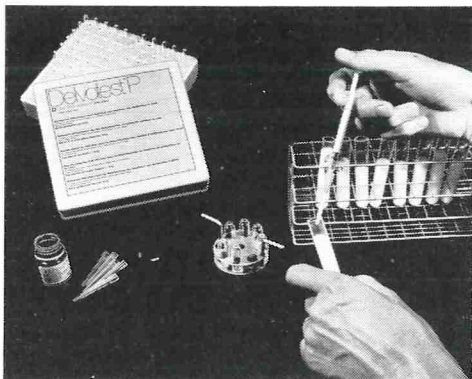


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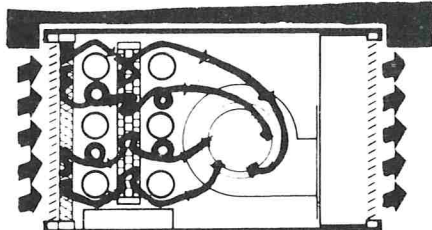


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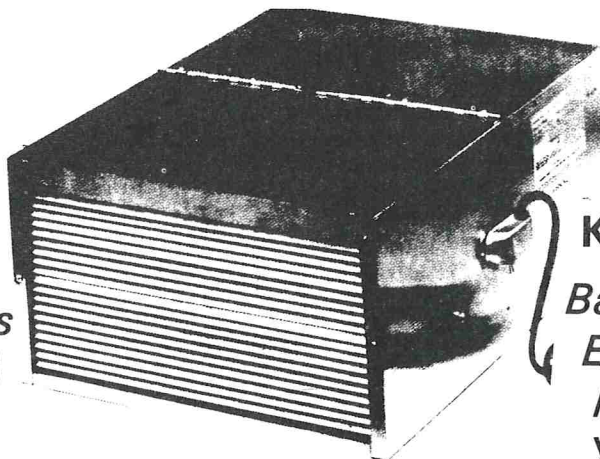
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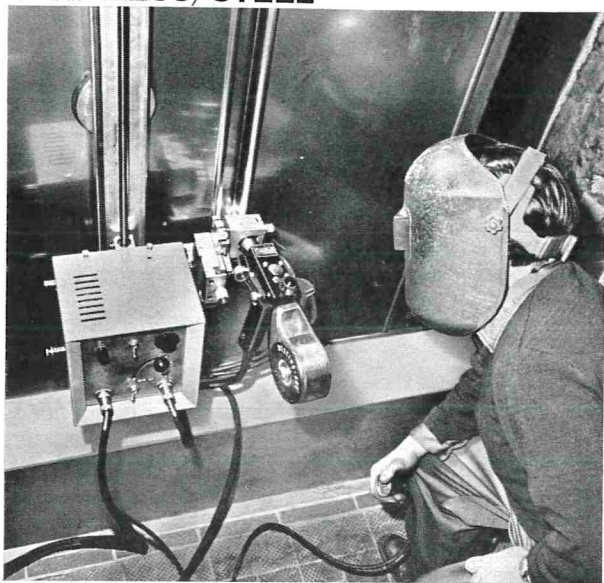
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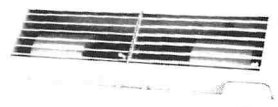
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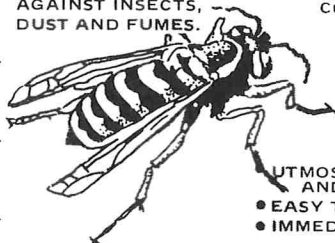
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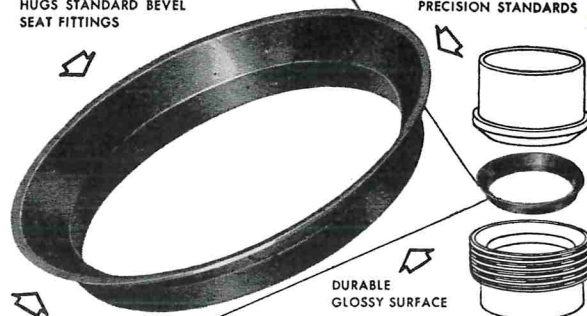
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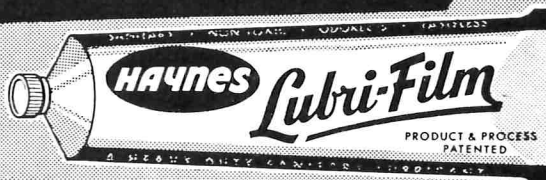
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Dairy authorities speak out on better cow milking



Stephen B. Spencer
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What's your score on vacuum?

