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**Technology**

**61ST ANNUAL MEETING**

**ST. PETERSBURG HILTON**

**August 12, 13, 14, 1974**

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(Reservations Page 1)

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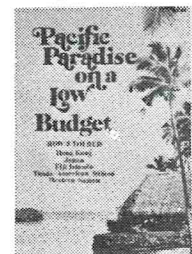
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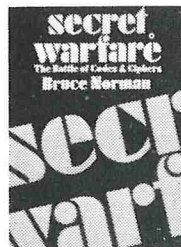
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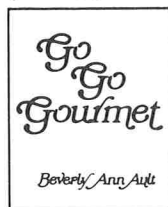
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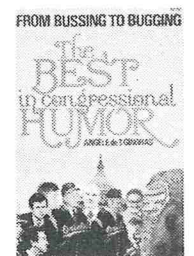
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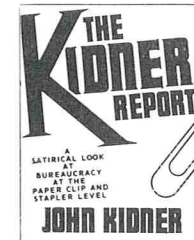
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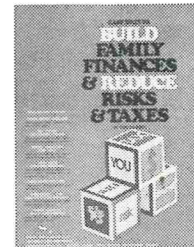
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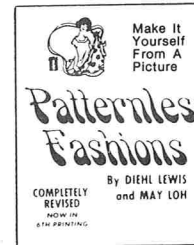
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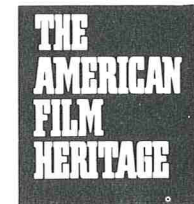
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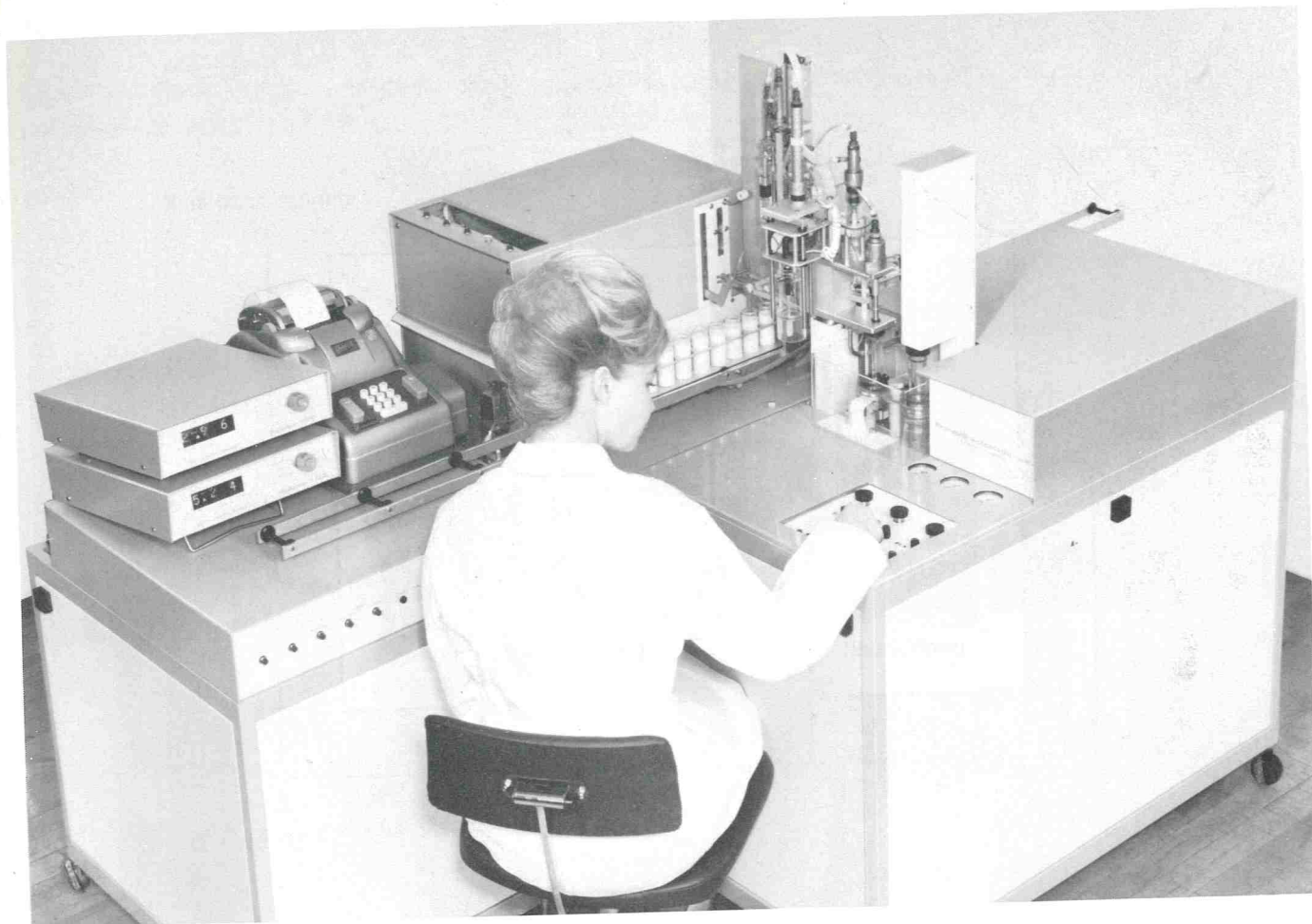


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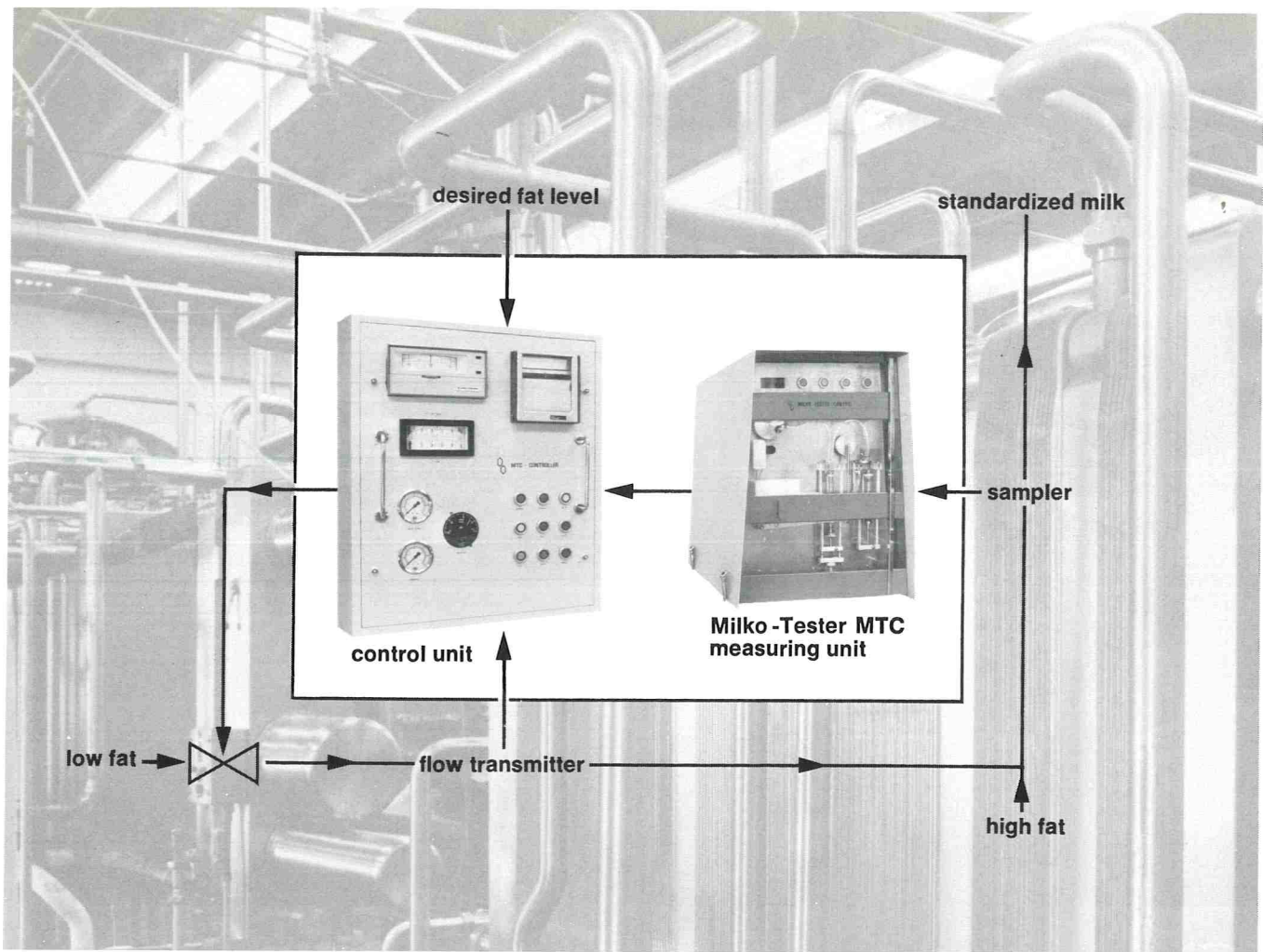
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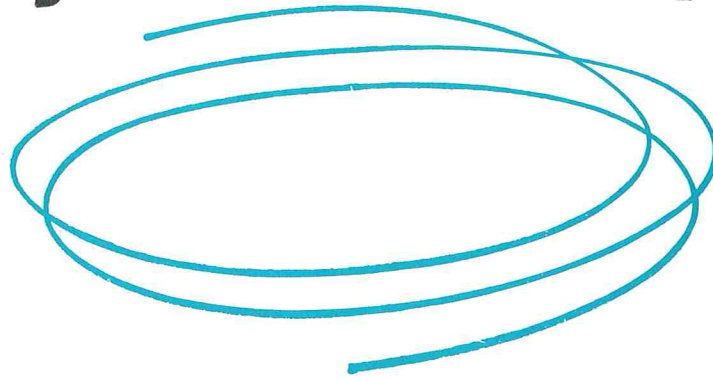
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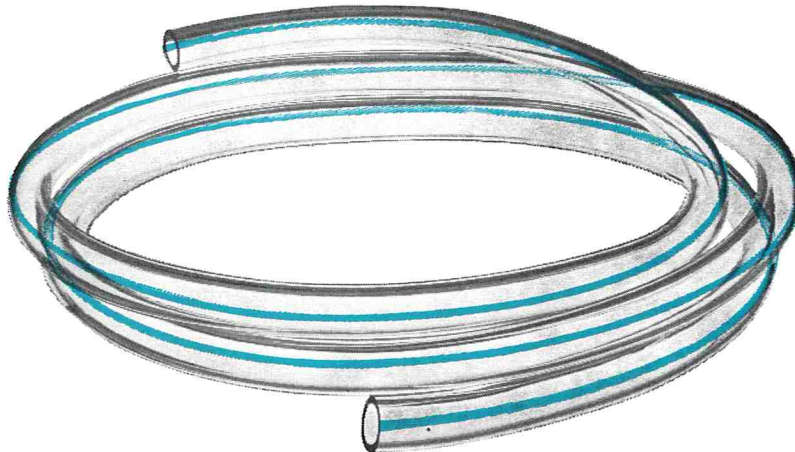




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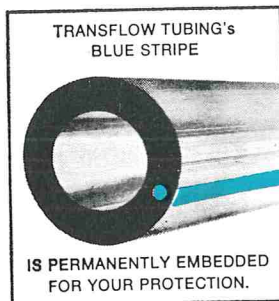


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### ABSTRACT

The practical application of sanitation and food protection principles by foodservice operators depends on their understanding what is necessary for achieving adequate protection of their customers and how this protection can be accomplished. The operator and, in turn, his personnel must be motivated by knowing the basic "why" for each requirement by understanding the direct relationship of the unsatisfactory procedure or condition to the possibility or likelihood of its causing illness. The foodservice operator needs practical tangible aids to guide and assist him in training, supervising, and reminding his personnel and to facilitate his initiating and carrying on an effective self-evaluation of his operation's safety.

We have a mutual concern—that of protecting the dining public from those hazards which could result in customer illness. The restaurant owner and other food service operators have the moral obligation to serve safe and wholesome food in a clean establishment. Most operators are concerned also about the image which their operation creates in the mind-eye of the customer.

The Public Health Officer is responsible to the American Public—the citizens of his community—to protect them from any hazards to health. Thus, we have the common interest, though methods for achieving the end protection vary, and we certainly do not follow the same paths in motivating and guiding the actions that will result in the protection which the dining customer has the right to expect.

Much has been said and written about the responsibilities of both the foodservice operator and the public health official with regard to protecting the public. The pendulum has swung from the far left of the educational approach to achieving adequate food protection to the far right of severe enforcement actions including the publicizing of violators of sanitation regulations.

The National Restaurant Association (NRA) has never and will never condone, sanction, or protect unsanitary or unsafe foodservice operations. Our standards and efforts are dedicated to maintaining the highest degree of safe and sanitary food purchasing, receiving, storing, handling, production, and

service. We constantly encourage foodservice operators to work cooperatively with regulatory agency officials and educational organizations concerned with the health and welfare of the citizens in order to make certain the efficient accomplishment of food protection programs.

### EDUCATION AND MOTIVATION

Our mutual problem is, as we all know, finding the way to educate and motivate foodservice management and, in turn, foodservice employees toward utilizing the procedures and observing rules of safety that will bring about the continuing control, if not elimination, of all hazards to food safety. We know that our problem, in part, exists because we have not succeeded in getting the practical knowledge of what must be done to the owner, operator or manager, and to his employees. In some instances, the knowledge of what must be done is there, but the management concern for its importance is lacking. If the boss does not insist on sanitation and safe food handling practices, food protection will be lax, or non-existent, and employees either will not know what they should do to protect the food from contamination and danger, or will not care.

Most of you are familiar with the recommendations which resulted from the 1971 Food Protection Conference at Denver—particularly those which had to do with safe food handling in foodservice establishments. Three foodservice operator-directors of the National Restaurant Association and I participated as members of that particular panel. The Public Health and Safety Committee and the Board of Directors of the NRA reviewed the short and long range recommendations which were made, concurring on all of those which directly pertain to food protection in foodservice operations.

We had gone on record, even before this conference, as supporting the desirability of uniform sanitation regulations, and their uniform interpretation. We co-sponsored the recommendation that each foodservice operator be furnished with a copy of the sanitation regulations applying to his community, together with a layman's language explanation of how the requirements relate to the prevention of customer illness, why they are necessary, and how the compliance can be accomplished.

<sup>1</sup>Presented at the 60th Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, Rochester, New York, August 13-16, 1973.

We proposed the recommendation that enforcement agencies identify critical operational food protection deficiencies, especially those causing most foodborne illness outbreaks in foodservice establishments. Inspections must emphasize these operational deficiencies and the elimination of the hazards involved.

Some of the actions recommended were of such a nature that they could not be quickly implemented. I refer, for example, to the proposal that a foodservice operator or operating manager demonstrate that he possesses the minimum essential knowledge of safe food handling and food protection practices. I think we are all involved in the implementation of this one—through development of training courses and programs which will form the basis for certification of foodservice operators.

Our greatest concern, however, was, and is, with the immediate situation. The customer must be protected now, not later, and must receive this protection everytime he or she enters a foodservice establishment. Since the foodservice operator faces his customers daily, it is incumbent upon him that he know just how safe his foodservice is and to what extent his employees are failing to observe the rules of sanitation. Hence, we advocated a recommendation that every operator capable of carrying on a self-inspection program be encouraged to do so, and that a model be developed to provide him with the assistance and guidance he would need to initiate and carry on such a program. Many readers have seen the new NRA publication, *A Self-Inspection Program for the Food Service Operators on Sanitation and Safe Food Handling*. It includes guidelines on initiating a management inspection and self-evaluation program, and includes 23 inspection checksheets covering both practices and functional areas of an operation. It even includes special coverage on the subject of customer concern.

#### SELF-INSPECTION

This publication has been made available for use by a foodservice operator as a practical management tool to provide him with the information that he needs concerning food protection and sanitation deficiencies and hazards which need correction or elimination. The system is, of course, recommended to the operator on the basis that his responsibility to protect his customers applies every hour his es-

tablishment is open for business. The guidelines insure coverage of his operation by all categories of management personnel including his night managers and managers of satellite operations, and include coverage of personal safeness of food handlers, food handling practices, 16 functional areas of an operation and five general areas of customer concern. The latter are included because, though customer complaints and customer reactions do not always relate directly to food protection, they do reflect customer dissatisfaction with an operation. In many instances, the unsatisfactory conditions or practices observed are, in the customer's mind, indices which lead to the conclusion that the entire operation may be unclean and suffering from bad management.

The 23 separate inspection checksheets provide flexibility of benefit to a foodservice operator by, first, permitting use of those which specifically apply to the type of foodservice operation involved and, secondly, permitting the scheduling of the inspection of various practices or functional areas at a frequency appropriate to the relative extent of hazard.

#### NO SINGLE METHOD

There is no single way of achieving complete protection of the dining public—no miracle method of assuring the actions which will guarantee food safeness day in and day out. Adequate regulations conscientiously enforced won't do it. Education and certification of foodservice operators won't do it. Indoctrination, training, and supervision of personnel won't do it. Yet all of these are extremely important, because a lack or deficiency of any of these will weaken the combination of actions which must exist if the public is to receive the protection it expects and has the right to expect.

I repeat what I have said many times before—the foodservice operator must understand the great importance of sanitation and food protection to safeguard both the health and welfare of his customers and the success of his business. If he does not, and does not understand what must be done and why—enforcement efforts become frustrating and his operation is doomed to mediocrity, if not failure. Management must want and insist on a pattern of safeness which will assure diners of safe food, served in a clean establishment every single hour that that establishment is in operation.

# THE PROCESS CHEESE INDUSTRY IN THE UNITED STATES: A REVIEW<sup>1</sup>

## II. RESEARCH AND DEVELOPMENT

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### ABSTRACT

Research and development (R & D) in corporate laboratories of cheese processors and in other research facilities in the United States have contributed knowledge of analytical technology, microbiology, defects and their corrections, manufacturing techniques, equipment, emulsifiers, and the use of other ingredients. Methods of wrapping and protecting the product during distribution were improved. Cheese foods and spreads with higher moisture, lower fat, and altered consistency were developed. The technology of producing slices of processed cheese has been revolutionary. Packages of slices are replacing the original, and highly successful, 5-lb. loaf. Mechanically formed, individually wrapped, and sealed slices are produced at fantastic speeds. Trends of industry indicate greater production per plant, more low-fat, high protein cheese products, and use of vegetable fats and proteins. Trends of R & D indicate improvements in emulsifiers, packaging, keeping quality, new products, and more mechanization and automation for greater efficiency and improved protection of this valuable food and its related products. The industry provides opportunities for trained food scientists, engineers, marketing specialists, and business administrators.

The original United States patents described the first research and development (R & D) on process cheese in the United States. Most industrial R & D has occurred in the plants or laboratories of the processors. The numerous patents on products, emulsifiers, equipment, processes, and packaging show the productiveness and ingenuity of the workers. Organizations supplying ingredients, equipment, and supplies to processors have engaged in R & D which contributed directly to the progress and welfare of the industry (118, 217). Research in institutions or commercial research laboratories has often been initiated, or supported, or both, by industry. The National Cheese Institute or its members have usually provided such support.

All research which contributes to knowledge of cheese affects the process cheese industry directly or indirectly. This review will be limited of necessity to those developments which seem to us to be directed to the progress of the industry.

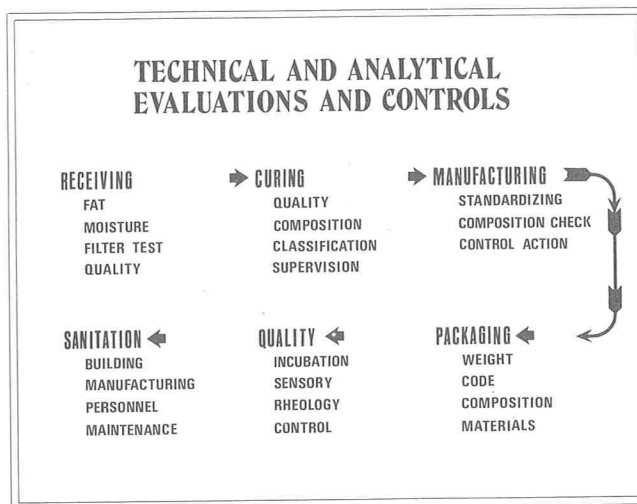


Figure 1. General functions of laboratories in process cheese operations.

### ANALYTICAL TECHNOLOGY

#### Laboratories

In the early decades of processing in the United States, only the larger operators maintained well equipped laboratories for routine analytical work and R & D projects. Now such laboratories perform many essential functions (Fig. 1).

Laboratory directors in large organizations were usually trained chemists, bacteriologists, or dairy technologists. Some had advanced degrees; others were usually called "Doc." They supervised and usually trained their own technicians to do the routine analytical work on cheese products and incoming supplies. These men with their help and facilities were the "trouble shooters" in production and merchandising and consequently often became involved in R & D.

Testing incoming and outgoing cheese for fat and moisture was a routine laboratory responsibility. The laboratory established standards for amounts of cream, color, salt, and emulsifiers as well as the optional ingredients used in pasteurized process cheese, cheese foods, and cheese spreads.

The laboratory checked packages leaving the fill-

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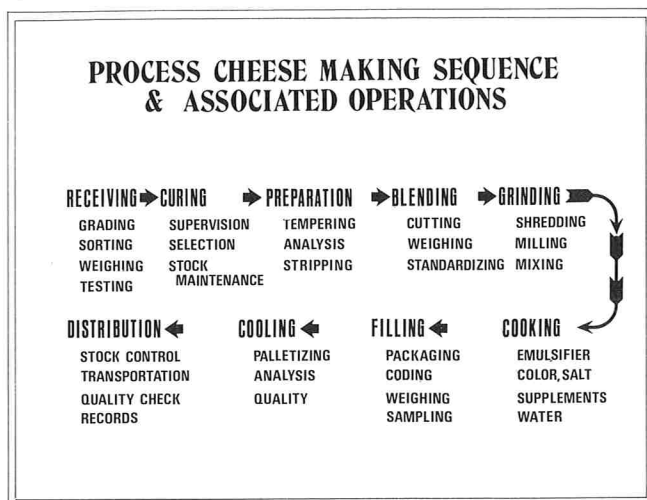


Figure 2. Flow sheet of process cheese making.

ing lines at regular intervals to measure weight, fat, moisture, salt, and sometimes pH and melting properties. Representative packages were reserved for storage at selected temperatures to measure effects of storage on body, flavor, color, slicing, and melting properties. Temperatures approximating 100 F were used commonly to check keeping quality and to detect faults in color, body, and flavor.

When extraneous matter in cheese was recognized as a serious problem, it became the laboratory's responsibility to check all incoming vat lots until eventually spot checking could maintain control. In addition to these analytical functions, the laboratory provided the routine inspections and sanitary control of plant operations.

Directors of laboratories were generally required to maintain liaison with regulatory agencies and their requirements. They provided the technical advice to company lawyers engaged in court actions and at legislative hearings, probably the most notable of which culminated in the modern Standards and Definitions of the Food and Drug Administration in the late 1940s. They were sometimes involved as expert witnesses in court actions (81).

#### Analytical control

The process cheese industry recognizes as standard the official analytical methods for determining fat and moisture. These methods were designated by the Food and Drug Administration as a part of Standards and Definitions of cheese and cheese products (27, 81). Modifications of standard methods have been developed in some respects to meet requirements of large scale operations.

Sampling cheese for analysis is complicated by the unpredictable distribution of moisture in such units as blocks or barrels as well as between units making up vat lots. A practical sample can only approximate

the "true" composition of the vat lot (188). For official inspection purposes or government transactions the official methods of sampling each particular style of cheese are followed (27). In government orders, the number of boxes or cans of cheese to be sampled are specified for each subgroup in shipments of different sizes.

The adaptation of the Babcock method for testing for fat in cheese is commonly practiced. The Mojonnier system for fat determinations is used less frequently. Moisture tests must be made on large numbers of samples each day; these consist of tests for payment on the moisture basis and routine checks on manufacturing operations. Consequently, large scale methods of drying have been developed which duplicate official tests very closely (188, 242). Rapid moisture tests to provide workable data in a few minutes, rather than hours, have been developed to furnish information for control of production operations. Methods vary between laboratories (113, 138, 144, 242, 253). Moisture tests on products containing large amounts of lactose require special procedures to obtain "constant" dry weight with minimum charring (59).

#### Microbiology

The improved keeping quality of processed cheese observed by the first producers in the United States was generally attributed to the "sterilization" of the cheese. The fact that bacteria did survive the heating process and did continue to change in numbers was known to some bacteriologists, but not generally appreciated despite the fact that the temperatures used were not high enough to produce sterilization (211). But the practical success of the temperatures used in improving keeping quality was well known (9, 224).

When process cheese was made in jacketed kettles and heated by indirect steam, the treatment resembled that of vat pasteurization of milk. Even when heating was accomplished in a few minutes by injecting steam directly into the cheese, temperatures maintained during filling of packages and stacking for cooling were adequate to fully justify the term "pasteurized." This fact was recognized eventually in the standards of the Food and Drug Administration. The standards held that the heating process should produce a practically negative phosphatase content in the finished cheese (81, 199, 200).

When gassy fermentations occurred in process cheese products containing pimentos, it became obvious that anaerobic spore-forming, gas-forming organisms could survive the heat treatments and produce undesirable effects (2). During and after World War II researchers investigated higher than normal pas-

teurizing temperatures for process cheese products in cans for military use (154-156). Studies concerning the survival of gas-forming, spore-forming anaerobes in process cheese products showed that low pH, high salt, and proper refrigeration decreased the survival rates as well as effects of their growth (3, 122-124). Development of large numbers of gram-positive bacilli in cheese spreads held 90 days at 90 F was associated with increased firmness, low pH, and large amounts of lactose from nonfat dry milk and whey solids in the formula (168).

