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Official Publication

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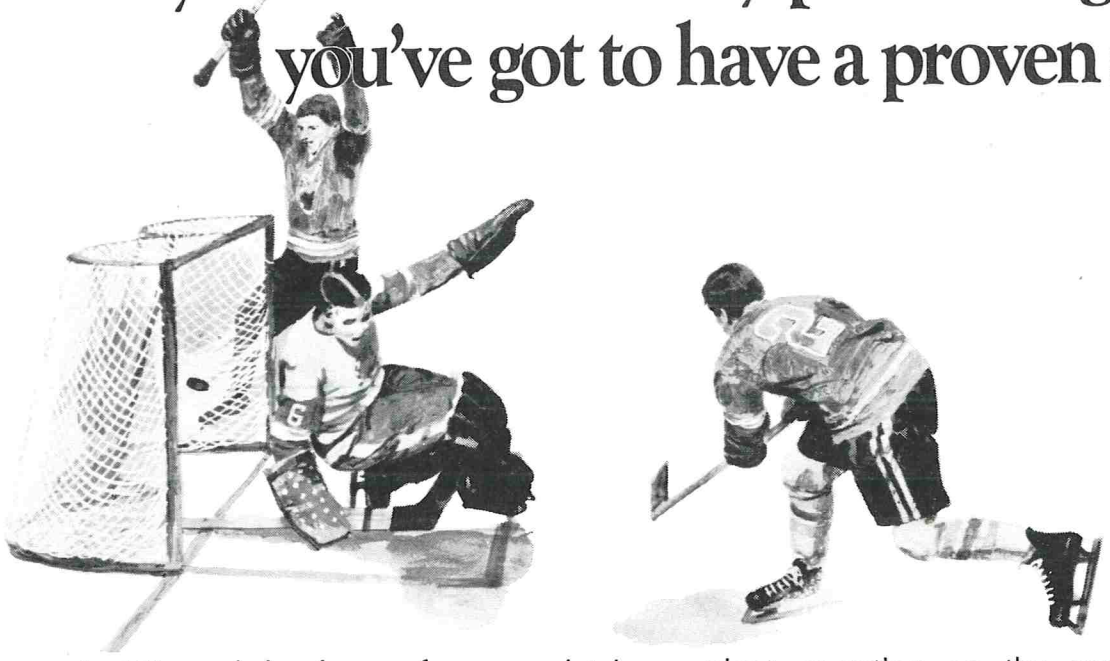

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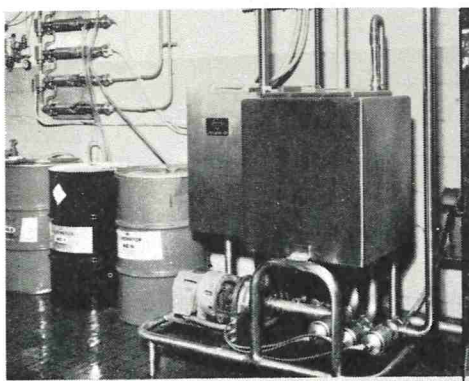

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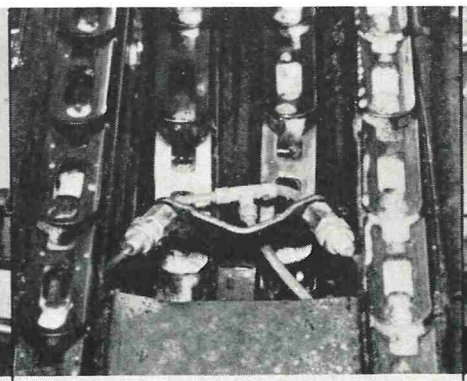

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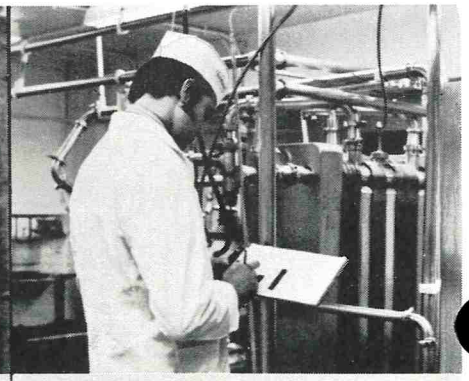

