December 1976
Vol. 39, No. 12
Pages 813-896
JMFTA 39(12):813-896 (1976)

Journal of
Milk and Food
Technology®

Journal of Food Protection™

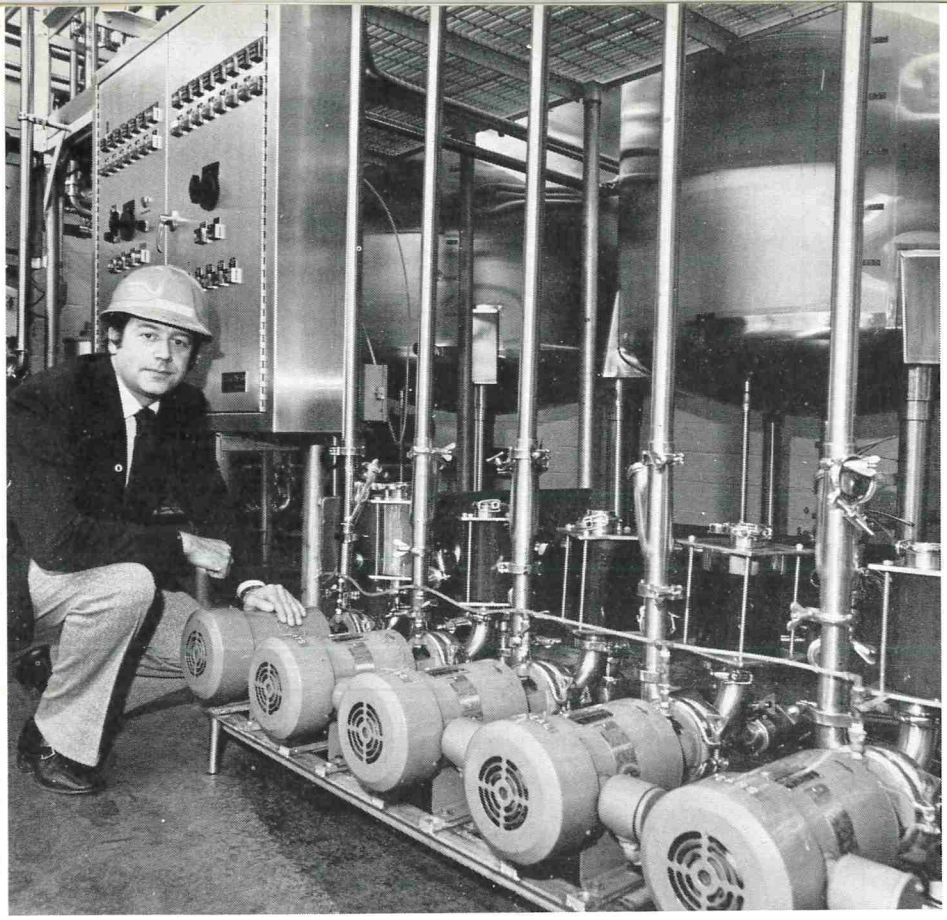
National Mastitis Council

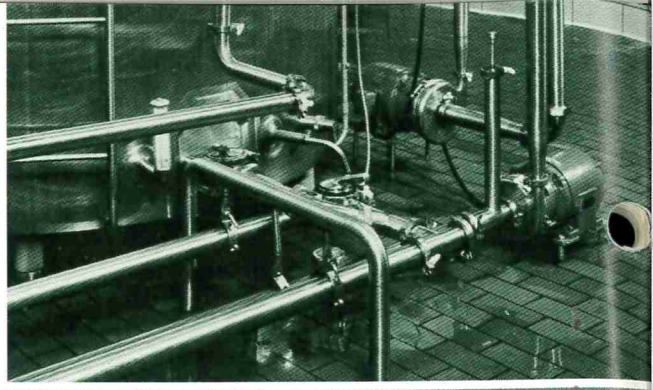
Annual Meeting

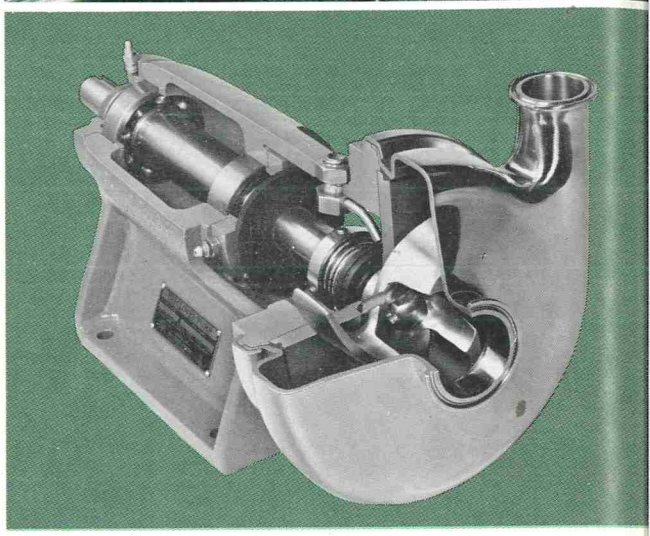
February 22-23, 1977

Executive Inn, Louisville, Kentucky

Official Publication







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NATIONAL MASTITIS COUNCIL ANNUAL MEETING

February 22-23, 1977

EXECUTIVE INN — LOUISVILLE, KENTUCKY

Everyone isnterested in prevention and control of bovine mastitis is cordially invited to attend the 16th Annual Meeting of the National Mastitis Council.

Vice President and Program Chairman James R. Welch has planned an outstanding program for this 16th Annual Meeting. Subject matter covers all segments of the mastitis problem, and this varied program will be of interest to veterinarians, researchers, extension dairy specialists, producers of products for treatment and control of mastitis, as well as dairy farmers.

Feature of the annual meeting will be program presentations by Dr. John Milne, National Dairy Laboratory, Ruakura Agricultural Centre, Hamilton, New Zealand. An international authority in the field of bovine mastitis, Dr. Milne will discuss natural defense mechanisms against mastitis which opens a different avenue of thought in the fight against this disease. Details of mastitis control in New Zealand will be discussed also.

Jack Linkletter, well known figure in sales promotion and consultant circles, who has wide experience in American agriculture, is the keynote speaker.

The role of milking machines in relation to the problem of mastitis will be handled by Steve Spencer, Extension Dairy Specialist, Penn State University. Dr. William Bickert, Michigan State Universtiy, will discuss automatic take-offs in relation to overmilking, and Dr. John H. Nicolai jr., Ellicott City, Maryland, will discuss vacuum level at the teat end.

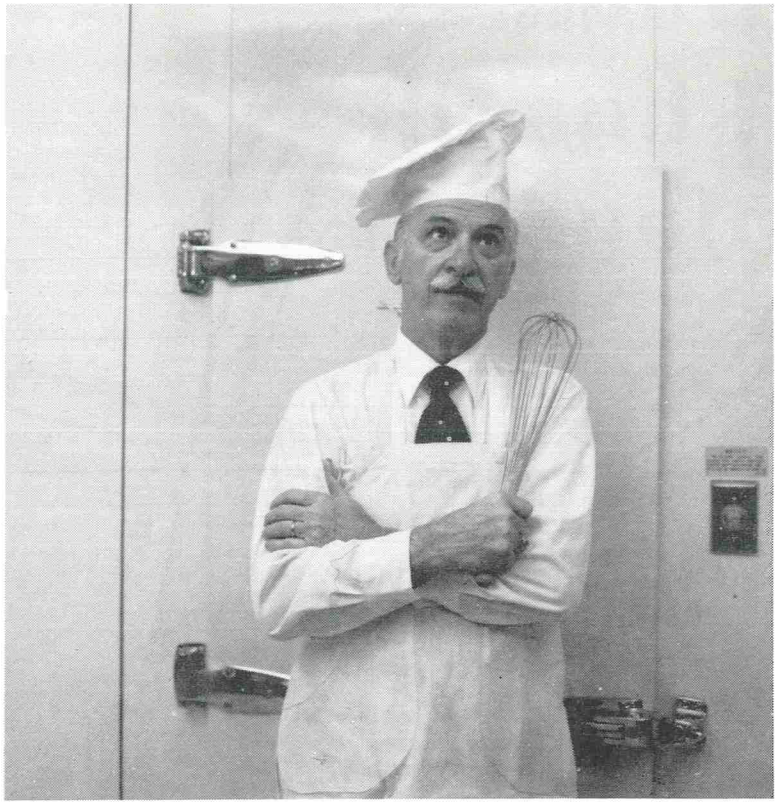
Other subjects of vast interest include discussions of the new FDA Treatment Guidelines by Dr. Ann Holt, BVM, FDA; interaction of factors predisposing to mastitis by Dr. Paul D. Thompson, USDA, ARS; milk quality from the processor point of view by Don Gregg; and quality premium programs and the dairyman by Melvin Leppo.

A panel on prevention and control of udder infections will include a veterinarian — Dr. Jan H. Pol; a dairyman — David Thuemmel; and dairy cooperative representatives — Sid Beale and Lowell Allen. The Tuesday evening program chaired by NMC Research Committee Chairman Dr. James W. Smith will include panelists Dr. E. V. Caruolo — Antibiotic Residues; Dr. R. J. Eberhart — Coliform; Dr. W. Nelson Philpot — Teat Dips; Dr. C. C. Miller — Infusion Products.

Make your plans to attend this excellent meeting. It will start at 8:45 a. m. on February 22 and will adjourn at noon on February 23. Request advance registration form from the National Mastitis Council, 910 — 17th Street, NW, Washington, DC 20006.

Send request for room reservation directly to the Executive Inn, Watterson Expressway at Fairgrounds, Louisville, KY 40213. Ask for NMC special rate.

R. D. Mochrie, PhD *President*
NATIONAL MASTITIS COUNCIL



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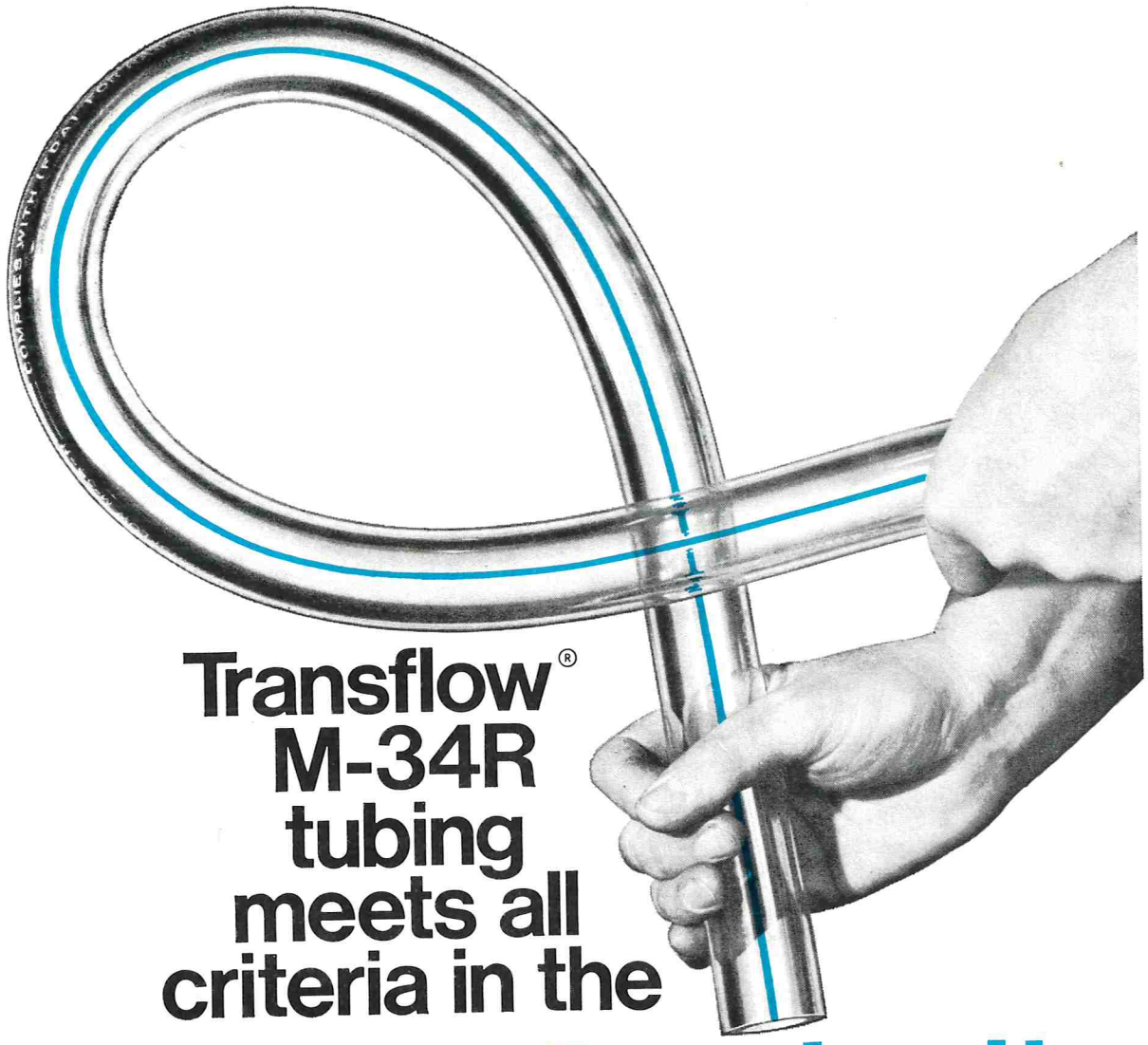

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Journal of Milk and Food Technology

INCLUDING MILK AND FOOD SANITATION

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Vol. 39 December 1976 No. 12

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The Journal of Milk and Food Technology is issued monthly beginning with the January number. Each volume comprises 12 numbers. Published by the International Association of Milk, Food and Environmental Sanitarians, Inc. with executive offices of the Association, 413 Kellogg Ave., P.O. Box 701, Ames, Ia. 50010. Printed by Heuss Printing and Signs, Inc., 204 N. Oak, Ames, Iowa 50010.

2nd Class postage paid at Ames, Ia. 50010.

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"Instruction to Contributors" can be obtained from the editor for the use of contributors of papers.

Orders for Reprints: All orders for reprints should be sent to IAMFES, Inc., P.O. Box 701, Ames, IA 50010.

Business Matters: Correspondence regarding business matters, advertising, subscriptions, orders for single copies, etc. should be addressed to Earl O. Wright (address above).

Subscription Rates: $32.00 per volume, one volume per year, January through December. Single copies $2.50 each.

Volumes on Microfilm are available from Xerox University Microfilms, 300 N. Zeeb Rd., Ann Arbor, MI 48106.

Membership Dues: Membership in the Association is available to individuals only. Dues are $16.00 per calendar year and include subscription to the JOURNAL OF MILK AND FOOD TECHNOLOGY. Student membership is $5.00 per year with certification.

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Journal of Milk Technology (1937-1946)

Journal of Milk and Food Technology (1947-1976)

Journal of Food Protection (1977-)

This is the last issue of the *Journal of Milk and Food Technology*. Next month (January, 1977) members of IAMFES and subscribers will receive the first issue of the *Journal of Food Protection*. This change affords an opportunity to briefly reflect on where we have been and where we hope to go in publishing a scientific journal.

JOURNAL OF MILK TECHNOLOGY

The International Association of Dairy and Milk Inspectors was organized in 1911. This organization, which became the International Association of Milk Sanitarians in 1935, published proceedings of its annual meeting for about 25 years. In 1937 the Association changed its policy and authorized publication of a bimonthly journal with the title *Journal of Milk Technology*. This was prompted by a report of the Association's Special Committee on Publication chaired by W. B. Palmer. Other members of the committee were C. S. Leete, J. J. Regan, J. H. Shrader, and J. A. Tobey. The first issue of the *Journal of Milk Technology* appeared late in 1937 and the other issues of Volume 1 were published in 1938.

The first issue of the journal carried an announcement which contained the following statement: "It is anticipated that the *Journal of Milk Technology* will prove to be a valuable medium to all persons and organizations concerned with any of the various phases of the milk and milk products industry and its many ramifications, whether from the standpoint of its official, industrial, regulatory, quality control, technical, or nutritional aspects. The journal will likewise be of interest to the general public and milk consumers." Although initially limited to the dairy industry, founders of the journal felt its coverage should be broad in scope. This breadth of coverage is reflected by subjects that were discussed in the first issue: technological problems in the ice cream industry, engineering of pasteurization, bovine tuberculosis, food value of milk and milk products, brucellosis, the resazurin test, and a comparison of tests for milkfat in ice cream.

Dr. J. H. Shrader served as the first Editor of the journal and W. B. Palmer as the first Managing Editor. The first Editorial Board consisted of 12 persons. Included was one woman (Sarah Vance Dugan of Louisville, Kentucky) and two Canadians (one was Dr. C. K. Johns who is still serving on the Editorial Board of the present journal).

As time went on professional activities of members of the International Association of Milk Sanitarians broadened and the organization, at its annual meeting in 1946, voted to change the name to the International Association of Milk and Food Sanitarians. At the same meeting the Executive Board authorized that the Association's publication become the *Journal of Milk and Food Technology*.

JOURNAL OF MILK AND FOOD TECHNOLOGY

The first issue of the journal with its new name and a new cover appeared in January, 1947. Dr. J. H. Shrader continued as Editor and the journal continued as a bimonthly publication.

Several noteworthy changes in journal operations were made during the early 1950s. These included: (*a*) change in page size (to 8½ × 11 inches) in 1950, (*b*) H. L. Thomasson became Managing Editor in 1951, (*c*) beginning in 1954 the journal appeared monthly instead of bimonthly, and (*d*) late in 1954 Dr. J. H. Shrader retired as Editor and Dr. J. C. Olson, Jr. began his service in that capacity.

For the first 9 years of its existence the journal was almost completely devoted to subjects of interest to the dairy industry. Dairy-oriented papers continued to predominate even after the title of the journal was changed. However, it was during Dr. Olson's tenure as Editor that papers dealing with non-dairy foods began to appear in the journal with greater frequency. Dr. Olson saw the need for a committee to develop policies for the journal. The Journal Management Committee resulted and it continues to guide the destiny of the journal.

Dr. Olson resigned in 1967 and was replaced by the present Editor, Dr. E. H. Marth. H. L. Thomasson retired as Managing Editor in 1974 and was succeeded by E. O. Wright who serves in that capacity at the present time. Ms. Barbara Lee became Assistant Managing Editor in 1976.

Recent years have seen: (*a*) a considerable increase in number of papers published annually, (*b*) changes in format made possible by a change in the printing company that prepares the journal, and (*c*) a change in journal content so that now papers dealing with non-dairy foods are more numerous than those with a dairy orientation.

The Journal Management Committee has long been concerned about the name of the journal. In 1975 the Committee proposed that the publication become the *Journal of Food Protection*. This recommendation was accepted by the Executive Board and the change will become effective in January, 1977.

JOURNAL OF FOOD PROTECTION

This international monthly journal in the English

(*continued on page 879*)

J. Milk Food Technol. Vol. 39, No. 12, Pages 819-822 (December, 1976)

Bacterial Spoilage of Citrus Products at pH Lower than 3.5[1]

B. J. JUVEN

Institute for Technology and Storage of Agricultural Products, ARO, The Volcani Center, Bet Dagan, Israel

(Received for publication April 1, 1976)

ABSTRACT

A strain of *Lactobacillus brevis*, L-3, was isolated from a blown can of grapefruit segments in sugar syrup; it caused spoilage of citrus products having pH values lower than 3.5. When inoculated into orange (pH 3.38) and grapefruit (pH 2.99) juices, after 5 h at 30 C L-3 produced 15 and 22 μg diacetyl/ml, respectively, and off-flavor was detectable. L-3 grew in APT broth acidified to pH 3.0 with citric, hydrochloric, phosphoric, or tartaric acid. However, its growth was inhibited at pH 3.6 if the acidulant was lactic acid, while with acetic acid inhibition occurred at a pH between 3.7 and 4.0. The thermal resistance of L-3 in orange serum (pH 3.4) was studied in the temperature range of 52 to 60 C; a *z* value of 8.3 was obtained. A simple and reliable capillary technique for studying the thermal resistance of gas-producing organisms in liquid foods and media is presented.

The lowest pH limit at which bacteria are able to grow and spoil food products is not clearly defined in the literature. Lactic acid bacteria, the most significant bacterial group connected with spoilage of high-acid foods (foods whose pH is 3.7 or lower), are referred to as being capable of growing at pH values down to 3.5 (*6*). Reviewing the role of spore-formers as food spoilage organisms, Ingram (*9*) summarized that "only yeasts and molds can spoil foods more acid than about pH 3.7." Jay (*10*) refers to the minimum pH value for the growth of *Lactobacillus* spp as 3.8 - 4.4. These two latter views appear to be in contradiction with the following data: (*a*) strains of *Lactobacillus* and *Leuconostoc* have been identified with a type of spoilage of citrus products (pH 3.5) known as "buttermilk off-flavor," characterized by the presence of diacetyl in the product (*13*); (*b*) strains of *Lactobacillus* isolated from orange products exhibiting this type of spoilage, grew in orange juice at 30 C (pH 3.4), giving generation times ranging from 6.8 to 8.8 h (*15*); (*c*) malo-lactic fermentation, induced in wines by certain lactic acid bacteria, has been shown to occur at pH values lower than 3.5 (*2, 14*). On the other hand, Faville et al. (*5*) showed that numbers of *Leuconostoc mesenteroides* remained unchanged during 7 h in orange juice (pH 3.68) at 30 C.

The isolation of a strain of *Lactobacillus* which caused gaseous spoilage of canned grapefruit segments and the study of some of its characteristics, among which is the ability of the organism to grow and cause spoilage at pH values lower than 3.5, are reported herein.

[1]*Contribution from the Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel. 1976 series, No. 147-E.*

MATERIALS AND METHODS

Test organsim

The organism used in these studies was isolated from a blown can of grapefruit segments in sugar syrup, by subculturing samples of the product both in orange serum broth (OSB, pH 5.4) and in APT broth (*4*). OSB has a composition identical to that of orange serum agar (OSA) (*12*) with the exception that in the former no agar is included. Identification of the isolate was made by cultural and biochemical tests (*16*).

Acidified media

Several acids were used for testing the minimum pH at which *L. brevis* L-3 would initiate growth in culture media. The medium used in these experiments was the APT broth acidified to selected pH values from 4.5 to 2.9 with filter-sterilized solutions (*2M*) of acetic, citric, hydrochloric, lactic, ortho-phosphoric, and D (+) tartaric acid. The basal medium, dissolved in reduced water amounts, was autoclaved at 121 C for 15 min. In the preparation of the acidified media a portion of the sterilized broth was used to determine the amount of each acid needed for achieving a certain pH, before inoculation of the remainder of the medium.

pH determinations

The pH was measured with a Radiometer PHM 26 pH-meter (Radiometer A/S, Copenhagen, Denmark).

Inoculation

An 18-h old culture of the test organism in APT broth at 30 C served as the source of the inoculum. Cells were harvested by centrifugation at 3200 × *g* for 15 min, resuspended in sterile 0.3 *M* sucrose aqueous solution to a final cell concentration of approximately 2×10^8 organisms per ml and enumerated on plates of APT agar. A quantity of ½ ml of this inoculum was used per 100 ml of medium. After inoculation, tubes containing either citrus juices or acidified media were sealed with a layer of sterile 2% agar. Growth of the inoculum was determined during a 7-day period by turbidity changes and by gas production as shown by the dislocation of the agar seal.

Growth and diacetyl plus acetoin production in citrus juices

Freshly extracted citrus (orange or grapefruit) juices were processed by heating them to boil, immediately pouring them aseptically into sterile glass containers, and cooling them rapidly. The pH values of the processed orange and grapefruit juices were 3.38 ± 0.05 and 2.99 ± 0.05, respectively. The total diacetyl plus acetoin content in inoculated juices was determined as diacetyl by the procedure described by Hill et al. (*8*).

Thermal resistance

Thermal death times of *Lactobacillus brevis* L-3 were determined in orange serum (pH 3.4 ± 0.05). Orange serum was obtained by heating freshly extracted orange juice to about 95 C, mixing it with filter aid

(30 g/1). and filtering it under suction through a Whatman No. 4 filter paper; it was autoclaved for 5 min at 121 C.

The method used for determination of thermal resistance of L-3 is described schematically in Fig. 1. Capillary tubes of 0.08-ml capacity (150 mm long and 0.83 mm internal diameter) were bent at one side with a gas flame (Fig. 1A) and then sterilized with dry heat. L-3 grown in

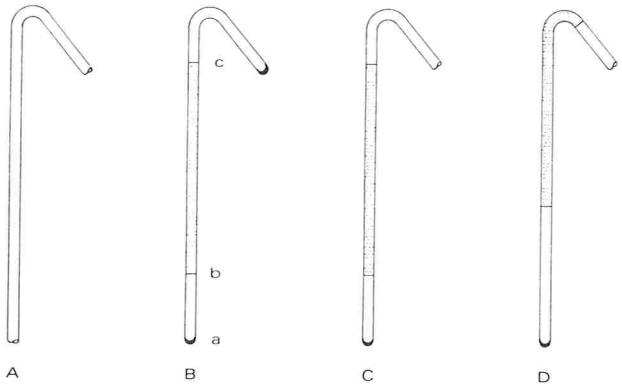

Figure 1. *The technique used for studying thermal resistance of* Lactobacillus brevis *L-3 (Schematic). (B)-The capillary tube (A) is partially filled with the suspension of L-3 in orange serum (bc) and sealed at both ends. (C)-Following the heat treatment, the end of the bent part of the tube is broken. (D)-Viable gas-producing organisms, after incubation, bring about a 2- to 4-fold increase in the height of the "bottom space" ab.*

OSB for 18 h at 30 C, was harvested by centrifugation at 3200 × *g*, washed twice with 0.3 *M* sucrose aqueous solution, and then suspended in sterile orange serum to give a final cell concentration of 10^6 per ml, enumerated on plates of OSA. The sterile capillary tubes were filled with 0.04 ml of the bacterial suspension, by immersing aseptically their straight end into a test tube containing the suspension. The tubes were then sealed at both ends (Fig. 1B) with a gas flame, care being taken not to heat the contents. Controls with uninoculated serum were done in parallel. The sealed capillaries were heated in a water bath at 52, 54, 56, 58, and 60 C for time intervals from 1 to 40 min and then cooled rapidly in water at 10 C. Less than 10 sec were required to equilibrate the temperature of the tube contents (as determined by thermocouples) with that of the heating/cooling bath. The extremity of the bent part of the tubes was broken aseptically (1C) and the tubes were then attached to millimeter paper by means of transparent adhesive tape, the open end being covered by the tape to reduce evaporation. The limits *b* and *c* of the suspension were recorded on the paper. The capillaries, with their bent ends up, were incubated at 30 C. Changes in the height of the "bottom space" *ab* were recorded during a 7-day period, although final results could be recorded after 3-4 days' incubation. No contamination of controls was observed throughout the incubation period. When the test organism was not destroyed by the heat treatment, it grew and produced gas, causing a 2- to 4- fold increase in the height *ab* (Fig. 1D).

RESULTS AND DISCUSSION

A short rod, identified as a heterofermentative lactic acid bacterium, was isolated from a blown can of grapefruit segments in sugar syrup. The pH of the spoiled product was higher (4.0) than that of unspoiled samples (pH 3.1 - 3.3). The spoilage organism was referred to as L-3. On the basis of cultural and biochemical characteristics, and employing the methods and criteria reported by Sharpe (*16*), L-3 was identified as *L. brevis*.

L-3 grew at both 15 and 45 C; it grew in the presence of 0.4% Teepol and produced ammonia from arginine; it did not hydrolyze aesculin. L-3 did not require riboflavin but did require folic acid for growth. It fermented ribose, glucose, maltose, arabinose, melibiose, raffinose, threalose, xylose, mannose, sucrose, and galactose, but not rhamnose, mannitol, sorbitol, salicin, lactose, melezitose, or cellobiose.

Freshly pasteurized citrus juices were inoculated with the organism: L-3 was able to cause gaseous spoilage of both orange (pH 3.38) and grapefruit (pH 2.99) juice. Spoilage was followed by a significant increase in pH. After 48 h of incubation at 30 C, the pH of the inoculated orange juice increased to 4.13 and that of the grapefruit juice increased to 3.23.

The minimum pH value at which bacteria would initiate growth appears to depend on the specific acid responsible for the reduced pH. Chung and Goepfert (*3*) showed that *Salmonella* would initiate growth in tryptone-yeast extract-glucose broth at pH 4.05 when citric acid was used as acidulant, but only at pH 5.50 when propionic acid was used. Table 1 shows the growth

TABLE 1. *Growth of* L. brevis *L-3 in APT broth*[a] *acidified to selected pH values by the addition of various acids*

pH	Acetic	Citric	Hydro-chloric	Lactic	Phosphoric	Tartaric
2.9	NT[c]	–[b]	–	–	–	–
3.0	NT	+[b]	+	–	+	+
3.2	NT	+	+	–	+	+
3.5	NT	+	+	–	+	+
3.6	–	+	+	–	+	+
3.7	–	+	+	+	+	+
4.0	+	+	+	+	+	+
4.5	+	+	+	+	+	+

[a] 10^6 cells/ml.
[b] + = Growth evident by increased turbidity and dislocation of an agar seal due to gas production; – = no growth.
[c] NT = Not tested.

of L-3 in APT broth, acidified with various acids to pH values from 4.5 to 2.9. L-3 was able to grow in the APT broth acidified down to pH 3.0 with any one of four of the six acids tested, namely: citric, hydrochloric, phosphoric, and tartaric. With lactic acid growth was inhibited at pH 3.6 and with acetic acid between 3.7 and 4.0. These two acids are in more or less common use as acidulants in the food industry and in laboratories for adjustment of pH of microbiological media. These data support the view that the pH of the medium is not the only factor governing bacterial growth at low pH values, but that the acid under study and/or its final concentration in the medium may also play a significant role.

The highest concentrations of the acids which allowed growth of the test organism when added to the APT broth were different: 0.074 *M* lactic, 0.14 *M* acetic, 0.13 *M* citric, 0.076 *M* phosphoric, 0.12 *M* tartaric, or 0.092 *M* hydrochloric.

The broth media were adjusted to the test pH, but not buffered at that value. Consequently, active growth of the inoculum would result in alteration of the pH. Citric acid is the main factor responsible for the low pH of citrus juices. When citric acid was added to the broth to give initial pH values between 2.9 and 3.7, along with the

growth of L-3 there was an increase in pH toward values of about pH 4.0.

The use of agar media acidified to pH 3.5 for enumeration of yeasts and molds (*1*) is based on the assumption that bacterial growth is inhibited at this pH value and therefore a selective enumeration of fungi is achieved. However, many data have been published which contradict this assumption. Koburger (*11*) recovered the lactic acid bacterium *Leuconostoc citrovorum* (*Leuconostoc cremoris*) from orange juice, using potato dextrose agar acidified to pH 3.5 with either citric or hydrochloric acid, but not with tartaric, lactic, sulfuric or phosphoric acid. Therefore, it may be concluded that the acid used is a very critical factor when the acidification of media is concerned. Table 1 shows that APT broth acidified to pH 3.5 was not inhibitory to L-3 when the acidulant used was citric, tartaric, phosphoric, or hydrochloric acid, but it was inhibitory if lactic acid was employed. The inhibitory effect of acetic acid at pH 3.7 may be explained on the basis of the relatively high acid concentration required to obtain this pH value in the broth, i.e., ca. 0.30 *M*, and on the basis of the antimicrobial action of the undissociated molecule. With lactic acid, since the concentration needed to inhibit growth (pH 3.6) is relatively low, it appears that the anion plays a major inhibitory role.

Lactobacilli produce diacetyl as one of their more important volatile metabolic products. Diacetyl has been shown to play a significant role in development of an off-flavor of the buttermilk-type and in the spoilage of citrus products by lactic acid bacteria (*7*). Figure 2 shows the accumulation of diacetyl plus acetoin in orange and grapefruit juices inoculated with *L. brevis* L-3 and incubated at 30 C. Diacetyl production followed the same pattern in both juices, and after 5 h reached a concentration of about 15 and 22 μg per ml in orange and grapefruit juices, respectively, and off-flavor was detectable.

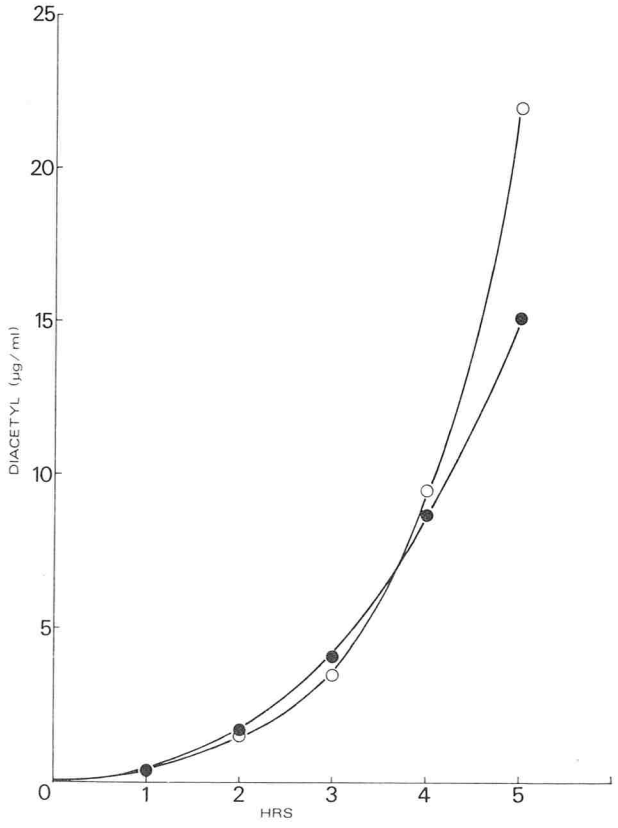

Figure 2. *Accumulation of diacetyl plus acetoin in citrus juices inoculated with* Lactobacillus brevis *L-3 (10⁶ cells/ml) and incubated at 30 C. Results were calculated as diacetyl. Circles, grapefruit juice; black dots, orange juice.*

The thermal death time (TDT) curve for *L. brevis* L-3 in orange serum (pH 3.4, 10^6 cells per ml) in the temperature range of 52 to 60 C is presented in Figure 3. From the measured z value (*ca.* 8.3), it appears that the thermal resistance characteristics of L-3 are similar to those of other lactic acid bacteria reported in the literature (*17*). The capillary technique used in this study, for determination of thermal resistance, was found to be a reliable one when gas-producing organisms were concerned. The technique is simple and does not require special equipment; it has the advantage of giving a very rapid temperature equilibrium between the tube contents and the heating/cooling medium. There is no need to subculture the tube contents since the remaining viable cells can be detected by gas production in the tube itself; therefore there is very little hazard of contamination of the contents. The technique was found to be useful for liquid foods, media, and thin food homogenates.