An inadequate vacuum pump can affect the operation of the milking units drastically. Excessive "drop-off," slower milking, incomplete milking and an increase in the incidence of mastitis are likely results.

As a dairyman, your net dollar income depends on the vacuum pump and it probably is used more hours each year than most farm tractors.

Few people would hitch six plows behind a four plow tractor and head for the field to do a day's work. Yet many of these same people milk cows with a short vacuum supply and never question whether the pump is adequate.

Vacuum pumps used to be rated according to the number of units that could be operated. Today we measure the pump capacity in terms of Cubic Feet of air per Minute (CFM). Just as "horsepower" is more reliable than "plow rating" as an indication of tractor size, "CFM" is more reliable than "unit rating" when sizing a vacuum pump.

CFM output depends upon vacuum level. As vacuum level increases, the CFM output decreases. It's important to operate the system at the vacuum level specified by the manufacturer or the output of the pump will be altered.

The important consideration of any vacuum pump is the CFM output at the milking vacuum level. The pump must have adequate CFM output to meet the vacuum requirements of the system and provide sufficient reserve to maintain a constant vacuum level.

Vacuum Requirement

The milking unit is the most important of the machine components which admit air into the system. The air consumption of milking units varies depending upon shell and inflation size, pulsation rate and length and size of pulsated air tubes. Typically, the air requirement of a milking unit while it is not milking is three to four CFM. The pulsator consumes 50 to 70% of this volume. Considerably larger air pumping capacity (a reserve) must be provided to make a milking system operational. Other components which consume air are such things as vacuum operated door openers, milk metering devices, and the vacuum regulator. The requirements of each component must be added together to determine the system requirements.

Vacuum Reserve

The vacuum reserve is the air pumping capacity which remains after the vacuum requirement of all components has been satisfied. That's the problem. We've thought in terms of the vacuum reserve as the amount of CFM capacity that's *left over*. We really should be thinking in terms of a *base reserve* for the operator(s) before we begin to compute the system requirements.

The reserve is all-important in order to maintain vacuum stability. The reserve is necessary in order to make allowances for operator usage and possible leaks in the system or other contingencies.

The most important reason for an adequate vacuum reserve is to provide for the amount of air that the operator

will use. The operator is the largest user of the vacuum reserve. Some operators are very wasteful of the available reserve. This occurs as units are being attached and removed. Improper unit adjustment is also a significant factor. When teat cups start to leak and "squeal" during milking, the vacuum reserve is depleted rapidly.

Some operators may deplete vacuum reserve as much as 30 or more CFM for short periods of time. The careful operator will use but half that amount during the milking process. The real test of any milking system is when a milking unit falls off. It takes huge reserves of air just to keep the remaining units on the cows. Reserve tanks aid a little during these occurrences but basically the vacuum pump must be relied upon to maintain vacuum level. It all adds up to the fact that an adequate pump is a *must* for every dairyman.

Research in Ireland, Wisconsin, Pennsylvania and California indicates that inadequate vacuum reserve is associated with higher leucocyte counts. In plain language it means that mastitis can result if your vacuum pump isn't large enough.

What's your Vacuum Score?

Don't make a mistake and just assume that your pump is putting out enough air. Have it checked with an air flow meter once a year. Many dealers are equipped to do this for you.

And how will you know for sure that they're not just trying to sell you a pump? Frankly, I've found most dealers to be very reliable in this respect.

There have been many different recommendations about pump sizes. It's hard to give one that's exactly right for each system. Here's a guide for you to check your vacuum needs. It's based upon the New Zealand Standard. The American Standard would give values equal to one-half of the New Zealand Standard.

For bucket users:

Allow 4 CFM per unit + 20 CFM base reserve.

For pipeline users:

Allow 5 CFM per unit + 40 CFM base reserve for the first operator and 20 CFM for each additional operator.

The resulting CFM values would give you the minimum size vacuum pump capacity. If your system has more than this, fine. If you have less vacuum capacity than this you should carefully investigate your vacuum needs.

This method of determining vacuum capacity is different from what you may have seen before. A 50 percent reserve is commonly used. While a 50 percent reserve may be satisfactory on a system of six or more units, our field studies indicate that using a 50 percent reserve is not adequate for the smaller system.

Remember this: There is no substitute for an adequate vacuum system. Make sure you know your score on your vacuum needs.

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This is one of a series of topics developed by noted Dairy authorities. For a complete set write for a free booklet.