

JANUARY, 1971

Vol. 34

P. 1-62

No. 1

*Journal of*

# MILK and FOOD TECHNOLOGY

**58TH ANNUAL MEETING**

August 16, 17, 18, 19, 1971

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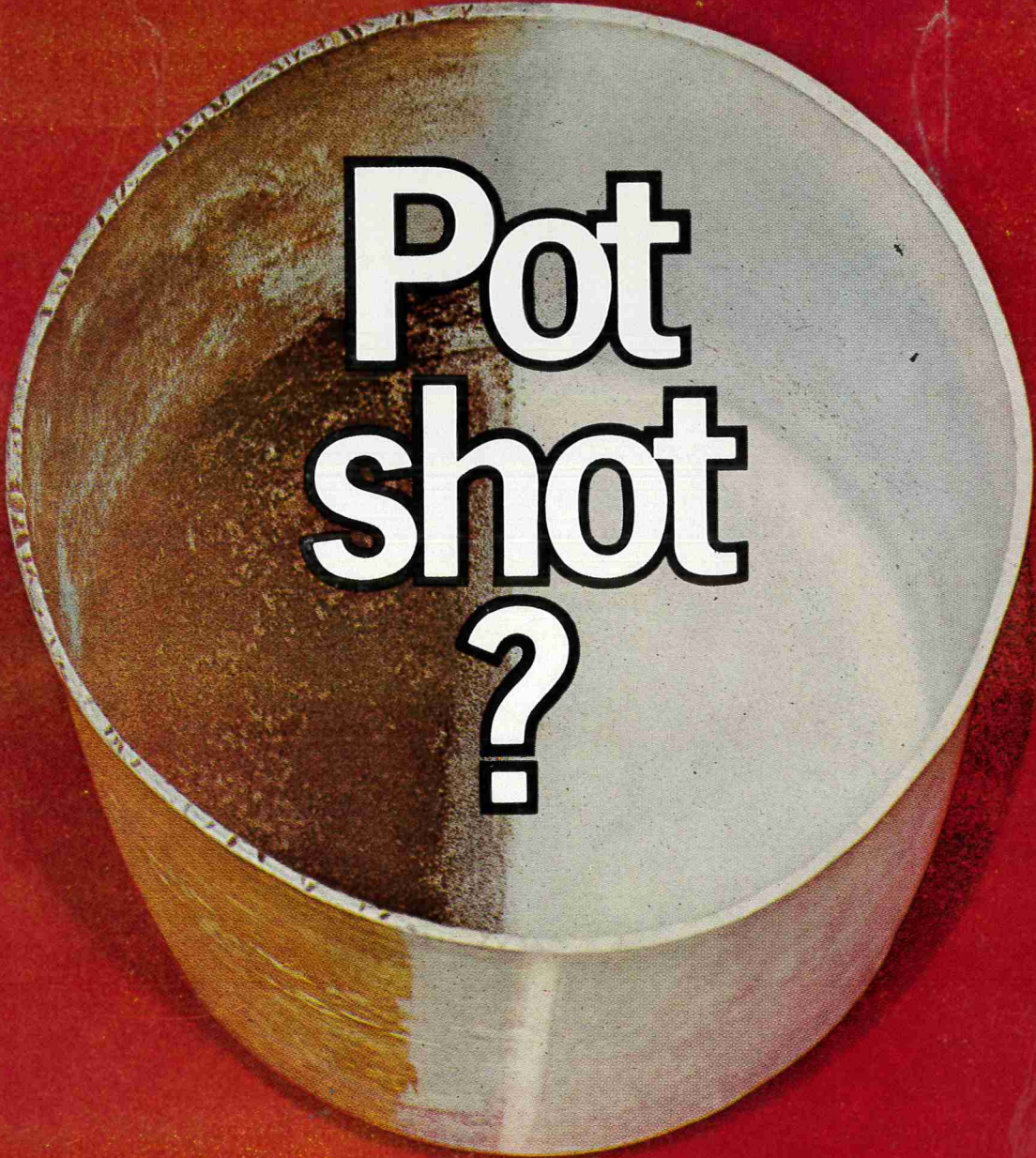
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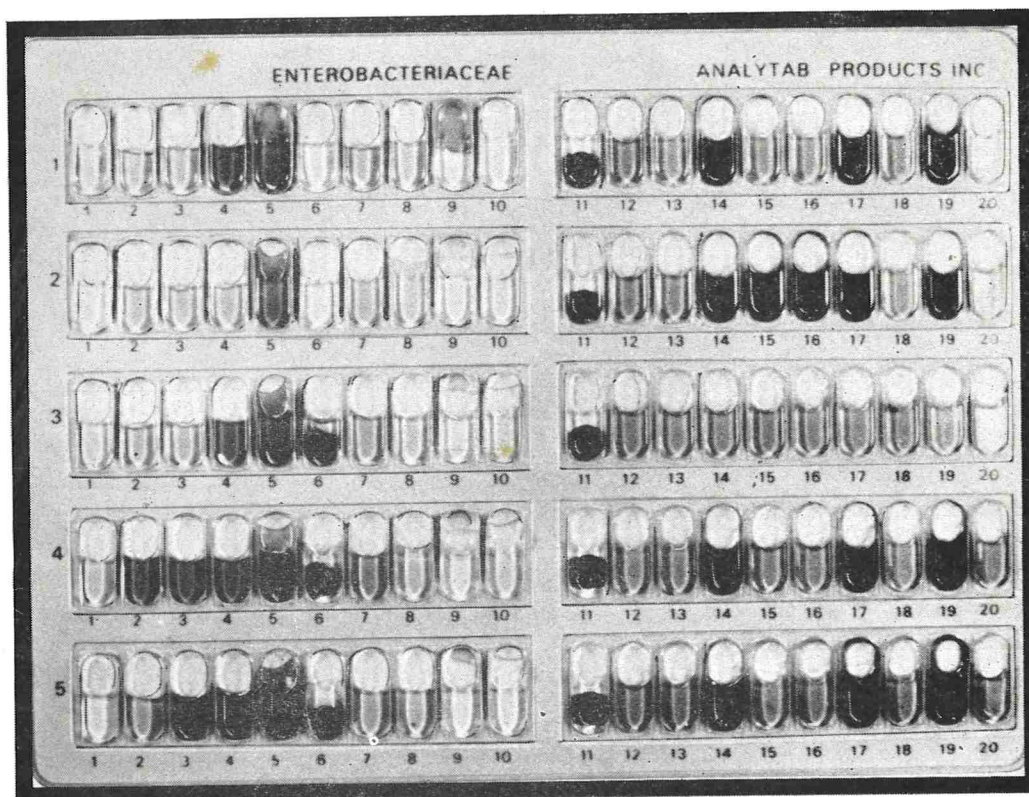
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The Journal of Milk and Food Technology is issued monthly beginning with the January number. Each volume comprises 12 numbers. Published by the International Association of Milk, Food and Environmental Sanitarians, Inc. with executive offices of the Association,

Journal of

# MILK and FOOD TECHNOLOGY

INCLUDING MILK AND FOOD SANITATION

Official Publication

International Association of Milk, Food and Environmental Sanitarians, Inc.

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 2nd Class postage paid at Shelbyville, Indiana 46176.

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*An Expanded Journal . . . . .**. . . . . An Enlarged Editorial Board*

The founders of the *Journal of Milk and Food Technology* (Volume 1, No. 1, page 4) believed that readers of the *Journal* should be familiar with animal husbandry, bacteriology, chemistry, physics, mechanical and electrical engineering, transportation, advertising, public relations, regulatory interpretation, and technology as these disciplines apply to the food and dairy industry. It was their intent that the *Journal* would provide information in all these areas.

In the past this *Journal* has been most concerned with the public health aspects of dairy products and other foods. We intend to continue and expand our efforts in this area. However, in keeping with the mandate of the founders, the needs of our readers, and the needs of research workers, we will, effective immediately, consider for publication papers in all areas of food and dairy science and technology. Examples of topics which are appropriate include:

- Food, dairy, and environmental sanitation and hygiene
- Foodborne disease hazards (microbiological, chemical, etc.)
- Food and dairy microbiology, including methodology
- Food and dairy chemistry, including methodology
- Food and dairy engineering
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- Food additives (intentional and unintentional additives)
- Food service and food administration
- Food and dairy fermentations
- Waste disposal and pollution
- Quality control and assurance in the dairy and food industry
- Food and dairy regulatory programs
- Agricultural sciences (animal, dairy, and poultry science; entomology; agronomy; horticulture; soil science; etc.) as they relate to food production, quality, safety, and processing.

The Executive Board of IAMFES authorized expansion of the Editorial Board so that papers in all areas of food and dairy science and technology can be evaluated promptly. The Editorial Board now includes approximately 40 bacteriologists, chemists, sanitarians, and technologists from industrial, university, and government laboratories. These scientists are familiar with a wide array of foods such as dairy products, meats and meat products, poultry and poultry products, fish and other seafoods, fruits, vegetables, cereals, fermented products, food ingredients, confectionery products, etc.

Institution of a page charge (\$25.00 per printed page) in 1969 has permitted expansion of the *Journal* so that acceptable research papers can be published within six months (or less) after submission.

Research workers in all areas of food and dairy science and technology are invited to submit research and review papers. They will be handled promptly and, if acceptable, will be published with dispatch. Membership in IAMFES is not necessary for publishing papers in the *Journal of Milk and Food Technology*. Interested authors can obtain "Instructions to Contributors" from the Editor.

E. H. MARTH  
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# TECHNOLOGY AND THE WORLD FOOD PROBLEM: CHALLENGES WITH INDIGENOUS FERMENTED FOODS<sup>1</sup>

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(Received for publication July 3, 1970)

## ABSTRACT

Microorganisms can contribute to the world's food supply if used to modify presently unacceptable raw materials so they become palatable; to convert wastes or other inedible materials to edible proteins, fats, and carbohydrates; and to preserve foods for later consumption. Challenges in the use of microorganisms for these purposes include: use of available native raw materials, selection of microorganisms, further processing of fermented foods or of microbial proteins, potential public health hazards, acceptance of new foods by undernourished persons, and cost of production and distribution as related to the ability of potential consumers to purchase the needed nutrients.

Through the ages, man has utilized microorganisms to manufacture many foods such as cheeses, cultured milks, bread, alcoholic beverages, vinegar, cocoa, and other products (1). Although the role of microorganisms in food fermentations was not recognized until late in the nineteenth century, processes for production of these foods were developed empirically. Discovery of microorganisms and of methods to cultivate them has led man to a greater, although still incomplete, understanding of fermentation processes which yield certain desired food products.

Use of microorganisms to assist in meeting present and future demands on the world's food supply has been suggested by numerous research workers and others who have been concerned with problems of malnutrition (9). It is generally conceded that microorganisms can contribute to the world's food "bank" in several different ways. First, they can be used to modify flavor, texture, and appearance of presently unacceptable materials so that such products can be included in the food supply. Second, microorganisms might find application in converting waste materials and other inedible substrates into edible proteins, fats, and carbohydrates. Finally, certain microbial processes could help to preserve for later consumption some highly perishable foods which are produced seasonally.

Successful and widespread application of fermentation processes to aid in meeting present and

impending world-wide food shortages offers a number of challenges, some of which will be explored in the following discussion. Specifically, the challenges which will be considered are: (a) availability of native materials, (b) selection of microorganisms, (c) further processing of fermented foods or microbial proteins, (d) potential public health problems, (e) acceptance of fermented foods or products derived from fermentations by persons most in need of dietary supplements, and (f) cost of production and ability of potential consumers to purchase foods derived from fermentations.

## AVAILABILITY OF NATIVE MATERIALS

Many of us who are concerned with food fermentations are too parochial in our approach to the problem. We have spent too much time dealing with fermentations which use rather conventional substrates such as milk or grains and hence we attempt to use these processes in areas where they may be unsuitable because entirely different raw materials are available.

Before the fermentologist can make significant contributions to feeding the world's undernourished populations, he must become familiar with the materials available which can serve as substrates for fermentations in a given region suffering from a depressed food supply. He must be familiar with native crops which are high in carbohydrate content and hence might be candidates for nutritive upgrading through fermentation. He also must give consideration to waste materials which contain nutrients that presently are unavailable to the area's population. Can some of these wastes be upgraded into edible products through fermentation? If not, can some of the wastes serve as substrates for the production of microorganisms which can be harvested and converted into edible products? Can crops able to furnish substrates for fermentations but presently not indigenous to a food-deficient area be introduced and successfully cultivated? What about indigenous plants presently considered as weeds? Can they be cultivated and can fermentable substrates be recovered from their leaves, stems, roots, or seeds?

It is apparent from the preceding questions that

<sup>1</sup>Presented at the Sixty-second Annual Meeting of the American Institute of Chemical Engineers, Washington, D. C., November 16-20, 1969.

to maximize his impact the expert in food fermentation must deal with a variety of problems outside of his field. Cooperation between agronomists, horticulturists, soil scientists, and experts in fermentation is necessary if maximum use is to be made of materials indigenous to a particular area where the population suffers from a lack of adequate and proper nutrients.

#### SELECTION OF MICROORGANISMS

The choice of a microorganism for use in a food fermentation is governed by ability of the microorganism to: (a) utilize the available substrate and (b) efficiently produce the desired change in or yield from a given substrate. Depending on conditions, the microorganism might be selected from among the molds, yeasts, or bacteria, since all types are employed either singly or in combination in certain food fermentations.

Unfortunately, selection of a microorganism to do a particular fermentation job is not as simple as might be suggested by the previous comments. Consideration must be given to the following before a decision is made.

(a) Frequently a desired change can be accomplished by more than one species in a given genus or by species in several genera. Comparisons must then be made to determine which particular species will do the desired job best under the set of environmental conditions which exist.

(b) A variety of strains of a given species may exist; some may be more suitable than others for use in a given fermentation. Again, comparative studies must be conducted to determine the most desirable strain for use under a given set of conditions.

(c) Microorganisms are biological entities which are subject to variation (2). Since this tendency does not appear to be uniform, it may be possible to select "stable" strains and thus be reasonably assured of dependable performance by the microorganism. Consideration also must be given to the possibility of genetic manipulation to develop strains of microorganisms which produce a desired end product, are efficient, or can utilize a particular substrate.

(d) Certain bacteria are subject to infections by bacteriophages. When this happens, a given culture invariably loses its ability to function and the fermentation is not completed (12). Selection of bacteriophage-resistant species or strains, if possible, or creation of environmental conditions which minimize this hazard must be considered if this problem becomes apparent (12, 16).

(e) Modification of the substrate may permit use of different microorganisms. It is possible that ad-

justing the pH, changing the degree of aeration, adding nutrients, or varying the heat treatment given may make the medium suitable for growth of a variety of microorganisms (11). Consequently, treatment of the substrate will, in part, govern the choice of the microorganism to carry out a given fermentation.

Although other factors may be of concern in certain fermentations, those which have been cited suffice to demonstrate the challenges associated with selection of the proper organism to do a given job.

#### FURTHER PROCESSING OF FERMENTED FOODS OR MICROBIAL PROTEINS

When food fermentations are discussed, we are generally concerned with two approaches. In the first, a microorganism is introduced into a substrate principally to bring about a desired change. Acid production in cultured dairy products is an example of this approach. The second procedure is that of introducing a microorganism into a substrate, allowing it to grow, harvesting the organism, and using it as a source of nutrients. Products obtained from the use of either procedure may benefit from further processing. It is conceivable that a more palatable product with desired characteristics may be obtained if the fermented material is subjected to heating, drying, freezing, concentration, a second fermentation, or another process. Both the initial substrate and changes brought about by the microorganisms will govern whether or not further processing is possible and the kind of technology which may prove useful.

Further processing is of greater significance when one is concerned with finding food applications for proteins produced by algae, molds, yeasts, or bacteria. For purposes of this discussion, we will consider that suitable methods exist for harvesting microorganisms from their substrate, usually a liquid, and this is not to be thought of as further processing.

We are told that dried preparations of algae such as *Chlorella vulgaris* and *Scenedesmus obliquus* contain approximately 40% crude protein (1). In contrast to this, yeasts such as *Candida utilis* and *Saccharomyces cerevisiae*, when dried, contain approximately 50 to 60% protein (1, 4, 8). The protein content of bacteria, on the basis of dry weight, will often be higher, ranging from 63% for *Bacillus subtilis* to 87% for *Lactobacillus fermentans* (1). Dried preparations of molds generally contain less protein, ranging from 19% for *Aspergillus flavus* to 38% for *Penicillium notatum* (1). In addition to information on the total protein content of these microbial preparations, we have some information on amino acid composition, particularly for dried yeasts, and on biological value, again mainly for proteins derived from yeast. Our

knowledge about the technology of handling these proteins is woefully inadequate. At this point suggested uses for these materials are generally limited to supplementing other foods at relatively low concentrations.

In contrast to this, a substantial body of knowledge has and is continuing to accumulate on milk, meat, and certain plant proteins. This information makes possible the processing of these proteins into foods which differ from the raw material in odor, flavor, texture, and appearance. Similarly, an understanding of the nature of microbial proteins is needed before they can be satisfactorily processed into foods which are palatable as well as nutritious—a condition which must precede the widespread use of these nutrients in the diet.

#### PUBLIC HEALTH PROBLEMS

Food products of microbial origin or made with the aid of microorganisms must not only be nutritious but also must be safe for consumption. The possibility of public health problems appears to be associated with (a) composition of the substrate used for the fermentation, (b) composition of the organisms being harvested, and (c) contamination with known toxigenic microorganisms which can proliferate and elaborate toxins during the fermentation process.

As was pointed out earlier in this discussion, if fermentation processes are to contribute markedly to the supply of available nutrients, then use of substrates indigenous to a deficient area but not now being employed as foods must be considered. Use of such media needs to be evaluated in the light of whether or not they contain substances which may be toxic to the consumer.

The presence of poisonous substances in certain plants is well recognized. Examples include: (a) the goitrogens such as the thiocyanates, derivatives of 2-thiooxazolidone, 3-indolylacetonitrile, and polysulfides of the *Brassicaceae* (cabbage and related plants) (14), (b) the estrogens genistein, genistin, and daidzein of soybeans (13), (c) alkaloids in a large variety of plants (10), (d) the carcinogenic substances associated with cycads (palm-like trees and their nuts) (10), (e) lathyrogens derived from certain peas and other plants in the genus *Lathyrus* (4), and (f) many others too numerous to cite in this discussion.

Organisms which are grown to supply nutrients also must be free of hazardous substances. It is necessary to demonstrate that potentially harmful compounds present in the medium at low levels are not concentrated by the microorganisms. This is of particular importance when sewage or other waste materials serve as the substrate.

It also is necessary that the microorganisms grown on a satisfactory substrate do not contain cellular components which are hazardous to health. Some investigators believe that the consumer of yeasts might experience harmful effects attributable to the high content of cellular purines (1). It has been suggested that the intake of an excessive amount of purines may cause a corresponding increase in the concentration of uric acid in the blood. By calculation it can be determined that 15 to 20 g of dried yeast would provide sufficient purines to give the maximum safe concentration of uric acid in blood. Some experts are not willing to accept this value since an increase in purine consumption does not necessarily result in a corresponding increase in blood uric acid (1). The answers to questions about the hazards of excessive purine compounds in the diet must be supplied before these suspicions will be laid to rest.

It also has been reported that yeasts grown on certain substrates produced liver necrosis when fed to rats, but the same yeasts grown on other media were entirely satisfactory for use in the diet (1). Other reports in the literature have suggested toxicity with food supplements derived from algae and from fungi such as *Penicillium* and *Aspergillus* species (1).

Finally, it is necessary that the substrate undergoing fermentation be protected from contamination by pathogenic microorganisms which can grow concurrently with the bacteria, yeasts, or molds responsible for the desired food product. It is well recognized that in certain dairy fermentations, contamination of milk with staphylococci can be hazardous since these bacteria produce an enterotoxin which, if present, can cause illness in consumers (15). This toxin is rather heat stable and once present in food can survive conditions which are detrimental to the organism responsible for its production. Staphylococci are widespread in nature, especially in and on animals and human beings. Consequently, contamination of a food can easily occur unless proper precautions are exercised.

Another group of organisms, the salmonellae, are ubiquitous and their presence on many substrates is not unusual. The more than 1300 species in the genus *Salmonella* are all believed to be pathogenic to man and most cause gastrointestinal disturbances (7). Recent studies have shown that these bacteria can proliferate during the manufacture of certain fermented foods and that they can survive for many months in the fermented product (3).

Mycotoxins, which have not been studied intensely until the last eight or nine years, also can occur in fermented foods which are contaminated with cer-



tain molds. Molds in the genera *Aspergillus*, *Penicillium*, *Stachybotrys*, *Cladosporium*, *Fusarium*, and others have been found able to produce toxic compounds which more often than not are hepatocarcinogens (6). Molds are often used to produce certain fermented foods. It is obvious that they must be carefully selected so that toxigenic strains are not employed in the fermentation.

From the foregoing discussion, it is evident that great care must be exercised before new fermented foods are consumed. It must be established that the substrates and microorganisms employed are harmless and that toxigenic or other pathogenic microorganisms do not enter and grow in the substrate during the fermentation process.

#### ACCEPTANCE OF NEW FOOD PRODUCTS

It is obvious that producing and stockpiling fermented foods or microbial nutrients is useless unless they are consumed by persons who are suffering from malnutrition. Introduction and acceptance of a new food may meet great resistance when persons have a low degree of literacy and a high degree of attachment to native ancestral and cultural ways. Education and advertising programs necessary to attain acceptance may be expensive added costs of production. It may be necessary initially to develop foods which closely resemble native dishes, hence a study of dietary habits in a given area would be in order. Acceptance of the new foods by governmental and other influential leaders in a community may aid in their utilization by the rest of the population. Although this is not an exhaustive treatment of this subject, it is one which cannot be ignored when development of new fermented foods is being considered.

#### COST OF PRODUCTION AND ABILITY TO PURCHASE

The per capita income of many persons in food-deficient areas of the world is less than \$200 per year and of many others it is below \$1000 per year. This fact must be realized before plans are made to supply nutrients via the route of fermentation. The question that must be asked is: "Can foods be produced economically enough to permit their purchase by these persons and at the same time provide a profitable operation?"

It is here where some of the real challenges can be found. The process must be made to operate as efficiently and economically as possible. The substrate must be cheap, the process simple, the micro-

organism efficient, the equipment inexpensive, and the finished product must be safe, nutritious, palatable, and inexpensive. Thought also must be given to waste disposal problems if large quantities of spent media result from fermentations used to produce microorganisms. Obviously, participation of governments in these programs may markedly alter the economic outlook.

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# A SELECTIVE MEDIUM AND PRESUMPTIVE PROCEDURE FOR DETECTION OF SALMONELLA IN DAIRY PRODUCTS

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(Received for publication July 6, 1970)

## ABSTRACT

A culture medium and testing procedure were developed to detect and differentiate *Salmonella* in pure culture study and for presumptive detection of *Salmonella* in dairy products. Most pure cultures of *Salmonella* were easily differentiated from other members of the *Enterobacteriaceae* group after 18 hr incubation at 37 C in a neutral red-lysine-iron-cystine broth. *Salmonella* changed neutral red in the medium from red to yellow and most strains turned the medium black through formation of a massive black precipitate. Species of *Enterobacter*, *Citrobacter*, *Proteus*, *Shigella*, and *Pseudomonas* intensified the red color of the medium and failed to blacken it. Species of *Klebsiella* and *Escherichia* usually changed the medium from red to yellow in 18 hr but none developed a black precipitate. The few nonhydrogen sulfide producing strains (no medium blackening) of salmonellae were differentiated from *Escherichia* and *Klebsiella* sp. by continued incubation to a total of 42 hr followed by the use of a second indicator, brom thymol blue. Only salmonellae gave an alkaline reaction and converted the medium from yellow to green or blue. The medium provided for rapid detection of most salmonellae after 18 hr incubation, as characterized by medium blackening or color change from red to yellow. Related enteric bacteria, other than *Arizona* strains, were readily differentiated.

For dairy products a slight modification in medium formula and use of novobiocin and trypsin were required. Novobiocin selectively inhibits growth of most interfering spore formers and gram-positive bacteria and also certain strains of *Escherichia coli* and *Proteus*. Trypsin was used to digest casein added with the dairy product sample. A positive presumptive test for *Salmonella* in dairy products was indicated in the medium by a color change from red to yellow and/or production of a massive black precipitate of iron sulfide after 24 hr incubation. Absence of salmonellae was indicated by no color change or no medium blackening. Results from testing several dairy products indicated that the procedure may be of value in the rapid screening of these foods for salmonellae. Although confirmation and serological identification are still essential, the test eliminates preenrichment and gives presumptive evidence of salmonellae contamination after 24 hr.

Recent outbreaks of food poisoning have resulted in the intensive scrutiny of all food products for salmonellae by public health officials and have prompted food processors to reevaluate processing procedures and factors that affect product quality.

Rapid and reliable means are urgently needed whereby contamination can be detected during processing and/or in the finished product. A food processor, especially where a perishable product is con-

cerned, cannot afford to wait from 4 to 7 days for analyses.

It is generally agreed that methods to detect and enumerate *Salmonella* are complex and time consuming. Recently, efforts to simplify these procedures have been reported (1, 8, 12). Many of the liquid media in current use were designed for isolating these organisms from feces and are by nature somewhat toxic to the bacteria. The liquid preenrichment medium prescribed by North (10) is widely used but its use may be questioned as it tends to favor lactose fermenting types.

Since most salmonellae decarboxylate lysine and produce hydrogen sulfide, it appeared that these biochemical characteristics could be utilized to develop a rapid presumptive test. Several decarboxylase media have been proposed for differentiating members of the *Enterobacteriaceae* group (2, 5). Edwards and Fife (4) proposed a lysine-iron agar for *Arizona* strains; however, it failed to distinguish between shigellae and many strains of *Escherichia coli*.

The objectives of the present study were threefold: (a) to develop a culture medium that could be used to differentiate *Salmonella* from other *Enterobacteriaceae* and also from organisms that are commonly found in dairy products, (b) to adapt the medium for use as a presumptive test for *Salmonella* in dairy products, and (c) to combine preenrichment with the presumptive test, thus shortening the period required for identification. Future work would attempt to use the fluorescent antibody technique (FAT) for confirming results from the presumptive test.

## MATERIALS AND METHODS

In preliminary studies various media were compared for their ability to selectively differentiate salmonellae from other enteric bacteria on the basis of lysine utilization and hydrogen sulfide production. The medium of Falkow (5) was compared with a lysine-iron broth medium similar in composition to the lysine-iron agar formula proposed by Edwards and Fife (4) for the detection of *Arizona* cultures. Attempts were made to improve the selectivity of the lysine-iron broth by adding or substituting various dyes, carbohydrates, iron and sulfur compounds, and anti-metabolites in the formula.

*Detection and Differentiation of Cultures**Medium*

The medium which showed the greatest potential for differentiating salmonellae from other genera contained: L-lysine — 10 g, tryptone — 5 g, yeast extract — 3 g, lactose — 5 g, glucose — 1 g, salicin — 1 g, ferric ammonium citrate — 0.5 g, sodium thiosulfate — 0.1 g, L-cystine — 0.1 g, neutral red — 0.025 g, and distilled water — 1 liter. The medium was adjusted to pH 6.2, dispensed in 8 to 10 ml portions in screw cap tubes, and autoclaved at 121 C for 15 min.

*Cultures*

Pure cultures of salmonellae and related types were tested in the selective medium. These included 64 *Salmonella* cultures representing serological groups A through I. Particular attention was given to reactions obtained from those serotypes most frequently isolated from contaminated nonfat dry milks (NDM), namely, *Salmonella cubana*, *S. anatum*, *S. monteveido*, *S. oranienburg*, *S. tennessee*, *S. worthington*, *S. newington*, and *S. new brunswick*. Representative strains of *Shigella dysenteriae*, *Shigella flexneri*, *Escherichia coli*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Citrobacter freundii*, *Proteus vulgaris*, *P. mirabilis*, *P. morgani*, *Paracolobactrum arizonae*, *P. aerogenoides*, *Providencia stuartii*, and species of *Klebsiella* and *Pseudomonas* were tested. Spore formers, such as *Bacillus subtilis*, *B. cereus*, *B. megaterium*, and *B. stearothermophilus*, which are frequently found in milk powders also were tested.

*Test procedure and evaluation*

Tubes containing 8 to 10 ml of test medium were usually inoculated with a 3 mm standard loop from an 18 to 24 hr broth culture. In tests with unknown isolates, growth of individual colonies on selective agars frequently served as an inoculum.

Cultures were incubated at 37 C for 18 hr and observed for neutral red color change, and medium blackening. Salmonellae were readily detected after 12 to 18 hr growth by neutral red color change and production of a massive black precipitate. A few diphasic salmonellae will not produce the black precipitate but do change the medium from red to yellow. Species of *Shigella*, *Enterobacter*, *Proteus*, *Citrobacter* and most gram-positive types intensify the red color of the medium and do not yield a black precipitate. Even those *Proteus* species such as *P. vulgaris* and *P. mirabilis* that produce hydrogen sulfide in TSI agar do not cause the medium blackening or change the medium color. Species of *Escherichia* and *Klebsiella* change the medium color from red to amber or yellow without blackening. Differentiation of the few nonblackening salmonellae strains in the medium, namely *S. sendai*, *S. abortusovaequina*, and diphasic *S. choleraesuis*, from species of *Escherichia* and *Klebsiella* required an additional period of incubation. Therefore, all cultures showing a medium color change from red to yellow after 18 hr at 37 C are reincubated an additional 24 hr at 37 C (total 42 hr). After the second incubation, 0.1 ml of 0.3% brom thymol blue solution is added to each tube, and the color recorded. Salmonellae strains produce an alkaline reaction, changing the medium from yellow to green or blue. Blue color is more intense at the top of the tube on standing but color differences are immediately obvious. *Klebsiella* and *Escherichia* species allow medium to remain bright yellow. Brom thymol blue solution (50% alcoholic) was prepared by mixing 0.3 g of brom thymol blue indicator powder (Nutritional Biochemical Corp.)<sup>1</sup> with 2 ml of 0.1N NaOH and diluting to 100 ml with 50% ethyl alcohol in distilled water.

*Presumptive Procedure for Detecting Salmonella in Dairy Products**Dairy product samples*

Commercial samples of dried milks were kindly furnished by the Dairy Division, Consumer and Marketing Service, USDA and the Food and Drug Administration. Because some difficulty was encountered in collecting positive commercial products, many of the test products had to be prepared experimentally in the dairy pilot plant. The processing milk was artificially contaminated with salmonellae prior to product manufacture. Products prepared included NDM, milk concentrates, whey, raw and pasteurized milk, and Cottage and Cheddar cheese. Milk powders containing approximately 1 to 10 salmonellae per 100 g were prepared to determine the sensitivity of the proposed medium. Milk concentrates and raw milks containing salmonellae, were prepared by seeding the milks with 18 hr broth cultures and diluting with milk to obtain a level of approximately 1 *Salmonella*/ml. Cheeses were prepared according to previously described methods and the salmonellae content was followed throughout storage and curing (6, 9). Cheeses containing less than 10 salmonellae/g were used. Whey from these cheeses containing low levels of salmonellae also were tested.

*Salmonella* strains added to or found in the dairy products included *S. senftenberg*, *S. typhimurium*, *S. cubana*, *S. anatum*, *S. new brunswick*, *S. oranienburg*, *S. monteveido*, *S. newington*, and *S. choleraesuis*.

*Medium for testing dairy products*

When the test medium as developed for differentiation of pure cultures was applied to detect salmonellae in dairy products, certain adjustments in the medium and test procedure were essential. First, selectivity for salmonellae had to be increased in tests with mixed populations and growth of certain gram-positive types had to be suppressed. Second, casein carried over with dairy product samples interfered with color changes and this problem had to be overcome.

*Novobiocin*. A number of antibiotics were screened for ability to selectively inhibit growth of interfering microorganisms in the presence of *Salmonella*. These included novobiocin, erythromycin, kanamycin, neomycin, and sodium oxacillin. They were added aseptically to the medium at levels ranging from 3 to 20 µg/ml. Preliminary evidence indicated that novobiocin (novobiocin, sodium) albamycin<sup>®</sup>, Upjohn Co.<sup>1</sup> was the most effective antibiotic in suppressing the growth of spore formers and species of *Proteus* and *Escherichia*. The optimum concentration of novobiocin in the test medium was determined with pure and mixed cultures of salmonellae and with salmonellae-positive dairy products. Concentrations tested were 2, 5, 10, and 15 µg/ml. A stock solution was prepared by adding 100 mg of crystalline novobiocin to 100 ml of sterile distilled water. The stock solution was stored at 4 C and discarded after two weeks.