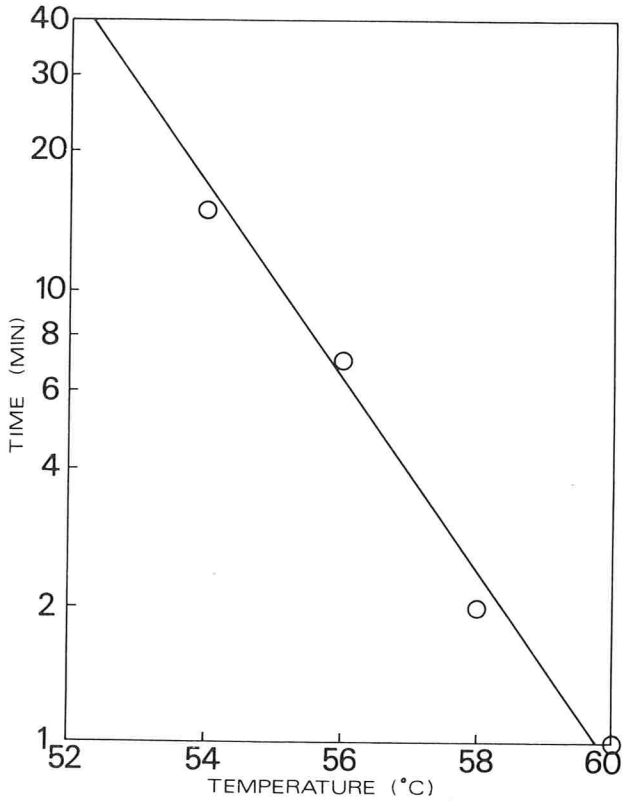

Figure 3. *Thermal-death-time curve for* Lactobacillus brevis *L-3; 10^6 cells per milliliter in orange serum, pH 3.4 (z is approximately 8.3).*

ACKNOWLEDGMENT

Our thanks are expressed to Mrs. Hannah Weisslowicz for technical assistance.

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J. Milk Food Technol. Vol. 39, No. 12, Pages 823-829 (December, 1976)

Microbiology of Fresh Comminuted Turkey Meat

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(Received for publication April 12, 1976)

ABSTRACT

Standard plate counts, coliform plate and most probable number (MPN) counts, *Escherichia coli* plate and MPN counts, *Staphylococcus aureus* MPN counts, and fecal streptococcus counts were determined for 75 samples of fresh ground turkey meat purchased from retail markets in the San Francisco Bay Area. The presence of *Clostridium perfringens* was determined by both direct plate count and enrichment techniques. Salmonellae were isolated using selective enrichment procedures. Samples were screened for presence of enteroviruses. Aerobic gram-positive and gram-negative organisms were isolated and identified. *Clostridium perfringens* and *Salmonella* sp. were isolated from 52% and 28% of the samples, respectively. The mean standard plate count was 84,000,000 per gram. The mean count for *E. coli* determined by the MPN method was 19 per gram. Fecal streptococci were isolated from 95% of the samples with a mean count of 18,000 per gram. *Staphylococcus aureus* was isolated from 80% of samples analyzed with a mean count of 34 per gram.

The amount of poultry consumed per capita in this country has steadily increased since the 1930's. This increase can be attributed to improvement in poultry production through breeding, nutrition, and disease control as well as processing procedures within the industry.

The economic need to use as much of the fowl as possible and improvements in processing techniques have resulted in marketing of several types of cut-up and processed turkey products. Further processed products, prepared from deboned turkey meat range from turkey rolls to ground or comminuted turkey meat.

It is estimated that nearly 50% of the beef consumed in this country is in the comminuted form. Much of this is consumed in a semicooked condition (*13*). Due to the popularity and economical aspects of meals prepared with comminuted beef, the consumer is being encouraged to try other comminuted meat products such as turkey. Turkey, in the comminuted form, is advertised as being low in cost, fat, and calories, yet high in some essential daily dietary components such as protein, niacin, and riboflavin (*29*).

The literature contains reports of two studies dealing with the microbiology of poultry meat (*27, 30*). The meat used in these studies was not purchased at the retail level but was obtained from either university or commercial poultry processing plants (*27, 30*).

Comminuted turkey, a further-processed product prepared from the dark meat of the fowl, is deboned by hand or by machine depending on the wholesale supplier (*23, 36*). Fresh or frozen turkey is obtained as a coarse grind and is generally reground at the retail level before being offered for sale in either prepackaged units or by open-try selection. Comminution of a product greatly increases the surface area, distributes the bacteria throughout, and oxygenates the product. Comminuted products, if mishandled, have long been recognized for their potential to support and promote rapid growth of microorganisms.

Several reports have implicated poultry and poultry products as important sources of organisms belonging to the tribe *Salmonellae* (*9, 11, 12, 19, 20, 37*). On several occasions, meat from turkeys has been implicated in outbreaks of salmonellosis (*4, 35*). Bryan et al. (*12*) demonstrated that dissemination of *Salmonellae* by turkeys begins on the farm. Their study indicated that during processing procedures, equipment and meat become contaminated with the organisms carried by the turkeys in their feces and on their feet and feathers.

Surveys of dressed poultry from processing plants and retail stores have revealed *Salmonellae* contamination present in 1 to 50% of the samples tested (*18, 32, 39*). Since comminuted turkey is a new product, its level of bacterial contamination is not reflected in retail market surveys conducted before this study.

Consumer awareness of potential public health problems and consumer generated pressures for protective legislation have resulted in the May 1973 enactment of Oregon's microbiological standards for meat products. The adopted standards for ground and whole cuts of red meats established a limit of 5 million organisms per gram for the total aerobic plate count and a limit for *Escherichia coli* of 50 per gram determined by the most probable number (MPN) method (*13*). Canadian officials have proposed microbial standards for ground meat items. This proposal establishes a limit for the standard plate count of 10 million per gram while the limit of 50 per gram was set for *E. coli* (*3*). With standards such as these already in existence for red meat items, consumer pressure may influence legislative

bodies to enact appropriate standards for poultry products. Therefore, studies of the microflora of comminuted poultry products purchased at the retail level are necessary before adoption of standards for these items.

MATERIALS AND METHODS

Samples

Refrigerated samples, in approximately 1 lb lots, were procurred at various retail markets in the San Francisco Bay Area. They were transported to the laboratory in a Freez Safe Styrofoam Case (GloBrite Foam Plastics Co., Chicago, IL) using Blue-Ice cold storage usits (Divajex Co., Santa Ana, CA) to provide a refrigerated atmosphere of approximately 8 C. Samples for bacteriological analysis were either analyzed immediately or after holding at 4 C for not longer than 48 h. Samples virological analysis were placed in sterile 50-ml centrifuge tubes and stored at -80 C until analysis was initiated.

Bacteriological analyses

Various media were used to indicate the numbers and types of organisms present. Aerobic plate counts were determined using Standard Methods Agar and incubation at 32 C for 72 h.

Estimates of coliforms were made by the plate count procedure with Violet Red Bile (VRB) agar as well as by the Most Probable Number (MPN) technique as described in *Standard Methods for the Examination of Dairy Products* (*1*).

Selenite and Tetrathionate broths, for detection of salmonellae, were incubated at 37 C for 24 h. The broths were then streaked to plates of *Salmonella-Shigella*, Bismuth Sulfite, and Brilliant Green Sulfadiazine agars and incubated for 24 h at 37 C. Colonies exhibiting positive reactions on these media were verified biochemically and serologically following procedures outlined in *Identification of Enterobacteriaceae* (*17*).

Determination of the *Staphylococcus aureus* MPN was done using the method outlined by the Association of Official Analytical Chemists (AOAC), with the substitution of Tellurite Polymyxin Egg Yolk agar for Vogel and Johnson agar. The AOAC procedure was used for determination of coagulase production (*7*).

Procedures from the *Bacteriological Analytical Manual for Foods* (*5*) were used for enumeration of fecal streptococci. In addition to these procedures, colonies from KF Streptococcal agar were transferred to Ethyl Violet Azide broth for confirmation. After incubation at 37 C for 48 h, colonies were considered confirmed if a yellow color developed and sediment was observed in the tube.

The presence of *Clostridium perfringens* was determined by aseptically removing five aliquots of turkey meat, 1 to 2 g each, and placing these in tubes containing Fluid Thioglycollate medium. Tubes were incubated for approximately 6-8 h in water bath set at 46 C. After this incubation period, cultures exhibiting profuse gas production were transferred to Cooked Meat Medium and incubated overnight at 37 C. Fluid Thioglycollate tubes showing little or no gas production after the 46 C incubation period were also incubated overnight at 37 C. If, after overnight incubation, gas was observed, the culture was transferred to Cooked Meat Medium. Tubes not exhibiting evidence of gas production were discarded. Presumptive *C. perfringens* isolates were purified and verified using Sulfite-Polymyxin B-Sulfadiazine agar (SPS), Liver Veal Egg Yolk agar, as well as gram stain, Iron Milk, and Nitrite Motility medium. The serum neutralization mouse assay technique was used for typing isolated strains (*16*).

Isolation and identification of aerobic bacteria present in each turkey sample were accomplished using the following procedures: Gram-negative organisms were isolated from the remaining sample by inoculating 10 ml double strength GN broth with 10 ml of the 10^{-1} dilution originally prepared for the total plate count procedure. After 24 h of incubation at 35 C, the sample was inoculated onto *Salmonella-Shigella*, Bismuth Sulfite, Brilliant Green Sulfadiazine, Hektoen Enteric, MacConkey's, and Eosin-Methyline Blue (EMB) agar plates. Following incubation at 37 C for 24 h, representative colony types were subcultured to EMB agar to assure purity. Identification of gram-negative isolates was made using the API 20E *Enterobacteriaceae* system (Analytab Products, Inc.). The Analytical Profile Index was used to assign species designations to isolates identified by this procedure.

Gram-positive organisms were isolated from the sample by inoculating 10 ml double strength Trypticase Soy Broth (TSB) containing 4.0 mg potassium tellurite/ml with 10 ml of the original 10^{-1} food slurry. In addition, 1 ml of the original food slurry was inoculated into 10 ml of TSB. Tubes were incubated overnight at 35 C before being inoculated onto Mueller-Hinton agar plates containing 5% defibrinated sheep blood. Representative colony types were subcultured to blood agar plates to assure culture purity. After overnight incubation at 37 C, isolates were separated into two categories on the basis of catalase demonstration in 3% hydrogen peroxide.

Catalase positive organisms exhibiting coccal morphology were examined for their oxidative-fermentative capabilities in O-F Basal medium containing 1% glucose. In addition, mannitol utilization and nitrate reduction were determined. Ioslates utilizing mannitol and exhibiting fermentative ability were tested for coagulase production. Rod-shaped catalase positive organisms were inoculated into a primary battery of media including purple broth base with 1% glucose, Simmon's Citrate agar slant, Indole-Nitrite-Motility medium, and a Trypticase Soy agar plate.

Streptococci were speciated using a multipoint inoculator (Melrose Machine Shop, Woodlyn, PA) and inoculating isolates onto the following agar media: blood, 40% bile, glucose, 6.5% sodium chloride, bile-esculin-azide, gelatin, starch, arabinose, glycerol, lactose, mannitol, raffinose, sorbitol, melibiose, and melezitose. Isolates were also examined for their ability to grow at 10 and 45 C.

Virological analysis

Samples of comminuted turkey, approximately 4 g, were put into 40 ml of skim milk and centrifuged for 20 min at 2,000 rpm. Supernatant fluid was treated with 100 μg of gentamicin and 5 μg of fungizone per ml. A 0.2-ml inoculum of supernatant fluid was transferred into each of two tubes of the following cell lines: HEK, VMK, PRMK, and WI-38. Cell cultures were incubated at 33 C in L-15 maintenance medium and read twice weekly on days 2 and 4 for cytopathogenic effect. On the seventh day, a blind passage was made and allowed to incubate 11 days before regarding the sample as negative.

RESULTS

The distribution of standard plate counts can be seen

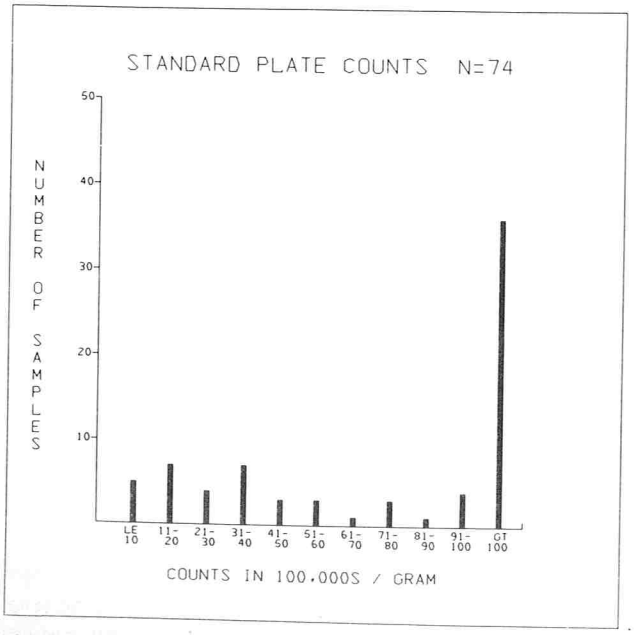

Figure 1. *Distribution of plate counts when samples contained up to 10,000,000 bacteria/g.*

in Fig. 1. Due to a laboratory accident, standard plate counts were recorded for only 74 of 75 samples. The counts ranged from 7.4×10^4 to 3.0×10^9 per gram with a mean count of 8.4×10^7 per gram. Thirty-five percent of the samples had aerobic plate counts of 5.0×10^6 or less. Fifty-one percent of the samples tested had aerobic plate counts of 1.0×10^7 per gram or less. Figure 2 shows the distribution of the 36 samples which had standard plate

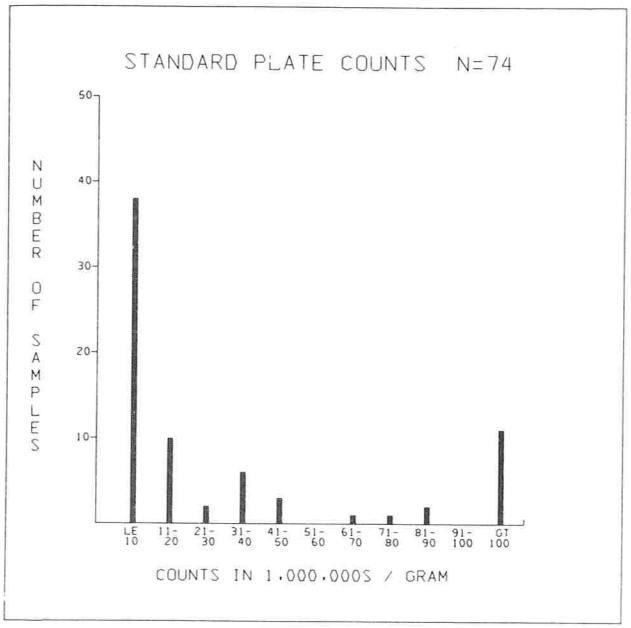

Figure 2. *Distribution of plate counts when samples contained more than 10,000,000 bacteria/g.*

counts greater than 1×10^7 per gram. Thirty-one percent of these samples had counts greater than 1×10^9 per gram.

Results of coliform determinations made by both the plate count and the MPN methods, can be seen in Fig. 3 and 4, respectively. Analysis by the coliform plate count

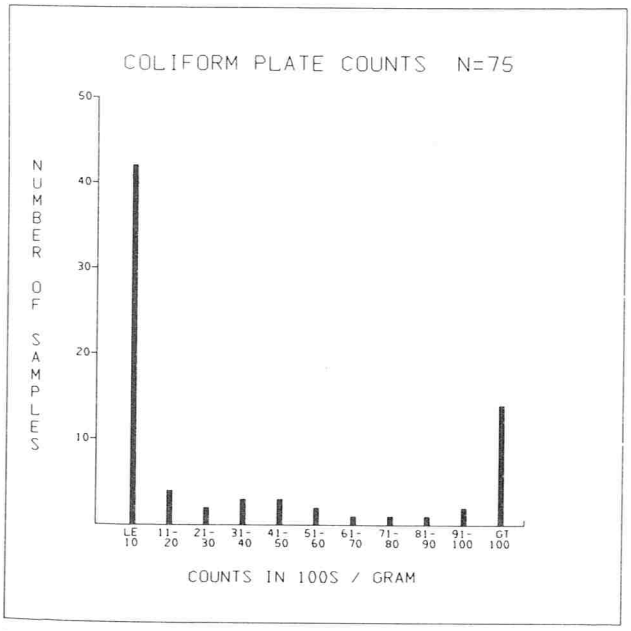

Figure 3. *Distribution of coliform plate counts.*

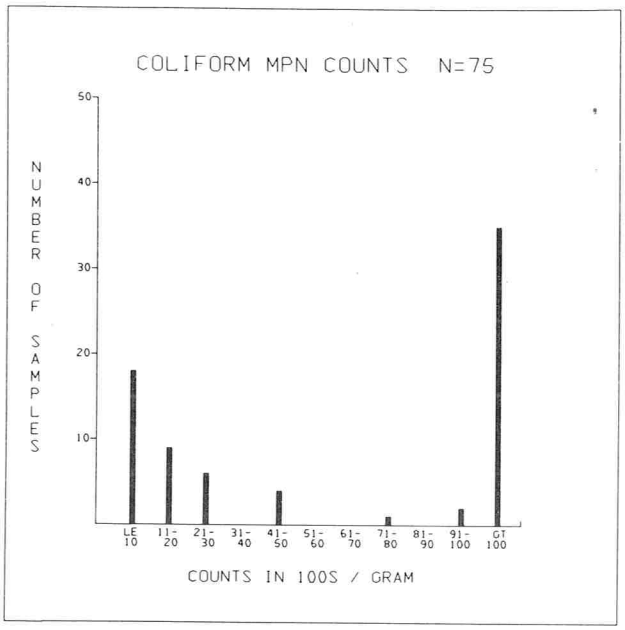

Figure 4. *Distribution of coliform MPN counts.*

method yielded counts ranging from 5.0×10^0 to 4.8×10^5 with a mean of 2.0×10^4 per gram. Using this technique, coliforms were not detected in 12% of the samples. Using the MPN technique, counts for the 75 samples ranged from 2.3×10^1 to 1.1×10^7 with a mean count of 6.3×10^4 per gram. Coliforms were detected in all but one sample with this testing procedure.

Use of the MPN technique facilitated detection of *E. coli* in 41% of the samples. Confirmed *E. coli* counts ranged from 3.6×10^0 to 7.5×10^2 with a mean count of 1.9×10^1 per gram. By the plate count procedure, *E. coli* was detected in only 8% of the samples with counts ranging from 5.0×10^0 to 6.0×10^3 with a mean of 8.7×10^1 per gram.

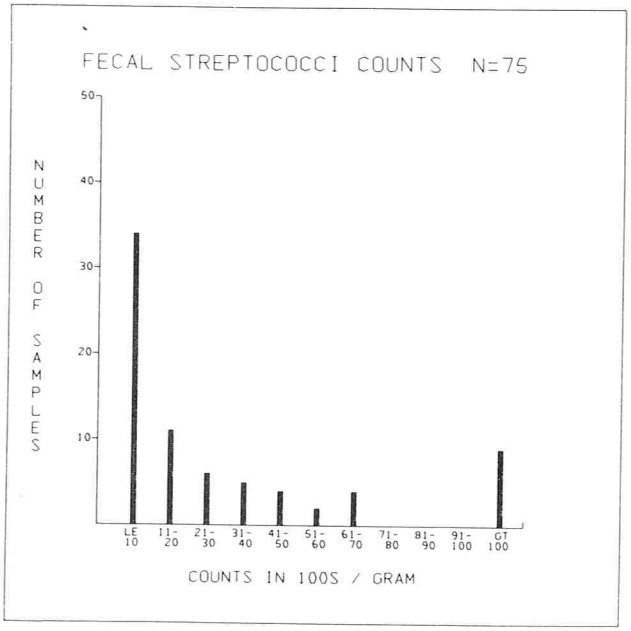

Figure 5. *Distribution of fecal streptococcus counts.*

Figure 5 shows the distribution of counts for fecal streptococci detected in 95% of the samples. Counts from positive samples ranged from 4.0×10^1 to 3.0×10^5 per gram. The mean count for the 75 samples was 1.8×10^4 per gram.

Staphylococcus aureus was detected in 69% of the samples tested by the MPN techique. Distribution of counts for these samples can be seen in Fig. 6. Counts ranged from 3.6×10^0 to 1.1×10^3 with a mean of 3.4×10^1 per gram.

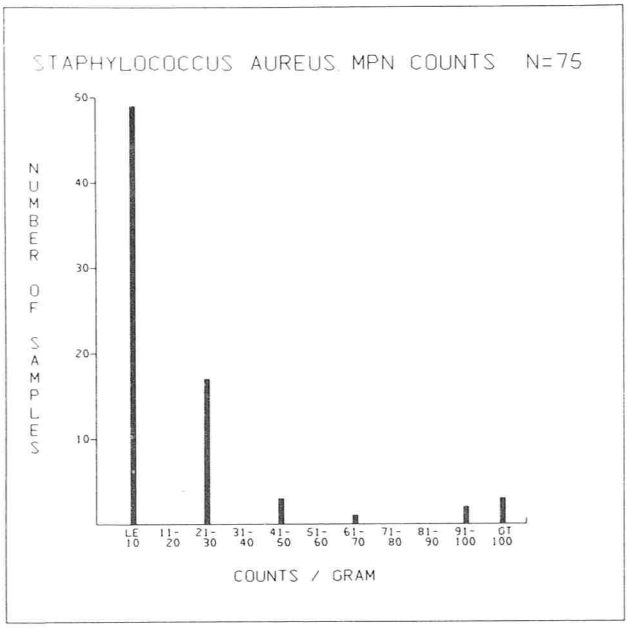

Figure 6. *Distribution of* Staphylococcus aureus *MPN counts.*

Twenty-one samples yielded isolates belonging to the genus *Salmonella.* Most of these isolates (52%) were *Salmonella enteriditis* bioserotype *pullorum.* Of the salmonellae isolated, 33% were identified as *Salmonella arizonae* while *Salmonella enteriditis* serotype *Saint Paul, Salmonella enteriditis* bioserotype *Miami* and *Salmonella enteriditis* serotype *Derby* each respresnted 5% of the salmonellae isolations.

The enrichment procedures used in this study yielded a variety of isolates. Table 1 lists the number of isolations and the percentage of samples represented for each of the aerobic organisms as well as *C. perfringens.* The most frequently isolated organisms were *Citrobacter freundii, Hafnia alvei* (*Enterobacter hafniae*), and *Escherichia coli* isolated from 87, 83, and 81% of the samples, respectively.

The enterovirus isolation procedure used in this study yielded no isolates.

TABLE 1. *Bacterial flora isolated from fresh comminuted turkey meat*

Organism	Number of isolations	% of samples
Gram-positive Isolates		
Bacillus polymyxa	1	1
Bacillus sp.	2	3
Clostridium perfringens	39	52
Corynebacterium sp.	1	1
Micrococcus sp.	26	35
Staphylococcus aureus	60	80
Staphylococcus epidermidis	50	67
Streptococcus acidominimus	2	3
Streptococcus agalactiae	1	1
Streptococcus anginosus	1	1
Streptococcus bovis	2	3
Streptococcus cremoris	2	3
Streptococcus dysgalactiae	1	1
Streptococcus equi	1	1
Streptococcus equisimilis	2	3
Streptococcus faecalis	35	47
Streptococcus faecalis var. *liquefaciens*	55	73
Streptococcus faecium var. *Casseliflavus*	2	3
Streptococcus faecium var. *durans*	1	1
Streptococcus lactis	20	27
Streptococcus *sp.*	*3*	*4*
Gram-negative Isolates		
Acinetobacter calcoaceticus var. *anitratum*	11	15
Acinetobacter calcoaceticus var. *lwoffi*	1	1
Alcaligenes sp. (*Achromobacter* sp.)	6	8
Citrobacter diversus	4	5
Citrobacter freundii	65	87
Enterobacter aerogenes	6	8
Enterobacter agglomerans	11	15
Enterobacter cloacae	52	69
Enterobacter liquefaciens (*Serratia liquefaciens*)	19	25
Escherichia coli	61	81
Flavobacterium sp.	1	1
Hafnia alvei (*Enterobacter hafniae*)	62	83
Klebsiella pneumoniae	48	64
Proteus inconstans (*Providencia alcalifaciens*)	3	4
Proteus mirabilis	11	15
Proteus morganii	12	16
Proteus rettgeri	1	1
Proteus vulgaris	4	5
Pseudomonas aeruginosa	16	21
Pseudomonas fluorescens	5	7
Pseudomonas fluorescens grp.	5	7
Pseudomonas maltophilia	2	3
Salmonella arizonae (*Arizona hinshawii*)	7	9
Salmonella enteriditis bioser. *Miami*	1	1
Salmonella enteriditis bioser. *pullorum*	11	15
Salmonella enteriditis ser. *Derby*	1	1
Salmonella enteriditis ser. *Saint Paul*	1	1
Serratia marcescens	5	7
Yersinia enterocolitica	3	4

DISCUSSION

The mean standard plate count reported in this study is considerably higher than counts for other comminuted meat products which have been reported by several investigators (*22, 24, 33*). Maxey et al. (*27*), reported counts ranging from 1×10^5 through 1×10^6 per gram of comminuted poultry meat including chicken. Ostovar et al. (*30*), compared immediately processed and delayed processed deboned poultry meat samples. In their study, standard plate counts of 3.3×10^5 per gram for the immediate process product and 7.1×10^5 per gram for

the product produced by the delayed processing procedure were reported.

The high standard plate counts reported in this study are most likely due to the additional grinding and handling as well as the temperature and length of refrigerated storage of the product before purchase at the retail level. The use of skin in the comminuted product probably contributes to high bacterial counts observed. Walker and Ayres (*37*) reported recovery of 4,700 organisms/cm^2 from the skin of live turkeys. They found that following processing, the counts increased to 44,000/cm^2 of skin surface. Current manufacturing procedures include use of 8-14% skin in the preparation of the product (*23*). This procedure provides the turkey meat with an inoculum of spoilage organisms as well as organisms of public health significance.

It is noteworthy that if the bacterial standards for ground beef items adopted in Oregon (*13*) and those proposed in Canada (*3*) were applied to the samples in this study, only 35% of the samples tested would comply with the Oregon standard for total bacterial count while 51% of the samples would comply with the proposed Canadian standards.

Using the MPN technique, *E. coli* was isolated from 41% of the samples while the plate method resulted in only eight isolates. This is evidence for the lack of sensitivity inherent with the plate count procedure for enumeration of this organism.

The fact that most streptococci isolated were identified as *Streptococcus faecalis* (25%) or its variety *liquefaciens* (40%) is in accord with the findings of Wilkerson et al. (*40*). They reported *Streptococcus faecium* to be the most prevalent enterococcus recovered from turkeys before processing while following processing, *S. faecalis* was found to be the most frequent enterococcus isolated.

In discussion of the importance of enteric bacilli in foods, it is necessary to consider the history, method of processing, and treatment the product receives before consumption. Since techniques for aseptic removal of the intestinal tract have not yet been developed, enteric bacilli will be found on the surfaces of red meat and poultry products in abattoirs and processing plants. Cooked red meat products with their natural enteric flora have rarely been associated with illness in man (*15*). Poultry and poultry products, on the other hand, are the most frequently incriminated sources in foodborne outbreaks of *Salmonella* infections (*31*).

Good processing procedures may result in low numbers of contaminants during the slaughtering and chilling operations in poultry processing. However, at temperatures above freezing, surface growth of aerobic, psychrotrophic organisms will occur. Comminution of the poultry meat greatly increases the surface area and creates a new environment for the organisms by distributing them throughout the product. Additionally, comminution increases the chances of contamination by providing further opportunity for product contact with surfaces of counters, machinery, knives, and hands of workers.

As a rule, properly cooked meats are not associated with gastro-intestinal illness in man. The consumer is being encouraged to try comminuted turkey as a substitute in recipes usually calling for hamburger (*2, 29*). Considering the bacterial load as reported in this study, the consumer should also be advised not to consume comminuted turkey in the rare or raw state as is often done with hamburger.

Comminuted turkey may also serve as the vehicle carrying organisms directly from the animal to a food-preparation establishment or indirectly, via the hands of the kitchen worker, to another food product which may be subsequently consumed in an uncooked state.

Indirect aspects of transmission become important when one considers the percentage of samples (see Table 1) yielding isolates of *Enterobacter* sp., *Klebsiella* sp., *S. aureus, C. perfringens,* and *Salmonella* sp.

The *Klebsiella-Enterobacter-Serratia* group of organisms, implicated as the predominant group of *Enterobacteriaceae* on spoiled poultry (*14*), represented 52% of the *Enterobacteriaceae* isolates from this study. *Klebsiella pneumoniae,* isolated from 64% of the samples studied, can be commonly associated with human respiratory and genitorinary infections (*8*). *Enterobacter cloacae,* found in 69% of the samples, has been associated with bacteremias as well as urinary tract infections (*8*).

Most *Salmonella* isolates in this study were *S. enteroditis* bioser. *pullorum,* an organism characterized as having a low level of infectivity for man (*20, 21*). *S. enteriditis* ser. *St. Paul, S. enteriditis* bioser. *Miami, S. enteriditis* ser. *Derby* and *S. arizonae* isolated in this study are all readily capable of causing human infection (*4, 21*).

Coagulase-positive staphylococci, recovered from 80% of the samples, can suggest the existence of carrier states among the birds and the personnel processing the product. Since *S. aureus* is known to be a weak competitor for growth in the presence of large numbers of other bacteria, it is doubtful that this product would be involved in outbreaks of staphylococcal intoxication. However, if sodium chloride were added and the product subsequently mishandled through lack of refrigeration, the rapid rate of cell replication might result in a product containing large numbers of enterotoxin producing staphylococci. This product would remain harmful even after cooking as some of the enterotoxins have been characterized as heat stable (*10*).

Clostridia are commonly found in our environment and their wide distribution in poultry processing plants has been demonstrated (*26, 28*). These organisms enter the slaughter plant on the feet, feathers, intestinal contents, and dirt associated with live birds. After scalding and plucking, however, few of these organisms can be recovered from the fowl surface (*28*). Lillard (*25*) has demonstrated that submersion of poultry in scald tanks can result in internal contamination of carcasses. She found that internal carcass contamination is not

washed off and may not be as easily destroyed by cooking as are surface contaminants.

By using enrichment techniques and five, 1 to 2-g portions of meat, *C. perfringens* was recovered from 52% of the samples. By the direct plating techniques, plating a 0.2-g sample with SPS agar, *C. perfringens* was not detected in any of the samples. Lack of isolation by this method may be due to low levels of contamination in conjunction with a small sample size, cell injury sustained during exposure to high and low temperatures, and increased susceptibility to Sulfadiazine and Polymyxin B. Of the *C. perfringens* isolates obtained in this study, 36 of 36 strains typed were identified as type A by the serum neutralization mouse assay method. The remaining three strains were lost through a laboratory accident, before typing.

Several bacterial organisms have, from time to time, been proposed for use as indicators of the hygienic quality in food products. As can be seen in the literature, none of these has met all criteria established for indicator organisms. With this in mind and knowing that enteroviruses have been isolated from samples of comminuted beef (*34*), it was decided to screen turkey samples for the presence of enteroviruses as possible indicators of contamination. Tissue culture under fluid media was the assay system chosen. This culture system is sensitive to a wide variety of viruses, observable for a long period, and may be carried through several passages. Failure to detect virus present in any of the samples may have been due to either the small sample size chosen for analysis or the absence of virus in the product.

Poultry products with high bacterial counts have been termed undesirable as far as keeping quality, public health aspects, and general esthetic principles are concerned (*3, 26*). Comminuted turkey meat purchased fresh at the retail level is a product with a high degree of bacterial contamination, limited keeping quality, and the ability to convey salmonellae and other enteric bacilli, staphylococci, and clostridia to the surfaces of equipment and the hands of kitchen workers in homes, hospitals, and factories.

ACKNOWLEDGMENTS

The authors express their gratitude to Richard Okoluk, John Dacey, Joseph Higuera, Matthew Leatherwood, and James Foster for their technical assistance and to Karen Trefz for the typing of the manuscript.

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J. Milk Food Technol. Vol. 39, No. 12, Pages 830-836 (December, 1976)

Destruction of Food Spoilage, Indicator and Pathogenic Organisms by Various Germicides in Solution and on a Stainless Steel Surface[1]

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(Received for publication December 22, 1975)

ABSTRACT

Representative food spoilage, indicator, and pathogenic organisms were exposed to newly developed imported iodophors and widely used representative domestic germicides that included iodophors. The organisms used in these experiments included *Salmonella derby, Escherichia coli,* yeast of the genus *Candida, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus lactis,* and spore of *Bacillus licheniformis.* Results of the germicide experiments showed generally similar effectiveness by iodophor and hypochlorite. Both were superior to the quaternary ammonium compounds (QAC) when used at much lower concentrations (12.5 and 25 for the halogens and 50 and 200 ppm for the QAC). The iodophors exhibited a consistently greater rate of destruction of yeast cells than the hypochlorite when low concentrations of germicide were used. A procedure to more closely simulate actual use of germicides in food and dairy industries also was employed in this study. Special polished metal strips were inoculated with organisms and exposed to an iodophor, a sodium hypochlorite, and a QAC for 15, 30, 60, and 300 sec. Again the halogen compounds were superior to the QAC in destroying the bacterial cells, especially against gram-negative species including *Salmonella.*

The practical application of any germicide is dependent on several considerations including bactericidal activity against various micro-organisms, degree of cleanliness of the surface, compatibility with associated or added compounds, and technique used in applying the disinfectant.

Many test methods have been proposed which were designed to demonstrate germicidal efficacy under actual use conditions. The number of procedures are too numerous to review each. However, a brief summary of some of the work should be mentioned.

Much of the early work with quaternaries demonstrated that the standard Food and Drug Administration phenol coefficient test for determining germicidal activity is not a suitable method for testing these compounds. As a result, many investigators attempted to develop a modification of the FDA method or in many instances a totally new procedure.

Stedman et al. (*12*) in a series of studies demonstrated the efficacy of several types of disinfectants on stainless steel surfaces. All of the products tested showed adaquate bactericidal activity when increased concentrations were used.