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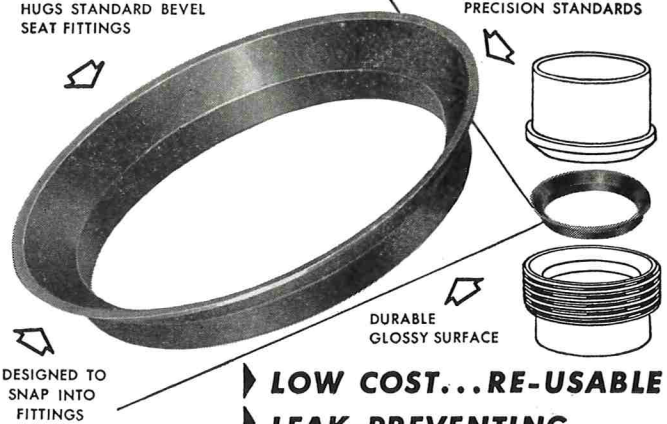

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COMPARATIVE GROWTH OF SALMONELLAE, COLIFORMS, AND OTHER MEMBERS OF THE MICROFLORA OF RAW AND RADURIZED GROUND BEEF[1]

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(Received for publication March 24, 1972)

Abstract

The population of salmonellae in raw ground beef at 5 C was stable during 6 days while that of the total microflora increased markedly. Radurization reduced the numbers of salmonellae and of the total population. The buildup of numbers also was retarded by radurization so that the total count was $< 10^6$ per gram after 6 days while the number of salmonellae remained constant. Ground beef incubated at 25 C showed a rapid increase in numbers of salmonellae, coliforms, and in the total microflora. While radurization reduced the numbers of these microorganisms, it did change the general pattern of competitive growth. Highly contaminated samples contained some coliforms able to grow at 5 C. Unirradiated samples as well as samples immediately following irradiation showed the coliforms to consist predominately of *Escherichia coli,* but during storage there was an increase in the proportion of intermediate types and of *Aerobacter.*

Fresh ground meats are subjected to considerable delay between production and consumption as well as exposure through packing, transportation, reprocessing in the supermarket, and extended household storage. Contamination with food spoilage microorganisms and a few pathogens is inevitable. The microbial population increases with time and each processing operation.

Salmonellae have been found in retail meat products *(5, 6, 17, 18)* and their frequent presence is a cause for alarm to regulatory sanitarians. Several studies have been made on survival of salmonellae in various food products *(1, 3, 13).* Goepfert and Chung *(7)* recently reported that salmonellae survived for more than 42 days in luncheon meats stored at 5 C, while they actually multiplied at room temperature (25 C). However, there has been no report on the relation between growth of salmonellae and the total microflora of ground beef.

Fresh ground meat in presently acceptable commercial form is not amenable to heat pasteurization, therefore indicating the need for an alternate process of microbial destruction. Low dose gamma-irradiation for radurization (radiation pasteurization) has potential for an acceptable process *(11, 16).* However, radurization results in a yet unstudied microflora, which is of particular interest because of potential survival and growth of organisms of public health significance.

This investigation was undertaken to examine the behavior of salmonellae and coliforms as examples of *Enterobacteriaceae* in the presence of the natural microflora of raw and radurized ground beef. Both common refrigerated storage at 5 C and gross mishandling of room temperature storage (25 C) were considered.

Materials and Methods

Source of ground beef

Machine-dispensed units of ground beef weighing approximately ¼ lb., as prepared for hamburgers, were obtained from a local commissary. This source was selected because it was known to provide a high quality, low microbial count product. For comparative purposes, samples were obtained from a local supermarket which consistently sold products of unusually high microbial count.