*Trypsin*. The enzyme trypsin was tested in the medium as a clarifying agent for milk casein. Sterile stock solutions were prepared by mixing 1 g of trypsin (1-300) Nutritional Biochemical Corporation<sup>1</sup> in 100 ml of distilled water and filtering through a Seitz bacterial filter. This solution was usually dispensed in tubes, frozen at -17 C, and then thawed and used as needed. Levels of trypsin tested for casein digestion and possible toxic effect on the growth of salmonellae varied from 1 to 10 ml of stock solution per 100 ml of test medium.

<sup>1</sup>Mention of brand or firm names does not constitute an endorsement by the Department of Agriculture over others of a similar nature not mentioned.

**Medium formula.** The medium developed for detecting salmonellae in dairy products was essentially the same as for pure cultures but was modified to contain: L-lysine — 10 g, tryptone — 5 g, yeast extract — 3 g, lactose — 5 g, glucose — 2 g, ferric ammonium citrate — 0.5 g, sodium thiosulfate — 0.1 g, L-cystine 0.3 g, neutral red — 0.025 g, and distilled water — 1 liter. No adjustment of pH was required. The medium was usually dispensed in 500 or 1000 ml portions in large mouth flasks, autoclaved, and stored at 4 C until used. Freshly prepared autoclaved medium with the oxygen expelled was not used until the following day or until after oxygen equilibration because false-positives were encountered under these conditions. Stock solutions of novobiocin and trypsin were added aseptically to the medium just prior to testing or at the time of mixing with dairy samples. The concentration of novobiocin was 5 µg/ml of medium or 0.5 ml of stock solution to each 100 ml. Stock trypsin was added at the rate of 2 ml for each 100 ml of medium.

**Evaluation and sensitivity.** The sensitivity of the procedure was established by testing milk powders known to contain low levels of sublethally impaired salmonellae (1-10 per 100 g). Additionally, milk powders known to contain salmonellae and related types such as *E. coli*, *Enterobacter*, *Citrobacter*, and *Proteus* were tested.

#### Assay of dairy products

**NDM.** NDM powder was added to sterile medium in the proportion of 1 to 10 (w/v). Lesser amounts of powder to medium may be used without harming the sensitivity but this limit should not be exceeded. Both 50 and 100 g samples were tested in the development of the procedure. One hundred gram samples were slowly mixed with 1000 ml of medium in a sterile Waring blender and after initial slow mixing, the solutions of novobiocin (5 ml) and trypsin (20 ml) were added and mixed. Mixtures were then returned to the original flasks. To allow resuscitation of sublethally impaired cells in dried products such as milk powder, the mixed samples were immediately warmed to 30 C and held for 6 hr. After 6 hr preincubation, flasks were placed in a 39 C water bath and held for an additional 18 hr. After incubation (total 24 hr) the mixtures were observed for color changes and/or blackening.

**Milk concentrates.** Milk concentrates from condensing (25 to 50% solids) were added directly to flasks or bottles of medium containing the prescribed amounts of novobiocin and trypsin (0.5 ml and 2 ml/100 ml, respectively). Usually 10 g of 50% concentrate or 20 g of 25% concentrate was added per 100 ml of medium. (Lesser amounts of concentrate to medium may be used). Mixtures were incubated at 39 C for 24 hr and observed for color changes as with NDM. Preincubation at 30 C was not essential with liquid dairy products.

**Cheddar and Cottage cheese.** Cheese samples of 11 g were blended in a Waring blender with 100 ml of sterile distilled water containing 0.2 g sodium citrate. Usually 10 or 20 ml aliquots of the cheese slurry were added to 100 ml of test medium. Novobiocin and trypsin were added and incubated as previously described for milk concentrates.

**Whey, raw and pasteurized milk.** Whey and milk samples were added to the medium at the ratio of 1 to 10 or 10 ml per 100 ml of medium. Trypsin and novobiocin were added, mixed, and the mixtures incubated at 39 C.

After 24 hr incubation, flasks or bottles were removed from the water bath and recorded for medium color changes. A positive presumptive test for the presence of salmonellae was indicated by medium blackening and/or color change from red to yellow. A negative test was indicated by the absence of color change or blackening. All salmonellae will change the medium color from red to yellow in 24-hr and in

addition most will form a massive black precipitate. Growth from all positive samples should be positively identified as containing salmonellae.

**Confirmation.** Samples from the presumptive test at 24 hr were removed and confirmed for the presence or absence of salmonellae by standard cultural and serological procedures; e.g., growth on differential agars and serological tests with specific antisera. Limited tests were made in which confirmation was made by FAT (11). To determine the effectiveness of the presumptive medium, duplicate samples of the dairy products were assayed by conventional lactose pre-enrichment, cultural and serological procedures (Bacteriological Analytical Manual).

## RESULTS AND DISCUSSION

### Differentiation of cultures

The medium as developed in pure culture study proved extremely useful in the detection and differentiation of unknown strains of salmonellae from other genera of the *Enterobacteriaceae* group. Salmonellae were readily differentiated after 18 hr incubation from species of *Shigella*, *Citrobacter*, *Enterobacter*, *Proteus*, and *Pseudomonas*. Unknown colonies of salmonellae picked from selective agars were also readily identified. All test strains with the exception of a few *S. pullorum* grew rapidly in the neutral red broth in 12 to 18 hr at 37 C and changed it in color from red to yellow or black. Most salmonellae decolorized neutral red and formed a massive black precipitate which obscured yellow color in 18 hr. Medium blackening was usually evident after 7 to 8 hr incubation. Typical medium reactions obtained with representative *Salmonella* serotypes are shown in Table 1. Only three serotypes tested failed to produce the characteristic black precipitate, e. g., *S. sendai*, *S. abortusovae*, and diphasic *S. choleraesuis*. These strains could still be identified as salmonellae and differentiated from other genera by continued incubation for 24 hr followed by addition of brom thymol blue. Of two *S. paratyphi* A strains tested, one failed to produce H<sub>2</sub>S and give the typical reaction of most salmonellae. Two of six strains of *S. pullorum* grew poorly at 37 C and could be differentiated more readily when grown at 30 C. Lactose-positive *Salmonella* produced typical reactions in the medium, e.g., decolorization and blackening. The reactions obtained with microorganisms other than salmonellae are shown in Table 2. Species of *Shigella*, *Enterobacter*, *Citrobacter*, *Proteus*, and *Pseudomonas* usually remained bright red in color after 18 hr incubation and did not blacken the medium. *Enterobacter*, *Proteus*, and *Citrobacter* species usually lower the medium pH, thus intensifying its red color. Even those *Proteus* strains which produce hydrogen sulfide on TSI agar, such as *P. mirabilis* failed to blacken the medium and remained red, thus easily distinguishing them from salmonellae.

TABLE 1. PURE CULTURE REACTIONS OF *Salmonella* IN TEST MEDIUM

Serotype	Serological group	Medium changes — 37 C		
		18 hr	42 hr <sup>a</sup>	
		Color	Black ppt.	Color
<i>Salmonella paratyphi</i> A	A	Y*	+± <sup>b</sup>	Y
<i>S. typhimurium</i>	B	BL**	+++	GB***
<i>S. schottmuelleri</i>	B	BL	+++	GB
<i>S. derby</i>	B	BL	+++	GB
<i>S. chester</i>	B	BL	+++	GB
<i>S. cholerae-suis</i>	C <sub>1</sub>	Y	—	GB
<i>S. cholerae-suis</i> var. kunzendorf	C <sub>1</sub>	BL	+++	GB
<i>S. montevideo</i>	C <sub>1</sub>	BL	+++	GB
<i>S. oranienburg</i>	C <sub>1</sub>	BL	+++	GB
<i>S. tennessee</i>	C <sub>1</sub>	BL	+++	GB
<i>S. blockley</i>	C <sub>2</sub>	BL	+++	GB
<i>S. newport</i>	C <sub>2</sub>	BL	+++	GB
<i>S. sendai</i>	D <sub>1</sub>	Y	—	GB
<i>S. typhi</i>	D <sub>1</sub>	BL	+++	GB
<i>S. enteritidis</i>	D <sub>1</sub>	BL	+++	GB
<i>S. pullorum</i>	D <sub>2</sub>	BL <sup>c</sup>	+++ <sup>c</sup>	GB
<i>S. anatum</i>	E <sub>1</sub>	BL	+++	GB
<i>S. meleagridis</i>	E <sub>1</sub>	BL	+++	GB
<i>S. newington</i>	E <sub>2</sub>	BL	+++	GB
<i>S. new brunswick</i>	E <sub>2</sub>	BL	+++	GB
<i>S. illinois</i>	E <sub>3</sub>	BL	+++	GB
<i>S. senftenberg</i>	E <sub>4</sub>	BL	++	GB
<i>S. worthington</i>	G	BL	+++	GB
<i>S. albuquerque</i>	H	BL	+++	GB
<i>S. gaminara</i>	I	BL	+++	GB

\*Y = Yellow

\*\*BL = Black from ppt — Neutral red decolorized to pale yellow

\*\*\*GB = Green to blue

<sup>a</sup>After adding brom thymol blue<sup>b</sup>50% of the *S. paratyphi* A strains formed a black ppt.<sup>c</sup>Four out of six strains gave blackening at 37 C — 2 grew poorly.

Many species of *Pseudomonas*, such as *P. fragi*, failed to grow in the medium at 37 C. Salmonellae and most *E. coli* and *Klebsiella* strains decolorized or irreversibly changed neutral red from red to yellow without a marked pH change, thus inactivating it as an indicator. None of the other test strains had this effect on neutral red. As indicated previously, the nonhydrogen sulfide strains of salmonellae required a longer incubation period and use of brom thymol blue to distinguish them from *E. coli* and *Klebsiella* species. Only salmonellae cultures gave an alkaline reaction changing brom thymol blue from yellow to green or blue. Lysine and lactose utilizing cultures such as *E. coli* and *E. aerogenes* sufficiently lowered medium pH and remained yellow. Thus culture differentiation in the medium is based upon pH changes as indicated by neutral red and brom thymol blue, decolorization of neutral red, and the production of iron sulfide or medium blackening. Cultures utilizing lysine are known to yield an alkaline reaction. Lac-

tose and salicin provided a carbohydrate source for microorganisms other than salmonellae which yield an acid reaction. Salicin also provided a carbohydrate source for slow or non-lactose utilizing strains such as *Proteus*. All *E. aerogenes*, *Klebsiella* and *Proteus* species, and most *E. cloacae* and *Escherichia* species fermented salicin. It has been reported by Edwards and Ewing (3) that all salmonellae produce hydrogen sulfide on TSI agar with the exception of *S. paratyphi* A, *S. sendai*, *S. berta*, *S. senftenberg*, *S. choleraesuis* (diphasic), and *S. abortivoequina*. Addition of L-cystine to the neutral red medium intensified blackening with most species, and resulted in medium blackening with strains of *S. senftenberg* and *S. berta* and some *S. paratyphi* A. Apparently L-cystine is more easily utilized than sodium thiosulfate or adds to the sulfur source for these strains. Test strains of *P. arizonae* changed medium color and gave blackening, making it impossible to differentiate them from salmonellae. *Paracolobactrum aerogenoides* could be distinguished by indicator changes.

Several advantages were found in the use of the cultural test medium: (a) rapid results were obtained in 12 to 18 hr with most salmonellae, (b) one medium was sufficient for differentiation from related types, and (c) hydrogen sulfide producing strains of *Proteus* and *Citrobacter* were less likely to be confused with salmonellae than on TSI agar or lysine iron agar.

#### Assay of dairy products

The medium was effective in isolating and detecting salmonellae in most known positive dairy products. Dairy products containing salmonellae either turned the medium very black or changed its color from red to bright yellow depending upon the strain's ability to form hydrogen sulfide. A negative test was indicated by the absence of color change or medium blackening. Only a limited number of dairy products could be made with different *Salmonella* serotypes; however, all but a few made with *S. choleraesuis* caused medium blackening in 24 hr. A strain of *S. senftenberg* and lactose positive *S. tennessee* developed lesser amounts of iron sulfide. Milk powders prepared to contain approximately 1-10 salmonellae cells per 100 g were usually positive by the presumptive test. Medium sensitivity for dairy products was increased by changing the cystine content from 0.01 to 0.03% and raising the incubation temperature to 39 C. (Low levels of sublethally impaired salmonellae in NDM failed to give the characteristic reactions when samples were placed immediately at 39 C). It was found that a preincubation period of 6 hr at 30 C was sufficient to allow resuscitation of injured cells and then the samples could be incubated at 39 C. Test strains of *E. coli*, *E. aerogenes*, *P. vulgaris*, *P.*

TABLE 2. MEDIUM REACTION OF PURE CULTURES OTHER THAN SALMONELLAE

Strain	Medium changes — 37 C		
	Color	18 hr	42 hr <sup>a</sup> Color
<i>Shigella dysenteriae</i>	Red	—	red to brown <sup>b</sup>
<i>Shigella flexneri</i>	Red	—	red to brown
<i>Paracolobactrum arizonae</i>	Black*	+++	green or blue
<i>Paracolobactrum aerogenoides</i>	Yellow	+	yellow
<i>Escherichia coli</i>	Amber to Yellow	—	yellow
<i>Enterobacter cloacae</i>	Red	—	red to brown
<i>Enterobacter aerogenes</i>	Amber to Red <sup>I**</sup>	—	red to brown
<i>Citrobacter freundii</i>	Amber to Red <sup>I</sup>	—	red to brown
<i>Klebsiella pneumoniae</i>	Yellow	—	yellow
<i>Klebsiella ozaenae</i>	Yellow	—	yellow
<i>Proteus vulgaris</i>	Red <sup>I</sup>	—	red to brown
<i>Proteus mirabilis</i>	Red <sup>I</sup>	—	red to brown
<i>Proteus morgani</i>	Red	—	red to brown
<i>Providencia stuartii</i>	Red	—	red to brown
<i>Pseudomonas fragi</i>	Red <sup>NG***</sup>	—	red to brown
<i>Pseudomonas fluorescens</i>	Red <sup>NG</sup>	—	red to brown
<i>Pseudomonas aeruginosa</i>	Red	—	red to brown
<i>Bacillus cereus</i>	Red	—	red to brown
<i>Bacillus megaterium</i>	Red	—	red to brown
<i>Bacillus subtilis</i>	Red	—	red to brown

\*Black from precipitate — Neutral Red decolorized

\*\*I — Red color usually intensified

\*\*\*NG — No growth

<sup>a</sup>After addition of brom thymol blue

<sup>b</sup>Red tubes at 18 hr need not be reincubated or treated with brom thymol blue

*mirabilis*, and *P. morgani* in the dairy products failed to give medium color changes or blackening after 24 hr incubation. Strains of *E. coli* did change the medium color to yellow with longer incubation periods (48 hr).

The antibiotics, novobiocin and erythromycin at levels of 5 and 10  $\mu\text{g}/\text{ml}$ , respectively were equally effective in isolating *Salmonella* in the presence of gram-positive microorganisms; however, novobiocin was slightly superior in suppressing growth of many *E. coli* and *Proteus* strains. Advantages of novobiocin in isolation of salmonellae from fecal samples have been shown by Jeffries (7). Test strains of *B. subtilis*, *B. megaterium*, *B. cereus*, and *B. stearothermophilus* were inhibited with 5  $\mu\text{g}/\text{ml}$  of novobiocin. All species of *Shigella* and many strains of *E. coli*, *Proteus*, and *Pseudomonas* were suppressed by 5  $\mu\text{g}$  of novobiocin in the medium. Novobiocin was required in the medium before a positive test could be obtained when NDM samples contained *Salmonella* and *E. coli* in a ratio of 1 to 10 per gram of powder. Obviously large numbers of lactose fermenters such as *E. coli* may overgrow salmonellae unless suppressed. Trypsin was effective as an agent to digest casein and to clarify the medium and thus enhanced detection of color changes and medium blackening with all dairy products tested. Digestion of casein was usually complete after 12 hr. Addition of trypsin to

the medium did not appear to inhibit growth or recovery of stressed cells of salmonellae (heating and drying) in NDM or other dairy products. Five to 10 times the prescribed level of trypsin in the medium had no effect on the growth of salmonellae or medium reactions.

Detection of salmonellae in dairy products was based upon irreversible color changes of the neutral red and blackening of the medium by salmonellae. Excessive aeration of cultures and the use of anaerobic conditions are to be avoided when conducting the presumptive test. For example, false-positive reactions were frequently encountered, (medium change from red to yellow), when freshly autoclaved medium with oxygen expelled was used. As a result autoclaved medium was not used until the following day. Shaking and excessive aeration during incubation prevented blackening and appeared to inhibit growth of stressed cells of salmonellae in NDM. The number of salmonellae in some commercial NDM samples was obviously low because difficulty was encountered in detection even with repeated sampling by standard cultural procedures. NDM made in the pilot plant to contain approximately 1 *Salmonella* and 10 *E. coli* per gram was useful as a positive control and in demonstrating the effect of medium variables. Limited trials have shown that the test is sufficiently sensitive to detect between 1 and 10

TABLE 3. DAIRY PRODUCTS ANALYZED FOR *Salmonella*

Product	No. of samples	Standard cultural procedures		Presumptive test	
		+	-	+	-
NDM Commercial	21	9	12	8	13
NDM <sup>a</sup>	10	10	0	10	0
NDM <sup>b</sup>	2	2	0	2	0
NDM <sup>c</sup>	2	0	2	0	2
NDM <sup>d</sup>	4	0	4	0	4
NDM <sup>e</sup>	1	1	0	1	0
Milk concentrate (25 to 50%)	3	3	0	3	0
Cheddar cheese	16	14	2	15	1
Cottage cheese	5	5	0	5	0
Raw milk	15	14	1	12	3
Whey	21	20	1	20	1

<sup>a</sup>Prepared with *Salmonella* serotypes producing hydrogen sulfide

<sup>b</sup>Prepared with *S. choleraesuis* and *S. senftenberg*

<sup>c</sup>Prepared with *E. coli*

<sup>d</sup>Prepared with species of *Proteus*

<sup>e</sup>Prepared with *S. new brunswick* and *E. coli*

salmonellae per 100 g sample. The procedure was slightly more sensitive when lesser amounts of powder to medium could be used, for example, 50 g of NDM to 1 liter of medium. Stressed cells of salmonellae in NDM were found to recover as fast or faster in the neutral red broth than in reconstituted milk. Recovery was determined by a direct plating procedure. As might be expected, the procedure was more sensitive for detection of salmonellae in liquid products and cheese than in NDM. In tests with raw milk, 1 *Salmonella*/ml could easily be detected when the total bacterial count did not greatly exceed 200,000/ml. In a few instances salmonellae were detected when the total count exceeded 1 million/ml. Raw milks of high counts obviously contained large numbers of lactose fermenters which overgrew salmonellae even when the medium contained novobiocin. *Salmonella* was readily detected in pasteurized milk and wheys. Lactic streptococci in the wheys were inhibited by novobiocin.

Results of tests with dairy products are shown in Table 3. Although 100% correlation was not obtained between the 24-hr presumptive test and the 4-day completed cultural and serological procedure, the number of positive samples at 24 hr was extremely high. Results to date indicate that the presumptive test cannot be solely relied upon for *Salmonella* detection. Even though confirmation of *Salmonella* in the growth medium is still required, the total time required for the completed test is reduced. The medium eliminates the usual preenrichment and is

more selective for the growth of *Salmonella* than reconstituted milk.

Full evaluation of the neutral red lysine-cystine medium and test procedure cannot be determined until many products have been assayed and the results compared with those of established test procedures. However, results from the various dairy products tested to date would indicate that the isolation procedure has definite potential as a rapid screening procedure that may be used by processors and technicians.

Preliminary tests with the FAT indicate that the growth from the presumptive test may be applied directly for FAT confirmation. Iron sulfide deposits were generally removed during the normal rinse procedures and did not interfere with fluorescence. Presumptive evidence of *Salmonella* and confirmation by FAT was obtained in a total of 26 hr. Results of FAT tests will be reported at a later date.

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# OCCURRENCE OF *BACILLUS CEREUS* IN SELECTED DRY FOOD PRODUCTS<sup>1, 2</sup>

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## ABSTRACT

One hundred seventy samples of dried food products in national distribution were examined for the incidence and level of contamination by *Bacillus cereus*. Twenty-five per cent of the samples yielded *B. cereus* at a level not exceeding 4000 per gram. Mannitol-egg yolk-polymyxin (MYP) agar was used as a presumptive test for the presence of *B. cereus* in the food samples. Various biochemical tests for the confirmation of suspicious colonies appearing on MYP agar were evaluated. A precipitin test employing spore precipitinogens was investigated as a confirmatory test for *B. cereus*. The possible role of *B. cereus* in outbreaks of foodborne disease in the United States is discussed.

The first report of confirmed cases of *Bacillus cereus* food poisoning appeared in 1950 (4). In the ensuing years, additional reports appeared in the literature confirming the role of *B. cereus* in foodborne disease (5, 8, 9, 10). A recent report by Ormay and Novotny (13) placed *B. cereus* as the third leading cause of food poisoning in Hungary during the period 1960-1968. These investigators stated that meat dishes (53.8%), vegetables (10.6%), milk and cocoa (9.6%), and miscellaneous foods (17.6%) were the most common food vehicles in the outbreaks of food poisoning studied. Mossel et al. (9) listed mashed potatoes, minced meat, liver sausage, rice dishes, puddings, and soups as the most common causative foods of *B. cereus* poisoning in The Netherlands. Although these investigations by European workers have indicated that a problem exists, the possible role of *B. cereus* in foodborne outbreaks in this country has largely been ignored.

Earlier this year, the first well-documented outbreak of *B. cereus* food poisoning in the United States was reported by Midura et al. (8). The outbreak, in which meat-loaf was the vehicle, occurred among the members of a fraternity in California. The mean incubation period was 10 hr and the predominant symptoms were abdominal pain, diarrhea, nausea, and some vomiting. Quantitative microbiological analyses of the remaining meat-loaf revealed an aerobic plate count of  $76 \times 10^6$ /g and a *B. cereus* count of  $70 \times 10^6$ /g.

Several other reports of *B. cereus* food poisoning in

the U. S. have appeared but the details are sketchy or totally lacking (1).

This study was undertaken to assess the frequency and level of *B. cereus* contamination in selected food products purchasable at the retail level. In addition, existing methods for isolation and identification of *B. cereus* from food products were evaluated.

## MATERIALS AND METHODS

### Samples

All food samples were products in national distribution that were purchased at various retail outlets in Madison, Wisconsin. Since all products were dried foods, they were kept at room temperature until sampling and analysis.

### Isolation and enumeration of *B. cereus* from foods

Ten grams of food sample were aseptically removed from its package and suspended in 90 ml sterile 0.1% peptone water in a screw-capped milk dilution bottle. The food suspension was then shaken vigorously 25 times through an arc of 1 ft in 7 sec. Further dilutions were made by sequential transfer of 1 ml to 9 ml 0.1% peptone water. One-tenth milliliter aliquots of appropriate dilutions were surface plated on the mannitol-egg yolk-polymyxin (MYP) medium of Mossel et al. (9). Plates were examined for colonies evidencing an egg yolk reaction after incubation for 24 hr and 42 hr at 32 C.

### Biochemical confirmatory tests of food isolates

Hemolysin production was determined by streaking a broth culture of the isolate on plates of trypticase soy agar containing sheep red blood cells (5%). Plates were incubated at 32 C for 24 hr.

Gelatin hydrolysis was determined by stabbing a loopful of a 24-hr broth culture into tubes of nutrient gelatin. After incubation for 48 hr at 32 C, tubes were immersed in an ice-water bath. Failure to solidify indicated that gelatin had been hydrolyzed. Negative cultures were reincubated and retested at 168 hr. Growth on phenethyl alcohol agar was determined by the method of Knisely (6).

Casein hydrolysis was determined by inoculating tubes of litmus milk with a loopful of a 24-hr broth culture. Tubes were examined after 48 hr at 32 C. Negative cultures were retested by streaking across the center of a plate of nutrient agar containing 1% sodium caseinate.

Growth at 49 C was measured by inoculating pre-tempered tubes of trypticase soy broth and incubating for 7 days in a thermostatically controlled water bath. The inoculum level was  $10^4$  cells/ml and tubes were considered positive for growth if turbidity became evident during incubation.

Growth in 7% NaCl was measured by inoculating trypticase soy broth + 6.5% added NaCl with  $10^4$  cells per milliliter. A positive reaction was recorded if the tubes showed turbidity after 7 days at 32 C.

DNase production was measured by streaking a loopful of broth culture once across the center of a DNase test agar (Difco) plate. After incubation at 32 C for 48 hr, the plate was flooded with a solution of 1% HCl. A clear zone around

<sup>1</sup>Published with the approval of the Director of the Research Division, College of Agricultural and Life Sciences.

<sup>2</sup>Presented in part at the 70th Annual Meeting of the American Society for Microbiology, April 26-May 1, 1970, Boston, Mass.



TABLE 1. INCIDENCE AND LEVEL OF EGG YOLK-POSITIVE AND 'NARROW-ZONE' FORMING ORGANISMS IN VARIOUS DRIED FOOD PRODUCTS

Product	Egg yolk-positive organisms		'Narrow-zone' organisms	
	No. samples +/- No. tested	Level of contamination per g	No. samples +/- No. tested	Level of contamination per g
Dry potatoes	8/20	100-4000	3/20	500-1800
Soup mixes	1/22	700	11/22	100-9000
Gravy mixes	2/23	100-1000	9/23	100-4000
Spaghetti sauce	6/16	100-1000	10/16	100-107,000
Cream, BarBQ sauces	1/16	100	5/16	100-900
Spices	10/25	100-1000	9/25	200-1,300,000
Seasoning mixes	11/20	100-1200	9/20	300-38,000
Puddings and custards	0/14	—	0/14	—
Flour and starch	1/6	100	0/6	—
Skimmed milk powder	3/8	200-600	0/8	—

the streak indicated a positive test for DNase production.

Starch hydrolysis, acetylmethylcarbinol (AMC) production,  $\text{NO}_3$  reduction and glucose fermentation were determined by methods described by Smith et al. (14).

Sensitivity to 201 phage was determined by spotting 0.01 ml of phage solution ( $6 \times 10^8$  phage/ml) on the center of a lawn of young cells (4 hr) growing on nutrient agar. A clear area against a surrounding turbid region of cell growth indicated sensitivity to the phage.

#### Serological procedures

*Preparation of antiserum.* Thoroughly washed spores of *B. cereus* T were generously supplied by Dr. R. Hansen, Department of Bacteriology, University of Wisconsin. Spore suspensions were autoclaved at 121 C for 30 min. The autoclaved spore suspension (15 mg/ml) was inoculated into the ear vein of rabbits according to the schedule of Norris and Wolf (11). After the precipitin titer against homologous antigen reached 1:256 the rabbits were bled from the ear artery and the immune serum was harvested. Globulins were removed from the serum by precipitation with  $(\text{NH}_4)_2\text{SO}_4$  and resuspended in phosphate buffered saline (pH 7.2).

*Preparation of precipitinogen.* Spores of the egg yolk positive and 'narrow zone' test strains were prepared by growing the cultures in G medium (3) with shaking at 32 C for 4 days. Spores were harvested by centrifugation and washed repeatedly with distilled water until free of vegetative cell debris as determined microscopically and by a negative catalase test. Seventy-five milligrams of spores were extracted with hot formamide according to the method of Norris and Wolf (11). Extracted material was diluted to 0.5 mgm/ml in phosphate buffered saline prior to use in the precipitin test.

*Precipitin tests.* Equal volumes of antigen and 0.5% agar were mixed at 45 C prior to placing in glass tubing (3 mm internal diameter) that was sealed at one end. A 1:16 dilution of the globulin fraction of the immune serum was mixed with an equal volume of 0.5% agar before layering over the antigen portion in the glass tubing. The tubes were then incubated at 25 C for 1-2 days before examination. A positive reaction was evidenced by the formation of a precipitate in either layer or at the interface between antigen and antibody layers.

## RESULTS

One-hundred seventy samples of dried food products were examined for the incidence and level of contamination by *B. cereus*. Results are summarized in Table 1. Colonies on the mannitol-egg yolk-poly-myxin agar plates were considered to be *B. cereus*

if they were flat, dry, and opaque and were surrounded by a large zone of precipitate. These colonies were always readily countable after 24 hr at 32 C. When the plates were incubated for an additional 18 hr as suggested by Mossel et al. (9), there were numerous colonies, usually quite moist in appearance, that showed a small zone of precipitation in the agar. In most instances, this zone was confined to the area directly beneath the colony, but occasionally it was observed to extend 1-2 mm around the colony. Although the colonial morphology was not typical of *B. cereus*, it was impossible to be certain that these were not weak egg yolk-positive strains of *B. cereus*. For this reason, representative colonies of this type were picked for further biochemical analysis.

Data in Table 1 show that the incidence of *B. cereus* in these selected products was 25.3%. The products most frequently contaminated were seasoning mixes (55%), spices (40%), dry potatoes (40%), milk powder (37.5%), and spaghetti sauces (37.5%). Although the incidence of contamination was fairly high, the level of contamination in any positive sample did not exceed 4000 *B. cereus* per gram and, in most instances was <1000 per gram. The incidence of 'narrow zone' colonies was somewhat higher (32.9%) and the level of contamination ranged up to a maximum of  $1.3 \times 10^6$  per gram. Several trials were conducted initially to determine whether heat shocking (75 C, 10 min) the 1:10 dilution of food would enhance isolation of *B. cereus*. An increase in recoverable *B. cereus* was not effected by heat shock indicating that spores germinated readily on the MYP agar. Consequently, the remaining samples were examined without employing a heat shock.

In an effort to identify the 'narrow-zone' organisms and to evaluate the biochemical tests most often applied to characterize *B. cereus*, a total of 51 colonies of egg yolk-positive organisms and 52 colonies of 'narrow-zone' types were isolated and examined. Table 2 summarizes results of these examinations. The only

properties that were shared by all 51 strains of *B. cereus* were hemolysis of sheep RBC and hydrolysis of gelatin. Other characteristics usually attributed to *B. cereus* were variable and do not appear to be reliable confirmatory tests. The 'narrow-zone' organisms shared many characteristics with *B. cereus* and could not be separated from *B. cereus* on the basis of these biochemical tests.

In 1961 Norris and Wolf (11) described a precipitin test that was purported to be species specific for members of the genus *Bacillus*. Formamide extracts were made of spores of 13 strains of wide zone organisms and 6 strains of 'narrow-zone' organisms. Each extract was then tested in Oudin tubes against the globulin fraction of serum prepared against autoclaved spores of *B. cereus* T. In initial trials with both types of organisms three distinct bands of precipitate were formed. The strongest band appeared at the interface between the agar layers and weaker bands were observed at the extremes of both the antigen and globulin layers. Absorption of the globulin fraction with vegetative cells of *B. cereus* T resulted in elimination of the band in the antibody-containing layer. The two remaining bands were found in each trial where a positive reaction occurred, i.e. with 10 of 13 and 4 of 6 strains of egg yolk-positive and 'narrow-zone' strains, respectively. These results indicated that either (a) the precipitin test was not specific for *B. cereus*, or (b) the 'narrow-zone' organisms were indeed strains of *B. cereus*. In either event, the data showed that there was no single formamide extractable precipitinogen that was present in all strains of *B. cereus*.

#### DISCUSSION

The incidence of *B. cereus* in the food products examined in this study was somewhat lower than the

TABLE 2. CHARACTERISTICS OF EGG YOLK POSITIVE (WIDE ZONE FORMING) AND 'NARROW-ZONE' ORGANISMS ISOLATED FROM FOOD PRODUCTS

Characteristic	Egg yolk positive organisms No. positive/ No. tested	Narrow zone organisms No. positive/ No. tested
Hemolysis	51/51	0/52
Gelatin hydrolysis	51/51	52/52
Starch hydrolysis	29/51	49/52
DNase production	29/51	49/52
Growth at 49 C	15/51	52/52
NO <sub>3</sub> reduction	48/51	47/52
Growth on PEA Agar	42/51	52/52
Grwoth in 7% NaCl	48/51	52/52
Fermentation of glucose	48/51	52/52
Casein hydrolysis	48/51	52/52
AMC production	47/51	52/52
Sensitivity to 201 phage	13/51	0/52
Precipitin reaction with T sera	10/13	4/6

47.8% incidence reported by Nygren (12) after his examination of 3888 samples of food products manufactured in Sweden. This difference could result from a difference in the sensitivity of detection methods used in the two studies. The lower limit of detection by the method employed in this investigation was 100 cells per gram compared to a sensitivity of 1 cell per gram by Nygren's method. The upper limit of contamination in the positive food samples examined in this study is in good agreement with that reported by Mossel et al. (9) and Nygren (12).

This method of detecting *B. cereus*, i.e. surface plating on mannitol-egg yolk-polymyxin agar, employs fermentation of mannitol and production of a precipitate in the agar as the differential principle. Formation of this precipitate is usually attributed to the action of lecithinase produced by the *B. cereus* organism. However, in the absence of a direct test for the presence of phosphorylcholine, the role of lecithinase in the formation of the precipitate cannot be established with certainty. Kushner (7) reported that *B. cereus* produced factors other than lecithinase that would cause turbidity to develop in egg-yolk broth. We have received six cultures of lecithinase-negative *B. cereus* from Dr. H. DeBarjac, and observed that each strain produced a zone of precipitate on the medium of Mossel et al. (9). For this reason, we prefer to designate the wide-zone organisms as egg yolk-positive rather than lecithinase-positive.