In other tests (*13*) these workers showed, as may be expected, that longer exposure times are necessary to reduce significantly the microbial populations on porous surfaces than nonporous surfaces and that the overall efficacy of disinfection obtained on a porous surface is a function of the particular germicide employed.

Vinson and Dickinson (*14*), in studies utilizing a variety of surfaces, demonstrated that various products representing disinfectants and a sanitizer were similar in action on hard surfaces like steel, painted plasterboard, and ceramic tile, but the products differed in their action on porous surfaces. These results are typical of much of the work done testing disinfectants vs. microorganisms on various surfaces.

This study was undertaken to compare several newly developed imported iodophors with five widely used domestic preparations, including two iodophors, a sodium hypochlorite, and two quaternary ammonium compounds. The organisms tested were representatives of spoilage types, pollution indicators and foodborne pathogens.

An attempt also was made to develop a procedure that more closely simulates actual use of germicides in food and dairy industries. Most dairy processing equipment, pipelines and vats, as well as many surface areas in meat and poultry plants are constructed of stainless steel. Using a modification of the method suggested by the American Association of Analytical Chemists for seeding penicillin cups (*2*), special polished stainless steel strips were inoculated with organisms and exposed to different concentrations of germicides for various time periods.

MATERIALS AND METHODS

Bacterial species tested included: *Salmonella derby* and *Staphylococcus aureus* which represent foodborne pathogens; spores of *Bacillus licheniformis* which show high resistance to germicides;

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Streptococcus lactis, a typical dairy contaminant; yeast of the genus *Candida* was used as a food spoilage yeast. *Escherichia coli* represented enteric bacteria used as an indicator of fecal contamination in food and water. *Pseudomonas aeruginosa*, responsible for skin infections in man and animals was known to be similar to germicidal characteristics to *Pseudomonas florescens*, a common psychrotroph associated with spoilage of perishable refrigerated foods. The *Staphylococcus, Pseudomonas, Micrococcus, Escherichia, Bacillus,* and *Candida* species had been isolated from meat and poultry processing plants. The *S. lactis* and *S. derby* were from the OSU stock culture collection and had previously been isolated from food products.

With the exception of *S. lactis*, the bacteria and yeast were inoculated on bottle slants prepared by using Standard Methods (SM) agar (*1*) and incubated for 18 to 24 h. Lactic agar (*4*) was used for *S. lactis*. Spores were obtained by inoculating 2 liters of Schaeffer's sporulation medium (*10*) with *B. licheniformis* and incubating on a shaker for 6 days at 30 C. The culture was centrifuged, spores collected, resuspended in physiological saline solution, and recentrifuged. The suspension was washed three times in this manner and finally diluted in physiological saline solution to obtain the desired concentration for the germicide tests. Spore suspensions were heat shocked before each test. The bacteria were washed off the bottle slants with sterile phosphate-buffered water adjusted to pH 7.2 (*1*) and filtered through sterile Whatman no. 2 filter paper. Turbidimetric adjustment yielded organism counts of approximately 10×10^9/ml. One ml of this suspension into 99 ml of germicide solution gave approximately 100×10^6 cells/ml.

The germicides studied included a sodium hypochlorite (NaClO); two quaternary ammonium compounds (QAC), one, an n-alkyl dimethyl dichloro-benzyl ammonium chloride (QAC-B), the other an n-alkyl dimethyl ethyl-benzyl ammonium chloride with 30% phosphoric acid (QAC-A). Three iodophors which represented combination detergent sanitizer products consisting of nonionic wetting agent combined with iodine were imported products and designated as Iodophor A, Iodophor B, and Iodophor C. Two were domestic products, Iodophor D consisting of nonylphenoxypoly (ethylenenoxy) ethanol iodine comples with 6.75% phosphoric acid and 14.0% glycolicacid, and Iodophor E consisting of butoxypolypropoxy polyethoxy ethanoliodine complex with 6.5% phosphoric acid.

The Chamber's method (*3*) of evaluating bactericidal agents was used in this study with several modifications. After the desired exposure periods, a 1-ml sample of the germicide-organism mixture was transferred into petri dishes containing 2 ml of the appropriate inactivator. Germicides were inactivated by using the method of Humphreys and Johns (*9*). Plates were slightly tilted to insure that all of the germicide solution would mix directly with the neutralizer. The contents were then further mixed immediately by swirling, and agar was poured. The available iodine in the iodophors was determined by titration to a colorless end point with standard thiosulfate. The iodometric method (*1*) was used to determine the amount of available chlorine in sodium hypochlorite. The concentration of the QAC compounds was assayed by the method of Furlong and Elliker (*6*).

Germicide solutions were tested in distilled water and U.S.D.A. buffered synthetic hard water, 500 ppm $CaCo_3$ (*5*). The iodophors and hypochlorite were used in concentrations of 12.5 and 25 ppm. QACs were tested at 25, 50, 100 and 200 ppm. Higher concentrations (200 and 300 ppm) were used in spore tests. Germicide trials were done at 25 C.

METAL STRIP PROCEDURE

A procedure to more closely simulate actual use of germicides in food and dairy industries was attempted in this study. Much of the surface that foods contact consists of metal, and it is of great importance that these areas be effectively sanitized.

TABLE 1. *Destruction of* Salmonella derby *by iodophor, hypochlorite, and QAC germicides*

Germicide	Conc. ppm	Test water	pH Germicide solution	Average number of surviving organisms[a]			
				15 sec	30 sec	60 sec	300 sec
Iodophor A	12.5	Distilled	3.40	0	0	0	0
	12.5	USDA[b]	6.70	TNC	0	0	0
	25	Distilled	3.05	0	0	0	0
	25	USDA	6.40	0	0	0	0
Iodophor B	12.5	Distilled	2.75	TNC	21	2	0
	12.5	USDA	3.30	TNC	TNC	TNC	0
	25	Distilled	2.50	TNC	11	0	0
	25	USDA	2.70	TNC	TNC	TNC	0
Iodophor C	12.5	Distilled	3.20	60	2	0	0
	12.5	USDA	6.90	TNC	TNC	TNC	TNC
	25	Distilled	2.90	0	0	0	0
	25	USDA	6.25	TNC	TNC	TNC	TNC
Iodophor D	12.5	Distilled	3.30	3	0	0	0
	12.5	USDA	6.70	79	2	0	0
	25	Distilled	3.05	0	0	0	0
	25	USDA	4.80	0	0	0	0
Iodopher E	12.5	Distilled	3.50	0	0	0	0
	12.5	USDA	6.70	2	0	0	0
	25	Distilled	3.25	0	0	0	0
	25	USDA	6.70	2	0	0	0
Hypochlorite	12.5	Distilled	5.60	0	0	0	0
	12.5	USDA	7.20	2	0	0	0
	25	Distilled	6.25	0	0	0	0
	25	USDA	7.20	0	0	0	0
QAC-A	50	Distilled	3.00	TNC	TNC	TNC	TNC
	50	USDA	5.92	TNC	TNC	TNC	TNC
	100	Distilled	2.80	TNC	TNC	TNC	TNC
	100	USDA	3.65	TNC	TNC	TNC	0
QAC-B	50	Distilled	4.50	11	3	0	0
	50	USDA	6.30	TNC	TNC	TNC	TNC
	100	Distilled	5.20	0	0	0	0
	100	USDA	7.70	TNC	TNC	TNC	TNC

[a]Initial number of cells 29.5×10^9 per ml.
[b]U.S. Department of Agriculture, 500 ppm ($CaCO_3$).

The metal strips inoculated in this procedure consisted of special 18 gauge, 1 × 3 inch, 304 no. 7 mirror finish metal with deburred edges and 1/8 inch hole 3/8 inch from one end. They were individually wrapped in aluminum foil and sterilized with the other materials in the autoclave.

Test organisms used included *E. coli, P. aeruginosa, S. aureus, S. derby,* and yeast of genus *Candida.* The germicide solutions were transferred into individual sterile Coplin staining jars before each experiment. The iodophor was used at concentrations of 25 and 50 ppm, the hypochlorite at 50 and 100 ppm, and the QAC at 50 and 200 ppm.

Germicides and organisms were prepared via the Chambers method (*3*) of evaluating bactericides. A modification of the AOAC method (*2*) for inoculating stainless cylinders was used for inoculating the strips. Filtered organism suspensions were transferred into a sterile duplex staining dish capable of holding 20 metal strips. Sufficient filtrate was added to completely cover the strips. Using flamed stainless steel forceps, the metal strips were placed in the organism suspension. After a 15-min contact period, strips were removed using forceps and supported individually on glass rods in petri dishes to allow for evaporation of excess fluid from both sides of the slides and further dried at 37 C for 30 min. The inoculated strips were then aseptically removed from the dishes using flamed forceps and placed in Coplin jars containing the germicide solutions. After the desired exposure periods, strips were placed in centrifuge bottles containing 99 ml of appropriate inactivator. Each bottle was shaken vigorously 50 times and 1 ml of this inactivator-organism solution was transferred into petri plates and agar was poured. The metal strips were exposed for 15, 30, 60, and 300 sec.

RESULTS

The overall variation in rate of activity between different iodophors is shown in Table 1 and lower rate of destruction in most QAC trials were considered significant in view of the interest in *Salmonella* contamination of food handling equipment and foods. The pH of the germicidal solution appeared to play a role in affecting some iodophor and QAC compounds in these tests. Some manipulation of QAC activity through pH adjustment might be possible as pointed out by Soike and Elliker (*11*) but the effect of pH varies with species of organism involved. The pH values of distilled and hard water varied considerably. Iodophor C at pH 6.0 and above was consistantly ineffective against test organisms in suspension. However, at lower pH values (2.8 to 3.2) it showed germicidal activity similar to the other iodophors. QAC-B was also more effective at lower pH values especially against *Salmonella.* The hypochlorite and iodophors A, D, and E appeared consistently most active against *S. derby.* Iodophors B and C and QAC-B appeared to be affected adversely by hard water salts. In most test solutions the buffered water resulted in a significantly high pH in use dilution. As might be anticipated, results with *E. coli* shown in Table 2 were, in general, similar to those of *S. derby.* It is important in evaluating such data to consider the general pattern of destruction as exceptions and skips are difficult to avoid partly because of the low levels of bactericide, variation in pH of solutions, and numbers of organisms recovered.

TABLE 2. *Destruction of* Escherichia coli *by iodophor, hypochlorite and QAC germicides*

Germicide	Conc. ppm	Test water	pH Germicide solution	Average number of surviving organisms[a]			
				15 sec	30 sec	60 sec	300 sec
Iodophor A	12.5	Distilled	3.51	TNC	0	0	0
	12.5	USDA[b]	7.31	TNC	TNC	150	0
	25	Distilled	3.20	3	0	0	0
	25	USDA	6.91	TNC	TNC	2	0
Iodophor B	12.5	Distilled	2.59	TNC	TNC	TNC	0
	12.5	USDA	3.00	TNC	TNC	TNC	0
	25	Distilled	2.39	TNC	TNC	TNC	0
	25	USDA	2.50	TNC	TNC	TNC	1
Iodophor C	12.5	Distilled	3.25	TNC	TNC	0	0
	12.5	USDA	6.69	TNC	TNC	TNC	TNC
	25	Distilled	2.89	TNC	3	0	0
	25	USDA	6.25	TNC	TNC	TNC	TNC
Iodophor D	12.5	Distilled	3.10	TNC	0	0	0
	12.5	USDA	6.59	TNC	264	0	0
	25	Distilled	3.01	23	0	0	0
	25	USDA	4.10	TNC	0	0	0
Iodophor E	12.5	Distilled	3.31	TNC	0	0	0
	12.5	USDA	7.61	TNC	0	0	0
	25	Distilled	3.20	3	0	0	0
	25	USDA	6.90	TNC	1	0	0
Hypochlorite	12.5	Distilled	7.90	TNC	27	0	0
	12.5	USDA	7.70	1	0	0	0
	25	Distilled	7.75	0	0	0	0
	25	USDA	8.60	0	0	0	0
QAC-A	50	Distilled	3.00	TNC	TNC	TNC	TNC
	50	USDA	6.20	TNC	TNC	TNC	TNC
	200	Distilled	2.60	TNC	TNC	0	0
	200	USDA	2.65	TNC	0	0	0
QAC-B	50	Distilled	8.18	TNC	TNC	29	7
	50	USDA	8.39	TNC	TNC	TNC	TNC
	200	Distilled	5.22	0	0	0	0
	200	USDA	7.45	TNC	17	3	0

[a]Initial number of cells, 8×10^9 ml.
[b]U.S. Department of Agriculture, 500 ppm ($CaCO_3$).

The iodophors destroyed all yeast cells within 15 sec at concentrations of 12.5 ppm in soft water (Table 3). The sodium hypochlorite at the low concentrations used in these trials was particularly ineffective against the yeast cells requiring 5 min of exposure for total kill. However with higher concentrations of bactericide hypochlorites were comparatively more active against the yeast (Table 6). QAC-B at 50 ppm also required 5 min of exposure for complete destruction and QAC-A was less effective.

S. aureus (not shown) was one of the more resistant organisms tested. At 12.5 ppm, 5 min of exposure was required for total kill by the iodophors. With sodium hypochlorite at 25 ppm 60 sec of exposure was required for complete destruction. When QAC-B was used in distilled water, the destruction time was 5 min and 60 sec with 25 and 200 ppm, respectively.

The iodophors and hypochlorite at 12.5 ppm proved to be very effective against *Pseudomonas*. QAC-A at 200 ppm killed all cells within 15 sec. QAC-B at that same concentration required a 60-sec exposure.

The iodophors with the exception of Iodophors B and C were more effective against *S. lactis* when used in U.S.D.A. hard water. In hard water 12.5 ppm reduced or completely destroyed all cells. The same concentration in distilled water required 5 min of exposure. QAC-A at 25 ppm was also more effective in hard water causing total kill within 5 min. QAC-B at 25 ppm in distilled water destroyed all *S. lactis* cells within 30 sec. In hard water the killing time was extended to 60 sec. Hypochlorite at 12.5 and 25 ppm destroyed all cells of *S. lactis* within 60 sec. Rate of destruction by iodophors A, D, and E was comparable to that of hypochlorites. Iodophors B and C again were slower in activity as was QAC-A.

Spores of *B. licheniformis* were tested against all eight germicides at higher concentrations (200 and 300 ppm) and exposure time was extended to a maximum of 20 min. The results reflected the high resistance of spores to all germicides tested. Only two of the products tested exerted any destructive effect on the spores under these test conditions. Sodium hypochlorite at 200 ppm destroyed all spores within 20 min in distilled water and substantially reduced the number of viable spores after 10 min of exposure. Iodophor C was the only iodine preparation that showed any destruction of spores causing total kill after 20 min in distilled water. QAC was relatively ineffective against the spores.

Studies by Hays (*8*) demonstrated that hypochlorites could be manipulated by lowering pH to provide a more effective germicide against spores. Reduction of pH from 9.0 to 4.0 generally resulted in greatly accelerated activity of hypochlorites against spores of *Bacillus subtilis* (*globigii*), but it was not possible to accelerate activity of

TABLE 3. *Destruction of yeast of the genus* Candida *by iodophor, hypochlorite, and QAC germicides*

Germicide	Conc. ppm	Test water	pH Germicide solution	Average number of surviving organisms[a]			
				15 sec	30 sec	60 sec	300 sec
Iodophor A	12.5	Distilled	3.14	0	0	0	0
	12.5	USDA[b]	6.80	18	1	0	0
	25	Distilled	2.90	0	0	0	0
	25	USDA	6.30	0	0	0	0
Iodophor B	12.5	Distilled	2.40	0	0	0	0
	12.5	USDA	3.02	254	4	0	0
	25	Distilled	2.71	1	0	0	0
	25	USDA	2.60	TNC	0	0	0
Iodophor C	12.5	Distilled	3.06	0	0	0	0
	12.5	USDA	6.80	TNC	TNC	TNC	TNC
	25	Distilled	2.80	0	0	0	0
	25	USDA	6.00	TNC	TNC	TNC	5
Iodophor D	12.5	Distilled	3.10	0	0	0	0
	12.5	USDA	3.30	110	0	0	0
	25	Distilled	2.90	0	0	0	0
	25	USDA	4.00	5	0	0	0
Iodophor E	12.5	Distilled	3.31	0	0	0	0
	12.5	USDA	7.30	0	0	0	0
	25	Distilled	3.10	0	0	0	0
	25	USDA	6.80	0	0	0	0
Hypochlorite	12.5	Distilled	5.50	TNC	TNC	TNC	0
	12.5	USDA	8.00	TNC	TNC	TNC	0
	25	Distilled	6.50	TNC	TNC	56	0
	25	USDA	8.10	TNC	TNC	TNC	0
QAC-A	50	Distilled	3.20	TNC	TNC	TNC	TNC
	50	USDA	7.00	TNC	TNC	TNC	2
	200	Distilled	2.65	TNC	TNC	TNC	TNC
	200	USDA	3.50	TNC	TNC	4	0
QAC-B	50	Distilled	5.60	152	15	1	0
	50	USDA	8.00	TNC	TNC	TNC	3
	200	Distilled	5.90	20	0	0	0
	200	USDA	8.81	37	0	0	0

[a]Initial number of cells, 3×10^8 ml.
[b]U.S. Department of Agriculture, 500 ppm ($CaCO_3$).

iodophors or quaternaries sufficiently by pH manipulation to produce destruction approaching that of hypochlorites when this organism was used as a test agent in a concentration of approximately 10 to 20 × 10^4. Results in the present study suggest the same trend except for Iodophor C which, for some unexplained reason, showed greater destruction than anticipated based on its effect against other organisms. It should be pointed out also that the iodophors and quaternaries were used in the present studies in higher concentrations than usual use dilution while the hypochlorite was used at 200 ppm. The hypochlorite still, therefore, would be considered the germicide of choice for destruction of bacterial spores, an observation that was noted previously in the study by Hays.

Tables 4 through 8 show the destruction rates of an iodophor, a hypochlorite, and a quaternary ammonium compound against organisms inoculated on specially polished metal strips.

All of the test organisms, when inoculated on metal strips, showed greater resistance to each class of disinfectant. In all but one instance, the concentration of product and the exposure period had to be increased to show results similar to those seen in the first part of this study. The exception was hypochlorite against *Candida.* The QAC was consistently less effective requiring high concentrations and longer exposure periods to approximate the bactericidal activity of the other germicides tested.

TABLE 4. *Destruction of* Salmonella derby *on metal strips*

Germicide	Conc. ppm	Test water	pH of Germicide solution	Average number of surviving cells[a]			
				15 sec	30 sec	60 sec	300 sec
Iodophor E	25	Distilled	2.90	TNC	TNC	122	3
	25	USDA	4.00	TNC	TNC	TNC	25
	50	Distilled	2.70	TNC	TNC	245	0
	50	USDA	3.11	TNC	TNC	TNC	16
Hypochlorite	50	Distilled	7.25	TNC	76	206	5
	50	USDA	7.90	TNC	TNC	TNC	12
	100	Distilled	7.81	219	164	14	0
	100	USDA	8.40	TNC	TNC	39	0
QAC-B	50	Distilled	7.85	TNC	TNC	TNC	TNC
	50	USDA	8.65	TNC	TNC	TNC	TNC
	200	Distilled	6.61	TNC	TNC	TNC	30
	200	USDA	8.60	TNC	TNC	TNC	TNC

[a]Average number of cells per strip, before exposure 1.6 × 10^7.

TABLE 5. *Destruction of* Eschrichia coli *on metal strips*

Germicide	Conc. ppm	Test water	pH of Germicide solution	Average number of surviving cells[a]			
				15 sec	30 sec	60 sec	300 sec
Iodophor E	25	Distilled	3.10	TNC	318	274	6
	25	USDA	4.15	TNC	TNC	TNC	2
	50	Distilled	2.70	TNC	TNC	6	2
	50	USDA	3.38	TNC	TNC	37	10
Hypochlorite	50	Distilled	6.91	TNC	8	4	4
	50	USDA	7.51	TNC	TNC	53	4
	100	Distilled	8.14	17	2	1	0
	100	USDA	8.43	19	6	2	0
QAC-B	50	Distilled	8.27	TNC	TNC	TNC	256
	50	USDA	8.85	TNC	TNC	TNC	TNC
	200	Distilled	7.55	TNC	TNC	431	16
	200	USDA	8.60	TNC	TNC	TNC	TNC

[a]Average number of cells per strip, before exposure 2.1 × 10^7.

TABLE 6. *Destruction of yeast of the genus* Candida *on metal strips*

Germicide	Conc. ppm	Test water	pH of Germicide solution	Average number of surviving cells[a]			
				15 sec	30 sec	60 sec	300 sec
Iodophor E	25	Distilled	2.90	TNC	8	20	6
	25	USDA	4.31	TNC	TNC	TNC	0
	50	Distilled	2.62	TNC	14	17	0
	50	USDA	3.38	116	9	0	0
Hypochlorite	50	Distilled	7.35	TNC	4	0	0
	50	USDA	7.50	151	4	2	0
	100	Distilled	8.25	25	5	0	0
	100	USDA	8.61	8	1	0	0
QAC-B	50	Distilled	7.80	TNC	TNC	TNC	51
	50	USDA	8.60	TNC	TNC	TNC	39
	200	Distilled	6.85	TNC	TNC	130	0
	200	USDA	8.59	TNC	TNC	148	10

[a]Average number of cells per strip, before exposure 7.7 × 10^5.

DISCUSSION

The eight different germicides in this study consisted of widely used commercial products and, therefore, were representative of common types available on the market. The two domestic iodophors, Iodophor D and E, showed high destruction rates in all tests. Both were very effective against all vegetative cells except *S. aureus* which was the most resistant vegetative bacterial culture tested. Iodophor C was also effective when used in distilled water, but in almost every test this compound was less effective in USDA hard water (500 ppm $CaCO_3$). The least effective iodophor was Iodophor B. Since iodine content and pH in use dilution of the different iodophors approximate each other, the difference in germicidal activity may be related to nonionic surface active agent complexes with iodine and consequently rates of release of iodine in use dilution. The imported products are compounded as much for detergent as for bactericidal activity.

The hypochlorite in low concentrations (12.5 ppm) was very effective against most vegetative cells causing complete destruction in less than 5 min of all bacterial cells except *S. aureus*. The iodophors exhibited a consistently greater rate of destruction of yeast cells than the hypochlorite especially at low bactericide concentrations. Possibly, the large cell mass of yeasts created a greater chlorine demand that had to be satisfied before the chlorine could destroy the yeast cells. Hypochlorites generally were the most effective germicides tested against spores.

Quaternary ammonium compounds (QAC) were the least effective germicides used in this study. The QACs, in most instances, showed significant bactericidal activity only at the highest concentration (50 and 200 ppm). Two QACs were tested, QAC-B, a basic quaternary ammonium compound, and QAC-A, an acidic preparation. QAC-B was significantly affected by the presence of 500 ppm $CaCO_3$, particularly against the gram-negative bacteria. *S. derby* showed resistance to QAC-B when used in hard water. At 50 ppm, it was totally ineffective and at 100 ppm, a 5-min exposure was required to kill all *S. derby* cells. The results on *Salmonella* are considered highly significant because of the importance of this type of organism and related species in food products. It is apparent that the hypochlorite and iodophors should provide more efficient destruction of *Salmonella* and related species when used as sanitizers in food plants.

In veiw of the bactericidal efficiency of both hypochlorite and iodophor compounds in low concentrations, either type of germicide appears to be well suited to a wide range of applications in dairy and food processing industry. Hays et al. (*7*) in a study similar to this work, reported that the combined action of hypochlorite followed by iodophor treatment served to apply the advantages of both germicides, effecting nearly

TABLE 7. *Destruction of* Pseudomonas aeruginosa *on metal strips*

Germicide	Conc. ppm	Test water	pH of Germicide solution	Average number of surviving cells[a]			
				15 sec	30 sec	60 sec	300 sec
Iodophor E	25	Distilled	2.95	TNC	TNC	TNC	61
	25	USDA	4.24	TNC	TNC	TNC	22
	50	Distilled	2.70	TNC	55	0	0
Hypochlorite	50	Distilled	7.30	59	19	62	0
	50	USDA	7.70	TNC	105	58	0
	100	Distilled	8.18	12	8	20	0
	100	USDA	8.70	186	22	3	0
QAC-B	50	Distilled	7.29	TNC	TNC	TNC	TNC
	50	USDA	8.60	TNC	TNC	TNC	TNC
	200	Distilled	6.85	TNC	TNC	TNC	TNC
	200	USDA	8.80	TNC	TNC	TNC	TNC

[a]Average number of cells per strip, before exposure 6.0×10^7.

TABLE 8. *Destruction of* Staphylococcus aureus *on metal strips*

Germicide	Conc. ppm	Test water	pH of Germicide solution	Average number of surviving cells[a]			
				15 sec	30 sec	60 sec	300 sec
Iodophor E	25	Distilled	3.00	TNC	TNC	274	0
	25	USDA	4.80	TNC	TNC	TNC	TNC
	50	Distilled	2.70	TNC	TNC	373	62
	50	USDA	3.49	TNC	TNC	TNC	0
Hypochlorite	50	Distilled	7.70	TNC	TNC	13	0
	50	USDA	8.20	TNC	TNC	237	0
	100	Distilled	8.50	225	95	21	0
	100	USDA	8.80	171	31	15	0
QAC-B	50	Distilled	7.09	TNC	TNC	TNC	TNC
	50	USDA	8.83	TNC	TNC	TNC	TNC
	200	Distilled	6.87	TNC	TNC	TNC	0
	200	USDA	8.33	TNC	TNC	TNC	TNC

[a]Average number of cells per strip before exposure 2.0×10^8.

complete destruction of the organisms tested in simulated beverage bottle sanitization treatments.

Results in this study demonstrate the adverse effects that hard water salts exert on antibacterial compounds. Each class of disinfectant showed reduced activity when used in 500 ppm of hard water. This effect was seen when products were tested both in solution and on stainless steel surfaces.

In tests designed to more closely simulate actual use of germicides on food and dairy equipment involving inoculated stainless steel polished metal strips, the iodophor and hypochlorite provided appreciable destruction in almost every case after exposure periods of 1 to 5 min or less. The QAC, used at must higher concentrations (200 ppm), was significantly less effective against organisms tested in these trials. Results with this technique appear to offer promise of a superior method for evaluating germicides for sanitization of food handling equipment and utensils. Possible improvements would be to standardize the number of organisms used in the inoculating suspension, the treatment of the slides between inoculation and exposure to germicide, and to develop a better method of removing the organisms from the strips.

This method also has the great advantage of using concentrations of germicides actually employed to sanitize metal surfaces of equipment and utensils and, therefore, provides a more accurate picture of effects of germicides under actual operating conditions. Tests done with lower concentrations than are used in plant practice always leave some doubt as to efficiency of germicides under actual use conditions.

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J. Milk Food Technol. Vol. 39, No. 12, Pages 837-840 (December, 1976)

The Microflora of Parsley

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(Received for publication May 4, 1976)

ABSTRACT

Commercially available parsley is a heavily contaminated product. The possible public health significance of this observation is discussed. Parsley with the roots intact is also heavily contaminated. However, microorganisms indicative of fecal contamination could be found much less frequently and less numerously. For this reason it is concluded that standards for *Escherichia coli* and/or D-streptococci might serve as a useful public health precaution.

Parsley belongs to those herbs, which are very commonly used in the preparation of commercial and homemade foods. In various types of food it is, as a rule, added to the food after cooking. Therefore, the parsley itself is not subjected to any heat treatment; the microbiological load of it is neither killed nor reduced. Most food, to which parsley is added, can, because of available nutrients, be an almost ideal medium for microbial proliferation. In many instances, this food will be stored at temperatures which will allow, if not encourage, this proliferation. Presumably this is especially true for food service operations catering to a large number of people.

In this study, we have examined the microbiological condition of parsley to find out whether the microbial load of it might be of any significance, from the public health point of view. Another reason for undertaking this study was the claim of one branch of the food industry that vegetables and products like parsley would harbor *Enterobacteriaceae* autochtonously; the examination of their products for *Enterobacteriaceae* and/or *Escherichia coli* for the purpose of judging the hygienic condition and wholesomeness of this commodity would therefore make no sense.

EXPERIMENTAL

Material and methods of sampling

Examination of the following specimens was carried out: (*a*) fresh unpacked parsley (11 samples), as obtainable by consumers in retail groceries; the different samples were bought from several different groceries; (*b*) fresh unpacked parsley (9 samples) after washing it under running cold potable water, as it is customarily done by housewives; (*c*) fresh, unpacked parsley after blanching (6 samples); blanching was done by immersing 25 g of parsley in a sieve into 2 liters of boiling water for exactly 60 sec; after that, the parsley was rapidly cooled using cold running potable water; (*d*) frozen parsley in cartons (14 samples), as they are obtainable by consumers in retail groceries out of the freezer; samples were taken from two large food manufacturers; (*e*) dried parsley (12 samples), as it is obtainable by consumers in retail groceries; samples were from four different food manufacturers, (*f*) fresh, unpacked parsley harvested under aseptic conditions by ourselves (65 samples). Two large market gardeners, one in the north west and the other in the south east of Berlin, permitted us to take samples from their crops. Before sowing the parsley seed in March/April, soil had been dressed with poultry and cattle manure. During the growing season, 2-3 top dressings with chemical fertilizers had been applied. Two different surveys were undertaken with this parsley. Twelve samples were tested as described in the chapter "Microbiological Techniques" (microbiological profile). Another 26 samples (about 1 g each) were examined with the P/A-test for *E. coli* Type I and the remaining 27 samples (about 1 g each) were examined with the P/A-test for D-streptococci (indicator organisms).

Microbiological techniques

Twenty-five gram aliquots of each sample were placed into a flask containing 225 ml of dilution fluid (*9*). Maceration was carried out using a mechanical blender (Ultra Turrax) for about 45 to 60 sec at 2000 revolutions/min. The time of 30 to 45 sec, as suggested by Barrand et al. (*1*) for maceration of meat and meat products was not sufficient to grind parsley, especially its stems. From this 1:10 stock dilution further decimal dilutions were prepared as appropriate to the expected degree of contamination.

Aerobic mesophile colony count. Using the pour-plate-method, 1 ml of suitable dilutions were plated in duplicate using Plate-Count-Agar (Oxoid CM 325) and incubated for 3 days at 30 ± 1 C.

Enumeration of Enterobacterioceae. Using the pour-plate-method, 1 ml of suitable dilutions were plated in duplicate using Violet-Red-Bile-Glucose- Agar (VRBG), (Difco 0012-01 plus 1% Glucose). After solidification the plates were covered with a second layer of VRBG to suppress strictly aerobic gram negative rods (*9*), and incubated for 18-24 h at 30 ± 1 C. Purple colonies, larger than 0.5 mm and surrounded by purple halos were counted.

Enumeration of yeasts and molds. Using the pour-plate-method, 1 ml of suitable dilutions were plated in duplicate using Oxytetracycline Glucose Yeast Agar (*5*), and incubated for 5 days at 24 ± 3 C.

Presnce/Absence-test(P/A) for Escherichia coli Type I (E. coli). Ten milliliters of the stock dilution and 1 ml of other suitable dilutions were suspended in 90 and 9 ml of resuscitation broth (CASO broth, Merck No. 5459), respectively. Repeated shaking of this suspension at room temperature (24 ± 3 C) for about 6 h was followed by adding 100 and 10 ml of double concentrated enrichment medium (Brilliant green-Bile-Lactose-Broth, Oxoid No. CM 31), respectively. This mixture was incubated for 18-24 h at 30 ± 1 C.

Cultures showing turbidity were subcultured in pre-warmed tubes containing the above mentioned enrichment medium and Tryptone-Water (*10*) and immediately transferred to a water bath at 44 ± 0.1 C for incubation for 24 to 48 h.

Only those isolates were identified as *E. coli* which showed the IMVEC pattern ++–+– (*4*), and which were LDC-positive and H_2S-negative (using Costin's LSA-medium, Merck No. 5266). Two strains of H_2S-positive *E. coli* were also regarded as being Type I.

P/A-test for D-streptococci. Duplicate aliquots of 1 g of material and 1 ml of suitable-dilutions were transfered into 9 ml of Streptococcus Enrichment Broth (*5*) and incubated for 24 h at 37 C. If blackening of the medium was observed, subculturing onto Streptococcus Confirmatory Agar (*5*) was done. Presence of enterococci was recorded if tiny colonies surrounded by black halos were to be seen on plates after 24 h of incubation at 37 C.

P/A-test of sulfite reducing clostridia. Duplicate aliquots of 1 g of material and 1 ml of suitable dilutions were transferred into tubes with Kelch's Liver Broth (Merck No. 5464) which were sealed with sterile paraffin and incubated for 24 h at 37 C. About 0.5 ml of broth from tubes showing gas production were transferred into new tubes and mixed with about 5 ml of SPS-Agar (Merck, No. 10235), tempered at approximately 48 C. After sealing with sterile paraffin, tubes were incubated for 24-48 h at 37 C. Blackened tubes were regarded as being positive for sulfite reducing clostridia.

P/A-test for Salmonella. Twenty-five grams of parsley were placed into a flask containing 225 ml of buffered peptone water and incubated for 18-24 h at 37 C. Suplicate 100-ml portions of this pre-enrichment broth were subcultured in pre-warmed Muller-Kauffmann-broth (Oxide CM 343) with incubation at 43 ± 1 C. After 18-24 h and 48 h subculturing was done onto Brilliant green-Phenol red-Lactose-Saccharose Agar (in petri dishes having a diameter of 15 cm) (Oxoid CM 7237) and Water blue-Metachromyellow-Lactose-Agar (Merck No. 1282). Incubation was at 37 C for 24 h. Suspect colonies were tested serologically with officially approved *Salmonella* antisera.

P/A-test for coagulase positive staphylococci. Duplicate aliquots of 1 g of material and 1 ml of suitable dilutions were transferred into tubes with 15 ml of enrichment broth (*3*) which were incubated for up to 48 h at 37 C. If blackening of the medium was observed after incubation, subculturing onto Baird-Parker's Medium (Oxoid CM 2751) and incubation for 24 h at 37 C was carried out. Typical colonies that were coagulase-positive were enumerated as staphylococci.

For dried parsley, as described under "Experimental" (*e*), the amount of material used was 1/5 that of fresh parsley (water content of fresh parsley is roughly 80% of the total weight). In this way, the bacteriological data refer to 1 g of rehydrated parsley.

RESULTS

Salmonellae and coagulase-positive staphylococci were not isolated from any of the specimens tested.

Table 1 summarizes the microbiological profile of the various kinds of parsley tested. Fresh parsley, as it is obtainable by consumers in retail shops, is a heavily contaminated product. Fecal contamination (*E. coli*) was present in all of the 11 samples tested. Rinsing the parsley, as is customarily done by housewives, had only little influence on the microbiological load of the product. Even the fecal contamination was demonstrable in two thirds of the rinsed specimens.