Detection of Type B botulism toxin in a process cheese spread led to extensive studies financed by the National Cheese Institute (88, 243). Staphylococcal enterotoxin A, which has been found in cheese, is not destroyed by the heat of cheese processing. When 2,112 vats of cheese were suspected of containing the toxin, a cheese processor initiated an intensive laboratory program to detect contaminated vat lots (254, 255). The presence of the enterotoxin was demonstrated in some lots even after two years of storage.

These and other studies have alerted the industry to all aspects of ingredient quality. Control has now been extended to the type of material, sanitation of the cheese milk, composition, manufacturing methods of the individual ingredient, and storage of both ingredients and finished products.

#### Color

The color of blends of process cheese which are made with both colored and uncolored natural cheese has to be adjusted for uniformity. Cheese color was commonly used in the early years of the industry. Eventually butter color was sometimes added to increase the yellow tint and, hopefully, to minimize the pinking effect of acid cheese (96). Other colors were suggested, such as Apo-8'carotenal (44). Canthaxanthin (4,4'diketo-beta-carotene) in diacetylated monoglyceride was patented for this same use (194).

An unnatural pink defect was often attributed to the use of acid cheese. It also was produced experimentally by prolonged heating or incorporation of semidry cheese adhering to the sides of an unwashed kettle when subsequent batches were cooked in it (183). Overheating was also identified with this defect (224).

Browning of process cheese was associated in the 1940s with the use of dried whey, dry skim milk, and malt sugar or glucose (111). When the browning appeared as a ring in the packaged cheese, it was believed to be caused by delayed cooling to under 90 F or to storing at room temperature or higher (76); some thought that penetration of oxygen caused the rings (91). Brown spots, which appeared only when the process cheese was cut, were observed in cheese food containing Swiss whey concentrate. Growth of such

spots resembled colonies of chromogenic bacteria sometimes observed in the cut surfaces of Swiss cheese, but some suspected the spots were brown particles in the concentrates used (91).

#### Crystals

Crystals were detected in "sandy" or "gritty" process cheese in the 1920s. These crystals were isolated and identified in 1930 (215) and again in 1949 (48, 142), as calcium tartrate formed when Rochelle salts were used as emulsifier.

Packages of process cheese in slices in recent years have sometimes developed crystals or "bloom" on the surfaces of slices. The formation was sometimes mistaken for mold growth. X-ray diffraction studies have identified these crystals as the alpha form of  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$  (203). It was found that formation of crystals of disodium phosphate on process cheese in storage depended on temperature, humidity, pH, and abnormally high ortho-phosphate levels (201, 203). Later analysis by X-ray and infrared detected tricalcium citrate tetra-hydrate, which appeared under the microscope as a fine network of slender crystals (202). Formation of calcium citrate crystals on packaged cheese in other studies was most apparent on areas exposed to pressure, and was eliminated by avoiding the use of citrate as emulsifier (164). Certain emulsifiers ( $\text{Na}_2\text{P}_4\text{O}_{12}$ ) have been discovered partially undissolved in process cheese (229).

#### Extraneous matter

As early as 1925, processors had discovered foreign materials in their raw cheese. They found such things as ball bearings, pieces of metal, hooks, bolts, nuts, glass, and even a watch (65). Today X-ray examination of processed cheese is used to detect X-ray opaque materials. But the presence in cheese of materials associated with insects and rodents was critical under the terms of the Food, Drug, and Cosmetic Act of 1938 (79). The definition of adulterated food in Section 402 of the Act clearly indicated the vulnerability of the food industry.

Research disclosed that certain solvents such as sodium citrate, phosphoric acid, and even some enzymes under proper conditions could disperse the cheese and release insoluble, critical material with minimum destruction. Such dispersions could then be filtered to disclose the insoluble material (209, 218, 236).

Studies sponsored in part by the National Cheese Institute evaluated methods of sampling and measurements for inspection and laboratory control purposes (161, 187). These studies recommended regular and frequent sampling of cheese from each factory, the use of nondestructive 50-g samples, dissolving the samples in 10% sodium citrate solution at 140-

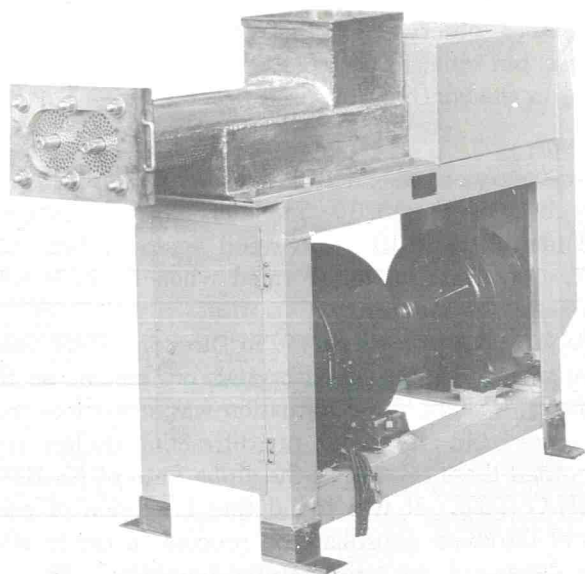


Figure 3. Cheese grinder for small operations, circa 1930. (Courtesy of C. Doering and Sons).

150 F, filtering through poplin disks and microscopic examination to identify residues. Such examinations made possible the routine and regulatory evaluation of the sanitary history of cheese (80).

Processors commonly used the 227-g sample to test for critical material. Taking samples of this size or larger with cheese triers was so destructive of finished cheese that some buyers required the saving at the factory of samples of salted but unpressed curd to be delivered with the cheese from each vat lot. To avoid destructive plugging of cheese before curing, a special knife was designed to cut a V-shaped section in one slice across the flat surface of the cheddar before waxing (74).

The effects of regular inspection and testing definitely improved sanitary practices of milk production and cheese manufacturing. Equipment was modified and contents of vats were protected from falling oil, dust, or other material from vat agitators. Factories and warehouses were made rodent proof and doors and windows more carefully fitted with screens and closures. Buildings were fumigated when necessary. At some warehouses, cheese in boxes was fumigated under canvas tarpaulins with methyl bromide before being accepted for waxing and storage. Even clothing used by factory workers was required to have no pockets from which pencils or other small objects might drop into milk or curd. Sanitary inspections and control operations became more efficient.

#### MANUFACTURE

##### *Formulation*

The compounding of process cheese developed

first in the United States as an art described in the original patents. The objectives were to produce the sensory characteristics approximating natural cheese, to extend keeping quality, and to deliver it in convenient form to please the consumer. The controls established by law in Wisconsin and later by the Federal Government imposed limitations on composition, combinations of varieties, and additives (71, 238).

The effects of blending several lots of cheese of various ages, physical properties, and composition opened new possibilities of composition and quality control. Techniques varied according to the manufacturer's idea of what was economical and acceptable to consumers. High flavors, for example, which were important to some manufacturers, were sometimes rejected by some consumers with preferences for milder products (36, 37, 39, 42, 192). In the 1920s body characteristics were modified in the development of cheese foods and spreads by adding nonfat milk solids or whey solids, and stabilizer, as well as emulsifiers (225, 230). Combinations of highly flavored cheese like Limburger and Blue with Cream or Neufchatel cheese required introduction of homogenization and stabilizing guns. These mixtures became popular in the 1930s (34). Eventually spreads with maximum moisture and semiliquid consistency were developed (31, 33, 35, 38, 41, 168, 204).

The earliest, systematic and published studies in the United States of composition and its relation to the characteristics of process cheese and cheese spreads began in 1927 and considered emulsifiers, temperature of treatment, moisture variation, and age of cheese. Principal observations of the finished products included measurements of moisture, pH, titratable acidity, body, slicing properties, color, keeping quality, appearance of the foil wrapper, and the relation between water-soluble nitrogen in the original and the processed product (217, 224, 227, 230). These studies provided some of the technical explanations of the art practiced in the United States or described in U. S. patents.

##### *Emulsifiers*

Much of the art of processing cheese lies in the areas of blending and emulsifying. The underlying principles of blending are generally well known while the use of emulsifiers is still something of a mystery with new emulsifiers and new combinations appearing almost annually, some with great success and others with alarming and costly failures.

Process cheese can be made without emulsifiers if the blend of natural cheese is carefully selected, heated, and stirred (71, 130). Before emulsifying salts were generally used, process cheese showing oiling



off, mealy body, and faulty consistency was not uncommon.

The documented use of emulsifying salts for process cheese in the United States began with the early work of Eldredge and Carpenter (55, 66). Subsequent studies and patents mentioned different salts and advanced explanations of their varied actions.

The effectiveness of emulsifiers at first was attributed to fat emulsifying ability; and to solvent action on cheese proteins (217) perhaps because processing increased the water-soluble nitrogen in the cheese (224). Later, electrophoretic studies of proteins in the natural and processed cheese showed no significant differences (95, 114). Some salts dispersed fat and were therefore considered to be good emulsifiers, but they did not always give the best body characteristics in the finished cheese. Other salts which produced desirable body characteristics did not act efficiently during heating and mixing of the blended cheese (114).

Studies of salts for process cheese have considered changes in water-soluble nitrogen, fat leakage, compressibility, knit, and flavor of the finished product. The results have indicated in general that satisfactory emulsifiers all have trivalent anions, form alkaline solutions, and precipitate or sequester calcium. But since all such compounds do not perform satisfactorily, the choice of salts or combinations of salts in practice has generally been determined by ability to disperse fat, to promote uniform melting and blending during processing, and to give the desired flavor, consistency, and melting properties in the finished cheese. In addition, the choice of salts, or combinations of them for processing, has also been influenced by the cheese chosen for processing.

Rochelle salts were used in the early days of commercial practice (71, 217), but the sodium salts of phosphates and citrates were more generally preferred. Other potential emulsifying agents have been mentioned including sodium and calcium gluconates, sodium mucate, lactates, and malates (55, 222), ammonium salts (115, 223), gamma and delta gluconic lactones (177), injections of carbon dioxide and hydrogen (85), and the use of proteolytic enzymes (196, 197).

Studies of sodium citrate showed that it was a good emulsifier which, when compared to disodium phosphate, decreased fat separation during heating and produced cheese with firmer body and slightly preferred flavor (216, 217, 224, 232).

Generally the industry has moved away from the original and almost universal (often exclusive) use of sodium citrate to the phosphates, which include disodium orthophosphate, trisodium orthophosphate, sodium metaphosphate, tetra-sodium pyrophosphate,



Figure 4. Grinding cheese, circa 1940 (Courtesy of Borden Foods, Cheese Division, The Borden Co.).

and sodium polyphosphates (217, 223). The versatility of phosphate salts has been indicated in numerous industrial patents which claim in general that certain salts can be used to control consistency (206) and to give the special melting properties desired for baked goods, meat products, frozen specialties, and other food products (256). Sodium metaphosphate, for example, has been indicated for making non-melting cheese desirable for novelty meat loaf (205). Other combinations of phosphates and protein peptizing agents have been proposed for preparing emulsified cheese for drying (221). In recent years the use of a complex of sodium aluminum orthophosphate (Kasal) has been developed for rapid heating with less fat leakage and less tendency for formation of surface crystals in packages (22, 140, 141).

Use of phosphates is not without peril. Crystallization with orthophosphates is a prevalent and often experienced defect, particularly with some of the newer products and packages. The defect is related to the pH of the finished product—generally higher usage can be tolerated with a lower pH.

More recently there seems to be a trend back to use of sodium citrate to improve body. Generally it is used in combination with other emulsifiers. Such use in some of the newer sliced products does, however, often cause a defect known as citrate haze. This appears as a fine precipitation of calcium citrate on the surface and is probably related to physical abuse of the product as it is being processed and packaged (164). Then, too, when sodium citrate is used in combination with other emulsifiers, there is a very narrow usage range—exceeding the limits can seriously damage the melting and body characteristics.

Loss of melting properties when process cheese is

reworked into a new blend may be corrected by use of a small amount of surface active agent like phosphorylated stearyl monoglyceride to supplement the emulsifier (139).

References to studies in other countries have not been included in this section in accordance with the limitations of this review. The reader must not assume that developments in the United States have proceeded unilaterally (62, 178, 246, 247). The Germans, particularly, have had much success with the higher polyphosphates. A leader in this field seems to be Joha-Benckiser-Knapsack GMBH at Ludwigshafen-Rhein. No attempt has been made to describe practices which have not been reported in the U. S. literature. Much work is being done on emulsifiers and improved methods are certain to follow.

### Ingredients

Cheddar and Swiss were the basic varieties of cheese used for processing when the industry was being established in the United States. Other blends of varieties soon followed in which Muenster, Limburger, and Brick cheese were used (12). Emulsifying salts, color, sodium chloride, and water were the first non-cheese ingredients commonly used. Other additives were gradually adopted: most of them were commercial innovations; some came from industrial or academic research; some were advocated and abandoned.

Melting properties, firmness, plasticity, and fluidity have been as important as flavor in the search for improvements. These characteristics have been controlled to a great extent, but not entirely, by the choice of the cheese, its variety, composition, acidity, and extent of curing. To these controls of properties have been added the actions of emulsifiers and effects of condensed or dried skimmilk, milk, whey, or whey proteins alone or in combinations (26, 35, 38, 41, 43, 102, 103, 226, 227, 239).

Low-fat cheese and skimmilk cheese have been used to make low-fat products desired by some customers and to decrease costs (31, 71, 102, 103, 119, 147). Skimmilk cheese, probably more than any other ingredient, aroused the intense antagonism to process cheese manufacture before standards were established.

Curd from whole-milk cheese was conditioned by some treatments to prepare it for immediate processing, such as the mixing of finely divided curd with an alkaline solution (127, 176), and the treating of granular or cheddared curd with acid (47, 110, 136). Hard rind of Swiss, after grinding and milling into small particles, was recommended for processing (53).

Unusual ingredients have been suggested. Cheese made slightly rancid by a special lipolytic enzyme

imparted an improved flavor in the processed product, according to some researchers (29). Injection of green mold veins into melted cheese before packaging small portions altered its appearance (145). Flavors were modified with chocolate (105) or condiments (156, 228, 244).

Ingredients-not-cheese first used in processing in addition to emulsifiers, color, salt, and water were chosen to modify composition, or flavor, stability, and consistency, and included different sources of milk fat, vegetable gums, stabilizers, lecithin, acids, liquid smoke, spices, chocolate, meat, and vegetable products. Substances which might simulate cheese flavor, like red pepper, were excluded by regulation.

As demand has developed for products to serve special diet needs, or to provide different physical characteristics, new and different emulsifiers and food materials or combinations of them have been introduced. All have been examined by regulatory officials as well as industry and, when acceptable to all interests, have been included in the 1950 Definitions and Standards of the Food and Drug Administration (81) and in subsequent revisions of those regulations.

### Equipment

The equipment first used in processing was typical of that used in the food industry; it was adapted to processing cheese. Advances since then represent the combined efforts, observations, and ingenuity of processors who saw the needs, the engineers who developed solutions, and fabricators who aided in perfecting specialized equipment. Fig. 2 indicates the diversity of operations which depend on mechanical devices.

In the early 1920s, the cheese selected for processing was placed on tables where wax and bandages were stripped from it by hand. Early operators some-

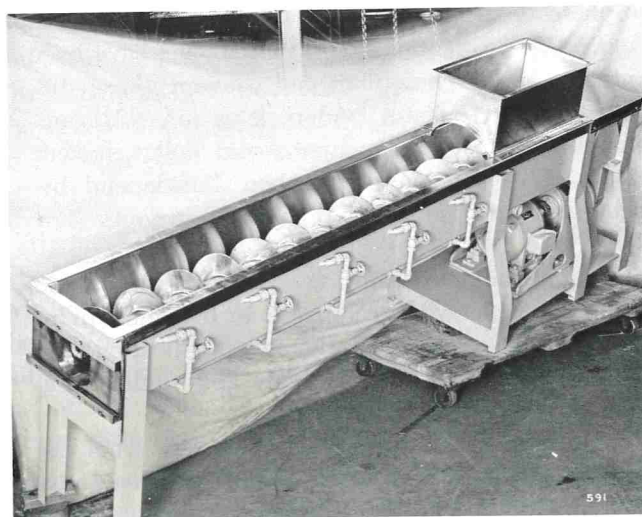


Figure 5. Lay-down cheese cooker, circa 1935. (Courtesy of E. C. Damrow Brothers Co.).

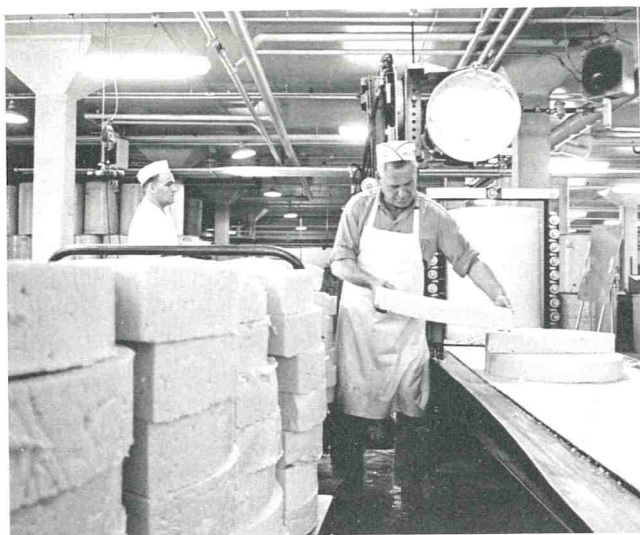


Figure 6. Cutting "barrel cheese" for blending, circa 1960. (Courtesy of Borden Foods, Cheese Division, The Borden Co.).

times dipped the cheese into hot water to facilitate stripping bandages. Sharpened scrapers and hand knives were used to remove hard rind and damaged spots or mold. The placing of cheese on roller conveyors instead of tables simplified cleaning and conveying cheese to the next operation.

Development of flexible wrappers (1, 126) to cover 20- and 40-lb. blocks simplified the cleaning operations. Wrappers easily stripped from blocks and blocks required little or no other preparation for subsequent operations. Ultimately, development of pressing cheese curd in barrels for curing further simplified preparation of cheese for processing and greatly reduced labor cost and minimized surface cleaning.

After cleaning, cheese was divided into pieces to fit the hoppers of the meat grinders which were first used to comminute the cheese. Dividing was done by pulling a wire through the cheese first by hand; later this was done by machine.

Meat grinders were replaced with powerful mills or shredders which could accept cheese in 20-lb. pieces and eventually much larger, as units of barreled cheese replaced cheddars. Capacities up to 50,000 lb. per hour are now possible with motors of 100 hp and over.

From the grinders, the product went first to steam-jacketed kettles. In the 1920s these were equipped with double acting agitators and scrapers. Later models injected steam to heat the cheese during the mixing process. At first the cheese was drawn from the kettles directly into foil-lined 5-lb wooden boxes. Later, as production speed was increased, the 200- to 400-lb. batches were conveyed or dropped into hoppers of fillers.

Kettles for large scale operations with greater capacity and more efficient stirring and heating were

designed by processors in the 1920s, but subsequently much improved in mechanical efficiency and sanitary construction. The horizontal or "lay-down" kettle consisted essentially of a covered, horizontal, round-bottomed trough in which the cheese was heated by direct steam injected through side openings. Cheese was stirred by helical type screws which mixed and forced it from the hopper opening to the opposite, discharge end. As the hopper filled and the cheese melted, the cheese flowed back above the horizontal screw toward the hopper end and was thoroughly mixed and homogeneous before it was released to flow to the hoppers of the filling machines. The time to complete the filling-to-discharge cycle approximated 3 to 6 min. Several modifications of the "lay-down" kettle were proposed as the industry developed (49, 133, 153, 180, 182, 217, 248). The design and capacities of "lay-down" kettles allowed continuous operation, but in practice these kettles were operated by the batch method with the hot cheese delivered into the hoppers of the filling machines and held with gentle agitation until packaged. Continuity was achieved by using several kettles to deliver in regulated sequence to the filling machines.

In 1925 a novel method of heating was patented (173-175) in which the cheese was emulsified by forcing it through staggered rows of heated tubes or by heating it by passing an electrical current directly through the cheese itself as it passed between the rows of tubes. Recently, another system has been developed to heat, mix, and move the cheese in a closed system from intake to packaging line (49).

Small capacity kettles have been designed for experimental work (137, 217, 224, 227); some have been of the vertical type with provisions for steam injection, others of the horizontal type. Much of the early research work was done with such small devices. Some modern processors have small kettles designed and built especially for research and development work to duplicate the action of the commercial models.

Highly sophisticated equipment has been developed to deliver the exact amounts of hot, fluid cheese into packages, pouches, or envelopes. These packages may be formed continuously immediately before filling and are mechanically closed, sealed, and placed in containers for merchandising. Such machines are dramatically different from the simple operations of the pioneer industry when boxes were nailed together, lined with foil shaped on a mandrel to fit the box, all by hand. Boxes were filled with a hand-operated gate, check-weighed, and closed by hand-folding the foil over the hot cheese, then passing the filled box to the operator who nailed on the cover.

Today's package filling operations represent highly



Figure 7. Filling 2-lb. paper boxes lined with semi-transparent, wax-coated Cellophane, circa 1940. (Courtesy of Borden Foods, Cheese Division, The Borden Co.).

technical solutions to the problems of labor costs, product flow, sanitation control, accurate measurement, exclusion of air, sealing, assembling, and labeling containers without hand labor and all at the speed required by modern standards of economy and efficiency. Packaging equipment is so diversified to meet the requirements of the type of cheese, size, shape, shelf-life, and packaging material that it is possible in this review only to suggest the complexity of the developments attained in these efficient operations. All operations in the production of process cheese, beginning with procurement, classification, and curing of natural cheese to provisions for holding before shipping to the merchandising outlet depend on efficient use of the capacity for packaging.

Movement of raw materials and finished products are an integral part of processing. In the early days, the cheese at all stages of processing was lifted, carried, and placed on trucks and moved by men. In today's operations manual labor is minimized with mechanical devices, palletizing, power-operated mechanical conveyors, and power lift trucks. Conveyors and sorting systems which are controlled by computers can now be used to mechanize the movement of cheese from packaging lines, through storage or holding areas, and finally into the trucks.

Packaging of natural cheese has assumed an important place in the operations of the processing plant. The processor has developed expertise and experience in selection and use of wrapping materials which can protect and display cheese for merchandising. The processor can provide and operate the elaborate machinery required to wrap, seal, and containerize the consumer-size units; he has the system established for merchandising the finished product; and finally he can make the fullest use of odd-shaped pieces and

remnants from cutting and check-weighing operations without waste.

#### Wrappers

The rapid market acceptance of process cheese resulted, in large part, from the tinfoil-lined wooden boxes used to package the cheese. The hot cheese filled the boxes and excluded air; the foil clung tightly to all sides of the loaf when the package was closed. Properly filled boxes were practically mold-free during normal merchandising. The sandwich-size cross section could be easily sliced. This package was far better for the U. S. market than the cans and glass containers that were tried first (131, 190).

Foil had been used for wrapping natural cheese as early as 1904. It was being used successfully for wrapping 3-oz. packages of Club cheese (Cold Pack) in 1915. The Kraft patent which specified metal foil which would stick to the cheese, not the box, might have been the most valuable patent owned by the first of the U. S. processors. It commanded valuable royalties (131).

Tinfoil tended to blacken in the container, especially when phosphates were used and when the pH of the cheese exceeded 5.8. Uncoated aluminum foil tended to pit; coated aluminum foil developed in the 1930s resisted corrosion and pitting but was difficult to manipulate in the packaging machines. Lead foil discolored the cheese in a few days. Non-metal films, like Pliofilm and Cellophane, were first tried with little success (233).

World War II demands for tin were critical. A substitute was essential. Research workers at the



Figure 8. Filling 4-oz. tin cans with process cheese for the Armed Forces during World War II. (Courtesy of Borden Foods, Cheese Division, The Borden Co.).

Marathon Paper Mills began to develop the semi-transparent modern film by coating Cellophane with a layer of Parafilm, a mixture of wax and latex. The first films tended to taint the surface of cheese. Eventually this fault was eliminated and the new coated film produced showed the necessary clinging properties and imperviousness to gas transmission required to protect the product. It was manufactured in rolls and preformed bags to line the boxes. This film was licensed exclusively to a leading manufacturer until a comparable film with essentially equivalent properties was developed and sold by General Felt Products Company. By 1940 the transparent films were being used with complete success and were regarded as satisfactory or even better than the original foil (72, 73).

Combinations of wrapping materials were made to obtain the best qualities of each in laminated film. Such combinations became widely used in packaging sliced processed cheese and natural cheese. They provided the impermeability to oxygen, nitrogen, and carbon dioxide, with strength and transparency essential for the U. S. market (195, 252).

Sliced processed cheese in packages sometimes developed mold when small air cavities were formed in packaging. By one method, stacks of slices were successfully heat-sealed with pressure against the sides and ends to expel the air (77). Packaging sliced cheese eventually involved various methods for preventing mold growth such as vacuum (61, 116); vacuum released with nitrogen, carbon dioxide, or specific ratios of mixtures of these gases (58); film combinations (61, 252); mold inhibitors (61, 101, 172); and heat shrinkable film (172).

Some of the research and development work was directed to protect cheese between the factory and the processing-packaging operations. In addition to the wrappers for covering blocks of cheese, films were applied to line barrels (78); impervious films were developed for use in combination with porous material to remove whey in pressing and to close openings in the surfaces (214).

The desirability of screening out ultraviolet light for retarding oxidation of process cheese was attempted in 1958 by incorporating a light-screening additive to Cellophane (69). Such problems had been anticipated and investigated at least 20 years earlier (163).

As the production of cheese spreads expanded in the 1930s, glass containers with vacuum closures were used. The vacuum which held the caps on the glasses was produced by flowing steam under the caps to exclude air, closing the containers, and cooling to condense the trapped steam to make the vacuum-sealed glass. Plastic cups and aluminum containers with tight closures were used. Sausage-shaped links

of process cheese were also tried in this same period (100).