Since there are other species of *Bacillus* that are also egg yolk-positive, i.e. *B. cereus* var. *mycoides*, *Bacillus anthracis*, *Bacillus thuringiensis* and *Bacillus laterosporus*, the presence of a zone of precipitate surrounding a colony on the MYP medium is not sufficient evidence to confirm that the colony is *B. cereus*. Reaction of the various colonies on mannitol is most often impossible to ascertain due to masking of non-fermentative colonies by the acid generated by proximal actively fermenting colonies. For these reasons, this method must be considered as a presumptive test and suspicious colonies should be tested further before a precise identification is possible.

These observations led to the assessment of various tests commonly employed to characterize *B. cereus*. Unfortunately, most of the presumably definitive characters were found to be quite variable and not reliable for positive identification. These tests did point up some interesting information. First, none of the egg yolk-positive isolates failed to grow at 37 C. Although the upper temperature limit for growth of *B. cereus* has been reported to be 48 C (2), 15 of 51 strains isolated in this study were able to grow at 49 C. Because of this, and the observation that egg yolk-positivity was readily apparent after 24 hr incubation, we suggest that the time and temperature of incubating the plates be changed from

the 32 C - 42 hr advocated by Mossel et al. (9) to 35-37 C - 24 hr. Such a change would not only accelerate the test but also would lessen the difficulty encountered with the 'narrow-zone' organism that occurs when plates are examined at 42 hr.

The 201 phage reported by Smith et al. (14) to be specific for and lyse all strains of *B. cereus* was inactive against 36 of 51 egg yolk-positive isolates. It would seem that this phage is more strain specific than species specific.

In toto, the biochemical characteristics tested were not adequate either to prove that the egg yolk-positive cultures were *B. cereus*, or that the 'narrow-zone' organisms were not. Differentiation of the 'narrow-zone' organisms from *B. cereus* was accomplished in this study by demonstrating that the width of the vegetative cells of the 'narrow-zone' organisms was never equal to or greater than 0.9  $\mu\text{m}$  (14). These organisms, with two exceptions, were group I bacilli (i.e. sporangia not swollen), and may possibly have been *Bacillus licheniformis* or other common food contaminants.

The precipitin test recommended by Norris and Wolf was evaluated as a confirmatory test for *B. cereus*. Antisera prepared against autoclaved spores of *B. cereus* T failed to provide definitive confirmation of isolates as *B. cereus* for reasons of specificity and sensitivity. Three of the wide-zone organisms failed to react with these sera while four 'narrow-zone' forming organisms showed a positive reaction. Thus it appears that spores of *B. cereus* T do not possess a formamide extractable antigen that is common to all egg yolk-positive *B. cereus* strains and that at least one precipitinogen is shared with species other than *B. cereus*. Recent investigations in this laboratory employing fluorescent staining of exosporium material have indicated that this approach may yield the desired sensitivity and specificity. These data are preliminary in nature and further evaluation is necessary.

It is apparent that contamination of food products by *B. cereus* is not uncommon. The foods examined in this study were by necessity limited in variety and number. It is quite probable that other types of food products, e.g. poultry and meats, have an incidence of contamination greater than reported here for dried foods. This possibility receives considerable support from the data reported by Ormay and Novotny (13) that meat and meat dishes were responsible for 53.8% of the *B. cereus* outbreaks that occurred in Hungary over an 8-year period. It would appear that more work on not only the incidence of *B. cereus* but also some of the properties of the organism and its mechanism of pathogenicity is warranted.

It is also quite probable that the mild nature of *B. cereus* poisoning (symptoms rarely last longer than

12 hr and no fatalities have been reported) has contributed to the lack of attention that study of this type of food poisoning has received. Since *B. cereus* is not shed in large numbers in the feces of affected persons, and because examination of food vehicles for *B. cereus* is not a routine practice, it is not surprising that more outbreaks of this type of food poisoning have not been recorded. Nonetheless, it is not prudent to ignore this organism as a casual agent in food poisoning cases, particularly, when there exists a simple and rapid presumptive detection procedure. It would not be surprising if the number of food poisoning outbreaks listed as 'etiology unknown' were to diminish after the incorporation of quantitative *B. cereus* determinations in the routine analysis of suspect food vehicles by the various investigating agencies.

#### ACKNOWLEDGMENT

This study was funded in part by contributions from the food industry to the Food Research Institute.

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# EFFECT OF MALATHION AND TRICHLORFON ON GROWTH<sup>1</sup> AND MORPHOLOGY OF LACTIC CULTURE ORGANISMS

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## ABSTRACT

Addition of 100 ppm of malathion or trichlorfon had little effect on lactic acid production in reconstituted non-fat dry milk by *Streptococcus diacetilactis* or *Streptococcus thermophilus*.

Variation in cell size of *Streptococcus cremoris* 3, *Streptococcus lactis* W 8 and several strains of *S. thermophilus* occurred following repeated transfers in litmus milk to which 100 ppm of malathion or 500 ppm trichlorfon had been added. The *Lactobacillus bulgaricus* A culture, after 15 serial transfers in litmus milk containing 500 ppm of trichlorfon, exhibited increased frequency of chain formation and cell structure 30 to 50  $\mu$  long with few visible cross walls. A similar effect was observed with *L. bulgaricus* A and *L. bulgaricus* GA after repeated transfers in litmus milk which contained 100 ppm malathion. One of five strains of *L. casei* studied showed similar changes in morphology after repeated transfers in litmus milk which contained 100 ppm malathion. Adding 500 ppm trichlorfon produced a lesser effect. Changes in morphology of this strain of *L. casei* were not permanent since cells resumed their usual appearance following three to five transfers in plain litmus milk.

Research on the influence of insecticides on microorganisms has been concerned primarily with the effect of such residues on soil microorganisms and has shown that, in general, such organisms are not greatly affected (1, 3, 5, 7, 8, 11, 15, 16, 17, 18). Although some concern has been expressed about the effect of insecticides on cultures used to manufacture dairy foods, little research has been published in this area. Kim and Harmon (10) reported that dieldrin and heptachlor, when added to whole, sterile milk in concentrations of <0.01 to 100 parts per million (ppm), had little or no effect on growth or fermentation characteristics of certain strains of *Streptococcus lactis*, *Streptococcus cremoris*, *Streptococcus diacetilactis*, and *Lactobacillus casei*. A similar lack of effect was found when milk containing <0.1 to 100 ppm of methoxychlor or malathion was inoculated with *S. lactis* or *L. casei* and incubated 48 hr at 32 C. Bradley and Li (4) found, however, that dieldrin, in a concentration of 2.15 to 2.37 ppm/g milk fat in cheese milk, reduced acid development by a commercial mixed strain culture during the manufacture of Cheddar cheese. These investigators also reported that *S. lactis*, *S. cremoris*, and *S. diacetilactis* pro-

duced less acid in milk containing dieldrin than in milk free of this insecticide.

Although the organophosphates generally are not found in milk, they can gain access through post milk-ing contamination and both malathion and trichlorfon have been detected in the milk of dairy cows exposed to these insecticides (2, 6, 9).

The purpose of this study was to determine if malathion or trichlorfon affect growth characteristics of some lactic culture organisms used in the dairy industry.

## MATERIALS AND METHODS

The lactic cultures used in this study included *S. cremoris*, *S. diacetilactis*, *S. lactis*, *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, and *L. casei*. Sources of the cultures studied are given in Table 1. All cultures were maintained in plain litmus milk as a control medium. Litmus milk to which 100 ppm malathion<sup>1</sup>, 100 ppm trichlorfon<sup>2</sup> or 500 trichlorfon had been added prior to being heat sterilized at 12 lb/in<sup>2</sup> for 12 min was used as a growth medium. Preliminary experiments indicated that lesser amounts of these two pesticides had no effect on the organisms used. To minimize the effect of heat on these insecticides (12, 14), litmus milk also was prepared and the pesticides added as an ethanol solution to the previously sterilized milk. The final concentration of alcohol was 1%, a concentration Kim and Harmon reported as having no effect on the lactic cultures used in their study (10). Serial transfers of the cultures, using a 1% inoculum, were made at 24 to 48 hr intervals for a total of 25-30 transfers. Control tubes of litmus milk with no added insecticide also were inoculated at each transfer period. Tubes were incubated at an appropriate temperature (32 or 37 C) and, upon coagulation of milk, were stored at 4 C until the subsequent transfer. Gram-stained preparations were made of the initial stock culture and at every fifth subsequent transfer to determine any changes that occurred in morphology.

The effect of malathion and trichlorfon on the rate of acid production by *S. diacetilactis* and *S. thermophilus* in plain, reconstituted, nonfat dry milk (NFDM) also was studied, by determining change in pH. The trichlorfon (10 ppm) was added to the sterile reconstituted NFDM (10% solids). The medium containing malathion was prepared by dissolving the NFDM in water containing 100 ppm malathion, followed by heat sterilization. Reconstituted NFDM, without added insecticides, was used as a control medium. These media were

<sup>1</sup>Journal Paper No. JA438 of the Wyoming Agricultural Experiment Station, Laramie, Wyoming. Project No. 930.

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<sup>1</sup>Liquid malathion, 99.5% pure, from American Cyanamide Company, Agricultural Division, Princeton, New Jersey.

<sup>2</sup>Crystalline trichlorfon, 99% pure, from Chemagro Corporation, Kansas City, Missouri.

TABLE 1. SOURCES OF LACTIC CULTURE STRAINS STUDIED.

Organism	Strain designation	Source
<i>S. cremoris</i>	3	Oregon State University
<i>S. lactis</i>	W 8	Purdue University
<i>S. diacetilactis</i>		Chr. Hansen's Laboratories
<i>S. thermophilus</i>	5	Purdue University
<i>S. thermophilus</i>	B	Chr. Hansen's Laboratories
<i>S. thermophilus</i>	F	Chr. Hansen's Laboratories
<i>S. thermophilus</i>	GH	Chr. Hansen's Laboratories
<i>S. thermophilus</i>	C 3	Oregon State University
<i>S. thermophilus</i>	RS 1	North Carolina State University
<i>L. bulgaricus</i>	A	Chr. Hansen's Laboratories
<i>L. bulgaricus</i>	GA	Oregon State University
<i>L. bulgaricus</i>	B	Purdue University
<i>L. bulgaricus</i>	C	University of Wisconsin
<i>L. casei</i>	A	University of Wisconsin
<i>L. casei</i>	B	Chr. Hansen's Laboratories
<i>L. casei</i>	C	Michigan State University
<i>L. casei</i>	ATCC 334	Oregon State University
<i>L. casei</i>	ATCC 393	Oregon State University

prepared in flasks and inoculated with 1% or 10% active cultures of *S. diacetilactis* or *S. thermophilus* and incubated at 32 and 45 C, respectively. The pH of the contents of each flask was determined at 30 min intervals for the first 10 hr and then a final pH determination was made after 24 hr of incubation.

#### RESULTS AND DISCUSSION

The lactic cultures employed showed a varied response to the presence of organophosphate insecticides depending upon the insecticide and its concentration, the solvent, and the culture strain used.

Rate of acid production in reconstituted milk, as measured by change in pH, by cultures of *S. diacetilactis* or *S. thermophilus* was not materially affected by the presence of either malathion or trichlorfon in the concentrations used. Tables 2 and 3 present data representative of those obtained in the trials conducted.

During the first hours of incubation, acid production occurred at a slower rate following a 1% inoculum than with a 10% inoculum, particularly with *S. diacetilactis*. The difference in pH was less apparent after 10 hr incubation and was negligible, in most instances, after 24 hr incubation. Any reduction in the rate of pH caused by the presence of these two insecticides was most evident with *S. thermophilus* during the period of most rapid acid development, namely from the fourth to the tenth hour of incubation. Malathion, at a level of 100 ppm, prolonged but slightly the time required to coagulate milk by *S. thermophilus* B when a 1% inoculum was used. No consistent increase in coagulation time was observed when a 10% inoculum was used or 100 ppm trichlorfon was added. Because any differences noted in acid production by these two cultures were small and

TABLE 2. EFFECT OF ADDED INSECTICIDE ON CHANGES IN pH IN RECONSTITUTED SKIM MILK<sup>1</sup> INOCULATED WITH *S. diacetilactis*.

Incubation time (hr) at 32 C	Control No insecticide Inoculum level		100 ppm malathion added Inoculum level		100 ppm trichlorfon added Inoculum level	
	1%	10%	1%	10%	1%	10%
	(pH)					
0	6.75	6.30	6.70	6.29	6.70	6.31
1	6.59	6.19	6.51	6.15	6.55	6.15
2	6.51	5.95	6.50	5.99	6.50	5.95
3	6.39	5.65	6.39	5.71	6.39	5.69
4	6.11	5.35	6.15	5.40	6.10	5.35
5	5.85	5.09	5.91	5.11	5.81	5.10
6	5.50	4.85	5.55	4.90	5.50	4.85
7	5.30	4.71	5.40	4.75	5.25	4.71
8	5.00	4.65	5.11	4.65	5.00	4.65
9	4.80	4.61	4.90	4.61	4.79	4.60
10	4.65	4.59	4.75	4.55	4.70	4.60
24	4.30	4.35	4.31	4.35	4.31	4.31

<sup>1</sup>10% solids

TABLE 3. EFFECT OF ADDED INSECTICIDE ON CHANGES IN pH IN RECONSTITUTED SKIM MILK<sup>1</sup> INOCULATED WITH *S. thermophilus* B.

Incubation time (hr) at 45 C	Control No insecticide Inoculum level		100 ppm malathion added Inoculum level		100 ppm trichlorfon added Inoculum level	
	1%	10%	1%	10%	1%	10%
	(pH)					
0	6.55	6.31	6.52	6.22	6.55	6.31
1	6.50	6.29	6.50	6.21	6.50	6.29
2	6.49	6.10	6.49	6.05	6.48	6.10
3	6.25	5.92	6.30	6.00	6.25	5.90
4	6.10	5.62	6.25	5.65	6.05	5.70
5	5.70	5.41	5.85	5.45	5.80	5.50
6	5.49	5.31	5.62	5.38	5.59	5.40
7	5.29	5.15	5.41	5.20	5.32	5.22
8	5.15	5.10	5.29	5.12	5.21	5.15
9	5.01	5.00	5.15	5.01	5.10	5.02
10	4.95	4.95	5.01	5.00	5.05	5.00
24	4.45	4.60	4.65	4.62	4.60	4.60

<sup>1</sup>10% solids

previous investigators (10) found little or no effect of malathion on acid production of lactic cultures; no other cultures were used in this phase of the study.

When grown in the presence of malathion or trichlorfon, *S. diacetilactis* showed little or no consistent changes in morphology or chaining characteristics. As shown in Table 4, twenty-five consecutive transfers in litmus milk which contained 100 ppm malathion or 500 ppm trichlorfon produced no consistent change in the morphology of this culture. No great or consistent changes were noted with the other streptococci studied although variations in cell size occurred.

Following 25 to 30 transfers in litmus milk with

100 ppm added malathion, individual cells of *S. cremoris* 3 averaged  $0.4 - 0.5 \mu$  in diameter, somewhat smaller than cells in the control milk which ranged from  $0.5 - 0.7 \mu$  in diameter. A similar effect on *S. lactis* W 8 was found. The cells, slightly elongated, were  $0.8 \mu$  wide in the control medium but varied from  $0.6 - 0.8 \mu$  wide when grown in the presence of malathion. Trichlorfon produced no morphological changes in *S. cremoris* 3 but was slightly inhibitory to growth after 15 serial transfers. *Streptococcus*

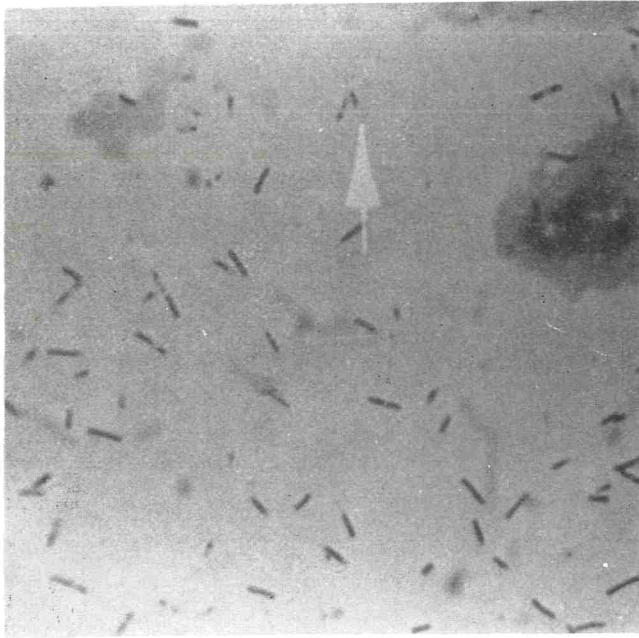


Figure 1. *Lactobacillus casei* A grown in plain litmus milk. (Head of arrow  $10 \mu$  long, shaft  $5 \mu$  long).

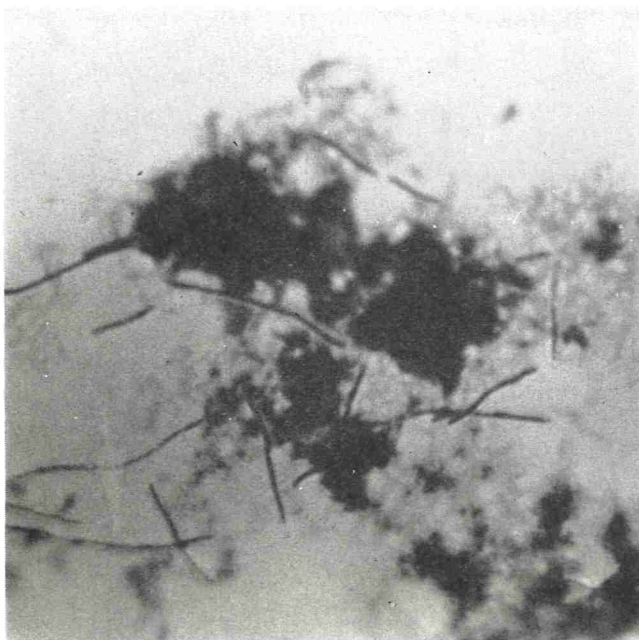


Figure 2. *Lactobacillus casei* A grown for 10 transfers in litmus milk with 100 ppm malathion added before sterilization.

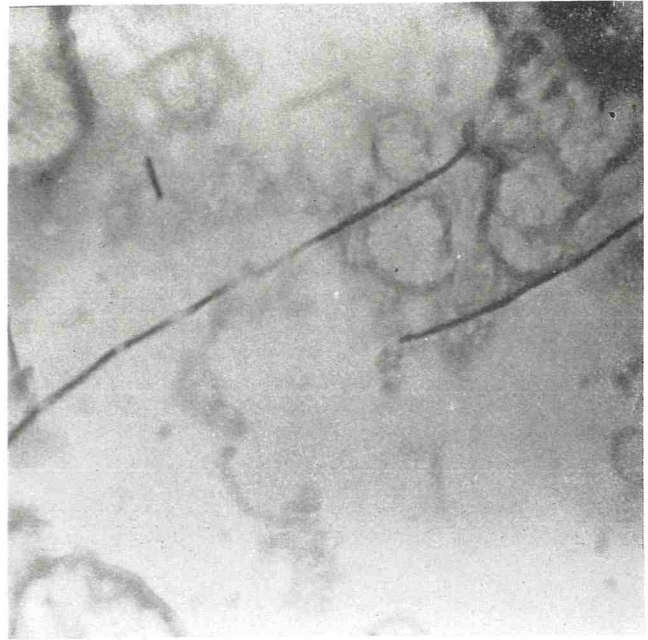


Figure 3. *Lactobacillus casei* A after 30 transfers in litmus milk with 100 ppm malathion added before sterilization.

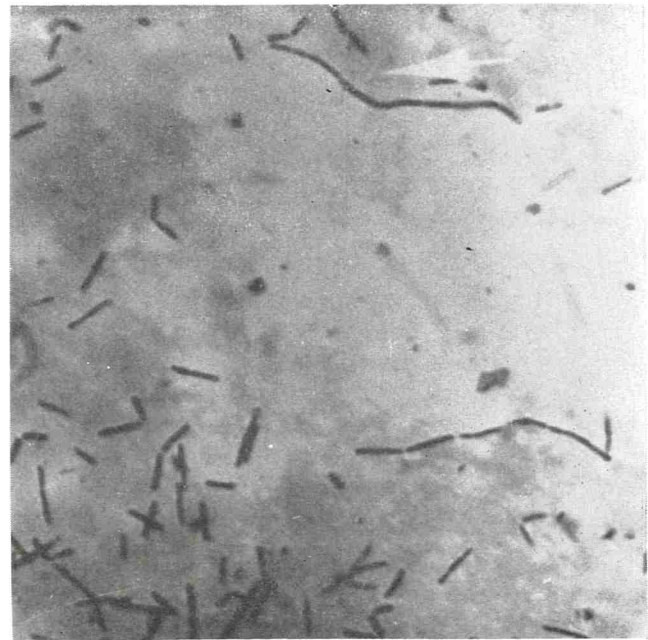


Figure 4. *Lactobacillus casei* A after 25 transfers in litmus milk with 500 ppm trichlorfon added before sterilization.

*lactis* W 8 evidenced more variation in cell size with cells measuring from  $0.6 \mu$  to  $1 \mu$  in width following repeated transfers in litmus milk with added trichlorfon.

As shown in Table 4, small differences in cell size, were observed among the strains of *S. thermophilus* grown in litmus milk containing malathion or trichlorfon. Cells of strains 5, F, and GH appeared smaller after culturing in milk with malathion than when cultured in pesticide-free milk. Cells of strains C 3 and RS 1, under the same conditions, appeared larg-

er in some, though not all, stained preparations. These same strains of *S. thermophilus*, when grown in the presence of trichlorfon, showed, with some exceptions, effects similar to those induced by malathion. Strain B did produce longer chains of cells following the tenth consecutive transfer in litmus milk containing trichlorfon, an effect not noted with malathion. It also was observed that adding these insecticides as an

alcoholic solution to previously sterilized litmus milk rather than as an aqueous solution prior to heat sterilization, did not increase the effect of the insecticide on the morphology of the strains of *S. thermophilus* grown in such litmus milk.

The four strains of *L. bulgaricus* did not undergo similar changes in morphology when grown in litmus containing 100 ppm malathion or 500 ppm trichlorfon added prior to heat sterilization. As indicated

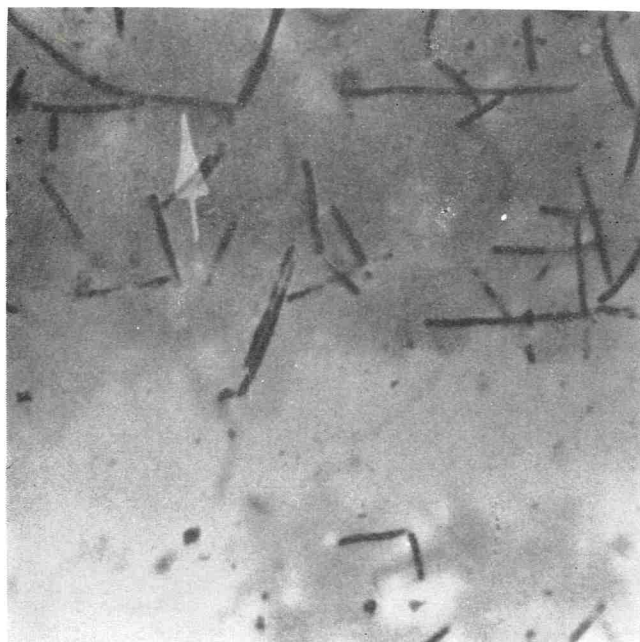


Figure 5. *Lactobacillus casei* A after 15 transfers in litmus milk with 500 ppm trichlorfon added as an alcoholic solution after milk was sterilized.

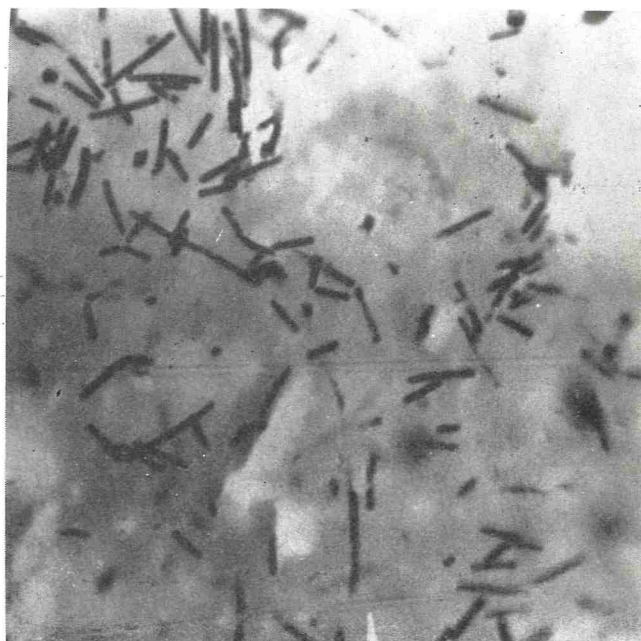


Figure 7. *Lactobacillus casei* A after 15 transfers in litmus milk with 1% ethyl alcohol added after milk was sterilized.

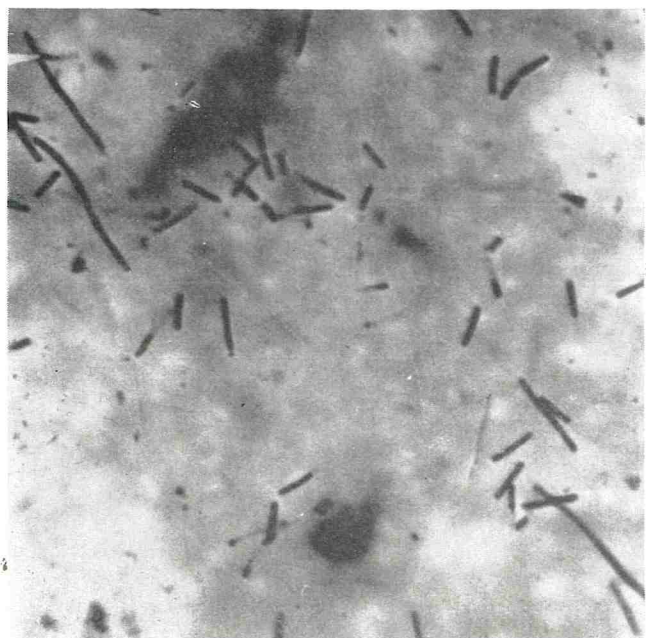


Figure 6. *Lactobacillus casei* A after 15 transfers in litmus milk with 100 ppm malathion added as an alcoholic solution after milk was sterilized.

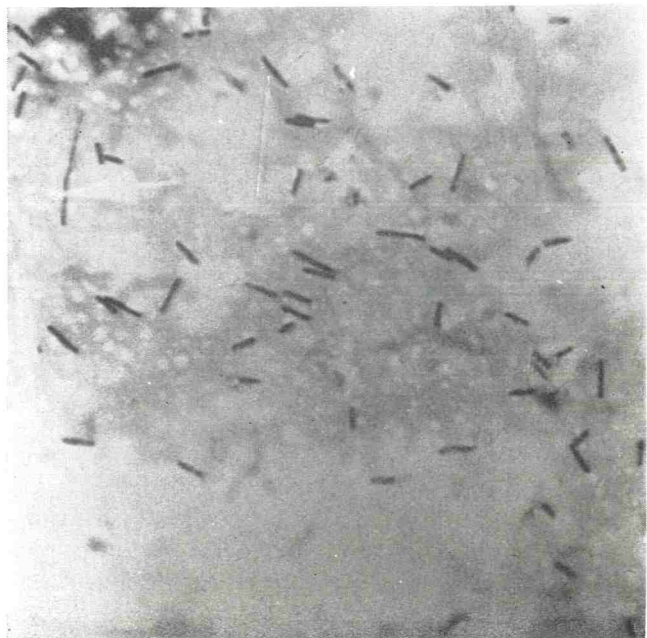


Figure 8. *Lactobacillus casei* A after 3 transfers in plain litmus milk following 30 transfers in litmus milk with 100 ppm malathion added before sterilization.

TABLE 4. EFFECT OF INSECTICIDES MALATHION AND TRICHLORFON ON MORPHOLOGY OF SELECTED LACTIC STREPTOCOCCI GROWN IN LITMUS MILK.

Organism	Strain	Temp. of incubation (C)	No. of serial transfers	Effects noted with insecticide	
				Malathion <sup>1</sup>	Trichlorfon <sup>2</sup>
<i>S. diacetylactis</i>		32	30	No definite effect.	No definite effect.
<i>S. lactis</i>	W 8	32	30	Cells appear smaller at 30th transfer.	Presence of larger cells noted at 25th and 30th transfer.
<i>S. cremoris</i>	3	32	30	Cells appear smaller at 25th and 30th transfer.	No effect. Growth slightly inhibited from 15 - 30th transfer.
	5	37	30	Smaller cells noted from 5th transfer on. Increased chaining seen after 30th transfer.	Variation in cell size observed. Usually smaller cell than in control medium.
	B	37	30	Little consistent effect.	Longer chains of cells observed from 12th transfer on.
	F	37	26	Smaller cells noted from 10th transfer on.	Smaller cells noted in most transfers.
<i>S. thermophilus</i>	GH	37	26	Decreased cell size observed from 5th transfer on. Occasionally long chains of cells seen.	Variation in cell size in the same chain of cells at 20th and 26th transfer.
	C 3	37	25	Increase in cell size noted from 10th to 25th transfer.	Increase in cell size found at 10th to 20th transfers—not seen at 25th transfer.
	RS 1	37	25	Little effect noted until 25th transfer when larger cell size was apparent.	Large cells found at 10th and 15th transfer—no effect noted at 20th and 25th transfer.

<sup>1</sup>100 ppm malathion added to litmus milk prior to heat sterilization.

<sup>2</sup>500 ppm trichlorfon added to litmus milk prior to heat sterilization.

TABLE 5. EFFECT OF INSECTICIDES MALATHION AND TRICHLORFON ON MORPHOLOGY OF SELECTED LACTOBACILLI CULTURES GROWN IN LITMUS MILK.

Organism	Strain	Temp. of incubation (C)	No. of serial transfers	Effects noted with insecticide	
				Malathion <sup>1</sup>	Trichlorfon <sup>2</sup>
<i>L. bulgaricus</i>	A	37	30	Longer chains of cells at 30th transfer.	Inconsistent variation in cell size.
	GA	37	30	Inconsistent variations in cell size.	No definite effect.
	B	37	27	No consistent effect.	No definite effect.
	C	37	25	No definite effect.	No definite effect.
<i>L. casei</i>	A	32	25	Increased numbers of chains of cells. Long chains of cells at 10th and subsequent transfer.	Increased number of chains of cells at 20th and 25th transfer. Less effect than with malathion.
	B	32	20	No definite effect.	No definite effect.
	C	32	25	No definite effect.	No definite effect.
	ATCC 334	32	20	No definite effect.	No definite effect.
	ATCC 393	32	20	No consistent effect.	No consistent effect.

<sup>1</sup>100 ppm malathion added to litmus milk prior to heat sterilization.

<sup>2</sup>500 ppm trichlorfon added to litmus milk prior to heat sterilization.

in Table 5, culturing *L. bulgaricus* A in the litmus milk containing trichlorfon for 15 transfers or more resulted in a greater variation in cell size than was observed in insecticide-free milk. This effect was not noted with the other three strains of *L. bulgaricus*. Cell structures 30 to > 50  $\mu$  long resembling long

chains of cells, but with few or no clearly defined "septae" or cross walls were observed after 30 serial transfers of *L. bulgaricus* A in litmus milk plus malathion. Similar structures, 20 to 30  $\mu$  long, also were seen in the *L. bulgaricus* GA culture following the 15th and 20th serial transfer in this same medium.



Four of the five strains of *L. casei*, namely B, C, ATCC No. 334, and ATCC 393 showed no consistent changes in morphology when grown in litmus milk which contained either 100 ppm malathion or 500 ppm trichlorfon added as aqueous solutions prior to heat sterilization. With these strains the individual cells making up the chains of organisms usually were distinct with the "septae" easily visible.