Blanching, however, markedly reduced the microbiological load and converted the product into less contaminated food. Frozen parsley was a heavily contaminated product. In comparison to the fresh parsley, the geometric mean of the enumerated microorganisms was only reduced by one log cycle. More than 50% of the samples showed fecal contamination.

As could be expected, dried parsley appeared to be more hygienic. Most likely, the microorganisms

TABLE 1. *The microbiological profile of parsley*

Samples	Mesophilic aerobic colony count	Count of *Enterobacteriacea*	Count of Yeasts and molds	Titer	Number of samples positive (or negative) for *E. coli*	D-streptococci	Sulfite reducing clostridia
Fresh parsley 11 samples	Min.: 1.4×10^5	1.7×10^4	9×10^3	neg. in 1g	0	0	0
	Max.: 3.7×10^8	2×10^8	7.8×10^5	pos. in 1g	4	—	3
	GM[a]: 3.7×10^7	4.7×10^6	7.4×10^4	pos. in 0.1g	3	—	4
				pos. in 0.01g	2	3	4
				pos. in 10^{-3}g	1	3	0
				pos. in 10^{-4}g	1	3	0
				pos. in 10^{-5}g	0	2	0
Fresh parsely washed 9 samples	Min.: 5.3×10^6	6.8×10^5	3.6×10^3	neg. in 1g	3	0	2
	Max.: 2.9×10^8	8.8×10^7	2.5×10^5	pos. in 1g	—	—	3
	GM: 1.9×10^7	3.8×10^6	4.7×10^4	pos. in 0.1g	2	1	3
				pos. in 0.01g	3	2	1
				pos. in 10^{-3}g	1	5	0
				pos. in 10^{-4}g	0	1	0
Fresh parsely blanched 6 samples	Min.: 1×10^2	$< 10^2$	$< 10^2$	neg. in 1 g	5	4	3
	Max.: 4.4×10^5	2.5×10^4	6×10^2	pos. in 1g	1	1	3
	GM: 5.1×10^3	about 4×10^2	about 10^2	pos. in 0.1g	0	0	0
				pos. in 0.01g	0	1	0
Frozen parsley 14 samples	Min.: 5.1×10^5	1.3×10^4	1×10^2	neg. in 1g	6	0	11
	Max.: 5.0×10^6	1.9×10^6	3.1×10^4	pos. in 1g	2	—	3
	GM: 2.6×10^6	1.8×10^5	7.8×10^3	pos. in 0.1g	3	—	0
				pos. in 0.01g	2	5	0
				pos. in 10^{-3}g	1	8	0
				pos. in 10^{-4}g	0	1	0
Dried parsley 12 samples	Min.: 10^3	10^2	< 10	neg. in 1g	10	3	4
	Max.: 9×10^5	1.7×10^5	1.7×10^4	pos. in 1g	2	—	2
	GM: 5.6×10^4	7.7×10^3	7.5×10^2	pos. in 0.1g	0	4	6
				pos. in 0.01g	0	2	0
				pos. in 10^{-3}g	0	2	0
Fresh parsley aseptically harvested 12 samples	Min.: 3.4×10^4	3.8×10^3	5×10^2	neg. in 1g	10	8	0
	Max.: 2.9×10^6	7.1×10^5	1.6×10^5	pos. in 1g	1	4	—
	GM: 1.8×10^5	3.7×10^4	1.0×10^4	pos. in 0.1g	1	0	9
				pos. in 0.01g	0	0	3

[a]GM = Geometric mean

TABLE 2 *Occurrence of indicator organisms in parsley*

Indicator	Harvested under aseptic conditions			Fresh, washed, and frozen parsley		
	Number of samples examined	Samples positive for / negative for the indicator (in 1 g)		Number of samples examined	Samples positive for / negative for the indicator	
E. coli Type I	38 (100%)	2*[a] (5%)	36 (95%)	35 (100%)	26 (74%)	0 (26%)
D-streptococci	39 (100%)	12 (30%)	27 (70%)	34 (100%)	34 (100%)	0 (0%)

[a] 2* in one sample (No. 106) *E. coli* Type I pos. in 0.1 g.

originally present in the fresh product had gradually died off.

A comparison between the aseptically harvested parsley and the fresh parsley bought in retail shops is of interest. The colony count and the count of *Enterobacteriaceae* was much lower (2 log cycles) in aseptically harvested specimens but there was little difference with regard to the count of yeasts and molds and the number of clostridia. The most significant difference was the occurrence of the indicator organisms *E. coli* and D-streptococci in aseptically harvested parsley and in commercially available parsley (see Table 2): While *E. coli* apparently only occurred in exceptional cases in parsley with the roots intact (contamination probably due to fecal material of wild life origin: birds, rodents), commercially available parsley was frequently (Table 2) and heavily (Table 1) contaminated with this organism. It is concluded that this contamination may be of human fecal origin.

A similar situation prevailed with regard to occurrence of D-streptococci. Only 30% of the samples harvested by ourselves showed this form of contamination while all the commercial specimens were frequently (Table 2) and heavily (Table 1) contaminated.

DISCUSSION

Results obtained indicate that commercially available parsley may indeed harbor a potentially unsafe microflora. The frequent and heavy contamination with *E. coli* should be considered with concern, since there is a good chance for this contamination to be of human origin. In this case, the *E. coli* contamination may also be indicative of other pathogenic organisms of human origin, e.g. *Salmonella* spp., causative agents of infectious hepatitis, etc. In fact, cases of viral hepatitis have already been reported as caused by vegetables (tossed salad, cold sandwiches containing lettuce) (*6, 7*).

It was demonstrated that blanching reduced the microbiological load appreciably. It may safely be assumed that blanching could be recommended as a reasonable process to render this product into safe food.

There seemed to be little difference between fresh parsley and aseptically harvested parsley with regard to the count of yeasts and molds and the number of clostridia. Postharvest handling did not seem to have an impact upon any of these parameters. In contrast to this observation is the fact that there was a 2-log cycle difference in the geometric mean, as already mentioned, with regard to the colony count and the count of *Enterobacteriaceae.* However, both these parameters do not appear to be too useful for hygienic evaluation of the fresh product since counts were high even in aseptically harvested parsley. For this purpose, *E. coli* seems to be more useful. It could be demonstrated in 74% of commerically available parsley samples but only in 5% of aseptically harvested samples of parsley, indicating that contamination may be of human origin (see Table 2). Not as clearcut is the situation with regard to D-streptococci. Although all of the commercially available specimens carried this type of contamination, 30% of the aseptically harvested ones were also positive for D-streptococci (see Table 2). If one wants to use D-streptococci as a hygienic parameter one probably has to determine the number (see Table 1). While the D-streptococcus contamination in commercially available parsley was very substantial, it seemed to be rather minor in aseptically harvested parsley.

CONCLUSION

We are in agreement with Fowler and Foster (*2*) that the definition of microbiological standards or guidelines for fresh vegetable products on the basis of colony count, count of *Enterobacteriaceae* (or coliforms), counts of yeasts and molds and/or number of clostridia seems to be debatable. However, standards for *E. coli* and/or D-streptococci might serve as a useful public health precaution.

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J. Milk Food Technol. Vol. 39, No. 12, Pages 841-844 (December, 1976)

Effect of Loin Quality on Discoloration of Pork Chops During Retail Display

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ABSTRACT

Pork chops from pale and soft loins were extremely susceptible to discoloration during retail display and had very limited retail caselife in comparison to chops from bright grayish-pink and firm loins. Pale and soft chops developed green discoloration on the second or third day of retail display. The green color of affected chops persisted through the interior of the longissimus muscle suggesting that physical or chemical changes, rather than bacterial growth, were responsible for the green pigmentation. Though the mechanism by which such discoloration occurs is not clear, it is apparent that some pale and soft loins react unfavorably to stress associated with storage, shipment, retail cutting, or display. Such loins should be identified at the time of carcass cutting, segregated and directed to an end-use or market outlet which does not necessitate retail display as a fresh product.

Pork muscles vary widely in color, firmness, physical structure, and marbling. Coincidentally, all of these characteristics have been shown to be related to muscle quality, consumer acceptance, palatability, cooking loss, and/or processing yields (*5, 24*). Stringer (*22*) reported that firmness, color, and texture have very limited effects on consumer acceptance; however, other researchers (*4, 7*) concluded that the physical appearance of beef retail cuts in the display case is the most important factor determining retail selection of meat products.

The color of pork plays an important role in determining consumer acceptance (*23*) and is a critical factor in determining the desirability of pork for processing (*9*). The importance of color in determining consumer acceptability has increased during the last decade because of greater use of transparent packages for self-service merchandising of fresh meat (*8*). Firmness of pork muscle is important for good keeping quality and to allow for more uniform response to curing (*9*). The effects of marbling and firmness on consumer acceptability of loin chops have been investigated (*17, 25*) and results indicate that consumers prefer firm, adequately marbled chops to soft, inadequately marbled chops.

Several problems have been associated with pale, soft, and exudative pork: increased processing losses (*3, 18*); decreased palatability (*2, 10*); increased cooking losses (*10, 18*); increased drip losses in retail cuts (*14*); increased incidence of bacterial spoilage (*15*); and decreased color stability (*23*). While conducting studies related to storage-life (*20*) and retail caselife (*19*) of fresh pork cuts, certain loin chops were observed which developed pale green or green muscle colors during retail display. This report relates surface discoloration and retail caselife to quality characteristics (color and physical structure) of fresh pork loins.

MATERIALS AND METHODS

Thirty-six blade or center sections of fresh pork loins were assigned color and structure scores by use of University of Wisconsin (*24*) pork quality standards. Loins were assigned to six quality groups, I, II, III, IV, V and VI on the basis of color and structure scores of 4 and 4, 3 and 4, 3 and 3, 2 and 3, 2 and 2, and 1 and 1, respectively. One chop was removed from each fresh loin section by use of a band saw to provide 36 individual chops. The remaining portion of each section was crust-frozen by one of three procedures: (*a*) completely covering the section with carbon dioxide pellets for 7 min, (*b*) submerging the section in liquid nitrogen for 10 sec, and (*c*) storing the section in a −23 C blast freezer for 90 min. Crust-freezing results in formation of a layer of frozen tissue approximately 3.2 mm in thickness around the entire wholesale cut and is used to assure rigidity of the cut during mechanical cleaving. One chop was removed from each crust-frozen section by use of a Bettcher power cleaver to provide 36 additional chops (12 from each crust-freezing procedure).

Individual chops were placed on styrofoam trays immediately after cutting, and overwrapped with a retail packaging film (polyvinyl chloride). Packaged chops were displayed in a 1 ± 1 C retail case. The lighting interval was 12 h daily of 82 ft-c incandescent light.

A trained panel, consisting of three members, evaluated each chop for overall appearance (8 = extremely desirable; 1 = extremely undesirable) and surface discoloration (8 = no discoloration; 1 = 100% discoloration) daily during 6 days of retail display. Traits considered in assigning overall appearance scores were freshness of external fat, freshness of bone surfaces, as well as color, surface discoloration, and peripheral discoloration of the muscle surfaces.

Psychrotrophic plate counts were obtained, following retail display, from 24 chops. Bacterial samples were obtained by swabbing a 6.45-cm² area (by use of a sterile aluminum template) on the surface of the cut. Aliquots of the initial solution were used to prepare appropriate dilutions for use in enumeration by the pour plate technique. The plates were incubated at 5 C for 7 days. Following retail display, 10-g samples of longissimus muscle were removed from certain chops, freed of all epimysium and subcutaneous fat, and thoroughly blended with 40 ml of deionized, distilled water in a high speed homogenizer. The pH of the meat homogenate was determined with a Corning Model 12 pH meter.

Data were analyzed using analysis of variance (*21*) while mean separation analyses were done using a modification (*11*) of the multiple range test (*6*).

RESULTS AND DISCUSSION

Surface discoloration scores and bacterial counts for chops from loins in each quality group are presented in Table 1. In all six quality groups (I through VI), surface discoloration increased as retail display time increased (from day 1 to day 6). The extent of discoloration during retail display was greatest for chops in groups V and VI and was least for chops in Group II. Adams and Huffman (*1*) reported changes in muscle color of pork chops from the desirable grayish-pink to a less desirable brownish or tan color as storage time increased and they attributed these color changes to metmyoglobin formation.

Chops from loins in quality groups I, II, III, and IV did not differ in surface discoloration after 1, 2, 4, and 6 days of retail display, but had less ($P < .05$) surface discoloration than chops from loins in group VI at all display intervals. Chops from loins in quality group V were light grayish-pink on day 1 and light gray or light tan on the fourth and sixth days of retail display. Chops from quality group VI were very light grayish-green on day 1, light green on day 2, and green to greenish-brown on the fourth and sixth days of retail display. Topel et al. (*23*) compared chops from dark, normal, and pale pork loins and reported that pale chops were the most unstable and developed a greenish-gray cast after 2 to 3 days of retail display.

Discoloration of muscle could result (*12*) from several causes: (*a*) myoglobin can be oxidized to brown metmyoglobin, (*b*) myoglobin can combine with H_2S, produced by bacteria, to form green sulfmyoglobin, (*c*) myoglobin can be degraded to form yellow or green bile pigments by microbially-produced hydrogen peroxide, or (*d*) microorganisms can elaborate foreign pigments which are green, blue, or luminescent. Production of slime, greenish discoloration, fluorescent pigments, white areas, and colored spots have been associated (*13*) with the growth of *Pseudomonas, Achromobacter,* and *Flavobacterium* on fresh meat.

Data in Table 1 indicate that there was no difference in psychrotrophic bacterial counts among chops from loins in the six quality groups. The lack of difference in total bacterial counts (Table 1) does not necessarily preclude bacteria as the source of the green color, since total counts do not account for possible differences in genera, species, or strains on normal versus discolored pork nor do they account for differential rates of bacterial growth. In a recent study (*16*) dark pork chops evidenced more rapid growth of microorganisms than did normal or PSE pork chops.

Immediately following termination of the 6-day retail display, chops of differing color were subjected to detailed examination. Chops which were green or greenish-brown were green on the underside (adjacent to the styrofoam backing board) and the green color persisted through the muscle as successive layers were removed by slicing across the planar surface. Chops which were light gray were likewise light gray on the bottom surface and throughout the longissimus muscle. Chops which were greenish-brown, light tan, brownish-pink, or brown did not evidence browning of interior pigments, suggesting that visual browning was a surface phenomenon, probably associated with development of metmyoglobin. The fact that the green color persisted through the interior of the muscle in green or greenish-brown chops strongly suggests that the green discoloration was of physical or chemical origin, rather than from the metabolic activity of bacteria (especially those which are aerobic). Conversely, limitation of brown discoloration to the outermost surfaces of pork chops supports the idea that oxidation of myoglobin (to form metmyoglobin) was caused by prolonged exposure to oxygen or by the activity of aerobic bacteria.

Development of brown metmyoglobin on the muscle surface during prolonged retail display was of greatest detriment to chops which were light gray or light green in color (perhaps because of the color contrast) and of least consequence to chops which were bright grayish-pink (Table 1). In very pale pork muscle, the very rapid fall in muscle pH exposes myoglobin to conditions which cause denaturation and/or more rapid oxidation to metmyoglobin (*19*). The green discoloration of muscle caused by bacteria results from myoglobin denaturation and destruction in which the heme ring is split from the protein portion of the pigment (*12*). Conversely,

TABLE 1. *Surface discoloration scores and bacterial counts for chops from loins in each of six quality groups*

Quality score[a]			Number of loin sections[b]	Surface discoloration score[c]				Bacterial count[d] (log_{10})
Group	Color	Structure		Day-1	Day-2	Day-4	Day-6	
I	4	4	4	7.1^{e}	6.9^{e}	5.7^{ef}	5.0^{ef}	3.4
II	3	4	6	7.4^{e}	7.1^{e}	6.3^{e}	5.8^{e}	3.9
III	3	3	8	7.3^{e}	7.0^{e}	5.8^{ef}	4.9^{ef}	4.1
IV	2	3	8	7.1^{e}	6.6^{e}	5.7^{ef}	5.0^{ef}	3.8
V	2	2	6	7.0^{e}	6.5^{e}	5.1^{f}	4.2^{f}	3.8
VI	1	1	4	5.9^{f}	4.8^{f}	3.4^{g}	2.7^{g}	3.8

[a]Quality scores were assigned by a three-member panel using pork quality standards of the University of Wisconsin (*24*).
[b]Two chops were obtained from each loin section.
[c]Means based on an 8-point scale (8 = no discoloration, 4 = 50% discoloration, 1 = 100% discoloration).
[d]Mean psychrotrophic counts (log_{10}) per 6.45 cm^2 taken from chops following 6 days of retail display.
[e,f,g]Means in the same column bearing different superscripts are significantly different ($P < .05$).

formation of green pigment verdohemoglobin in blood pigment is an intermediate step in conversion of hemoglobin to biliverdin or bilirubin and is not of bacterial origin (*26*). The latter reaction occurs as a result of denaturation and opening of the heme ring to form verdoglobin or choleglobin, both of which are green.

In the present study, representative chops which were green (n = 4), pale-green (n = 6), light-gray (n = 5), brownish-pink (n = 6), and bright grayish-pink (n = 7) were used for pH analyses. Chops which were green had a mean pH of 5.1 while those which were pale-green had an average pH value of 5.3; chops of light-gray, brownish-pink, and bright grayish-pink had mean pH values of 5.4,5.6, and 5.8, respectively. These data suggest that denaturation of myoglobin could occur in muscles of very low pH, and that this denaturation could result in formation of green pigment. Further research is needed to identify the mechanism by which the green color is formed and to clarify relationships of such discoloration to bacterial, physical, or chemical circumstances.

Since the time green-colored pork chops were first noticed in this laboratory, we have observed such chops in several instances in retail meat markets. Moreover, several packers have reported that some, but not all, pale and soft loins produce chops which turn green within 6 to 12 h following cutting; such chops are considered unsalable at the retail level. Previous research (*23*) has documented development of a gray-greenish cast on pale chops after 5 to 6 h of display and has determined that consumers were very sensitive to abnormal color change in selection of pork chops. Though the mechanism by which such discoloration occurs is not clear, it is apparent that certain pale and soft loins react unfavorably to some stress associated with retail cutting or display. Such loins can be expected to have a relatively short retail caselife. Correspondingly, pale and soft loins should be identified by plant personnel at the time the carcass is cut and (*a*) directed to markets where retail display is not required (e.g., the H.R.I. trade), (*b*) used for processed meat items (e.g., Canadian bacon), or (*c*) displayed at retail as quickly as possible and concurrent with peak demand periods.

Overall appearance scores and expected retail caselife for chops from loins in each of six quality groups are in Table 2. Appearance scores were highest for chops from loins in quality group II, but such chops did not differ from those in quality groups I, III, or IV at any display interval. Chops from loins in quality group VI were decidedly inferior ($P < .05$) in overall appearance to chops from dark colored, firm loins. These data suggest that loins with muscle structure scores (*24*) lower than 3 will produce chops which can be expected to have a retail caselife of 4 days or less. Chops from loins with a color score of 1 and a muscle structure score of 1 will likely be acceptable in appearance only on the first day of retail display, because they are extremely susceptible to discoloration and may be unsatisfactory in color shortly after cutting.

ACKNOWLEDGMENT

Technical Article 12376, Texas Agricultural Experiment Station. This study was partially supported by the Meat Science Research Laboratory, Agricultural Marketing Research Institute, Agricultural Research Service, U.S. Department of Agriculture, under cooperative agreement 12-14-100-11, 365(51).

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TABLE 2. *Overall appearance scores and expected retail caselife for chops from loins in each of six quality groups*

Quality score[a]			Number of loin sections[b]	Overall appearance score[c]				Expected retail caselife[d] (days)
Group	Color	Structure		Day-1	Day-2	Day-4	Day-6	
I	4	4	4	7.0[e]	6.8[e]	6.0[ef]	5.3[ef]	6.0
II	3	4	6	7.4[e]	7.1[e]	6.6[e]	6.0[e]	6.0
III	3	3	8	7.4[e]	7.0[e]	6.1[ef]	5.1[ef]	5.5
IV	2	3	8	6.8[e]	6.5[e]	5.9[ef]	5.1[ef]	5.7
V	2	2	6	6.7[e]	6.2[e]	5.3[f]	4.3[f]	4.0
VI	1	1	4	5.2[f]	4.5[f]	3.4[g]	2.7[g]	1.0

[a]Quality scores were assigned by a three-member panel using pork quality standards of the University of Wisconsin (*24*).
[b]Two chops were obtained from each loin section.
[c]Means based on an 8-point scale (8 = extremely desirable, 4 = slightly desirable, 1 = extremely undesirable).
[d]Expected caselife was defined as the last day (of a 6-day retail display period) at which total desirability was "slightly desirable" (a score of 5 or higher).
[e,f,g]Means in the same column bearing different superscripts are significantly different ($P < .05$).

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J. Milk Food Technol. Vol. 39, No. 12, Pages 845-847 (December, 1976)

Mathematical Model Describing the Relationship of Fat, Protein, and Lactose to the Total Solids—Its Application in Milk Testing

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(Received for publication April 2, 1976)

ABSTRACT

California dairy farmers producing and selling Grade A milk for pasteurization receive payment based on milkfat and solids-not-fat content of raw milk. In 1970, the concept of using electronic methods for testing of milk and its components was accepted by the California Department of Food and Agriculture. The InfraRed Milk Analyzer (IRMA) with its ability to measure fat, protein, and lactose, using a predetermined factor for the unmeasured solids, appeared to fill the need for a rapid, accurate, and economical method. After comparing IRMA results with chemical tests of known accuracy for several seasons, it appeared the ash factor could vary seasonally and geographically, as well as with individual dairy herds. It was decided to study the accuracy and calibration stability of InfraRed Milk Analyzers (IRMA), as well as the use of a constant value for the ash factor. By applying a multiple regression technique to milk sample data, it was possible to generate a model describing the relationship of fat, protein, and lactose to total solids (and solids-not-fat).

From the beginning of the Milk Testing Program by InfraRed Analyzers (IRMA), the question often has arisen as to the accuracy and calibration stability of IRMA equipment (*1*). Of particular interest, also, was the ash factor used throughout the State and whether this factor should be changed from time to time.

The California Food and Agriculture Code provides that testing for fat and solids-not-fat could be done by the buyer, the seller, or a third party which would include either State personnel or commercial laboratories. Producers were reticent to accept third-party testing by existing conventional analytical methods and preferred to accept analyses by instrumentation, more specifically, the InfraRed Milk Analyzer (IRMA).

Presently, four laboratories conduct milk testing with IRMA equipment in California. Two of these laboratories are operated by dairy processing plants, and two others are independent commercial laboratories.

The Bureau of Milk and Dairy Foods Control, California Department of Food and Agriculture (CDFA), is supervising calibration of IRMA units. Milk samples (eight samples per calibration set) are distributed to the IRMA laboratories throughout the State and tested for fat, protein, lactose, and total solids by the control laboratory, CDFA. The results of comparative analyses are evaluated by CDFA. This paper describes the methods used by CDFA in this program with these stated goals: (*a*) to maintain calibration uniformity and stability of IRMA units in the State; (*b*) to give an assurance of stability of IRMA units in the State; (*c*) to give an assurance of analytical accuracy for the calibration samples; and (*d*) to develop a reliable mathematical model for computing the total solids from measured values of fat, protein, and lactose.

MATERIALS AND METHODS

The control laboratory, CDFA, uses the following procedures (*2, 3*) in testing milk components: (*a*) fat-Mojonnier (Roese-Gottlieb), (*b*) protein-Udy (dye binding), (*c*) lactose-Polarimeter, and (*d*) Total solids-AOAC Method. The solids-not-fat (SNF) content is computed from the equation:

$$SNF = TS - F$$

Where, TS = Total Solids (%)

F = Fat (%)

The IRMA equipment measures the percentage content of fat, protein, and lactose. The content of solids-not-fat is computed, using the measured values of fat, protein, and lactose, in the following way:

$$SNF = P + L + K$$

Where, P = Protein (%)

L = Lactose (%)

K = Ash factor (%)

The total solids content, for IRMA equipment, is calculated from:

$$TS = F + SNF$$

$$= F + P + L + K$$

Presently, the values of K = .53% and K = .50% are used for milk testing in two milk production regions.

Results of chemical tests for 165 milk samples were used in a numerical analysis to investigate the relationship of fat, protein, lactose, and ash factor to total solids.

RESULTS AND DISCUSSION

The composition of tested milk samples is described in Table 1. The data presented in Fig. 1 and 2 indicate the

TABLE 1. *Milk composition of test samples*

Component	Mean x	Standard deviation
Fat	3.837	.481
Protein	3.384	.224
Lactose	4.849	.086
Total solids	12.680	.698
Solids-not-fat	8.843	.252

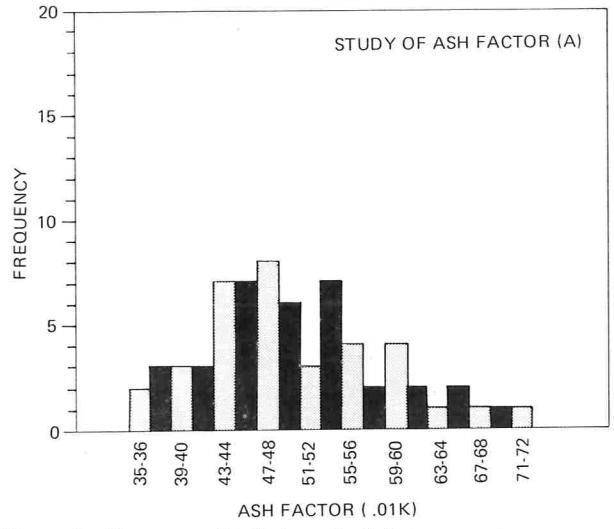

Figure 1. *Frequency distribution of ash factor.*

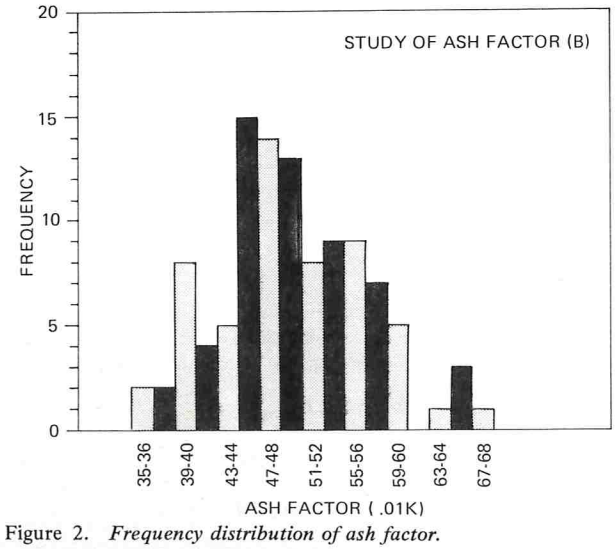

Figure 2. *Frequency distribution of ash factor.*

frequency distribution of ash factor values. The ash factor in milk is affected by breed of herd, feed, geographical location, and seasonal climatological conditions, and other variables. After studying the variation of ash factor both within and between the sets of milk samples, errors caused by applying a constant value of ash factor were realized by the CDFA.

The relationship of fat, protein, lactose, and total solids, described by Fig. 3, indicates a significant

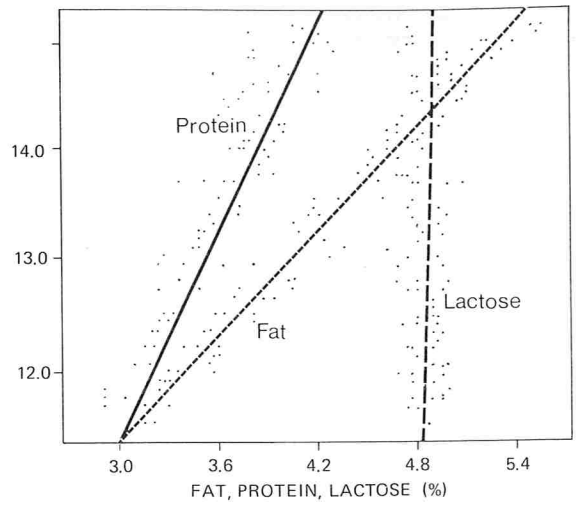

Figure 3. *Relationships between fat, protein, lactose, and total solids.*

correlation between fat, protein, and total solids, and a low correlation between lactose and total solids. The computed correlation coefficients are presented in Table 2.

TABLE 2. *Correlation coefficients*

	r
Total solids - Fat	.976
- Protein	.906
- Lactose	.173

After establishing the correlation levels between the milk components, four different mathematical models were tested:

1. $TS = a + (bF) + (cP) + (dL)$
2. $TS = a + (bF) + (cP)$
3. $SNF = a + (bF) + (cP) + (dL)$
4. $SNF = a + (bF) + (cP)$

It was expected that the total solids could be computed from the measured values of fat and protein only due to the low correlation between total solids and lactose. However, the results indicate a closer fit between the measured and computed values of TS when all three milk components (F, P, and L) are considered. The results of the multiple regression analysis are presented in Table 3. The following final equation was generated for computing TS and SNF:

$$TS = 2.76914 + (1.03111\ F) + (1.00097\ P) + (.52934\ L)$$

The above equation was applied to compute the values of TS for 864 tests (8 samples × 18 sets × 6 IRMA units) performed during the program of IRMA calibration. The computed values of TS and SNF (= TS - F) were compared with the results of the tests conducted by the control labortory, CDFA. A very good fit of tested and computed values was achieved. The differences between the computed data and the results of chemical analyses

TABLE 3. *Multiple regression analysis-output data (Dependent variable = TS)*

Coefficient of determination		.994
Coefficient of correlation		.997
Variance of estimate		.003
Std. error of estimate		.053
F Value		9423.508
Std. error of regr. coef.	- F	.015
	- P	.032
	- L	.049
Computed t-value	- L	69.728
	- P	31.327
	- L	10.817
Partial corr. coef.	- F	.984
	- P	.927
	- L	.649
Sample size		165

were significantly lower than between the obtained IRMA test data (using the ash factor of .53 or .50, respectively) and the CDFA control labortory tests.

The method of computing the values of TS (and SNF), by using a mathematical model, from the fat, protein, and lactose tests by IRMA equipment, gives more accurate results than the presently used system based upon constant values of the ash factor.

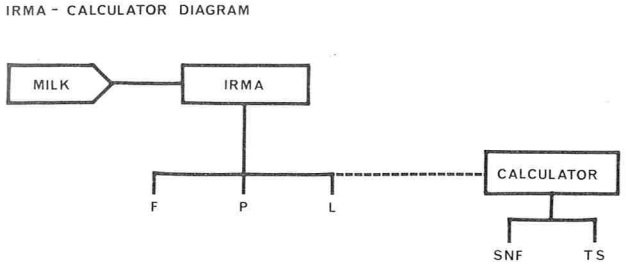

Figure 4. *Principle of the method proposed in this report.*

Consideration is being given to the use of the formula in connection with the infrared milk analyzers. The accuracy and reliability of the developed mathematical formula is being verified for different levels of milk composition. The principle of the proposed method is presented in Fig. 4.

The CDFA is using this method for checking the analytical accuracy of milk testing in the IRMA calibration program. Test results are used as the input for the mathematical model; the computed and measured values of total solids are then compared. Thus, this approach assures reliability for calibration of milk test equipment.

CONCLUSIONS

By computing the values of total solids (and solids-not-fat) by using the authors' mathematical model from data measured by IRMA equipment, the authors' theory supports the greater accuracy obtained from the multiple regression technique than by the current system of predetermined constant values for the ash factor.

ACKNOWLEDGMENTS

The authors thank the California Cooperative Creamery (Petaluma), Dairy Products Laboratory (San Francisco), Dairymens Cooperative Creamery Association (Tulare), and Daylin Laboratories (Los Angeles) for their cooperation in this program.

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J. Milk Food Technol. Vol. 39, No. 12, Pages 848-851 (December, 1976)

Interval Plating: A Simplified Method to Determine Suitability of Distilled Water as a Dilution Fluid

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(Received for publication April 19, 1976)

ABSTRACT

A simplified procedure has been developed for routine evaluation of distilled water used in milk, food, and water service laboratories. It is simple to do, requires no additional equipment or reagents, and can be done along with the routine daily workload. It requires no "control" water, and has a sensitivity which is appropriate for the intended use of the distilled water.

In both *Standard Methods for the Examination of Dairy Products* (*1*) and *Standard Methods for the Examination of Water and Wastewater* (*2*), recognition is given to the fact that in bacteriological determinations the quality of distilled water used to prepare media and dilution blanks is of considerable importance. To assist laboratories in determining the acceptability of their distilled water supply for use in Standard Methods bacteriological determinations, a procedure known as the Distilled Water Suitablility Test (DWST) (*3*) is detailed in both of the publications just mentioned. Neither publication requires that the DWST be done as a component of standard bacteriological procedures, but by virtue of stating that the distilled water source be acceptable, and by including only one procedure to determine this, the DWST by implication almost becomes required.

Certainly any requirement, whether stated or implied, which provides a reliable indication of the quality of a laboratory's distilled water contributes to continued assurance that laboratory analyses are being done competently. However, the DWST has some features which may make its inclusion in Standard Methods publications more of a disservice to users than a service.

The DWST basically involves incorporating the distilled water in question into a minimal growth medium, doing the same with a "control" double distilled water, inoculating both with a strain of *Enterobacter aerogenes*, and comparing the two for total amount of growth. This procedure lacks applicability in most small laboratories doing routine analyses because (*a*) the length and tedium of the procedure is prohibitive, (*b*) the quality of double-distilled "control" water varies with each laboratory doing the DWST, and (*c*) the DWST is probably too sensitive for the applications required in most routine milk and water analysis laboratories (*6*).

We are reporting the results of efforts to develop a more simple, valid, and applicable system for determining the quality of distilled water. The method is an Interval Plating Procedure, and is intended to measure the water quality in a more meaningful manner in relation to the procedure for which the distilled water will be used, i.e., routine Standard Methods bacteriological counts.

MATERIALS AND METHODS

Interval plating procedure

The distilled water to be examined was made into stock phosphate buffer by the procedure described in *Standard Methods for the Examination of Dairy Products* (*1*). This was then dispensed in 99-ml amounts into standard dilution bottles and autoclaved at 121 C for 15 min and stored at room temperature.