Cultures

Salmonella typhimurium, Salmonella enteritidis, and *Salmonella heidelberg* were obtained from the Center for Disease Control, Atlanta, Georgia. *Escherichia coli,* two strains, was from the departmental stock culture collection. Cultures were maintained on Plate-Count Agar (PCA; Difco) slants. Organisms were subcultured twice in nutrient broth at 37 C to provide 18 hr cultures for experimental purposes.

Inoculation and radurization of meat

Dilutions of the culture were prepared in sterile buffered phosphate diluent *(2)* and inoculated into ¼ lb. meat to obtain approximately 10^2-10^3 salmonellae or *E. coli* cells per gram in the control samples. In the samples to be radurized, the inoculum was increased to obtain a population level after irradiation comparable to that of the control. At this level of inoculation, the number of inherent salmonellae or *E. coli,* if any, was rendered insignificant. The inoculated meat was passed five times through a sterile meat grinder to distribute uniformly the inoculated bacteria. Inoculation and mixing were at 5 C. The inoculated meat in ¼-lb. quantities was put into sterile polyethylene bags. Irradiation was at room temperature using a ^{60}Co source providing 17 Krad of gamma-radiation per minute. During this short exposure of irradiation the temperature of the meat did not increase more than 1 C. The physical facilities were essentially those reported by Teeny and Miyauchi *(14).*

[1]Paper No. 3000 Journal Series, Nebraska Agricultural Experiment Station, Lincoln.

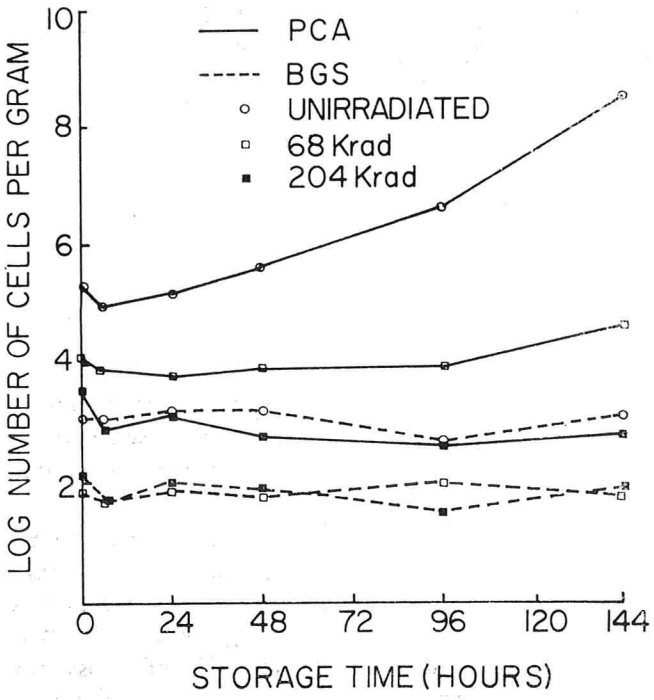

Figure 1. Comparative behavior of S. *typhimurium* and other members of the flora in raw and radurized ground beef at 5 C.