The greatest effect on morphology was noted with *L. casei* A. In litmus milk used as a control medium this organism occurred primarily as a single cell measuring 0.7 to 0.8  $\mu$  wide and 2.5 to 3  $\mu$  long as shown in Fig. 1. Cells in pairs and a few short chains of cells 10 to 15  $\mu$  were seen. When this culture was grown in litmus milk which contained 100 ppm malathion, an increase in length and incidence of chain formation was noted, as pictured in Fig. 2 and 3. Chains 15 to 30  $\mu$  long were observed by the 5th to 10th transfer and after 15 to 20 transfers chains 30  $\mu$  long or longer with indistinct "septae" or cross walls were found. The greatest change in appearance was represented by "chains" or structures 50 to 100  $\mu$  in length with few "septae" or cross walls visible. Growth of this culture in litmus milk containing 500 ppm trichlorfon resulted in increased chaining, but the effect was less pronounced even after 25 transfers, as shown in Fig. 4, than that observed with malathion.

It has been reported (13) that malathion could be isomerized by heat and the resulting isomalathion was a stronger inhibitor than malathion of human serum and erythrocyte cholinesterase. If an isomer of malathion was produced in the litmus milk prepared by adding malathion prior to heat sterilization either the isomer or the unconverted malathion may have caused the morphological changes which were found, to varying degrees, with some of the strains of lactic cultures used in this study. Malathion and trichlorfon were dissolved in ethyl alcohol and added to the previously sterilized milk used as the growth medium for *L. casei* A. As shown in Fig. 5, the addition of trichlorfon in this manner increased the effect of this insecticide and resulted in increased cell size and longer chains of cells after fewer transfers than when the trichlorfon was added to litmus milk prior to sterilization of the medium. This increased effect was not found with malathion (Fig. 6). The addition of 1% ethyl alcohol to litmus milk to serve as control medium resulted in an increase in cell size of *L. casei* A but had no appreciable influence on chain length (Fig. 7).

Changes observed in morphology and chaining pattern of *L. casei* A were not permanent since three subsequent serial transfers in insecticide-free litmus milk resulted in formation of cells similar in appear-

ance to those of the control culture grown in plain litmus for 25 to 30 transfers (Fig. 8).

It would appear from results of this study that the insecticides malathion and trichlorfon when added to milk in concentrations of 100 ppm and 500 ppm, respectively, had little or no consistent effect on the growth or morphology of the streptococcus cultures employed. Some effect, particularly upon chain formation, on *L. casei* and *L. bulgaricus* was noted. The degree of change in these latter organisms was dependent upon the culture strain and the insecticide used, and the insecticide levels required to induce such changes was far above those previously reported as having been found in milk.

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### ISM ANNOUNCES ITS NEW "SCHOOL OF ENVIRONMENTAL SANITATION MANAGEMENT"

The Institute of Sanitation Management is pleased to announce the development of its School of Environmental Sanitation Management at the University of Illinois, Champaign-Urbana. A bold, new step forward in ISM's continuing education and career development efforts, this four-year program presents an opportunity for managers of environmental sanitation to enter and continue an academic program which has been specifically developed and tailored to improve their managerial and technical skills and knowledge. Conducted by the Bureau of Business Management of the College of Commerce and Business Administration, Division of University Extension, in cooperation with ISM, the School will

run one week each year; the first year's curricula being presented from February 28 to March 5, 1971. The following year (1972), the First and Second Year Program will be presented, with the Third and Fourth Year Program being added in 1973 and 1974 respectively.

Those participants completing the first year's program will then be eligible for the second year; those completing the second year go on to the third, and so on, so that at the end of four years he or she will have had a concentrated academic course in Environmental Sanitation Management—one which will provide much of the essential matter necessary to meet this decade's growing demands on the Work Environment.

### HOW TO DELUDE THE PUBLIC!

An editorial under the title "Non-Science" in the British "Review of Nutrition and Food Science" comments as follows:

"If the public took much notice of the newspapers they might be seriously worried about their food. After several years of doubts, warnings and scares covering almost every aspect of life, cyclamate, certain pesticides and sodium glutamate were withdrawn in rapid succession.

"Legitimate doubts and warnings are one matter but from time to time a scaremonger reaches the headlines through sheer nonsense. For example, a recent report from America stated that yogurt causes cataracts in rats, with the obvious suggestion that it might do the same for man.

"The basis of the story was a piece of sheer nonsense—a 'scientist' had fed rats on nothing but yogurt. Other 'scientists' have damned skim milk powder because when fed to rats as their sole food they died.

"It has often been pointed out that if animals are fed excessive doses of water or even vitamins they, too, will die. Certainly any incomplete diet must, by definition, cause harm.

"The public are not in a position to make a judgment but fortunately they remember little of what they read in the newspapers."

Lest it be thought that it is only lay reporters and broadcasters who are responsible for misleading the public with respect to alleged hazards of food and environmental chemicals, here are some rules that appear to be followed by scientists who are somewhat less than objective in designing and drawing conclusions from animal experiments:

*Rule 1.—Give heroic doses.*

The results of animal tests are applied to man, so play it safe. Determine the effects of massive doses and conclude that ordinary use levels *may* have the same effect in man, only it *may* take longer or *may not* be recognized as causally related.

*Rule 2.—Inject, instead of feed.*

Ignore the factors involved in oral administration, such as rate and extent of absorption, gastrointestinal changes, systemic biotransformations, blood and tissue levels, and the normal excretory routes. Instead pump a large dose under the skin or into the muscle, vein, or abdomen of an animal (one will do). Then conclude that the effect observed *may* occur in women who are—or who *may not* know they are—pregnant.

*Rule 3.—Dose fasting animals.*

To get best results give large doses of the chemical substance to animals on an empty stomach. Forget the fact that in normal use, food chemicals are consumed in food, and generally in only low concentration. (By following this rule, common salt will be found to be more lethal than most additives.)

*Rule 4.—Feed a single food as the total diet.*

Disregard the fact that animals, just like people, need balanced diets with adequate proteins, calories, vitamins, minerals, etc. See how far they can get on only a single food regardless of how nutritionally incomplete it may be. Never mind that the only single complete food that nature has provided is breast milk for infants, and that ordinary bread or milk (or yogurt) will not sustain normal health without added supplementation.

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# MICROBIOLOGY OF POULTRY PRODUCTS<sup>1, 2</sup>

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## ABSTRACT

Changes in poultry processing and marketing over the past few decades have resulted in production of many convenience food items. These products are subjected to much handling and require that strict attention be given to sanitation measures.

Many types of microorganisms are present on poultry products as a result of contamination from feathers, feet, and intestinal contents of the birds. Equipment and personnel on processing lines also contribute to the spread of bacteria. The bacterial flora may be significant in causing spoilage, or may represent a public health hazard unless controlled by proper sanitation and cooking or low temperature. Trends in bacterial numbers during processing vary with different plant practices and the adequacy of plant sanitation; examples of these differences are described. Several investigations are reviewed on microorganisms present on poultry from the farm through the finished product, including retail store practices. Sources and control of salmonellae and other potential pathogens are discussed.

Per capita consumption of poultry meat in the United States is probably greater than in any other country. Research has produced great improvements in breeding, nutrition, disease control, management, and processing. Within the past 35 to 40 years, the result has been an increase in consumption from about 12 lb. of poultry meat per capita per year to more than 40 lb. per capita. The broiler industry, which produces meat in about 8 weeks, has experienced tremendous growth, but turkey production also has had a marked increase. Along with these advances in production, poultry processing into various types of consumer products has followed similar trends. Large scale commercial processing of poultry has demanded that greater attention be given to plant sanitation and wholesomeness of the products. If we compare the poultry processing industry today with that of a few decades ago, we note that many rather drastic changes have occurred. Formerly, poultry was shipped to consuming areas as "New York dressed," with only the blood and feathers removed, and the head, feet, and viscera remaining with the carcass (19). Today, carcasses are eviscerated, cut up,

packaged, and, in some instances, made into further processed convenience items. Poultry rolls, roasts, logs, steaks, and segments receive considerable handling. Cooked boned poultry meat may present an additional source of contamination if treated improperly during its production. Products such as frozen pies, poultry stuffing, and salads all are potential sources of excessive microorganisms. The greater the amount of handling and processing, the greater the possibility of contamination with pathogens and spoilage organisms. Hence, the increased emphasis on control by adequate sanitation procedures in processing plants. Processing methods, including actual line operations and cooking, as well as freezing and storage conditions, have been the subjects of many investigations by research workers and examination by the federal government (2).

## TYPES OF BACTERIA ON PROCESSED POULTRY

Several surveys have been made of microorganisms in processing plants, and on processed poultry at the plant and retail levels. These investigations have provided much worthwhile information on numbers and types of organisms. Table 1 lists types of bacteria that have been isolated from processed poultry by various workers. There are 24 different genera given here and probably more could be listed on further

TABLE 1. GENERA OF BACTERIA ISOLATED FROM POULTRY

Genus	Investi- gators	Genus	Investi- gators
<i>Pseudomonas</i>	1, 2, 3	<i>Flavobacterium</i>	1, 2
<i>Alcaligenes</i>		<i>Paracolibacterium</i>	
<i>Achromobacter</i>		<i>Microbacterium</i>	
<i>Escherichia</i>		<i>Streptomyces</i>	
<i>Aerobacter</i>		<i>Haemophilus</i>	1
<i>Proteus</i>		<i>Gaffkya</i>	
<i>Salmonella</i>		<i>Neisseria</i>	2, 3
<i>Micrococcus</i>		<i>Actinomyces</i>	
<i>Staphylococcus</i>		<i>Streptococcus</i>	
<i>Sarcina</i>		3	<i>Brevibacterium</i>
<i>Corynebacterium</i>			<i>Arthrobacter</i>
<i>Bacillus</i>			<i>Lactobacillus</i>

<sup>1</sup>Journal Paper No. J-6665 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Project 1749. Center for Agriculture and Economic Development, cooperating.

<sup>2</sup>Presented at the 57th Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, Cedar Rapids, Iowa, August 17-20, 1970.

<sup>1</sup>Gunderson, M. F., K. D. Rose, and M. J. Henn. (13)

<sup>2</sup>Ayres, J. C., W. S. Ogilvy, and G. W. Stewart. (3)

<sup>3</sup>Salzer, R. H., A. A. Kraft, and J. C. Ayres. (21)

examination of the literature. The significance of many of these types regarding public health or spoilage is known; no one can argue that the genus *Salmonella* has not been in the limelight as a pathogen commonly found on poultry products. More recently, we've been hearing about *Clostridium perfringens*, one of the organisms that doesn't appear on the earlier list of Table 1, probably because most investigators did not look for anaerobes.

Microorganisms on poultry products may be classified as pathogenic, or at least potentially pathogenic, and non-pathogenic. Among non-pathogens, organisms of importance are those that cause spoilage. In the list of genera given in Table 1, *Pseudomonas* is probably the most significant of the gram-negative non-spore forming rods associated with spoilage of poultry meat (1). At the time when spoilage becomes evident by off-odor and slime formation, these bacteria also may have produced a green fluorescent pigment visible under ultraviolet light. Their biochemical activity also is demonstrated by changes in proteins and fats which lead to undesirable odors.

The significance of numbers and types of the various organisms in relation to poultry products will be discussed in greater detail, but there are a few points that should first be recognized regarding public health hazard and sanitary quality. The list of genera applies only to bacteria from poultry that was not processed beyond the carcass or cut-up stage. Certainly, the flora might be expected to change with heat, freezing, drying, certain packaging methods, or with preparation of further-processed products. So it is important to know the type or stage of treatment of the product in attempting to interpret microbiological results; this of course applies to all food products. Further, in considering the microbiology of poultry products, as these items finally reach the consumer, attention should not be focused on the processing plant alone. More significance should be placed on production methods on the farm, retail store handling of products, and the ultimate use in the consumers' kitchen.

#### BACTERIAL LOADS DURING PROCESSING

When we do consider bacterial numbers on poultry during processing, we may encounter appreciable variation in total counts. Gunderson et al. (12) reported loads of about 4800 per cm<sup>2</sup> on freshly killed, warm eviscerated chicken, and up to 60,000 per cm<sup>2</sup> for ice-chilled birds eviscerated when cold. In a survey conducted in Iowa, Walker and Ayres (25) found about 1500 organisms per cm<sup>2</sup> on the skin of live birds, and an increase to about 35,000 per cm<sup>2</sup> on carcasses immediately after processing. The range of counts was 20,000 to 160,000 at the latter stage. This gen-

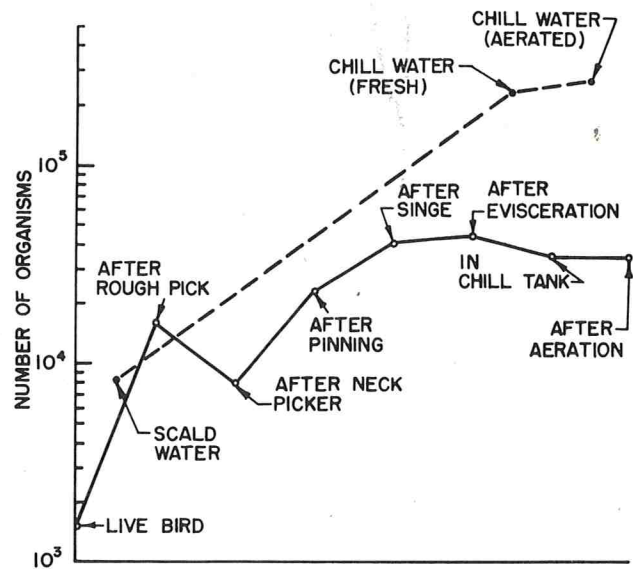


Figure 1. Numbers of bacteria on the skin of chickens during processing and in scald and chill waters (Walker and Ayres, 25).

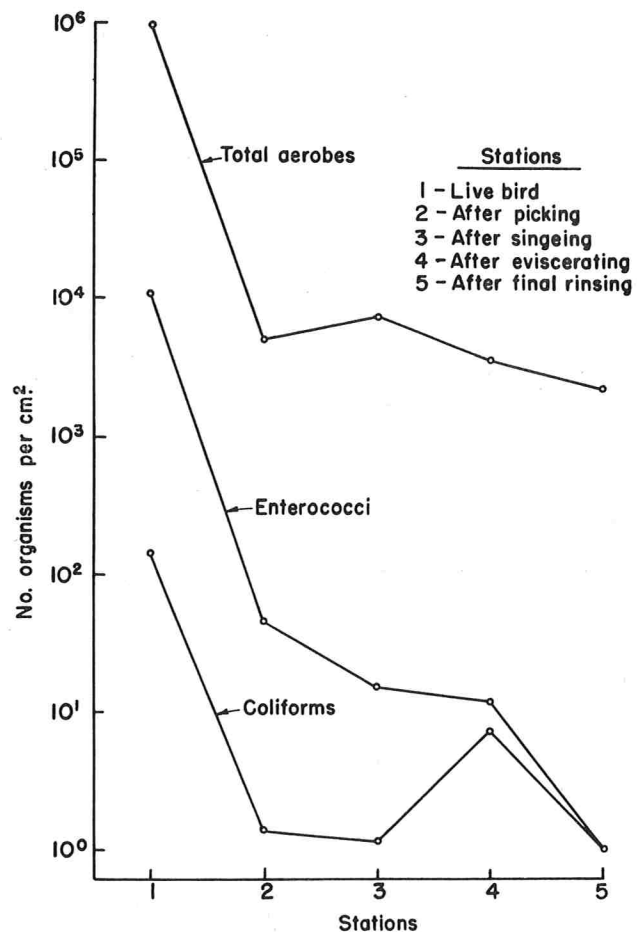


Figure 2. Counts of bacteria from the skin of turkeys during processing (Wilkerson et al., 26).

TABLE 2. RECOVERIES OF SALMONELLAE FROM FARM SOURCES<sup>1</sup>

Source	No. of samples	No. positive	% positive
Feed on 59 farms	97	9	9.3
Feed ingredients	9	1	11.1
Drinking water in troughs	29	12	41.5
Droppings on farms	34	23	67.6
Droppings in coops on trucks	34	13	38.2

<sup>1</sup>From Bryan et al. (4)

eral increase in bacterial numbers during processing may be noted in Fig. 1. The trend in counts differed from that of other workers who observed reductions during processing operations (9, 12, 26). However, these differences may be related to efficiency of sanitation measures in the plants. For example, the results of Wilkerson et al. (26), shown in Fig. 2, indicate decreases in numbers of total aerobes, enterococci, and coliforms from the live bird to the carcass after rinsing. These data were gathered from only one turkey processing plant. The weather also played a role in loads of organisms on the live bird. Total numbers of organisms were higher during rainy or snowy weather than during dry days. In more recent work (6), plant differences again were pointed out. Chilled eviscerated turkey carcasses in one plant yielded counts of about 500 to 900,000 total aerobes per cm<sup>2</sup>, whereas in another plant the range was approximately 400 to 11,000,000.

#### EFFECT OF PLANT PRACTICES ON BACTERIAL NUMBERS

Aside from differences in plant sanitation, variations in processing procedures among plants may cause differences in bacterial loads on the carcasses. Kotula et al. (16) observed that numbers of bacteria on chickens and in the chill tanks increased during chilling for 6 hr in standard chill tanks with no agitation. When a low-agitation continuous chiller was used, counts changed very little before and after chilling, but with a tumble-type, higher-agitation continuous chiller, counts decreased significantly. The manner in which the tumble type chiller was operated

also made use of the greatest amount of fresh chilling medium, thus resulting in less contamination of carcasses. A greater dilution effect then resulted in decreased bacterial numbers.

With turkey giblets, Salzer et al. (21) found that the amount and rate of change of wash water influenced bacterial loads on the giblets. Washing decreased numbers of total aerobes and indicator bacteria at least ten-fold. Results for total aerobic counts expressed as percentage of samples having different bacterial loads are shown in Fig. 3. In all four plants surveyed, washing caused a reduction of counts from the range of 10-1,000 organisms per cm<sup>2</sup> of giblet surfaces down to 0-100 for the majority of samples. Beneficial effects of addition of water at several stages in processing are rather strikingly shown by a comparison of bacterial levels in Plants B and C. Both plants used flume-conveying of giblets from the evisceration station to the packaging location. The difference in operation was that in Plant B, water was introduced and drained at three points on the flume, whereas in Plant C, only one change in water was employed. It is significant that when three changes of water were used, none of the giblets had counts greater than 100 organisms, but with only one addition of water, many samples still had bacterial numbers in the range of 100 to 10,000 per cm<sup>2</sup>. Similar effects are documented by other studies. For example, Galton et al. (11) made only one isolation of *Salmonella* from tables or trays containing edible viscera when running water was available at the tables, but 50 isolations were made in plants where the wash water was changed only when pans containing the edible viscera were filled. In the study of Salzer et al. (21), coagulase positive staphylococci were present on 62 of 360 livers before washing, or 17%, and on only three after washing, or less than 1%. When 240 giblets were sampled for *Salmonella*, 11 recoveries were made from unwashed livers, and only two from washed livers. Undoubtedly, several other investigations of a similar nature could be cited, but the point of all this discussion relates to adequate sani-

TABLE 3. ISOLATIONS OF SALMONELLAE AND STAPHYLOCOCCI DURING PROCESSING OF TURKEYS

Processing stage	Salmonellae <sup>1</sup>			Staphylococci <sup>2</sup>		
	No. of samples	No. positive	% positive	No. of samples	No. positive	% positive
After picking	46	29	63	24	11	46
After washing	33	6	18	—	—	—
After evisceration, washing, and spin-chilling	50	5	10	12	3	25
After overnight chilling	58	10	17	20	10	50

<sup>1</sup>From Bryan et al. (4)

<sup>2</sup>From da Silva (8)

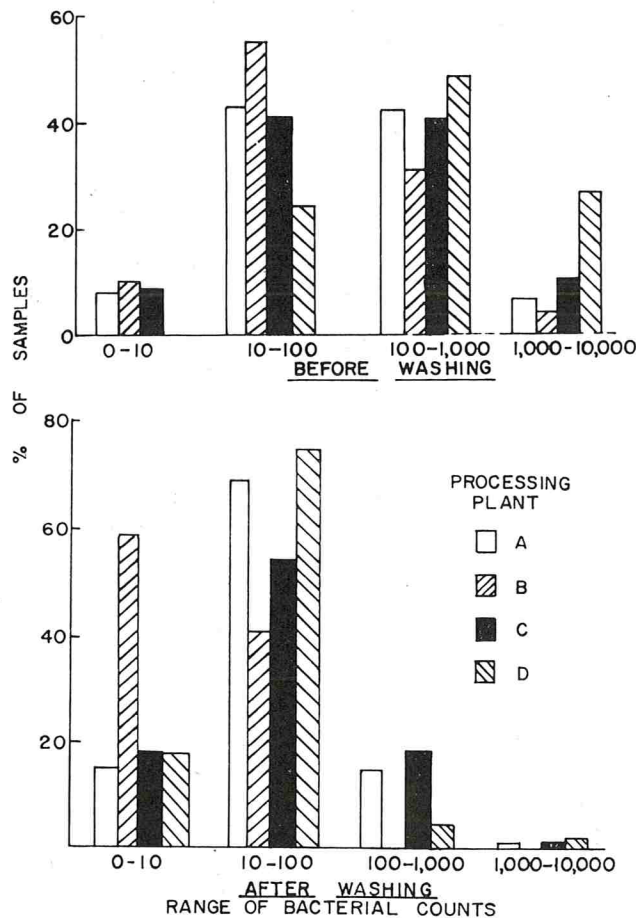


Figure 3. Incidence of total aerobic bacteria on turkey giblets from four processing plants (Salzer et al., 21).

tation and treatment of product during processing in order to reduce potential health hazard and retard onset of spoilage.

#### SOURCES OF BACTERIA ON POULTRY MEAT

Sources of contamination of poultry meat are the soil and filth on feathers and feet of live birds, since the predominant organisms are similar to those found in soil and water, and intestinal contents of the birds (25). Other environmental influences affect bacterial loads on poultry meat during processing operations. Organisms which cause spoilage or food-borne disease, are either present at the time of slaughter or are introduced to the product during processing by equipment, cutting tools, plant personnel, or possibly air and water (2) contaminated with bacteria from some of the other sources mentioned. With this in mind, it is not difficult to understand that further processed products with excessive handling are liable to also become "further contaminated."

Before going on to further processed poultry products, it may be desirable to review some work on salmonellae and staphylococci, by expanding on the

sources contributing to their presence on the products. Both types of bacteria are very important in public health; the salmonellae situation with regard to poultry has been reviewed and emphasized time and time again. In 1969, staphylococci were causative agents of about 25% of the 370 outbreaks of all food borne diseases, including the Chinese restaurant syndrome. This was an increase of about 1% from 1968. Salmonellae were responsible for approximately 13% of the outbreaks in 1969, and about 12.5% in 1968. Of the total number of food borne disease outbreaks in 1969, salmonellosis from turkeys and chickens accounted for 4.5%, and staphylococcus food poisoning outbreaks from these poultry items somewhat less than 5% (24). These values represent a relatively small proportion of the total. Nevertheless, when one considers the many different types of foods available, these percentages assume greater significance. It can be calculated that poultry meat was responsible for about one-third of the salmonellosis outbreaks, and about one-fifth of the staphylococcus outbreaks.

In a survey of nine federally inspected chicken processing plants, Surkiewicz et al. (22) recovered salmonellae from about 20% of the carcasses after evisceration. However, *Escherichia coli* was isolated from every carcass sampled, possibly pointing up the over-efficiency of this organism as an indicator of contamination by salmonellae.

Going on with a study conducted by Bryan et al. (4) to determine sources of salmonellae on turkey products, examinations were made of the farms supplying the birds, including feed and trough water, turkeys and their droppings, beds of delivery trucks, and turkeys and equipment during processing. Recoveries of salmonellae isolated from farm environments are shown in Table 2. When 97 samples of feed were taken from 59 farms supplying turkeys to the processing plant, salmonellae were found in 9 samples of pre-mixed feed which came from 6 different mills. Drinking water in troughs on ranges and in brooder houses was positive for 12 of 29 samples, and droppings in and around the troughs and feeders showed 23 positive samples from 34 flocks. Samples of fresh droppings taken from delivery trucks harbored salmonellae from 13 of the 34 flocks. On autopsy, 10 of 20 dead turkeys collected from the farms also had salmonellae present in their

TABLE 4. SALMONELLAE ON CARCASSES AND RAW TURKEY PRODUCTS EXAMINED IN TWO PROCESSING PLANTS<sup>1</sup>

Product	No. of samples	positive No.	% positive
Carcasses	208	24	11.5
Partially processed meat	49	8	16.3
Finished raw products	336	90	26.8

<sup>1</sup>From Bryan et al. (5)

viscera.

Table 3 shows trends in isolations of salmonellae and staphylococci during processing; the latter group of organisms were determined in another survey by da Silva (8). These results are for turkeys after picking, up to the packaging operation, and obviously do not include all processing steps to that point. The greatest incidence of salmonellae occurred after feather removal (63% positive); recoveries of salmonellae decreased after washing (18%), again showing the value of water. A further decrease to 10% occurred after evisceration, more washing, and spin chilling. After overnight chilling in tanks, the incidence increased to about 17%. Occurrence of staphylococci showed similar trends but these organisms were generally present in a greater percentage of samples than were salmonellae. Chilling overnight in tanks increased incidence of staphylococci on carcasses.

Type of picking equipment was important in contamination of carcasses with salmonellae and staphylococci. Spiral automatic pickers evidently spread such contamination to a greater extent than other kinds of defeathering equipment. As many as 12 of 14 samplings (86%) taken from the spiral picker were contaminated with salmonellae in this plant; clean-up operations decreased the incidence to 2 of 12 samples (16.5%). When samples were taken of evisceration equipment, about 30% were positive for salmonellae and 50% for staphylococci. Much of the contamination with staphylococci, in particular, was associated with the hands of workers.

#### FURTHER PROCESSED PRODUCTS

Further processed poultry products are, as the term implies, items of poultry meat processed beyond the eviscerated carcass stage. These products may be as simple as "segments," which are merely pieces cut from the carcass, or may consist of boned meat fabricated into any of several other forms. Bryan et al. (5) listed some turkey products as follows: cooked, ready-to-eat rolls; uncooked, frozen rolls; logs; roasts; and steaks or patties. The processing plant work described earlier also included further processed turkey products, and the investigation involved 48 visits to 2 processing plants over a period of 1.5 years. Tests for salmonellae were made on eviscerated chilled carcasses or carcasses that had been previously frozen and then thawed before use, line samples of meat at various stages of processing, uncooked finished products such as rolls and roasts, and equipment in contact with the meat. Results for frequency of salmonellae isolations in both plants are expressed as percentages of samples tested (Fig. 4). In all instances, more recoveries were made when meat used for preparing the products came from freshly

killed turkeys after overnight chilling than from frozen carcasses. These differences, although not statistically significant, are still important in considering the use of fresh vs. frozen turkeys for further processing. Statistics do not necessarily cause food borne disease, but salmonellae do. Not only were the intermediate samples or the final uncooked product more often contaminated with salmonellae, but the processing equipment was also. Certain items of equipment in further processing operations provided relatively high incidence of salmonellae and staphylococci; these included work tables, scales, conveyors, knives, cutting boards, and pans.

Data showing differences between carcasses and meat during processing to finished products are given in Table 4. The greater number of recoveries of salmonellae from finished uncooked products was statistically significant at the 1% level compared with carcasses. Although differences in finished products between the two plants were also significant, for purposes of comparison of carcasses and the products, the data have been combined here.

Salmonellae and staphylococci were present in raw turkey products, but these organisms were not re-

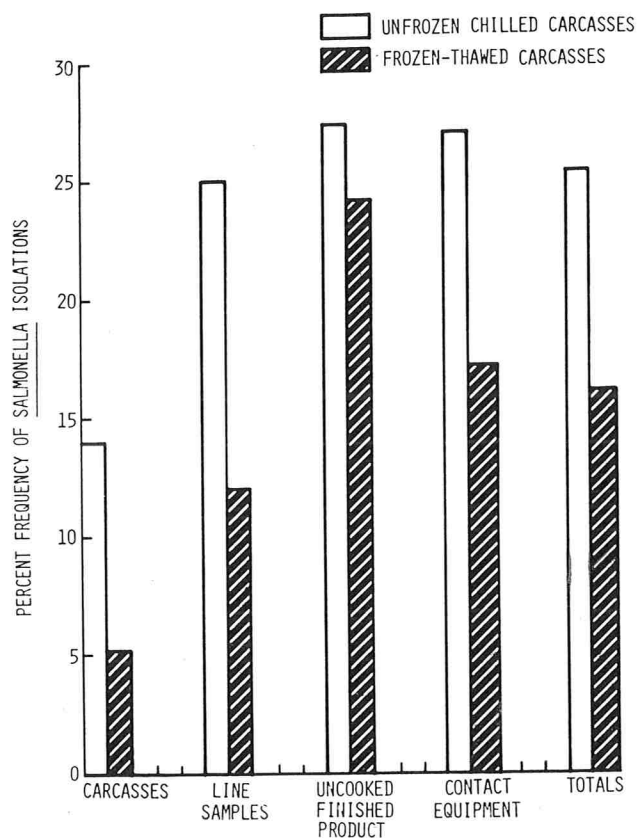


Figure 4. Incidence of salmonellae on turkey meat from frozen or chilled carcasses and from processing equipment (data from Bryan et al., 5).

covered from commercially cooked turkey rolls. Commercial cooking operations, as practiced in the plants surveyed, were adequate to destroy the potential pathogens. According to regulations of the U. S. Department of Agriculture (23), cured and smoked poultry rolls must be heated to an internal temperature of at least 68.3 C (155 F) and all other poultry rolls to at least 71.1 C (160 F). The findings that there is little likelihood of survival of these bacteria at such temperatures should be emphasized, and cooking procedures must be adequate to insure destruction. Presence of salmonellae after such heat treatment must logically indicate post-cooking contamination. However, work reported by Kinner et al. (15) indicates that temperatures as high as 82 C (180 F) may be necessary for destruction of coliforms and enterococci in the juice-spice mixtures used to manufacture Eastern-type turkey rolls. Later work reported by Mercuri et al. (18) showed that no salmonellae were recovered from cooked Eastern-type turkey rolls during storage for 30 days at 5 C. Bryan et al. (6) found no salmonellae on Western-type rolls cooked in a water bath in one plant at 74 C (165 F) for 5.5 hr, or in another plant at 85 C (185 F) for 4.5 hr. In earlier investigations, other workers concluded that temperatures of 71.1 C (160 F) or 73.9 C (165 F) were adequate, or at least minimum for safety of products such as roast turkey (10), stuffing (7, 20), and turkey rolls (27).

From the work that has been reported, it is evident that salmonellae and staphylococci may be spread throughout processing operations, but that adequate heating will serve to destroy these organisms. It is possible that in the future, more attention will be given to cooking a greater variety of products in the plant, rather than depending on low temperature alone to control these pathogens.

#### CLOSTRIDIUM PERFRINGENS

An organism that was mentioned earlier as becoming more prominent in food-borne disease is *C. perfringens*. This organism accounted for about 17.5% of the outbreaks and 65% of the patients in 1969. As far as number of victims is concerned, *C. perfringens* was a very potent causative agent. However, of a total of 72 outbreaks of *C. perfringens* food poisoning, only 20 were associated with turkeys and chicken. Most of the outbreaks were traced to beef. Hall and Angelotti (14) sampled frying chickens obtained from retail stores in the Cincinnati area. About 58% of 26 samples of chicken examined yielded *C. perfringens*. All five samples of chicken liver were positive. As the authors pointed out, a high incidence of contamination of the raw product leads to strong possi-

bilities of contamination after the product is cooked if the same work areas are used in preparation.

#### RETAIL STORE SURVEYS

Because of the proximity of one of our federal public health organizations, Cincinnati was also the subject of an earlier investigation by Wilson et al. (28) for salmonellae in retail poultry products. They found an incidence of 17% (88 positives of 525 samples) from poultry in various retail outlets.

A study was conducted by May (17) to determine the level of "gross bacterial contamination" of chicken during cutting at processing plants compared with retail stores. As may be expected, bacterial numbers varied widely on the carcasses, and increased during cutting operations. Initial contamination and amount of handling during removal of parts contributed to differences in numbers. Counts increased approximately 6-fold in the six plants studied, and about 8-fold in the five retail stores during cutting and packaging. Most of the contamination was attributed to work surfaces and manual contact. Surfaces of meat blocks in stores were the greatest source of contamination. In one store where the block was scrubbed daily with a brush and detergent, counts were considerably lower than in other stores where only a wire brush was used. Similarly, in one plant where a spray washer was used on the conveyor carrying the parts to the packaging area, bacterial numbers were considerably lower than those in plants where spray washing of the belt was not performed. Information such as this, of the common sense variety, should be used to advantage in the industry at all phases of operations.

#### CONCLUSIONS

Although this review is by no means complete, an attempt has been made to present the microbiology of poultry products particularly with regard to the modern industry and with an eye on future trends toward more convenience-type products. Commercial practices must of necessity have continued stringent surveillance by the processor, since his operation is at present the most feasible to control. However, the other ends of the marketing chain also may be subject to closer scrutiny. Farm production methods, transportation of products, retail store practices, institution management, and consumer education are all areas in need of attention to insure wholesomeness of poultry products.