Various antimicrobials were used by industry to protect wrapped packages of slices. These mold inhibitors were used in the process cheese itself in prescribed amounts or on the wrappers, providing the absorption of antimicrobial from the wrapper did not exceed the limit prescribed by the FDA. Dichloromethyl succinate was used but abandoned when the FDA did not approve its use. Sorbic acid and its calcium salt proved to be acceptable and effective (213); the products formed when they were decomposed by mold action were harmless. Propionic acid and calcium and sodium propionates were generally effective and were accepted by the FDA but imparted a slight Swiss-like flavor.

Wrappers have been modified in strength, permeability to O<sub>2</sub>, CO<sub>2</sub>, and N<sub>2</sub>, transparency, sealing properties, and behavior in packaging machines. These modifications have improved keeping quality of the cheese and increased the protective properties and sales appeal of the packages, simultaneously adapting them to the requirements of complicated, high-speed machines made to deliver units in a multiplicity of sizes and shapes. The complicated interrelationships solved cooperatively by processors and manufacturers of wrappers and machines are a tribute to their combined research and development programs and practical know-how of production methods and standards.

#### CHEESE PRODUCTS

##### *Cheese foods and spreads*

Process cheese foods and spreads were developed in the 1920s. E. E. Eldredge, formerly with Phenix Cheese Company, began research for Fred Pabst, Sr. at Pabst Farms, Oconomowoc, Wisconsin, and in 1922 started a processing operation with Swiss cheese made at the Farms. Whey disposal was a problem. Eldredge precipitated the whey protein to put into the process cheese; then he concentrated whey in the Pabst brewery in Milwaukee and used the concentrate in the processing operation. The resulting cheese was soft in body, pleasing in flavor, and was first offered under the name Cheesette, but when this name was not allowed, it was called Pabst-ette and was soon marketed widely in the U. S. (68). Competitors introduced Nukraft (Kraft Cheese Co.) and Phen-ett (Phenix Cheese Company (12)). Within a few years Velveeta displaced Pabst-ette after Pabst had been purchased by the Kraft Company.

By 1947 the production of cheese foods and cheese spreads was well established, and industry was cooperating with the FDA of the Federal Security Agency to develop definitions and standards for these and other cheese products. The definitions recognized

that cream and concentrates of milk, skimmilk, and whey produced softer body and mild flavors when added to process cheese. These additions were desirable when moisture did not exceed 44% with fat not  $< 23\%$ , except when the mixture contained fruits, meat, or vegetables. Such mixtures were designated "Pasteurized Process Cheese Foods." "Spreads" were defined with not over 60% moisture (but over 44%) and with not  $< 20\%$  fat. In making these process cheese foods, the blends were to contain  $> 50\%$  cheese in the finished products. Emulsifiers were permitted within limits, as in process cheese, and so were acids, water, salt, and color.

The pasteurized process cheese spreads were like the cheese foods except for lower fat, higher moisture, and use of sweetening agents and gums. Definitions and standards of cheese foods and spreads promulgated by the Food and Drug Administration of the Federal Security Agency in 1950 established specific limits for these products in composition and ingredients (81).

The earliest cheese foods and spreads sometimes developed "sandiness" when excessive amounts of whey or skimmilk concentrates were used. Insolubility of lactose at concentrations exceeding 16.9% of the moisture in the cheese was demonstrated (82, 83, 168, 226). Excessive moisture, low fat, and lack of acidity were also associated with defects in body and keeping quality (225).

When the restrictions of the original processing patents began to expire in 1938, new manufacturers were looking for information on the manufacture of all pasteurized process cheese products. Trade papers began to feature discussions on formulation, ingredients, composition, reaction, methods of heating, use of gums and emulsifiers (33, 34, 38, 41, 54), and have continued to do so as new blends and products for aerosol dispensers have developed (31, 98, 146, 147).

Research during and after World War II on stability and quality of spreads for use by the Armed Forces produced specifications and much useful information on ingredients, average age of blend, special flavors, and methods to prolong keeping quality with high temperature short-time sterilization and aseptic canning of spreads (154, 156, 157). Studies of combinations of Cheddar and semisoft cheese in process cheese spreads showed that acidity, age, and ratios of solids-not-fat to fat affected the melting properties and firmness of the finished cheese (117, 168, 169, 239).

#### Slices

In the late 1930s the appeal of pre-sliced cheese for merchandising was recognized. Cheddar and Swiss cheese were being sliced and packed in gas-venting cans (249). Research workers at Marathon Paper

Mills were wrapping and sealing slices of natural cheese in Parafilm.

In 1944 a new method was invented which formed hot processed cheese in a thin sheet on a cooling drum. This sheet was cut continuously into ribbons and removed from the drum. The ribbons were superimposed mechanically and then cut to form stacks of sliced cheese for packaging. The packages of slices were very well accepted (19, 134). The process was not licensed for use by other manufacturers.

By 1950 several other methods of making sliced cheese were being used or had been invented or proposed. Some operators cast cheese in blocks and sliced it for packaging. Placing hot cheese into compartments, sized to hold one slice, was followed with several variations (108, 235). Cheese was spread or extruded in thin layers and chilled on moving belts to form ribbons for cutting to the desired size (171). Spinning hot cheese in a bowl, while cooling it to the solidifying temperature, formed a ribbon of cheese as it crept up the sides of the bowl until it could be removed and sliced to the proper size (109).

Slices of cheese, with some exceptions (19, 134), tended to stick together. Some operators prevented this by interleaving slices with non-sticking films (108, 158). Heating the surfaces of slices prevented sticking. This was accomplished by passing slices or slabs between hot rollers and then cool rollers by one method (89) or exposing them to radiant heat to form a thin layer of oil on the surfaces. This free oil had the added advantage of inhibiting mold growth (92, 167).

Multi-ribbon formation of non-sticking slices was further improved by a machine which chills both sides of the cheese ribbons. The hot cheese is pumped through a manifold with 8 to 12 nozzles extruding ribbons of cheese on the upper of two stainless steel belts where it is partially cooled, then inverted onto the lower belt to complete the chilling operation. The cooled ribbons are superimposed to make 6, 8, or 12 layers. These are cut to form stacks of slices of the desired dimensions (24).

The most modern method of producing individually wrapped slices was developed by L. D. Schreiber Cheese Company, Inc. Highly sophisticated automatic equipment forms the wrapping material into a tube into which the hot cheese is extruded continuously. This tube is sealed transversely to form a chain of packets which is then cooled. Each packet is cut from the chain transversely through the center of the seal to form individually wrapped and sealed slices. This is done at a speed of 400 to 600 slices/min (50).

Making packages of sliced cheese involved preventing mold growth in the interstices of the packages where small volumes of air had been trapped

in wrapping. Packaging with N<sub>2</sub> or CO<sub>2</sub>, or mixtures of these gases, was a successful solution (181). Another method advocated spraying sides and bottom of the package with liquid wax and then applying a polyvinylidene film as a top cover (245).

Development of the antimicrobials, sorbic acid and potassium sorbate, proved to be effective and safe for protecting sliced cheese from mold growth in packages. The treatment was most effective with tight clinging wrappers which excluded air, because the antimicrobial itself was attacked by mold, if any were present, before the cheese itself was affected. Research showed that the sorbic acid was superior to the vacuum pack in prolonged storage. It was usually applied to the inner surface of the thermoplastic-coated film used for wrapping (213). Machines were devised for applying the sorbic acid powder to all cheese surfaces in a stream of pressurized air (97).

Studies showed that molds isolated from moldy process and natural cheese which had been previously treated with potassium sorbate would grow on an artificial medium containing 1,800 to 5,400 ppm potassium sorbate. *Penicillium roqueforti* was most resistant (152).

Packages of process cheese in slices are replacing the original 5-lb. loaf. Kraftco announced in June 1973 that it had stopped making the 5-lb. loaf which changed the course of the cheese industry in the 1920s.

#### Cans and bars

Processed cheese was first placed in cans in the U. S. by J. L. Kraft in the summer of 1915; it was first marketed in 1916. In 1917 large orders were placed by the Federal Government for military use and by 1918 the cheese was being shipped worldwide. This cheese was prepared from cured Cheddar, shredded, blended, heated to a dough, and run to a filler. This filler lined 1/4-lb. cans with paper, filled and covered them at the rate of 18,000 units per hour. Then this canned cheese was heated under pressure at 250 F for 30 min, chilled and packed 24 per case (13). The J. L. Kraft and Bros. Cheese Company of Chicago became known as the producer of sterilized cheese in cans (5), although Phenix Cheese Company was also working with this product (13). After 1918 the commercial acceptance of the 5-lb. loaf quickly discouraged production of canned cheese for domestic use.

Research and development on the canning of process cheese again became important with the expansion of military forces in 1940. Canned cheese in 4-oz. tins was designated an important part of field rations for the troops. Larger 7-lb. cans were needed for field kitchens as well as military bases.

The 7-lb. cans are still produced for institutional use.

These products were made for the military forces to conform to specifications of the Quartermaster Corps, Food and Container Institute. Information from commercial laboratories, civilian advisers, and experiments of the National Cheese Institute were all used to prepare specifications for manufacturing. Problems of canning cheese varied. Exteriors of tins were coated with non-reflecting, dark-colored lacquer. Interiors of cans were lacquered to prevent discoloration or corrosion, and were lined with parchment paper for easy emptying. Palatability was a prime concern. Special combinations of process cheese were submitted to taste panels. Successful combinations selected for production included such flavors as pimiento, smoke flavor, and cooked bits of bacon or ham. The optimum composition of the cheese was determined in the same way. It was found that products with higher moisture were preferred to the regular process cheese. These products also were better adapted to canning and sterilization when it was practiced eventually. High nutritive value was required in some of these rations so butter was added.

These products had to endure long periods of storage at temperatures of 100 F or sometimes higher. Maximum temperatures of heating during processing approximated 195 F at first, but eventually sterilization in the can was practiced before the Korean War.

Research and development on canned cheese spread for military use continued after 1945 at the Food and Container Institute of the Quartermaster Corps. These projects showed that process cheese in cans exposed to a heat treatment of 240 F for 75 min in a steam-pressure retort was not acceptable, whereas process cheese spreads with and without meat added, stood this treatment satisfactorily to give good keeping quality and palatability (155). The age of blends of Cheddar influenced the stability of spreads (157).

The durability of process Cheddar in tin cans was tested by storage for up to 7 years at 100, 70, 47, 32, 0, and -20 F. Effects were measured by changes in color, texture, flavor, vitamins, and container damage. Cheese at 100 F softened, oiled off, and swelled the tins within 6 months. At 70 F and below freezing, the product was relatively stable, but cheese stored at 32 F was best (56).

Swelling of cans of process cheese spreads after long storage at high temperatures indicated that anaerobic spore-forming, gas-forming organisms had survived the heating treatments. Studies with spores of such an organism, PA 3679, under test conditions, showed that heat resistance of the spores decreased with lower pH and higher brine concentration (121).

Further research disclosed that heating and cooling lags in thermal death time increased with the level of cheese in the can and when the can was in a flat position rather than on edge (124).

Related to the problem of preserving the food value of cheese in cans for the Armed Forces was the development of process cheese food bars. Military specifications for cheese-flavored bars had been proposed in December 1961. The bar consisted of Blue cheese, dehydrated potatoes, potato starch, shortening, uncreamed Cottage or Bakers cheese, starter distillate, color, and water. Bars made of this mixture and freeze-dried until crisp had a composition of 13-15% fat, 7.7-9% protein, 70-75% carbohydrate, 0.7-1.4% salt, and a maximum of 2% moisture. This bar was patented by the Secretary of the U. S. Army (125).

#### *Dried process cheese*

Drying of natural cheese has been practiced for an unknown length of time; for military use it seemed especially attractive during World War II. It was tried, but not generally used (60). Spray drying a cheese emulsion was studied in the 1960s.

Spray drying of cheese began with selection of cured cheese with the desired flavor. Cheddar and Blue were commonly used. The cheese was dispersed to make a slurry in water and heated with sodium citrate or disodium phosphate to 100-180 F. It was then homogenized and spray dried at temperatures approximating 160 F. Foam spray drying of cheese was accomplished by dispersing cheese in water at 180 F with sodium citrate, homogenizing, injecting N<sub>2</sub> between a high pressure pump and the atomizing nozzle, and then spray drying the foamed mixture. The resulting product, when canned under N<sub>2</sub>, was better in quality than non-foamed spray-dried cheese. Adding the antioxidant, Nordihydroguaiaretic acid, improved shelf-life but sometimes off-flavors appeared (51, 166, 193).

Agglomeration of particles of dried cheese was accomplished by suspending them in a fluidized bed with hot air or gas. A suitable agglomerating agent was then sprayed directly on the cheese so it was agglomerated and dried at the same time (25). Using different spraying nozzles produced desired control of particle size in usual spray-drying operations (166).

Gas chromatographic studies showed volatile flavors were lost to some extent in spray-drying cheese and new flavors formed when slurries were heated above 180 F. Foam spray-dried cheese retained flavor better (52).

Preparation of a process cheese-like mixture for coating popcorn involved adding vegetable fat. It met regulatory objections as a filled cheese. The manufacturer claimed it was not sold as cheese but merely to coat popcorn and thus manufacture of the

product was permitted. A process for making a similar coating was described in 1970. The ingredients were spray-dried Cheddar, blended hydrogenated vegetable oils, dry buttermilk and sweet whey, glycerides, lecithin, and salt. The mixture could be sprayed when heated (220).

#### TRENDS

This consideration of the future of the process cheese industry will be limited to aspects of production, research and development, and manpower during the next 25 years.

#### *Production*

*Trends.* Data in Table 1 show 4-year averages of the number of manufacturing plants and production of pasteurized process cheese, cheese foods, and cold pack cheese reported annually by the U. S. Department of Agriculture since 1956. The number of plants since 1956 has been fairly steady although a downward trend is suggested by the 4-year period ending in 1971. Production of processed cheese of all types shows an upward trend. Production per plant is increasing. The trend should continue with expected improvements in buildings, equipment, and markets.

Values in Table 2 show that combined production of processed cheese, cheese foods, spreads, and cold pack is equivalent to half the total cheese (excluding Cottage types) produced in the United States. Production of processed cheese, which is the processed equivalent of Cheddar cheese, equals about 60% of Cheddar production. It is interesting that this relationship is the same as the consumer preference for this product indicated almost 40 years ago by the studies of Hobson and Schaars (112). The relationship seems likely to endure.

Trend of production has exceeded the growth in population as per capita consumption of all kinds of cheese has increased from 7.9 lb. in 1954 to 13.2 lb. in 1972. It is probable that this increased consumption of processed cheese products should continue with the greater demand for economical, high-quality, protein foods in convenient packages.

*Cheese procurement.* Processed cheese operations have been located traditionally in or near the areas of maximum production. Many other factors affect choice of location such as proximity to markets, costs of transportation, and availability of skilled labor. Now there is a change taking place in areas of production which will affect the procurement policies if not the location of manufacturing operations. Data in Table 3 show total cheese production in the United States in 1956 and 1971. Percentage changes in production in five general areas of the country are shown.



TABLE 1. PROCESSED CHEESE, CHEESE FOODS, CHEESE SPREADS AND COLD PACK CHEESE. FOUR-YEAR AVERAGES OF PRODUCTION IN THE UNITED STATES FROM 1956 TO 1971 (237)

Years	Processed cheese		Processed cheese foods, spreads and cold pack <sup>1</sup>		Totals	
	Plant	Production	Plant	Production	Plant	Production
	(Number)	(1,000 lb.)	(Number)	(1,000 lb.)	(Number)	(1,000 lb.)
1956-'59	48	528,789	48	193,278	59	722,067
1960-'63	42	571,478	46	221,475	57	879,963
1964-'67	43	659,267	45	246,207	59	905,474
1968-'71	42	682,760	40	406,477	56	1,089,237

<sup>1</sup>Excluding spreads made from Neufchatel and Cream cheese.

In this 25-year interval all divisions of the U. S. increased cheese production except the South Central division. Production of whole milk Cheddar in this area actually declined.

The significant increase in importance of West North Central U. S. as a cheese producing area is evident from data in Table 3. In 1956 this section made only 35% as much cheese as the leading division, the East North Central; in 1971 it made 62% as much cheese as the leader. The leading area increased production  $84 \times 10^6$  lb. in the 25-year interval, whereas the West North Central division raised production  $193 \times 10^6$  lb.

Procurement of cheese for processing may be affected by the recent large scale amalgamations of farmer cooperative associations. Historically, cooperatives market fluid milk in the highest price classification. They have established their own manufacturing plants to handle surplus milk or have sold it instead to independent manufacturers. It seems unlikely at this time that processors will undertake the conversion of variable surplus supplies into cheese in their own factories although the possibility may be highly desirable as demands for fluid milk change.

*Milk substitutes.* Production of processed cheese-like foods from vegetable fats and proteins, or with such fats and proteins in combination with milk fat and milk proteins, seems probable. This trend will be encouraged by consumers who want to meet special dietary requirements. The making of low-fat, low-cost, cheese-like food can be expected for domestic and foreign markets that are demanding these low-cost protein-rich foods where shortages exist. Research is needed to make these products of acceptable quality. Industry is now studying possibilities and has demonstrated its ability to produce these products.

*Manufacturing.* Production data indicate the trend toward greater production per plant. Expanded and improved operations will require closed systems and mechanized operations to eliminate hand contacts and to reduce labor requirements at all stages of manufacturing. There will be less reliance on manual controls. Manpower will be used only for machine activation, inspection, engineering functions, and mechanical maintenance. Such changes depend in large

part on the improvements in grinders, cookers, and packaging machines. Closed systems will retain more of the volatile odors now lost in steam cooking operations. Consideration is now being given to achieving emulsification of the fat and protein of cheese with less dependence on chemical emulsifiers by using systems related to those described by Parsons in which cheese is emulsified under pressure at temperatures below that of pasteurization (173-176) or by application of colloid mill mixing and dispersing principles.

Analytical procedures using ultrasonic sound and electrometric methods of analysis are coming into use. These procedures can be adapted to in-line analysis of product flow. They offer methods for continuous standardizing and composition control of blends.

Uniformity of composition facilitates mechanization and automation of product movement during processing, packaging, and casing. The mechanical handling of the cased goods with computer controls is now practiced in modern plants and warehouses; such controls will be used extensively in advanced processing plants to simplify handling the diversity of products which are directed to many different destinations for storage and shipping.

*Packaging.* Although methods and materials for packaging have been greatly improved in recent years, they are still being changed. Wrapping materials now provide good protection, but experts are searching for methods and materials which will make antimicrobials unnecessary. Mold-free units must be made

TABLE 2. RELATION BETWEEN PRODUCTION OF NATURAL CHEESE AND PRODUCTION OF PROCESSED CHEESE, CHEESE FOODS, CHEESE SPREADS AND COLD PACK IN THE UNITED STATES (237)

Year	Total cheese <sup>1</sup>	PCP <sup>2</sup>	%	Cheddar	PC <sup>3</sup>	%
	-(million pounds)-			-(million pounds)-		
1971	2,373	1,230	52	1,225	751	61
1970	2,201	1,138	52	1,182	686	58
1969	1,990	1,017	51	1,058	650	61
1968	1,938	971	50	1,050	644	61
1967	1,913	1,010	53	1,103	724	65

<sup>1</sup>Total of all types excluding Cottage and full skim American.

<sup>2</sup>Production of pasteurized process cheese, cheese foods, cheese spreads and cold pack cheese.

<sup>3</sup>Production of pasteurized process cheese.

TABLE 3. COMPARISON OF CHEESE PRODUCTION IN THE UNITED STATES IN FIVE DIVISIONS IN 1956 AND 1971 (237)

Divisions of U.S.	Total all types*		Change %	Cheddar		Change %
	1956	1971		1956	1971	
	million pounds			million pounds		
New Eng. and Mid Atl.	131	260	+98	36	63	+75
E. North Central	809	1,181	+46	512	596	+16
W. North Central	214	594	+178	180	373	+107
South Central	144	147	+2	118	87	-26
Mt. and Pacific	84	159	+89	41	65	+58
Total	1,387	2,373	+71	888	1,225	+38

\*Total of all types excluding Cottage and full skim American.

to open easily and to reseal hermetically to prevent dehydration, odor absorption, and mold contamination in the household refrigerator. Packaging operations will be speeded to deliver more than 200 packages and over 1,000 slices in packages per minute.

*Expansion.* Process cheese manufacturers expect greater production in more complex manufacturing units, some of which will have capacities exceeding 100 million lb./year. Such growth will be accomplished by development of the engineering accomplishments and technical expertise now used in modern plants. There are other methods of corporate development now evident in this industry. Some processors are expanding by increasing production of processed cheese, cheese foods, cheese spreads, and packaged natural cheese. These operations have succeeded and will continue to expand because they specialize in efficient procurement, curing, processing, quality control, and merchandising.

A second type of growth is attracting attention. It is production and merchandising of closely related food products. These are products which need similar facilities, sanitation techniques, skilled labor, and technological control. They are products which can use the same or similar equipment for heating, sterilizing, cooling, packaging, warehousing, and distribution. Outlets for these related products can be served by the same sales force and methods. These products, for example, can be cheese-like foods made with vegetable fats and proteins alone or combined with cheese; they may be convenience foods using cheese as a chief ingredient. This type of expansion attracts more attention as the dairy industry becomes more aware of opportunities in other phases of food production and distribution in this country and abroad.

And then there are organizations which are growing by absorbing or combining with other enterprises. The complementary enterprises may not even be closely related to processing in either procedures or location, but are attractive as outlets for capital and management ability. Examples of such organizations are conglomerates which are processing cheese, handling dairy products, groceries, meats, confections, chemicals, and engaged in warehousing and agri-

business enterprises. In such organizations the identity of the processor may be obscured.

#### Research

It would seem highly desirable if technical, industrial, and economic problems could be studied in a Cheese Research Institute supported by industry through the National Cheese Institute. Such an institute could provide postgraduate training for top echelon technical management directors and provide a place to train laboratory technicians and quality control personnel.

Such an institute would attract staff members of high caliber to work on problems of processes, products, packaging, equipment, analytical procedures, and quality control. The institute might centralize and classify scientific publications, statistical information, and other knowledge related to the industry's labor relations, regulatory edicts and proposals, and commerce. Such information would be compiled for the direct support of public relations and service work of the National Cheese Institute.

A few objectives of research and development important at this time are listed here; they may serve to suggest the nature of other information needed to assist the development of this growing industry: (a) engineering to achieve better use of heat, more mechanization and automation in manufacturing, storage, and transportation; (b) simplification and mechanization for rapid, accurate, laboratory procedures to determine chemical composition, rheological properties, and to identify and quantify certain microorganisms, enzymes, antibiotics, toxins, and vitamins in natural cheese and the finished products; such information must be the basis of informative labeling to show composition and nutritive values; (c) improvement of emulsifiers and stabilizers and mechanical methods of dispersing, solubilizing and making blends of cheese homogeneous; (d) preservation of cheese flavors and development of new combinations of flavors and ingredients to make meat-like foods with a cheese base; (e) improvements of sanitary controls and quality to enhance keeping properties without mold inhibitors or preservatives; (f) improve-

ments of wrappers and packaging methods and equipment to increase efficiency and extend keeping quality; (g) development of precooked and packaged cheese-base foods or food combinations for convenience meals, (h) improvements in methods of controlling and stabilizing rheological properties ranging from meat-like consistency to free-flowing sauces and blends for industrial use in other foods and for dispensing mechanically in serving in fast-food establishments, (i) improved products for "instant" meals in homes and in hospitals and institutions with minimum facilities for preparing meals, (j) improvement and development of new types of low-fat cheese products, and (k) development of low-cost non-dairy foods or combinations of them with cheese to approximate composition, nutritive value, and usefulness of cheese.

*Manpower*

Manpower to operate the complex processing plants in the future will need special training. It is probable that technical schools can teach a basic understanding of sanitary control, simple analytical procedures, and quality evaluation. In-plant training, however, will be essential to develop the proficiency necessary to perform assigned duties.

The technical directors, research workers, and management personnel can be expected to have university training or its equivalent. But these men and women will require exposure to industrial procedures and conditions to achieve maximum efficiency. To maintain and develop skill, knowledge and judgment, we foresee more reliance on seminars with their peers. Such seminars may be conducted by industrial associations, business institutes, universities, and regulatory and governmental agencies. These seminars should discuss pertinent technical advances in chemistry, bacteriology, food science, environmental health and sanitation, as well as public relations, labor management, business law, banking, business cycles, foreign and domestic trade, and the like.

#### CONCLUSION

What began as a simple cooking, package-filling, and merchandising business has developed into a consumer-conscious, complex, and diversified industry. We expect its amazing progress of the past 50 years will be surpassed by future progress.

People are intensely aware of the problems and hazards of population explosions and food shortages in all parts of the world. The process cheese industry with greater resources than ever of scientific information and technological capabilities can contribute efficiently and effectively to provide the people of this country with convenient, healthful and nutritious foods.

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## MICROBIAL BETA-GALACTOSIDASE: A SURVEY FOR NEUTRAL pH OPTIMUM ENZYMES

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### ABSTRACT

Pure cultures of yeast, molds, and bacteria were screened for neutral pH optimum  $\beta$ -galactosidases (lactases) that would be suitable in dairy products applications. Only 2 of 125 identified and 10 of 250 unidentified cultures warranted further study. These cultures produced high levels of  $\beta$ -galactosidase with moderate galactose product inhibition. Characterization of the partially purified enzymes from unidentified cultures revealed that all required either  $\text{Na}^+$ ,  $\text{K}^+$  or  $\text{Mg}^{++}$  cation activation, were inhibited by  $\text{Cu}^{++}$ ,  $\text{Mn}^{++}$ , and  $\text{Fe}^{+++}$ , were most active around pH 6.8, and were unstable during storage (at either  $-196^\circ\text{C}$  or  $4^\circ\text{C}$ ) except in the presence of 0.5 M ammonium sulfate. Most of the enzymes compared favorably in performance with a commercially available  $\beta$ -galactosidase when tested in skim milk.