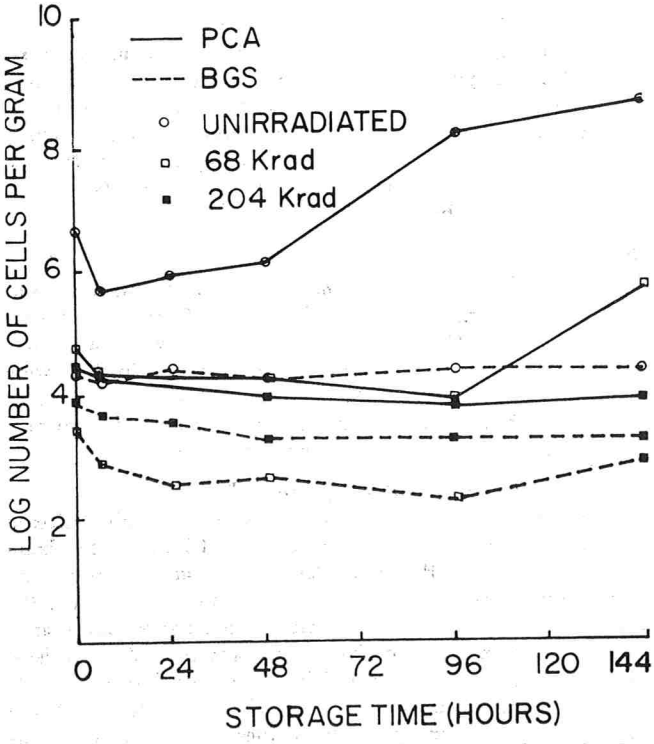

Figure 2. Comparative behavior of S. *enteritidis* and other members of the flora in raw and in radurized ground beef at 5 C.

Bacterial counts

Dilutions were prepared in sterile buffered diluent *(2)* using a Waring Blendor. For total counts, PCA was used with incubation at 32 C for 48 hr. Coliform or *E. coli* counts were made using Violet Red Bile Agar (VRBA; Difco) with incubation for 24 hr at 35 C. Salmonellae were determined by pour plating with Brilliant Green Sulfa Agar (BGS) (BBL) and incubated at 37 C for 48 hr. The counting involved differentiation of salmonellae from greenish-yellow coliform colonies. To differentiate *Salmonella* colonies from pseudomonads and *Proteus*, colonies were transferred to Triple Sugar Iron Agar *(12)*. Black colonies, which represented more than 99% of the colonies picked from BGS agar, were transferred into Urea Broth (Difco). Negative urease test confirmed the colonies of *Salmonella*.

Determination of the nature of coliform bacteria in retail ground beef

Twenty colonies from the countable VRBA plate were picked by random design into nutrient broth. Growth from nutrient broth was streaked on EMB plates and was also inoculated into another nutrient broth tube. The latter nutrient broth tube was incubated in a water bath at 44.5 C for 24 hr and observed for growth. The isolates were also subjected to indole, methyl red, Voges-Proskauer, and citrate (IMViC) tests *(8)*.

RESULTS

The fate of salmonellae and other members of the microflora of ground beef at 5 C.

An inoculum of S. *typhimurium* of approximately 10^3 cells per gram in ground beef, as determined by plating on BGS agar, showed no change in population density for 6 days at 5 C. Other members of the microflora grew to increase the PCA count which was 1.9×10^5 - 2.9×10^8. When samples were inoculated with S. *typhimurium* before radurization to provide

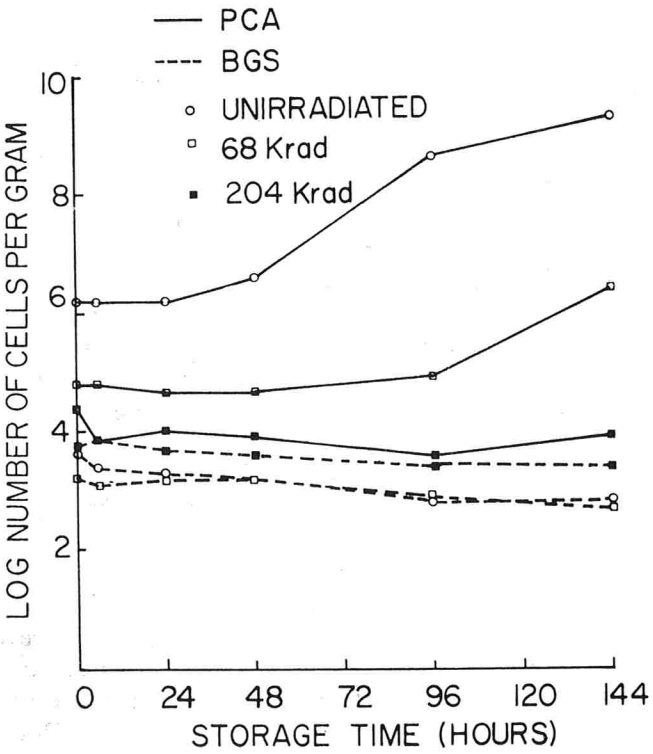

Figure 3. Comparative behavior of S. *heidelberg* and other members of the flora in raw and in radurized ground beef at 5 C.