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# GROWTH AND ACTIVITY OF LACTIC-ACID BACTERIA IN SOYMILK

## I. GROWTH AND ACID PRODUCTION<sup>1</sup>

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(Received for publication July 17, 1970)

### ABSTRACT

Soy milk with a protein content similar to that of cow's milk was prepared from soybeans (variety Chippewa 64). Soybeans were washed, soaked until 1 ml of water per gram of beans was absorbed, comminuted with water equivalent to 7.6 times their dry weight, and the mixture filtered through cheese cloth to obtain an aqueous extract free of large particles. Growth rates of 13 species of lactic-acid bacteria in sterile soy milk were generally greater than or comparable to those in cow's milk or Elliker's broth.

Acid production in soy milk was not always directly related to growth rates of the organisms. Substantial formation of acid was limited to those bacteria able to utilize the sugars in soy milk, e.g., *Streptococcus thermophilus*, *Lactobacillus delbrueckii*, *Lactobacillus pentosus*, and *Leuconostoc mesenteroides*. Sources of readily available nitrogen (e.g., protein digests), when added to soy milk, enhanced acid production by *S. thermophilus*, the *Leuconostoc* species, and *L. pentosus*; appeared inhibitory to *L. delbrueckii*; and had no apparent effect on the other test cultures. Addition of whey powder, glucose, or lactose to soy milk enhanced acid production by *Streptococcus lactis*, *Streptococcus cremoris*, *Streptococcus diacetylactis*, *Lactobacillus casei*, and *Lactobacillus helveticus*; whereas addition of sucrose was without benefit. The presence of 0.23-0.25% titratable acid, corresponding to a pH of 5.7, caused coagulation of the sterilized soy milk.

Utilization of soybeans offers potential opportunities for alleviating present and imminent worldwide shortages of food. The soybean outranks any other natural source in its plentiful and inexpensive supply of calories and protein (1, 8, 12). The nutritive quality of soybean protein is the best of those available from plant sources and is inferior to animal protein only because it is deficient in sulfur-containing amino acids (10).

Whole unmodified soybeans are relatively indigestible and unacceptable as food (29). However, other than use of certain molds, relatively little attention has been devoted to employing fermentation techniques to produce acceptable foods from soybeans. Use of lactic acid bacteria to prepare high-protein foods has possibilities.

Kellogg (24) was among the first to use a lactic culture in soy milk. He inoculated sterilized soy milk with *Lactobacillus acidophilus* to produce a buttermilk-like product. Gehrke and Weiser (17, 18) studied growth and activity of *Streptococcus lactis*, *Leuconostoc citrovorum*, and *Leuconostoc dextranicum* in soy milk. More recently patents have been issued on processes for preparing yogurt and cheese from soy milk (6, 25).

Preparation of cheese-like products from soy milk using *Streptococcus thermophilus* as a starter culture was described by Hang and Jackson (19, 20). The suitability of *S. thermophilus* for acid production in soy milk has been confirmed by Matsuoka et al. (27). Recently Yamanaka et al. (31) described a process for preparing a sour milk beverage from a mixture of amino acids, soybean protein, cow's milk, and a starter composed of *S. thermophilus* and *Lactobacillus bulgaricus*.

In spite of these attempts to produce fermented foods with lactic acid bacteria, there is no published information on the behavior of these bacteria in soy milk. Consequently a study was undertaken to determine (a) growth of and acid production by some lactic acid bacteria in soy milk, (b) suitability of soy milk subjected to various heat treatments as a culture medium for lactic acid bacteria, (c) ability of some lactic acid bacteria to hydrolyze soybean lipid, and (d) ability of some lactic acid bacteria to hydrolyze soybean protein. Growth and acid production will be considered in this report and subsequent papers will discuss other phases of this investigation (3, 4, 5). Preliminary reports on some of this work have been presented (15, 16).

### MATERIALS AND METHODS

#### Cultures

Thirteen species of lactic acid bacteria, listed in Table 1, were used for this study. Stock cultures were grown and maintained in agar slabs of a medium consisting of 0.25% (w/v) glucose, 0.50% (w/v) yeast extract, 0.01-0.05% (w/v)  $K_2HPO_4$ , and 1.5% (w/v) agar. Cultures to be used for experiments were grown in sterile litmus milk and were then stored at 5 C between semimonthly transfers. Each culture

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

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TABLE 1. SPECIES, SOURCES, AND INCUBATION TEMPERATURE OF THE LACTIC-ACID BACTERIA USED IN THESE EXPERIMENTS.

Species	Sources	Incubation temperature (°C)
<i>Streptococcus lactis</i> II	Dr. G. W. Reinbold	30
<i>Streptococcus cremoris</i> 40-990	Moseley Laboratories	30
<i>Streptococcus thermophilus</i> Mc	Dr. N. F. Olson	45
<i>Streptococcus diacetylactis</i> 8-6264	Chr. Hansen's Lab.	30
<i>Leuconostoc mesenteroides</i> 512F	NRRL <sup>1</sup>	30
<i>Leuconostoc citrovorum</i> Da3	Marschall Laboratory	30
<i>Pediococcus cerevistae</i> 1325	NRRL	30
<i>Lactobacillus delbrueckii</i> Ld. <sub>3</sub>	Dr. Elizabeth McCoy	37
<i>Lactobacillus casei</i> 1445	NRRL	30
<i>Lactobacillus helveticus</i> 1842	NRRL	37
<i>Lactobacillus fermenti</i> 42-7	Dr. Elizabeth McCoy	37
<i>Lactobacillus pentosus</i> 124-2	Dr. Elizabeth McCoy	30
<i>Lactobacillus brevis</i> 1834	NRRL	30

<sup>1</sup>Northern Regional Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, Peoria, Illinois.

was regularly examined microscopically for purity.

Before a test, the culture was transferred from litmus milk into the experimental medium. It was then transferred in the same medium at least twice at daily intervals. A 12-14-hr-old culture resulting from the last transfer was used in each experiment. An inoculum of 1% (v/v) was used in all tests.

#### Preparation of soymilk

Dry, mature, whole soybeans (variety Chippewa 64) were thoroughly washed and then soaked in distilled water for 6-8 hr at 5 C, until absorbed water was 1 ml per gram of dry soybeans. A ratio of one part of such soaked beans to 7.6 parts of water resulted in a medium with a protein content approximately equal to that of cow's milk.

The soybean-water mixture was comminuted in a Waring blender for 3 min, filtered twice through 3 thicknesses of cheesecloth, and the residue was discarded. The resultant soymilk was dispensed into containers and autoclaved for 15 min at 120 C.

A proximate analysis of the autoclaved soymilk was made (Table 2). Protein content was determined by a semimicro Kjeldahl method (30); the nitrogen-to-protein factor used was 5.71 (22). Fat and moisture contents were measured by Mojonnier methods (28) and ash by AOAC procedures (7).

#### Growth and acid production in soymilk

Two hundred milliliters of soymilk in a 250-ml screw-cap Erlenmeyer flask were inoculated with 2 ml of a 12-14-hr-old culture prepared as described above. The inoculated medium was incubated quiescently at the temperature optimum for the species used (Table 1).

At the time of inoculation (0 hr) and at selected time intervals extending over a 16- or 24-hr period, two samples of the growth medium were aseptically withdrawn from the flask. One was used for estimation of growth and the other for measurement of acidity.

Growth was estimated from viable cell counts (2) using Elliker's lactic agar (13). Duplicate plates were incubated up to 48 hr at the optimum temperature of the species under study. Viable cell counts per milliliter of medium were plotted on semilogarithm paper against time in hours. Slopes of the curves during the exponential phase were calculated. Generation times were determined from such slope values (26).

Acid development was measured by titration of 1-g samples

with 0.02N NaOH using phenolphthalein as the indicator. Changes in pH were followed with a Beckman pH meter (model H2) equipped with a glass-reference combination electrode. Measurements were made on 10-ml portions which had been stored in screw-cap tubes at -40 C immediately after withdrawal from the culture flask. These samples were subsequently thawed at 60 C for 15 min and then cooled to 20-25 C before testing for pH and acidity.

All experiments were duplicated and average values are reported.

#### Comparative growth and acid production in cow's milk and broth

Growth and acid production in low-fat milk and in an enriched broth were studied concurrently with tests using soymilk. Low-fat milk was prepared by mixing raw whole (3.7% fat) and raw skim (0.09% fat) milks in proportions which gave a final fat content of 1.8-1.9%. This was the amount of fat found in the soymilk prepared above. Elliker's broth (13) was used since it is a suitable medium for both lactic streptococci and lactobacilli. Low-fat milk and Elliker's broth were treated as was the soymilk. Viable counts and acidity were determined by methods described above.

#### Effect of additives on acid production in soymilk

The relationship between nutritional adequacy of soymilk and acid production by lactic acid bacteria in this medium was studied. The following substances were added to soymilk: whey powder, peptone, caseitone, glucose, sucrose, and lactose. Ten per cent solutions of these materials were prepared and then autoclaved (whey, peptone, and caseitone) or filter-sterilized (glucose, sucrose, and lactose). One part of each solution was added to nine parts autoclaved soymilk to give a final concentration of 1%. Acid production by lactic cultures was determined after 16 hr of incubation using the methods described earlier. Results are expressed as per cent developed acidity to eliminate small differences in initial acidity caused by the various additives.

## RESULTS AND DISCUSSION

### Comparative growth of lactic acid bacteria in soymilk, milk, and Elliker's broth

Data on growth of lactic acid bacteria in the three media are summarized in Table 3. Their growth

TABLE 2. ANALYSIS OF SOYMILK.

Component	Per cent
Protein	3.58
Fat	1.90
Ash	0.43
Moisture	91.25
Carbohydrate (by difference)	2.84

TABLE 3. GENERATION TIMES (MINUTES) OF LACTIC-ACID BACTERIA DURING GROWTH IN SOYMILK, MILK, AND ELLIKER'S BROTH<sup>1</sup>.

Organism	Soymilk	Milk	Elliker's broth
<i>S. lactis</i> II	40.8	56.4	60.0
<i>S. cremoris</i> 40-990	45.0	64.8	34.2
<i>S. thermophilus</i> Mc	34.0	36.0	51.6
<i>S. diacetilactis</i> 8-6264	48.6	48.6	56.4
<i>L. mesenteroides</i> 512F	56.4	75.0	58.2
<i>L. citrovorum</i> Da3	82.2	78.6	100.2
<i>P. cerevisiae</i> 1325	50.4	106.2	47.4
<i>L. delbrueckii</i> Ld <sub>3</sub>	85.8	126.0	82.2
<i>L. casei</i> 1445	112.8	112.8	94.8
<i>L. helveticus</i> 1842	78.6	69.8	78.6
<i>L. fermenti</i> 42-7	78.6	75.0	66.6
<i>L. pentosus</i> 124-2	66.6	69.6	112.8
<i>L. brevis</i> 1834	94.8	94.8	69.6

<sup>1</sup>Values represent averages of duplicate experiments.

TABLE 4. RATE OF ACID PRODUCTION BY LACTIC-ACID BACTERIA IN SOYMILK, MILK, AND ELLIKER'S BROTH.

Organism	$\mu$ moles acid per g per hr <sup>1,2</sup>		
	Soymilk	Milk	Elliker's broth
<i>S. lactis</i> II	1.1	4.9	2.9
<i>S. cremoris</i> 40-990	0.7	6.3	3.8
<i>S. thermophilus</i> Mc	3.9	5.0	0.7
<i>S. diacetilactis</i> 8-6264	0.3	2.4	3.0
<i>L. mesenteroides</i> 512F	2.4	0.1	3.6
<i>L. citrovorum</i> Da3	0.2	0.1	0.2
<i>P. cerevisiae</i> 1325	0.4	0.1	2.0
<i>L. delbrueckii</i> Ld <sub>3</sub>	2.7	0.1	2.1
<i>L. casei</i> 1445	0.2	0.2	2.0
<i>L. helveticus</i> 1842	0.1	0.8	0.9
<i>L. fermenti</i> 42-7	0.2	0.0	0.1
<i>L. pentosus</i> 124-2	2.4	0.1	1.2
<i>L. brevis</i> 1834	0.2	0.0	0.7

<sup>1</sup>Average rate of acid production determined from the slope of curve of titratable acid (as lactic acid) against time in hours.

<sup>2</sup>Values are averages of duplicate experiments.

patterns are shown in Fig. 1 to 4.

*Streptococcus species.* *Streptococcus lactis* (Fig. 1) and *S. thermophilus* (Fig. 1) exhibited higher growth rates in soymilk than in milk or broth (Table 3). The maximum population in milk attained by *S. lactis* was about 4-fold higher than in either of the

other media. *Streptococcus thermophilus* also reach a higher (3-fold) maximum population in milk than in soymilk. *Streptococcus cremoris* (Fig. 1) exhibited a higher growth rate or shorter generation time in soymilk than in milk, but its growth rate in broth was highest (Table 3). The maximum population attained in broth also was higher than in milk and soymilk. The growth rate of *S. diacetilactis* (Fig. 1) was similar in soymilk and in milk; and slightly lower in broth.

*Leuconostoc species.* *Leuconostoc mesenteroides* (Table 3, Fig. 2) grew at nearly equal rates in soymilk and in broth and markedly more rapidly in both, than in milk. The maximum population was 10-fold greater in both soymilk and broth than in milk. *Leuconostoc citrovorum* (Table 3, Fig. 2) grew at nearly equal but rather slow rates in soymilk and in milk; growth in broth was somewhat poorer. In all three media, maximum populations attained by *L. citrovorum* were nearly the same ( $>10^7$ /ml).

*Pediococcus.* Growth rates of *Pediococcus cerevisiae* (Table 3, Fig. 4) in soymilk and in broth were nearly the same; that in milk was one-half as fast. There were pronounced differences in the maximum populations attained after incubation in the three

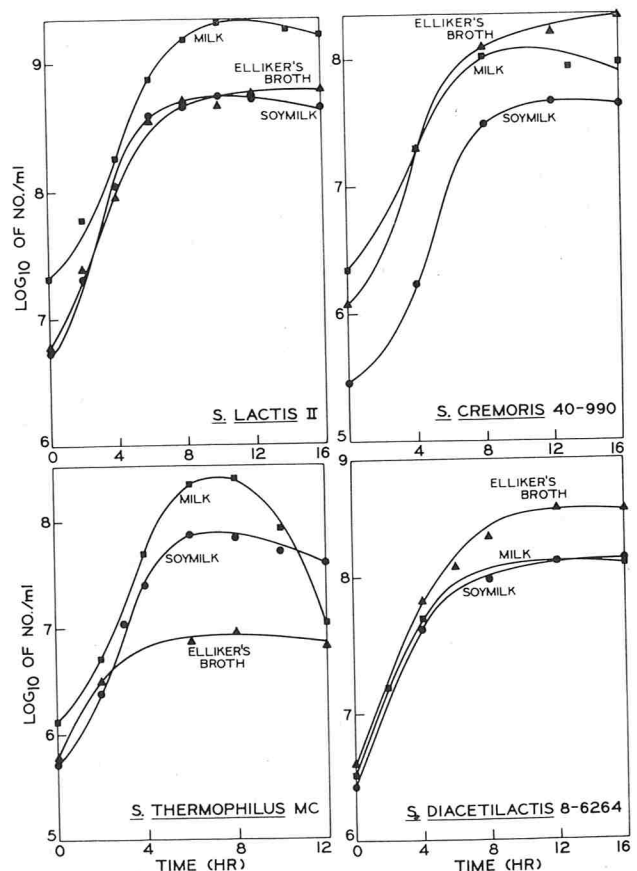


Figure 1. Growth of *Streptococcus lactis*, *Streptococcus cremoris*, *Streptococcus thermophilus*, and *Streptococcus diacetilactis* in soymilk, milk, and Elliker's broth.

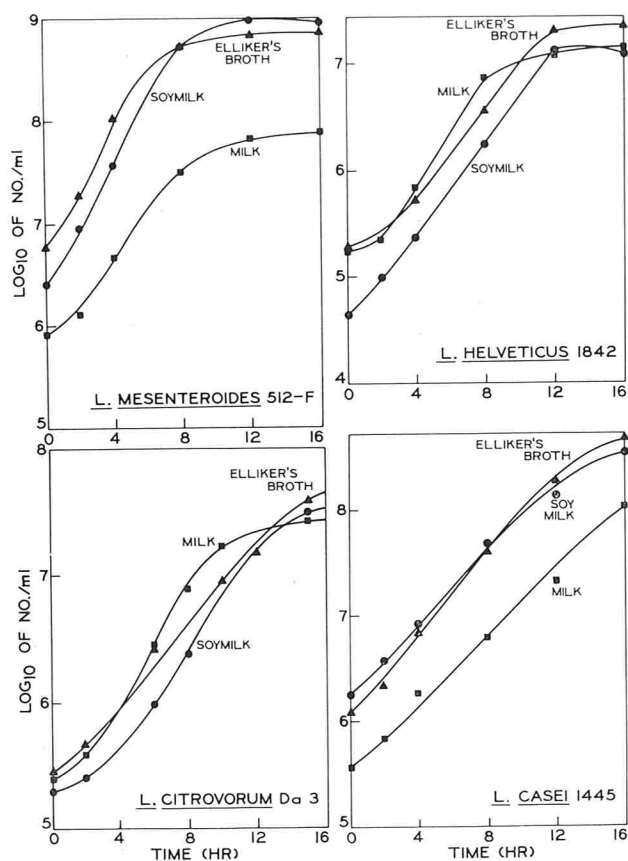


Figure 2. Growth of *Leuconostoc citrovorum*, *Leuconostoc mesenteroides*, *Lactobacillus casei*, and *Lactobacillus helveticus* in soymilk, milk, and Elliker's broth.

media; in soymilk and broth they were nearly 100-fold higher than in milk.

*Lactobacillus species.* Growth rates and maximum populations of *Lactobacillus delbrueckii* (Table 3, Fig. 3) were similar in both soymilk and broth. In comparison, the growth rate in milk was slower and maximum numbers in this medium were 10-fold less. *Lactobacillus helveticus* (Table 3, Fig. 2) grew slightly faster in milk than in soymilk and broth. Maximum populations of the organism in all three media were similar. *Lactobacillus casei* (Fig. 2), *L. fermenti* (Fig. 3), *L. pentosus* (Fig. 3), and *L. brevis* (Fig. 3) grew at similar rates in soymilk and in milk (Table 3). Maximum populations attained by all four organisms were about 10-fold higher in soymilk than in milk. Except for *L. pentosus*, growth rates and maximum numbers of the latter organisms were higher in broth than in the other two media.

#### Comparative rate of acid production in soymilk, milk, and Elliker's broth

Rates of acid production by lactic acid bacteria in the three media are recorded in Table 4. Rate of acid production in broth was, in most instances, equal to, or greater than that in the other media. *Streptococcus lactis*, *S. cremoris*, and *S. diacetylactis* formed acid most rapidly in milk, but comparatively slowly in soymilk. *Lactobacillus helveticus* behaved

similarly only to a lesser extent. Rate of acid production by *S. thermophilus* was highest in milk, but that in soymilk, although less than in milk, was remarkably high. The organism showed an unusually low rate of acid production in broth. *Leuconostoc mesenteroides*, *L. delbrueckii*, and *L. pentosus* produced acid at relatively high rates in soymilk, whereas in milk acid production was practically nil. Acid formation by *P. cerevisiae*, *L. casei*, *L. citrovorum*, *L. fermenti*, and *L. brevis* was comparatively slow and low in both soymilk and milk.

Table 5 presents data on the amount of acid produced by lactic acid bacteria and on the reaction of media at the end of the incubation period. A rather close and consistent relationship between pH and titratable acidity is evident. Slight deviations may be caused by differences in kinds of acid produced, e.g., proportion of weakly dissociated acids (14).

Data in Table 5 show that after 16 hr. *S. lactis*, *S. cremoris*, and *S. thermophilus* produced 4 to 5 times as much acid in milk as was initially present, bringing the pH of the medium to about 4.0. *Streptococcus diacetylactis* produced acid in milk to a lesser extent (3 times that of the initial acidity, pH 5.25). In contrast, these *Streptococcus* species formed less acid in soymilk after 16 hr. The reduction in pH accompanying such acid production by *S. lactis*, *S. cremoris*, and *S. diacetylactis* was slight and not suf-

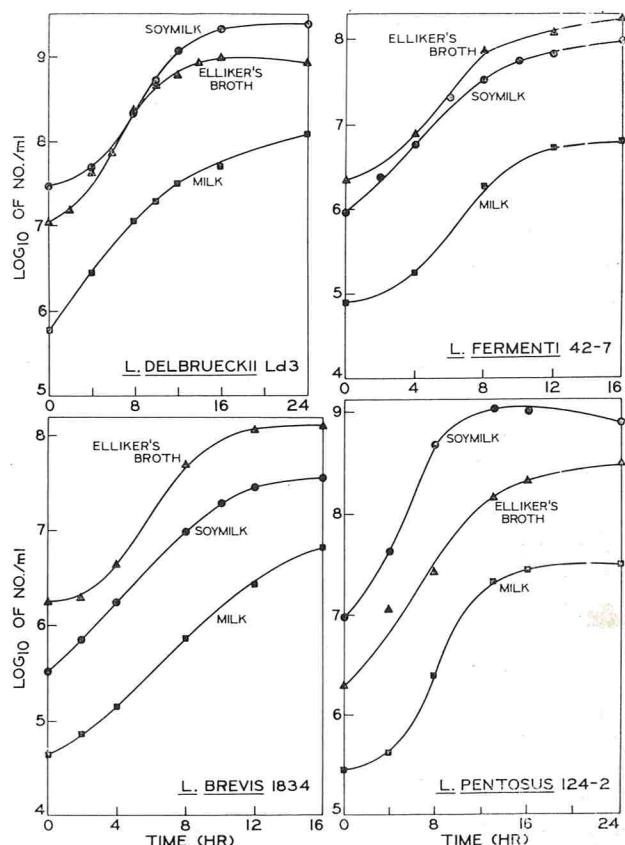


Figure 3. Growth of *Lactobacillus brevis*, *Lactobacillus delbrueckii*, *Lactobacillus fermenti*, and *Lactobacillus pentosus* in soymilk, milk, and Elliker's broth.

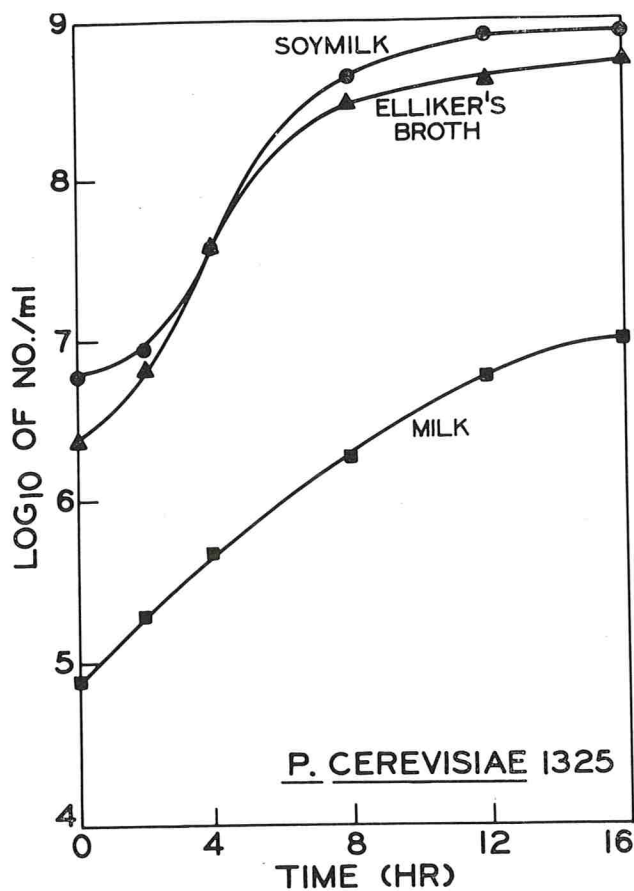


Figure 4. Growth of *Pediococcus cerevisiae* in soymilk, milk, and Elliker's broth.

ficient to cause coagulation of soymilk. *Streptococcus thermophilus* reduced the pH of soymilk as much as of milk and caused both to coagulate. *Leuconostoc citrovorum*, *P. cerevisiae*, *L. casei*, *L. fermenti*, and *L. brevis* did not induce any significant change in pH or titratable acidity in soymilk or milk. The

strain of *L. casei* used in this study, like most strains of this species (9), produced acid slowly and caused coagulation of milk in three days, at the earliest. *Lactobacillus helveticus* produced a very slight increase in titratable acidity of milk but none in soymilk.

*Lactobacillus delbrueckii*, *L. pentosus*, and *L. mesenteroides* produced considerable amounts of acid in soymilk and comparatively less in milk. As with *S. thermophilus*, acid production by these organisms was sufficient to cause coagulation of soymilk. Approximately 0.23-0.25% titratable acidity was needed for soymilk to coagulate. This occurred at a pH of about 5.7, and under conditions of these experiments, between 4 to 8 hr of incubation.

Reports on the isoelectric pH of various soybean fractions are conflicting (11) and, in part, this is related to dependence of isoelectric points on the method of protein isolation and on the ionic strength of the buffer used when determinations were made. Jones and Csonka (21) assigned a pH value of 5.2 to the isoelectric point of a glycinin fraction of soybean protein. The pH value reported herein for coagulation of soymilk seems reasonable since autoclaving must cause some degree of protein denaturation which can affect isoelectric properties.

#### Effect of additives on acid production in soymilk

Acid production is dependent on numbers of bacteria, their growth rate, and their ability to utilize the carbohydrate available in the medium. Soybeans are rather low in their content of simple sugars; the monosaccharides arabinose and glucose are minor constituents (0.0015% and 0.006%, respectively, on a dry basis). The bulk of sugar in soybeans occurs

TABLE 5. PER CENT TITRATABLE ACIDITY (% T.A.)<sup>1</sup> AS LACTIC ACID, AND pH OF SOYMILK, MILK, AND ELLIKER'S BROTH INOCULATED WITH LACTIC-ACID BACTERIA AND INCUBATED FOR 16 HOURS.

Organisms	Soymilk		Milk		Elliker's broth	
	pH	% T.A.	pH	% T.A.	pH	% T.A.
Initial value (Range)	6.50	0.14-0.18	6.25	0.16-0.18	6.40	0.18-0.20
<i>S. lactis</i> II	5.93	0.30	4.40	0.76	4.28	0.57
<i>S. cremoris</i> 40-990	6.08	0.27	4.35	0.92	4.33	0.68
<i>S. thermophilus</i> Mc	4.40	0.49	4.40	0.74	4.10	0.31
<i>S. diacetylactis</i> 8-6264	5.83	0.27	5.25	0.56	4.25	0.63
<i>L. mesenteroides</i> 512F	4.80	0.42	6.15	0.19	4.35	0.58
<i>L. citrovorum</i> Da3	6.20	0.18	6.22	0.19	5.67	0.22
<i>P. cerevisiae</i> 1325	6.13	0.19	6.20	0.18	4.40	0.46
<i>L. delbrueckii</i> Ld <sub>3</sub>	4.80	0.42	6.23	0.24	4.60	0.54
<i>L. casei</i> 1445	6.18	0.17	6.13	0.19	4.30	0.48
<i>L. helveticus</i> 1842	6.40	0.16	5.70	0.25	4.65	0.38
<i>L. fermenti</i> 42-7	6.40	0.18	6.38	0.18	6.03	0.21
<i>L. pentosus</i> 124-2	4.58	0.50	6.23	0.26	4.83	0.41
<i>L. brevis</i> 1834	6.35	0.16	6.23	0.16	5.65	0.27

<sup>1</sup>Values are averages of duplicate experiments.

TABLE 6. EFFECTS OF ADDITION OF WHEY, PEPTONE, CASITONE, GLUCOSE, SUCROSE, AND LACTOSE ON ACID PRODUCTION BY LACTIC-ACID BACTERIA IN SOYMILK.

Organism	Soymilk	Soymilk with added					
		Whey	Peptone	Casitone	Glucose	Sucrose	Lactose
		(% developed acidity after 16 hr) <sup>1</sup>					
<i>S. lactis</i>	0.04	0.41	0.01	0.05	0.40	0.02	0.37
<i>S. cremoris</i>	0.02	0.28	0.01	0.03	0.27	0.03	0.25
<i>S. thermophilus</i>	0.41	0.43	0.50	0.59	0.42	0.39	0.42
<i>S. diacetylactis</i>	0.03	0.32	0.02	0.05	0.28	0.03	0.30
<i>L. mesenteroides</i>	0.22	0.22	0.38	0.42	0.31	0.25	0.21
<i>L. citrovorum</i>	0.05	0.10	0.14	0.17	0.12	0.06	0.09
<i>P. cerevisiae</i>	0.03	0.07	0.03	0.06	0.21	0.04	0.05
<i>L. delbrueckii</i>	0.31	0.31	0.11	0.24	0.61	0.31	0.28
<i>L. casei</i>	0.02	0.12	0.05	0.07	0.24	0.04	0.08
<i>L. helveticus</i>	0.01	0.19	0.01	0.02	0.14	0.01	0.02
<i>L. fermenti</i>	0.01	0.00	0.00	0.01	0.02	0.00	0.01
<i>L. pentosus</i>	0.21	0.22	0.44	0.48	0.24	0.23	0.20
<i>L. brevis</i>	0.00	0.02	0.00	0.02	0.03	0.01	0.01

<sup>1</sup>Values are averages of duplicate experiments.

as (a) oligosaccharides: 5.0% sucrose, 1.1% raffinose, 3.8% stachyose; and (b) hemicelluloses, e.g., araban and arabinoglactan (23). Since some lactic acid bacteria cannot utilize these carbohydrates, supplementation of soymilk with other carbohydrates may be necessary to insure adequate acid production. Tests were conducted to determine whether added nitrogen and carbon sources would facilitate formation of acid. Lactic-acid bacteria were grown in soymilks fortified separately with 1% whey powder, peptone, casitone, glucose, sucrose, and lactose. Data on acid production in such soymilks are recorded in Table 6.

Acid production by *S. lactis*, *S. cremoris*, and *S. diacetylactis* was enhanced by glucose, lactose, or whey powder added to soymilk. Added sources of nitrogen had no beneficial effect on acid production by these bacteria. Acid development by *S. thermophilus* was unaffected by added sugars but was increased when soymilk was fortified with protein digests. This suggests that the carbon source(s) in soymilk is not the limiting factor for *S. thermophilus* but instead the nitrogen sources are not optimal.

The *Leuconostoc* species produced more acid when protein digests were added to soymilk. Added glucose, lactose, or whey powder enhanced acid production by *L. citrovorum*, whereas only glucose produced a similar effect with *L. mesenteroides* and *P. cerevisiae*.

*Lactobacillus delbrueckii* developed most acid when glucose was present in soymilk, whereas other added sugars or whey powder were essentially without effect. Protein digests added to soymilk resulted in reduced acid production by this organism. Addition of glucose, whey powder, and lactose caused an increase in acid production by *L. casei* and *L. helveticus*, whereas the protein digests were without ef-

fect. *Lactobacillus pentosus* produced more acid when peptone and casitone were present, but not when soymilk contained the other additives. Acid production by *L. fermenti* and *L. brevis* was not affected by any of the additives.

Results of this investigation indicate that, in general, soymilk is a satisfactory growth medium for most lactic-acid bacteria. Supplementation of soymilk with certain fermentable sugars or partially degraded proteins can lead to higher yields of acid from some of the organisms.

#### ACKNOWLEDGMENTS

The authors thank Dr. C. A. Kust, Department of Agronomy, University of Wisconsin for supplying the soybeans used in this study. This work was supported in part by funds from the Graduate School of the University of Wisconsin.

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## EGG INDUSTRY COMPLETES TWO STANDARDS

New sanitary standards for fittings, valves, spray dryers and pumps, were adopted by the E-3-A Sanitary Standards Committee at its November 5 meeting in Houston, Texas.

E-3-A is a cooperative effort by representatives of five groups—Institute of American Poultry Industries, Dairy & Food Industries Supply Association, U. S. Department of Agriculture, International Association of Milk, Food and Environmental Sanitarians and U. S. Public Health Service—to establish voluntary criteria for cleanability of processing equipment and product protection.

Standard No. E-0800 will provide for sanitary fittings and valves for liquid egg processing.

Spray dryers for egg products are covered by the new E-3-A Accepted Practices for Egg Products Spray Drying Systems, Serial No. E-60700. The

Practices offer criteria for installation, operation, and cleaning of spray drying equipment. Publication of the documents is scheduled for the fall of 1971 in the Journal of Milk and Food Technology. Standards become effective one year after official adoption. Reprints from the Journal are available from the offices of IAPI and DFISA.

Also completed at the semi-annual meeting was an amendment to the Pump Standard to provide for an optional material.

Other items on the agenda were referred back to the originating DFISA Task Committees for further revisions. Scheduled for the next E-3-A meeting in April, this business will include drafts of tentative standards for egg breakers, egg washers and HTST Pasteurization Practices.