Renewed interest in enzymatic hydrolysis of lactose in milk and dairy products has been stimulated by recent studies demonstrating the prevalence of lactose intolerance among certain groups of consumers; (3, 7, 11). Furthermore, the pollution problem posed by disposal of enormous quantities of cheese whey containing large amounts of lactose has forced food scientists to seek more meaningful uses for whey. Increased use of whey in foods and feed, however, will necessarily be limited by consumer lactose tolerance and will consequently require some degree of lactose hydrolysis.

The feasibility of enzymatically hydrolyzing lactose in milk and dairy products has been reported by several laboratories (8, 14, 15). These studies have been carried out largely with yeast enzymes. Microbial  $\beta$ -galactosidase, E.C. 3.2.1.22, (lactase) would appear to have the greatest commercial potential primarily because of ease of production. Although a broad variety of microorganisms are known to produce  $\beta$ -galactosidase, (1, 9, 10, 13, 16, 17), very few have properties that would make them suitable for commercial use. Consequently we initiated a screening program to search for new sources of neutral pH optimum  $\beta$ -galactosidases that would be suitable for dairy product applications.

### MATERIALS AND METHODS

#### Cultures and media

One hundred twenty-five identified yeasts, molds, and bacteria were obtained from the culture collection of the Northern Regional Research Center, Peoria, Illinois. Cultures of the following genera were included: *Penicillium*, *Aspergillus*, *Absidia*, *Cunninghamella*, *Mucor*, *Rhizopus*, *Circinella*, *Blakeslea*, *Chlamydomucor*, *Streptomyces*, *Actinomyces*, *Actinopycnidium*, *Debaryomyces*, *Kluyveromyces*, *Pichia*, *Schwanniomyces*, *Bullera*, *Brettanomyces*, *Candida*, *Cryptococcus*, *Rhodotorula*, *Torulopsis*, *Escherichia*, and *Bacillus*. Additionally, 250 unidentified organisms were isolated by enrichment culture techniques from soil samples taken from locations used for dairy waste disposal.

Yeasts were maintained on yeast extract-malt extract (YM) agar slants (5) having the following composition in grams per liter: yeast extract, 3.0; malt extract, 3.0; peptone, 5.0; glucose, 10.0; and agar, 25.0. Molds were maintained on Czapek Dox agar (5). Bacteria were maintained on either trypticase-soy agar (Baltimore Biological Lab) or yeast extract-peptone-lactose agar (YPL) having the following composition in grams per liter: yeast extract, 2.0; peptone, 10.0; ammonium sulfate, 4.0; lactose, 10.0; salts solution, 20.0 ml (2); and agar, 15. Cultures were stored at  $2^\circ\text{C}$  after incubation.

#### Enrichment cultures

Enrichment cultures were grown in either YPL broth or in YM broth with lactose substituted for glucose. Each 1-2 g of soil sample was inoculated into 200 ml of broth contained in a 1-liter flask. Samples were incubated at  $28^\circ\text{C}$ . Samples were periodically removed, diluted, and spread on appropriate agar plates. Isolated colonies were picked and purified by isolation from additional spread plates. Isolates were identified as yeast, mold, or bacterium by gram strains and phase contrast microscopy of wet mounts.

#### Screening for $\beta$ -galactosidase production

Two hundred-milliliter broth cultures contained in 1-liter flasks that were incubated for 24 to 48 h on a gyratory shaker at  $28^\circ\text{C}$  were used for cell production during screening. The total mass of yeasts and bacteria was harvested by centrifugation, that of molds by filtration. Cells and mycelia were washed once in 35 ml of 0.05 M potassium phosphate buffer pH 6.8, and resuspended in fresh buffer to a 12-ml volume; extracts were prepared by using a Sonifier cell disruptor, Model W 185 (Heat Systems - Ultrasonics, Inc., Plainview, N. Y.). Sonication was done in an ice-water bath at 90-95 watts power setting. Particulate matter was removed by centrifugation, and extracts were stored in liquid nitrogen.

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### Enzyme assay

$\beta$ -Galactosidase activity of crude extracts was determined by incubating the following reaction mixture for 30 min at 35 C in screw cap tubes: enzyme; phosphate buffer pH 6.8, 0.07 M; lactose, 0.139 M; and water in a total volume of 3.0 ml. Parallel reactions were conducted in which 0.139 M D-galactose was included to estimate product inhibition. Reactions were stopped by boiling 5 min. The amount of glucose liberated was then determined according to the method described by Jasewicz (6). o-Nitrophenol- $\beta$ -D-galactopyranoside (ONPG) (Calbiochem) was used as substrate for purification, thermal stability, pH optimum, activator, and inhibitor experiments. A unit of enzyme was defined as that amount which produced one  $\mu$ mole of glucose or O-nitrophenol per minute under the reaction conditions specified above. Specific activity was defined as the number of units per mg protein. Protein was determined by the Biuret method (4).

### Enzyme purification

Selected cultures were grown in 2-liter quantities, and extracts of cells were prepared by using a French pressure cell operated at 16,000 psi. Partial purification was done by fractional ammonium sulfate precipitation followed by DEAE sephadex chromatography using a NaCl gradient elution in pH 7.0, 0.05 M phosphate buffer.

### Enzyme characterization

Thermal stability of partially purified enzymes was determined by heating microgram quantities of enzyme in 1.0 ml of water in screw cap tubes at the desired temperature for 10 min. Samples were immediately cooled in an ice bath and the remainder of the reaction ingredients were added. Reaction mixtures were then brought to 35 C, ONPG was added, and hydrolysis rates were determined from kinetic traces by using a recording Beckman DB spectrophotometer at 420 nm.

A variety of mono- and divalent cations were tested for activator or inhibitor effects on the selected, partially purified enzymes. These tests were conducted in tris-hydroxymethylamino methane chloride buffer pH 7.0 to avoid phosphate precipitates.

Product inhibition studies with partially purified enzymes were performed in which glucose or galactose was included in the standard reaction mixture at a final concentration of 0.139 M. This corresponded to their concentration if total lactose hydrolysis had occurred in milk.

pH optimum experiments were done in 0.05 M phosphate buffer over the range of 6 to 8. Results were normalized for differences in nitrophenol extinction coefficients at the various pH levels tested by reference to standard curves.

## RESULTS AND DISCUSSION

### Screening

The screening procedure used in this study was effective in selection of microorganisms producing high levels of  $\beta$ -galactosidase with moderate product inhibition and neutral pH as optima. Although many of the identified organisms were from genera that had not been previously reported to produce  $\beta$ -galactosidase, the hope for a  $\beta$ -galactosidase with unique properties did not materialize. Only two of the identified organisms surveyed produced substantial

levels of enzyme, and both were inhibited by galactose in excess of 53%. These organisms, *Klyveromyces lactis* NRRL Y-1118 and *Klyveromyces fragilis* NRRL Y-1109, are known  $\beta$ -galactosidase producers and the enzyme from Y-1109 has been well studied in dairy products applications (15).

The remaining cultures producing substantial quantities of  $\beta$ -galactosidase were isolated from enrichment cultures. Crude extract specific activities, galactose inhibition, enzyme yields, and morphological type of the 10 most active organisms are given in Table 1. Generally, yeast enzymes were more susceptible to galactose inhibition than were bacterial enzymes. Since no attempt was made to determine optimal cultural conditions, it is possible that higher yields might have been obtained with other media.

Failure to find any promising mold enzymes was not surprising since most mold  $\beta$ -galactosidases have acid pH optima and would not have been active at the screening pH.

Although microbial  $\beta$ -galactosidases are generally of intracellular origin, the advantages of processing an extracellular enzyme dictated screening of spent broths for activity. No  $\beta$ -galactosidase activity was detected in any of the spent broths.

Microscopic examination of unidentified selected cultures showed that all the bacteria were gram-negative rods. Yeasts were observed to be ellipsoidal budding types.

### Enzyme purification

Ammonium sulfate fractionation of selected culture crude extracts generally resulted in precipitation of

TABLE 1.  $\beta$ -GALACTOSIDASE PRODUCTION BY ENRICHMENT CULTURE ISOLATES

Culture	Morphological type	Specific activity*	% Inhibition by D-galactose	Units/200 ml culture
MTA-7	Bacterium	1.68	24.2	492
MTB-28	Bacterium	.476	29.3	116
MTA-1	Bacterium	1.54	31.0	473
105 <sub>b</sub>	Bacterium	3.40	36.6	1027
Bel-17	Bacterium	1.91	13.2	600
Bel-15	Bacterium	1.22	29.1	411
MTB-18	Bacterium	1.38	38.0	224
227	Yeast	2.14	51.6	534
217	Yeast	3.00	52.3	864
204	Yeast	3.71	49.5	682

\*Crude extracts.

the bulk of  $\beta$ -galactosidase in the 30-50% saturation salt-cut. DEAE-sephadex chromatography of the enzymes resulted in elution between the 0.30 and 0.35 M portion of the NaCl gradient in all instances. Purifications varied among the enzymes ranging from 8- to 15-fold relative to crude extract specific activities with recoveries ranging from 60 to 80%.

TABLE 2. THERMAL STABILITY OF SELECTED  $\beta$ -GALACTOSIDASES

Source of $\beta$ -galactosidase	Percent			
	activity remaining 35 C	activity remaining after 40 C	10 min heating at 45 C	50 C
MTA-7	76.6	58.6	17.2	—*
MTB-28	89.0	57.4	23.5	0
MTA-1	97.0	66.7	17.9	—
105 <sub>b</sub>	98.5	76.5	0	—
Bel-17	88.7	37.1	0	—
Bel-15	66.2	0	—	—
MTB-18	79.3	18.5	—	—
227	97.0	32.2	8.9	—
217	100	63.0	74.3	32.9
204	39.1	0	—	—

\*Not determined.

TABLE 3. EFFECT OF pH ON ONPG HYDROLYSIS BY SELECTED  $\beta$ -GALACTOSIDASES

Source of $\beta$ -galactosidase	Specific activity*		
	pH 6.0	pH 6.8	pH 8.0
MTA-7	6.6	11.0	7.1
MTB-28	2.0	6.6	4.0
MTA-1	6.6	27.6	20.0
105 <sub>b</sub>	7.1	17.2	11.4
Bel-17	5.3	21.0	1.0
Bel-15	5.8	11.5	5.1
MTB-18	8.5	28.5	12.2
227	2.1	4.0	0.7
217	4.8	8.5	5.2
204	16.7	38.1	23.8

\*Specific activity = number of units per mg protein.

#### Thermal stability

Most of the enzymes studied were essentially inactivated by 10 min heating at 45 C (Table 2). The enzyme from culture 217 was the exception retaining 75% activity at 45 C. Commercial application of  $\beta$ -galactosidase for neutral pH dairy products will require good thermal stability. Ideally the enzyme should function optimally either at 4 C and below or above 45 C to minimize the deleterious effects of microbial contaminant growth. The results for  $\beta$ -galactosidases in this study indicate that none would satisfactorily meet the high temperature specification. Activities of our enzymes at 4 C or below were not determined.

#### Storage stability

Storage stability is another commercially important enzyme characteristic. All of the partially purified  $\beta$ -galactosidases in this study were unstable during storage in dilute buffer at 4 C or frozen in liquid nitrogen. Storage stability could be greatly extended for at least one month by addition of ammonium sulfate (0.5 M). Although other methods of stabilization were not tested it is possible that some may be applicable.

#### pH optimum

The specific activities of partially purified  $\beta$ -gal-

actosidases at various pH levels are given in Table 3. All enzymes were most active at pH 6.8.

#### Influence of cations

The influence of cations on the activity of  $\beta$ -galactosidases varies with the origin of the enzyme, the substrate, and the pH of the reaction (13).  $\beta$ -Galactosidases of bacterial origin in this survey were similar to the *Escherichia coli* enzyme showing the greatest stimulation with Na<sup>+</sup> (0.1 M) and lesser stimulation with K<sup>+</sup> (0.1 M) and Mg<sup>++</sup> (0.1 M). No activity was observed in Tris-Cl buffer in the absence of the above cations. Contrary to the findings of Wallenfels (13), a low concentration of Mn<sup>++</sup> (10<sup>-4</sup> M) or Fe<sup>3+</sup> (10<sup>-4</sup> M) did not cause additional stimulation in the presence or absence of Na<sup>+</sup>. Instead, inhibition was observed. The source of enzymes or the assay buffer (Wallenfels used 0.04 M imidazole buffer pH 6.8 containing 0.04 M NaCl) might explain these observations. Enzymes of yeast origin were activated most by Mg<sup>++</sup> (0.1 M) and to a lesser extent by Na<sup>+</sup> (0.1 M) and K<sup>+</sup> (0.1 M); this activation was similar to that of other yeast enzymes (15). Mn<sup>++</sup> and Fe<sup>+++</sup> also inhibited the yeast enzymes. All the  $\beta$ -galactosidases studied were greatly inhibited by Cu<sup>++</sup> (10<sup>-4</sup> M); this suggests that they are of the sulfhydryl type. Inhibition experiments were conducted in the presence of the appropriate activating ion.

A comparison of ONPG hydrolysis inhibition by glucose and galactose at concentrations equal to total lactose hydrolysis in milk is shown in Table 4. Generally, bacterial enzymes were inhibited by both sugars except for MTA-1 which was not inhibited by galactose. In contrast, yeast enzymes were not inhibited by glucose; in fact, cultures 217 and 227 were stimulated to the extent of 28% and 47%, respectively. Inhibition of lactose hydrolysis by glucose and transgalactosidation activity were not determined.

#### Comparison of lactases with commercial enzyme

Shortly after initiation of this work a neutral pH optimum  $\beta$ -galactosidase produced by *K. lactis*, and marketed as "Maxilact" (Enzyme Development Corp., New York, N. Y.<sup>2</sup>) became commercially available. Consequently, it became necessary to compare the performance of our enzymes to Maxilact in skim milk. Reaction mixtures were set-up containing 30 units of enzyme (based on lactose hydrolysis), 5.0 ml double strength reconstituted dry skim milk, and water to a final volume of 10.0 ml. Reactions were conducted at 35 C and 1-ml samples were taken at 1-h intervals

<sup>2</sup>Mention of a trademark or proprietary product does not constitute a guarantee of warranty of the product by the U. S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

TABLE 4. INHIBITION OF ONPG HYDROLYSIS BY 0.139 M GLUCOSE AND GALACTOSE

Source of $\beta$ -galactosidase	Percent inhibition	
	Glucose	Galactose
MTA-7	25.0	9.9
MTB-28	28.2	35.5
MTA-1	34.0	0
105 <sub>b</sub>	31.1	41.8
Bel-17	27.3	32.8
Bel-15	46.2	35.6
MTB-18	33.8	28.4
227	0	20.0
217	0	32.5
204	0	48.3

TABLE 5. A COMPARISON OF SKIM MILK LACTOSE HYDROLYSIS BY UNIDENTIFIED  $\beta$ -GALACTOSIDASES WITH A COMMERCIALY AVAILABLE ENZYME

Source of $\beta$ -galactosidase	% Lactose hydrolyzed 3 h., 35 c
MTA-7	50.5
MTB-28	45.8
MTA-1	49.2
105 <sub>b</sub>	45.4
MTB-18	49.6
Bel-17	46.5
Bel-15	40.4
227	38.2
204	48.5
Maxilact	51.4

over a 3-h period. Reactions were stopped by boiling 5 min. Protein was removed by the Symogii method (12), and the amount of glucose released was determined as previously described. The results after 3-h incubation as shown in Table 5 indicated that most of the enzymes compared favorably with Maxilact. Although not shown in the table it should be noted that the rate of hydrolysis was substantially slower after the first hour of incubation.

Commercial utilization of  $\beta$ -galactosidase may be facilitated by immobilizing the enzyme on insoluble supports. The effects of immobilization on our enzymes are currently being evaluated and will be reported later.

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## A Research Note

USE OF ACETIC AND LACTIC ACID TO CONTROL THE QUANTITY OF MICROORGANISMS ON LAMB CARCASSES<sup>1</sup>H. W. OCKERMAN<sup>2</sup>, R. J. BORTON<sup>2</sup>, V. R. CAHILL<sup>2</sup>, N. A. PARRETT<sup>2</sup>, AND H. D. HOFFMAN<sup>3</sup>*The Ohio State University, Columbus, Ohio 43210 and The Ohio Agricultural Research and Development Center, Wooster, Ohio 44691*

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## ABSTRACT

Numbers of bacteria were significantly (statistically) reduced on ovine carcasses, refrigerated for 12 days, by spraying with a solution of acetic or lactic acid. However the biological significance of this small reduction is of questionable value when good storage conditions are maintained.

Microorganisms are introduced into and onto meat carcasses during conventional slaughter procedures (1, 7, 9, 12) and some means to control their numbers would be beneficial to the meat packing industry. Various methods of control have been attempted, such as washing the animal (6), washing and drying the carcass (11), washing the carcass with 200 ppm chlorine solution (3, 5) and using antibiotics in the washing solution (4, 13). Recently various organic acids have been utilized in an attempt to control microbial growth of various types. Mountney and O'Malley (10) studied the effect of some of the acids on the general flora of poultry carcasses and found acetic, adipic, and succinic acids effective for reducing bacterial numbers. Thomson et al. (13) reported citric and succinic acids effective for reducing *Salmonella typhimurium* contamination. Biemuller et al. (2) and Carpenter (3) reported various acids controlled growth of *Salmonella enteritidis* as well as total flora on pork carcasses.

This study examined the effect of four concentrations (6, 12, 18, and 24%) of acetic and lactic acid sprays, on the number of microorganisms on lamb carcasses during a 12-day refrigerated storage period.

## MATERIALS AND METHODS

Thirty-two sheep were slaughtered, dressed, split, and washed with water. After washing, one-half of each carcass was sprayed with 50 ml of an acid solution and the other half was sprayed with 50 ml of distilled water. The acid solutions used are shown in Table 1. There were four replications of each treatment.

Swabs were taken on the treated and control halves at

TABLE 1. STRENGTH AND pH OF ACID SOLUTIONS

Lactic acid		Acetic acid	
Strength	pH	Strength	pH
6%	1.8	6%	2.4
12%	1.6	12%	2.2
18%	1.5	18%	2.1
24%	1.4	24%	1.9

six locations [the leg (stifle area), crown (posterior area of leg), loin, flank, shoulder, and neck] after storage for 0, 2, 7, and 12 days at  $3 \pm 1$  C. Swabbing was done by using a water soaked cotton bud and a 12.3 cm<sup>2</sup> circular template. After swabbing, cotton buds were immediately placed in a 1% solution of Bacto-Peptone broth and held at 4 C until plating was accomplished. After appropriate dilution, the organisms were incubated at 25 C for 72 h with Tryptone Glucose Extract Agar as the incubation medium. Colonies were counted and, after considering dilution, the numbers/cm<sup>2</sup> were recorded. A logarithmic transformation of these counts was then analyzed using Harveys' Least-Squares Maximum Likelihood General Purpose Program (8).

## RESULTS AND DISCUSSION

Figure 1 shows the effect of acetic acid on the quantity of microorganisms. All concentrations of acetic acid reduced microbial numbers on the lamb carcasses through 12 days of storage. It was found that the 18% concentration was most effective ( $P < .01$ ) in reducing microbial counts when the means at each level were compared. Also the effect of the 18% acetic acid treatment was most consistent over the 12-day storage period. Overall the lowest microbial levels were found after 2 days of storage ( $P < .01$ ) when the means at each storage period were compared.

Figure 2 shows the effect of lactic acid on the quantity of microorganisms. The results are not as consistent as with acetic acid, but it can be seen that over the 12-day storage period the 12% level of lactic acid was the most effective ( $P < .01$ ) for reducing the bacterial level but after 7 days of storage was very similar to the 18% lactic acid concentration.

Overall there was a nonsignificant ( $P > .05$ ) difference between the bacterial levels found with the two acids and also a nonsignificant ( $P > .05$ ) difference between the six carcass sample locations.

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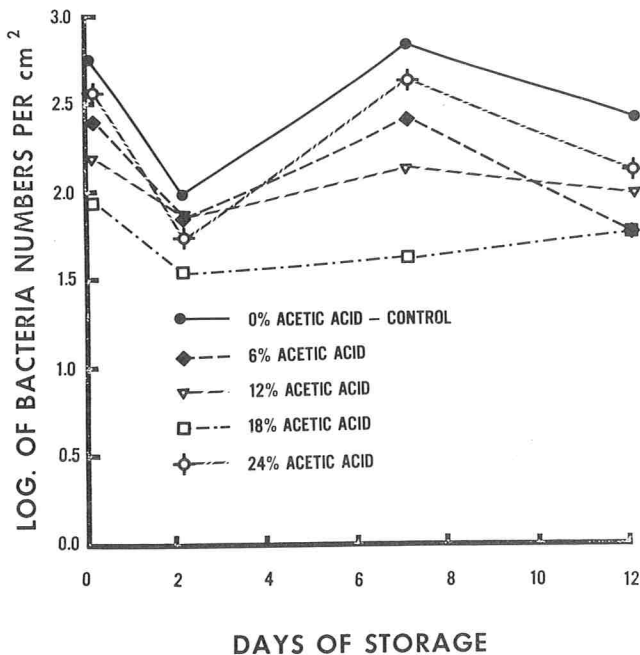


Figure 1. Effect of four concentrations of acetic acid on microorganisms on lamb carcasses.

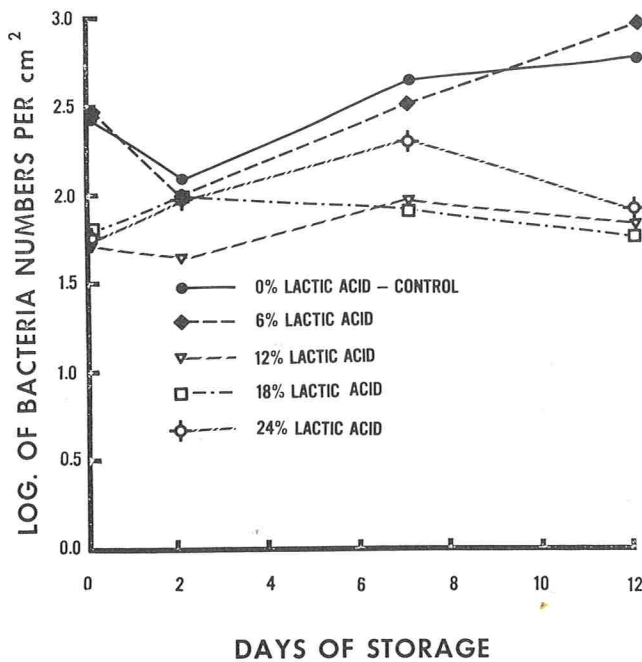


Figure 2. Effect of four concentrations of lactic acid on microorganisms on lamb carcasses.

Bleaching of the carcass was observed at the 12% and higher concentrations of acetic acid and at the 18% and 24% concentrations of lactic acid.

In this study both the acetic and lactic acid significantly (statistically) ( $P < .01$ ) reduced the microbial numbers on these ovine carcasses during 12 days of refrigerated storage, but the biological significance of this small reduction may be of little advantage when animals are slaughtered and stored under ideal conditions (from a microbiological content standpoint) used in this study. This experiment relied on natural contamination and growth and much of the organism's growth was controlled by fairly low humidity ( $81 \pm 3\%$ ), rapid air flow ( $50 \pm 2$  meters/min), and low temperatures ( $3 \pm 1$  C) in the storage cooler. To evaluate these acids and their levels, at higher numbers of bacteria, further studies should be done under less ideal storage conditions.

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# PHYSICAL-CHEMICAL TREATMENT OF WASTE EFFLUENTS FROM CANNING OF LEAFY GREEN VEGETABLES

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## ABSTRACT

Suspended solids in waste effluents from the canning of leafy green vegetables were effectively reduced by segregation and separate physical-chemical treatment of the unit wastes. Turbidity in the dunker washer effluent from spinach was reduced by treatment with 0.04 mg of an anionic polymer per liter at pH 4.0. The most concentrated effluent (from the tumbler fillers) contained 11% of the total suspended solids load and 16% of the COD in only 1.3% of the waste water flow. Treatment with 20 mg cationic polymer per liter, 80 mg ferric sulfate per liter, and 2 mg anionic polymer per liter reduced the suspended solids concentration from 1,348 mg/l to 63 mg/l—a reduction of 95%. The tumbler filler effluent from turnip greens and diced roots was treated on a pilot-scale with 70 mg cationic polymer per liter, 80 mg calcium chloride per liter and 10 mg anionic polymer per liter to reduce the suspended solids from 1,148 to 140 mg/l. The composite effluent from canning of turnip greens and diced roots was treated with polymeric flocculating agents and activated carbon in a physical-chemical treatment system. The suspended solids concentration was reduced by 92% and the COD by 19% through flocculation and settling. The clarified effluent was applied to a downpass column of activated carbon which adsorbed 89% of the COD applied.

Physical-chemical methods of treatment are being increasingly applied to municipal (1, 3) and industrial wastes (2, 9). Chemical methods of treatment applied to food processing wastes have most commonly employed inorganic salts such as lime, alum, or iron sulfate to coagulate suspended solids (13). Few studies on the use of polymeric chemical agents to treat food wastes have been reported. Hopwood and Rosen (10) investigated use of sodium lignosulphonate on slaughterhouse and poultry packing wastes. Bough and Shewfelt (6) reported on the use of synthetic polyelectrolytes to treat pimiento canning wastes.

The number of applications of carbon treatment to food processing wastes have also been few. Carbon treatment to remove dissolved wastes has been applied to olive brines (14) and cherry brines (17). Preliminary studies on application of carbon treatment to potato, collard greens, pimiento, and poultry rendering wastes were reported by Shewfelt (16).