TABLE 1. THE EFFECT OF STORAGE AT 5 C ON THE DISTRIBUTION OF *Aerobacter*, *E. coli*, AND INTERMEDIATES IN RAW AND RADURIZED GROUND BEEF FROM A RETAIL STORE.

Irradiation dose	Without storage: Microbial count per gram PCA	VRBA	Number of isolates	Distribution of isolates in per cent: *E. coli*	Intermediates	*Aerobacter*
0	3.7×10^7	3.8×10^4	100	77	16	7
68 Krads	2.2×10^6	1.4×10^3	89	82	12	6
136 Krads	1.2×10^6	70	26	96	4	0

Irradiation dose	After storage for 144 hr: Microbial count per gram PCA	VRBA	Number of isolates	Distribution of isolates in per cent: *E. coli*	Intermediates	*Aerobacter*
0	5.3×10^9	7.8×10^4	94	44	52	4
68 Krads	2.3×10^8	2.8×10^2	73	74	23	3
136 Krads	3.3×10^6	<10	10	100	0	0

TABLE 2. THE EFFECT OF STORAGE AT 25 C ON THE DISTRIBUTION OF *Aerobacter*, *E. coli*, AND INTERMEDIATES IN RAW AND RADURIZED GROUND BEEF FROM A RETAIL STORE.

Irradiation dose	Without storage: Microbial count per gram PCA	VRBA	Number of isolates	Distribution of isolates in per cent: *E. coli*	Intermediates	*Aerobacter*
0	7.6×10^6	7.2×10^3	60	70	30	0
68 Krads	7.4×10^5	1.7×10^2	57	67	33	0
136 Krads	2.2×10^5	6.7×10^1	23	91	9	0

Irradiation dose	After storage for 24 hr: Microbial count per gram PCA	VRBA	Number of isolates	Distribution of isolates in per cent: *E. coli*	Intermediates	*Aerobacter*
0	1.8×10^9	2.5×10^8	60	97	3	0
68 Krads	6.2×10^8	8.2×10^7	60	93	7	0
136 Krads	4.6×10^8	5.7×10^7	60	92	8	0

10^2 - 10^3 cells per gram after radurization at 68, 136, or 204 Krads, the counts on BGS showed no change during 6 days at 5 C. The PCA count increased slightly during 6 days at 5 C in the samples radurized at 68 Krad. Comparative growth is shown in Fig. 1. There was no apparent increase in count in samples radurized at 136 or 204 Krad. The results with 136 Krad were similar to those with 204 Krad and for the sake of simplicity were not included in Fig. 1.

Salmonella enteritidis and S. *heidelberg* gave similar results to those obtained with S. *typhimurium* (Fig. 2-3).

Growth of salmonellae and other members of the microflora of ground beef at 25 C.

Salmonella typhimurium was inoculated into raw ground beef to obtain a population of approximately 10^3 cells per gram. When incubated at 25 C for up to 24 hr, the BGS count increased at near the same rate as did the numbers of other members of the microflora as measured by PCA count (Fig. 4). Samples inoculated with S. *typhimurium* before radurization to provide 10^2 - 10^3 cells per gram after radurization at 68, 136, or 204 Krad showed a similar pattern of growth to that obtained with inoculated unirradiated product. However, the growth lag was less in unirradiated samples than in radurized samples. Results from only two dose levels were included in Fig. 4 to show the general trend and to avoid excess data in one figure.

Repetition of the above experiments but with S. *enteritidis* or S. *heidelberg* gave similar results to those obtained with S. *typhimurium* (Fig. 5-6).