# A RESPONSE TO THE ENVIRONMENTAL CHALLENGE<sup>1</sup>

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## ABSTRACT

Rapid industrialization, growing populations, and an ever expanding technology have generated a new and well founded concern with the quality of our environment. Currently some 360 million tons of solid waste must be collected and disposed of each year. Methods of accomplishing this huge task are still in their infancy. Some 70% of municipal disposal facilities are open dumps. Only 6% of so-call sanitary landfills meet official standards. Collection techniques are still labor oriented. Nevertheless, some remarkable technological developments are taking place which suggests that methodology and technology in this area will cease to be a problem in the near future. The big factors, yet to be resolved, are the economic and the people problems. Lessons learned and progress achieved by milk and food sanitarians, pioneers in environmental control, lead one to believe that they have much to contribute in achieving solutions to these aspects of the broad ecological problem. The lesson appears to be this: despite the sensationalism that is characteristic of modern communications media, a vast number of energetic and talented people are quietly formulating methods and programs which will produce reasonable solutions to these great issues.

Ecology means the relationship of living things to their environment. Environment means the aggregate of surrounding things, conditions, and influences. Under these definitions ecology involves, for people, not only physical conditions but political, social, economic, and spiritual things as well. Thus, war is a factor in our ecological problem. Campus unrest is a factor. Rudeness and uncivil behavior are elements in the problem. However, as a practical matter, the current popular concern with ecology is limited to physical considerations; primarily, air, water and land pollution.

## POLLUTION

It is a little difficult to establish the dimensions of the problem in these terms because pollutants have a way of shifting back and forth from one category to another. For example, the particulates from imperfect combustion that go up a smokestack and into the atmosphere are solids but are considered as air pollutants as long as they are in the air. If, however, they are removed from the combustion products by scrubbers or electrostatic precipitators in the stack

they are obviously solid waste that becomes a land pollutant. Similarly solid waste that goes to an open dump is a land pollutant. But, if at the dump, it is burned much of it becomes an air pollutant. The Federal Bureau of Solid Waste Management uses the following numbers to describe the volume of waste generated annually in the United States. These figures were for 1967. They are divided into five categories on the basis of the generating source. These categories and volumes are

Urban waste	-----	250 million tons
Industrial waste	-----	110 million tons
Agricultural waste	----	2,280 million tons
Mining waste	-----	1,700 million tons

All of this adds up about 4 billion 340 million tons of waste. Indicative of the problem of public understanding is the impression that most people get from these figures. I often ask people what they visualize 4 billion 340 million tons of solid waste to be. Inevitably it means garbage and rubbish that goes to the dump, whereas in reality, garbage and rubbish constitute only about 6% of the total solid waste.

Another way of looking at the problem is the materials flow concept. This approach holds, quite logically, that all materials which enter into production of material things ultimately end up as waste in one form or another. The in-puts are fuel, food, and raw material. The out-puts are finished goods. Weight of finished goods plus weight of processing waste equals the in-put. However, during processing, oxygen is taken out of the atmosphere so that the final weight of the out-put, which ultimately becomes waste, even for finished goods, is greater than the in-put. According to a study made by Ayres and Kneese, the active in-puts in 1965 amounted to 2,492,000,000 tons of active materials.

The authors of the study report that three quarters of the over-all weight of the active in-put is eventually discharged into the atmosphere as carbon and hydrogen which combine with oxygen to produce carbon dioxide and water. The remaining residuals are either gases such as carbon monoxide, nitrogen dioxide, and sulfur dioxide; dry solids such as rubbish; wet solids such as garbage and sewage; and industrial wastes suspended or dissolved in water. The authors go on to say, "Looking at the matter this way clearly reveals a primary interdependence

<sup>1</sup>Presented at the 57th Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, Cedar Rapids, Iowa, August 17-20, 1970.

between various waste streams. In a sense, the solids are the irreducible limiting form of waste."

#### LIMITED RESOURCES

There are two aspects of the problem that these numbers point up. Recognizing that the earth and its atmosphere, vast as it may be, is, nevertheless, finite it follows that there is a limit to the resources that are available for human use and a limit to the capacity of the earth and the atmosphere to absorb the waste products that the use of those resources produces. Consequently the first and probably the most important step toward an improved environment is conservation. Obviously a reduction in the volume of material that goes into the waste stream means a more effective use of resources. It also means that pollution is reduced because the volume of pollutants is less. This philosophy parallels the approach that has been developed with respect to milk. Much of this work is devoted to production of good milk on the farm. To be sure, modern equipment in the milk plant can do wonders with raw milk in terms of improving it through clarification and pasteurization. But the best way to get a good finished product is to start with a good raw product. As a result you emphasize low bacteria counts in the raw milk in order to reduce the subsequent problem.

*Full utilization of raw material*

The conservation approach to waste management has three major facets. The first is full utilization of the raw material. Perhaps the most familiar expression of this concept is the meat packing industry's boast that they use all of the pig but the squeal. Another example, more familiar to those of us associated with milk business, is the problem of whey. The old method of disposing of whey by dumping it into the creek created a water pollution problem. Present thinking on this question considers whey as material with a significant use potential. Thus, instead of dumping, we attempt to recover the whey and use it for animal feed or some other practical purpose. In this way, not only do we make more effective use of the original raw material but reduce or eliminate the pollution problems that were caused by discharging whey into the stream.

Industry is particularly effective in this area, primarily because of the economics involved. Indeed, in most industrial operations the difference between red and black ink on the profit and loss statement is often the effectiveness with which waste has been reduced and raw material utilized. A friend who operates a saw mill in Maine told me that he couldn't stay in business were it not for the additional income he realized from the sale of sawdust, chips, slabs, and even bark.

#### *Re-cycling and re-use*

The second phase of the conservation approach to waste management is in re-cycling and re-use. I make a distinction between the two terms. Re-cycling to me, means taking the material in a product that has been used and reprocessing it back into its original form so that it can be used over again for the same or similar purpose. For example, the metal in a worn out automobile can be recovered and used again as iron or lead or copper. Glass can be re-cycled back into new glass. Paper can be repulped and made into new paper. It should be noted, however, that materials derived from re-cycling are usually inferior to those made from virgin stock.

Re-use, on the other hand, means using the material in a worn out product for some other purpose. Glass manufacturers have developed a process for making a road building material out of used glass containers. Paper, which constitutes slightly more than 50% of the material in municipal dumps, makes an excellent fuel which can be used to produce BTUs. Indeed, one of the exciting new promises in waste management is a unit known as the CPU 400. This unit is a sophisticated, highly efficient incinerator that approaches complete combustion. Hot gases from the burning process are used to activate a turbine which turns a generator. This unit is able to handle up to 400 tons of rubbish a day and can generate 15,000 kilowatts of power during that period. That is enough power to meet about 10% of the needs of a community of 150,000 people. Sale of the power reduces the cost of waste disposal from 4 or 5 dollars a ton to somewhere between 1 and 2 dollars a ton. The volume of residual waste going into the waste stream is greatly reduced and use of a resource is greatly extended. A new and needed product is created and the dollars and cents cost of waste disposal is reduced substantially. It is this kind of practical, down to earth, imaginative thinking that makes solid waste management such an exciting field of activity today.

The CPU 400 was developed by Combustion Power Company, Inc., of Palo Alto, California under a grant from the Bureau of Solid Waste Management. The unit is still in the developmental stage and some of the inevitable bugs that are found in any new piece of equipment have yet to be resolved. It is expected, however, that a full-scale unit will be in operation by 1972. For our purposes, the important point, illustrated by the CPU 400, is the concept of re-use as opposed to re-cycling. Both are sound approaches to the waste management problem. Both are actually being employed at the present time and both offer the opportunity for refinement and increased effectiveness.

### *Collection of waste*

The most familiar part of waste management and the most expensive is collection. This is particularly true of urban waste. The waste generated by an industrial operation remains under the plant's control until enough volume is accumulated to make re-cycling or re-use economically feasible. Waste from individual dwellings, offices, and apartment buildings has to be collected in relatively small volumes and transported, generally substantial distances, to the disposal site. Richard Vaughn, Director of the Bureau of Solid Waste Management, points out that collection methods have changed little in centuries. The basic technique of a receptacle mounted on wheels is about the same whether the rig is moved by a horse or an internal combustion engine. Somebody has to empty the contents of a trash can into a wagon and then drive the wagon to the dump. Collection, Mr. Vaughn says, is labor oriented. It is, therefore, generally rather inefficient and expensive. As a rough rule of thumb, collection costs two or three times as much as disposal. In New York, collection costs are reported to be twelve to thirteen dollars a ton while disposal costs are 4.5 to 5 dollars a ton. Litter is the most expensive of all forms of solid waste to collect, costing as much as \$88.00 a ton.

Collection is not only difficult but it also complicates the disposal problem. Each kind of waste material lends itself to a "best way" of disposition. In order to realize this "best way" the material has to be separated from other materials. Trash arrives at the disposal site as a mixed-up hash of food wastes, paper, metal, plastic, lawn trimmings, glass—you name it. To separate these materials into categories so that they can be re-cycled or re-used, even disposed of in a manner best suited to their physical characteristics, is very difficult and expensive. Some communities try to accomplish this separation at the point of collection—put the cans in one trash can, glass in another, garbage in another and so on. Some people will do this kind of separation but most people find it irksome to the point where it is politically difficult to get such a program adopted.

If for no other reason than cost, collection is an area that cries out for a new technology. Some gestures have been made toward improvement in the form of home compactors. Such devices reduce volumes and lead to less frequent collections and better pay loads but they do not seem to me to be an answer to the problem. Garbage disposals that grind organic material and mix it with water to make a slurry that can be discharged into conventional sewer lines may point the way. Whatever the ultimate solution to this problem, clearly it is a critical element in the overall problem that inhibits effective employ-

ment of re-cycling techniques and constitutes a major portion of waste management costs.

### FINANCIAL ARRANGEMENTS

Financing waste management programs is another matter of critical importance. In a free market economy cost of disposal is not built into the price of a product. Raw material costs, production costs, distribution costs are all incorporated into the price of an item. The cost of disposal, rightly or wrongly, has been assumed by the community and paid for out of tax dollars. Since people do not like to pay taxes, the most politically expedient approach to financing waste management activities has been to spend as little money as possible on the activity. This is the major reason that 70% of the solid waste disposal facilities in American municipalities are still open dumps. It is why scarcely 6% of the municipal incinerators meet acceptable standards. It is why only 12% of the so-called sanitary landfills are satisfactory. Indeed, a dramatic illustration of the point from which our current attack on waste begins is the fact that one of the objectives of the Bureau of Solid Waste Management is simply to reduce the number of open dumps by 5000 in the next 5 years. The principal barrier is money.

This situation has naturally encouraged public officials and legislators to seek out new methods of financing waste management. There are several proposals under consideration, some of which have been put into practice. One method, aimed at industrial operations, establishes waste disposal standards to which all manufacturing establishments in a given product category must conform. Providing the standards are realistic this tactic has considerable merit. It would force manufacturers to provide waste disposal facilities for their manufacturing operations and thereby make that element of the waste management problem a part of the cost which would be reflected in the price. It is important, however, that the requirement be uniform otherwise it would give competitive advantage to one company that did not have to incur expenses that another company was obliged to assume. My own company has assumed this burden voluntarily. A few months ago our President, Mr. Hinman announced that the International Paper (I.P.) Company would spend in excess of \$100,000,000 to equip all of our 26 paper mills with the best available devices for water and air treatment. It is believed that by 1974 we will return water to the rivers that is in a better condition than it was before we used it. Even the odor associated with paper making will be almost completely eliminated.

A second method to achieve the objective of building disposal costs into a product is through a direct

tax on the product—I suppose a gasoline tax earmarked for highways is something of a precedent. Many states are considering proposals along this line. There are problems associated with this idea, one of the most important being uniformity. State taxes raise the specter of 50 different kinds and degrees of taxation. Problems we have encountered in labeling and duplicate inspections in the milk industry are ample illustrations of the kind of problems that could be encountered.

At this time the most significant new development of financing such programs has been utilization of Federal government financial resources. I have already mentioned research grants. Of more immediate impact is the encouragement offered to municipalities through direct federal financial support for construction of new waste disposal facilities.

If I have been successful in my exposition up to this point it should be apparent that despite the magnitude of waste management problems the broad vision of how to meet the challenge that it represents has been described. The necessary technology is being developed and, as always happens, improvements in technology will follow rapidly once the first bold moves have been made. Furthermore, we have seen that both the public and private sectors have recognized the costs involved and have either adopted measures or are creating measures that will satisfy that part of the equation. Most important of all is the inspiring fact that many men and women of ability and energy are devoting their talents to a resolution of this problem. Yet, in spite of the demonstrable progress that has been made and the exciting prospects that are likely to be realized in the near future, the major and most difficult part of the problem still remains. For when all is said and done, when all the technology is reviewed and all the numbers are analyzed the basic issue stands in all its formidable simplicity. That issue is people. Pollution, on the surface, is a physical problem, a problem of gases and solids and liquids in the waste stream. But behind this physical aspect of the problem are people, people with their attitudes, their understanding, their emotions, their strengths, and their weaknesses but above all with their will.

#### PEOPLE ARE CHALLENGED

In his monumental work, *A Study of History*, the British historian, Arnold Toynbee, identifies 21 civilizations that have come into existence, gone through a cycle of growth, maturity, decline, and in most instances disappearance. Only one of the 21 exists today as a living, driving force. That, of course, is our own western civilization that has spread its influence over all the earth.

Professor Toynbee sought an answer to the birth, growth, and death of civilizations. Why did they come into being in the first place? Why did they grow? Why did they stop growing and die? He concludes that societies move out of a primitive state into the more advanced stage called civilization as the result of a successful response to a challenge. Something happened that forced people to adopt new ideas, to create new technologies and new institutions. There was a challenge that demanded an appropriate response. Where that response was forthcoming growth took place. Growth led to new challenges requiring new responses. This sequence of challenge and response resulting in growth continued until the society encountered a challenge to which it was unable to formulate an adequate response. At that point the society ceased to grow and began the long decline that ended in oblivion.

The fascinating thing about this challenge and response sequence is that in the beginning challenges were external, generally physical, outside of the society. As these external challenges were met an environment favorable for growth of people took place. Gradually, almost imperceptibly, the nature of the challenges changed from outside the society to within the society. Instead of physical problems they became people problems, problems of human relationships. It was this challenge that all previous civilizations failed to meet. The most casual review of our problems today shows that we, in our turn, are face to face with the great challenge of human relationships.

One of the most critical elements in the existence of any society, large or small, is leadership. Toynbee calls this the Dominant Creative Minority. This is the leadership group. When the Dominant Creative Minority no longer commands the confidence of the majority that it leads, society begins to disintegrate and a new one takes its place.

#### TODAY'S CRISES

What I find most disturbing in our national life today is not the caliber of the responses that we have formulated and are formulating to the extraordinarily difficult challenges that we face. What is disturbing is the apparent failure of the majority to understand the significance or effectiveness or even the existence of those responses. The result is a loss of confidence in the Dominant Creative Minority. If one is to believe the daily doses of evil tidings we receive from the communication media nothing good ever happens any more. In its foreign relations the government is an imperialist aggressor dominated by a military-industrial complex. Domestically it is in the service of corporate powers and unresponsive to the needs

of the people. The business world is preoccupied with profits. Our educational institutions are archaic dealing in irrelevant educational programs. The environment is polluted beyond repair. It is little wonder that, confronted with this catalogue of woes, large numbers of people, particularly young people, are disenchanted with the leadership or the older generation or the establishment that has brought about these conditions. As a result there is a lack of confidence in our ability to find answers to these problems through the existing institutions. The rise of the technique of massive demonstrations, of direct action of picketing or petitions or political activism is an indication of this lack of confidence. In its more extreme form the direct action technique becomes riots, bombing, and violent confrontation.

Recently I had a conversation with a young man who was a graduate student at one of our eastern universities. We discussed the phenomenon of campus unrest and, as you might expect, ultimately got to the subject of war and Vietnam. He held the popular view of the military-industrial complex conspiracy, economic imperialism, and a leadership insensitive to the will of the people. I suggested that there was another side to the story and that he might find it illuminating to read the documents, speeches, reports, and position papers relating to Vietnam. I pointed out that they were readily available from the State Department. His answer, devastating in its implication, was, "Well, I don't believe much of anything that comes out of the State Department." Now bear in mind that this was no wild-eyed kook but a fine, intelligent young man with 17 or more years of high priced education behind him. Yet he would not accept an official statement from a Department of the United States government as something that he could believe. Call it the "generation gap" or the "credibility gap" or what you will. The awesome fact is that he no longer had any confidence in the leadership of the existing Dominant Creative Minority. How widespread, how deep this attitude is I do not know. Certainly the incidence of campus unrest, the commentaries of journalists and television celebrities, as well as the general bad connotation of such terms as "bureaucracy" and "politician" suggest that the attitude is widespread and deep. Possibly one of the most eloquent expressions of this strange phenomenon was given by a popular actress last winter. She was in the audience at a panel discussion staged by a popular talk show on the occasion of one of the moratoriums. Comments were invited from the audience and the actress, her voice strained with emotion, said, "Don't you understand. Millions of young people are trying to tell you that you've made a goddamn mess of the world."

Yet of all the strange aspects of this strange and

baffling period is the fact, and it can be documented beyond any shadow of doubt, that probably no other period in the history of mankind has seen such progress as that which has been achieved during the past four decades. In technology, in economics, in the arts, in the social sciences, achievements have been little short of spectacular. Poverty, often called the "shame of America" has been reduced to the lowest point in our national history. And last year alone the number of people officially classified as "poor" was reduced by 5%. In government, creative stimulating responses to enormously difficult challenges have been made in the vast number of areas of which the present system of Federal Milk Marketing orders is but one small illustration. Achievements in the field of public health is another example. A whole new body of jurisprudence, called Administrative law has come into being. No matter where one looks, creativity, imaginative responses, inspiring new developments have combined to establish a record of unparalleled achievement. But opposed to this real record of remarkable responses to complex challenges is the doctrine of despair, of pessimism, of doubt, and disbelief.

There are many reasons why this situation exists. It seems to me, however, that a principal one is the error of considering existence of problems an indication of failure. The point that needs to be grasped is the relationship between the challenge and the response. It is not the challenge that is the measure of success or failure, it is the response that is crucial. I am not particularly concerned with the existence of problems in our society for there have always been and always will be problems. My concern is with the nature of the responses to those challenges.

Possibly a basic cause of equating problems with failure rather than looking at the nature of the response is the fact that we live always on the utmost extremities of human experience. No one has ever gone beyond this point in time. Beyond is the vast eternity of the unknown. It is this that gives validity to the statement that the hindsight of the simplest schoolboy is often superior to the foresight of the most exalted statesman.

So it is with ecology and with the nature of the environment in which we shall live. It is the nature of men that in solving one problem new problems will be created which require new solutions. The bulk farm tank, for example, was an excellent solution to problems in milk handling but utilization of bulk tanks generated a whole new set of problems, problems in measurement, in quality control, in financing, in logistics. But the fact that these new problems came into being did not vitiate the virtues of the bulk tank as a solution to a problem. It is easy to

look back on the events of yesterday and say we should have done this or that. But the thing that separates the men from the boys is the will to attack the new problems rather than to withdraw into a cocoon of despair simply because the problems exist.

Our problems of water, air, and land pollution are finite, as the numbers I cited demonstrate. That there can be solutions to these problems is apparent as some of the exciting new developments such as the CPU 400 indicate. Technically, although the problem is Bunyanesque in scope, it can be solved. There is no question in my mind whatsoever that the technology is emerging that can meet the challenge. The real problem is a problem of will. We shall not fail for want of technical competence. We shall only fail for want of the will to succeed. I am an optimist by nature. I cannot review the long, torturous history of mankind without being impressed by the folly and tragedy that marks that laborious journey. And yet in spite of all the bitter, terrible nature of that

story there always emerges in all its wonder and glory the tenacity and strength and soaring courage of the human spirit. I, no more than you, can look into the dim reaches of tomorrow and define with certainty the shape of things to come. I only know, as you must know, that out there is where our destiny lies. We can never turn back. All that we can do is sail on and on, forever on uncharted seas. And so I cannot embrace the philosophy of despair. I stand, as must we all, aware of the great challenges but ready and able to meet them as did our fathers and their fathers before them. The great response to the ecological problems of our industrial society will not come from the outside, they will come from within ourselves. For it matters not how dark the gate, how crowded with punishment the scroll. What matters is that we believe in our competence and strength, that we believe in ourselves, that we understand that we and we alone are the masters of our fate, that we are the captains of our souls.

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### REPORT OF THE EXECUTIVE SECRETARY AND MANAGING EDITOR, 1969-1970

This marks the end of the 19th and the beginning of the 20th year I have been making these reports at our Annual Business Meeting. I hope you will forgive me for making a personal observation in this report. The most outstanding occurrence this year, as far as I am concerned, was the action taken by the Executive Board in setting up a retirement plan for me. I am very grateful and hope definite plans will be instituted to provide such a plan when the person who replaces me is employed.

I am happy to report that we are still in excellent condition financially. Our net income for the past year is over \$3000. While this is not as large as last year because of the economic situation, it is still satisfactory. Our total income was slightly higher than last year which set a record for us but increased costs reduced our net.

Our affiliate membership dropped some but our direct membership and subscriptions increased thus maintaining the circulation of the *Journal* at over 4000 copies per month. Affiliate collection of dues was not as good as last year and continues to be a problem.

The considerable expansion of the *Journal* has increased its value to the membership without any increase in cost to them. The increase in food articles has begun to attract more and more people in all areas of the food industry and, as I have predicted many times, will lead to increased membership

in this area. The August, 1970 *Journal* will be the largest we have ever published.

Our work with the egg industry on E-3-A Sanitary Standards is progressing rapidly and we hope to initiate the 3-A program in other areas within the next year. I have made three recommendations to the Executive Board: (a) set up a very active committee on membership (this is hard to do but I do need help in this); (b) provide a committee to work with me on advertising-with the considerable amount of material on food in the *Journal*, we should attract more advertising by the food industry; and (c) make definite plans for hiring and training my successor.

I attended the usual number of meetings, published 12 issues of the *Journal*, and performed all the routine chores which are necessary to the job but which becomes quite repetitious to report each year.

With the added emphasis on all phases of sanitation and prevention of contamination there appears to be a very bright future for the Association and all our members. May we discharge our obligations with distinction.

Again for the 19th time it has been a privilege to serve you.

Respectfully submitted,

H. L. "RED" THOMASSON

Executive-Secretary, IAMFES

Managing Editor, *Journal of Milk and Food Technology*

## A DIFFERENTIAL BROTH FOR SEPARATING THE LACTIC STREPTOCOCCI<sup>1</sup>

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(Received for publication September 17, 1970)

### ABSTRACT

Arginine degradation and citrate utilization, the major differentiating characteristics among lactic streptococci, formed the basis of a differential broth for separating *Streptococcus cremoris*, *Streptococcus lactis*, and *Streptococcus diacetylactis* strains in pure cultures.

The medium contains milk as the sole source of carbohydrate (lactose), arginine and sodium citrate as specific substrates, and a suitable pH indicator (bromocresol purple), in addition to other ingredients. The pH of the medium is adjusted to  $6.2 \pm 0.05$  (which becomes  $6.15 \pm 0.05$  after sterilization) to increase citrate utilization and the broth is dispensed into test tubes containing Durham fermentation tubes. *Streptococcus cremoris* produces a yellow reaction (acid) in the broth. *Streptococcus lactis* initially turns the broth yellow, but on liberation of  $\text{NH}_3$  reverses the color back to the original violet hue. *Streptococcus diacetylactis* produces a violet reaction, and  $\text{CO}_2$  accumulates in the Durham fermentation tubes from the fermentation of sodium citrate.

The most commonly used starter microorganisms in the dairy industry belong to Sherman's lactic group of the genus *Streptococcus* (2). *Bergey's Manual of Determinative Bacteriology* (1) recognizes only *Streptococcus lactis* and *Streptococcus cremoris* as members of Sherman's lactic group. Sandine et al. (7) have shown that *Streptococcus diacetylactis* also should be included in this group. Routinely, members of this group are differentiated by several biochemical tests (4, 5, 6); the major tests being arginine hydrolysis (6) and King's test for diacetyl and acetoin (4). Among the lactic streptococci most strains of *S. lactis* and *S. diacetylactis* can deaminate arginine; *S. cremoris* lacks this ability. *Streptococcus diacetylactis* is the only species able to rapidly utilize citrate to produce  $\text{CO}_2$ , diacetyl, and its reduced  $\text{C}_4$  compounds. Niven et al. (6) were the first to differentiate *S. lactis* from *S. cremoris*, on the basis of arginine hydrolysis. This differentiation was based on detection of  $\text{NH}_3$  liberated from arginine with Nessler's reagent. Later, Mikolajcik (5) described a broth in which arginine hydrolysis could be demonstrated by incorporating an acid-base indicator.

A disadvantage of the media described by Niven et al. (6) and Mikolajcik (5) was that *S. diacetylactis*

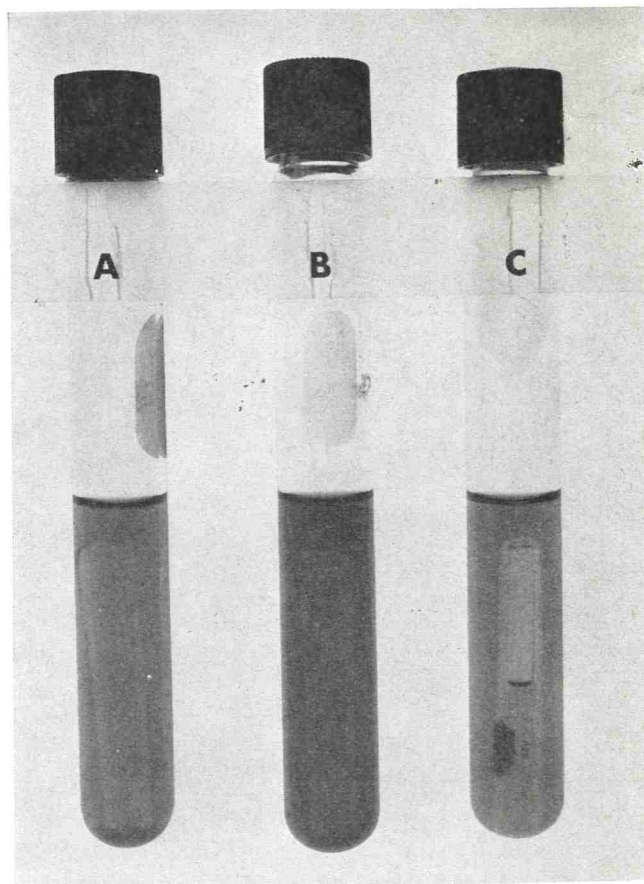


Figure 1. Photograph taken with yellow-sensitive film showing reactions in the differential broth by *S. lactis* C<sub>2</sub>F-violet indicator color and no gas (left), *S. cremoris* W-yellow indicator color and no gas (middle), and *S. diacetylactis* 18-16-violet indicator color and gas (right), after 48-hr incubation at 30 C.

also gave a reaction similar to *S. lactis*. Sandine et al. (7) described another broth that could be used to separate the citrate-fermenting lactic streptococci from those that do not utilize the tricarboxylic acid, by detection of  $\text{CO}_2$  produced in the broth.

Our investigation was undertaken to develop a single test broth that could differentiate between all three species in Sherman's lactic group, namely *S. lactis*, *S. cremoris*, and *S. diacetylactis*. We tried to combine the principles underlying the differential broths of Mikolajcik (5) and Sandine et al. (7) in the development of such a medium. Suitable modifi-

<sup>1</sup>Journal Paper No. J-6727 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa. Project No. 1839.

TABLE 1. MAJOR BIOCHEMICAL CHARACTERISTICS OF REPRESENTATIVE LACTIC *Streptococcus* AND FOUR *Leuconostoc* STRAINS AND THEIR REACTIONS IN THE DIFFERENTIAL BROTH

Strains	NH <sub>3</sub> in Niven's broth <sup>a</sup>	King's test <sup>b</sup>	Coagulation of milk in 48 hr at 30 C	Reactions in broth		
				Acid <sup>c</sup>	NH <sub>3</sub> <sup>d</sup>	Gas <sup>e</sup>
<i>S. lactis</i>						
7963	+	—	+	—	+	—
C <sub>2</sub> F	+	—	+	—	+	—
E	+	—	+	—	+	—
7962	+	—	+	—	+	—
<i>S. cremoris</i>						
HP	—	—	+	+	—	—
ML <sub>4</sub>	—	—	+	+	—	—
SC <sub>1</sub>	—	—	+	+	—	—
SC <sub>3</sub>	—	—	+	+	—	—
<i>S. diacetilactis</i>						
18-16	+	+	+	—	+	+
26-2	+	+	+	—	+	+
DRC <sub>3</sub>	+	+	+	—	+	+
31-2	+	+	+	—	+	+
<i>Leuconostoc citrovorum</i>						
CAF-B	—	— <sup>f</sup>	—	NC <sup>g</sup>	—	—
Da <sub>3</sub>	—	— <sup>f</sup>	—	NC <sup>g</sup>	—	—
<i>Leuconostoc dextranicum</i>						
L.D.	—	—	—	GR <sup>h</sup>	—	+1
1145	—	—	—	NC <sup>g</sup>	—	+1

<sup>a</sup>Tested with Nessler's reagent.

<sup>b</sup>Colorimetric detection of diacetyl-acetoin with  $\alpha$ -naphthol.

<sup>c</sup>Yellow color.

<sup>d</sup>Reversal of yellow color to violet (purple).

<sup>e</sup>Collection in Durham fermentation tubes (roughly quantitative).

<sup>f</sup>Tested up to 72 hr of incubation.

<sup>g</sup>No change.

<sup>h</sup>Growth and very slight acid.

<sup>i</sup>Minute amounts of gas observed only after 3 days of incubation.

cations of Mikolajcik broth (5) were made to accommodate utilization of citrate in the presence of the highly basic guanidyl amino acid. To readily observe CO<sub>2</sub> production in the medium, Durham fermentation tubes were placed in the broth in the test tubes.

#### MATERIALS AND METHODS

##### Cultures

Fourteen *S. cremoris*, 10 *S. lactis*, 13 *S. diacetilactis*, 2 *Leuconostoc citrovorum*, and 2 *Leuconostoc dextranicum* strains were included in this investigation. They were obtained from culture collections at the Departments of Food Technology, Iowa State University; Microbiology, Oregon State University; and Food Science, University of Wisconsin.

##### Media

The differential broth contained 0.5% tryptone, 0.5% yeast extract, 0.1% K<sub>2</sub>HPO<sub>4</sub>, 0.5% arginine, 2% Na citrate, and 0.002% bromocresol purple. Three and one-half milliliters of 11% reconstituted Matrix milk (Galloway-West Co., Fond du Lac, Wis.) were added to every 100 ml of broth and steamed for 15 min. The lactose content of the broth was considered approximately equal to 0.175%. The pH of the mixture was adjusted to 6.2  $\pm$  0.05 after it was cooled to room temperature. The broth, in 7-ml quantities, then was dispensed into screw capped test tubes (length 126 mm and neck 10 mm internal diameter,

Pyrex No. 9825 containing Durham tubes and was sterilized at 121 C for 15 min.

One loop-full amounts of active 18-hr milk cultures were used to inoculate the tubes. At the time of inoculation the pH of the medium should be 6.15  $\pm$  0.05. The test tube caps were tightly closed to prevent escape of liberated ammonia and CO<sub>2</sub>, and incubation was at 30 C. Indicator color reactions and CO<sub>2</sub> accumulation were observed at 24-hr intervals for 72 hr.

#### RESULTS AND DISCUSSION

In most instances, reactions were complete in 24 to 48 hr. After 24-hr incubation, all tubes were tapped a few times to liberate residual gas from the medium.

*Streptococcus lactis* turned the broth color first to yellow by production of lactic acid and later reversed the reaction to violet because of liberation of NH<sub>3</sub>. *Streptococcus cremoris*, on the other hand, produced a deep-yellow color. In addition to reversing the indicator color, *S. diacetilactis* produced copious amounts of CO<sub>2</sub> within 48 hr, which collected in the Durham fermentation tubes. Further, *S. diacetilactis* produced a more intense purple than *S. lactis*. The reactions in the broth are shown in Fig. 1.



*Streptococcus diacetylactis* failed to produce gas when the pH values were high; i.e., pH  $\geq 6.8$ . Adjusting the pH of the cooled medium to  $6.2 \pm 0.05$  before sterilization resulted in copious gas production and good differentiation. This is in keeping with the pH optima for citrate permease of *S. diacetylactis* and *Leuconostoc* species as described by Harvey and Collins (3).