Escherichia coli (ATCC 25922) was used as the test organism. On the day before the interval plating procedure was to be done, 2-3 ml of Nutrient Broth (Difco) was inoculated from a nutrient agar slant culture and incubated at 32 C for 18 h. One-tenth milliliter of the broth culture was then added to 100 ml of Nutrient Broth in a flask and incubated for 4 h to obtain a culture in the mid-log phase of growth. The 4-h culture was then diluted serially to 10^{-6} in three of the dilution blanks prepared as indicated above. The 10^{-6} dilution was immediately plated as the "zero" time dilution by measuring 2 ml of the dilution into each of five plates and mixing with Violet Red Bile Agar (Difco) maintained in a water bath at 45 C. The culture was allowed to remain in the dilution water at room temperature for 60 min, and then plated again using the same protocol as for the "zero" time plating. Plates were then incubated at 32 C for 18-24 h. Colonies were enumerated and the following calculation done:

$$\frac{\text{Mean colony count (60 min)-Mean colony count (0 min)}}{\text{Mean colony count (0 min)}} \times 100 = \%\ \text{change between 0-60 min}$$

Arbitrarily we have established that the percent change in colony count between zero and 60 min should not exceed ± 15% for consideration of the distilled water as acceptable for application in routine milk, food, and water bacteriological analysis. Figure 1 illustrates schematically the Interval Plating Procedure.

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[2] *State Chemical Laboratory.*

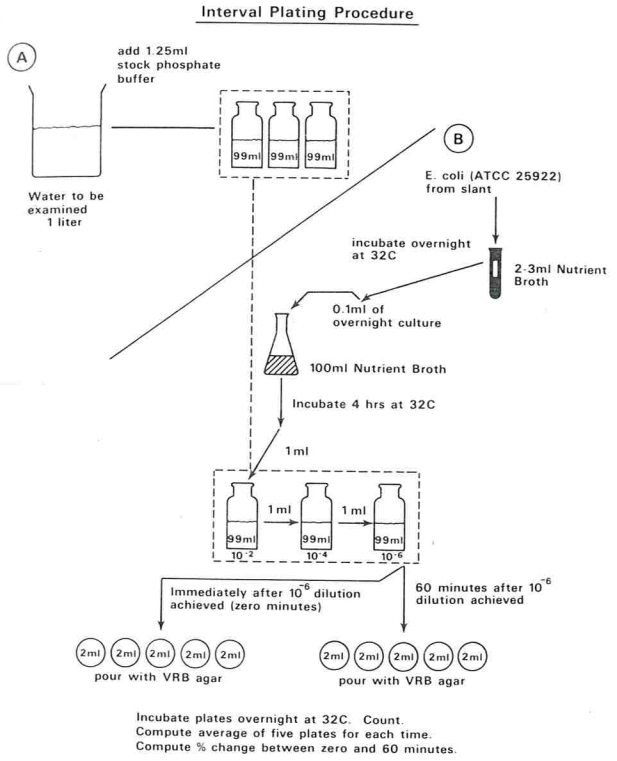

Figure 1. *Interval Plating Procedure.*

Comparison of the Distilled Water Suitability Test and the Interval Plating Procedure

To compare the DWST with the Interval Plating Procedure two identical comparative studies were done. Distilled water samples were requested from 19 milk testing laboratories approved by the Iowa State Department of Agriculture. These were representative of the water supply used in routine standard plate counts and other bacteriological testing. The water samples were submitted by mail, split, and evaluated on the same day by both methods.

RESULTS

Interval Plating Procedure

As a preliminary indication of the ability of the Interval Plating Procedure to detect toxic qualities in distilled water, several waters of known source and quality were analyzed. Table 1 shows the sources of distilled water and the results of interval plating. One of the distilled waters (Water #5) used to prepare dilution blanks in an unrelated experiment, caused bacterial population reduction after remaining at room temperature for 45 min. This water was from a building distribution line and was passed through a mixed-bed resin long overdue for recharge. Water #2 was the same water as #5 after recharging the resin column. Water #1 was the same building water passed through a Millipore Super Q (Millipore Corporation, Bedford, MA 01730) water treatment unit. Water #3 was again the same water but specially processed for use in tissue culture, and Water #4 was the untreated building line distilled water.

TABLE 1. *Detection of toxic properties of distilled water by the Interval Plating Procedure*

Water	Ohms specific resistance	%Change 0-60 min
1[a]	1,200,000	— 1.6
2[b]	630,000	— 4.5
3[c]	500,000	—10.3
4[d]	320,000	—99.9
5[e]	200,000	—99.9

[a]Building line water, through Millipore Super Q.
[b]Building line water, through recharged resin.
[c]Building line water, specially processed for tissue culture.
[d]Building line water, untreated.
[e]Building line water, through exhausted resin, suspected as toxic from previous experience.

Results of the interval plating showed that the procedure agreed with subjective judgements, essentially ohms specific resistance data previously made on the quality of distilled water used to prepare dilution water blanks and correlated directly with a population reduction.

As assurance that VRB Agar should be used in the procedure rather than a general purpose medium such as Plate Count (PC) Agar, known "good" and "bad" distilled waters were subjected to interval plating using both SPC and VRB Agars. Results of this evaluation are presented in Table 2.

TABLE 2. *Use of Violet Red Bile Agar vs Plate Count Agar in Interval Plating Procedure*

Water	Medium	%Change, 0-60 min
"Good" water (1,200,00 ohms-cm²)	PC[a]	— 6.1
	VRB[b]	+ 4.8
"Bad" water (330,000 ohms-cm²)	PC	— 5.8
	VRB	—98.4

[a]PC = Plate Count Agar.
[b]VRB = Violet Red Bile Agar.

As data in Table 2 show, PC Agar produced comparable results for both qualities of water, while the use of VRB Agar provided greatly divergent values. This appears to indicate that remaining in the diluent for 60 min did not cause cell death, but apparently injury resulted, preventing growth in the more selective VRB Agar. Since VRB Agar is apparently more capable of detecting toxic effects, while PC Agar permits growth regardless of injury, VRB Agar was chosen for use in the procedure.

Comparative study between DWST and the Interval Plating Procedure

Results of two separate comparative studies between the DWST and the Interval Plating Procedure are presented in Table 3. The results of trial #1 point out one of the inherent deficiencies of the DWST in that there are no standard specifications for the control water other than being double-distilled. In trial #1, all 17 waters by the DWST appeared to be extremely toxic (acceptable ratio is 0.8-3.0). That this is indeed the case from a practical standpoint is highly unlikely. Also, water #10 was submitted from the State Hygienic Laboratory as one of the approved milk laboratories in the state and was taken after passage through the Millipore Super Q system where it had a resistance measurement in excess of 1,000,000 ohms before shipment to the State

TABLE 3. *Comparative study between the Distilled Water Suitability Test and Interval Plating Procedure*

Water	Trial 1		Trial 2	
	DWST ratio	Int plating % , 0-60 min	DWST ratio	Int plating % , 0-60 min
1	0.04T[a]	–50.6T	0.82	+ 8.8
2	0.03T	–42.7T	1.15	–63.4T
3	0.01T	–99.9T	1.09	+ 4.7
4	0.08T	+80.0T	1.96	– 1.5
5	0.01T	–99.9T	0 T	–99.9T
6	0.02T	+2.6	2.42	+ 4.0
7	0.02T	+18.7T	1.28	+ 6.5
8	0.01T	–99.9T	0.65T	–99.9T
9	0.01T	+53.6T	2.5	– 7.8
10	0.02T	– 5.6	2.13	+ 4.6
11	0.01T	–41.4T	0.18T	–19.1T
12	0.05T	+10.5	0.93	+ 5.7
13	0.01T	–99.9T	0.19T	–91.6T
14	0.14T	+12.8	0.26T	– 6.1
15	0.01T	– 4.8	0.16T	–42.3T
16	0.01T	–73.6T	0.17T	–10.4
17	0.03T	+ 8.5	0.21T	+ 1.2
18			0.26T	– 2.7
19			0.10T	–24.0T

[a]T indicates unacceptable by established DWST criteria (either toxic or nutritive) or by suggested Interval Plating criteria (not exceeding ± 15% change at 60 min).

Department of Agriculture's laboratory. Thus it appears that the control water used in the DWST contained growth promoting substances making all of the samples appear to be extremely toxic.

In trial #2 the control distilled water used in the DWST appeared to have returned to normal, since a likely range of ratio values resulted. Only one sample was judged "false positive" by the Interval Plating Procedure because the DWST result indicated an acceptable ratio. Four water specimens were toxic by the DWST and acceptable by interval plating. The interval plating and DWST methods agreed upon the quality of 14 of the samples (74% agreement).

DISCUSSION

Any service laboratory, regardless of size, whose users rely upon the results generated is bound professionally and ethically to routinely do quality assurance procedures. Ideally, these should be done not monthly or yearly, but daily as an incorporated part of the customary workload. There are many quality control procedures, some more expensive and time consuming than others, but the method sought should give the most significant results for a given application for the least amount of expense and effort.

The quality of culture media used and the performance of laboratory personnel has been monitored to various degrees for some time. However, the quality of distilled water used to prepare media and, specifically, dilution water blanks is for the most part taken for granted, although it has been suggested in some jurisdictions that periodically small laboratories submit samples of their distilled water to a central laboratory for evaluation by the DWST (*4*). Poor water quality can tend to mask, complicate, or misinterpret results just as much as a poorly performing technician or unacceptable culture medium, and only occasional surveillance certainly cannot give an adequate view of the over-all quality of a distilled water source.

The routine users of Standard Methods publications use distilled water primarily to prepare dilution blanks and media. Culture media provide considerable protection from toxic effects of any water (*6*); therefore, the primary interest in distilled water quality is in its use as a dilution fluid. If distilled water is to be used for production of a minimal medium for bacterial cultivation, then the DWST would be justified for that particular application. However, we are concerned whether the dilution system will leave cells uninjured for a reasonable period, not whether or not the water in question will permit actual growth.

The DWST is complicated, time-consuming, relies on essentially another "unknown" water as a control, and is too sensitive biologically for the use to which the water will be put in routine water and milk analysis.

Interval plating, on the other hand, is simple and can be easily included in the daily routine and requires no additional materials. With the Interval Plating Procedure, the unknown distilled water is applied to a standard system and its effect on that system determines its acceptability, not how an unknown and a so-called "control" compare to each other in their effect on a standard system. Finally, the Interval Plating Procedure is a modification of the very system it was designed to monitor—survival in dilution blanks and, therefore, does not assess unnecessary limits of sensitivity.

Specific chemical parameters (specific resistance, copper, chlorides, etc.) provide an indication of quality, but overlook the potential presence of any number of both recognized and as yet unidentified factors which may either adversely affect a biological system or provide undesired nutritive supplements. The best way to determine biological suitablility is by biological assay.

The use of a pure culture for this procedure rather than a naturally contaminated source, such as raw milk, is justified since it is an attempt to develop a system as standardized as possible. The use of organisms other than *E. coli* was not evaluated and this particular organism was selected because of ease of cultivation and wide availability. However, a study has been done (*5*) in which *Staphylococcus aureus, Streptococcus pyogenes, E. coli,* and *Salmonella typhimurium* were allowed to remain in various diluents for 60 min. The *E. coli* strain used in that study was considerably less affected by any toxic effects in the dilution systems. Therefore it can be assumed that one of the less sensitive organisms has been used in this study, and that the distilled water in question is at least being given the benefit of the doubt.

A potential argument to undermine the importance of distilled water quality in dilution blank preparation is the suggestion that in an actual analytical situation the presence of the food or milk itself will tend to protect the organism while in the diluent. Straka and Stokes (*7*) have

shown that the flora of frozen meat and turkey pies, when diluted to between 10^{-3} and 10^{-5} underwent considerable decline when allowed to remain in phosphate buffered dilution blanks for 60 min. Thus, protection of organisms by the product itself is still in doubt.

To conclude, an interval plating method is suggested as an alternative to the DWST for use in milk, food, and water analysis laboratories. It is simple to do, can be easily included in the daily routine, does not rely on comparison with a "control" water, and is more meaningful for the evaluation of the distilled water for use as a dilution fluid.

ACKNOWLEDGMENT

The authors would like to thank Mrs. Vera Remmes for her extended technical assistance.

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J. Milk Food Technol. Vol. 39, No. 12, Pages 852-853 (December, 1976)

A Research Note

Contribution of KH_2PO_4 to Toxicity in Phosphate Buffered Dilution Water Systems

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(Received for publication April 19, 1976)

ABSTRACT

It appears that different sources of KH_2PO_4 used to prepare phosphate buffered dilution water have various effects on the ability of the dilution water to maintain a stable bacterial population. When the water used to prepare the dilution water is of either very good or very poor quality, the KH_2PO_4 source is of no consequence. However, if water of an intermediate quality is used, the KH_2PO_4 source becomes of consequence in the over-all quality of the system.

There have been various degrees of concern over the years about the best dilution system to use for various bacteriological applications. Interest has been primarily in the areas of which buffering and/or protective agents to use (*2, 5, 7*) or the quality of the distilled water (*3, 6*).

During a study to develop a simplified method to determine distilled water suitability (*4*), it became apparent that yet another factor may play a role in determining the ability of a dilution system to maintain a given level of a bacterial population over time. That factor is variability between lots, brands, and conditions of the dihydrogen potassium phosphate (KH_2PO_4) used to prepare the stock phosphate buffer solution. This research note reports our preliminary data on the subject.

TABLE 1. *Identification of various sources of KH_2PO_4 studied*

Study code	Supplier	Lot no.	Size	Comments
G	Mallinckrodt Chemical Works	XJX	1 lb	sealed bottle
H	Matheson Coleman and Bell	25	1 lb	unsealed bottle
I	Fisher Scientific Company	740075	¼lb	unsealed bottle uneven crystals
J	Fisher Scientific Company	735003	1 lb	sealed bottle powdery & lumpy
K	Mallinckrodt Chemical Works	NDT	1 lb	unsealed bottle
L	Mallinckrodt Chemical Works	MXX	1 lb	unsealed bottle used for prescriptions
M	Mallinckrodt Chemical Works	XTP	1 lb	unsealed bottle
N	Mallinckrodt Chemical Works	TCH	1 lb	unsealed bottle
X	University of Iowa Pharmaceutical Services	—	—	Stock buffer solution

MATERIALS AND METHODS

Various lots and brands of KH_2PO_4 were obtained and used to prepare a number of stock buffer solutions as described in *Standard Methods for the Examination of Dairy Products* (*1*). The sources of the KH_2PO_4 are presented in Table 1.

Stock solutions were used to prepare standard 99-ml dilution blanks in combination with three different qualities of distilled water, and any differences in toxicity were evaluted by the Interval Plating Procedure as previously described (*4*). The three qualities of distilled water used are indicated in Table 2.

RESULTS

Using the suggestion in the Interval Plating Procedure

TABLE 2. *Characteristics of distilled water studied*

No.	Specific resistance (ohms)	Treatment
1	1.2×10^6	Water From Central Distillation Unit passed through Millipore Super Q[a]
2	0.63×10^6	Water From Central Distillation Unit passed through mixed-bed resin
3	0.32×10^6	Water From Central Distillation Unit

[a]Millipore Corp., Bedford, Mass.

TABLE 3. *Effect of interaction of different quality waters and various sources of KH_2PO_4 on interval plating survival*[a]

KH_2PO_4 Source	Percent change in bacterial population		
	Water 1	Water 2	Water 3
G	+ 2.3	–49.8	–53.5
H	+ 8.5	– 1.3	–78.3
I	– 1.2	–21.4	–44.0
J	– 1.7	–44.3	–68.3
K	–12.6	–16.9	–81.9
L	– 5.7	– 6.3	–83.8
M	– 9.4	–33.3	–49.4
N	+13.1	–14.4	–45.9
X	– 7.2	–26.2	–64.6

[a]% change in *E. coli* (ATCC 25922) population when allowed to remain in dilution system for 60 min.

(4) that a change in bacterial population after standing 60 min in buffered diluent should not be greater than± 15% it will be noted from Table 3 that none of the diluents exceeded this range when water of 1.2×10^6 ohms-cm^2 specific resistance was used. However, only three of the diluents were within the specified range with water of 0.63×10^6 ohms specific resistance and none qualified with the lowest quality distilled water.

These results suggest that when water of a high quality is used the quality of the KH_2PO_4 source appears to be of no consequence in contributing to toxic effects. When a poor quality distilled water is used to prepare the dilution system, the system appears to be toxic, also regardless of the KH_2PO_4 source. Therefore, very good or very poor qualities of distilled water seem to be the dominant factor in determining the toxicity, or lack of it, in phosphate buffered dilution systems with the quality of phosphate source not being of consequence. However, when water of an intermediate quality is used, the KH_2PO_4 source produces differences, with some of the systems being acceptable by interval plating, and others not.

DISCUSSION

At this preliminary stage it is difficult to speculate on the significance of these results. It seems that distilled waters at the two extremes of quality, very good or very poor, dominate the situation, and mask any effect of an inferior KH_2PO_4 source. Ideally, the answer would be to always have available a distilled water source of high and consistent quality whereby the need to be concerned about toxic factors in the dihydrogen potassium phosphate (KH_2PO_4) would be eliminated or at least considerably reduced. However, most small laboratories are fortunate if their distilled water is in the intermediate quality range (500,000 - 600,000 ohms specific resistance), thus making it entirely possible that the buffering agent is adversely affecting the quality of the buffer system.

Of the nine sources examined, only three, in combination with the intermediate quality water, (sources H, L, N) were able to meet the criteria established in the Interval Plating Procedure. Sources I and J, which were questionable by visual inspection, did indeed produce an unacceptable dilution system, but the remaining sources produced results which cannot be correlated with any of the descriptive information.

This report, as mentioned previously, is preliminary. Additional studies must be undertaken before the situation inferred in this note is seriously evaluated. However, this research note should alert those involved in laboratory quality control that another factor, previously taken for granted, may well be a significant variable in enumeration systems.

ACKNOWLEDGMENT

The authors would like to thank Mrs. Vera Remmes for her technical assistance.

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J. Milk Food Technol. Vol. 39, No. 12, Pages 854-858 (December, 1976)

Electronic Somatic Cell Count—Chemical Method, DMSCC, and WMT Tests for Raw Milk

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(Received for publication April 16, 1976)

ABSTRACT

The Direct Miscroscopic Somatic Cell Count — field method (DMSCC), Wisconsin Mastitis Test (WMT), and Electronic Somatic Cell Count (ESCC) were studied to determine variability and relationship to each other. The coefficients of variation computed at a DMSCC count near one million were 15.6% (DMSCC), 6.3% (WMT), and 4.2% (ESCC). Linear regression equations were determined for predicting DMSCC results by WMT and ESCC. The approximate width of the 95% confidence intervals for ESCC predicting DMSCC were ± 275,000 and for WMT predicting DMSCC were ± 600,000. The prediction of square root and log transformations of DMSCC by WMT exhibited narrower confidence intervals for low somatic cell counts, but wider intervals for high counts (greater than 1,000,000).

The chemical method of Electronic Somatic Cell Count utilizing a Coulter Counter (ESCC) is used as an official procedure in Canada and several European countries. As yet, it has not been recognized as an official test in the U.S., but evidence of its applicability appears to be mounting (*2, 3, 5-9*) with a need perhaps only to refine details of operation. McCauley et al. (*4*) obtained good results in a working government laboratory over a year of routine testing. The procedure was found to be rapid and the results agreed well with the Direct Microscopic Somatic Cell Count (DMSCC). In the U.S., the Wisconsin Mastitis Test (WMT) is used extensively and is offically recognized as a screening test for abnormal milk. While the WMT has been rather extensively studied, it has not been compared with the ESCC for precision and accuracy over the range of counts ordinarily found in milk. The DMSCC field method has not been analyzed for variability (as has the "reticle strip" procedure) but it is an official confirmatory test widely used throughout the U.S.

The work reported herein was designed to further demonstrate the applicability of the ESCC to routine counting of somatic cells, and to compare it with and determine its relationship to DMSCC and WMT results. Further, the work was designed to evaluate variability of the three procedures, the DMSCC both at a level of one million and also over the broad range of counts noted in commercial laboratories.

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MATERIALS AND METHODS

Milk samples

Raw milk samples used in this study were obtained from the on-going operation of Dairy Quality Control Institute, Inc., laboratory. Samples arrive at the laboratory either in Whirl-Pak bags or in 50-ml plastic, single-service rigid container. Each working day throughout the duration of the experimental work, 10 samples were isolated from the day's supply by means of a set of random number charts prepared by computer. On any given day, sample numbers may range between 150 and 250. Three tables of random numbers were applied; one for 100-150 samples, a second for 150-200 samples, and a third for a sample intake of 200-250. This arrangement allowed a random selection of samples depending upon the total received, and was used over 3 months of study. Because an insufficient number of milk samples with high somatic cell count (1.5 million and higher) were available for evaluation, all samples showing WMT results of 22 and above were held aside for testing during 1 month.

For regression analyses a total of 405 samples was analyzed by the three different methods: ESCC, WMT, and DMSCC. For the latter two methods randomly selected samples were agitated by the method outlined in *Standard Methods for the Examination of Dairy Products* (*1*). Iced or refrigerated milk samples for ESCC evaluation were vigorously shaken before sampling. This was done in multiple sample racks. The Whirl-Pak bags were opened before shaking. The rack of samples was agitated in a vibrating manner and with such vigor that foaming occurred. Thorough distribution of fat was necessary to secure a representative aliquot for Coulter Counter analysis. Additional mixing also took place when pipettes (10 ml, wide-tipped) were rinsed two times in the next sample of milk. Ten ml of milk were placed in thin-glass, snapcap medicine vials (7 dram, 66 mm in height, 25 mm outside diameter; Demuth Glass Works, Div., of Brockway Glass Co., Inc., Parkersburg, W. VA). The cap was punctured in the center to prevent pressure buildup during heating.

WMT and DMSCC

The Wisconsin Mastitis Test precedure outlined in *Standard Methods for the Examination of Dairy Products* (*1*) was used in this study.

The DMSCC "field method" outlined in *Standard Methods* (*1*) was used in this study. This method was selected because many labortories in the United States are using it for counting somatic cells. Ten fields, separated by approximately 1 mm, were counted horizontally and 10 fields vertically across the film. The miscroscopic factor was 500,000. When 50 fields were counted, as required of counts of 300,000 or less, 25 fields were counted both horizontally and vertically, but separted by somewhat less distance. Levowitz-Weber stain was used.

ESCC

The ESCC has not been adopted as a standard in the United States so this method will be outlined in detail. In this study a Model Z_F Coulter Counter, fitted with both a Size Distribution Analyzer and a Coulter X-Y Recorder II were used (Coulter Electronics, Hialeah, Florida). Initally the machine was standardized using the Size Distribution Analyzer and polystryene particles of 9.69-μm diameter (polystyrene, Coulter Electronics, Hialeah, Florida). The equipment was checked for standarization once each month during three months of use. Each time it was found to have maintained its original calibration.

The settings established for these trials were an aperture current (I) of 1 ma, and amplification (A) of ½, and a lower threshold (T_L) of 34. The constant K, 2.64, for this machine was applied in the formula

$$K = \frac{V}{I \times A \times T_L}$$

to calculate a lower threshold value for any given somatic cell volume, V. In this study, the equipment was set to record particles of 44.55 μm^3 and larger. This corresponds to cell diameters of 4.4 μm and greater.

There are two ways of establishing the size below which particles will not be counted, and thereby excluding "debris" from counts. The Coulter X-Y Recorder II may be used to identify the lower threshold (T_L) by determining the low point or valley on the chart. This valley separates the debris from the cells. Alternately the T_L may be established by measurement of cell diameter and use of appropriate calculations.

The first step in the ESCC is to add 3 drops of Somafix (Coulter Electronics, Hialeah, Florida), a fixative, to 10 ml of milk in the glass vial. Somafix is an acid medium, and coagulation of milk can occur unless samples are shaken after the fixative is added. Fixative was added to no more than three samples before they were mixed in a Vortex Jr. Mixer for 2-3 sec.

Samples were then put in racks of 24 and heated in a water bath at 60 C ± 1. The samples were held 6.5 min in the water bath to assure 5 min of treatment at 60 C. Following heating, samples were cooled rapidly in a water bath to 20 C and immediately diluted.

The second step of this method is dilution of milk samples. Each sample was mixed on the Vortex Jr. Mixer for 3-5 sec and replaced in the racks. After mixing all 24 samples in one rack, each sample was given one sharp shake, the lid was removed from the vial, and the sample was diluted in a Coulter Diluter. The diluter extracted 0.1 ml of sample, which was then discharged with 9.9 ml of dilutent, Somaton (Coulter Electronics, Hialeah, Florida).

Foam will prevent proper digestion during the heating step, and thus it is essential to avoid forming a foam layer during dilution. Samples were discharged from the diluter down the side of the accuvette (sample container, Coulter Electronics), keeping the discharge tube above the level of liquid. Diluted samples were not mixed.

Diluted samples were heated to 80 C ± 1 C for 10 min. The water level was maintained about 2 cm above the level of sample to assure heating of the entire mixture. Samples were then cooled in a water bath at 20 C. Samples must not be held more than 1 h before counting.

The final step in the ESCC method is counting of cells. One count was taken on positive polarity, one on negative. An aperture opening of 100 μm was used, and 100 μl of diluted sample were tested on each polarity.

Experimental design

To determine the variability of the DMSCC, each of four technicians were asked to prepare 12 films from a milk sample known to have a count near 1.0 million/ml. Each technician then counted the somatic cells on each film, his own as well as films prepared by the other three technicians. The total number of films counted by each technician was 48, thus a total of 192 counts were made. An analysis of variance of these counts provided an indication of test variability at that critical level of count where action is usually taken in mastitis control programs.

To further pinpoint variability of the three tests over a wide range of somatic cell counts, 10 samples were selected to represent milk with counts from 100,000 to 600,000 cells per ml from one day's supply at the laboratory. One technician counted all samples on the Coulter Counter in duplicate, two counts each on positive and negative polarity. In the study of variability, WMT tests were done singly by one technician. For the DMSCC test, two technicians prepared films and two technicians counted each film. Results of these tests were evaluated by an analysis of variance.

For regression analysis 10 randomly selected samples were analyzed each day by each of the three procedures. Also, 199 samples with WMT values over 22 were analyzed one month to increase the number of samples with high somatic cell counts. A total of 405 samples was analyzed. For the regression study WMT tests were done in duplicate, one test per sample being done by each of two technicians. ESCC tests were also made in duplicate, one analysis each at positive and negative polarity. Single DMSCC counts were made on all samples. Regression analysis was utilized to determine the relationship between the counting procedures.

Time study

Time required to count somatic cells in a large number of samples by the ESCC method was also studied. The ESCC method was included with the regular laboratory procedures for each sample of milk received one day. One person added the fixative and heated the 142 samples of milk received that day. A second person diluted the samples, and a third person used the Coulter Counter to count the somatic cells. The technician time required for each of these steps was recorded.

RESULTS AND DISCUSSION

Variability of DMSCC

The sample of milk from which 48 films were prepared and 192 counts were made exhibited a mean count of 1,130,000. The analysis of variance for this experiment was calculated with the BMDO2V program from the Health Sciences Computing Facility at UCLA. Results are shown in Table 1.

TABLE 1. *Analysis of variance of 192 Direct Microscopic Somatic Cell Counts*[1]

Source of variation	Degrees of freedom	Sums of squares (10^9 omitted)	Mean square (10^9 omitted)
Milk subsample	11	1008	92
Film preparation	3	599	200
milk × film	33	1023	31
Counting	3	1257	419
count × milk	33	1165	35
count × film	9	207	23
residual	99	1838	19
Total	191	7098	

[1]All subsamples were drawn from one sample of milk having a DMSCC of 1.13 million.

The sums of squares from the interaction of counting × milk subsample, counting × film preparation and the residual were pooled as representative indicators of the pure error. This was justified by similar size of these error terms and the lack of any obvious reason for a systematic error in these interactions. The resulting estimate of pure variance, s_p^2, is 22.7×10^9. The mean square due to counting was then partitioned into a pure error component and a variance due to counting according to the following equation.

$$Ms_c = s_p^2 + 48s_c^2$$

where

Ms_c - is the mean square due to counting.

s_c^2 - is variance due to counting.

The variance of a sample mean can be determined from the pure error variance and the variance due to counting as follows:

$$s^2 = \frac{s_c^2}{e} + \frac{s_p^2}{fe}$$

s^2 - is the variance of the sample mean.
e - is the number of examinations with one examination equivalent to counting the cells in 20 fields.
f - is the number of films prepared.

The estimated variance due to counting for these examinations was 8.25×10^9. Thus, the variance of the

sample mean is estimated by the following equation.

$$s^2 = \frac{8.25 \times 10^9}{e} + \frac{22.7 \times 10^9}{fe}$$

The field method requires only one film and one observation, so both denominators, e and f, are 1. The resulting standard deviation is 176,000 which is equivalent to a coefficient of variation of 15.6% and 95% confidence interval of ± 30.6% of the mean. This coefficient of variation compares favorably with the previously reported value of 17% (*10*). The variation of these results also compares favorably with an earlier study of the reticle strip method (*11*) when the values of e and f are adjusted to reflect the greater area counted on each film and the use of two films per sample of milk. The surface area to be counted on each film, when utilizing the reticle strip method, corresponds to an e value of 3.33. This yields an estimated coefficient of variation of 6.8%.

Variability of DMSCC and ESCC

The results of the direct microscopic counts of the 10 samples of milk ranging in somatic cell counts from 100,000 to 2,000,000 were analyzed with the same anlysis of variance program. The grand mean of these sample counts was 730,000. The variance of these counts was analyzed (Table 2) to determine if it was influenced by the range of cell concentrations.

TABLE 2. *Analysis of variance of DMSCC on milk samples of various somatic cell counts*[1]

Source of variation	Degrees of freedom	Sums of squares (10^9 omitted)	Mean squares (10^9 omitted)
Milk sample	9	7664	852.
Film preparation	1	0.72	0.72
milk × film	9	157.	17.5
Counting	1	28.6	28.6
count × film	1	274.	274.
count × milk	9	294.	17.5
residual	9	115.	12.8
Total	39	8534	

[1]Ten samples of milk in the range of 100,000 to 2,000,000 somatic cells per ml.

The very large interaction term between counting and film preparation prohibits use of this term as part of the pure error and the correspondingly small value for the sum of squares due to film preparation essentially eliminate any possible partitioning of the error terms. However, the mean and standard deviation of each of the four direct microscopic counts on each sample of milk were computed. The standard deviation was observed to be proportional to the square root of the mean. This observaton suggests that the coefficients of variation computed from the previous data will vary with the inverse square root of the cell count for counts between 300,000 and 3,000,000.

A one-way analysis of variance program, BMDO1V from the Health Sciences Computing Facility at UCLA was used to analyze the four counts by the Coulter Counter on each of the 10 samples of milk. The coefficient of variation for these tests varied with the inverse square root of the cell count, but was 3.5% for samples with a count mean one million. The 95% confidence limits exhibited the same trend and had a value of 12% of the mean for samples with counts near one million.

The predicted results from the Coulter Counter and direct microscopic counts on these 10 samples of milk were compared to determine if the counts were biased by either procedure. For each sample of milk the mean and 95% confidence limits for the ESCC techniques were contained within the 95% confidence limits of the DMSCC in that sample.

Prediction of DMSCC by WMT and Coulter Counter

Regression analysis was utilized to compare estimates of the somatic cell count of the 405 random samples collected in the laboratory to determine the relationship between the three methods. All regression equations were linear, but when the WMT was utilized as the independent variable, equations were generated for predicting the dependent variable, the square root of the dependent variable and the logarithm of the dependent variable (Fig. 1). A regression equation utilizing ESCC values as a predicator of the DMSCC was also determined. No transformations were attempted for this relationship.

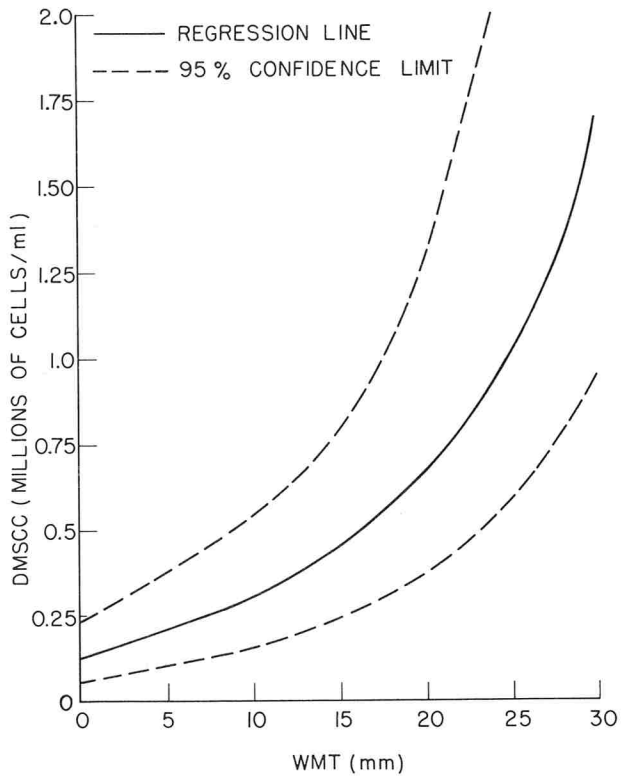

Figure 1. *Logarithmic relationship between the WMT and the DMSCC results.*

The results of the regression analysis are summarized in Table 3.

TABLE 3. *Summary of the regression analysis of DMSCC, WMT, and Coulter Counter*

Variables		Correlation coefficient	Percent of variation explained	Approximate 95% confidence Interval
Independent	Dependent			
Coulter Counter	DMSCC	0.95	89	± 275,000
WMT	DMSCC	0.76	56	± 600,000
WMT	DMSCC (sq. rt. transform)	0.83	69	± 200,000 + 0.3$\hat{y}$
WMT	DMSCC (log transform)	0.85	72	± 0.7$\hat{y}$
WMT	Counter Counter	0.76	56	± 575,000
WMT	Coulter Counter (sq. rt. transform)	0.85	72	± 200,000 + 0.3$\hat{y}$
WMT	Coulter Counter (Log transform)	0.88	77	± 0.6y

Note: $\hat{y}$ is the predicted dependent variable value.

The correlation coefficients for prediction of the direct microscopic count of somatic cells by the WMT agree very favorably with corresponding value published earlier (*12*). The column of figures headed "Percent of Variation Explained" are the R^2 values from the regression analysis multiplied by 100. The most important measure of the effectiveness of any of these relationships is the 95% confidence interval about the predicted value. The expressions in this column of the table are approximations of the confidence intervals statistically calculated with the following equation.

$$CI = \hat{y} \pm t(n-2,\ 1-\tfrac{\alpha}{2}) * s * \left[1 + \frac{1}{n} + \frac{(X_k - \bar{X})^2}{\Sigma (X_i - X)^2}\right]^{1/2}$$

where

CI - is the confidence interval.