The theoretical bases of waste treatment with polymeric flocculating agents, as described by LaMer and Healy (11) and O'Melia (15), were previously discussed (6). Recent books by Culp and Culp (7) and Weber (18) contain much useful information on basic and

applied aspects of physical-chemical treatment systems.

The objective of this study was to investigate the application of physical-chemical methods of treatment to reduction of wastes in effluents from canning of leafy green vegetables. Previous characterization studies (5) suggested that certain concentrated unit effluents should be segregated and treated separately to reduce the suspended solids load. Treatment of the composite effluent with polymeric flocculating agents followed by carbon adsorption was also investigated.

## MATERIALS AND METHODS

### Effluent samples

Samples of waste effluents were collected from the commercial leafy greens canning operation described previously (5). The unit effluents collected for treatment were those from the dunker washers and tumbler fillers. Physical-chemical treatment of the total or composite effluent was also investigated. The same procedures of collection as employed previously were followed (6). A flow diagram of processing operations and unit effluents was included in the characterization study (5).

### Laboratory studies

The different synthetic flocculating agents tested in this study were WT-3000, an anionic polymer; Natron 86, a cationic polyacrylamide; and Atlasep 105c, another cationic polymer. The inorganic salts employed were calcium chloride, alum, ferric sulfate, and ferric chloride. Variations of polymer-salt concentrations and combinations were tested under different pH conditions.

A Phipps and Bird laboratory stirrer was used to mix 500-ml volumes of waste effluents contained in 800-ml beakers with the desired chemical agents for a maximum time of 4 min at 100 rpm. When a treatment employed both a cationic and anionic polymer, the cationic polymer and salt (if appropriate) were mixed with the waste effluent for 2 min at 100 rpm; the anionic polymer was then added to the system and mixed for another 2 min. After mixing, treated waste samples were allowed to settle for 60 min. Supernatant liquids were decanted and analyzed for turbidity (8) and, where indicated, suspended solids and chemical oxygen demand (COD) (12). Turbidity values, expressed as formazin turbidity units (FTU), were determined with a Hach 2100A turbidimeter with an accuracy of  $\pm 2\%$  of full scale deflection. Scale settings of 0-1.0, 0-10, 0-100, and 0-1,000 were used in these experiments. Values shown for suspended solids and COD are averages of duplicate determinations.

### Pilot-scale studies

The apparatus and procedures described previously (6)

TABLE 1. EFFECT OF pH AND CONCENTRATION OF POLYMER ON REDUCTION OF TURBIDITY IN DUNKER WASHER EFFLUENT<sup>1</sup> FROM CANNING OF SPINACH

WT-3000 (mg/l)	Turbidity values, FTU pH			
	3.0	4.0	5.0	6.0
0.0	14	12	—	31
0.04	—	3.1	17	58
0.1	—	3.8	14	60
0.2	—	4.2	11	57
0.3	—	3.7	—	58
0.4	—	3.9	19	58
2.0	—	5.2	22	58

<sup>1</sup>The pH of the raw effluent was 6.5

TABLE 2. FLOCCULATION OF FILLER EFFLUENT FROM CANNING OF KALE GREENS WITH NATRON 86, FERRIC SULFATE AND WT-3000 AT pH 6.0 TO REDUCE TURBIDITY<sup>1</sup>

Natron 86 (mg/l)	Fe <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> (mg/l)					
	0	20	40	80	120	160
	(Turbidity)					
0	190 <sup>2</sup>	140	115	92	87	83
2	190	58	57	86	85	83
5	185	43	21	47	34	37
10	125	43	13	24	13	15
20	98	18	13	12 <sup>3</sup>	12.5	12.5
40	110	27	13	14	14	14
60	165	77	37	24	19	17
80	180	95	50	37	22	18

<sup>1</sup>While the amounts of Natron 86 and Fe<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub> were varied as indicated, every treatment contained 2 mg/l of WT-3000.

<sup>2</sup>Turbidity values shown are expressed in formazin turbidity units (FTU).

<sup>3</sup>Corresponds to a 95% reduction in suspended solids concentration from 1,348 to 63 mg/l

were used for pilot-scale treatment of waste effluents from leafy greens. Conditions investigated were those shown to be optimum in laboratory studies on the different effluents for removal of turbidity and suspended solids.

After treatment with the desired chemical agents and settling to remove suspended solids, the clarified supernatant liquid was decanted from the settling tank and filtered through activated carbon to adsorb soluble waste components. A column (5 × 100 cm) containing 2 lb of granular activated carbon was fed at a rate of 75 ml/min which corresponded to a liquid residence time of 10.3 min. The column effluent was collected in successive 1-gal batches.

## RESULTS AND DISCUSSION

The unit effluent from the dunker washers in the processing of leafy green vegetables was previously found to contain an average of 2.3 lb suspended solids/ton and 4.7 lb COD/ton in a waste flow of 563 gal/ton (5). Considerable variation was observed in the suspended solids load of this effluent, as shown by the standard deviation value of 1.6 lb/ton. This variation was primarily due to intermittent dumping

of the washers based upon a subjective decision of an operator about the condition of the greens. Table 1 shows that the concentration of soil and plant materials causing turbidity in the dunker washer effluent was easily reduced by treatment with only 0.04 mg of the anionic polymer WT-3000 per liter at pH 4.0. Treatments at pH values between 4.0 and 6.0 were less effective in reducing turbidity.

The most concentrated unit effluent produced in the canning of leafy greens issued from the tumbler fillers. It contained 11% of the total suspended solids load and 16% of the total COD load in only 1.3% of the waste water flow (5). Segregation and separate treatment of this effluent was recommended. Table 2 shows results obtained by treating the tumbler filler effluent from Kale greens with 2 mg of WT-3000, an anionic polymer per liter, and various concentrations of Natron 86, a cationic polymer, and ferric sulfate at pH 6.0. Turbidity of the raw effluent was effectively reduced from 190 to 12 FTU by a combination of WT-3000 (2 mg/l), Natron 86 (20 mg/l), and ferric sulfate (80 mg/l). This corresponded to reduction of the suspended solids concentration from 1,348 mg/l to 63 mg/l—a reduction of 95%.

Results in Table 2 show that concentrations of Natron 86 greater than 20 mg/l caused resuspension of the precipitate and increased turbidity values. Restabilization of solids from the coagulated into the suspended phase is a common phenomenon associated with treatment with polyelectrolytes and is caused by overdosing (15).

When turnip roots are diced and packed together with turnip greens, a starch-containing effluent is

TABLE 3. FLOCCULATION OF TUMBLER FILLER EFFLUENT<sup>1</sup> FROM CANNING OF TURNIP GREENS AND DICED ROOTS WITH NATRON 86, WT-3000, AND CALCIUM CHLORIDE TO REDUCE TURBIDITY

Trial no.	Natron 86 (mg/l)	CaCl <sub>2</sub> (mg/l)	WT-3000 (mg/l)	Turbidity (FTU)
I	0	0	0	23
	20	80	20	12
	50	80	20	4.3
	70	80	20	4.3
	100	80	20	3.2
II	70	40	14	5.3
	70	80	14	5.3
	70	120	14	5.1
	70	200	14	5.4
III	70	80	2	8.2
	70	80	4	8.3
	70	80	6	3.7
	70	80	10	3.0 <sup>2</sup>
	70	80	14	3.2
	70	80	20	3.3

<sup>1</sup>The pH of the raw effluent was 6.4; the suspended solids concentration was 1,100 mg/l.

<sup>2</sup>The suspended solids concentration of this decanted supernatant was 125 mg/l.



TABLE 4. PILOT-SCALE TREATMENT OF TUMBLER FILLER EFFLUENT FROM CANNING OF TURNIP GREENS AND DICED ROOTS

Treatment conditions	Sample	Turbidity (FTU)	suspended solids (mg/l)	COD (mg/l)
Natron 86, 70 mg/l; CaCl <sub>2</sub> , 80 mg/l; followed by WT-3000, 10 mg/l; at pH 6.4	Raw effluent, pH 6.4	180	1,148	11,466
	Floc. and settled, 0.5 h	90	319	
	Floc. and settled, 1.0 h	52	161	
	Floc. and settled, 1.5 h	50	140	9,114

<sup>1</sup>Sampled after flocculation and settling by decantation from lowest port on side of settling tank.

produced. Table 3 gives results on this effluent collected from the tumbler fillers and treated with various concentrations of a cationic polymer, calcium chloride, and an anionic polymer at pH 6.4. The greatest reduction in turbidity, from 23 to 3.0, was obtained with 70 mg Natron 86, 80 mg calcium chloride, and 10 mg WT-3000 per liter. This combination of chemical agents was then tested on a pilot-scale, as shown in Table 4. Under these conditions, the suspended solids concentration was reduced from 1,148 to 140 mg/l (88%) and the COD from 11,466 to 9,114 (20%).

The average composite waste load from canning of leafy greens was 20.2 lb COD/ton, 2.8 lb suspended solids/ton, and 2,666 gal waste water/ton (5). Flocculation of waste materials in the composite effluent produced from canning of turnip greens and diced roots is shown in Table 5. Treatment with 10 mg Natron 86 and 40 mg ferric sulfate per liter at pH 4.0 reduced the turbidity from 36 to 3.5 FTU. These conditions were then tested in a pilot-scale study as shown in Table 6. Treatment No. I with 10 mg Natron 86 and 40 mg ferric sulfate per liter at pH 4.0 reduced the suspended solids concentration from 134 to 11 mg/l—a reduction of 92%. Removal of the flocculated material also reduced the COD by 19% from 569 to 460 mg/l. Further treatment of the clarified effluent by filtration through activated carbon removed most of the COD. The

COD concentration observed in the fifth gallon of effluent was 83 mg/l. The total amount of COD removed by carbon adsorption amounted to 89% of COD applied. A second treatment applied to the composite effluent at pH 7.0 combined 20 mg Atlasep 105c, a cationic polymer, 40 mg ferric sulfate, and 2 mg WT-3000 per liter. Table 6 shows that treatment No. II followed by flocculation and settling reduced the concentration of suspended solids and COD by 92% and 17%, respectively. Carbon treatment of the clarified effluent removed 84% of the total COD applied.

The dosage of carbon that would be necessary to purify the composite effluent cannot be determined from the experiments just described because an excess of carbon was necessary to achieve purification in the single-pass down-flow column. It would be necessary to utilize a series of columns as described by Culp and Culp (7) to determine the minimum contact time, carbon dosage, and economics of treatment by carbon adsorption. Drawing on the experience of the widely publicized treatment plant at South Lake Tahoe, California that uses carbon adsorption in tertiary treatment of municipal wastes, the adsorption capacity of 8 × 30 mesh Calgon Filtrasorb 300 carbon in upflow countercurrent columns was 0.5 lb of COD per pound of carbon (7). This is the same carbon product employed in the present study.

It is unlikely that the economics of carbon treatment for such concentrated effluents as vegetable canning wastes would be favorable under present conditions. Longer carbon contact times and increased capital costs are to be expected when wastes contain over 60 mg COD/l as compared to an operation like South Lake Tahoe which uses carbon adsorption as a tertiary treatment system to reduce the COD typically from 24 mg/l down to 12 mg/l (7). In the present study, the COD was reduced from 460-470 mg/l to 83-113 mg/l by carbon treatment of a waste that had been clarified by flocculation and settling.

Results of this study have shown that the loads of suspended solids in effluents from the canning of leafy green vegetables can be reduced by segregation and separate treatment of concentrated unit

TABLE 5. FLOCCULATION OF COMPOSITE EFFLUENT FROM CANNING OF TURNIP GREENS AND ROOTS<sup>1</sup>

Trial	pH	Natron 86 (mg/l)	Salt, (mg/l)	Turbidity (FTU)
I	4.0	—	—	36
	4.0	30	CaCl <sub>2</sub> , 40	17
	6.0	30	CaCl <sub>2</sub> , 40	36
	8.0	30	CaCl <sub>2</sub> , 40	23
	9.8	30	CaCl <sub>2</sub> , 40	52
II	4.0	1	CaCl <sub>2</sub> , 40	46
	4.0	10	CaCl <sub>2</sub> , 40	12
III	4.0	10	CaCl <sub>2</sub> , 40	4.8
	4.0	10	FeCl <sub>3</sub> , 40	4.5
	4.0	10	Alum, 40	5.2
	4.0	10	Fe <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> , 40	4.5
IV	4.0	5	Fe <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> , 40	10
	4.0	10	Fe <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> , 40	3.5
	4.0	30	Fe <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> , 40	6.2

<sup>1</sup>The pH of the raw effluent before adjustment was 9.8.

TABLE 6. PILOT-SCALE TREATMENT OF COMPOSITE EFFLUENT FROM CANNING OF TURNIP GREENS AND ROOTS

Treatment conditions	Sample no. and description	Turbidity (FTU)	Suspended solids (mg/l)	COD (mg/l)
I. Natron 86, 10 mg/l; Fe <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> , 40 mg/l; pH 4.0	1. Effluent adjusted to pH 4.0	57	134	569
	2. Floc. and settled, 0.5 h	10	18	—
	3. Floc. and settled, 1.0 h	8.5	11	460
	4. Carbon filtrate, <sup>1</sup> 1st gal	0.1	0	0
	5. Carbon filtrate, 2nd gal	0.3	0	29
	6. Carbon filtrate, 3rd gal	0.2	0	59
	7. Carbon filtrate, 4th gal	0.4	0	74
	8. Carbon filtrate, 5th gal	0.5	0	83
II. Atlasep 105c, 20 mg/l; Fe <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> , 40 mg/l; followed by WT-3000, 2 mg/l; pH 7.0	1. Raw effluent	57	134	569
	2. Floc. and settled, 0.5 h	2.0	14	—
	3. Floc. and settled, 1.0 h	1.5	11	470
	4. Carbon filtrate, <sup>1</sup> 1st gal	—	0	10
	5. Carbon filtrate, 2nd gal	0.2	0	59
	6. Carbon filtrate, 3rd gal	0.3	0.3	88
	7. Carbon filtrate, 4th gal	0.3	0.2	103
	8. Carbon filtrate, 5th gal	0.4	0.5	113

<sup>1</sup>Filtered through a 2-lb column of activated carbon at a rate of 75 ml/min (liquid residence time, 10.3 min).

effluents. Laboratory and pilot-scale experiments on the composite effluent suggest that physical-chemical methods could be used for complete treatment of the wastes from leafy greens, but flocculation of concentrated unit effluents appears to be a more promising approach to significant reduction of the processing waste load.

#### ACKNOWLEDGEMENTS

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## THE INFLUENCE OF TIME AND TEMPERATURE OF INCUBATION ON THE PLATE COUNT OF MILK<sup>1</sup>

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### ABSTRACT

The geometric mean bacterial count of 131 raw milk samples, using plates incubated 48 h at 30 C, was 15% higher than the geometric mean when the bacterial counts were determined at 32 C incubation. When 72 h of incubation were used, the geometric mean of samples using the lower incubation temperature was 20% higher. The mean for all 72-h raw milk counts was 31% higher than that for 48 h counts. There was no significant difference between geometric means of 51 pasteurized milk samples using plates incubated at 30 or 32 C after 48 h incubation. After 72 h of incubation, the geometric mean obtained at 30 C was 10% higher. For all 72-h counts on pasteurized milk the mean was 55% higher than for 48 h. A number of both raw and pasteurized samples showed little or no increase in count following Preliminary Incubation at 12.8 C for 18 h while others "blew up" to high levels, suggesting undesirable contamination. The International Dairy Federation procedures tended to reflect the latter better than the SPC.

The lack of direct relationship between the Standard Plate Count (SPC) and public health is now readily admitted (14, 19). However, most dairy microbiologists believe that it should give some indication of the conditions under which milk has been produced and handled. Its inadequacy for this purpose is generally recognized (5). Of seven bacteriological tests for raw milk, Hartley et al. (9) found only the Psychrotrophic Bacteria Count (PBC) showed a significant correlation with farm conditions. Since some psychrotrophs fail to form colonies with the current SPC procedure (10, 14, 16), and the PBC is too time-consuming for a routine control procedure, a lower incubation temperature and longer incubation period should increase the usefulness of the SPC in reflecting substandard farm practices.

In 1958 the International Dairy Federation accepted the report of an international committee recommending incubation at 30 C for 72 h for fluid milk, and for 120 h for dry milk (12). As the IDF procedure for fluid milk has been widely adopted outside North America, it seemed desirable to compare

it with the current SPC procedure (1). This paper reports the results of such a study.

### MATERIALS AND METHODS

Between November, 1972 and March, 1973, 169 samples of raw and 76 of pasteurized milk collected for routine analysis at Orillia were utilized in the study. All samples were plated and incubated at 30 and 32 C for both 48 and 72 h, both before and after Preliminary Incubation (PI) (13) at 12.8 C for 18 h. In addition the PBC was determined after 10 days at 5 C on all but 23 raw and 12 pasteurized samples.

For statistical analysis, all plates showing 30 or more colonies were included; this left 131 raw and 51 pasteurized samples. Plate counts were converted to logarithms and averaged; the antilogarithm of this average gave the geometric mean.

### RESULTS AND DISCUSSION

As only single plates in two dilutions were used, the information necessary to calculate the experimental error for these data was not available. On the basis of previous research (8) a conservative estimate of experimental error of 0.01 was used to do the Duncan's Multiple Range Test on the means. For 131 raw milks differences as small as 5.7% in geometric means could be detected with 95% confidence. All differences among the means of the four time-temperature combinations on the non-PI samples were significant. The geometric mean of counts after 48 h incubation at 30 C was 15% more than for 32 C; after 72 h this had increased to 20%. The geometric mean of all plates incubated for 72 h was 31% higher than that for plates incubated for 48 h.

For the 51 pasteurized samples, differences as small as 9.7% could be detected with 95% confidence. No significant difference was found between counts at 32 and 30 C after 48 h. However, after 72 h of incubation, the difference between means was significant. The geometric mean at 30 C was 10% greater than for plates incubated at 32 C. For all 72-h counts the geometric mean was 55% more than for those at 48 h. All analyses were made on data from non-PI samples.

A more simplistic approach has been to determine the number of times counts at 30 C exceeded those

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at 32 C, and vice versa. With raw milks before PI, 76 were higher at 30 C, 38 at 32 C. After PI the relative numbers were 66 and 29. Pasteurized milk counts were higher at 30 C for 41 samples compared with 25 higher at 32 C before PI.

A further means of comparison is shown in Table 1 for raw, and Table 2 for pasteurized samples. In Table 1 the percentage of initial counts under 5,000/ml, which might be regarded as indicating unusually careful production, decreases as the incubation temperature is lowered and as the period is prolonged to 72 h. After PI there is also an increase in counts over 300,000/ml with lower temperature and longer incubation period, as well as a sharp decrease in counts of <10,000/ml.

In Table 2 the distribution of counts from 76 pasteurized samples is shown. Here the longer incubation period shifts over 13% of samples with initial SPC counts of under 300/ml into the higher categories before PI and 9.0% after PI. The influence of incubation temperature is less clear, but the effect is evidently less than on raw milk samples. Of interest is the high percentage of samples with PI counts over 30,000/ml; such increases are doubtless due to the growth of gram-negative organisms, indicative of post-pasteurization contamination. PI would be a useful procedure for indicating potentially poor shelf-life.

With the growing interest in PI in several areas, some of the raw data are presented in Table 3. A

fair proportion of these milks showed little or no increase on PI, suggesting they had been carefully produced. Several others (Nos. 4-6, 4-11 and 4-16) evidently contained organisms (presumably psychrotrophs) that grow much better at 30 C than at 32 C. While the SPC for none of the 21 samples exceeded 20,000/ml, nine of them exceeded this limit at 30 C for 72 h. And despite their reasonably low SPC's, eight of the 21 "blew up" on PI, suggesting external contaminants capable of active growth at 12.8 C. Although PBC's were obtained with incubation at 5 C for 10 days, and thus may be lower than they would have been at 7 C (1), in a number of samples (Nos. 4-5, 4-6, 4-11, 4-16, 76-7, 76-9, 76-15) these exceeded the initial plate counts on all four procedures. And, interestingly, a number of these failed to "blow up" on PI to the degree expected. Unfortunately PBC's were not obtained for samples Nos. 26-3 to 26-12, where initially low count samples did show impressive multiplication during PI. Since the udder flora rarely if ever multiply at 12.8 C for 18 h (13), such large increases in count point strongly to unsanitary production practices. And if the main purpose of the bacteriological examination of raw milk is to assure the consumer that milk was produced under acceptable sanitary conditions, one wonders how the extremely lenient SPC standards currently in force can be justified.

Randolph et al. (18) recently reported that incu-

TABLE 1. PERCENTAGE DISTRIBUTION OF PLATE COUNTS FOR 169 RAW MILK SAMPLES

Preliminary Incubation:	None				12.8 C for 18 h			
	48		72		48		72	
	30	32	30	32	30	32	30	32
Incubation period (h):								
Incubation temp (C):								
(Count Range)	(Percentage of samples in each count range)							
<5000	11.4	15.3	5.3	9.2	2.3	6.9	1.6	1.6
5,000 - 10,000	14.5	12.9	12.2	13.8	6.9	6.9	2.5	9.2
11,000 - 20,000	21.4	21.4	18.3	19.8	9.9	6.1	12.2	7.6
21,000 - 50,000	19.8	22.1	28.2	25.6	13.0	16.1	13.0	13.7
51,000 - 100,000	18.3	15.3	19.1	18.3	16.1	13.7	13.7	15.3
110,000 - 300,000	11.6	9.2	12.2	9.2	15.3	16.1	15.3	17.6
>300,000	3.1	3.8	4.6	3.8	36.8	34.4	41.3	35.0

TABLE 2. PERCENTAGE DISTRIBUTION OF PLATE COUNTS FOR 74 PASTEURIZED MILK SAMPLES

Preliminary Incubation:	None				12.8 C for 18 h			
	48		72		48		72	
	30	32	30	32	30	32	30	32
Incubation period (h):								
Incubation temp (C):								
(Count Range)	(Percentage of samples in each count range)							
<300	48.6	44.6	27.0	31.1	17.6	18.9	12.2	10.8
310 - 500	6.8	9.5	9.5	6.8	10.8	8.1	8.1	4.1
510 - 1,000	16.2	17.6	21.6	21.6	10.8	13.5	9.5	14.9
1,100 - 2,000	16.2	13.5	23.0	20.3	12.2	13.5	17.6	13.5
2,100 - 5,000	2.7	8.1	9.5	10.8	13.5	13.5	20.3	23.0
5,100 - 10,000	1.4	0.0	2.7	2.7	5.4	2.7	1.4	2.7
11,000 - 30,000	4.1	2.7	2.7	2.7	4.1	6.8	6.8	8.1
31,000 - 100,000	2.7	2.7	2.7	2.7	17.6	15.0	16.2	14.9
110,000 - 300,000	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
>300,000	1.4	1.4	1.4	1.4	8.1	8.1	8.1	8.1

TABLE 3. PLATE COUNTS ( $\times 10^{-3}$ ) OF SELECTED RAW MILK SAMPLES BEFORE AND AFTER PRELIMINARY INCUBATION

Incubation time (h): Incubation temp (C):	Initial Count				Count after PI				Psychrotroph count
	48		72		48		72		
	30	22	30	32	30	32	30	32	
(Sample no.)									
4-2	6	7	6	7	9	4	10	6	6
4-5	50	16	54	19	>300	>300	>300	>300	55
4-6	7	2	10	7	73	4	74	7	34
4-8	11	2	31	12	11	8	17	8	8
4-9	2	2	5	3	8	1	9	1	1
4-11	30	20	33	22	>300	96	>300	120	54
4-16	6	5	12	8	61	5	67	10	100
4-20	11	6	15	8	2	4	2	6	2
6-14	15	7	21	13	30	21	60	38	2
26-3	23	13	40	30	1600	>300	1000	>300	—
26-4	5	3	6	3	1300	>300	1300	>300	—
26-6	14	11	16	20	600	250	750	450	—
26-7	6	5	10	5	600	630	800	850	—
26-12	8	8	9	17	1000	800	1100	880	—
76-1	13	8	16	8	140	110	150	120	8
76-6	7	3	11	4	3	3	12	7	11
76-7	12	7	22	10	38	16	54	20	37
76-9	7	7	30	8	44	26	53	26	46
76-10	7	5	10	6	92	51	92	55	11
76-15	12	3	25	3	10	10	27	13	15
114-13	18	17	28	20	>300	>300	>300	>300	55

bation at 27 C does not appear to offer significant advantages over the 32 C incubation for raw milk counts. The present studies do not support this view; had an incubation period longer than 48 h been used by Randolph et al. the lower temperature very probably would have given significantly higher counts (4). These differences may be caused by regional or climatic factors, or because 30 C is closer to the optimum temperature for raw milk platings found by Smith et al. (21) and Babel et al. (4). In the present studies the increase in count for raw milk samples following an additional 24-h incubation at both 30 and 32 C was considerably greater than that reported for 32 C incubation by Huhtanen et al. (11) or by Johns (14). And with pasteurized milks the increase with 72-h incubation was even more marked, no doubt due to the greater recovery of stressed organisms during the extra 24 h of incubation.

Our findings indicate that at least for this particular geographical area, switching to 30 C incubation would enhance the usefulness of the SPC in reflecting production and handling practices, while incubation for 72 h would be beneficial for both raw and pasteurized samples, especially the latter. Colony size was larger after 72 h, making for much greater ease of counting.

If future studies show little or no increase in count beyond 72 h, samples could be plated from Mondays to Fridays inclusive and all plates counted on regular working days, avoiding the necessity for counting on

Saturdays and Sundays as many non-regulatory laboratories are doing.

Thomas et al. (22) 1963, also indicated that a temperature lower than 32 C for incubation periods longer than 48 h may have some distinct advantages for enumeration of pasteurization-resistant bacteria in milk. Studies on the effect of temperature and time of incubation need to be conducted by many workers in many areas and throughout all seasons in order that those responsible for the next edition of *Standard Methods for the Examination of Dairy Products* will have adequate data on which to base their decisions on modifications in the SPC procedure.