The fate of coliform organisms and other members of the microflora of ground beef at 5 C

For the observation on raw product an inoculum of *E. coli* was added to obtain a coliform count

(VRBA count) of approximately 0.2% of the total population (PCA count). During storage at 5 C, the PCA count increased, but the coliform count remained approximately the same (Fig. 7). Since *E. coli* was more sensitive to radiation than many other members of the microflora, it was necessary to adjust the inoculum for the samples to be irradiated. The inoculum of *E. coli* was increased to provide approximately 10^2 - 10^3 cells per gram. After radurization at 68 or 136 Krad, the coliform count showed a reduction during 6 days at 5 C. The reduction was greater in radurized samples at 136 Krad. Two strains of *E. coli* gave similar results.

Growth of coliform bacteria and other members of the microflora of ground beef at 25 C

Coliform organisms in the raw and the radurized product grew rapidly at 25 C and approximately at the same rate as other members of the microflora (Fig. 8).

Nature of coliform bacteria in retail ground beef

The above data were obtained using ground beef from a central processing operation. These samples consistently had a low total and coliform count. Somewhat different results were obtained when ground beef was obtained from an atypical retail store *(15)* (two years of unreported results), which continually supplied a product with a high total and

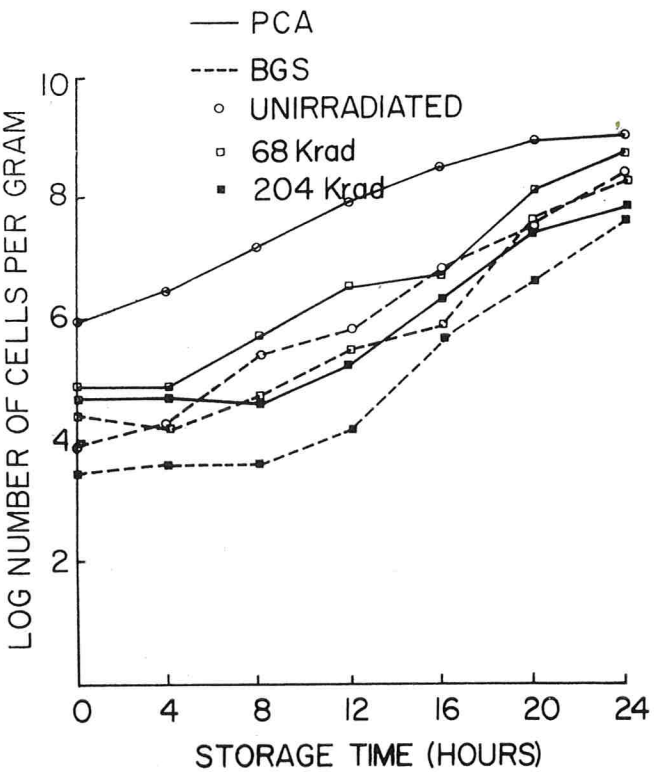

Figure 5. Comparative growth of *S. enteritidis* and other members of the flora in raw and in radurized ground beef at 25 C.

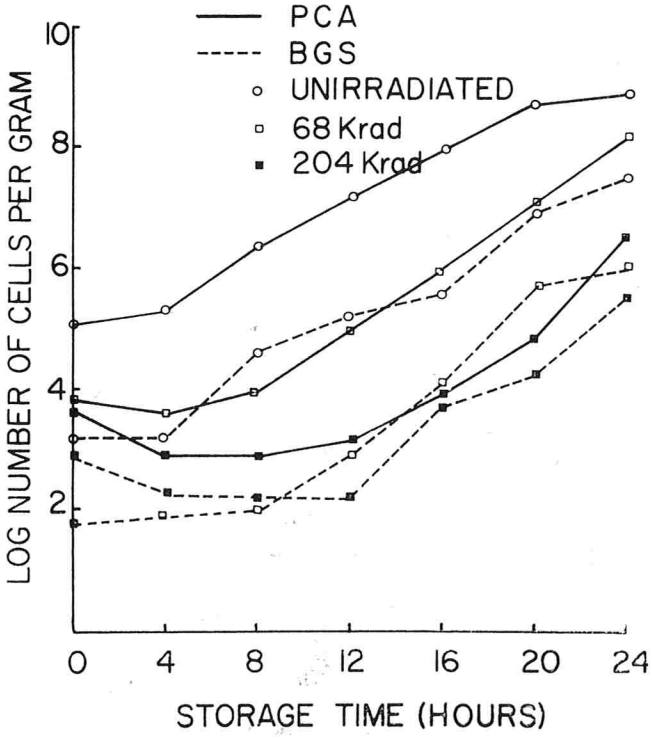

Figure 4. Comparative growth of *S. typhimurium* and other members of the microflora in raw and in radurized ground beef at 25 C.