$t(n-2,\ 1-\frac{\alpha}{2})$ - is the student t value with n-2 degrees of freedom and $1-\frac{\alpha}{2}$ probability.

s - is the estimate of the standard deviation.

n - is the number of observations.

X_k - is independent variable value at which the confidence interval is being determined

$\bar{X}$ - is the mean of the independent variable values.

X_i - is the i^{th} independent variable value.

y - is the predicted somatic cell count.

* - implies multiplication.

The approximate width of the 95% confidence intervals exhibit different characteristics because of the transformations of the dependent variables. In each case the predicted 95% confidence interval may not accurately represent the actural confidence interval because the above equation pools all of the variation about the regression line. Therefore, the actual 95% confidence intervals for DMSCC and ESCC values for the milk samples exhibiting each possible level of WMT were calculated. The results of these calculations indicate that the actual 95% confidence interval on either the predicted DMSCC or the ESCC value for a milk sample of any given WMT value averages ± 55% of the predicted count.

The slope and intercept for the regression equation between ESCC and DMSCC were 0.89 and 90 000 respectively (Fig. 2). The hypothesis that the slope is 1.0

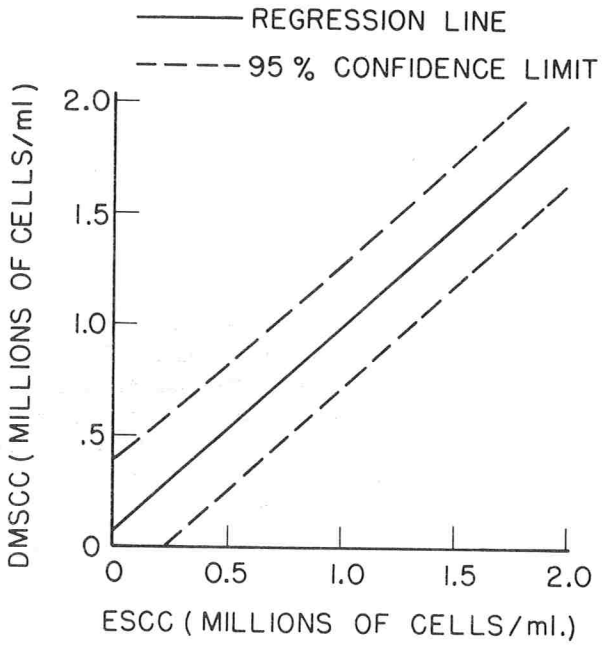

Figure 2. *Linear relationship between the ESCC and the DMSCC results.*

and that the intercept is 0.0 was statistically rejected. This suggest that the ESCC method may underestimate the DMSCC on samples with low counts and overestimate the DMSCC on samples with high counts.

The confidence limits on WMT results were estimated on the duplicate WMT evaluations of the random milk samples. For these data, the confidence limits or repeatability on the Wisconsin Mastitis Tests were ± 2.2. This represents 95% confidence limits for the true WMT value of approximately ± 12% of the measured value or a 6.3% coefficient of variation. Thus, the large variation in somatic cell counts predicted by WMT is not caused by lack of precision, but rather a bias particularly for larger WMT values.

Time study

An average of 2.39 min of technician time was required per sample to estimate the somatic cell count by the ESCC-chemical method on 142 milk samples. This time includes 0.49 min per sample for addition of the fixative and 0.95 min both for the diluting and counting steps. Although the technicians had previously been using this method on selected samples, the only time it was utilized for routine analysis was during the time study. Therefore, we anticipate that this time interval would be reduced by additional experience allowing approximately 350 samples to be analyzed by two technicians in and 8-h day.

CONCLUSIONS

Three methods of estimating numbers of somatic cells, the DMSCC field method, the WMT and the ESCC-chemical method, have been evaluated in this study in terms of precision and bias. The precision of the data presented in this paper when expressed as the coefficient of variation for counts near one million is 3.5% (ESCC-chemical method) 6.3% (WMT), and 15.6% (DMSCC-field method). However, these figures only represent the extent to which each method can reproduce itself.

Another essential characteristic of any technique is lack of significant bias. The ESCC and WMT were compared to the accepted standard, DMSCC, to evaluate this characteristic. Repeated tests by both ESCC and DMSCC on 10 samples selected to represent the range of 100,000 to 1,600,000 cells per ml indicated that both the mean and 95% confidence limits of the ESCC values are within the 95% confidence interval of the DMSCC field method for a given sample of milk.

The relationship between the ESCC values and DMSCC values on 405 samples of milk was linear, but suggest that the ESCC may underestimate the DMSCC on samples with low cell counts and overestimate the DMSCC on samples with high somatic cell counts. Because of the human element in the DMSCC counts, we believe that this characteristic may be a bias in the DMSCC values rather than the counts by the ESCC method. The predictive capability of the ESCC-chemical method suggest that it is an adequate alternate to the DMSCC.

The relationship between the WMT and DMSCC or ESCC in non-linear. Furthermore, the large confidence interval ± 55%, about the mean cell count predicted by the WMT, cannot be explained by lack of precision in the methods. This indicates that the WMT is biased by one or more factors in addition to the cell concentration in the sample. Hence, the WMT is not a suitable predictor of DMSCC.

The WMT is currently accepted as a screening procedure. One of the limiting WMT values, 22 mm, that is used for screening milk for over 1,500,000 cells per ml is very close to the upper 95% confidence limit of the regression curve of the WMT and DMSCC. This means that with these limits there should be very few false negative WMT results. Only one of the 405 random samples evaluated in this experiment showed a false negative WMT result by this standard. Thus, the WMT is a safe screening method, but a poor substitute for the DMSCC.

A time study on a single trial of 142 samples indicated that Coulter Counter estimates of somatic cell concentrations can be completed in 2.4 min of technician time per sample.

ACKNOWLEDGMENTS

Approved for publication as Scientific Journal Series Article No. 9419 of the Minnesota Agricultural Experiment Station.

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J. Milk Food Technol. Vol. 39, No. 12, Pages 859-863 (December, 1976)

Foodborne Disease Outbreaks Traced to Poultry, United States, 1966-1974

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(Received for publication June 15, 1976)

ABSTRACT

To determine the epidemiologic characteristics of foodborne disease outbreaks traced to poultry, we reviewed records of all 352 such outbreaks reported to the Center for Disease Control 1966-1974; 217 (62%) outbreaks were traced to turkey, 129 (37%) to chicken, 5 (1%) to both turkey and chicken, and 1 (0.3%) to cornish hen. Outbreaks from poultry accounted for 12% of all foodborne disease outbreaks reported from 1966 through 1974, but the number and percentage has been decreasing since 1969; these outbreaks involved 30,606 cases of gastrointestinal illness (20% of all cases of foodborne disease) and 14 deaths. Food-service establishments were responsible for mishandling the food in 79% of outbreaks, homes in 19%, and food-processing establishments in 2%. In 85% of the outbreaks, the food-handling error was storage of food at improper holding temperatures. In outbreaks reported 1972-1974 in which an etiologic agent was indentified, *Salmonella* spp. were responsible for 44%, *Clostridium perfringens* for 26%, and *Staphylococcus aureus* for 26%. The number of reported outbreaks from turkey increased dramatically during the Thanksgiving-Christmas holiday season. The downward trend in the number and percentage of poultry outbreaks since 1969 may reflect public education efforts.

This is the first major review of outbreaks traced to poultry (excluding eggs) since inception of foodborne disease surveillance at the Center for Disease Control (CDC) in 1966. It has been undertaken to determine epidemiologic characteristics of such outbreaks and to provide guidance as to how they might be better controlled.

METHODS

To prepare this report, records of all 2,920 foodborne disease outbreaks reported to the CDC Foodborne Disease Outbreaks Surveillance Activity from 1966 through 1974 were reviewed. A foodborne disease outbreak is defined as an incident in which two or more persons experience a similar illness after ingesting a common food, which epidemiologic data implicate as the source of illness. There are two major exceptions to this definiton—with botulism or chemical poisoning one case constitutes an outbreak. The records of foodborne disease outbreaks consist primarily of summaries of investigations conducted by state and local health departments, occasionally with the assistance of CDC. Some of the data emanate from the Food and Drug Administration (FDA), the U.S. Department of Agriculture (USDA), and the U.S. Armed Forces.

The number of outbreaks of foodborne disease reported to the Surveillance Activity is believed to represent only a small fraction of the total number that occur. Interstate outbreaks, large intrastate outbreaks, outbreaks caused by mishandling of food in food-processing establishments, and outbreaks of serious illness are more likely to be reported than small localized outbreaks or outbreaks of very mild or brief illness. The completeness and quality of the outbreak investigations vary widely among reporting sources.

RESULTS

From 1966 through 1974, 352 outbreaks traced to poultry were reported to CDC—217 (62%) to turkey, 129 (37%) to chicken, five (1%) to both turkey and chicken, and one (0.3%) to cornish hen (Table 1). The number of poultry outbreaks reported in a year rose from 27 and 26 in 1966 and 1967, respectively, to peak levels of 61 and 67 in 1968 and 1969; thereafter, the number per year gradually fell to 23 and 28 in 1973 and 1974 (Table 1 and Fig. 1). The 352 outbreaks from poultry accounted for 12% of the 2,920 foodborne disease outbreaks reported from 1966 through 1974 and 15% of the 2,329 outbreaks in which a specific food vehicle was identified. During the peak years, poultry outbreaks comprised 21% of all outbreaks in which a food vehicle was identified; in 1973 and 1974, they comprised only 10%. Peak reporting years for outbreaks traced to turkey and chicken coincided.

The 352 outbreaks involved 30,606 cases of gastrointestinal illness (Table 2). These cases accounted for 20% of all cases of foodborne disease reported from 1966 through 1974 (Fig. 1). The number of cases in any one outbreak ranged from one to 1,364 with a mean of 87. Outbreaks from turkey involved 70% of the cases or a mean of 100 cases per outbreak, while outbreaks from chicken had a lower mean of 56 cases per outbreak. By

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TABLE 1. *Outbreaks transmitted by poultry by year of occurrence, 1966-1974*

Year reported	Foodborne disease outbreaks						
	Total reported	Total with vehicle identified	Total poultry (%)	Turkey	Chicken	Turkey and chicken	Cornish hen
1966	181	169	27 (16)	11	14	2	0
1967	273	230	26 (11)	19	7	0	0
1968	345	302	61 (20)	40	19	2	0
1969	371	318	67 (21)	45	22	0	0
1970	366	295	51 (17)	31	19	0	1
1971	320	247	38 (15)	20	18	0	0
1972	301	247	31 (13)	19	12	0	0
1973	307	211	23 (11)	16	7	0	0
1974	456	310	28 (9)	16	11	1	0
Total	2,920	2,329	352 (15)	217	129	5	1

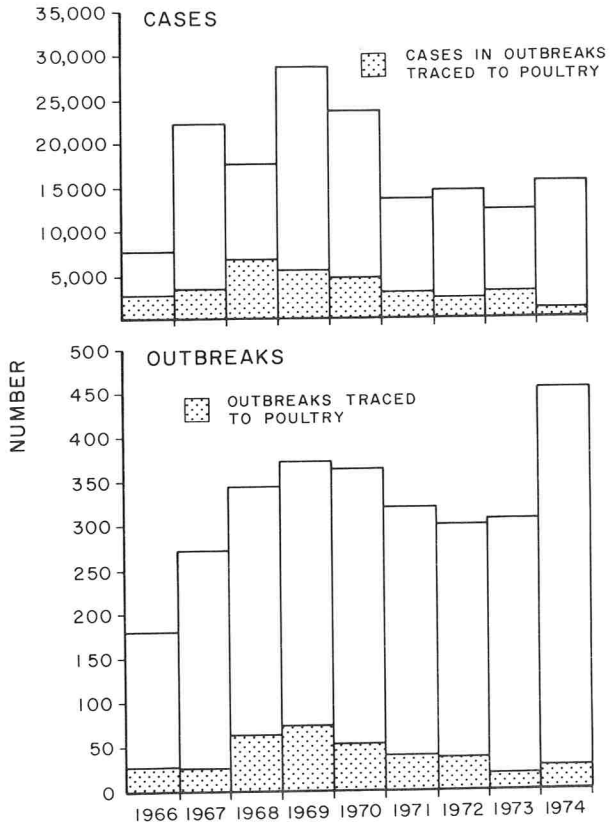

Figure 1. *Foodborne disease outbreaks and cases reported to Center for Disease Control, 1966-1974.*

comparison, the mean number of cases per outbreak for all foodborne disease outbreaks reported from 1966 through 1974 was 53. The case fatality ratio in the 30,606 cases was 0.05%. There were nine deaths in outbreaks traced to chicken (case-fatality ratio 0.1%) and five deaths in outbreaks traced to turkey (case-fatality ratio .02%).

In most of the outbreaks from poultry (62% of those from turkey and 50% of those from chicken), the meat itself was epidemiologically incriminated (Table 2). In the remainder, poultry in combination with or in addition to other foods was suspect. For turkey, the most common combinations were turkey with stuffing (15%) and turkey with gravy (10%). For chicken, the most common combination was chicken salad (16% of outbreaks). In 18 (8%) outbreaks traced to turkey and 33 (26%) outbreaks traced to chicken, a non-poultry food was also incriminated.

In most poultry outbreaks (Table 3), the incriminated

TABLE 2 *Outbreaks transmitted by poultry, cases and deaths, 1966-1974*

Poultry	Outbreaks	Cases	Deaths	Cases per outbreak
Turkey				
Turkey only	135	16,314	3	121
Turkey (sliced) sandwich	2	52	0	26
Turkey salad	8	1,277	0	160
Turkey with gravy	22	1,987	0	90
Turkey with stuffing	32	1,451	2	45
Turkey and other food[a]	18	668	0	37
Subtotal	217	21,749	5	100
Chicken				
Chicken only	64	2,503	6	39
Chicken salad	21	2,598	0	41
Chicken gravy	7	292	0	42
Chicken livers or gizzard	4	29	1	7
Chicken and other food[b]	33	1,820	2	55
Subtotal	129	7,242	9	56
Turkey and chicken	5	1,375	0	275
Cornish hen	1	240	0	240
Total	352	30,606	14	87

[a]Includes turkey a la king, creamed turkey.
[b]Includes chicken a la king, creamed chicken, chicken casserole, chicken soup.

food was eaten at home (22%), at school including the school cafeteria (20%), or at a restaurant (21%). By comparison, in all foodborne outbreaks reported from 1966 through 1974, the incriminated food was eaten at home in 35%, at a restaurant in 28%, and at school in only 8%. In outbreaks caused by turkey from 1966 through 1974, the incriminated food was most frequently eaten at school (26% of outbreaks).

The place where the food responsible for an outbreak was mishandled was specified in 190 (54%) outbreaks (Table 4). In analyzing food-handling errors, we recognized that poultry is commonly contaminated with potential enteric pathogens and we focused here not on the causes of this contamination but on the factors that subsequently contributed to outbreaks. Of the 190 outbreaks, food-service establishments (locations where food is prepared for public consumption, such as restaurants, cafeterias, caterers, hospitals, and industrial plants) were responsible in 79%, homes in 19%, and food-processing establishments (locations where a food is prepared for market) in 2%. By comparison, in all

outbreaks reported from 1966 through 1974 for which a place of mishandling was specified, food-service establishments were responsible in 61%, homes in 30%, and food-processing establishments in 10%.

Of the four outbreaks attributed to the mishandling of poultry in food processing establishments, pre-cooked "ready-to-eat" turkey meat was incriminated in three and chicken livers in one (Table 5). *Salmonella* organisms of different serotypes caused the three outbreaks from turkey and *Clostridium botulinum* type A caused the one from chicken livers. The four outbreaks affected 56 persons; the mean of 14 cases per outbreak was considerably lower than the mean of 87 cases per outbreak for all poultry outbreaks. One death occurred—in the outbreak of botulism. All four outbreaks occurred in 1968 and 1969.

In 138 (39%) of the outbreaks, one or more food-handling errors contributing to the outbreak were reported (Table 6). The most common one, reported in 117 (85%) outbreaks, was the storage of food at improper temperatures. Inadequate cooking, contaminated equipment, and poor personal hygiene were each

TABLE 3. *Outbreaks transmitted by poultry by place of acquisition, 1966-1974*

Poultry	Home	School	Restaurant	Church	Camp	Not specified or other[a]	Total
Turkey							
Turkey only	25	42	26	6	3	33	135
Turkey (sliced) sandwich	0	0	2	0	0	0	2
Turkey salad	2	3	1	2	0	0	8
Turkey with gravy	2	6	5	0	0	9	22
Turkey with stuffing	11	4	8	1	0	8	32
Turkey and other food	8	1	4	1	1	3	18
Subtotal	48	56	46	10	4	53	217
Chicken							
Chicken only	18	5	15	1	0	25	64
Chicken salad	2	4	4	4	0	7	21
Chicken gravy	0	1	4	0	0	2	7
Chicken livers or gizzard	1	0	1	0	0	2	4
Chicken and other food	9	4	4	2	2	12	33
Subtotal	30	14	28	7	2	48	129
Turkey and chicken	0	1	1	0	0	3	5
Cornish hen	0	0	0	0	0	1	1
Total	78	71	75	17	6	105	352

[a]Includes outbreaks in hospitals, nursing homes, prisons, banquet halls, fraternity houses, and on picnics

TABLE 4. *Outbreaks transmitted by poultry by place where food was mishandled, 1966-1974*

Poultry	Food-service establishment	Home	Food-processing establishment	Unknown	Total
Turkey					
Turkey only	65	8	3	59	135
Turkey (sliced) sandwich	2	0	0	0	2
Turkey salad	4	1	0	3	8
Turkey with gravy	10	3	0	9	22
Turkey with stuffing	8	7	0	17	32
Turkey and other food	5	5	0	8	18
Subtotal	94	24	3	96	217
Chicken					
Chicken only	22	2	0	40	64
Chicken salad	14	1	0	6	21
Chicken gravy	3	0	0	4	7
Chicken livers or gizzard	0	0	1	3	4
Chicken and other food	15	8	0	10	33
Subtotal	54	11	1	63	129
Turkey and chicken	2	0	0	3	5
Cornish hen	1	0	0	0	1
Total	151	35	4	162	352

TABLE 5. *Outbreaks transmitted by poultry caused by mishandling of food in food-processing establishments, 1966-1974*

Year	Month	State	Vehicle of transmission	Cases	Deaths	Place of exposure	Etiologic agent	Reference
1968	Oct.	California	Bottled chopped chicken livers	1	1	Home	*Clostridium botulinum* Type A	(2)
1968	Nov.	Washington	Pre-cooked turkey[a]	33	0	Motel	*Salmonella reading*	(4)
1969	Jan.	Tennessee	Smoked Turkey*	11	0	Home	*Salmonella infantis*	(3)
1969	Dec.	Washington	Smoked Turkey*	11	0	Home	*Salmonella saint-paul*	(5)

[a]These turkeys were sold "ready-to-eat."

reported as contributing factors in 37 (27%) outbreaks. The frequencies of factors contributing to outbreaks from chicken and turkey were similar.

Criteria for confirming the etiologic agent of an outbreak of foodborne disease were established in 1972 (*6*). Of the 82 poultry outbreaks reported from 1972 through 1974, an etiologic agent was confirmed in 34 (Table 7). In all of these outbreaks, the agent was bacterial. Salmonellae of various serotypes were responsible for 15 (44%) of these outbreaks, *C. perfringens* and staphylococci were each responsible for nine (26%) and *Shigella flexneri* was responsible for one (3%). *C. perfringens* was a more prominent cause of outbreaks from turkey (33%) than from chicken (18%). By comparison, in all outbreaks of foodborne disease of confirmed etiology reported from 1972 through 1974, bacterial etiologies accounted for 65%—salmonellae for 23%, staphylococci for 21%, *C. perfringens* for 7%, shigellae for 3%, and other bacteria for 11% (*8*).

Outbreaks from chicken were more or less evenly distributed throughout the year, except perhaps for a decrease during the winter months, while the number of reported outbreaks from turkey were dramatically increased in November and December (Fig. 2). By comparison, outbreaks of all foodborne disease tend to be distributed more or less evenly throughout the year; however, outbreaks caused by salmonellae and staphylococci occur more frequently in the summer months.

DISCUSSION

Outbreaks transmitted by poultry comprised a substantial percentage of the total outbreaks reported during the period 1966-1974 but it is noteworthy that the percentage has been decreasing from its peak of 21% in 1968 and 1969. We do not know the cause of this recent

TABLE 6. *Outbreaks Transmitted by poultry by contributing factors, 1966-1974*

Poultry	Number of reported outbreaks	Number of outbreaks in which factor(s) reported	Improper holding temperature	Inadequate cooking	Contaminated equipment	Poor personal hygiene	Other
Turkey							
Turkey only	135	48	36	21	11	10	2
Turkey (sliced) sandwich	2	2	2	1	2	1	0
Turkey salad	8	2	2	0	0	0	0
Turkey with gravy	22	11	11	2	3	3	0
Turkey with stuffing	32	11	11	2	3	3	1
Turkey and other food	18	9	7	1	1	2	1
Subtotal	217	83	69	27	20	19	4
Chicken							
Chicken only	64	20	19	5	5	6	2
Chicken salad	21	14	10	0	3	6	1
Chicken gravy	7	2	2	0	0	0	0
Chicken livers or gizzard	4	1	1	1	0	0	0
Chicken and other food	33	18	16	4	9	6	0
Subtotal	129	55	48	10	17	18	3
Turkey and chicken	5	0	0	0	0	0	0
Cornish hen	1	0	0	0	0	0	0
Total	352	138	117	37	37	37	7

TABLE 7. *Outbreaks Transmitted by poultry by Etiologic agent, 1972-1974*

Poultry	*Salmonella*[a]	*C. perfringens*	*Staphylococcus*	Unknown	Total
Turkey					
Turkey only	5	6	2	19	32
Turkey (sliced) sandwich	0	0	0	0	0
Turkey salad	1	0	1	1	3
Turkey with gravy	0	1	0	3	4
Turkey with stuffing	2	0	2	3	8[b]
Turkey and other food	0	0	0	4	4
Subtotal	8	7	5	30	51
Chicken					
Chicken only	2	2	3	11	18
Chicken salad	3	0	0	1	4
Chicken gravy	0	0	0	0	0
Chicken livers or gizzard	0	0	0	0	0
Chicken and other food	1	0	0	6	7
Subtotal	6	2	3	18	29
Turkey and chicken	0	0	1	0	1
Cornish hen	1	0	0	0	1
Total	15	9	9	48	82

[a]Includes two outbreaks of *S. reading* and 1 outbreak of *S. san diego, S. chester, S. manhatten, S. saint paul, S. kottbus,* and *S. oranienburg.*
[b]Includes one outbreak of *Shigella flexneri.*

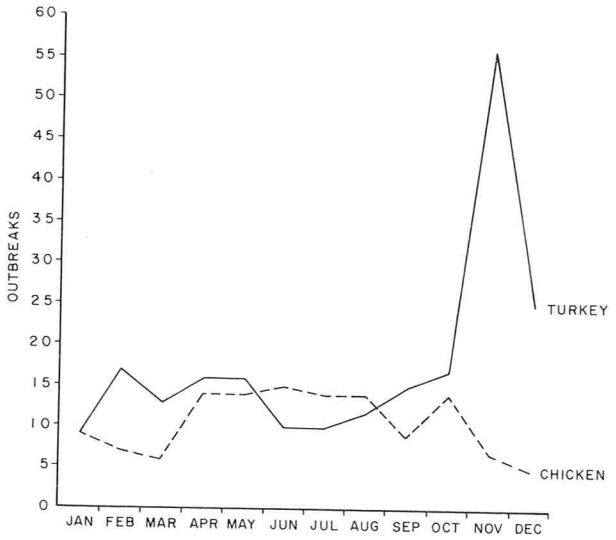

Figure 2. *Foodborne disease outbreaks transmitted by turkey and chicken, by month of occurrence, 1966-1974.*

reduction, but we wonder if it reflects a favorable impact of public education efforts to control salmonellosis conducted in this country following the 1969 recommendations of the National Research Council's Committee on *Salmonella* (*7*). The data suggest that public health education may be particularly important during the Thanksgiving-Christmas holiday season.

Outbreaks from turkey greatly outnumbered outbreaks from chicken, although far more chicken than turkey is consumed in the United States. This may be because turkeys are often prepared a day or more before serving (*1*) and are often cooked whole and stuffed, which renders adequate cooking and refrigeration somewhat more difficult.

Outbreaks from turkey were not only more numerous but they also affected larger numbers of people. The mean number of persons per outbreak was double that found in chicken outbreaks and double that found in all outbreaks reported from 1966 through 1974. This is probably because turkey is often served to large gatherings of people particularly during the Thanksgiving-Christmas holiday season, when a dramatic increase in reported outbreaks traced to turkey occurred. Deaths were relatively rare in outbreaks from poultry as in foodborne disease generally.

In a large proportion of outbreaks traced to poultry, the food was eaten at school. The proportion occurring in schools was more than double that found in all foodborne disease outbreaks reported from 1966 through 1974; in outbreaks from turkey, schools were a particularly frequent place of acquisition, implicated in over one-quarter of these outbreaks. Bryan and McKinley have noted the problem of outbreaks from turkey in schools and have recommended procedures for preparing, thawing, cooking, cooling, and reheating turkeys in a school kitchen (*1*).

Food-service establishments were almost always responsible for the food-handling error that resulted in the outbreaks. Homes were sometimes the place where the food-handling error took place; food-processing establishments were rarely responsible.

The major food-handling error contributing to outbreaks was storage of food at improper temperatures, i.e. between 44 F (7 C) and 140 F (60 C). Inadequate cooking, contaminated equipment, and poor personal hygiene were less frequently reported.

The major etiologic agents found in poultry outbreaks of confirmed etiology reported from 1972 through 1974 were salmonellae, *C. perfringens,* and staphylococci. Raw turkeys have often been found contaminated with these three organisms (*1*), and improper food-handling practices may allow these bacteria to multiply to numbers capable of causing illness. Food-handlers have also been a potential source of foodborne pathogens, particularly staphylococci.

ACKNOWLEDGMENTS

We thank Frank L. Bryan, Cheif, Foodborne Disease Training, Instructional Services Division, Bureau of Training, CDC; E. M. Foster, Director, and J.M. Goepfert, Food Research Institute, The University of Wisconsin, Madison, Wisconsin; and Arthur J. Mercuri, Chief, Animal Products Laboratory, Agricultural Research Service, USDA, Athens, Georgia, for their helpful comments on the manuscript.

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J. Milk Food Technol. Vol. 39, No. 12, Pages 864-871 (December, 1976)

Coagulation of Milk with Immobilized Proteases: A Review

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ABSTRACT

Enzymatic coagulation of milk by immobilized proteases, and their potential application to cheese manufacture, is reviewed. Particular emphasis is given to the immobilized protease catalyst and to the reactor design for coagulation of milk. Pepsin and chymotrypsin retained more activity and greater stability than the other immobilized proteolytic enzymes. Porous glass beads, several anion exchange resins, and the copolymer ethylene-maleic anhydride gave best results among the support materials that were evaluated. Covalent attachment of enzyme to support is preferable to adsorption techniques but may be too costly. Perhaps the best catalyst is one using a lengthy procedure for covalent immobilization of enzyme on glass beads but good results were also obtained with simpler adsorption techniques. Catalysts varied greatly in initial activity but all lost activity upon exposure to milk. Stirred tank, packed bed, and fluidized bed reactor designs were used. Continued research is required to make enzymic milk coagulation with immobilized proteases economically feasible.

A relatively new field, immobilized enzymes encompass surface and biological chemsitry, providing an interface for imaginative applications, be they theoretical or practical. Since about 1960 (*70*), immobilized enzymes have been discussed in over 1,500 research papers, many trade journal articles, numerous reviews (*1,50,61,65,73, 74,77,78,86-89,95*), several books (*40,57,68,100*), and various patents. Immobilized enzymes can offer certain advantages over soluble enzymes in areas such as the study of enzymes, analytical biochemistry, preparative pharmacology, and industrial processing — including food processing (Table 1). For example, in industrial processing, immobilized enzymes are reusable, generally more stable and more suited to continuous processing design than soluble enzymes. Specific uses of immobilized enzymes illustrating their wide variety of applications include a cobalamin binding study (*84*), elucidation of the casein micelle structure (*4, 15*), steroid transformation (*60*), leukemia therapy (*21*), urea-selective electrode (*39*), chill-proofing of beer (*96*), producing lactose-free milk (*25,98*), and reduction of nitrate and nitrate in waste treatment (*58*). Although immobilized enzymes have gained usage in a few commercial operations, there is considerable work remaining to fulfill their potential applications.

TABLE 1. *Soluble versus immobilized enzymes in food processing: Advantages and disadvantages*[1]

Disadvantages of soluble enzyme

1. Large amount of enzyme can remain in product to participate in further (undesirable) reaction.
2. Re-use of enzyme precluded.
3. Extent of reaction limited by product inhibition.
4. Precise control difficult.
5. If necessary, enzymatic reaction generally stopped by heating, which can be detrimental to the food as well as add another processing step.
6. Largely a batch process.

Advantages of immobilized enzyme

1. Enzyme reusable.
2. Reaction easily terminated by separating substrate from enzyme.
3. More precise control.
4. Less product inhibition.
5. Greater pH and temperature stability.
6. Can use enzymes presently unusable for various reasons.
7. Potential operation over greater pH range by modifying charge characteristics of support.
8. Continuous or batch use.
9. Greater reactor design flexibility.

Disadvantages of immobilized enzyme

1. Lower specific activity.
2. Inactivation with continued operation.
3. Cost of support and immobilization procedure.

[1]Advantages of the soluble enzyme are not listed. The soluble enzyme is the presently used form and thus implicitly has advantages.

An immobilized enzyme requires a support material, a method of immobilization, and an enzyme. A variety of enzymes have been immobilized (*100*). Support materials for enzyme immobilization are quite diverse (*73,74*) including: (*a*) *polymers* (*51*) such as polystyrene (*9, 33*), nylon (*30,37,44,59,81*), ethylene-maleic anhydride copolymer (*52*), and Teflon (*34*); (*b*) *inorganic substances* (*10,56,92*) such as alumina (*13*), glass (*72,83,90,93,94, 97*), stainless steel (*12,13,41*), and sand (*47,83*); (*c*) *natural products* such as cellulose (*49*), collagen (*22,35,45*), and chitin (*79,80*); and (*d*) *modified materials* such as DEAE-cellulose (*99,100*) and DEAE-Sephadex (*99,100*). The physical form of these supports is also quite diverse; they can exist as powders, particles, granules, sponges, gels, spheres, fibers, and membranes.

Several immobilization methods are available which may be placed in three categories: (*a*) *adsorption,* enzyme physically adsorbed to a water insoluble matrix; (*b*) *covalent attachment*, chemical bond formed between enzyme and support material; and (*c*) *entrapment*, chemical and/or physical localization of enzyme in water insoluble matrix. Examples from each category include physical adsorption of phosphatase on hydrophobic derivatives of cellulose (*11*), lactase covalently coupled to porous glass (*97*), and invertase entrapped in fibers (*53*).

Immobilized enzymes allow greater flexibility in reactor design than do soluble enzymes, which may be their most significant advantage. Common reactor designs for using immobilized enzymes are packed bed, fluidized bed, and stirred tank reactors (*5,8,27,50,89*). These reactors permit either batch or continuous processing but continuous processing is inherently more efficient than batch. Continuous operation would be the ideal usage of immobilized enzymes, particularly in food processing.

Many elegant covalent attachment and polymeric entrapment procedures using expensive, functionalized support materials have been published. Food processing with immobilized enzymes, however, probably will require simple immobilization procedures and inexpensive support materials for the foreseeable future. Unless exceptional performance is obtained with the more costly catalysts, the added expense of immobilization cannot be justified in a food processing system. Both commercially successful food processes (see below) using immobilized enzymes employ adsorption (a simple immobilization procedure) on inexpensive DEAE-modified matrices (*3,7,55,75,77*).

Thus, the ideal food processing application of immobilized enzymes will apparently require an inexpensive support, a simple preparation, and a reactor design permitting good substrate-enzyme contact in a continuous, free-flowing operation. Ultimately, use of immobilized enzymes will be an economic decision, based as much on catalyst cost and performance as on processing changes.

Examples of food processes that currently use immobilized enzymes are limited (*57,65,77,91*). However, immobilized enzymes are used as an integral part of two commercial food processing systems. The resolution of L-amino acids by immobilized L-amino acid acylase is a commercial process in Japan (*75,77*). The immobilized enzyme selectively removes the acetyl group from the optically active L-isomer of a racemic mixture of the acetylated amino acid. The resulting free L-form is easily separated from the acetylated D-form, which is then racemized chemically to regenerate more L-amino acid. Converting to this continous process from the previous batch operation reduced costs about 40%.

Glucose isomerase, one of the more important soluble enzymes in the food industry, is used in the other commercial immobilized enzyme system. Clinton Corn Processing Company received the 1975 IFT Food Technology Industrial Achievement Award for developing a process to produce high fructose corn syrup using immobilized glucose isomerase (*55*). Other companies—Novo Enzyme Corporation, Penick and Ford Corporation, and Corning Glass Works—have developed similar systems. The enzymatically converted cornstarch costs less than does invert sugar produced from sucrose, and has comparable sweetness. The commercial process involves liquefying raw cornstarch, saccharifying to dextrose, isomerizing to fructose, and refining (*3,7,77*). This is the first large scale commercial application of immobilized enzymes in a continous process in the world and is still the largest. Many other potential food processing applications of immobilized enzymes have been studied or proposed but none have become commercially viable (*57,65*).

COAGULATION OF MILK WITH IMMOBILIZED PROTEASES

Clotting milk with immobilized proteases may find application in cheesemaking (*48,66,67,71*). Current research in the cheese industry is attempting to make the traditional, labor-intensive, batch process more efficient and ultimately continous (*48,54,85*). Retaining the batch process, the industry has advanced through using larger batch units (*48*), thereby achieving an economy of scale. Mechanization (*64,85*)—and even automation (*63*)—of the various batch steps has also improved efficiency. New enzymes have been used to supplant the dwindling supply of rennet (*62*). Direct acidification has been researched as a way to avoid use of starter cultures (*69*).

A fully continuous cheesemaking system is still in the developmental stage. A continous process for making cottage cheese has been reported but is not being used (*28*). However, several commercial units are available that make individual batch steps continuous. For instance, equipment for continuous cheddaring and salting have been developed (*19,23,24,63*). A continuous coagulator using soluble milk clotting enzyme in a cold renneting step has also been reported (*48*).