Studies of the relative values of different procedures would be far more valuable if concurrent farm inspections were made by competent personnel as was done by Hartley et al. (9). Unfortunately, in the present studies this was not possible. Based on their experience with PI, several workers (2, 3, 6, 7, 15, 17, 23, 24) in this field have reported their belief that it has definite advantages in indicating unacceptable farm practices, even though several survey-type studies have failed to show its superiority for this purpose. However, the findings of Reinbold et al. (19) indicate the need for standardizing the ratio of volume to surface area of the sample if comparable results are to be obtained using PI.

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*A Research Note***INCIDENCE OF SALMONELLA IN BEEF AND CHICKEN**

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## ABSTRACT

A survey was undertaken to determine the incidence of *Salmonella* in retail purchases of beef, ground beef, and chicken fryers. *Salmonella* were isolated from 3 of 36 (8.3%) fresh whole chicken fryers. No *Salmonella* were detected in 129 quarters of carcass beef or in 100 samples of ground beef. The failure to detect *Salmonella* in beef products is discussed.

Meat and meat products play a role in *Salmonella* outbreaks in humans (1, 7). In the United States poultry has received more surveillance than other food animal species because of disease outbreaks involving egg products and recognition of the importance of salmonellosis in these animals (2). Poultry meat also is involved in more "reported" *Salmonella* outbreaks than red meat (1). Even though the organism has been shown to be present in cattle populations (2, 3, 7), limited data are available concerning the potential infectiveness and/or contamination of beef and beef products. Felsenfeld et al. (4) found only a 0.2% positive incidence in 512 beef samples obtained from retail stores. Weissman and Carpenter (8) found a 74% incidence when sampling 50 beef carcasses from five different processing plants. They concluded there was no particular area on the carcass that was more likely to be contaminated than any other area.

Reports of the *Salmonella* incidence in chicken carcasses vary from 2.7% (5) to 15% (9). Chicken fryers have been reported to be contaminated 26% of the time when sampled over a 4-day period (6).

This research note is presented to compare the incidence of *Salmonella* isolated from beef and chicken from a retail establishment in Denver, Colorado with previously reported figures.

## MATERIALS AND METHODS

Beef carcasses, represented by 129 quarters (108 forequarters and 21 hindquarters) from 3 different processing plants, and 36 fresh, whole chicken fryers were sampled immediately upon receipt at the retail establishment. Approximately 1 ft<sup>2</sup> at either the cervical or anal regions of the beef quarters and the entire surface area of the chicken fryers were swabbed. Cotton swab applicators dipped into mannitol broth (1% mannitol in 0.3% nutrient broth; Difco) were used for swabbing, then placed in 10 ml of mannitol broth, transported to the laboratory within 60 min, and incubated overnight at 35 C. One milliliter was transferred to each of 10 ml of selenite-cystine broth (BBL) and 10 ml of tetrathion-

TABLE 1. INCIDENCE OF SALMONELLA FROM BEEF CARCASS QUARTERS, GROUND BEEF, AND FRESH WHOLE CHICKEN FRYERS

Sample	No. samples tested	No. samples positive	% Samples positive
Beef forequarters	108	0	0
Beef hindquarters	21	0	0
Chickens	36	3	8.3
Ground Beef	100	0	0

ate broth (BBL) and incubated at 35 C for 24 h. Material from each tube was streaked on MacConkey agar (BBL), brilliant green sulfadiazine agar (BBL), and bismuth sulfite agar (BBL) plates. These plates were incubated at 35 C for 24 h (48 h for bismuth sulfite) and any suspect *Salmonella* colonies were examined for appropriate biochemical reactions and screened with polyvalent O *Salmonella* antisera (Difco) for confirmation.

One hundred 1-lb. samples of ground beef were obtained from the same retail establishment over a 5-month period. A 25-g portion from each sample was placed into 225 ml of mannitol broth and incubated overnight at 35 C. One milliliter was transferred to each of 10 ml of selenite-cystine broth and tetrathionate broth and handled similarly as with the carcass swabs.

## RESULTS AND DISCUSSION

Results of the study, in Table 1, revealed an 8.3% incidence of *Salmonella* spp. isolated from 36 fresh whole chicken fryers and a 0.0% incidence from 129 beef quarters and 100 samples of ground beef.

The incidence of *Salmonella* contamination of fresh whole chickens was found to be in the range of previously reported results, 3 to 26% (5, 6, 9). Failure to recover *Salmonella* from beef carcasses correlates closely with the very low (0.2%) incidence reported by Felsenfeld et al. (4) who sampled beef at the retail level. This is in contrast to the 74% incidence reported by Weismann and Carpenter (8) who tested carcasses at the processing plant. A possible explanation for the negative results of the present study may be the time from the time of processing to the time of arrival at the retail establishment. A period of approximately 7 days was the usual time lapse, with carcasses being held at between 33 and 35 F. Storage temperature, plus degree of drying that occurs, may have caused any contaminating *Salmonella* to decrease to nondetectable levels. The time was

approximately the same for the chicken fryers, but these were wrapped in plastic bags so that little drying occurred.

Differences in sampling and analysis may have affected the results. Weismann and Carpenter swabbed an "entire section" or about 12% of the total surface area of the carcass as compared to 1 ft<sup>2</sup> in the present study. They also did not indicate any serological tests for confirmation of *Salmonella* isolates.

Another possible reason for the seemingly conflicting results of *Salmonella* contamination in beef products is the level of sanitation of the processing plants. The sanitary conditions of a plant probably control to a great extent the degree of contamination of the carcasses that are processed. The present study might indicate that the 3 plants which processed the beef quarters tested had a high level of sanitation. Unfortunately, this can only be hypothesized since plant sanitation conditions were not available.

Another factor possibly responsible for a disparity in results from those of Weismann and Carpenter (8) is the dissemination of contaminated beef carcasses from the processing plant to the retail level. At the processing plant it would appear the contaminated carcasses should be relatively concentrated in groups because of slaughtering of pens of beef which have been together for a period. From this point on if the beef is dispersed to retail markets at various locations, contaminated carcasses would become less concentrated at any one specific retail establishment. It would appear that the frequency of isolating an organism, such as *Salmonella*, would be less at the retail level than at the processing plant.

The inability to detect *Salmonella* in 100 samples of ground beef indicates that the raw materials and

the retail processing area and personnel were relatively free of *Salmonella* contamination.

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## WINE RESEARCH AT THE NEW YORK STATE AGRICULTURAL EXPERIMENT STATION<sup>1</sup>

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### ABSTRACT

The New York State Agricultural Experiment Station is uniquely qualified for an effective wine research program by its departmental structure and facilities. Cooperative research carried out by geneticists, viticulturists, entomologists, pathologists, and enologists is directed toward developing and selecting grape varieties for New York that will be suitable to the climate, resistant to pests, productive, and yield a high quality wine. The relation of vineyard site selection, cultural practices, and wine making techniques to wine quality are also under study. Enological research is the responsibility of chemists, microbiologists, and engineers who are studying the relation of wine composition, fermentation, and processing methods to wine quality. An outstanding variety for white wine was introduced in 1972. Under the selection number GW-3, the new variety, named Geneva White, has gained wide acceptance in preliminary tests by growers and wine makers. Promising new red varieties for New York are also under development at the Geneva Experiment Station.

To include the topic of wine research at a meeting of scientists long dedicated to a flourishing milk industry — even to the point of substituting “milk breaks” for “coffee breaks” — seems like sacrilege indeed.

But, enologists and dairy scientists have much in common. The dairy industry learned much of its microbiology from enology, and now the wine industry is turning to the milk industry for inspiration in the area of sanitary engineering.

If our common patron saint, Louis Pasteur, were alive today he would be over 150 years old. American school children learn that Pasteur developed the pasteurization process for milk. What they rarely hear is that he discovered the process while studying the problem of wine spoilage and the fermentation process. Although the merits of pasteurization are still debated by wine connoisseurs, the process was more readily adopted by the milk consuming public.

The wine industry has been attracting world-wide attention in recent years, and New York is no exception. In fact, grapes are the most rapidly expanding fruit of New York's agricultural economy.

Annual wine consumption in the U. S. has reach-

ed over 350,000,000 gal. Of the wine consumed, 12 to 14% is imported, two-thirds comes from California, about 10% from New York, and the other 12% is scattered over 9 states, predominately Washington, Michigan, and Pennsylvania. Although overshadowed by California in total wine production, New York until recently nearly matched California in production of sparkling wines. But, in the wine boom since 1967, California has tripled its sparkling wine production, and New York State now only produces about 20% of the domestic bubbly!

A rapidly developing agricultural industry of this magnitude deserves the support of its state's agricultural research facilities, and Cornell has met the responsibility through facilities of the New York State Agricultural Experiment Station at Geneva.

### VITICULTURE

At Geneva, viticultural research is primarily the responsibility of the Pomology and Viticulture Department. “Viticulture” was added to the department title in 1972 in recognition of the expanding importance of grapes in a department concerned with all commercially important fruits grown in New York State. New York viticulturists have unique problems, advantages and disadvantages, that relate to climatic conditions differing from those of California. The colder New York climates have resulted in selection of commercial varieties that include winter-hardy American species in their genetic background.

The search for grapes of higher wine quality, that combine cold resistance with productivity, shapes a research program that is distinctive to New York. With expansion of vineyard acreage in a temperature-limiting geographic area, site selection is of paramount importance. Interrelations of climate, soils, and suitable varieties are under study, and the choice areas are being defined and sought out.

The management of vine (pruning, training); selection of root-stocks; and phylloxera, insect and disease resistance are all receiving attention. Research demonstrates that grape productivity and quality are related to leaf area, sunlight exposure, and temperature. These are related in turn to spac-

<sup>1</sup>Presented at the 60th Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, Rochester, New York, August 13-16, 1973.

ing of rows, vines, canes, and shoots.

The costs of labor must be kept to a minimum. Cornell research developed the mechanical harvesting concept that now accounts for over 85% of the grapes harvested in New York State. Next on the agenda is development of a mechanical pruning procedure that will cut the enormous costs of individually cutting and tying of each cane.

The wine grape breeding and selection program has been shifted from the fresh-fruit market development of the past to introduction of more suitable wine grapes. The goal of this program is to develop grapes that will be hardy and productive in New York, and at the same time have less of the pronounced American species flavors than do the present commercial grapes.

The desired type of grape is characterized now by the family of French-American hybrids, developed in France and now gaining rapid acceptance in Northeastern U. S. Harvests have increased from 100 tons per year in 1954 to possibly over 10,000 tons in 1973, an increase of 30% per year. It is painting with a broad brush to include all of these hybrids in one set of statistics, since they vary widely in productivity, growth habits, hardiness, and wine quality. But they do have a common quality: they yield a more neutral wine; i.e., they have less of the American pronounced fruitiness often described as grapey or "foxy."

The Geneva hybrids will be particularly adapted to New York growing conditions. A white wine grape, named Cayuga White and released officially in 1972, is the first introduction of this program. This is a highly productive white grape that produces a characteristic dry white wine of excellent quality. It is a cross between one of the better quality French Hybrids, "Seyval," and a former Geneva product, "Schuyler," a grape containing a high proportion of *vinifera* in its genetic background.

Grape pests and insects such as the grape berry moth are under study. Sex attractants (pheromones) in traps are a promising control method for the moth. Again, disease and insect problems are distinctive for each geographic area, and entomology and pathology programs must be designed to meet New York State problems.

#### ENOLOGY

Working cooperatively with the viticultures to cover the "enology" side of the wine research program are staff members of the Department of Food Science and Technology. Under "enology" (the Greek-derived term for the science of wine making) studies are made in such subjects as evaluation of wine

grape introductions and selections for wine quality, color and flavor chemistry, causes and prevention of haze and turbidity, microbiology of wine fermentation and spoilage, production of grape concentrates, effect on wine quality of mechanical harvesting and many other engineering operations.

Pigment studies may be cited as an example of a unique situation in New York State enology. The red anthocyanin color is made up of a family of at least 20 closely related compounds. These compounds vary in degrees of methoxylation, glucosidation, and acylation. Each of the chemical variants is under study for its effect on color stability and hue.

A bane of the wine maker's existence is the occurrence of sulfide aromas in the fermented wine. Our studies have traced the cause to use of sulfur as a fungicide in the vineyard and poor fruit condition. The intensity of the problem has been related to certain strains of yeast. Sedimentation and decanting of musts before fermentation has been found to alleviate the problem.

Mentioned earlier was the rise to predominance of mechanically harvesting grapes in New York. Related enological studies have been concerned with oxidation rates, their measurement and prevention. In short, our results have shown that the rapid handling of grapes by the mechanical harvester offsets the oxidative effects brought about by bruising. In fact, oxidation in grapes is reversible if not permitted to proceed too long.

Concentration of grape juice appears to have a particular advantage for New York State grapes. Their pronounced flavors, sometimes objectionably potent, can be considerably subdued in the concentration process. Concentrates offer the advantages of reduced shipping costs, and are especially appealing to the home wine maker, a rapidly burgeoning market.

The relation of cultural practices to wine quality is a fascinating study, since the chemistry of the grape responds with great sensitivity to its environment. Sunlight and temperature affect photosynthesis and respiration, and are responsible, therefore, for sugar and color development and acid metabolism. Pruning, training, grafting, fertilization, and other vineyard practices have major influences on the ratio of leaf area to fruit load, and result in impressive effects on fruit quality.

This discussion would be incomplete if no mention were made of our investigation into the alleged toxicity of French hybrid grapes. Dr. Hans Breider, a German geneticist, had reported that the feeding of these grapes to chickens caused low egg hatchability, bone malformations, weakness, and death. Since French hybrids were fast gaining acceptance,

we felt that the toxicity report should be thoroughly investigated.

Two years were spent in vain efforts to produce the symptoms. Accepted chick rations were used with the wine substituted for water in the diet. Finally, We procured some of the Kükengrütze used by Dr. Breider as his basal ration and we produced the reported defects, not only with wine but in the chicks drinking water as well. The conclusion was that we were dealing with nutrient deficiencies caused by diets that no poultryman would consider feeding without considerable supplementation.

#### CONCLUSION

The Geneva Experiment Station recognizes the need for varieties and cultural practices that will keep the state competitive for years to come. Research into the making of premium quality table wines will continue to receive major emphasis. The chemistry, physics, microbiology, and engineering expertise of the Food Science and Technology Department will supplement the research of the viticulturists, geneticists, soil scientists, entomologists, and pathologists to make a research program that should aid industry to develop and improve the quality and quantity of New York State wines.

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### TOWARD TOMORROW: A SYMPOSIUM OF ALTERNATIVES AT UNIVERSITY OF MASSACHUSETTS

The Division of Continuing Education of the University of Massachusetts/Amherst is proud to announce "*Toward Tomorrow: A Symposium of Alternatives*." This unique summer college program to be held from June 14 to July 6 will offer its participants a change to explore alternative technologies and life styles in a serious fashion.

"*Toward Tomorrow*" will cover five major areas:

Area A. *The self-sufficient arts*, including food production, fish culture, gardening, food preservation, bees, goats, chickens and rabbits, stalking and foraging for food, vegetarian cooking, organic gardening, etc.

Area B. *Shelter*. This includes domes, A-frames, cinva-rams, polyurethane spray insulation, inexpensive housing, and others.

Area C. *Alternative energy*, including solar power, windmills, methane digesters, solar voltage cells, etc.

Area D. *Ecology*, including futuristics, global survival and ecological studies, field trips, ethical issues, etc.

Area E. *Funky stuff*. This will include building electric cars, building steam cars, ESP, Bio-Feedback, sensitivity training, etc.

In addition to these one-week long courses, which will be repeated three times, outside speakers who are experts in their field will be frequent visitors. "*Toward Tomorrow*" will find a place in the life of all people who are concerned with their environment and their future.

Each seminar will emphasize the "how to" of the subject matter, so that participants can expect to come away from their experience with a practical working knowledge of how to build, measure, grow, etc. During the present energy crisis and period of inflationary prices, this timely series of courses will find a place in everyone's life.

For further information about this program please contact Mr. Francis Koster, Division of Continuing Education, 102 Hills House North, U Mass, Amherst, Massachusetts 0100, 413-545-3108.

# THE MILKO-TESTER AND PROBLEMS RELATED TO ITS USE<sup>1</sup>

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## ABSTRACT

The Milko-Tester is a relatively new milkfat testing device and is only now beginning to gain fairly wide adoption in industry and regulatory laboratories. Used properly, on representative samples of milk, it can provide consistent, precise results at efficient speeds necessary in today's high-volume laboratory operations. But it cannot be used in lieu of, or as a cover-up for poor sampling and testing practices. In fact, more than ever, proper sampling, proper calibration and standardization, and routine check-testing of results are required. Experiences in our laboratory indicate that only the most careful day to day control will assure reliable, useable results. In this paper are presented some pitfalls to be avoided, and also some routine controls we have found essential after 5 years of experience with Milko-Testers, both Mark II and Mark III models.

Over the years the Babcock and Gerber tests have become standard procedures for milkfat analysis when milk is purchased. And over the years results have been adequate for the needs of the dairy industry when good sampling and testing practices were followed. It has been only recently—and for a very short time as gauged against other methods—that electronic procedures for milkfat analysis have been available. Mark II and Mark III models of the Milko-Tester are excellent examples of space age electronic testers that can be of great value in the large-scale centralized laboratory operations common in today's industry. But at the same time introduction of new methods or new processes bring with them unique and oftentimes unexpected problems. Only experience, research, trial and error can serve to uncover such problems and provide specific techniques for their successful resolution. In a quality control laboratory where hundreds of thousands of dollars are at stake and where equitable treatment of producer and processor are a must, routine control and objective daily checks are essential. Our experience indicates that Milko-Testers can be used within the increasingly tighter standards required of today's laboratory. Our experience also indicates that they can be used only if greater—not less—control is exercised in routine sampling, maintenance, and check-testing procedures. It is my purpose to share with you some of the observations we've made and

techniques we employ in our laboratory to assure proper control of electronic milkfat testing.

## CARE AND IDENTIFICATION OF MILK SAMPLES

We will deal with sampling first. You can have perfect laboratory procedures, but if sampling techniques are poor, you will never know true test values for milk. In the Twin City market we have been on a universal sampling program for years. Across the country the universal sample is now being widely accepted. By definition it is a sample which can be used for any test, bacteriological or chemical. The hauler takes an aseptic sample each time he picks up milk at the farm, without knowing what test will be applied to the milk. It may be a test for bacterial content or it may be one for butterfat. In any event, under this system it is essential that the hauler agitate the milk in the tank sufficiently long to obtain a uniform blend. The hauler must be impressed with the fact that if he samples too soon, test results will not be correct. Like the rest of us, the milk hauler is pushed by today's economics. Hence he is in a hurry to get around that route and so from time to time must be reminded that milk must be mixed adequately.

Let me point out one very important item concerned with sample care. In Minnesota and Wisconsin we have extreme weather conditions. The temperature can vary as much as 120 F from one season to another. Under these conditions, samples must be protected so they are at 32-40 F at all times. Results obtained with the Wisconsin Mastitis test will be affected if the milk is frozen or is allowed to go over 40 F (1) and true test results cannot be obtained with the Milko-Tester if samples freeze (1) while they are being delivered to the receiving station. One way to keep samples cold in the summer and also from freezing in the winter is to have ice water up to the level of milk in the sample container.

Another important item is the space above the milk needed to properly mix the sample. I have seen samples come into the laboratory with no space at all, especially in whirl-pak bags. This happens when the bags are folded shut, rather than whirled. A technician, then, must unfold the open end of the bag and re-whirl it, wasting valuable time.

<sup>1</sup>Presented at the Sanitarians Conference, University of Minnesota, St. Paul, Minnesota 55101.

Another matter which causes problems is inadequate identification of samples. There are pens available which will mark plastic bags and the marking will not wash off. Identifying marks should be put on the bag before milk is added. Gummed labels also can be used. The important point is that samples must be legibly identified so that the right farm is charged with the test results. You have heard stories, just as I have, of farms being out of business for several months while still receiving test results. This is embarrassing for everyone concerned and indicates that the sample was not marked properly; hence another farmer is not receiving his test results.

#### SAMPLE TEMPERATURE BEFORE TESTING

Now I would like to discuss preparation of samples for butterfat testing. Samples should be heated to 100 F before they are tested. This is necessary to soften the cream layer which clings to the inside of the container. The sample temperature should not rise above 100 F since "oiling off" may result. This will lead to an inaccurate test result. AOAC (2) requires that the sample be poured from one container to another 3 or 4 times before testing. With automatic and semi-automatic Milko-Tester equipment this is not always practical. Still the mixing process cannot be slighted. Some people simply swirl the sample container and then pipette the milk. This is inadequate. If the sample is to be completely blended, and if it is not poured from one container to another, then the container must be inverted completely at least 6 times. This is why the space above the milk is so important. After mixing, the sample must be pipetted immediately.

#### PROPER USE OF THE REFERENCE METHOD

Milko-Testers have to be standardized against a reference method such as the Babcock, Gerber, or Mojonnier procedure (2) even though, in actual operation, they may be slightly more accurate than either the Babcock or Gerber method and also more precise. In the Twin-Cities market, and in most markets in this country, the Babcock method is used for standardization purposes. Once standardized, the Milko-Tester can be a very stable instrument. Later, I'll discuss our standardizing technique but first I would like to make a few comments about the Babcock test.

The laboratory technician must know how to do a Babcock test before he can properly standardize the Milko-Tester. While people have a state license for Babcock testing it is apparent that many do not properly perform this test. Of course, a technician is only as good as the equipment he has to use. Plant

operators should inspect their Babcock test equipment. Just because thousands of dollars were paid for a Milko-Tester, doesn't guarantee accurate test results. Keep in mind that another test, the Babcock test, is being used to establish Milko-Tester settings. It's the Babcock test then, that determines accuracy. I heard a comment in one plant that "our manager doesn't want to spend any money on Babcock equipment since he bought this Milko-Tester." He doesn't understand the problem!

I feel compelled to review the Babcock test as I have seen this test perverted so many times. Check the centrifuge; is it equipped with a speed indicator? is there a heater with a thermostat and a built in thermometer? is there a brake on the centrifuge? I was in one laboratory where a technician had to wait until the centrifuge stopped free-wheeling because the brake didn't work. In another laboratory the centrifuge was not balanced properly because of broken bottles stuck in centrifuge baskets. I thought the centrifuge would fly off the bench. Is the laboratory equipped with sufficient water baths? One laboratory I know used a single water bath to temper milk samples to 100 F and also to temper test bottles at 135 F. Does your laboratory have a thermostatically controlled water container so that water at 140 F can be added to Babcock bottles between periods of centrifuging, to bring the fat into the neck of the bottle? Does your laboratory have a test bottle shaker to mix the acid and milk so all fat is released? Does your laboratory have a magnifying reader equipped with light source to read the fat column? I was in a laboratory that had hand calipers so dull it was impossible to read to one-half point. Does your laboratory have an acid dispenser so acid can be measured accurately? If this equipment is not available, it must be purchased before \$12,000 are spent for a Mark III Milko-Tester.

Availability of proper Babcock equipment does not insure an accurate test result. After milk samples at 100 F are pipetted into the Babcock test bottle; this milk must be cooled to 50 F in a water bath. Now acid at 60 F is added to the test bottle and the bottle is immediately placed in a mechanical shaker and agitated at least 5 min. Some people try to shake samples in wooden bottle racks and this will not work satisfactorily. Samples in the middle of the rack are not agitated enough. I have seen technicians read charred samples, again inaccurate results occur. Not too long ago, I showed a young lady in a laboratory at a cheese plant how to do the Babcock test; she was delighted with the golden yellow fat columns only because, previously, she had always obtained charred fat columns. Temperature of milk and acid and proper mixing are exceedingly import-

ant. Also, laboratories must be equipped with accurate thermometers to determine if water in the tempering bath is 135-140 F. Overall results of the Babcock test still make the difference between financial loss or gain for a dairy plant operating with electronic testers.

#### STANDARDIZATION OF THE MILKO-TESTER

Now we are ready to discuss standardization of the Milko-Tester. I would like to quote from the 11th edition of the *Official Methods of the Association of Official Analytical Chemists* (2). On page 252, it describes standardization of the Milko-Tester as follows. "Test in triplicate 20 representative milks, ranging from 3 to 6% fat by the Roese-Gottlieb Method or Babcock Method and Milko-Tester. Calculate average for each sample by each method to nearest 0.01%. During any calendar day of use, make performance check consisting of comparison of results obtained on 1 milk bulk sample, using both Milko-Tester and Roese-Gottlieb Method or Babcock Method. If difference is  $>0.04\%$ , repeat determination on 3 additional samples. If average difference is  $>0.04\%$ , re-calibrate Milko-Tester."

The first question that comes to mind is probably, "How do you secure 20 different milk samples between 3-6% fat?" I will tell you briefly how we accomplish this at our laboratory. I have 10 plastic quart containers which are filled with commingled milk from a silo tank at a local dairy. This commingled milk should be as fresh as possible. The 10 quarts of milk are refrigerated over night, allowing cream to rise to the top. Cream is taken from some of the quarts and added to others to obtain samples in the range of 3-6% fat. If it is found that insufficient cream has been added, there are a few extra quart samples on hand to supplement that already added. I want to stress that you should not use cream which has gone through a separator. Our experience strongly suggests that cream of this kind undergoes physical changes that lead to erratic Milko-Tester results. We learned this the hard way as we used to build our standardizing samples with separated cream.