A total of 40 strains belonging to the species *S. lactis*, *S. cremoris*, and *S. diacetylactis* were tested in the broth in pure cultures. All gave good growth and excellent differentiation. In addition, four strains of starter *Leuconostoc* species also were tested in this medium. Minute amounts of gas production were observed with two strains of *L. dextranicum* after 3 days of incubation. The two *L. citrovorum* strains failed to produce gas even after prolonged incubation. No appreciable color change in the violet differential broth was observed with the *Leuconostoc* cultures. Results of the reactions in the differential broth along with reactions in previously described biochemical tests (4, 6) for 16 representative strains are summarized in Table 1.

Arginine hydrolysis by starter strains grown in this broth can further be confirmed by testing portions of the culture with Nessler's reagent on a porcelain spot plate. Positive results are indicated by a deep-red precipitate.

This differential broth has wide application in the starter-culture industry and in routine laboratory work. The broth also is suitable for qualitative differentiation of individual colonies developing on agar plates containing dilutions of several commercial starter cultures. It simplifies biochemical identification of lactic streptococci.

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### REPORT OF THE COMMITTEE ON FOOD PROTECTION, 1969-1970

In previous reports to the membership, this Committee has discussed the desirability of convening a National Conference on Food Protection. It is now possible to state that a National Conference on Food Protection will be held in Denver, Colorado in April 1971. The Food and Drug Administration has contracted with the American Public Health Association to conduct the Conference. A great deal of planning still remains to be done. However, the Conference will run about four days, and will be designed to be an action meeting. Specific proposals are expected to be forthcoming from the Conference to improve food protection programs in this country. Invitations to participate will be sent to all interested governmental, professional, and trade organizations.

Present plans call for work groups to study the following problem areas and recommend solutions.

- (a) Control of contamination of raw agricultural and marine products.
- (b) Control of contamination of processed foods.
- (c) Prevention of mishandling during preparation of foods in commercial and institutional food service operations.
- (d) Consumer education to minimize abuse of foods in the home.
- (e) Development of an improved system for detection, in-

vestigation, and reporting of microbial hazards associated with foods.

- (f) Coordination of regulatory activities among the governmental agencies and with industry control programs.
- (g) Training and utilization of professional and non-professional manpower.
- (h) Development of public acceptance and political support for food protection programs.
- (i) Selection and use of criteria for evaluating program effectiveness.
- (j) Needs for research, surveillance, and related technical activities in support of a national food protection program.

This, of course, can only be an interim report, since, much remains to be done in planning a Conference of this scope. The Chairman and Vice-Chairman of this Committee have been involved in the preliminary planning, and it is our hope that the entire Committee will become involved in the National Conference on Food Protection.

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# QUALITY AND ECONOMIC CONSIDERATIONS IN THE DATING OF MILK<sup>1</sup>

## IV. MILK HANDLING PRACTICES IN STORES. DIFFERENCES BETWEEN DATING AND NON-DATING MARKETS

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(Received for publication August 10, 1970)

### ABSTRACT

#### *New York City vs. Chicago*

Milk was found to be significantly less available in the dating market than in the non-dating market. A higher incidence of special deliveries was noted in the dating market than in the non-dating market. Milk inventories maintained in the dating market were significantly lower than milk inventories maintained in the non-dating market. Returns due to date or code expiration were identical for the dating and non-dating markets and so few as to be of no consequence.

#### *Birmingham vs. Montgomery*

Milk was found to be slightly less available in the dating market than in the non-dating market but the difference was not statistically significant at accepted confidence levels. A higher incidence of special deliveries was noted in the dating market than in the non-dating market. Milk inventories maintained in the dating market were slightly lower than milk inventories maintained in the non-dating market.

#### *Camden vs. Philadelphia*

Milk was found to be slightly less available in the dating market than in the non-dating market but the difference was not statistically significant at accepted confidence levels. Special deliveries were so few as to be of no consequence in either the dating or non-dating market. Milk inventories maintained in the dating and non-dating markets were identical. Returns due to date or code expiration were identical for the dating and non-dating markets and so few as to be of no consequence.

The principal objective of this phase of the study was to determine differences in store milk handling practices between dating and non-dating market stores, with particular emphasis placed on differences in milk availability.

Surveys of milk handling practices by stores were conducted in three paired sets of markets during the period from August to December 1969. Paired sets of markets surveyed included: New York City, New York (dating) and Chicago, Illinois (non-dating); Birmingham, Alabama (dating) and Montgomery, Alabama (non-dating); and Camden, New Jersey

(dating) and Philadelphia, Pennsylvania (non-dating). Of the dating markets surveyed, expiration date labeling is required in New York City and Birmingham (Jefferson County). Time of pasteurization dating is required in Camden (State of New Jersey).

Selection of stores (chain vs. independent) in the markets surveyed was based on shares of food sales in each of the markets in 1966 and the assumption that share of milk sales in stores would approximate share of food sales.

Store enumerations were conducted in each market by professional enumerators experienced in data collection. Enumerators were instructed in the use of the questionnaire prior to the store enumerations.

### RESULTS AND DISCUSSION

#### IN-STORE HANDLING PRACTICES:

##### NEW YORK CITY VS. CHICAGO

#### *Size of store and sales characteristics*

Thirty food stores in the dating market (New York City) were enumerated. During the calendar week (Sunday to Saturday) previous to the date of enumeration, the 30 stores reported weekly sales of 104,850 quart-equivalent units of whole homogenized milk, or average weekly sales of 3,495 quart-equivalent units per store (Table 1). Thirty food stores also were enumerated in the non-dating market (Chicago). During the calendar week previous to the date of enumeration, the 30 stores reported weekly sales of 153,167 quart-equivalent units of whole homogenized milk, or average weekly sales of 5,106 quart-equivalent units per store. The 30 stores in the dating market were open a total of 184 days (average of 6.13 days per week) as opposed to 202 days for the 30 stores in the non-dating market (average 6.73 days per week).

There were more small stores in the dating market sample than in the non-dating market sample (Table

<sup>1</sup>This study was financed by the Milk Industry Foundation.

TABLE 1. SALES OF WHOLE HOMOGENIZED MILK BY SAMPLE STORES IN NEW YORK CITY AND CHICAGO.

	New York City		Chicago	
	Dating market	Non-dating market	Dating market	Non-dating market
	(30 stores)	(30 stores)	(30 stores)	(30 stores)
	-(Quart-equivalent units)-			
Total weekly sales	104,850	153,167		
Average weekly store sales	3,495	5,106		
Range of weekly store sales	560 to 9,408	420 to 13,125		
Average daily store sales	570	758		
Range of daily store sales	80 to 1,568	60 to 1,875		

2). For example, over three-fourths of the stores in the dating market had daily sales of 800 quarts or less. In the non-dating market, only one-half of the stores had daily sales of 800 or fewer quarts.

A total of 21 different brands (including seven private label brands) packaged by 17 processors were enumerated in dating market stores. Dating market stores carried an average of 1.7 brands and were served by 1.4 processors per store. In non-dating market stores, a total of 12 different brands (including five private label brands) packaged by nine processors were enumerated. Stores in the non-dating market carried an average of 1.2 brands and were served by 1.1 processors per store.

*Delivery practices*

During the calendar week previous to the date of enumeration, the 30 stores in the dating market sample were open 184 days and received regular deliveries on 180 of those days. Thus, these stores received delivery on 97.8% of the days that the stores were open. During the same period, the 30 stores in the non-dating market sample were open 202 days and received regular deliveries on 173 of those days or 85.6% of the days that the stores were open.

All dating market stores received regular deliveries of milk 6 days per week. In the non-dating market sample, stores received regular deliveries of milk an average of 5.77 days per week. A comparative frequency distribution of delivery days per week for sample stores in the dating and non-dating markets is presented in Table 3.

*Milk availability and special deliveries*

Significant differences in milk availability were noted between dating and non-dating market stores. Fourteen of the 30 stores (46.7%) in the dating market reported selling out of whole homogenized milk one or more days during the calendar week previous to the date of enumeration (Table 4). These 14 stores reported selling out before closing a total of 30 times. During the same period, one of the 30 stores (3.3%) in the non-dating market reported selling out once prior to closing. Store closings without whole homogenized milk on hand—expressed as a percentage of store closings—yielded figures of 16.3% and 0.5% for dating and non-dating market stores, respectively. An analysis of the difference in store closings without whole homogenized milk on hand between the dating and non-dating markets indicated that the observed difference was statistically significant (the observed difference could have occurred due to random variation or chance less than one time in 100).

Special deliveries of whole homogenized milk during the calendar week previous to the date of enumeration numbered seven and one for dating and non-dating market stores, respectively. Special deliveries of whole homogenized milk—expressed as a percentage of the number of days that regular deliveries were received—yielded figures of 3.9% and 0.6% for dating and non-dating market stores, respectively.

*Milk inventories*

The previously cited differences in milk availability were also reflected in differences in inventories for dating and non-dating market stores. The 30 dating market stores reported average daily closing time inventories of whole homogenized milk totaling 1,581 quart-equivalent units and average daily sales (weekly sales/days open) totaling 17,265 quart-equivalent units during the calendar week previous to the date of enumeration. Twenty-nine non-dating market stores (one store would not provide inventory information) reported average daily closing time in-

TABLE 2. AVERAGE DAILY STORE SALES OF WHOLE HOMOGENIZED MILK BY STORE SIZE IN NEW YORK CITY AND CHICAGO.

Average daily store sales	New York City				Chicago			
	No. of stores	Dating market		No. of stores	Non-dating market		Cum.	
		% of stores			% of stores			
(Quart-equivalent units)		Class	Cum.		Class	Cum.		
1- 400	8	26.7	26.7	7	23.3	23.3		
401- 800	15	50.0	76.7	8	26.7	50.0		
801-1,200	6	20.0	96.7	12	40.0	90.0		
1,201-1,600	1	3.3	100.0	2	6.7	96.7		
1,601-2,000	—	—	—	1	3.3	100.0		

TABLE 3. DELIVERY DAYS PER WEEK FOR SAMPLE STORES IN NEW YORK CITY AND CHICAGO

Delivery days per week	New York City			Chicago		
	No. of stores	Dating market		No. of stores	Non-dating market	
		% of stores	Cum.		% of stores	Cum.
6	30	100.0	100.0	23	76.7	76.7
5	—	—	—	7	23.3	100.0

TABLE 4. PROPORTION OF STORE CLOSINGS WITHOUT WHOLE HOMOGENIZED MILK, NEW YORK CITY AND CHICAGO.

Item	New York City		Chicago	
	Dating market		Non-dating market	
	Number	Per cent	Number	Per cent
Stores in sample	30	100.0	30	100.0
Stores closing without whole homogenized milk on hand	14	46.7	1	3.3
Store closings	184	100.0	202	100.0
Store closings without whole homogenized milk on hand	30	16.3	1	0.5

ventories of whole homogenized milk totaling 10,997 quart-equivalent units and average daily sales totaling 21,680 quart-equivalent units during the calendar week previous to the date of enumeration. Average daily closing inventories of whole homogenized milk—expressed as a percentage of average daily sales of whole homogenized milk—yielded figures of 9.2% and 50.7% for dating and non-dating market stores, respectively.

#### Returns

Returns or disposals of whole homogenized milk due to date or code expiration were almost identical for the dating and non-dating markets. During the calendar week previous to the date of enumeration, two of the 30 dating market stores reported returns or disposals totaling 50 quart-equivalent units due to date expiration. During the same period three of the 30 non-dating market stores reported returns or disposals totaling 75 quart-equivalent units due to code expiration. Returns or disposals of whole homogenized milk due to date or code expiration—expressed as a percentage of weekly sales of whole homogenized milk—yielded identical figures of 0.05% for the dating and non-dating market samples.

Total returns reported in the processor survey for New York City were 2.6% (Table 4, Part III). Total returns (including returns due to date expiration) reported by sample stores in New York City were 0.33%. The differences between levels of returns noted in the two surveys may have been the result of different definitions of returns. In the store survey, all returns reported had to have been delivered

TABLE 5. SALES OF WHOLE HOMOGENIZED MILK BY SAMPLE STORES IN BIRMINGHAM AND MONTGOMERY

	Birmingham	Montgomery
	Non-dating market	Dating market
	(29 stores)	(30 stores)
	—(Quart-equivalent units)—	
Total weekly sales	91,584	58,073
Average weekly store sales	3,158	1,936
Range of weekly store sales	538 to 10,905	335 to 3,290
Average daily store sales	506	317
Range of daily store sales	77 to 1,818	54 to 548

to the store premises and subsequently returned to the processing plant or disposed of on the store premises. In the processor survey, on the other hand, the term returns might have been construed by respondents to include products which never left the truck as well as products which were returned from stores or otherwise disposed of. Another possible explanation for the higher level of returns reported in the processor survey is that the processor survey covered returns of all fluid dairy products while the store survey dealt only with returns of whole homogenized milk (see footnotes to Table 4, Part III).

#### Package marking in New York City

In conjunction with the store handling practices survey, a package marking survey was also conducted in New York City. Under the New York City dating statute, packages must bear the legend "may be sold until midnight of (followed by the first three letters of the month followed by the numeral or numerals constituting the appropriate calendar date)." The maximum allowable time between pasteurization and sale under the statute is 66 hr and predated is not permitted.

A total of 638 packages were selected at random by store enumerators in 20 New York City stores and examined to ascertain the package marking. All of the 638 packages examined were marked in conformance with the existing dating statute. None of the packages examined was found to be offered for sale after the expiration date marked on the package. None of the packages examined was predated.

#### IN-STORE HANDLING PRACTICES: BIRMINGHAM VS. MONTGOMERY

##### Size of stores and sales characteristics

Thirty-one food stores in the dating market (Birmingham) were enumerated. During the calendar week previous to the date of enumeration, 29 of the stores (two stores would not provide sales information) reported weekly sales of 91,584 quart-equivalent units of whole homogenized milk, or average weekly sales of 3,158 quart-equivalent units per store

TABLE 6. AVERAGE DAILY STORE SALES OF WHOLE HOMOGENIZED MILK BY STORE SIZE IN BIRMINGHAM AND MONTGOMERY.

Average daily store sales	Birmingham			Montgomery		
	No. of stores	Dating market		No. of stores	Non-dating market	
		Class	% of stores		Class	% of stores
(Quart-equivalent units)			Cum.			Cum.
1- 400	15	51.8	51.8	20	66.7	66.7
401- 800	9	31.0	82.8	10	33.3	100.0
801-1,200	2	6.9	89.7	—	—	—
1,201-1,600	2	6.9	96.6	—	—	—
1,601-2,000	1	3.4	100.0	—	—	—

TABLE 7. DELIVERY DAYS PER WEEK FOR SAMPLE STORES IN BIRMINGHAM AND MONTGOMERY.

Delivery days per week	Birmingham			Montgomery		
	Dating market			Non-dating market		
	No. of stores	% of stores	Cum.	No. of stores	% of stores	Cum.
6	—	—	—	1	3.3	3.3
5	31	100.0	100.0	29	96.7	100.0

TABLE 8. PROPORTION OF STORE CLOSINGS WITHOUT WHOLE HOMOGENIZED MILK, BIRMINGHAM AND MONTGOMERY.

Item	Birmingham		Montgomery	
	Dating market		Non-dating market	
	Number	Per cent	Number	Per cent
Stores in sample	31	100.0	30	100.0
Stores closing without whole homogenized milk on hand	4	12.9	1	3.3
Store closings	193	100.0	183	100.0
Store closings without whole homogenized milk on hand	5	2.6	1	0.5

(Table 5). Thirty food stores were enumerated in the non-dating market (Montgomery). During the calendar week previous to the date of enumeration, the 30 stores reported weekly sales of 58,073 quart-equivalent units of whole homogenized milk, or average weekly sales of 1,936 quart-equivalent units per store. The 29 stores in the dating market were open a total of 181 days (average of 6.24 days per week) as opposed to 183 days for the 30 stores in the non-dating market (average of 6.10 days per week).

There were more small stores in the non-dating market sample than in the dating market sample (Table 6). To illustrate, two-thirds of the stores in the non-dating had sales of 400 quarts or less per day. In contrast, slightly over one-half the stores in the dating market sold 400 or fewer quarts daily.

A total of five different brands (no private label brands) packaged by five processors were enumerated in dating market stores. Dating market stores carried an average of 2.8 brands and were served by 2.8

processors per store. In non-dating market stores, a total of three different brands (no private label brands) packaged by three processors were enumerated. Each non-dating market store carried the brands of all three of these processors.

*Delivery practices*

During the calendar week previous to the date of enumeration, the 31 stores in the dating market sample were open 193 days and received regular deliveries on 155 of those days or 80.3% of the days that the stores were open. In the dating market sample four stores reported receiving two regular deliveries per day (a.m. and p.m.) from one or more of the handlers serving these stores. During the same period, the 30 stores in the non-dating market sample were open 183 days and received regular deliveries on 151 of those days or 82.5% of the days that the stores were open. A comparative frequency distribution of delivery days per week for sample stores in the dating and non-dating markets is presented in Table 7.

*Milk availability and special deliveries*

Slight differences in milk availability were noted between dating and non-dating market stores. Four of the 31 stores (12.9%) in the dating market reported selling out of whole homogenized milk one or more days during the calendar week previous to the date of enumeration (Table 8). These four stores reported selling out before closing a total of five times. During the same period, one of the 30 stores (3.3%) in the non-dating market reported selling out once prior to closing. Store closings without whole homogenized milk on hand—expressed as a percentage of store closings—yielded figures of 2.6% and 0.5% for dating and non-dating market stores, respectively. An analysis of the difference in store closings without whole homogenized milk on hand between the dating and non-dating markets indicated that the observed difference was not statistically significant (the observed difference could have occurred due to random variation one time in four).

Special deliveries of whole homogenized milk dur-

ing the calendar week previous to the date of enumeration numbered nine and one for dating and non-dating market stores, respectively. Special deliveries of whole homogenized milk—expressed as a percentage of the number of days that regular deliveries were received—yielded figures of 5.8% and 0.7% for dating and non-dating market stores, respectively.

#### *Milk inventories*

The previously cited differences in milk availability were also reflected in slight differences in inventories for dating and non-dating market stores. Only 22 of the dating market stores enumerated provided the requested sales and inventory information. These 22 stores in the dating market reported average daily closing time inventories of whole homogenized milk totaling 1,798 quart-equivalent units and average daily sales totaling 8,920 quart-equivalent units during the calendar week previous to the date of enumeration. Only 25 of the non-dating market stores enumerated provided the requested sales and inventory information. These 25 stores in the non-dating market reported average daily closing time inventories of whole homogenized milk totaling 2,310 quart-equivalent units and average daily sales totaling 7,892 quart-equivalent units during the calendar week previous to the date of enumeration. Average daily closing inventories of whole homogenized milk—expressed as a percentage of average daily sales of whole homogenized milk—yielded figures of 20.2% and 29.3% for dating and non-dating market stores, respectively.

#### *Returns*

Accurate returns information was not available at the store level in either the dating or non-dating markets. As a rule, store personnel were unable to provide the requested information on returns inasmuch as processors in both markets routinely assumed complete responsibility for rotating stock in the store dairy case and replacing any date or code expired milk. Wilson (1) recently reported on a study, conducted in 1966, of the comparative level of wholesale returns in Jefferson County and Montgomery. Returns of whole homogenized milk expressed as a percentage of wholesale sales were 6.3% and 2.7% for Jefferson County and Montgomery, respectively.

#### *Package marking in Birmingham*

In conjunction with the store handling practices survey, a package marking survey also was conducted in Birmingham. Under the Jefferson County dating statute, packages must bear the legend "legal sale through date above (followed by a calendar day of the month)." The maximum allowable time between pasteurization and sale under the statute is 120 hr and predated is not permitted.

A total of 1,376 packages were selected at random by store enumerators in 20 Birmingham stores and examined to ascertain the package marking. Of the packages examined, 1,347 (97.9%) were marked in conformance with the existing dating statute. Two of these packages were offered for sale after the expiration date marked on the package. Twenty-nine of the packages (2.1%) examined were unmarked. None of the packages examined was predated.

#### IN-STORE HANDLING PRACTICES: CAMDEN VS. PHILADELPHIA

##### *Size of stores and sales characteristics*

Thirty-one food stores in the dating market (Camden) were enumerated. During the calendar week previous to the date of enumeration, 30 of the stores (one store would not provide sales information) reported weekly sales of 119,877 quart-equivalent units of whole homogenized milk, or average weekly sales of 3,996 quart-equivalent units per store (Table 9). In the non-dating market (Philadelphia) 30 food stores were enumerated. During the calendar week previous to the date of enumeration, 27 stores (three would not provide sales information) reported weekly sales of 108,472 quart-equivalent units of whole homogenized milk, or average weekly sales of 4,018 quart-equivalent units per store. The 30 stores in the dating market were open a total of 200 days (average of 6.67 days per week) as opposed to 168 days for the 27 stores in the non-dating market (average of 6.22 days per week).

There were more small stores in the dating market sample than in the non-dating market sample (Table 10). Four-fifths of the stores in the dating market sold 800 or less quarts daily; whereas in the non-dating market, slightly over 60% of the stores had daily sales of 800 or fewer quarts.

A total of 14 different brands (including six private label brands) packaged by 11 processors were enumerated in dating market stores. Dating market stores carried an average of 1.1 brands and were served by 1.1 processors per store. In non-dating mar-

TABLE 9. SALES OF WHOLE HOMOGENIZED MILK BY SAMPLE STORES IN CAMDEN AND PHILADELPHIA.

	Camden	Philadelphia
	Dating market	Non-dating market
	(30 stores)	(27 stores)
	—(Quart-equivalent units)—	
Total weekly sales	119,877	108,472
Average weekly store sales	3,996	4,018
Range of weekly store sales	560 to 12,440	300 to 9,340
Average daily store sales	599	646
Range of daily store sales	93 to 1,777	43 to 1,557

TABLE 10. AVERAGE DAILY STORE SALES OF WHOLE HOMOGENIZED MILK BY STORE SIZE IN CAMDEN AND PHILADELPHIA

Average daily store sales	Camden			Philadelphia		
	No. of stores	Dating market		No. of stores	Non-dating market	
		Class	% of stores		Class	% of stores
			Cum.			Cum.
(Quart-equivalent units)						
1- 400	10	33.3	33.3	7	25.9	25.9
401- 800	14	46.7	80.0	10	37.0	62.9
801-1,200	4	13.4	93.4	6	22.2	85.1
1,201-1,600	1	3.3	96.7	4	14.9	100.0
1,601-2,000	1	3.3	100.0	—	—	—

ket stores, a total of nine different brands (including five private label brands) packaged by seven processors were enumerated. Twenty-nine of 30 stores in the non-dating market received regular deliveries from one processor only and the remaining store received regular deliveries from two. In all instances, the number of processors serving stores and the number of brands received by stores were the same.

*Delivery practices*

During the calendar week previous to the date of enumeration, the 31 stores in the dating market sample were open 207 days and received regular deliveries on 161 of those days or 77.8% of the days that the stores were open. During the same period, the 30 stores in the non-dating market sample were open 187 days and received regular deliveries on 151 of those days or 80.7% of the days that the stores were open.

Stores received regular deliveries of milk an average of 5.19 days per week and 5.03 days per week in the dating and non-dating markets, respectively. A comparative frequency distribution of delivery days per week for sample stores in the dating and non-dating markets is presented in Table 11.

Differences exist between the average delivery frequency on wholesale routes reported in the processor survey (Table 2, Part III) for New Jersey and the average delivery frequency reported by dating market stores. An unweighted average of delivery frequencies for small, medium, and large wholesale stops in New Jersey yielded an average of 5.76 days per week as compared to the 5.19 days per week reported in the store survey.

This difference could have resulted from several factors. First, the processor survey covered processors located throughout New Jersey, whereas the store survey was conducted only in the Camden marketing area. Second, one of the larger processors included in the processor survey reported an average delivery frequency of 6.0 regular deliveries per week as opposed to an average of 4.8 deliveries per week calculated from information provided by stores served

by this processor. Finally, one other large processor serving the Camden market did not respond to the processor survey. The average frequency of regular deliveries calculated from information provided by stores served by this processor was 4.7 deliveries per week. These two processors accounted for 59% of the total volume of milk included in the store survey.

*Milk availability and special deliveries*

Slight differences in milk availability were noted between dating and non-dating market stores. Seven of the 31 stores (22.6%) in the dating market reported selling out of whole homogenized milk one or more days during the calendar week previous to the date of enumeration (Table 12). These seven dating market stores reported selling out before closing a total of 12 times. During the same period, four of the 30 stores (13.3%) in the non-dating market reported

TABLE 11. DELIVERY DAYS PER WEEK FOR SAMPLE STORES IN CAMDEN AND PHILADELPHIA.

Delivery days per week	Camden			Philadelphia		
	No. of stores	Dating market		No. of stores	Non-dating market	
		Class	% of stores		Class	% of stores
			Cum.			Cum.
7	—	—	—	1	3.3	3.3
6	10	32.3	32.3	9	30.0	33.3
5	19	61.3	93.6	14	46.7	80.0
4	—	—	93.6	2	6.7	86.7
3	2	6.4	100.0	4	13.3	100.0

TABLE 12. PROPORTION OF STORE CLOSINGS WITHOUT WHOLE HOMOGENIZED MILK, CAMDEN AND PHILADELPHIA.

Item	Camden		Philadelphia	
	Dating market		Non-dating market	
	Number	Per cent	Number	Per cent
Stores in sample	31	100.0	30	100.0
Stores closing without whole homogenized milk on hand	7	22.6	4	13.3
Store closings	207	100.0	187	100.0
Store closings without whole homogenized milk on hand	12	5.8	6	3.2

selling out before closing a total of six times. Store closings without whole homogenized milk on hand—expressed as a percentage of store closings—yielded figures of 5.8% and 3.2% for dating and non-dating market stores, respectively. An analysis of the difference in store closings without whole homogenized milk on hand between dating and non-dating markets revealed that the observed difference was not statistically significant (the observed difference could have occurred due to random variation more than one time in four).

Special deliveries of whole homogenized milk were so few as to be of no consequence in either the dating or non-dating markets. Special deliveries to enumerated stores during the calendar week previous to the date of enumeration numbered one and two for dating and non-dating market stores, respectively. Special deliveries of whole homogenized milk—expressed as a percentage of the number of days that regular deliveries were received—yielded figures of 0.6% and 1.3% for dating and non-dating market stores, respectively.

#### *Milk inventories*

The previously cited differences in milk availability were not further reflected in differences in inventories for dating and non-dating market stores. Thirty of the dating market stores enumerated provided both the requested sales and inventory information. These 30 dating market stores reported average daily closing time inventories of whole homogenized milk totaling 7,497 quart-equivalent units and average daily sales totaling 17,723 quart-equivalent units during the calendar week previous to the date of enumeration. Twenty-seven of the non-dating market stores enumerated provided both the requested sales and inventory information. These 27 non-dating market stores reported average daily closing time inventories of whole homogenized milk totaling 7,175 quart-equivalent units and average daily sales totaling 17,628 quart-equivalent units during the calendar week previous to the date of enumeration. Average daily closing inventories of whole homogenized milk—expressed as a percentage of average daily sales of whole homogenized milk—yielded figures of 42.3% and 40.7% for dating and non-dating market stores, respectively.

#### *Returns*

Returns or disposals of whole homogenized milk due to date or code expiration were almost identical for the dating and non-dating markets. Twenty-nine dating market stores provided information on returns or disposals of whole homogenized milk during the calendar week previous to the date of enumeration. Three of the 29 dating market stores reported returns

or disposals totaling 56 quart-equivalent units due to date or code expiration. During the same period, these 29 dating market stores reported weekly sales of 116,237 quart-equivalent units of whole homogenized milk.

The 27 non-dating market stores that reported weekly sales of 108,472 quart-equivalent units of whole homogenized milk also provided information on returns or disposals. Four of the 27 non-dating market stores reported returns or disposals of 65 quart-equivalent units due to code expiration during the calendar week previous to the date of enumeration. Returns or disposals of whole homogenized milk due to date or code expiration—expressed as a percentage of weekly sales of whole homogenized milk—yielded figures of 0.05% and 0.06% for the dating and non-dating market samples, respectively.

Total returns reported in the processor survey for New Jersey were 3.2% and 1.0% for the dating and non-dating sections of the market, respectively (Table 4, Part III). Total returns (including returns due to date or code expiration) reported in the store survey were 0.98% and 0.63% for the dating and non-dating markets, respectively. Of the 0.98% returns reported by dating market stores, 0.58% was accounted for by one store that reported returning an abnormally high number of packages (675 quart-equivalent units) due to improper sealing. Elimination of this one abnormal response would result in a level of total returns approximating 0.4 to 0.5% for the dating market. The differences between levels of returns noted in the two surveys may be the result of different definitions of returns. In the store survey, all returns reported had to have been delivered to the store premises and subsequently returned to the processing plant or disposed of on the store premises; in the processor survey, the term returns might have been construed by respondents to include product which never left the truck as well as product which was returned from stores or otherwise disposed of. Another possible explanation for the higher level of returns reported in the processor survey (both dating and non-dating markets) is the fact that the processor survey covered returns of all fluid dairy products while the store survey dealt only with returns of whole homogenized milk (see footnote to Table 4, Part III).

#### *Package marking in Camden and Philadelphia*

In conjunction with the store handling practices survey, package marking surveys were conducted in both the dating and non-dating markets. Under the New Jersey dating statute, packages must bear the legend "pasteurized during the 24-hour period ending 6:00 a.m. (followed by the day of the week at the end of this period)." Predating is permitted but the product cannot be offered for sale prior to 12:01



a.m. of the day of the week appearing on the package.

Ninety-nine packages were selected at random by store enumerators in the 31 dating market stores and examined to ascertain the package marking. Only 82 of the 99 packages (82.8%) examined were marked in conformance with the existing dating statute. The remaining 17 packages (17.2%) did not conform to the package marking requirements of the dating market; in addition, seven of the conforming packages were marked with tomorrow's date and offered for sale before 12:01 a.m. tomorrow. Thus, a total of 24 of the packages (24.2%) examined were in violation of the dating statute.

Ninety packages were selected at random by store enumerators in the 30 non-dating market stores and examined to ascertain the package marking. Eighty-seven of the 90 packages (96.7%) were marked with a coded marking and three packages (3.3%) were unmarked. All unmarked containers were packaged by the same processor.

#### REFERENCE

1. Wilson, L. E. 1969. A study of returned out-of-date milk products in Jefferson County, Alabama. Paper presented at a meeting of the Alabama Milk Control Board, Montgomery, Alabama. 22 pp.

### REPORT OF THE COMMITTEE ON FROZEN FOOD SANITATION, 1969-1970

The question of "Legal Standards" and "Bacterial Guidelines" for frozen foods has been a topic of discussion for approximately 10 years. The Committee on Frozen Food Sanitation has explored this subject without attempting to reach any conclusion. The following comments on the subject are not necessarily the unanimous conclusions of the Committee, but rather reflect the thinking of individual members.

#### LEGAL STANDARDS

Legal Standards would provide a quality and uniformity index for both regulatory agencies and industry; however, it is felt that there is a lack of flexibility with legal standards. Research may indicate the need for change and it is difficult to revise legally established standards. It also is felt that research and experience must demonstrate the need for the establishment of a legal standard.

The Association of Food and Drug Officials of the United States has edited and published *Recommended Bacterial Limits for Frozen Precooked Beef and Chicken Pot Pies*. At this time the Committee is aware of only one state which has established a 50,000 standard plate count and five coliform organisms per gram for all frozen foods.

The increased use of microwave ovens has created some concern that time and temperature relationships may not be adequate to destroy pathogenic organisms. Some members of the Committee feel that this problem creates a need for legal standards while other members do not.

#### BACTERIAL GUIDELINES

The Committee has expressed two viewpoints concerning guidelines. One is that guidelines could serve as a tool for both industry and regulatory agencies until legal standards

can be established. The other viewpoint is that guidelines will provide the consumer the protection he deserves, and that if a segment of the industry chose to avoid either guidelines or legal standards, it could do so without too much penalty. The Committee is not aware of established guidelines for all frozen foods, and most members feel that additional work is needed before a meaningful guideline can be established.

Available information indicates that the public health record of the frozen food industry has been satisfactory. Improper handling and preparation by the consumer has resulted in some problems. It has, therefore, been suggested that a consumer educational program on proper handling and preparation of frozen food products from time of purchase would be advisable.

#### COMMITTEE MEMBERS

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# FATE OF COLIFORMS IN YOGURT, BUTTERMILK, SOUR CREAM, AND COTTAGE CHEESE DURING REFRIGERATED STORAGE<sup>1</sup>

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(Received for publication September 8, 1970)

## ABSTRACT

*Aerobacter (Enterobacter) aerogenes* and *Escherichia coli* were inoculated separately into commercially produced samples of yogurt, buttermilk, sour cream, and cottage cheese. Inoculated products were stored at 7.2 C and were tested daily for up to 10 days to determine changes in numbers of coliforms and in pH values. The number of viable coliforms in yogurt declined dramatically and was markedly different from the initial value after only 24 hr of storage. Usually, survival of coliforms in yogurt did not exceed 3 days of holding. In buttermilk, most often a marked decline in numbers of coliforms was evident after 24 hr of storage. A substantial reduction in numbers (>50% of organisms present initially) of *A. aerogenes* B199 occurred in sour cream during the first 24 hr of storage, but a similar decline in numbers of *E. coli* and *A. aerogenes* FD was not evident until after 3 days of storage. Changes in numbers of *E. coli* and *A. aerogenes* in cottage cheese generally were not as rapid as in other products during the first days of storage. A few cottage cheese samples, however, did support rapid increases in test culture numbers. Because of the rapid decline in numbers of coliforms in yogurt, buttermilk, and sour cream, the provision in *Standard Methods for the Examination of Dairy Products* that permits examination of some of these products for up to 48 hr after manufacture seems inadvisable.