Enzymatic milk coagulation, a two-phase process, involves first the enzymatic cleaving of a phenylalanyl-methionine bond in *k*-casein; this splits off negatively charged peptides and thus destabilizes the casein micelle (*29*). The secondary phase is physical aggregation of micelles to form a coagulum. The large temperature coefficient (Q_{10}) of the secondary phase but low Q_{10} of the primary phase permits separation of the two phases by lowering the temperature. Thus, the immobilized enzyme retains sufficient activity at lower temperatures to complete the primary enzymatic phase but clotting would not occur until after milk is removed from the immobilized enzyme and warmed.

Enzymatic coagulation of milk with immobilized proteases has been reviewed (*65,66,67,70,71*). This review is concerned with the potential application to cheesemaking of the coagulation of milk with the immobilized proteases reported in the literature. Thus, the following information about each previous research study is important and will be discussed: (*a*) enzyme used, (*b*) support used, (*c*) method of immobilization, (*d*) activity of resulting catalyst, (*e*) inactivation rate of catalyst, (*f*) reactor design, and (*g*) contact time and flow rate.

The various results reported in the literature are difficult to compare since they were obtained and reported in different ways. Most papers did not give enough information on which to compare activity on the basis of milligrams of bound enzyme. Furthermore, some researchers coagulated milk at its normal pH, some

acidified milk before enzyme contact, and others acidified milk after enzyme contact. Both phases of enzymatic milk coagulation are pH dependent—and the secondary phase is highly pH dependent—making results from these different pH values difficult to interpret. Reactor design, contact times, and flow rates varied but a reasonable idea of the immobilized protease activity and the milk clotting system was obtained from each paper and compared to the others.

Enzyme used

Use of immobilized instead of soluble enzymes for milk coagulation offers some advantages from the standpoint of the enzyme itself. First, there is a worldwide shortage of veal rennet, prompting use of other suitable proteases in traditional cheesemaking systems. However, the substitutes have some limitations (*62*). Shortage of milk-clotting enzymes may be eased by employing immobilized enzymes which can be re-used. Second, since the immobilized enzyme does not contaminate cheese, other proteases could be used that are not suitable in the soluble form because of excessive proteolysis. In addition, lack of contamination of cheese with milk-clotting enzymes would allow more controlled, longer ripening times. Greater storage stability would be advantageous with high-moisture cheeses since greater amounts of these types could be manufactured during peak milk production and held until seasonal slumps in production. In these types and other cheese varieties controlled amounts and types of proteases can be added to obtain the desired rate of proteolysis. Proteases can be selected for their beneficial effects on cheese ripening rather than a compromise between milk-clotting and cheese ripening capabilities. It may be possible also to infuse these proteases into cheese curd and eliminate their loss into cheese whey. While use of immobilized milk-clotting enzymes appears promising, yield, flavor, and texture of cheese made with immobilized clotting enzymes need to be researched.

Several proteolytic enzymes have been immobilized for use in milk coagulation (Table 2). Direct comparison of catalyst performance on the basis of enzyme immobilized is unfair since the support material and immobilization method have a great effect on catalyst activity. But, taking that into consideration, pepsin appears to be the best immobilized protease for coagulation of milk that is reported in the literature. Pepsin, an enzyme with a low pH optimum and not particularly active nor stable at the normal pH of milk, is quite active and stable upon immobilization (*14,16,18*). Perhaps this is due to the negatively charged supports to which it has been attached and which effectively put pepsin in a lower microenvironmental pH. Rennet was found to be more active but less stable than pepsin (*16*). It has been reported that chymotrypsin was used with some success (*99*). The *Mucor miehei* proteases, papain, and trypsin were not as effective as other milk-clotting enzymes used (*16,20,36*).

TABLE 2. *Proteolytic enzymes used in immobilized enzyme coagulation of milk*

Enzyme	References
Chymotrypsin	*16,26,38,99*
Mucor miehei proteases	*16*
Papain	*20,46*
Pepsin	*16,17,18,32,36,43*
Rennin	*16,38*
Rennet	*16,17*
Trypsin	*36*

Supports used

For coagulation of milk with immobilized proteases to be commercially feasible, the support material must be evaluated for cost, physical characteristics suitable for particular processing conditions, extent of enzyme-substrate contact, toxicity, and flow characteristics. A variety of support materials have been used to immobilize proteases for milk coagulation (Table 3). Milk-clotting enzymes have been adsorbed or covalently bound to several anion exchange resins (*38,39*). Three polymers—agarose, ethylene-maleic anhydride (EMA), and polyacrylamide—were used with EMA being most satisfactory (*20,36,38*). Porous glass beads were also used successfully (*16,17,32,43*); coating of the glass with zirconium oxide improved performance (*18*).

Methods of immobilization

Covalent attachment and physical adsorption are the two major methods reported in the literature for immobilizing milk clotting proteases (Table 3). No entrapment procedure has been published since this method is applicable only to small molecular weight substates able to diffuse in and out of the entrapping material.

TABLE 3. *Support materials and immobilization methods of immobilized proteases used in the coagulation of milk*

Support	Method of immobilization	References
Agarose	Covalent	*38*
EMA resin	Covalent	*36*
CM-cellulose	Covalent	*20,38*
Polyacrylamide	Covalent	*20*
Porous glass	Covalent	*16,17,32,43*
ZrO_2-coated porous glass	Covalent	*18*
DEAE-cellulose	Adsorption	*38,99*
Amberlite GC 400 I	Adsorption	*99*
DEAE-Sephadex	Adsorption	*99*
(Enzyme polymer)	Glutaraldehyde cross-linking	*46*

The method of immobilization is quite important in analyzing applicability of catalyst to commercial milk coagulation. Questions to be answered include: (*a*) Is the method simple and inexpensive? (*b*) Does the method prevent desorption of enzyme? (*c*) How much enzyme is bound? (*d*) What is the specific activity? (*e*) Will the method be approved for food use? Physical adsorption is the simplest, least expensive method but generally allows desorption of enzyme. Green and Cruthchfield (*38*) adsorbed rennin to DEAE-cellulose but found all of the resulting activity due to leached, soluble enzyme. However, Yanushauskaite et al. (*99*) adsorbed chymotrypsin first to a chlorotriazine dye and then to

anion exchange resins and reported no desorption of enzyme.

Covalent attachment of the milk-clotting enzyme to the support material has been used in most research. This procedure is preferable for laboratory studies but may be too complicated and costly for preparing immobilized cheesemaking enzymes. Covalent bonding usually eliminates desorption of enzymes; this problem was reported in one study but was subsequently corrected (*18*). Minimizing desorption of immobilized milk-clotting enzyme is important to lessen catalyst inactivation and control proteolysis in cheese curd during aging.

Although important for scale-up calculations, few papers report enzyme bound on a unit basis. Specific activity, also important, is inadequately reported. To illustrate the range reported, Yanushauskaite et al. (*99*) found retention of only 2-5% of the specific activity of the bound chymotrypsin compared to the soluble form whereas Goldstein (*36*) observed 40% retention of specific activity of pepsin upon immobilization. The ideal is to have a method producing a catalyst with maximum specific activity and activity per unit of support to minimize the size of the reactor.

Catalyst activity

Catalyst performance depends upon both activity and stability. A catalyst with high initial activity and a slow rate of inactivation is preferred. Comparison of catalyst activities reported in the literature is difficult since activity was determined in different ways. Activity depends on temperature of reaction, amount of enzyme used, pH of milk, contact time, etc., and these parameters varied among papers. However, initial activities varied greatly, suggesting real differences in the catalysts and not just in experimentation. For instance, Green and Crutchfield treated 5 ml of milk at 30 C with 1.7 mg of bound enzyme and observed clotting in 115 min. This low activity required a long contact time (flow rate of 240 ml/3 days) in their packed bed reactor. Cooke and Caygill (*20*) also reported low activity with a packed bed reactor, containing 15 mg of bound enzyme, operating at 4 C and a milk flow rate of 3-4 ml/h. Effluent milk clotted in 5 min after warming to 37 C. Yanushauskaite et al. (*99*) obtained somewhat better results. Milk (5 ml) treated for 15 min at 3 C with 20 mg of bound chymotrypsin clotted in 30 sec upon warming to 37 C.

Cheryan et al. (*14, 16-18*) obtained high activity with pepsin covalently coupled to porous glass beads. Milk of normal pH and at 10 C was passed through a fluidized bed reactor containing about 5 mg of bound enzyme. A contact time of only 1 min resulted in immediate clot formation after adjusting the milk to pH 6.1 and 30 C.

Inactivation rate

All previous reports indicated a decline in milk clotting activity of the immobilized enzyme during continuous exposure to milk. Although details of inactivation rates were given in only a few papers, it appears that these rates varied. Inactivation rates are very important for commercial operation; slow obviously is desirable. Inactivation seems to be a characteristic of all immobilized enzymes but is greatly accelerated when the substrate contains protein. Cooke and Caygill (*20*) found that repeated use of their immobilized papain led to a gradual loss in activity. Yanushauskaite et al. (*99*) observed that an initial flow rate of 3-4 ml/h produced a clot in 5 min but only five column volumes of milk later this flow had to be reduced to 1.5 ml/h to obtain the same clotting time. Ferrier et al. (*32*) observed a slower decline of milk-clotting activity of pepsin covalently attached to glass beads that was dependent upon pepsin source and temperature of milk being treated but independent of flow rate. Using the same catalyst, Cheryan et al. (*14, 16, 17*) obtained a slightly better stability of the immobilized protease activity by using milk of normal pH in a fluidized bed reactor rather than a packed bed reactor. Subsequently, Cheryan et al. (*18*) further improved stability of the immobilized protease activity and reported a 40-70-h useful catalyst lifetime in their system. They used a ZrO_2-coated controlled pore glass support and glutaraldehyde as the coupling agent to precoat the support with bovine serum albumin before attaching pepsin. The labile imine bonds formed between the proteins and glutaraldehyde during immobilization were reduced with sodium borohydride.

Reactor design

One of the great advantages in using immobilized proteases to coagulate milk is the flexibility in reactor design. The reactor is that vehicle providing contact between enzyme and milk (*50*). Three common designs—fluidized bed, packed bed, and stirred tank—which are applicable to continuous processing have been reported in the literature (Table 4). It is important that the reactor provide good enzyme-substrate contact but also allow free flow. Reactor design and the physical form of the catalyst must be complementary.

TABLE 4. *Reactor designs used in the coagulation of milk with immobilized proteases*

Reactor	References
Packed bed	*20,32,38,43*
Stirred tank	*26,36,38,99*
Fluidized bed	*16,17,18*

Both stirred tank and fluidized bed reactors appear well suited to treat milk with immobilized proteases. Shear forces in stirred tank reactors limit types of catalysts to those having sufficient structural integrity. The stirred tank can be used on a batch basis if the enzyme is filtered out after the reaction has been completed. The packed bed reactor, which has been investigated in many applications of immobilized enzymes, is limited by milk coagulating and plugging the column. The fluidized bed reactor alleviates plugging of the packed bed and can be used with supports that would disintegrate in a stirred tank (*8, 27*).

Contact time and flow rate

Contact time (with a known flow rate) determines reactor size and amount of immobilized enzyme required and influences the economics of a commercial continuous coagulator. A high flow rate and short contact time is desirable so that a large quantity of milk can be clotted by a small amount of catalyst in a small reactor.

Reported contact times varied greatly (Table 5). The extremely long contact times reported are not suitable for cheesemaking.

TABLE 5. *Contact times and flow rates used in the coagulation of milk with immobilized proteases*

Flow rate or contact time	Initial clotting time	Reactor temperature (C)	References
250 ml/3 days	115 min	4	*38*
3-4 ml/h	5 min	4	*20*
15 min	.5 min	3	*99*
1.7 min	1.7 min	36	*26*
1 min	immediate	10	*16,17,18,32,43*

CONCLUSIONS

A summary of the research literature concerning milk clotting with immobilized proteases is presented in Table 6. Of the immobilized proteases cited, the best system reported appears to be one using the catalyst and reactor design described by Cheryan et al. (*16, 18*). This system includes pepsin covalently attached to a zirconium oxide coated porous glass bead, precoated with bovine serum albumin. These beads are used in a fluidized bed reactor at low temperature to separate the two phases of enzymatic milk coagulation. Effluent milk is acidified and warmed for coagulation. Short contact times and high activity were observed. Inactivation of catalyst resulted in an approximately 50-h useful catalyst life.

However, this system is far from the ideal, and preliminary calculations show the system to be economically infeasible at present. The glass support material is costly, lacks sufficient density for optimum fluidization, and the immobilization procedure is lengthy. The clotting activity exceeds that of other reported immobilized proteases, but a catalyst with higher activity and a slower rate of inactivation would be much more desirable. Further studies on cheaper, denser supports and simpler immobilization procedures that give catalysts with comparable or greater activity are necessary to allow more favorable economic projections.

Milk is nearly an ideal food with which to study use of immobilized enzymes. It is a fluid and enzymes are already used in various milk processes (*2, 48, 76*). However, the protein in milk appears to deposit on the immobilized enzyme leading to a rapid decline in activity of the enzyme catalyst. Regeneration of the catalyst activity has been only partially successful (*14, 16, 32*).

Other immobilized enzymes have been studied for use in milk (*14, 48, 66, 67, 71, 76*). These include immobilized catalase to destroy hydrogen peroxide used in cold pasteurization of milk (*6*), immobilized peroxidases as antimicrobial agents (*66*), immobilized lactase to hydrolyze lactose (*94, 98*), and immobilized sulfhydryl oxidase to eliminate cooked flavor from milk sterilized by the Ultra-High Temperature process (*77, 82*). Perhaps use of immobilized proteases in a continuous system will eventually be integrated into commercial cheesemaking.

TABLE 6. *Coagulation of milk with immobilized proteases: A summary*

Enzyme	Support	Immobilization method	Activity	Inactivation rate	Reactor	Contact time	References
Chymotrypsin and rennin	Agarose	Covalent	Poor	—	Packed bed and stirred tank	Long	*38*
Rennin	DEAE-cellulose	Adsorption	Poor	—	Packed bed and stirred tank	Long	*38*
Papain	None	Glutaraldehyde cross-link	—	—	—	—	*46*
Pepsin and trypsin	EMA resin	Covalent	Good	Gradual	—	—	*36*
Papain	CM-cellulose and polyacrylamide	Covalent	Poor	Rapid	Packed bed	Long	*20*
Chymotrypsin	Amberlite and DEAE-cellulose and DEAE-Sephadex	Adsorption	Poor	—	Stirred tank	Medium	*99*
Chymotrypsin	CM-cellulose	Covalent	Poor	—	Stirred tank	Long	*26*
Pepsin	Porous glass	Covalent	Good	Gradual	Packed bed	Short	*32,43*
Rennin and chymotrypsin and *Mucor miehei* proteases	Porous glass	Covalent	Poor	—	Fluidized bed	Short	*16*
Pepsin	Porous glass	Covalent	Very good	Gradual	Fluidized bed	Short	*16,17*
Rennet	Porous glass	Covalent	Excellent	Gradual but faster than pepsin	Fluidized bed	Short	*16*
Pepsin	Porous glass	Covalent	Excellent	Slowest	Fluidized bed	Short	*18*

ACKNOWLEDGMENTS

Research reported from the author's laboratories was supported by the College of Agricultural and Life Sciences, University of Wisconsin, Madison, and by grants from Cooperative Research Service, U.S. Department of Agriculture and Dairy Research, Inc., Arlington Heights, Illinois.

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J. Milk Food Technol. Vol. 39, No. 12, Pages 872-877 (December, 1976)

3-A Sanitary Standards for Silo-Type Storage Tanks for Milk and Milk Products

Number 22-04

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

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

A.

SCOPE

A.1

These standards cover the sanitary aspects of silo-type storage tanks for milk and milk products.

A.2

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

B.

DEFINITIONS

B.1

PRODUCT: Shall mean milk and milk products.

B.2

SILO-TYPE TANK: Any vertical tank in excess of 10 feet inside height for the storage or storage and cooling of milk or milk products.

B.3

CONTROL AREA(S): Shall mean the area(s) in which all appurtenances for the operation of the silo tank are located and vent lines terminate, except as provided in subsection D.11 and shall be a part of one or more of the following:

B.3.1

A processing area.

B.3.2

An area in the plant at least the equivalent of a processing area.

B.4

ALCOVE(S): Shall mean an extension of the control area(s) in which appurtenances and vent line openings are located.

B.5

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.6

SURFACES

B.6.1

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.6.2

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

C.

MATERIAL

C.1

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

C.1.1

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

C.1.2

Plastic materials may be used for bearings, gaskets,

[1]*The data for this series are contained in the following reference: AISI Steel Products Manual, Stainless & Heat Resisting Steels, April 1963, Table 2-1, pp. 16-17. Available from American Iron and Steel Institute, 150 East 42nd Street, New York, N.Y. 10017.*

[2]*Alloy Casting Institute Division, Steel Founders' Society of America, 21010 Center Ridge Road, Rocky River, Ohio 44116.*

seals, and parts used in similar applications. These materials shall comply with the applicable provisions of the "3-A Sanitary Standards for Multiple-Use Plastic Materials Used as Product Contact Surfaces for Dairy Equipment, Serial #20-00" as amended.

C.1.3

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

C.2

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

D.

FABRICATION

D.1

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

D.2

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

D.3

All product contact surfaces shall be easily accessible for cleaning, either when in an assembled position or when removed. Removable parts shall be readily demountable. Sight or light openings, when provided, shall be relatively flush and shall be located in a control area.

D.4

Means shall be provided for mechanically cleaning the product contact surfaces of the tank, piping and all non-removable appurtenances thereto.

D.5

All product contact surfaces shall be self-draining. The bottom of the lining, if flat, shall have a minimum slope of ¾ inch per foot toward the outlet or if the bottom of the lining is of the reverse dish-type the portion of the bottom adjacent to the side wall shall have a minimum slope of ¾ inch per foot toward the outlet.

D.6

Gaskets shall be removable. Any gasket groove or gasket retaining groove shall not exceed ¼ inch in depth or be less than ¼-inch wide. The minimum radius of any internal angle in a gasket groove or gasket retaining groove shall be not less than 1/8 inch.

D.7

The inside radii of all welded or permanent attachments shall be not less than 1/4 inch. Where the top head and the bottom join the vertical lining of the tank the radius shall not be less the 3/4 inch. The top head shall be dished or otherwise shaped so that it readily facilitates mechanical cleaning.

D.8

There shall be no threads on product contact surfaces.

D.9

Sanitary pipe and fittings shall conform with "3-A Sanitary Standards For Fittings Used On Milk And Milk Products Equipment And Used On Sanitary Lines Conducting Milk And Milk Products, Number 08-17," except that sanitary fittings made of optional metal alloy shall not be used.

D.10

Fittings to accommodate the temperature sensors of both an indicating thermometer and a recording thermometer shall be provided.

D.10.1

They shall conform to one of the following types:

D.10.1.1

Fittings conforming to "Supplement No. 1 to 3-A Sanitary Standards for Instrument Fittings and Connections Used on Milk and Milk Products Equipment, Number 09-07."

D.10.1.2

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

D.10.2

The fittings for temperature sensors shall be located not more than 24 inches above the bottom of the lining and the indicating or recording thermometer shall be located so that it is easily readable.

D.11

All openings in the tank lining shall be within a control area, except openings for mechanical agitators and openings for cleaning and/or vent line(s). Cleaning and/or vent line(s) shall terminate in a control area.

When the re-vent line method is used to prevent siphonage, the terminal ends of the cleaning and/or vent line(s) in the control area shall be arranged or means provided to prevent liquids or objects being drawn up in the re-vent line.

D.12

Unless otherwise specified, means for mechanical and/or air agitation of product shall be provided that when operated intermittently or continuously shall be sufficient to maintain the butterfat content of whole milk throughout the tank within a variation of plus or minus 0.1 percent as determined by an official AOAC Milk Fat Test[3]. The agitator, if not designed for me-

[3]*The method of making these tests will be found in the following reference: Official Methods of Analysis: Available from the Association of Official Analytical Chemists, P.O. Box 540, Benjamin Franklin Station, Washington, D.C. 20044.*

chanical cleaning, shall be readily accessible from a control area and shall be removable for manual cleaning. A mechanical agitator shall have a seal of a packless type, sanitary in design. A bottom support or guide, if provided for a vertical agitator, shall be welded to the tank and shall be readily accessible for inspection. It shall not interfere with the drainage of the tank. All internal angles shall have radii of at least 1/8 inch. When the agitator shaft has a cavity, the diameter of the cavity shall be greater than the depth.

D.13

A sanitary connection(s) of sufficient diameter to prevent back pressure during normal filling and to prevent vacuum during emptying shall be provided in or near the top of the tank as a vent connection. The vent line(s) from this connection(s) shall terminate in a control area and shall be provided with a perforated cover(s) having openings not greater then 1/16 inch diameter, or slots not more than 1/32 inch wide. This cover(s) shall be so designed that parts are readily accessible and easily removable for cleaning. Woven wire mesh shall not be used for this purpose. (See Appendix, Section I)

D.14

The outlet and inlet shall be located where they are readily accessible. The outlet shall be in a position that will provide complete drainage of the tank. The top of the terminal end of the outlet passage shall be lower than the low point of the bottom of the lining at the outlet. When tanks are located in the processing area or an area in the plant at least the equivalent of a processing area, inlets and outlets may be in the side or bottom of the tank. Means shall be provided for easy access to the valve(s) for cleaning and inspection purposes.

D.15

Inlet and Outlet conections in the tank shall be provided with welding stub ends, bolted or clamp-type flanges or 3-A sanitary threaded connections. The face of a bolted or clamp-type flange or a 3-A sanitary threaded connection shall be as close as practical to the outer shell of the tank. (See Appendix, Section L and Section M).

D.16

The control area and alcove, or if there is more than one, the lowest shall be at an elevation that will include the lowest vertical portion of the tank.

D.17

A manhole(s) shall be provided. If there is more than one control area, there shall be a manhole that is accessible from the lowest control area. The inside dimensions of the manhole opening shall be not less than 15 inches by 20 inches oval, or 18 inches diameter. A hand grip shall be mounted externally on the tank near the manhole in order to facilitate easy access to the tank interior.

The sleeve or collar of a manhole opening for an inside swing type of manhole cover shall be pitched so that liquids cannot accumulate.

D.18

The manhole cover shall be the inside or outside swing type. If the cover swings inside, it shall also swing outside away from the opening for disassembly and cleaning. No threads or ball joints shall be employed within the lining to attach the manhole cover and its appendages. The manhole cover and its appendages shall be removable without tools.

D.19

Equipment for producing and introducing air under pressure into the product and which is supplied as an integral part of the tank shall comply with the "3-A Accepted Practices For Supplying Air Under Pressure In Contact With Milk, Milk Products And Product Contact Surfaces, Number 604-03."

D.20

The tank shall be insulated with insulating material of a nature and amount sufficient to prevent freezing, or in 18 hours, an average temperature change of greater than 3 F. in the tank full of water when the average differential between the temperature of the atmosphere surrounding the tank is 30°F. above or below that of the water in the tank, provided that the insulating material shall be the equivalent of not less than 2 inches of cork in insulating value. Tanks installed partially or wholly outside of a building shall be insulated with insulating material having the equivalent of not less than 3 inches cork in insulating value over non-refrigerated areas. Insulation material shall be installed in such a manner as to prevent shifting or settling.

D.21

Tanks Supports (When the tank is installed in a processing or an area in the plant at least the equivalent of a processing area.)

D.21.1

Adjustable legs of round stock with sealed bases shall be provided of sufficient size and spacing to carry the tank when full and to raise the milk outlet sufficiently high to allow for adequate cleaning. The tank or bracing, whichever is lower, shall have a minimum clearance of 8 inches from the floor. Leg socket exterior shall be readily cleanable.

B.21.2

When tanks are mounted on a slab or island, that portion of the base within the processing area shall be effectively sealed.

D.22

The outer shell shall be smooth and effectively sealed. Outside welds need not be ground. A vent hole shall be provided in the outer shell of the tank and shall be located to provide drainage from the outer shell and shall be vermin proof.

D.23

Non-product contact surfaces to be coated shall be effectively prepared for coating.

E.

REFRIGERATION

E.1

Refrigerated tanks shall be capable of maintaining milk temperature of 40°F. or lower when the tank is full.

APPENDIX

F.

STAINLESS STEEL MATERIALS

Stainless steel conforming to the applicable composition ranges established by AISI[1] for wrought products, or by ACI[2] for cast products, should be considered in compliance with the requirements of Section C.1 herein. Where welding is involved the carbon content of the stainless steel should not exceed 0.08 percent. The first reference cited in C.1 sets forth the chemical ranges and limits of acceptable stainless steels of the 300 series. Cast grades of stainless steel corresponding to types 303, 304, and 316 are designated CF-16F, CF-8 and CF-8M, respectively. These cast grades are covered by ASTM[4] specifications A 296-68 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 here-in.

H.

SUGGESTED CLEANING PROCEDURES

One cleaning method found to be satisfactory is to pump the cleaning solution to the dome of the tank through stainless steel welded lines and distribute it in such a manner as to provide flooding over the entire inner dome, side walls and bottom.

Means should be provided for manual cleaning of all surfaces not cleaned satisfactorily by mechanical cleaning procedures.

I.

AIR VENTING

To insure adequate venting of the tank which will protect it from internal pressure or vacuum damage during normal operation, the critical relationship between minimum vent size and maximum filling or emptying rates should be observed. The size of the free vent opening of a tank should be at least as large as those shown in the table below:

Minimum Free Vent Opening Size (inches, I. D.)	Maximum Filling or Emptying Rate (gallons per minute)
1¾	175
2¼	300
2¾	400

The above sizes are based on normal operation and are sized to accommodate air only and not liquid. The diameter of the connecting vent pipe line between the vent opening in the tank and the control area should be no smaller than the inside diameter of the vent opening in the tank. The perforated vent cover should have a free opening area equal to at least 1½ times the area of the vent opening in the tank. Means should be provided to prevent siphonage, such as a re-vent line, or anti-siphon device (see illustrative sketches in Appendix). The vent piping of a tank outside of a building should be protected against freezing. The venting system covered in the preceding paragraphs is intended to provide the venting during filling and emptying; *however, it is not adequate during cleaning.* During the cleaning cycle, tanks when cleaned mechanically should be vented adequately by opening the manhole door to prevent vacuum or pressure build up due to sudden changes in temperature of very large volumes of air[5]. Means should be provided to prevent excess loss of cleaning solution through the manhole opening. The use of tempered water of about 95°F. for both pre-rinsing and post-rinsing is recommended to reduce the effect of flash heating and cooling. Provisions should be made to prevent overfilling with resultant vacuum or pressure damage to the tank.

J.

TEMPERATURE RECORDER

A temperature recorder should be provided on all tanks to record temperatures during the filling, storage, emptying and cleaning periods. This tempeature recorder should be accurate to plus or minus 1°F. within the temperature range for milk storage. The recorded elapsed time, as indicated by the chart, should be the true elapsed time over at least a seven-day period.

K.

PLACEMENT

If the tank is not in a processing area or an area in the plant at least the equivalent of a processing area or adjacent to the outside wall of one of these areas, a hallway should be constructed at least 7 feet high and 5 feet wide to provide easy access to the control area. Extension through the roof is permissible.

L.

INLET AND OUTLET CONNECTIONS

The distance between the nearest point on the outer shell of the tank to (1) the face of a bolted or clamp-

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

[5]*For example, when a 12,000 gallon tank (with 1600 cu. ft. of 135°F. hot air after cleaning) is suddenly flash cooled by 50°F water sprayed at 100 gpm the following takes place:*

Within one second, the 1600 cubic feet of hot air shrinks approximately 102 cubic feet in volume. This is the equivalent in occupied space of approximately 765 gallons of product. This shrinkage creates a vacuum sufficient to collapse the tank unless the vent, manhole, or other openings allow the air to enter the tank at approximately the same rate as it shrinks. It is obvious, therefore, that a very large air vent such as the manhole opening is required to accommodate this air flow.

type flange or (2) the face of a 3-A sanitary threaded connection on an inlet or outlet valve connection should not exceed the smaller of (a) twice the nominal diameter of the connection or (b) five inches.

M.

VALVES

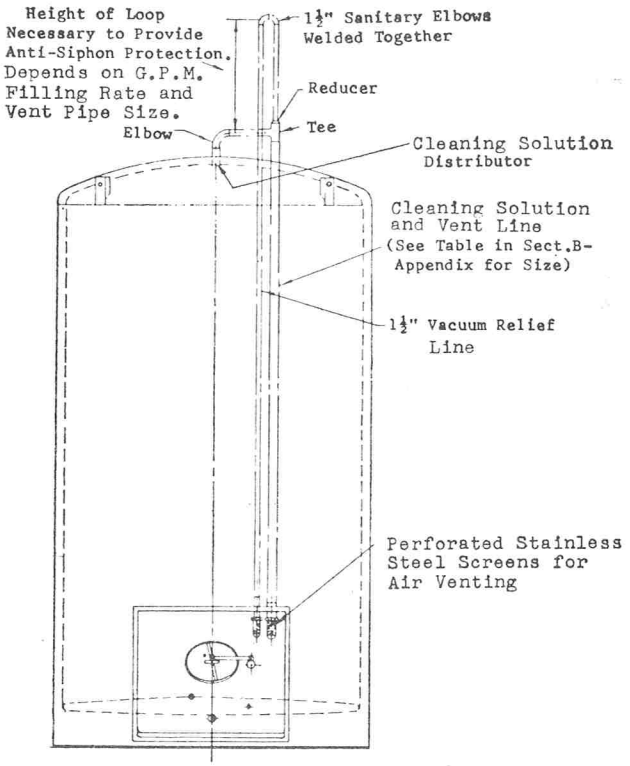
Valves on inlet and outlet connections on the tank should be of the close coupled plug-type or of the close coupled compression-type.

These standards are effective December 24, 1976, at which time the "3-A Sanitary Standards for Silo-Type Storage Tanks for Milk and Milk Products, Number 22-03" and the amendments thereto are rescinded and become null and void.

Appendix - Section I Continued

PIPING FOR AIR VENTING

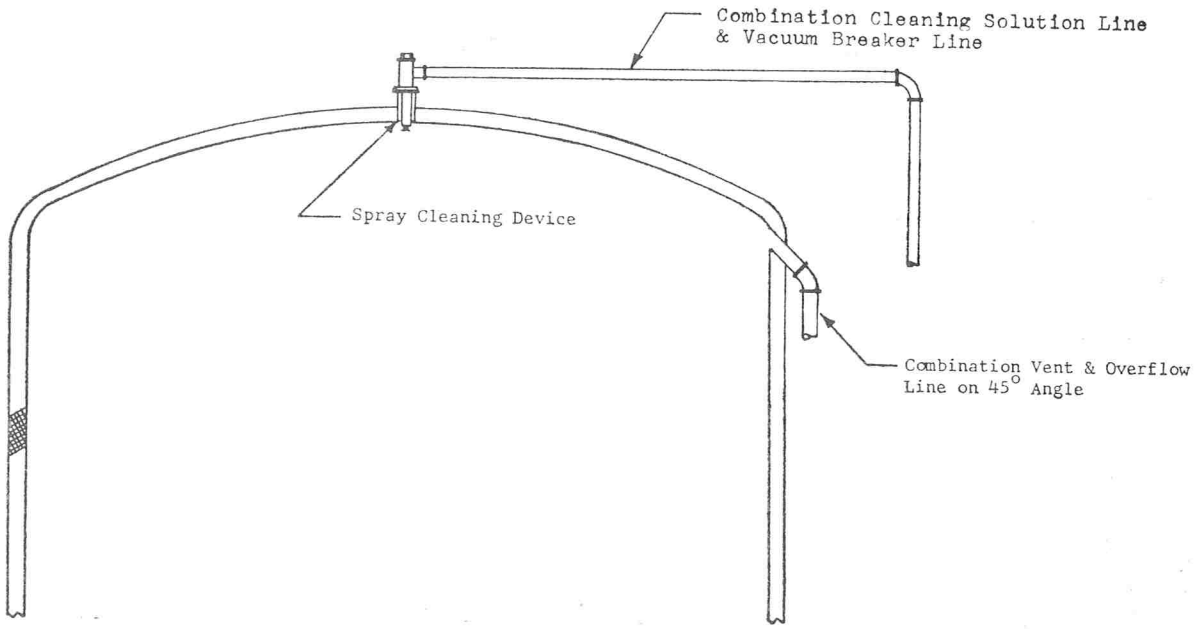
Example #1, a suggested method for venting



This design shows a loop-type syphon breaker. The loop is made so that its height above the vent line is sufficient to prevent milk rising in the vacuum relief line to the top of the loop when the tank is being filled at the maximum rate recommended by the manufacturer

Legend: In event of overfilling, milk enters the vent line through the opening in the top of the tank. The area of this opening is such that when the tank is being filled at the maximum rate recommended by the manufacturer, pressure in the tank will not exceed that for which the tank is designed. The vacuum relief line will prevent any siphoning action in the overflow line.

Example #2, a suggested method for venting.



Legend: The purpose of the opening at the juncture of the head and shell is to act as a vent during normal operation, and an overflow in case the tank is overfilled. In this event, the cleaning solution line acts as an auxiliary vent to break any siphoning action in the overflow line.

Report of the Editor
Journal of Milk and Food Technology
1975 - 1976

REVIEW OF VOLUME THIRTY-EIGHT

Volume 38 was completed when the December, 1975 issue was published. This was the first volume of the *Journal* that was printed by Heuss Printing and Signs, Inc. of Ames, Iowa.

Volume 38 was the largest ever published. It contained 868 pages (512 in 1967), 136 papers of all kinds (64 in 1967), 100 research papers (30 in 1967), 26 technical papers of general interest (11 in 1967), and 10 nontechnical papers of general interest (23 in 1967). Volume 38 exceeded Volume 35 (the largest volume published before 1975) in total number of pages, number of research papers, and pages devoted to Association Affairs and News and Events. The single largest issue (100 pages) ever to be published also appeared in Volume 38 (October, 1975). Details about the entire make-up of Volume 38 and of other recent volumes appear in Table 1.

As was true of other recent volumes, Volume 38 contained papers that dealt with a variety of topics. This is the third volume in which more papers considered non-dairy foods (56%) than dairy foods (44%). In Volume 37 the values were 48, 41, and 11% for papers dealing with non-dairy foods, dairy foods, and the environment, respectively. As indicated in the 1974-1975 report, the trend toward more papers that deal with non-dairy foods is likely to continue. This is true because of a shift in research interests, availability (or lack) of funds, and the greater need for reserach on foods of non-dairy origin.

PRESENT STATUS OF VOLUME THIRTY-NINE

The first six issues of Volume 39 consisted of 456 pages, including covers. This compares with 400 pages for the first six issues of Volume 38 and 264 pages for similar issues of Volume 30 (1967). The first six issues of Volume 39 contained 45 research papers, 20 technical papers of general interest, and 9 non-technical papers of general interest. This compares with 50, 11, and 4 papers in the same catgories for the first six issues of Volume 38. There was a total of 74 papers in the first six issues of Volume 39 compared with 65 papers in similar issues of Volume 38.