As I just quoted, AOAC (2) requires use of 20 samples, I also said we use 10 quarts. Why so much milk? The reason is that, in our laboratory we make duplicate samples by dividing each quart into 6 subsamples. Now we have three complete sets of 20 samples. Having made samples like this many times, I can tell you that it takes at least 1 h to do this task. You may ask, "Why three sets?" Remember, we are standardizing the Milko-Tester against a second method, and samples are needed for this test as well. In our routine we heat one set of samples to 100 F; Milko-Tester tests are then done in triplicate. Im-

mediately afterwards three samples from each of the 20 sample containers are removed for the Babcock test. This leaves two more complete sets for use in the event changes in diluent quantity are required for the Mark II, or a change in Shift value is needed in the Mark III. Such alterations necessitate a second set of analyses. In the end it is a grand average of 60 Babcock and 60 Milko-Tester tests that must be within 0.01% of each other. Once this is accomplished, the Milko-Tester is standardized.

I would like to emphasize here, that samples to be tested with any Milko-Tester should not be held at 100 F for longer than 30 min. Another point I want to make is that once samples are heated, they should not be reheated again and tested with the Milko-Tester. Results will be at least 0.05% low on reheated samples (3).

Now the Milko-Tester is standardized; how do you know if it will stay standardized from day to day? AOAC (2) requires daily check testing done in triplicate by both Milko-Tester and the reference method. If average results of the two methods differ by 0.04% or more, recalibration is necessary. I have seen our Milko-Tester go 5 or 6 months without need of calibration.

#### DAY TO DAY CALIBRATION CHECKS

I will now describe how we make sure our tester is properly calibrated. On Tuesday and Friday of each week we make up two samples, one with a 3.0% and one with a 4.5% milkfat. These two samples are made up in a quart container and each is split into 6 subsamples. We call these our A & B sample checks. We do one A sample and one B sample in triplicate by both the Milko and Babcock methods. We average results of the three tests and now we know the "true" value for the A and B sample. We do the A and B samples both in the morning and the afternoon on Tuesday, Wednesday, and Thursday. Friday we prepare a new set of A and B samples for use on Friday, Saturday, and Monday. Over and above this routine; and also on a daily basis, we purchase a quart of homogenized milk from a local dairy. First in the morning, before samples are tested officially, we test this sample of homogenized milk about 10 to 20 times making certain that replicates are within allowable tolerances. The average test is recorded and during the day this cold homogenized sample is tested after every 24 samples through the Milko-Tester. If the results deviate by  $>0.02\%$  from the average test value, we check the zero point on the Milko-Tester.

On Friday of each week the machine is checked for leaks; the homogenizer is oiled; the shift value is

checked along with water bath level and temperature, and the equipment cleaned on the exterior and interior. A special form is used to record observations made at that time. Each day results of check tests are recorded on another daily check test sheet. Also, any time a part is replaced on the machine, this fact is recorded on our daily check test sheet. These records then, are kept for the regulatory agency charged with the responsibility for checking machine operation.

The Milko-Tester is a good laboratory instrument, but will only give accurate results when the samples are adequate and when someone understands the

instrument, operates it properly, and gives it the needed care.

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### CRUMBINE AWARD IS REVIVED

The Samuel J. Crumbine Award for outstanding achievement in the development of a program of food and drink sanitation is being revived by the Single Service Institute after a suspension of five years.

The Crumbine Award was established in 1954 by the Paper Cup and Container Institute (now the Single Service Institute) to encourage improvement and stimulate public interest in food and beverage sanitation. The award was named in honor of Dr. Samuel J. Crumbine, public health pioneer, long time Kansas State Health Officer, former general executive of the American Child Health Association and at the time of his death, consultant to the Public Health Committee of PCCI.

• Eligibility for the Award is limited to local units of government (county, city, town, district, etc.)

which are legally and directly responsible for protecting the health of the consumer by assuring that public eating and drinking establishments meet established sanitation standards and observe proper sanitation procedures.

Presentations will be made at the American Public Health Association annual meeting in New Orleans, Louisiana, in October 1974. The following years it will be made at annual meetings of NEHA and IAMFES respectively.

Applications for the Award will be accepted from June 15 to August 31, 1974. Entries must be made on an official application form which may be secured from the Awards Jury.

Inquiries and requests for application forms may be addressed to the Crumbine Jury, C/O The Single Service Institute, Inc., 250 Park Avenue, New York, New York 10017.

# USING ATOMIC ABSORPTION SPECTROPHOTOMETRY TO MONITOR CALCIUM IN CLEANING SOLUTIONS FLOWING IN A MILK PIPELINE<sup>1, 2</sup>

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## ABSTRACT

Concentrations of calcium in mixtures of acid and alkaline detergents containing milk were determined by atomic absorption spectrophotometry. Four concentrations of milk (0.0, 0.01, 0.1, and 1.0%) were injected into flowing water containing four concentrations of acid or alkaline detergent (0.0, 0.03, 0.3, and 3.0%) at each of three temperatures (20, 45, or 70 C). The concentration of alkaline detergent was not a significant variable. Less calcium was detected in solutions of acid detergent at 20 C than in solutions at 45 or 70 C. An increase in concentration of milk from 0.01 to 0.1% resulted in an approximate 10-fold increase in the amount of calcium detected with both alkaline and acid detergents. When the concentration of milk was increased from 0.1 to 1.0%, the same 10 fold increase in the amount of calcium was noted for the acid detergent. However, 0.1 to 1.0% increase in the concentration of milk resulted in only a 7-fold increase in the amount of calcium detected in the alkaline detergent.

Residue on a milk-contact surface of storage and processing equipment is removed at a varying rate during the cleaning cycle. This rate of removal has been studied at different time increments (2). If the rate of removal of the residue could be monitored continuously, a more accurate analysis of a cleaning cycle could be made.

Anderson et al. (1) found that, with little interference from either individual chemical ingredients or from changes in the solution temperature, an atomic absorption spectrophotometer could be used to detect the amount of calcium in solutions that contained milk, ingredients of commercial detergents, and water.

The object of this study was to determine the ability of the atomic absorption spectrophotometer to de-

tect and measure the amount of calcium (milk) injected into alkaline and acid detergent solutions flowing in a piping system. The long-range objective of this research was to develop instrumentation for monitoring the rate of removal of constituents of residue from milk-contact surfaces during cleaning.

## MATERIALS AND EQUIPMENT

### Materials

A dry alkaline detergent (Klenzade HC-41, recommended concentration 1.0 - 3.0%) and a liquid acid detergent (Klenzade AC-3, recommended concentration 0.1%) were used as cleaning agents. A standard stock solution of 2000 mg calcium/l was prepared by dissolving 4.9945 g of dried CaCO<sub>3</sub> (analytical grade) in 6 N HCl and diluting the solution with distilled water to one liter.

To measure the amount of calcium in milk, standard solutions containing 0, 5, 10, and 15 mg calcium/l were prepared by diluting the standard stock solution with distilled water. Each standard solution and distilled water containing milk were aspirated into the spectrophotometer. Instrument responses were recorded and compared to determine the amount of calcium in the milk. For the acid detergent study, standard solutions containing 0.0, 0.16, 1.6, and 16.0 mg calcium/l were prepared by diluting the standard stock solution with acid detergent-water (DW) solution. These solutions were aspirated into the spectrophotometer, and instrument responses were recorded before and after each test to confirm the amount of calcium injected. For the alkaline detergent study, no standard solutions were used because the cleaning solution from the pipeline was force-fed into the spectrophotometer, and no method was available for force-feeding a standard solution at the same rate as the solution was aspirated during a test.

### Equipment

The simulated cleaned-in-place (CIP) system (Fig. 1) consisted of a variable speed sanitary pump (7½ hp motor) with controller, a 60-gal detergent-water-solution reservoir, a milk injector, a turbine flow meter with a read-out unit, 105 ft of 1½-inch stainless steel tubing, and three air-actuated, stainless steel valves.

The milk injection unit consisted of a solenoid-operated valve, several stainless steel fittings, and a 20-inch length of plastic tubing. The plastic tubing was marked to indicate the volume of milk-water (MW) solution at various depths of filling.

An atomic absorption spectrophotometer (Varian Techtron, Model 1000), equipped with a calcium hollow cathode

<sup>1</sup>Contribution from the University of Missouri Experiment Station, Journal Series No. 6808.

<sup>2</sup>Trade names and names of commercial companies are used in this publication solely to provide specific information. Mention of a trade name or manufacturer does not constitute a guarantee or warranty of the product by the U. S. Department of Agriculture or an endorsement by the Department over other products not mentioned.

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TABLE I. MEAN INSTRUMENT RESPONSE AND AMOUNTS OF CALCIUM FOR KNOWN CONCENTRATIONS OF MILK

Conc. of milk (%)	Alkaline		Acid	
	Calcium (mg/l)	Instrument response (mv)	Calcium (mg/l)	Instrument response (mv)
0.0	0.0	0.0	0.0	0.0
0.01	0.16	2.03	0.13	1.12
0.1	1.62	20.40	1.59	13.55
1.0	15.58	131.81	16.76	126.22

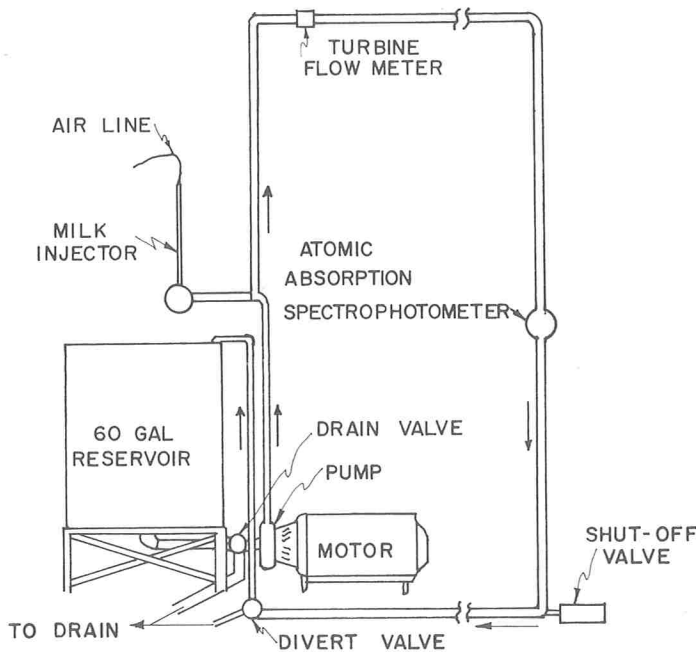


Figure 1. Schematic diagram of the simulated CIP system.

lamp, was used to measure the amount of calcium in solutions of milk-detergent-water (MDW). The dimensions of the nitrous-oxide burner were 6.324 cm × 0.457 mm. The slit width was 0.5 nm, and the lamp current was 6 ma. Acetylene was the fuel gas, and nitrous-oxide was the support gas. Signals from the spectrophotometer were fed into a two-pen, 10-inch chart laboratory recorder.

For the alkaline detergent study, a stainless steel needle and plastic tubing were used to force feed the MDW solution directly into the nebulizer by line pressure (Fig. 2). For the acid detergent study, a fluid control valve with assembly and plastic tubing were used to aspirate the MDW solution into the nebulizer (Fig. 3). The MDW solution was removed from the CIP return line via a 1/8-inch I.D. copper tube (directed upstream) attached to the fluid control valve (Fig. 3).

**Procedure**

The experimental design was a split-split plot with two replications. Each replication consisted of 12 series of four tests each. Four concentrations of milk (0.0, 0.01, 0.1, and 1.0%), four concentrations of detergent (0.0, 0.03, 0.3, and 3.0%), and three temperatures (20, 45, and 70 C) were used in all possible combinations.

The following procedure was used for each series of four tests: the reservoir was filled with softened water < 1 ppm calcium), and the required concentration (0.0, 0.03, 0.3, or 3.0%) of detergent was added. The DW solution was heated

to the required temperature while it was circulated through the system at a velocity of approximately 5 fps.

Diluted milk samples (0.0, 0.01, 0.1, or 1.0%) were placed in the milk injector. When the DW solution reached the desired temperature, a test was started by injecting the milk into the DW solution at a constant rate for 20 sec. After the MDW solution passed through the test section, it was diverted to the drain to prevent contamination of the DW solution in the reservoir.

Figure 4 shows typical instrument responses (tracings) for four milk concentrations injected into solutions of alkaline and acid detergents during the study. After the 20-sec injection period, the tracing for each of the concentrations of milk returned to the baseline, a condition which indicated that the instrument had performed properly, and test conditions were valid.

To obtain an average peak response, data points obtained at 1-sec intervals for each injection curve (Fig. 4) were read into the computer. Quantities of calcium (mg/l) that caused these responses were determined by using standard curves. Variances in average peak values were compared by analyses of variance (ANOVA). Totals of the three-way interactions from the ANOVA program were used with orthogonal multipliers and with error A, B, and C terms to obtain sums of squares that were due to each of the 47 components (6).

Those components that contributed significantly (P < 0.05) to the total sums of squares were used in a multiple regression computer program with mean values of the three-way interactions from the ANOVA program to obtain response surface equations for each detergent. Two equations, one linear and one polynomial, were required because the

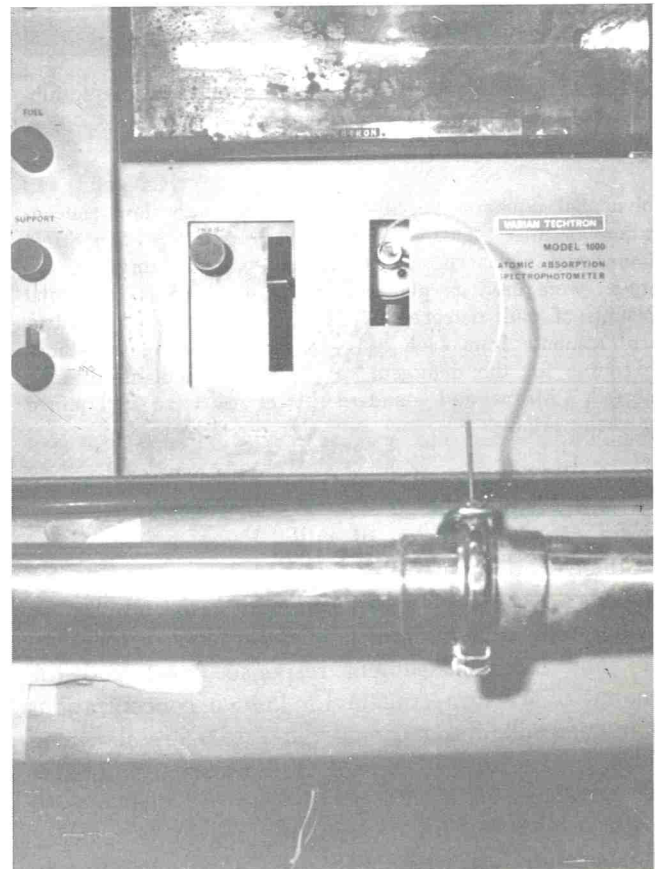


Figure 2. Stainless steel needle and plastic tubing used to force feed MDW solution into nebulizer.

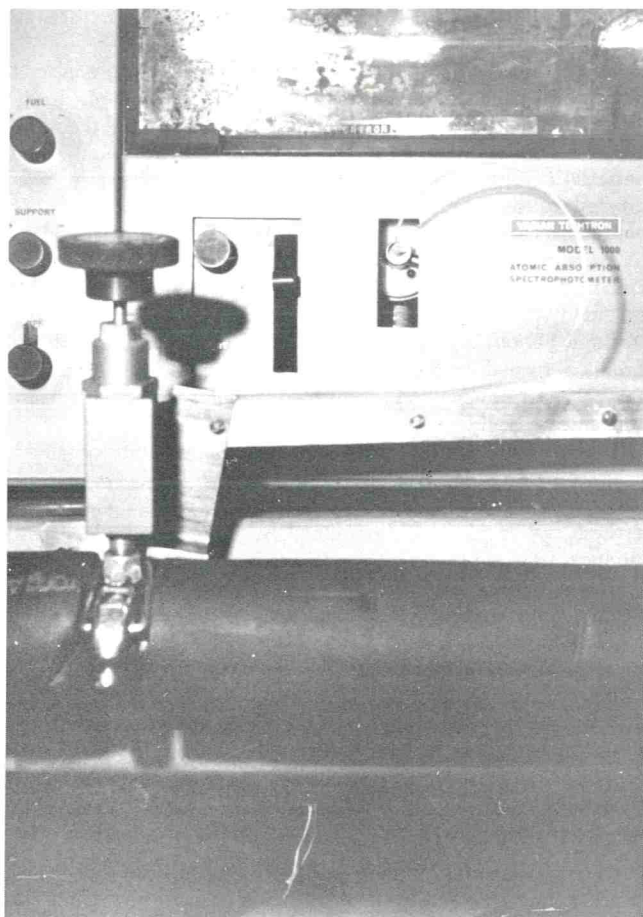


Figure 3. Fluid control valve with assembly and plastic tubing used to aspirate MDW solution into nebulizer.

polynomial equation became negative for very low concentrations of milk. These equations were used to obtain three-dimensional plots. Response surface equations for each detergent were used as standard curves to determine the total amounts of milk detected by the spectrophotometer. Instrument response from each test was analyzed using the standard curve for the detergent to predict the amount of milk injected. Means and standard deviations were determined for each variable.

## RESULTS AND DISCUSSION

### *Effect of concentration of milk*

Concentrations of milk tested were responsible for 95.4 and 97.1% of the experimental variations in the studies with alkaline and acid detergents, respectively. The mean instrument response (mv) and the amount of calcium (mg/l) for known concentrations of milk in alkaline and acid detergents are shown in Table 1. In the studies of both the acid and alkaline detergents, no significant differences ( $P < 0.05$ ) were noted in the amount of calcium (mg/l) in solution with no milk and with 0.01% milk. The average sensitivity of the instrument to calcium (defined as a signal to noise ratio of 2:1) was 0.055 and 0.048 mg/l

with solutions of 0.01% milk in alkaline and acid detergent solutions, respectively.

For the alkaline detergent studies, a 10-fold increase in the concentration of milk (from 0.01 to 0.1%) resulted in a 0.16 to 1.62 mg/l increase in the amount of calcium. This increase was equaled by a 10-fold increase (from 2.03 to 20.40 mv) in the response of the spectrophotometer. The next 10-fold increase in the concentration of milk (from 0.1 to 1.0%), which amounted to an increase in the amount of calcium from 1.62 to 15.58 mg/l, produced less than a 7-fold increase (from 20.4 to 131.8 mv) in the instrument response. The reduction in instrument response may have resulted from the increased viscosity of samples and the higher concentration of salts in the solution. Since salts crystallize in the nebulizer, instrument response is reduced (4).

For the acid detergent studies, an increase in the concentration of milk from 0.01 to 0.1% amounted to a 12-fold increase (from 0.13 to 1.59 mg/l) in the amount of calcium and a 12-fold increase (from 1.12 to 13.55 mv) in the instrument response. However,

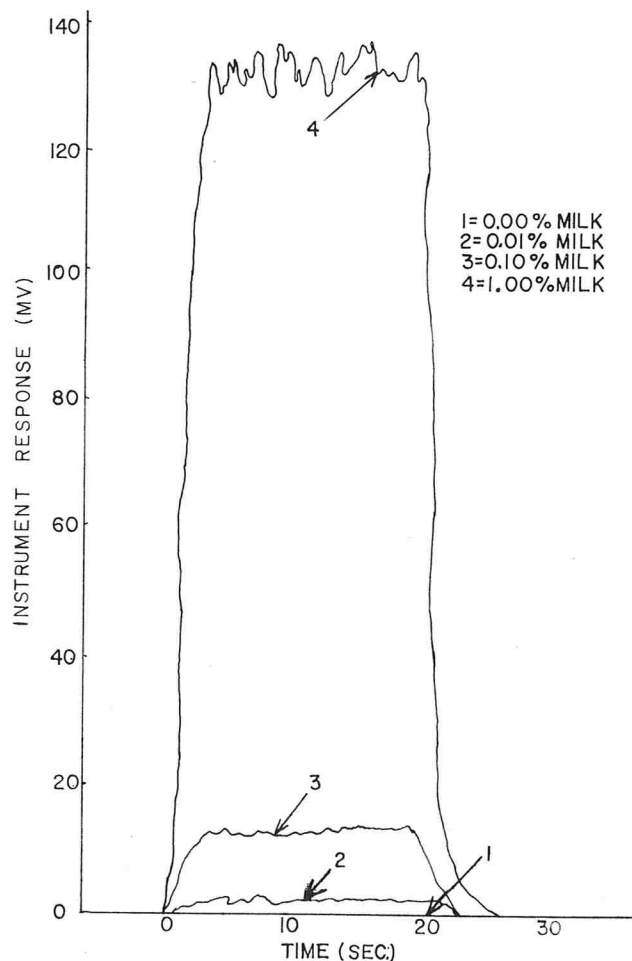


Figure 4. Typical instrument responses to four concentrations of milk injected into solutions of alkaline and acid detergents.

Response surface equations:

Above 20 mv

$$Y = 114.131 - 17.278 (\text{Log } D) + 160.879 (\text{Log } M) + 69.823 (\text{Log } M)^2 + 8.443 (\text{Log } M)^3 - 13.608 (\text{Log } D) (\text{Log } M) - 2.077 (\text{Log } D) (\text{Log } M)^2 + 0.090 (\text{Log } D)^2 (\text{Log } M)$$

Below 20 mv

$$Y = 202.96 (M)$$

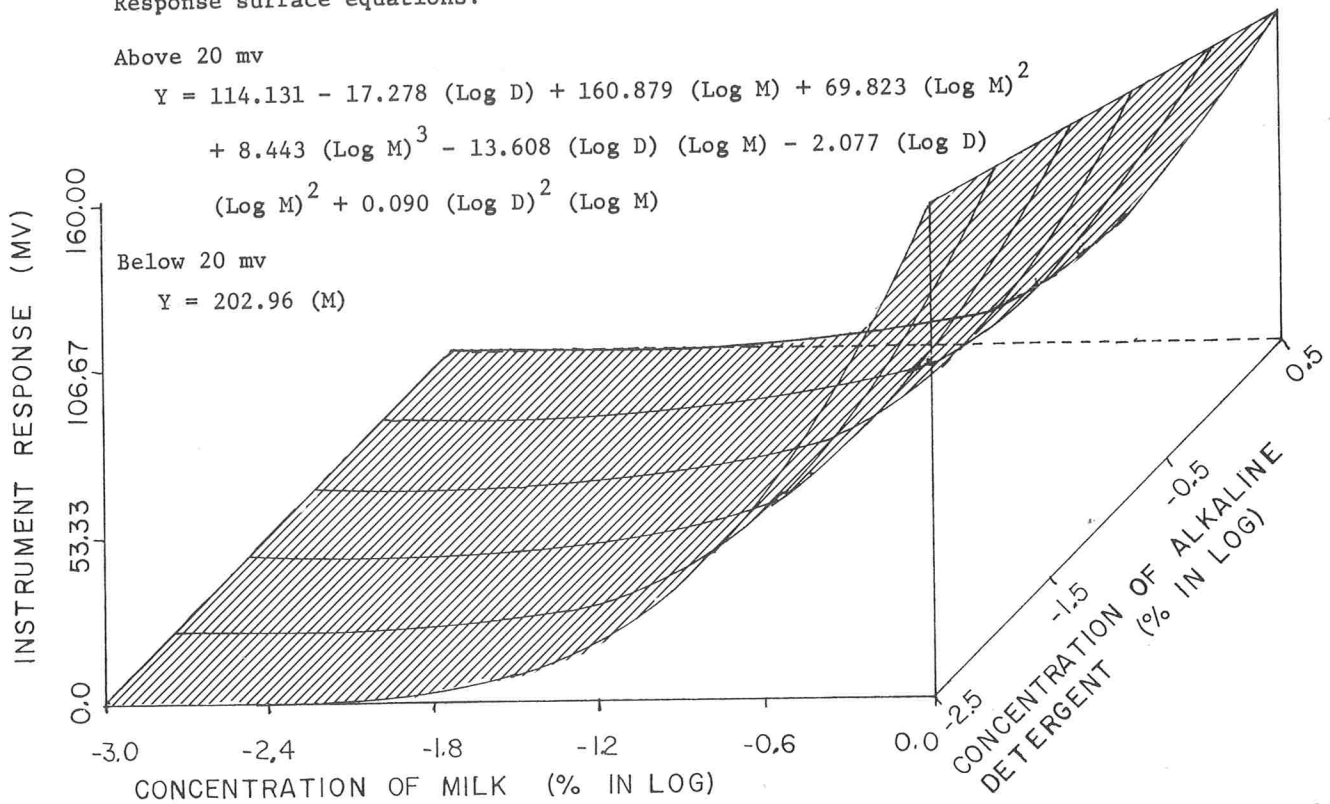


Figure 5. A plot of the response surface equation for instrument response (mv) as a function of concentration of milk (% in log) and concentration of alkaline detergent (% in log) using both straight line and polynomial equations.