*Standard Methods for the Examination of Dairy Products (1)* specifies that coliform tests on cottage cheese be done within 24 hr after manufacture and that cultured milks be tested for coliforms within 48 hr after pasteurization. This requirement has made it difficult for receiving communities to do meaningful testing on these products when shipping times of more than 24 or 48 hr are involved.

Although it is generally believed that coliforms will be inactivated in acid products such as yogurt, cultured buttermilk, and sour cream, and conversely, will multiply in cottage cheese, definitive data to indicate the rate of death or multiplication are lacking. The present study was undertaken to characterize

the growth or destruction of some coliforms when suspended in these foods and held at a refrigeration temperature.

## MATERIALS AND METHODS

### Cultures

Two cultures each of *Escherichia coli* and *Aerobacter (Enterobacter) aerogenes* were used throughout this study (*E. coli* H52 and FD, *A. aerogenes* NRRL-B199 and FD). The FD strains were recent isolates from dairy products. Inocula for *E. coli* H52 and *A. aerogenes* B199 were prepared from skim milk cultures. The FD strain inocula were prepared by harvesting growth from 24-hr-32 C-trypticase soy agar slants and then washing it 3 times in distilled water before inoculating the dairy products under test.

### Products: Selection, inoculation, and examination

Dairy products used were obtained from grocery stores or the university dairy plant, except some cottage cheese samples which were obtained from processing plants. All samples were pretested for coliforms and only those that had initial concentrations of <10/g were used in this study.

Products other than buttermilk were inoculated and then stirred manually until the inoculum was dispersed homogeneously throughout the food. With buttermilk, the inoculum was mixed into the product by shaking. After inoculation, each sample of product was divided into 2 portions and each portion placed into a sterile beaker covered with aluminum foil. Samples were held at 7.2 C and were tested daily for a 10-day period to determine pH values and numbers of coliforms. Coliform counts were carried out as described in *Standard Methods for the Examination of Dairy Products (1)*. Violet red bile agar was used as the coliform medium throughout this study. Data in tables represent averages of results from tests on the 2 subsamples prepared from each product.

## RESULTS AND DISCUSSION

### Yogurt

Data on the behavior of *A. aerogenes* and *E. coli* in yogurt are summarized in Tables 1 and 2. It is evident that all test cultures were rapidly inactivated in this product; in many instances coliforms were essentially undetectable after 24 hr of storage. In fact, inactivation of coliforms in yogurt was so rapid

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

TABLE 1. FATE OF *Aerobacter aerogenes* IN YOGURT DURING REFRIGERATED STORAGE.

Sample	No. <i>A. aerogenes</i> /gram after days					pH values of yogurt after days				
	0	1	2	3	4	0	1	2	3	4
<i>A. aerogenes</i> B199 <sup>1</sup>										
1	330	35	<1	—	—	4.10	4.10	3.95	—	—
2 <sup>2</sup>	700	420	170	40	7	4.40	4.40	4.25	4.20	4.18
3	65	<1	—	—	—	4.00	4.05	—	—	—
4	<10	<1	—	—	—	4.10	4.05	—	—	—
5	<10	<1	—	—	—	4.00	3.90	—	—	—
<i>A. aerogenes</i> FD										
1	660	<1	<1	3	<1	3.74	3.84	3.71	3.69	3.68
2	950	20	<1	<1	<1	3.95	3.68	3.85	3.87	3.80
3	1000	<1	<1	<1	<1	3.88	3.81	3.80	3.85	3.77
4	740	<1	<1	<1	<1	4.00	3.90	3.98	3.85	3.95
5	770	<1	<1	<1	<1	3.65	3.65	3.72	—	—

<sup>1</sup>A similar inoculum was used for all samples. Inactivation of *A. aerogenes* in the product was so rapid that only a fraction of the organisms added to some samples could be recovered initially.

<sup>2</sup>Peach yogurt.

TABLE 2. FATE OF *Escherichia coli* IN YOGURT DURING REFRIGERATED STORAGE.

Sample	No. <i>E. coli</i> /gram after days					pH value of yogurt after days				
	0	1	2	3	4	0	1	2	3	4
<i>E. coli</i> H52 <sup>1</sup>										
1	1100	260	23	<10	<1	4.20	4.05	4.05	4.05	4.05
2	110	1	<1	—	—	4.35	4.19	4.19	—	—
3	30	<1	—	—	—	4.04	4.00	—	—	—
4	28	<1	—	—	—	4.10	4.05	—	—	—
5	410	13	5	1	<1	4.20	4.10	4.10	4.00	4.00
<i>E. coli</i> FD										
1	110	<1	<1	1	<1	3.75	3.75	3.67	3.70	—
2	600	130	120	38	17	3.95	3.95	3.95	3.84	3.90
3	430	60	30	16	<1	3.91	3.88	3.88	3.77	3.79
4	490	110	20	31	15	3.90	3.90	3.95	3.85	3.92
5	150	<1	<1	<1	<1	3.65	3.65	3.65	3.62	3.78

<sup>1</sup>A similar inoculum was used for all samples. Inactivation of *E. coli* in the product was so rapid that only a fraction of the organisms added to some samples could be recovered initially.

TABLE 3. FATE OF *Aerobacter aerogenes* IN CULTURED BUTTERMILK DURING REFRIGERATED STORAGE.

Sample	No. <i>A. aerogenes</i> /gram after days										pH values of buttermilk after days					
	0	1	2	3	4	5	6	7	8	9	10	0	1	3	5	10
<i>A. aerogenes</i> B199																
1	890	640	580	420	350	310	280	220	160	65	35	4.90	4.70	4.45	4.40	4.40
2	850	670	600	440	420	340	270	250	170	100	33	4.85	4.65	4.45	4.45	4.40
3	880	640	520	460	450	345	260	210	170	140	70	4.85	4.80	4.60	4.60	4.60
4	750	680	470	380	370	290	170	100	40	18	13	4.80	4.70	4.45	4.45	4.45
5	840	690	510	410	380	340	280	200	160	110	130	4.70	4.60	4.40	4.40	4.40
<i>A. aerogenes</i> FD																
1	720	330	150	120	50	25	20	<1	<1	<1	<1	4.15	4.15	—	4.25	4.25
2	690	210	65	10	<1	<1	<1	<1	—	—	—	4.12	4.10	—	4.18	4.15
3	650	180	75	30	15	<1	<1	—	<1	<1	<1	4.15	4.10	—	4.15	4.18
4	740	320	220	170	160	80	60	25	10	—	<1	4.30	4.30	—	4.40	4.35
5	720	440	280	180	220	200	100	80	20	35	<1	4.35	4.35	—	4.42	4.58

TABLE 4. FATE OF *Escherichia coli* IN CULTURED BUTTERMILK DURING REFRIGERATED STORAGE.

Sample	No. <i>E. coli</i> /gram after days											pH values of buttermilk after days				
	0	1	2	3	4	5	6	7	8	9	10	0	1	3	5	10
<i>E. coli</i> H52																
1	1300	1100	950	980	780	570	350	260	120	35	17	4.30	4.30	4.30	4.20	4.20
2	1600	880	450	220	65	28	18	10	13	8	<1	4.80	4.60	4.55	4.55	4.50
3	1500	1300	1100	700	380	170	23	20	10	<1	<1	4.80	4.65	4.60	4.60	4.55
4	1400	940	750	380	310	100	28	28	25	13	7	4.70	4.50	4.45	4.45	4.45
5	1400	960	650	330	220	110	20	13	15	<1	<1	4.50	4.35	4.35	4.35	4.35
<i>E. coli</i> FD																
1	920	600	450	310	260	110	70	30	20	<1	<1	4.15	4.15	—	4.28	4.22
2	730	640	370	260	80	110	35	40	20	10	<1	4.12	4.10	—	4.20	4.15
3	520	380	280	200	170	110	65	210	20	10	<1	4.15	4.10	—	4.39	4.35
4	670	460	470	360	280	300	180	260	180	95	85	4.30	4.30	—	4.39	4.35
5	740	440	450	440	300	170	320	200	230	130	70	4.35	4.35	—	4.45	4.55

TABLE 5. FATE OF *Aerobacter aerogenes* IN SOUR CREAM DURING REFRIGERATED STORAGE.

Sample	No. <i>A. aerogenes</i> /gram after days									pH values of sour cream after days						
	0	1	2	3	4	5	6	7	8	9	0	1	3	5	7	9
<i>A. aerogenes</i> B199																
1	860	350	210	130	65	55	23	23	3	<1	4.50	4.40	4.30	4.30	4.30	4.30
2	870	550	230	140	50	30	10	5	<1	—	4.70	4.60	4.55	4.55	4.60	—
3	780	340	110	13	<1	—	—	—	—	—	4.50	4.40	4.35	—	—	—
4	780	210	50	<1	—	—	—	—	—	—	4.60	4.50	4.40	—	—	—
5	860	330	140	63	30	8	3	<1	—	—	4.65	4.55	4.50	4.0	4.00	—
<i>A. aerogenes</i> FD																
1	1000	520	180	30	15	25	2	<1	<1	<1	4.18	4.05	4.12	4.17	4.17	4.20
2	1400	1300	640	680	170	160	130	35	20	3	4.20	4.21	4.15	4.19	4.17	4.22
3	1000	720	490	280	160	40	5	<1	<1	1	4.25	4.25	4.19	4.20	4.25	4.35
4	1400	860	720	400	200	270	160	140	80	20	4.50	4.42	4.40	4.40	4.47	4.60
5	770	220	10	<1	1	<1	<1	<1	<1	—	4.35	4.25	4.10	4.30	4.25	—

TABLE 6. FATE OF *Escherichia coli* IN SOUR CREAM DURING REFRIGERATED STORAGE.

Sample	No. <i>E. coli</i> /gram after days											pH value of sour cream after days					
	0	1	2	3	4	5	6	7	8	9	10	0	1	3	5	7	10
<i>E. coli</i> H52																	
1	1700	1300	930	500	370	370	300	400	300	180	200	4.70	4.70	4.61	4.60	4.60	4.60
2	1700	930	590	260	73	110	85	100	63	85	18	4.60	4.60	4.57	4.50	4.50	4.50
3	1700	400	240	95	70	30	23	50	4	<1	—	4.60	4.60	4.55	4.50	4.50	—
4	1500	1400	1200	1100	1000	1000	1000	1100	1000	880	830	4.69	4.65	4.65	4.70	4.70	4.70
5	940	800	680	560	570	370	200	190	110	38	8	4.40	4.40	4.33	4.20	4.20	4.20
<i>E. coli</i> FD																	
1	1000	520	360	30	30	6	13	<1	<1	<1	<1	4.18	4.21	4.14	4.15	4.15	—
2	1300	1600	740	380	260	160	90	25	5	<1	<1	4.20	4.25	4.10	4.10	4.15	4.15
3	980	820	550	340	150	170	70	90	50	<1	33	4.25	4.25	4.15	4.20	4.25	4.40
4	1400	1000	740	420	600	280	200	200	100	140	200	4.50	4.50	4.40	4.45	4.55	4.80
5	680	350	60	10	10	4	2	2	1	1	<1	4.20	4.20	4.15	4.25	4.25	4.25

with *A. aerogenes* B199 that sometimes an initial number could not be detected and at other times only a limited number of those organisms added were recovered a short time (< 30 min) later. It is noteworthy that 6 samples of yogurt permitted somewhat longer survival of coliforms, but even here the numbers declined markedly during the first 24 hr of

storage. The pH value of products in which coliforms survived longer tended to be somewhat higher initially but then dropped during storage. This may have contributed to the demise of the coliforms.

#### Cultured buttermilk

The behavior of *A. aerogenes* and *E. coli* in butter-

TABLE 7. FATE OF *Aerobacter aerogenes* IN COTTAGE CHEESE DURING REFRIGERATED STORAGE.

Sample	No. <i>A. aerogenes</i> /gram after days										pH value of cottage cheese after days						
	0	1	2	3	4	5	6	7	8	9	10	0	1	3	5	7	10
<i>A. aerogenes</i> B199																	
1	1000	1100	980	700	700	800	550	590	500	410	410	4.93	4.93	5.00	4.88	4.76	4.90
2	1100	1300	950	750	640	690	620	350	250	180	75	4.94	4.95	5.00	4.79	4.74	4.69
3	1100	5900	20000	16000	15000	15000	13000	8200	7300	7300	3300	4.94	4.90	5.01	4.83	4.94	4.87
4	1100	960	920	850	800	770	680	590	620	400	370	4.94	4.95	4.93	4.83	4.81	4.78
5	1100	900	880	600	600	560	470	340	280	170	130	4.94	4.95	5.03	4.90	4.81	4.59
<i>A. aerogenes</i> FD																	
1	140	130	140	110	120	140	120	120	160	200	340	4.80	4.80	4.80	4.80	4.70	4.75
2	140	170	160	130	210	330	540	1200	1900	3200	7100	4.85	4.80	4.85	4.70	4.75	4.83
3	100	120	260	670	2600	4000	3200	7x10 <sup>4</sup>	3x10 <sup>5</sup>	7x10 <sup>5</sup>	5x10 <sup>6</sup>	5.00	5.05	4.98	4.95	4.90	4.85
4	110	130	340	440	1400	990	1800	1300	2600	2x10 <sup>4</sup>	2x10 <sup>4</sup>	4.95	4.95	4.78	4.75	4.70	4.70

TABLE 8. FATE OF *Escherichia coli* IN COTTAGE CHEESE DURING REFRIGERATED STORAGE.

Sample	No. <i>E. coli</i> /gram after days										pH value of cottage cheese after days						
	0	1	2	3	4	5	6	7	8	9	10	0	1	3	5	7	10
<i>E. coli</i> H52																	
1	2200	2200	2000	1900	2100	2000	1700	1600	1500	870	500	5.10	5.00	4.84	4.76	4.73	4.61
2	2100	2200	2200	2100	2000	2100	1900	1900	1700	1800	1800	5.10	5.02	4.99	5.03	5.18	5.10
3	6400	7900	8800	3x10 <sup>4</sup>	5x10 <sup>4</sup>	8x10 <sup>4</sup>	1x10 <sup>5</sup>	1x10 <sup>6</sup>	1x10 <sup>6</sup>	2x10 <sup>6</sup>	5x10 <sup>6</sup>	5.10	5.03	5.05	5.08	5.13	4.99
4	890	830	860	780	730	790	760	720	640	690	610	5.06	5.01	4.93	4.75	4.72	4.67
5	680	780	750	755	830	790	670	670	690	680	650	5.07	5.07	5.09	5.08	5.05	5.02
<i>E. coli</i> FD																	
1	140	190	180	160	150	190	150	190	260	340	360	4.80	4.80	4.85	4.80	4.70	4.75
2	140	125	180	170	160	170	140	160	160	180	120	4.85	4.80	4.78	4.70	4.80	4.85
3	110	85	140	120	150	130	120	140	160	170	120	5.05	5.02	4.85	4.95	4.85	4.80
4	140	115	140	175	200	150	160	170	140	220	150	4.95	4.92	4.85	4.80	4.80	4.75

milk is detailed by data presented in Tables 3 and 4. Four of the 20 samples produced greater than 50% reduction of the test organism during the first 24 hr of storage. Additional decline in numbers occurred during further storage so that, after 4 days, the population nearly always declined to less than 50% of the number initially present. In general, *E. coli* appeared to be somewhat more sensitive than *A. aerogenes* to the destructive effects of buttermilk.

#### Sour cream

The decline in numbers of *A. aerogenes* and *E. coli* during storage of inoculated sour cream is reported in Tables 5 and 6. In general, *A. aerogenes* appeared slightly more sensitive to the destructive effects of sour cream than did *E. coli*. The number of *A. aerogenes* B199 was nearly always reduced by at least 50% during the first 24 hr of storage, whereas only 2 samples containing *E. coli* exhibited a similar inactivation of the organism. *Aerobacter aerogenes* was not detectable after 7 days in 7 of the 10 sour creams under test. *Escherichia coli*, on the other hand, survived for 10 days in 6 of the 10 samples examined.

#### Cottage cheese

Tables 7 and 8 record data obtained when cottage

cheese was inoculated with coliforms and then stored. It is evident that survival of *E. coli* and *A. aerogenes* was greater (both in terms of time and numbers) in this product than in yogurt, buttermilk, or sour cream. In fact, growth of coliforms was evident in some of the samples tested. The pH values of most cottage cheese samples declined during storage, but this change often did not appear related to either survival or demise of coliforms in the product.

The maximum storage time before testing cultured milks and cream for coliforms is designated in *Standard Methods for the Examination of Dairy Products* (1) as 48 hr. Because of the rapid inactivation of the 4 test organisms under study when in yogurt and sour cream, meaningful tests could not be carried out after 24-hr storage. Although the reduction in numbers of test organisms in buttermilk after 24 hr was not as great as in yogurt or sour cream, the reduction was significant. After 48 hr of storage, the test organism concentration averaged one-half of the initial population.

For cottage cheese, *Standard Methods for the Examination of Dairy Products* (1) recommends that the coliform count be carried out within 24 hr after manufacture. Although most counts on cottage cheese

were relatively stable for 48 hr, 2 of the 18 samples tested supported a dramatic increase in test culture numbers within this period. If we could assume the growth of the test organisms studied to be typical of that for coliforms found in cottage cheese, the substantial increase in coliform count within 48 hr after manufacture in about 10% of the lots of cottage cheese could result in an adverse regulatory action being applied unfairly if testing were delayed to 48 hr after manufacture. For this reason, it would seem desir-

able to continue to test cottage cheese within 24 hr after manufacture.

#### ACKNOWLEDGMENT

This study was supported in part by Public Health Service Grant 86-68-158 to the American Public Health Association.

#### REFERENCE

1. Walter, W. G. (ed.). 1967. 'Standard methods for the examination of dairy products. American Public Health Association, New York.

## ASSOCIATION AFFAIRS

### REPORT OF THE EDITOR

### JOURNAL OF MILK AND FOOD TECHNOLOGY 1969-1970

#### REVIEW OF VOLUME THIRTY-TWO

Volume 32 of the *Journal of Milk and Food Technology* was completed with publication of the December, 1969 issue. This was a record-breaking volume in that it contained more pages (624) and more papers (87) than any preceding volume of the *Journal*. Forty-seven research papers and 14 technical papers of general interest were published. This material utilized approximately 45% of the total pages, whereas non-technical papers of general interest, association affairs, and news and events occupied 30% of the pages. Advertising, covers, index, standards, etc. made up the remaining 25% of volume 32. This distribution, when compared to that of volume 31, reflects an increase (5%) in space devoted to technical material and decreases in space utilized for non-technical material (2%) and for covers, advertising, etc. (3%). The composition of volume 32 is detailed more completely in Table 1 together with similar information for volumes 30 and 31.

Research papers in volume 32 dealt with a variety of topics including studies on the plate count method, oxidase method, resazurin test, changes during cooking of crab, tests for abnormal milk, composition of apples, egg pasteurization, fat tests for skim milk, cleaned-in-place systems, coliform bacteria, food-plant wastes, microbiology of meats, microbiology of feedstuffs, methods to enumerate molds, microbial quality of imitation dairy products, Cheddar cheese, low-fat spreads, salmonellae in cheese, methods to measure aflatoxins, salmonellae in butter, and other subjects. General interest papers dealt with such topics as interstate milk shipments, fluorescent antibody technique, imitation foods, methods for detection of viruses and of *Clostridium perfringens*, *C. perfringens* food poisoning, recirculation cleaning, vector control, milk powder, testing of milk, solid wastes disposal, and other topics.

#### PRESENT STATUS OF VOLUME THIRTY-THREE

The first six issues of volume 33 contained 25 research

papers, 7 general interest technical papers, and 8 non-technical general interest papers. This compares with 24, 7, and 10 papers in the same categories in the first six issues of volume 32. In addition, 48 pages of E-3A and 3A standards have appeared in volume 33. The first six issues of volume 33 contained 16 more pages than did similar issues of volume 32.

On July 15, 1970 there was a backlog of 48 papers ready for publication. This included 28 research papers and 20 general interest (technical and nontechnical) papers among which are 7 papers from the 1969 annual meeting and 1 from the 1970 annual meeting. In addition, on July 15 there were 16 research papers being reviewed or revised. The backlog would not be as great if 48 pages had not been devoted to standards. As a consequence, it will be necessary to add extra pages to most, if not all, remaining issues in volume 33 so that research papers can be published within six (or less) months after they are received.

#### REVIEW PAPERS

In the Editor's opinion, review papers are extremely helpful to busy people so they can keep up-to-date on developments in fields which are not their immediate concern. Consequently a number of persons (many suggested by members of the Editorial Board) have been asked to prepare reviews on timely topics. Three such review papers, dealing with activation of bacterial spores, nitrites and nitrates in the environment, and carcinogens in the environment, are awaiting publication. Additional review papers should be received in the near future.

#### EDITORIALS

Last year the Journal Management Committee suggested that consideration be given to the regular use of editorials. Consequently, the Editor contacted all members of the Editorial Board to determine their interest in and willingness to prepare editorials. There was no ground-swell of enthusiasm for editorials although some members of the Board favored their use. Many board members felt that editorials in technical journals often are a waste of space, especially if one has to appear each month and no one really has anything worthwhile to say. Some Board members felt that the "Letter to the Editor" could serve the same purpose since

TABLE 1. SUMMARY OF CONTENTS OF JOURNAL OF MILK AND FOOD TECHNOLOGY FOR 1967, 1968, AND 1969

Item	Volume 30 (1967)	Volume 31 (1968)	Volume 32 (1969)
1. Total pages, including covers	512	540	624
2. Research papers			
a. Number	30	32	47
b. Pages	137	142	205
c. Per cent of total pages	26.7	26.3	32.9
3. General interest papers-technical			
a. Number	11	16	14
b. Pages	47	74	87
c. Per cent of total pages	9.2	13.7	12.2
4. General interest papers-nontechnical			
a. Number	23	14	26
b. Pages	72	65	91
c. Per cent of total pages	14.1	12.0	14.6
5. Association affairs			
a. Pages	64	68	62
b. Per cent of total pages	12.5	12.6	9.9
6. News and events			
a. Pages	51	42	36
b. Per cent of total pages	9.9	7.8	5.8
Per cent of pages-technical material	35.9	40.0	45.1
Per cent of pages-nontechnical material	36.5	32.4	30.3
Per cent of pages-covers, advertising, standards, index, etc.	27.6	27.6	24.6

some of the letters really are "mini-editorials."

#### EDITORIAL BOARD

The Editorial Board has been expanded to include 25 specialists in university, government, and industry research organizations. During 1969 the following persons were added to the Board: Mr. E. F. Baer, Professor Herman Koren, Dr. B. J. Liska, Dr. D. S. Postle, Dr. W. D. Powrie, Dr. R. B. Read, Jr., Dr. D. F. Splittstoesser, and Dr. W. G. Walter. In spite of these additions, it has been necessary to send papers for review to persons not on the Board. Thus far in

1970 help has been received from: Dr. Owen Fennema, Dr. F. J. Francis, Dr. J. M. Goepfert, Dr. H. W. Jackson, Dr. L. H. Schultz, Dr. E. G. Stimpson, Dr. H. L. A. Tarr, Mr. G. S. Torrey, Mr. Leon Tumerman, Dr. J. H. von Elbe, and Dr. W. C. Winder. It is apparent to the Editor that further expansion of the Editorial Board is necessary to provide expertise in additional areas of food science, technology, and hygiene.

E. H. MARTH  
Editor

*Journal of Milk and Food Technology*

### ANNOUNCEMENT CONCERNING THE SANITARIANS AWARD FOR 1971

Announcement is made that nominations will be accepted for the annual Sanitarians Award until June 1, 1971, and the members of the International Association of Milk, Food and Environmental Sanitarians, Inc. are requested to give consideration to the nomination of individuals whose professional work in the field of milk, food, or environmental sanitation has been outstanding.

The Award consists of a Certificate of Citation and \$1,000 in cash, and is sponsored jointly by the Diversey Chemical Corporation, Klenszade Products, Inc., and Pennwalt Corporation. It is administered by the International Association of Milk, Food and Environmental Sanitarians, Inc., and is presented annually. The next presentation of the Sanitarians Award will be made at the 58th annual meeting of

the Association which is to be held at San Diego, Calif., in August 1971.

The Executive Board of the Association has established the following rules and procedures governing the Sanitarians Award.

#### *Eligibility:*

##### 1. *General Criteria*

To be eligible for nomination the Sanitarians Award offered annually by the International Association of Milk, Food and Environmental Sanitarians, candidates must:

- Have been a member of IAMFES in good standing for a period of five years prior to the date when the Award is to be presented;
- Be a living citizen of the United States or Canada who, at the time of nomination, is employed as a professional sanitarian in the field of milk, food, and/or environmental sanitation

by a county, municipality, state or federal government provided that in the odd years beginning with 1969 the Sanitarians Award will be limited to state and federal employees and the even years to county and municipal employees.

Members of the Executive Board, members of the Committee on Recognition and Awards of the International Association of Milk, Food, and Environmental Sanitarians, and industry members shall not be eligible for the Award. Race, sex or age shall not enter into the selection of the Award recipient.

- c. Have made a meritorious contribution in the field of milk, food or environmental sanitation, to the public health and welfare of a county, counties, district, state or federal government with the United States or Canada.
  - d. Have completed the achievements and contributions on which the nomination is based during the seven-year period immediately preceding January 1, of the year in which the Award is to be made.
2. *Additional Criteria*
- a. Co-workers are eligible for nominations if both have contributed equally to the work on which the nomination is based and each independently meets the other qualifications for nomination.
  - b. Where co-workers are selected to receive the Award, each shall receive a certificate and share equally in the cash accompanying the Award.
  - c. No person who has received, or shared in receipt of the Award, shall be eligible for re-nomination for this Award.

#### *Nominations*

Nominations of candidates for the Sanitarians Award may be submitted by the Affiliate Associations of the IAMFES, or by any member of the Association in good standing except members of the Executive Board, members of the Committee on Recognition and Awards, and employees of the sponsoring companies. Nominations from persons who are not members of the Association cannot be accepted. No member or Affiliate may nominate more than one candidate in any given year.

Each nomination must be accompanied by factual information concerning the candidate, a resume of his work and achievements, evidence supporting his achievements and if available, reprints of publications. A form for the submission of nominations may be obtained upon request from H. L. Thomasson, Executive Secretary, International Association of Milk, Food and Environmental Sanitarians, Inc.,

P. O. Box 437, Shelbyville, Indiana 46176.

#### *Submission of Nominations*

The deadline for submission of nominations is set annually, and all nominations and supporting evidence must be postmarked prior to midnight of that date. The deadline this year is June 1, 1971. Nominations should be submitted to Sam O. Noles, Chairman, Committee on Recognition and Awards.

#### *Selection of the Recipient*

The Committee on Recognition and Awards of the International Association of Milk, Food and Environmental Sanitarians, Inc., has full responsibility for selecting from among the candidates nominated the recipient of the Sanitarians Award. In judging the contributions of each candidate, the Committee will give special consideration to (a) originality of thought, mode of planning, and techniques employed, (b) the comprehensive nature of the candidate's achievements, and (c) their relative value as they affect the health and welfare of the area served by the candidate. The Committee will give consideration also to the efforts of the candidate to establish professional recognition in the area in which he serves, as well as to his research, administrative development, program operation and educational achievements. Additional information or certification of submitted information will be requested when considered necessary by the Committee. Testimonial letters in behalf of a candidate are not desired.

If after reviewing the nominations and supporting evidence, the Committee decides that the work and achievements of none of the candidates have been significantly outstanding, the Award shall not be made. In this connection, it is fundamental that if meritorious professional achievement cannot be discerned the Award shall be omitted for a year rather than to lower the standards for selections of a recipient.

Sam O. Noles, Chairman  
Committee on Recognition and Awards,  
State Board of Health,  
Jacksonville, Fla. 32201

### **CONFERENCE ON WASTE MANAGEMENT AND POLLUTION CONTROL IN FOOD PROCESSING**

Waste management and pollution control in food processing is the theme of a two-and-a-half-day conference at Virginia Tech during the 1971 Food Industry Week. Representatives from industry, government, and the University are expected at the conference Feb. 8-10 at the Donaldson Brown Center for Continuing Education. They will discuss problems and possible solutions concerning wastes from



dairy, fruit and vegetables, red meats and poultry and seafood processing. General and special sessions will deal with responsibility in pollution control, water quality requirements, in-plant waste management, waste treatment and disposal, and appraisal and solution to problems.

Invited speakers include Walter Mercer, director of Western Research Laboratory, National Canners Association, Berkeley, Cal.; John H. Litchfield, manager, biology and medical science section, Battelle Memorial Institute, Columbus, O.; Herbert A. Schlesinger, consultant, Lockwood Greene Engineers, New York City; Strother Smith, attorney-at-law, Abingdon; and A. H. Paessler, executive secretary, State Water Control Board, Richmond. Several faculty members from Tech's departments of biology and civil engineering are also on the program. J. David Baldock, assistant professor of food science and technology, and Edward B. Hale, associate professor of agricultural engineering, are conference directors. Both men are also extension specialists.

This conference is supported by a grant under Title I of the Higher Education Act of 1965 and is sponsored by the departments of food science and technology and agricultural engineering and Tech's Extension Division.

**FIRST ANNUAL FLORIDA DAIRY FOOD INDUSTRIES CONFERENCE**

The First Annual Florida Dairy Food Industries Conference will be held at the Robert Meyer Motor Inn, Orlando, Florida on February 24.

For additional information, please contact Dr. C. Bronson Lane, 203 Dairy Science Building, University of Florida, Gainesville, Florida 32601.

**COURSE IN SAFETY IN THE LABORATORY PHS-DHEW MARCH 29 - APRIL 2, 1971**

This course is intended to train safety managers, laboratory staff members and related workers in accidental injury control. The course provides an opportunity for existing staff members to be re-appraised of the facilities and methods at their disposal to minimize the occurrence of injury in the laboratory.

Administrative and organization aspects as well as design and construction of laboratory equipment and facilities will be discussed; including plumbing, electrical, and other appropriate standards. Methods of handling reagents, biological, pathological, radioactive materials, and various specimens will be reviewed. An Animal care and related facilities pro-

tection from extreme temperatures, and waste storage and disposal are included. In addition, the trainee will have opportunity to investigate ventilation systems, clean rooms, diseases transferable to man, emergency aid, noise, and other pertinent subject matter. For further information contact Chief, Training Branch, Bureau of Occupational Safety and Health, 1014 Broadway, Cincinnati, Ohio 45202.

**IAMFES PRESIDENT AND IMS CHAIRMAN TO ADDRESS FLORIDA ASSOCIATION MEETING**

Dick Whitehead, President of the IAMFES, and Shelby Johnson, IMS Chairman, will speak at the 1971 Florida Association of Milk, Food, and Environmental Sanitarians annual meeting. This event is scheduled for March 16-18 at the Langford Hotel in Winter Park, Florida.

Other confirmed speakers at this time include Dr. George Muck, Dean Foods, Rockford, Illinois; Dr. C. Bronson Lane, Associate Professor, University of Florida Department of Dairy Science, Gainesville, Florida; Mr. Bob Rutgerson, President of Foss-America, Inc., Fishkill, New York; Dr. H. H. Van Horn, Chairman of the University of Florida Department of Dairy Science.

An outstanding Florida dairy industry man and sanitarian will be recognized at the awards banquet on March 18, according to FAMFES President Dave Fry.

For further information, please contact: Dr. C. Bronson Lane, 203 Dairy Science Building, University of Florida, Gainesville, Florida 32601.

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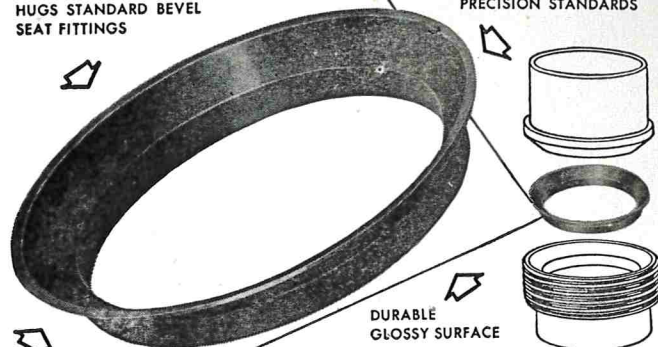
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