Awaiting publication on July 1, 1976 were 53 research papers, 6 technical papers of general interest, and 2 non-technical papers of general interest. This compares with 42, 16, and 3 papers in the same categories on July 1, 1975. In addition there were 36 research papers and 1 non-technical paper of general interest being revised or reviewed. This backlog is somewhat greater than at the same time in 1975. In all likelihood Volume 39 will contain more pages and more papers than did Volume 38.

REVIEW PAPERS

Volume 38 contained review papers on milking machines and mammary infection, vegetable proteins and dairy products, alkaline cleaner formulations, nutrition research, certification of foodservice managers, methods to detect salmonellae, wine technology, mercury contamination, botulism in commercially canned foods, most-probable-number technique, patulin, measuring water in gaseous systems, and anasakiasis. Additional review papers have appeared in Volume 39 and several are now awaiting publication.

Authors are encouraged to prepare review papers. They will be published promptly and without a page charge. Prospective authors are

TABLE 1. *Summary of contents of the* Journal of Milk and Food Technology, *1967, 1969, 1971-1975*

Item	Volume 30 (1967)	Volume 32 (1969)	Volume 34 (1971)	Volume 35 (1972)	Volume 36 (1973)	Volume 37 (1974)	Volume 38 (1975)
1. Total pages, including covers	512	624	728	832	752	752	868
2. Total papers published	64	87	102	132	108	102	136
3. Research papers							
a. Number	30	47	67	78	65	72	100
b. Pages	137	205	288	317	284	330	402
c. Percent of total pages	26.7	32.9	39.5	38.1	37.7	43.9	46.3
4. General interest papers-Technical							
a. Number	11	14	24	35	31	21	26
b. Pages	47	87	150	242	208	160	178
c. Percent of total pages	9.2	12.2	20.6	29.1	27.7	21.2	20.5
5. Equipment standards							
a. 3-A, pages	9	12	40	23	17	41	25
b. E-3-A, pages	—	7[a]	30	15[b]	—	—	2
c. Percent of total pages-all standards	1.7	3.0	9.6	4.5	2.2	5.4	3.1
6. General interest papers-Nontechnical							
a. Number	23	26	11	19	12	9	10
b. Pages	72	91	46	76	49	29	46
c. Percent of total pages	14.1	14.6	6.3	9.1	6.5	3.8	5.2
7. Association affairs							
a. Pages	64	62	45	47	84	75	67
b. Percent of total pages	12.5	9.9	6.3	5.6	11.2	9.9	7.7
8. News and events							
a. Pages	51	36	17	7	4	0	26
b. Percent of total pages	9.9	5.8	2.3	0.8	0.5	0.0	3.0
Percent of pages-technical material, including standards	37.6	48.1	69.7	71.7	67.6	70.5	69.9
Percent of pages-nontechnical material	36.5	30.3	14.9	15.5	18.2	13.7	16.0
Percent of pages-covers, adds, index, etc.	25.9	21.6	15.4	12.8	14.2	15.8	14.1

[a]These were Baking Industry Equipment Standards.

[b]Includes 1 page of Baking Industry Equipment Standards.

encouraged to contact the Editor if they have questions about the suitability of their material for publication.

INTERNATIONAL CHARACTER OF JOURNAL

In recent years the number of papers by authors outside of the U.S. has increased. This serves to make the *Journal* more valuable to domestic and foreign readers. The first six issues of Volume 39 have contained papers by authors in Canada, India, Israel, and The Netherlands. Awaiting publication are papers by authors in Canada, Germany (West Berlin), Iran, Ireland, Israel, Poland, Switzerland, and The Netherlands. It is hoped that this trend will continue and that more authors outside of the U.S. will consider the *Journal* as the medium for publication of their research findings.

CHANGE IN NAME OF JOURNAL

The Journal Management Committee met at the 1975 Annual Meeting of IAMFES and recommended that the title *Journal of Food Protection* should replace *Journal of Milk and Food Technology*. This recommendation was considered by the Executive Board with the result that a committee (Drs. E. H. Marth and K. G. Weckel) was appointed to outline advantages and disadvantages of changing the name of the *Journal*. The committee prepared such a document, and submitted it to the Executive Board for consideration at its meeting in November, 1975. At this time the Board unanimously approved the recommended change in name of the *Journal* and authorized the Editor and Managing Editor to implement the recommendation.

In response to this authorization, an application has been filed to obtain copyright protection for the new name. Furthermore, the new CODEN and ISSN have been obtained. A different cover also has been designed and should be completed in the near future. The change in name will become effective in January, 1977. The new title, in subdued type, has appeared on the cover of the *Journal* since March, 1976.

The Editor prepared a brief story about the change in name and sent it together with an appropriate cover letter to nearly 90 suitable domestic and foreign trade and scientific journals in the food, dairy, and health fields. It is hoped that many of these journals will carry the article and thus facilitate introduction of the new name.

EDITORIAL BOARD

The Editorial Board currently consists of 51 U.S. and Canadian scientists in academic, governmental, and industrial laboratories. Upon recommendation of the Editor and with approval of the Executive Board, 20 new members were added to the Editorial Board early in 1976. No further changes in membership of the Editorial Board are necessary at this time, although Dr. K. G. Weckel has resigned.

The following persons who are not members of the Editorial Board has reviewed manuscripts during the first six months of 1976: S. E. Barnard, N. L. Benevenga, R. L. Bradley, Jr., H. E. Calbert, D. O. Cliver, E. G. Hammond, D. R. Henning, H. W. Jackson, D. H. Kleyn, W. S. LaGrange, R. C. Lindsay, D. B. Lund, T. E. Minor, G. K. Murthy, V. S. Packard, T. Richardson, L. H. Schultz, M. L. Speck, E. C. D. Todd, and K. F. Weiss. Their help is acknowledged and appreciated.

Respectfully submitted,

ELMER H. MARTH
Editor
Journal of Milk and Food Technology
(Journal of Food Protection)

Journal of Food Protection (*continued from page 818*)

language will publish research and review papers on all topics in food science and on the food aspects of the animal (dairy, poultry, meat, seafood) and plant (cereals, fruits, vegetables) sciences.

Major emphasis of the journal will be on: (*a*) cause and control of all forms (chemical, microbial, natural toxicants) of foodborne illness, (*b*) contamination (chemical, microbial, insects, rodents, etc.) and its control in raw foods and in foods during processing, distribution, and preparing and serving to consumers, (*c*) causes of food spoilage and its control through processing (low temperatures, high temperatures, preservatives, drying, fermentation, etc.), (*d*) food quality and chemical, microbiological and physical methods to measure the various attributes of food quality, (*e*) the foodservice industry, and (*f*) wastes from the food industry and means to utilize or treat the wastes.

ELMER H. MARTH
Editor
Journal of Food Protection

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 7-9, 1977. STATISTICAL QUALITY CONTROL SHORT COURSES FOR THE FOOD PROCESSING INDUSTRY. Methods and techniques, applications and decision making. Mini Center, University of California, Davis. Registration fee: $115. For further information contact: Robert C. Pearl, Food Science & Technology Department, University of California, Davis, CA 95616. (916) 752-0980 or 752-6021.

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 Machinery and Supplies Association, 7758 Wisconsin Avenue, Washington, D.C. 20014.

February 15-16, 1977. DAIRY MARKETING FORUM. Sponsored by the University of Illinois. Ramada Inn Convention Center, Champaign, IL. Fee: $20.

March 3-5, 1977. BAKING INDUSTRY SANITATION STANDARDS COMMITTEE MEETING. Hyatt Regency Chicago Hotel, Chicago, IL.

March 21-25, 1977. MIDWEST WORKSHOP IN MILK AND FOOD SANITATION. 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.

March 29-31, 1977. WESTERN FOOD INDUSTRY CONFERENCE. Freeborn Hall, University of California, Davis, California. For further information contact: Robert C. Pearl or John C. Bruhn, Department of Food Science & Technology, Cruess Hall, University of California, Davis, CA 95616. (916) 752-0981 (Pearl) or 752-2192 (Bruhn).

April 5-7, 1977. NATIONAL CONTROLLED ATMOSPHERE RESEARCH CONFERENCE. Kellogg Center for Continuing Education, Michigan State University, East Lansing, Michigan. For further information contact: D. H. Dewey, Department of Horticulture, Michigan State University, East Lansing, MI 48824 or W. G. Chace, U.S. Department of Agriculture, ARS, Beltsville, MD 20705.

April 20-22, 1977. DAIRY AND FOOD INDUSTRIES SUPPLY ASSOCIATION 58TH ANNUAL MEETING. Beach Club Hotel, Naples, Florida. For information contact: DFISA, 5530 Wisconsin Ave., Washington, D.C. 20015. (301) 652-4420.

May 10-12, 1977. SECOND INTERNATIONAL POWDER & BULK SOLIDS HANDLING & PROCESSING SHOW. O'Hare International Trade & Exposition Center and the Regency O'Hare, Rosemont, Illinois. For information contact: Aaron Kozlov, Industrial & Scientific Conference Management, Inc., 222 West Adams St., Chicago, IL 60606. (312) 263-4866.

University of Minnesota Sponsors Training Course

The University of Minnesota is planning a training conference entitled "Pesticide Applications In Food Processing and Manufacturing Areas" which will be held January 24-26, 1977, at the Radisson Hotel in Minneapolis, Minnesota.

The primary topics covered at the conference will include: Pest Identification, Habits and Damage; Understanding Pesticide Labels; Pesticide Benefits and Hazards; Pest Management (Prevention and Control); Pesticide Application Techniques and Calibration; and Federal and State Pesticide Laws and Regulations.

The Minnesota Department of Agriculture has the responsibility to implement and enforce the Federal Insecticide, Fungicide and Rodenticide Act of 1972 in Minnesota. One of the major requirements of this Federal Law is that pesticide applicators must be certified to apply or supervise the application of "restricted use" pesticides after October 21, 1977.

It appears that most of the pesticides used in food or feed plants will be restricted. The University of Minnesota Agricultural Extension Service is the major source of training for the applicators. The conference described above is the training opportunity designed for personnel applying pesticides in food processing and manufacturing areas. Each state is expected to provide this opportunity for applicators through their own agencies or through reciprocity agreements with other state agencies.

According to the Minnesota State Plan, pesticide applicators in the food industry in Minnesota can be certified by passing the required State examinations. Attending a training session provided by the University or anyone else is *not* required. However, the examinations are based on the information covered in the University training conference or in the resource materials that will be provided.

Registration fee for the training conference is $50.00. Additional information may be obtained from Phillip Harein, Extension Entomologist, 226 Hodson Hall, University of Minnesota, St. Paul, MN 55108. (612) 373-1705. Further information regarding certification in Minnesota may be obtained from either Mike Fresvik or Leo Lehn, Minnesota Department of Agriculture, State Office Building, St. Paul, Minnesota 55155. (612) 296-8379.

Business Executives, Foreign Officials to Discuss World Environment Issues in New Organization

A new organization which brings together business executives with top foreign officials and experts to discuss environmental problems will soon be operational in New York.

The organization—International Environment Forum—is sponsored by the Center of International Environment Information, a private, non-profit institution that seeks to foster public understanding in the United States and Canada of global environmental problems.

In announcing the establishment of the Forum, Arthur Reef, vice president of Amax Inc. and Chairman of the Center's Advisory Committee, stressed that "it is designed to help major multinational corporations deal with fast-moving environmental developments in foreign countries and at the international level."

Charter Members of the Forum already include Procter & Gamble, Texaco, Alcoa, ITT, Amax Inc., and the Edison Electric Institute.

The Forum is being created in response to the need for information on international environmental issues that affect the operations of companies in fields such as energy production, mineral extraction and processing, chemicals, pulp and paper, automotive, food and agriculture and manufacture of pollution control and monitoring equipment.

The Forum meets five times a year for half day workshop/briefings conducted by senior environmental policy makers and experts from foreign governments, such intergovernmental organizations as the Common Market, the Organization for Economic Cooperation and Development (OECD), and the United Nations.

Subjects to be covered will vary with developments and the interests of members, but include the world-wide trend toward environmental impact statements, envrionmental regulations and enforcement in various countries, what lies ahead in Latin America, Asia, Africa and Europe.

A committee headed by Miles O. Colwell, M.D., Vice president, Health and Environment, Alcoa, advises the Forum on speakers and programs.

The Center for International Environment Information—a part of the UN Association of the USA—is located at 345 East 46th Street, New York, N.Y. 10017.

Statistical Quality Control Short Courses for the Food Processing Industry

Two concurrent intensive three-day Statistical Quality Control Short Courses for the food processing and related industries will be offered at the University of California at Davis during February 7-9, 1977. The SQC Short Courses are designed for professionals working in food production facilities i.e., managers; superintendents; food technologists; quality assurance personnel; key foremen and foreladies; and administrators who deal with quality control problems. Both courses will cover selected lecture material on statistical quality control and include practical examples and exercises. The "Methods and Techniques Short Course" will emphasize basic statistical approaches used in quality assurance. This course is designed for those who want to further develop their expertise. The "Applications and Decision Making Short Course" will show how applications of statistical methods solve management problems of quality assurance. (Certified Quality Engineers can earn three re-certification units by attending the SQC Short Courses.)

The registration fee is $115 which includes lunches, one dinner, and course materials. For further information contact either: Robert C. Pearl or Pamela Tom at the Food Science & Technology Department, University of California, Davis, CA 95616 Phone: (916) 752-0980.

Metric in a Nutshell

How the new metric law will affect Americans on the job is now revealed in a new pocketbook, *Metric in a Nutshell.* For the first time, voluminous material has been compressed into a straight forward, easy to understand, 100 page book which covers metric conversion from A to Z. It tells about the base and derived units, how to read a metric ruler, how to measure volume and weight, gives metric clothing and cooking details. The book covers metric in the shop, metric driving, federal guidelines, short cuts, rules of thumb and quick conversions. It containes over 50 pages of illustrations and tables.

The editors of the *American Metric Journal* have condensed four years of metric expertise into this book and included a metric dictionary along with scores of memory aids and even a metric shopping and price guide.

Readers may receive the book by sending $5.45 to: AMERICAN METRIC JOURNAL, *Metric In A Nutshell,* 18438 Oxnard Street, Tarzana, CA 91356, (213) 345-9305.

Dr. El-Ahraf Heads Environmental Task Force

Dr. Amer El-Ahraf has been appointed by The Chancellor's office, California State University and Colleges to head a Task Force on Environmental Health Manpower Education in California. The 100 page report, which has been recently completed, dealt with technical and academic planning issues concerned with educational program planning in the area of environmental health and formulated recommendations relevant to the following primary goals: (1) Improved number and distribution of needed environmental health personnel; (2) Improved distribution of environmental health academic programs; (3) Improved utilization of clinical/agency training sites; (4) Improved articulation between graduate, four-year and two-year academic programs in the

public and private sectors; and (5) Improved quality of environmental health programs.

The Environmental Health Task is a part of the Health Manpower Education Project headed by Dr. Ray Burwen. Dr. El-Ahraf is the Chairman, Department of Health Science and Human Ecology, California State College, San Bernardino, Editor of the California Journal of Environmental Health and past President, California Environmental Health Association.

Millipore Booklet Describes New Test for Bovine Mastitis

The new MF-DNA SomataCount Test for somatic cells in milk is described in a booklet now available from Millipore Corporation.

The test was developed at the University of Wisconsin, and Millipore assisted in refining it for routine laboratory use. A number of Dairy Herd Improvement Association (DHIA) and Agricultural Research Center laboratories now provide the test as a routine service to members.

The test is superior in many respects to other somatic cell counting or mastitis screening techniques. For example, it is faster and more reproducible than the DMSCC, it is more sensitive and more objective than current wet chemical methods, and it does not require fresh milk, but performs just as well with preserved or moderately aged samples. The test is rapid (one technician can perform 400 tests per day) and requires little training. Color and cell-count standards are readily available for verification of test accuracy and calibration of equipment.

Copies of Millipore AB815 on "The MF-DNA SomataCount Test for Bovine Mastitis" are available free, on request, from Millipore Corporation, Bedford, Massachusetts 01730. You can also ask for copies by calling Millipore's toll-free number: 800-225-1380. Ask for Technical Service.

NSF Tests New Seminar Series

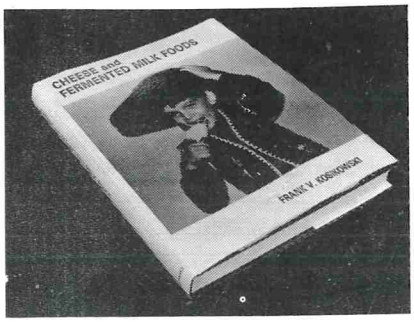
A new two-day seminar series was tested this fall by the National Sanitation Foundation in Dayton, Ohio and Anaheim, California. Based on the responses to the program received from seminar participants, the seminar series is planned for ten locations throughout the country in the first half of 1977.

The series consists of two seminars. The first day covers general sanitation requirements for food service equipment based on NSF standards. The second seminar considers the sanitation aspects of food service facility planning and plan review based on the NSF national conference series on the topic.

Participants may attend either one or both of the seminars, although the great majority of the participants this fall chose to attend both days. Proposed locations include: Memphis, Tennessee-January; St. Louis, Missouri - February; Montgomery, Alabama-February; New Orleans, Louisiana-March; Minneapolis, Minnesota-March; San Francisco, California-April; Phoenix, Arizona-April; San Diego, California-April-(first day only); Milwaukee, Wisconsin-May; Cincinnati, Ohio-June.

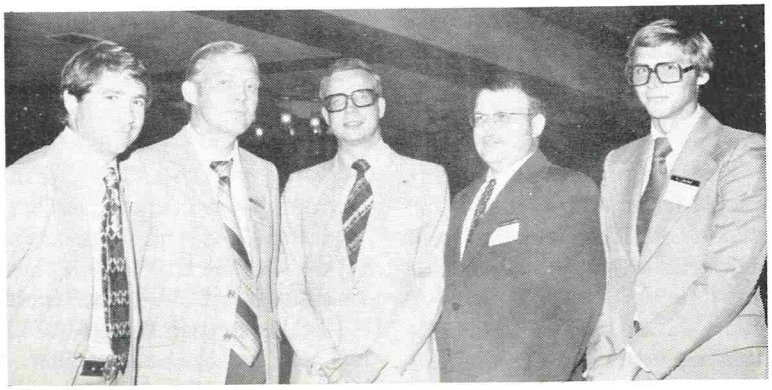
For more specific information, contact: Education Service, National Sanitation Foundation, NSF Building, P.O. Box 1468, Ann Arbor, MI 48106.

New Book on Cheese and Fermented Milk Foods



A completely revised and expanded new edition of *Cheese and Fermented Milk Foods* by Professor Frank Kosikowski, Cornell University, will become available, on or about January 1, through Edwards Brothers, Inc., Ann Arbor, Michigan.

The book, exhibiting almost twice the volume of material presented in the original, and presented in a clear manner with extensive explanations, contains up-to-date, comprehensive information on cheese and fermented milk characteristics, procedures, history, microbiology, public health and safety, nutrition, analysis, packaging and economics. Many of the author's personal experiences with cheese and yogurt making in developed and developing countries, and among nomadic peoples, are described.



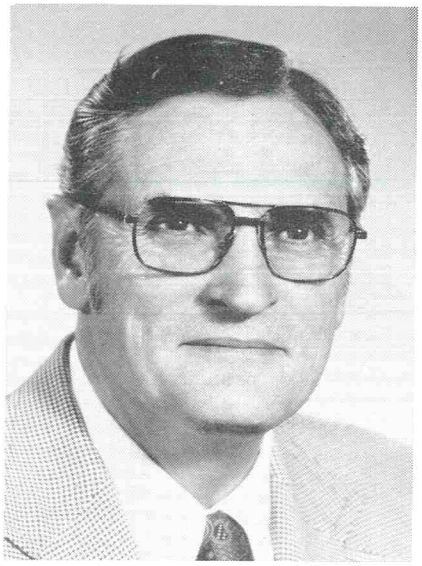
The American Cultured Dairy Products Institute held an International Symposium during its September 8-9 Annual Meeting and Conference in St. Louis, Missouri. Participants in this event included (left to right): Martin Levin, Yoplait North Central, Otsego, Michigan; Bent Andersen, BEPEX Corp., Santa Rosa, California; Dr. Hans Stamer, Molkerei Luneburg, Luneburg, West Germany; Dr. Rockseisen, Molkerei Luneburg, Luneburg, West Germany; Soren Jepsen, The Danish Sugar Corp. Ltd., Denmark. The conclave drew over 160 delegates from 28 states, Canada, Denmark, and West Germany.

Association Affairs

NOMINATION FOR OFFICE OF IAMFES INC. 1977-1978

(Notice to membership—ballots will be mailed to paid members as of December 21, 1976)

For Secretary-Treasurer



O. M. (Oak) Russell

O. M. (Oak) Russell is Manager of Production Services for the Dairy Division of Foremost Foods company.

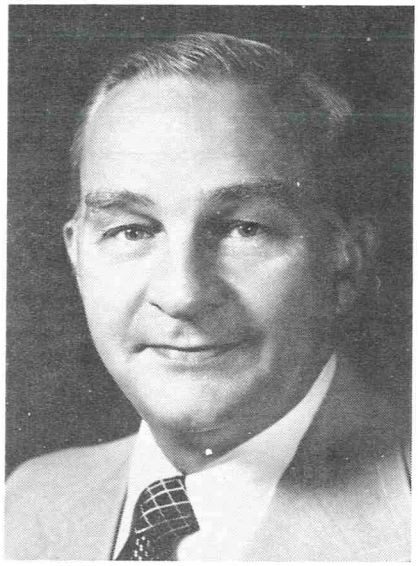
In this capacity he is responsible for the Division's quality assurance and control programs and for product formulation, package evaluation and food and drug liaison and compliance.

Mr. Russell has been associated with Foremost for 31 years and has held his present position since 1972.

During his career he has also served as Manager of Production and Engineering for the fluid milk, cottage cheese and ice cream operations of Foremost Dairies and as manager of the Company's Southern California operations.

He has also served with Foremost in Fresno, Tulare and Los Banos. He earned his B.S. Degree at Iowa State University and his M.S. Degree in Dairy Manufacturing at Pennsylvania State University.

Mr. Russell has long been active in industry affairs. He is a member, with ten years' service, of the 3A Standards, Sanitary Sub-Committee for the MIF, and is presently the industry representative of Region III on the Interstate Milk Shippers Conference Executive Board. He also participates in the California Dairy Institute technical committee.



William L. Arledge

William L. Arledge is Corporate Director of Quality Control and Related Services for Dairymen, Inc. in Louisville, Kentucky. He is responsible for administering policies and training related to the overall quality control programs and laboratories in Dairymen, Inc. and its subsidiaries, Flav-O-Rich and D. I. Manufacturing. He is also responsible for coordinating all supply and equipment programs for member dairymen as an adjunct to the Quality Program. Bill is also responsible for the Health and Safety Program of Dairymen, Inc. and subsidiaries.

Except for a term of active duty in the United States Army of four years, three months as an Army Aviator, Bill has spent his entire working life in the dairy industry. He was graduated from the University of Tennessee in Knoxville with a degree in Dairy Manufacturing.

Bill's work in the dairy industry started in 1945 with Mayfield Dairy in Athens, Tennessee, during his high school and college years. During college Bill worked also at the University of Tennessee Creamery. After a tour of active duty he returned to the University of Tennessee, followed by a year's experience selling dairy equipment and supplies. In 1960 he joined Southeast Milk Sales in Bristol, Virginia, as Director of Quality Control, remaining in this capacity until the formation of Dairymen, Inc. in September, 1968, at which time he was named to his present position.

Bill has been a member of IAMFES since 1962, having served as Chairman of two separate subcommittees of the Farm Methods Committee and as a member of the Applied Laboratory Committee.

Bill has also been very active in many other phases of the dairy industry. A member of the National Mastitis Council Board of Directors since 1964, Bill was NMC President in 1971. He has been an active participant of the Interstate Milk Shipments Conference and a member of its Executive Board since 1969. He now is Chairman of the Standards and Research Committee of the American Dry Milk Institute and Chairman of the IMS Committee of the National Milk Producers Federation.

Through the years Bill has also been active in Kiwanis Club and was the District Commissioner of Ocanosta District for the Boy Scouts. Bill is still actively engaged in the Army Reserve program, serving as Aviation Team Leader of the 100th Division Maneuver Training Command. The Arledges are active also in church work, having served as Sunday School teachers and Superintendant of Sunday Schools.

Bill and his wife Pat live at 10802 Tattenham Lane in Louisville, Kentucky, with their 12-year old daughter, Lea Ann. Their son Bill, Jr., 24, is manager of a men's clothing store in Columbus, Indiana, and their other son, Mike, 21, is in the United States Army at Fort Sill, Oklahoma.

Kansas Affiliate Meets

An enthusiastic group of approximately 75 sanitarians met in Emporia, Kansas, for the 1976 Annual Meeting of the Kansas Association of Sanitarians. The three-day conference was highlighted by plant tours of a Dolly Madison Bakery and an Iowa Beef Packers facility. Technical session topics included quality control in milk and food, food adulterants, insecticide use in food establishments and sanitation in far away places.

Officers elected during the business session were: James Pyles, President; J. A. Rogers, first vice-president; Warren Griffin, second vice-president; and John W. Zook, secretary-treasurer. Directors for the affiliate are Don Bechtal, past president; Galen Hulsing, General Sanitarians Section; John Moshier, Food Service Section; and Lowell Mueller, Dairy Section.

Service certificates were presented to Charles Fusseneggar, Jr., Sanitarian with the Dairy Division, Kansas Board of Agriculture; Al Hodges, sanitarian, City of Pratt, Kansas; and Harry Holzle, director, environmental section, Topeka-Shawnee County Health Department.

C. H. "Bud" Corwin was named Sanitarian of the Year. Corwin, Sanitarian for the City of McPherson, is a one-man department in charge of all phases of enforcement of sanitation practices for the city.

Affiliate Meetings

CONNECTICUT — January 1977. Hartford Areas.

FLORIDA — March 15-17, 1977. Kayhler Plaza, Orlando.

KENTUCKY — February 22-23, 1977. Stouffer's Inn, Louisville.

MISSOURI — April 1977. Ramada Inn, Columbia.

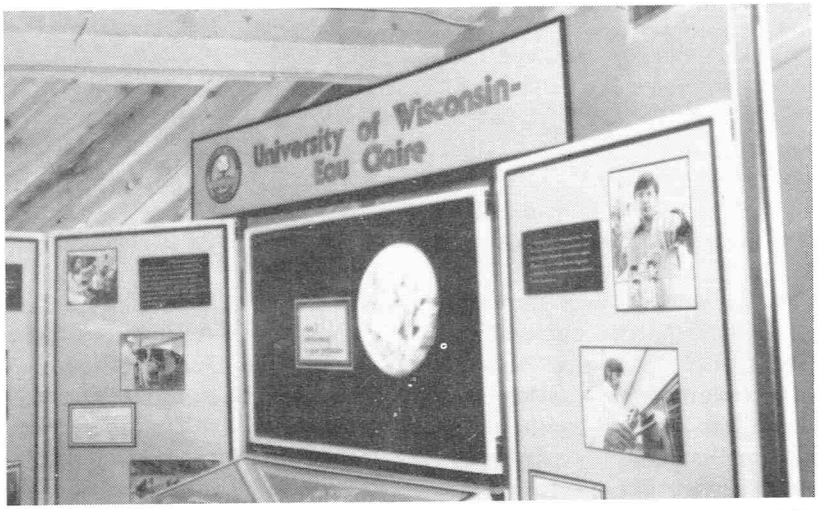
ONTARIO — March 1977. Holiday Inn, Highway #427, Etobicoke.

PENNSYLVANIA — June 13-15, 1977. State College.

VIRGINIA — March 8-9, 1977. Donaldson Brown Center, VPI & SU, Blacksburg.

Wisconsin Affiliate Holds Annual Conference

WAMFS officers are (back row, left to right): Don Raffel, secretary-treasurer; Dr. Elmer Marth, past president; Clifford Mack, president; (front row, left to right): Leonard Rudie, president-elect; and Norman Kirshbaum, second vice-president.



Display outside the University of Wisconsin-Eau Claire's environmental health exhibit.

The Wisconsin Association of Milk and Food Sanitarians held its 32nd Annual Educational Conference September 16-17 in Milwaukee. Meeting jointly with WAMFS were the Wisconsin Dairy Plant Fieldman's Association, the Wisconsin Institute of Food Technologists and the Wisconsin Dairy Technology Society. Approximately 200 persons attended the two-day conference.

Following a bicentennial theme, the meeting's keynote address was given by Dr. K. G. Weckel, who gave a review of the development of the food industry in Wisconsin. Speakers in the food and dairy technical sessions discussed current issues in food inspection, pollution control and equipment maintenance. A special feature of the meeting was the audio-visual presentation by the University of Wisconsin-Eau Claire, which emphasized the professional status of the sanitarian.

At its annual awards banquet, WAMFS presented a $300 scholarship to Margaret Uebele, an Environmental Health major at the University of Wisconsin-Eau Claire. Mr. Daniel Jindra, membership director of Lake to Lake Dairy Cooperative, was named WAMFS Sanitarian of the Year. Mr. Jindra has served on the Board of Directors of the American Dairy Association of Wisconsin, and is past-president of WAMFS.

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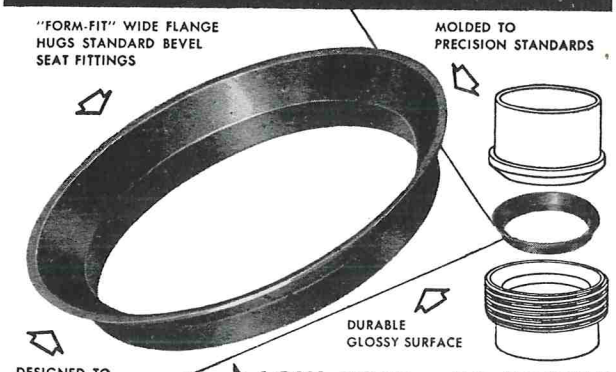

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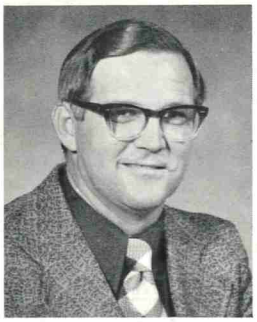
Dairy authorities speak out on better cow milking



Dr. Robert D. Appleman
Professor of Animal Science
University of Minnesota

Automatic take-off milking units: They can save and protect.

There are two primary reasons why an investment in more mechanized milking is being considered by many dairymen. One is to reduce labor. The other is to improve udder health and maintain production of high quality milk.

LABOR TAKES A BIG BITE

Labor accounts for 15 to 30 percent of all costs in a dairy operation. About 55 percent of this labor is expended in the milking operation. In general, the total labor cost to produce 100 pounds of milk in a herd averaging 12,000 pounds per cow annually when labor* is valued at $3.00 per hour approaches $2.50 per cwt. in 30-cow herds; $2.10 in 50-cow herds; $1.68 in 100-cow herds; $1.13 in 250-cow herds; and $.91 in 500-cow herds**.

With an investment to modernize milking parlors, including unit take-off, it is not unusual to substantially lower the labor costs of producing milk.

Many of the milking chores are repetitious and result in drudgery. According to our studies, 5 to 10 percent of the milker's chore time is spent removing the milking unit. On top of that, the typical milker spends from 12 to 30 percent of his time machine-stripping cows.

THE OPERATOR IS A BUSY MAN

Proper stimulation of cows in a milking parlor is important to good milk letdown. Recent New Zealand work shows there is a loss of up to 1,000 pounds of milk per cow yearly when cows are not properly stimulated. In many barns the milker cannot effectively handle as many milking units as today's economy demands. Frequently, washing and stimulation time is limited to less than 15 seconds per cow because the milker is too "busy" with machine stripping or handling other units. The result is slow milking combined with considerable overmilking. Automatic unit take-off should improve this situation. Addition of automated prep stalls will help even more, provided they function properly.

SOME RESEARCH RESULTS

Research studies comparing automatic take-off and conventional milking units involving 550 cows in a Louisiana herd resulted in these conclusions:

1. Automatic unit take-off significantly reduced the number of quarters infected with mastitis.
2. Automatic unit take-off reduced udder irritation as evidenced by lower CMT scores.
3. The men operating the automatic take-off units reduced their walking distance in the parlor by more than 25%.

Dr. Nelson Philpot, leader of this study, says one should not expect miracles. Automatic take-off units do not make a poor operator better. They do, however, allow a conscientious operator to do an even better job on more cows.

According to dairymen using this equipment, proper maintenance and proper operation of equipment is even more important with non-automated systems. The ability, cooperative attitude and location of your local serviceman should become a primary factor in deciding whether to install this more sophisticated and expensive equipment.

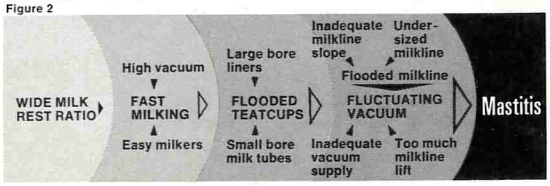
MASTITIS PREVENTION NOT SIMPLE

Dairymen should not necessarily expect a reduction in the number of cows requiring treatment for clinical mastitis (gargot). In a marginal system, more cases may result because the significance of a single variable is not the same in every milking system or in every situation.

Frank Smith, California milking system specialist, illustrates this point well. He indicates that too many researchers and educators have attempted to over-simplify the cause of mastitis. In turn, they have over-simplified its prevention. *The concept of a direct, independent relationship shown in Figure 1 is incorrect.* Figure 2 arranges these same variables in a manner which is sequential, additive, and interdependent.

Figure 1

Figure 2



As mentioned earlier, installing automatic unit take-off may allow one to milk cows faster and reduce overmilking. However, if such a change resulted in flooded milk lines and fluctuating vacuum, the incidence of mastitis might increase rather than decrease. Providing all other deficiencies in the system were corrected, automatic take-off would prove highly beneficial.

AUTOMATIC TAKE-OFFS A COMMON SIGHT?

Where cost of this mechanization is not excessive and such installations prove to be reasonably trouble-free over time, I'm sure that automatic take-off units will become an increasingly more common sight on dairy farms.

*For our purposes, the labor figures include all dairy chore labor, feeding labor, and the raising of offspring. Field labor isn't included.

**In 250-cow and 500-cow herds, we assume the existence of a parlor and a free-stall barn with mechanized feeding and waste handling.

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