Response surface equations:

Above 9.0 mv

$$Y = 92.446 + 0.738 (\text{Log } D)^2 + 0.655(T) + 159.091 (\text{Log } M) + 87.110 (\text{Log } M)^2 + 14.825 (\text{Log } M)^3 + 0.800 (\text{Log } D)^2 (\text{Log } M) + 0.189 (\text{Log } D)^2 (\text{Log } M)^2 + 0.638(T) (\text{Log } M) + 0.142(T) (\text{Log } M)^2$$

Below 9.0 mv

$$Y = 112.12 (M)$$

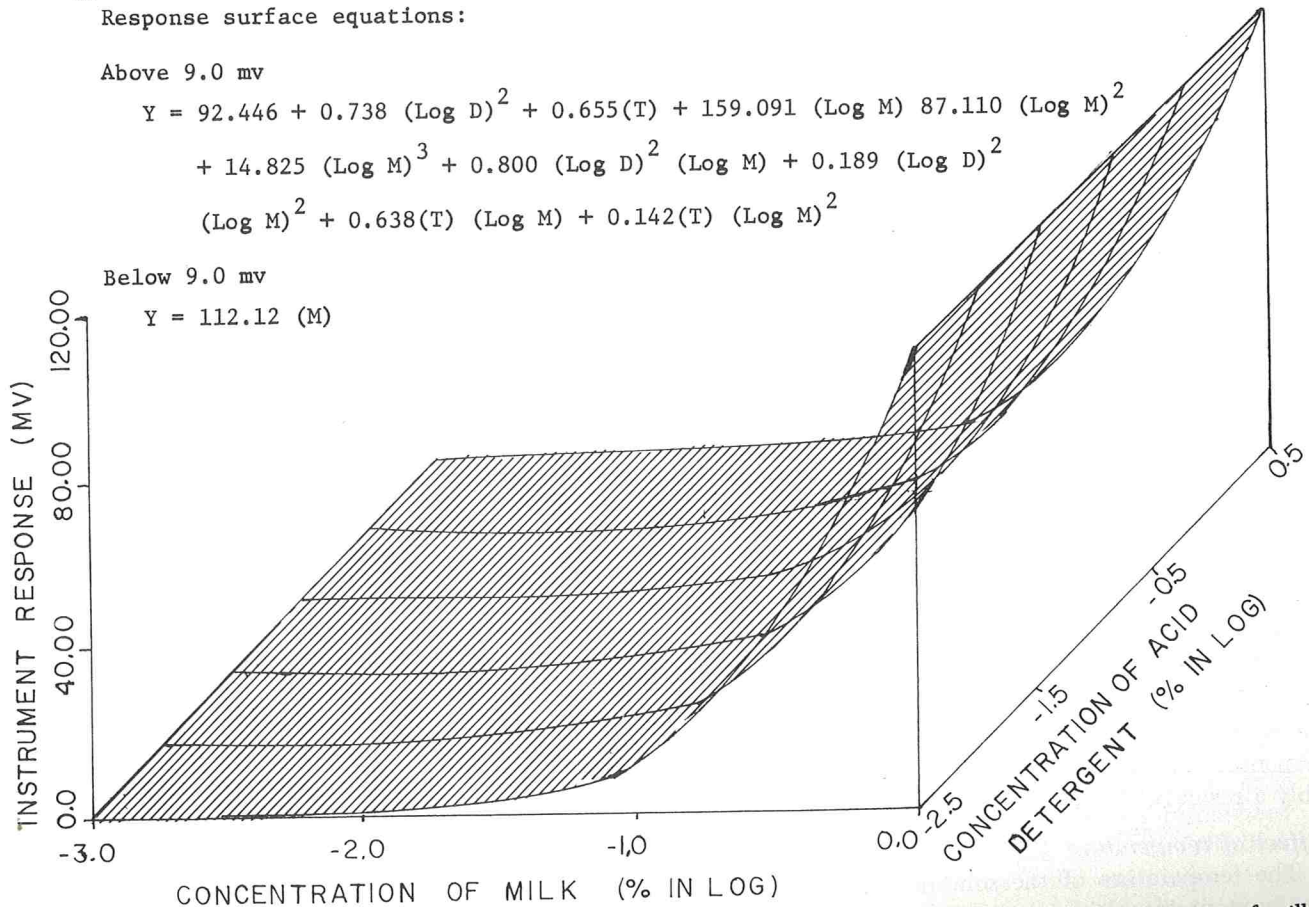


Figure 6. A plot of the response surface equation for instrument response (mV) as a function of concentration of milk (% in log) and concentration of acid detergent (% in log) using both straight line and polynomial equations.

TABLE 2. OVERALL MEAN AMOUNTS OF MILK SOLIDS INJECTED AND MEASURED (MG), WITH THEIR STANDARD DEVIATIONS, FOR THREE CONCENTRATIONS OF MILK AND FOUR CONCENTRATIONS OF ALKALINE DETERGENT, AVERAGED FOR THREE TEMPERATURES

Concentration of milk (%)	Concentration of det. (%)	Amount measured (mg)		Amount injected (mg)	
		Mean	Standard deviation	Mean	Standard deviation
0.01	0.0	384	34	390	69
	0.03	448	94	457	196
	0.3	400	36	544	90
	3.0	401	23	271	63
		(408) <sup>a</sup>		(416)	
0.1	0.0	4,180	591	2,620	421
	0.03	4,220	619	3,890	2,000
	0.3	4,050	487	5,300	504
	3.0	4,240	484	4,420	1,970
		(4,170)		(4,060)	
1.0	0.0	38,100	4,940	26,500	4,590
	0.03	41,900	9,190	42,900	10,400
	0.3	42,600	4,240	50,000	13,000
	3.0	39,900	3,140	35,500	10,800
		(40,600)		(40,700)	

<sup>a</sup>Numbers in parentheses are the average amounts of milk solids for each concentration of milk.

TABLE 3. OVERALL MEAN AMOUNTS OF MILK SOLIDS INJECTED AND MEASURED (MG), WITH THEIR STANDARD DEVIATIONS, FOR THREE CONCENTRATIONS OF MILK AND FOUR CONCENTRATIONS OF ACID DETERGENT, AVERAGED FOR THREE TEMPERATURES

Concentration of milk (%)	Concentration of det. (%)	Amount injected (mg)		Amount measured (mg)	
		Mean	Standard deviation	Mean	Standard deviation
0.01	0.0	402	69	415	101
	0.03	400	47	359	97
	0.3	413	60	447	69
	3.0	424	43	389	72
		(409) <sup>a</sup>		(402)	
0.1	0.0	3,650	610	3,610	617
	0.03	4,690	859	3,750	849
	0.3	3,840	362	4,160	311
	3.0	4,170	487	4,670	391
		(4,090)		(4,050)	
1.0	0.0	36,100	5,870	37,500	8,860
	0.03	39,000	3,300	36,600	6,540
	0.3	39,500	4,180	38,400	5,100
	3.0	43,600	3,250	43,000	4,650
		(39,600)		(38,900)	

<sup>a</sup>Numbers in parentheses are the average amounts of milk solids for each concentration of milk.

an increase (from 0.1 to 1.0%) in the concentration of milk, which resulted in an increase in the amount of calcium from 1.59 to 16.76 mg/l, caused a 9.3-fold increase (from 13.5 to 126.22 mv) in the instrument response. The reduced instrument response was probably a result of the increased viscosity of the samples.

#### Effect of temperature

The temperature of the solution had no effect on the amount of calcium measured in the alkaline detergent. The fact that the alkaline detergent sample

was force fed into the nebulizer, rather than aspirated, may have eliminated some of the effects of temperature. or the acid detergent studies, the amounts of calcium measured at 20 C were slightly but significantly lower than those measured at 45 or 70 C. This reduction in the amount of calcium measured may have been caused by an increase in the viscosity of the acid detergent samples at the lower temperature. Since the acid detergent samples were aspirated into the nebulizer, viscosity would have af-

ected rates of aspiration.

#### *Effect of detergent*

Except at a 1.0% concentration of milk, no significant differences were noted in instrument response (mv) due to concentration of alkaline or acid detergent. For 1.0% milk, the instrument response decreased as the concentration of alkaline detergent increased, and significant detergent-milk interaction occurred. The instrument response was 154.23, 144.16, 134.71, and 95.00 mv for 0.0, 0.03, 0.3, and 3.0% concentrations of alkaline detergent, respectively. The decrease in instrument response may have resulted from formation of thermally stable compounds in the nitrous oxide flame.

The response surface equations and the plot of the equations for instrument response (mv) as a function of the concentrations of milk (% log) were used to obtain the response surfaces for the alkaline detergents (Fig. 5) and acid detergents (Fig. 6). These instrument response surfaces were used to predict the amount of milk injected.

#### *Quantitation of milk injected—alkaline study*

Overall mean amounts of milk solids injected and measured (mg) and their standard deviations for three concentrations of milk and for four concentrations of alkaline detergent are averaged for three temperatures (Table 2). For a 0.01% concentration of milk, the mean amount of milk solids injected for the alkaline detergent was 408 mg, and the mean amount measured was 416 mg. The standard deviation for the amount of milk solids measured was greater than the standard deviation for the amount injected. This might be expected because the variation that occurred during injection should have been included in the variation of the amount measured. For a 0.1% concentration of milk, the mean amount of milk solids injected was 4,170 mg, and the mean amount measured was 4,060 mg. The coefficient of variation for amount of milk injected for each concentration of alkaline detergent varied from 10 to 15%. The amount of milk solids measured for a 0.0% concentration of alkaline detergent was low, 2,620 mg. For a 1.0% concentration of milk, the mean amount of milk solids injected for the alkaline detergent was 40,600 mg; the mean amount measured was 40,700 mg. The coefficient of variation for amount of milk solids injected for each concentration of alkaline detergent ranged from 8 to 15% except for the 0.03% concentration of alkaline detergent which had 43% coefficient of variation. The amount of milk solids measured for a 0.0% concentration of alkaline detergent was low, 26,500 mg. Coefficients of variation for amount of milk solids measured for each concentration of alka-

line detergent were all high and ranged from 17 to 30%.

#### *Quantitation of milk injected—acid study*

The overall mean amounts of milk solids injected and measured (mg) and their standard deviations for three concentrations of milk and for four concentrations of acid detergent are averaged for three temperatures (Table 3). For a 0.01% concentration of milk, the mean amount of milk solids injected for the acid detergent was 409 mg; the amount measured was 402 mg. The coefficient of variation for the amount of milk solids injected for each concentration of acid detergent ranged from 10 to 17%. The coefficient of variation for amount of milk solids measured for each concentration of acid detergent ranged from 15 to 27%.

For a 0.1% concentration of milk, 4,090 mg of milk solids were injected, and 4,050 mg of milk solids were measured. The coefficient of variation for the amount of milk solids injected ranged from 9 to 18%. The coefficient of variation for mean amounts of milk solids measured for each concentration of acid detergent ranged from 8 to 23%. When an average of 39,600 mg of milk solids was injected (1.0% milk), an average of 38,900 mg of milk solids was measured. The coefficient of variation for amount of milk solids injected ranged from 7 to 16%. The coefficient of variation for the amount measured ranged from 11 to 18%.

The above discussion indicates that considerable variations occurred in these data. These variations were most likely a result of differences in instrument adjustment and instrument drift. A slight change in flame setting gave a substantial change in instrument response. In atomic absorption spectrophotometry, standard solutions are usually tested before and after an unknown solution is analyzed to reduce errors that are due to variations in settings. In these tests, it was impossible to obtain the same setting each day. These variations in settings do not, however, detract from the method's potential use for monitoring the presence of calcium in a MDW solution on a continuous basis.

#### CONCLUSIONS

The following conclusions were reached: (a) concentration of milk accounted for most of the variation in the data; (b) the atomic absorption spectrophotometer can be used to measure the amount of milk flowing in a piping system; (c) the two formulated commercial detergents, one acid and one alkaline, did not significantly interfere with measurement of calcium; (d) because of the method of analysis used, a significant difference in quantities of calcium in

solutions with no milk and 0.01% milk was unable to be detected; and (e) temperature of the solution did not, for practical purposes, affect the amount of calcium measured.

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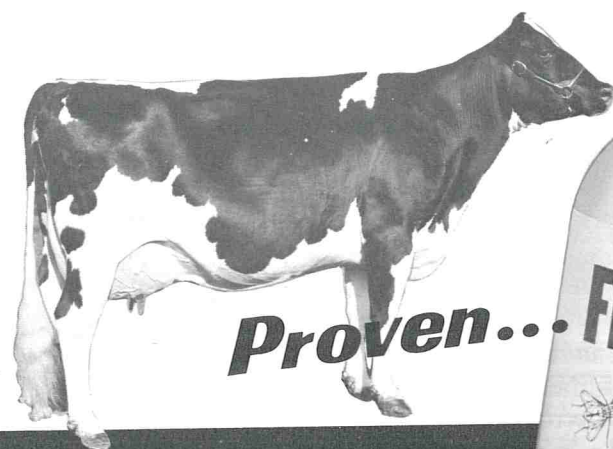
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He first served as laboratory director of the Gibbs Preserving Co. in Baltimore, inaugurating quality control, introducing new products and improving food-packing processes.

When World War I broke out, he was called to the U. S. Bureau of Plant Industry and initiated laboratories and research programs to utilize industrial food plant wastes and by-products. He increased production of vegetable oils and worked out the processes of manufacturing castor oil for airplane lubrication by the government.

During the 1920s he was appointed director of the bureau of chemistry and food for Baltimore City Health Department. He had full charge of the food control work of the city and had started the city's industrial hygiene program. He also directed investigations which led to law suits at that time.

During the same period he was an instructor of biochemistry at Johns Hopkins. He attained national prominence when he served as a "guinea pig" in an experiment testing the lethal aspects of illuminating gas.

During the 1930s he organized and directed the research laboratories of the National Dairy Products Corp. in Baltimore in association with Dr. E. V. McCollum, who was the discoverer of several vitamins. He then transferred to New York as corporation secretary of Sealtest Inc. and helped devise their milk control and laboratory control service.

He published "Food Control: Its Public Health Aspect" in 1939, just before he joined Eastern Nazarene as chairman of the division of science and mathematics and professor of chemistry.

Dr. Shrader was active at the college and spearheaded the effort to get the college accredited in the 1940s. He was also the organizer and first editor of "The Christian Scholar," a school publication. In 1959 the college's new science building was named in his honor. Dr. Shrader retired in 1961 and moved from Quincy to Waterville. He also summered in Mt. Vernon, Va.

He conducted and published research in 65 journal

articles about chemistry, food chemistry, dairy technology and environmental hygiene.

In 1971 he published "Cosmic Splendor: An Empiric Approach to Religion Through Modern Culture." He had edited a mimeographed journal entitled "Religious Inquiry" while at Eastern Nazarene and the book was the culmination of his interest in the correlation and interrelationship of science and religion in terms of modern thinking and expression.

He was a member of the American Association for the Advancement of Science, the American Chemical Society, the American Public Health Assn., the American Scientific Affiliation, the Institute of Food Technologists, the International Association of Milk and Food Sanitarians, the Institute of Religion in an Age of Science, and Sigma Tau, an honorary science society.

Husband of the late Annie A. (Hosley) Shrader, he is survived by three daughters, Mrs. Roger W. Mann, sister of former Eastern Nazarene College President Dr. Edward S. Mann, of Waterville, with whom he lived, Mrs. Jean S. Mullen of Boston and Miss Margaret M. Shrader of Orleans; 10 grandchildren and three great-grandchildren.

**Note: It was my privilege to know and work with Dr. Shrader. What a great and fine person he was!!**

"Red"

### KENTUCKY ASSOCIATION OF MILK, FOOD AND ENVIRONMENTAL SANITARIANS, INC.

#### Kentucky 1974 Educational Conference, Louisville, Kentucky

The 1974 Educational Conference for Fieldmen and Sanitarians sponsored by the Kentucky Association of Milk, Food and Environmental Sanitarians, Inc., was held February 26-27, 1974 at Stouffer's Inn, Louisville, Kentucky.

Over 300 (city, county and state sanitarians, milk and food industry fieldmen, plant managers, related service company representatives and university personnel) were registered. Twenty-four registrants from out-of-state included persons from Florida, Georgia, Illinois, Indiana, Iowa, Missouri, New York, Ohio, Tennessee, Texas and Washington, D. C.

The program was broken into general sessions, food and general sanitation sections and milk section. Fifteen papers were presented during the conference.

The following awards were given at the awards banquet by the Kentucky Association of Milk, Food and Environmental Sanitarians, Inc.

Outstanding Sanitarian Award: Harry A. Marsh, Lexington-Fayette County Health Department, Lex-





(Left to right: W. L. Cammack, Kraft Foods, Owenton, Kentucky, Outstanding Fieldman Award. Lyman C. Knierem, Jr., Zero Manufacturing, Louisville, Kentucky, Outstanding Service Award. Dudley J. Conner, Department for Human Resources, Frankfort, Kentucky, Past President's Award. Harry A. Marsh, Lexington-Fayette Co. Health Department, Lexington, Kentucky, Outstanding Sanitarian Award. Mrs. Emma W. Miller, (seated) Director, Milk & Water Laboratory (retired), Louisville, Kentucky, Honorary Membership.

ington, Kentucky.

Outstanding Fieldmen Award: (plaque donated by Sep-ko Chemical Company), W. L. Cammack, Kraft Foods, Owenton, Kentucky.

Outstanding Service Award: Lyman C. Knierem, Jr., Zero Manufacturing Company, Louisville, Kentucky.

Honorary Membership: Mrs. Emma W. Miller (Retired), Director, Milk & Water Laboratory, Kentucky State Health Department.

A Past Presidents plaque was also presented to Dudley J. Conner by newly elected president Dr. James C. Hartley.

New Officers and Directors elected at the business meeting were as follows: President, Dr. James C. Hartley, University of Kentucky, Lexington; President-elect, Bruce K. Lane, Louisville, Jefferson County Health Department, Louisville; Vice President, James Spillman, D.I., Kyana Division, Louisville; Secretary-Treasurer, Leon Townsend, Bureau of Human Resources, Frankfort.

Directors: North Central Region, Marvin Fornash, N.F.O., Georgetown, James T. Harrill, Bourbon County Health Department, Paris; South Central Region, William Gilreath, Southern Belle Dairy, Somerset; Eastern Region, J. B. Bowman, Lee-Owsley County Health Department.

### BERNARD J. SCHEIB RETIRES

Bernie Scheib, Sales Coordinator-Dairy Industry, West Agro-Chemical, Inc. and a long time member of International retired April 1.

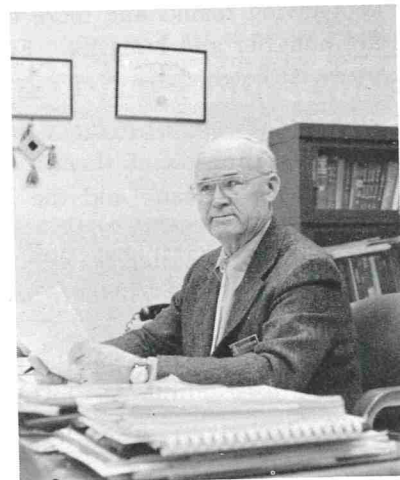
Bernie was a co-founder of Lazarus Laboratories and holds a patent on the use of phosphoric acid to formulate iodophors with a high acid to iodine ratio.

In his twenty-one years of service with West Chemical, Bernie has served in many capacities. After assisting in organizing the Lazarus Laboratories sales forces throughout the Eastern and Southern areas of the U. S., Bernie was named the Northeast Sales Manager, then Manager of Dairy Sanitation Technical Services, and most recently Sales Coordinator-Dairy Industry in the Midwest.

Bernie has always been active in local, regional and national dairy associations and most recently was active on several committee assignments with the National Mastitis Council.

Bernie has made a fine contribution during his years of service and leaves behind him a host of friends.

### CARL E. HENDERSON RETIRES



Carl E. Henderson, the dean of New Mexico environmentalists, is retiring Rebruary 28 after 40 years of service in public health within the state.

Henderson, 65, launched his career in public health as District Sanitarian in Eddy County in 1934. The native New Mexican attended Carlsbad High School and graduated from the University of New Mexico in 1931 with a bachelor of science degree.

Henderson, a quick-witted man who sports a sharp sense of humor and a seemingly endless variety of bolo ties, became Supervisor of Food and Milk Sanitation for the New Mexico Health Department in 1943. He held that post until 1971 when he became chief of the Food Quality Division of the Environmental Improvement Agency.

Henderson's public health vocation has been paralleled year for year with an avocation in scouting. During his years of leadership in New Mexico scouting, he served as Kit Carson Council Commissioner and

Chairman of the Council's Health and Safety Committee.

There is no clear line of demarcation between Henderson's professional life as a food and milk sanitation officer and his interest in working with young men in scouting. Henderson initiated the use of chlorine to combat bacterial contamination of utensils while camping out at Philmont.

A fellow long-time Health Department employee, John Gavahan of Las Vegas, testifies that Henderson's active membership in the Church of Christ has also come in handy during his public health career.

"We (Gavahan, Henderson and Charles Caldwell) had agreed to inspect the water system of a site the church was interested in buying. The site was isolated, and the preacher's wife packed us a big picnic lunch.

"It took us half a day just to get to the place, and we decided to eat before making the inspection. The preacher suggested we 'give thanks' before eating.

"Well, he kept giving thanks and more thanks and we kept getting hungrier and hungrier. Finally Carl (Henderson) solved the dilemma.

"He waited for the preacher to take a breath after one particular long expression of thanks; injected a quick 'Amen, Brother Freeman'; and the lunch was half devoured before the preacher could protest."

Henderson received his Master of Science degree in 1948 from the University of Missouri and became a Registered Professional Engineer the same year. He is a member of seven professional organizations including the New Mexico Public Health Association, the New Mexico Sanitarians Association and the Commissioned Officers Association of the United States Public Health Service. He is also a 33rd Degree Mason.

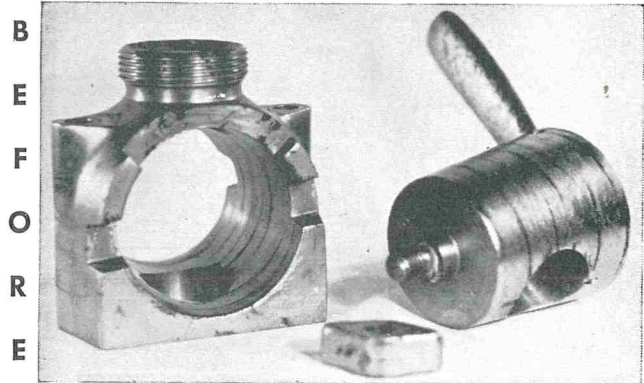
While serving with the New Mexico Health Department and the EIA, Henderson was instrumental in four acts of legislation including the New Mexico Restaurant Law and the New Mexico Food Act.

During his 40-year professional career, Henderson has been honored as Diplomat of the Intersociety Academy for Certification of Sanitarians and as Commander of the United States Public Health Service Reserve.

Aaron Bond, long-time acquaintance of Henderson and Director of the EIA, said, "A man of Carl's experience and knowledge is irreplaceable. He has been and I am sure will continue to be one of the most untiring and unselfish men I have ever known."

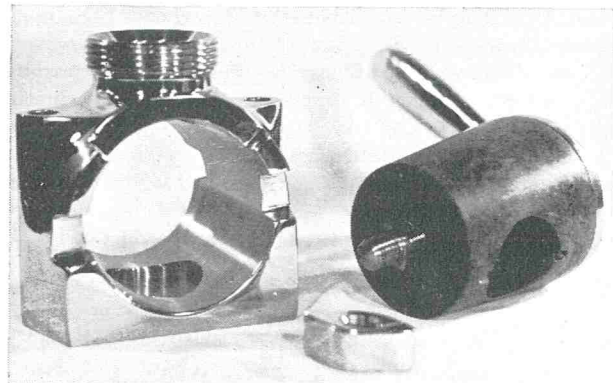
The EIA honored Henderson with a banquet Thursday (Jan. 24) at 7 p.m. in the F.O.E. Building, 833 Early Street.

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## Dairy authorities speak out on better cow milking



Dr. Elmer H. Marth  
Department of Food Science,  
University of Wisconsin/Madison.

# Rapid cooling can help maintain high quality of raw milk

The two most important things a dairyman can do to insure milk quality at his dairy are:

1. Put the cleanest possible milk into the bulk tank.
2. Cool it as quickly and efficiently as possible.

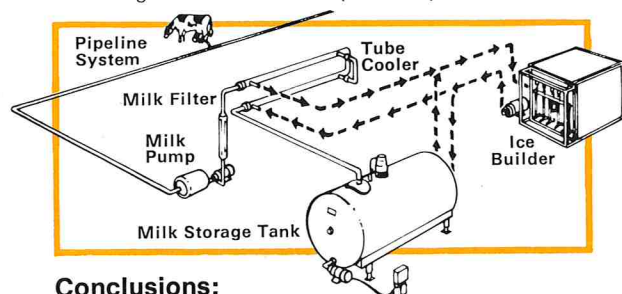
Much has been said about the necessity of sanitary milk handling through the entire milking operation. However, even under the most sanitary conditions, milk from a healthy cow will contain several hundred to several thousand bacteria per milliliter. Certain strains can cause undesirable conditions such as rancidity or other off-flavors unless their growth is retarded.

### Follow the rules

The best way to retard bacterial growth is by cooling milk as rapidly as possible, without freezing it.

1. *Milk must be cooled promptly.* Delays result in bacterial growth. Some of the bacteria in milk can multiply in as little as 20 to 30 minutes if the milk is warm.
2. *Cooling should be rapid,* so further appreciable bacterial growth does not occur during the cooling process. Care must always be exercised so that milk does not freeze.
3. *Milk must be cooled to and maintained at a safe temperature.* Cooled milk must be held at a 40° F. and preferably 36-38° F. This temperature must be maintained throughout the storage period. When freshly drawn milk is added to milk already in the bulk tank, the rise in temperature of the initial milk must be minimal and the temperature of all milk in the tank must be rapidly reduced to 36-38° F. (Again, milk must not be frozen in the process.)
4. *Raw milk should not be stored for excessive periods* and should be moved from the bulk tank to the tank truck under conditions which preclude additional microbial contamination.

The refrigerated bulk cooling tank is the most widely used device to cool milk on the farm today. However, it is limited in its ability to meet some of the demands outlined above. New equipment available makes it possible to "pre-cool" milk on the way to the tank. Instant coolers using chilled water from an ice-builder help make rapid cooling practical on the farm. This type of cooling also eliminates the possibility of freezing milk.



### Conclusions:

Even though all conditions needed for effective rapid cooling are met, some bacteria can still grow in refrigerated milk. Two points already discussed bear repeating. Be certain that good sanitary practices are followed during production of milk to insure that few bacteria of the kind able to grow at refrigeration temperatures are present. Do not hold raw milk refrigerated for excessive periods.

The successful marketing of milk depends on everyone doing his part at each step along the way. Even though you, as an individual dairyman, may not benefit *directly* from each of your efforts to improve sanitation and milk handling, you most certainly will benefit *indirectly* through greater total consumer acceptance of milk and dairy products thanks to fewer flavor problems and the absence of other negative factors caused by improper handling.

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