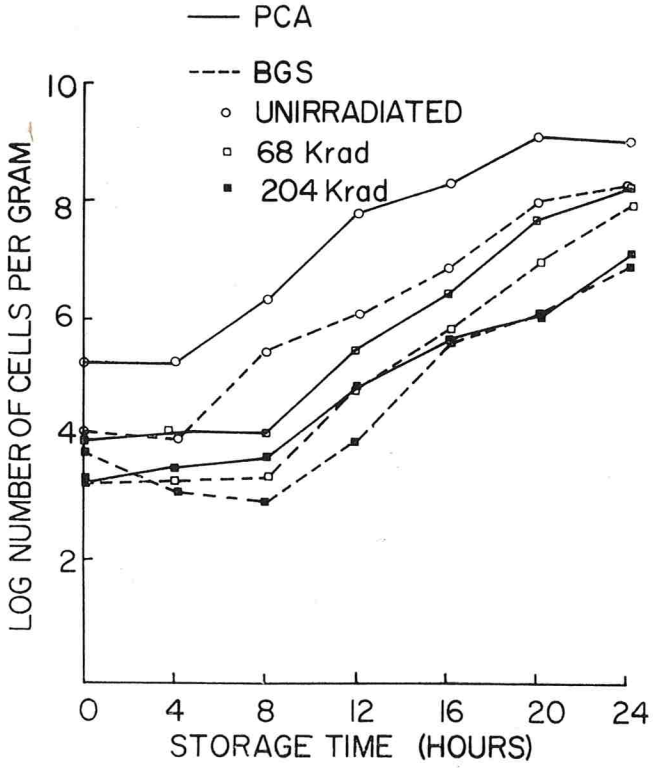

Figure 6. Comparative growth of *S. heidelberg* and other members of the flora in raw and in radurized ground beef at 25 C.

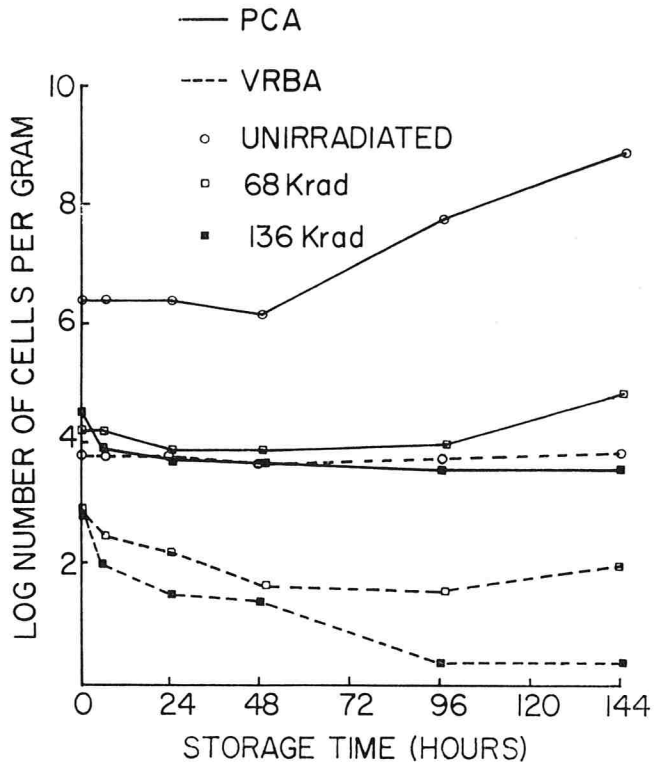

Figure 7. Comparative growth of *E. coli* and other members of the microflora in raw and in radurized ground beef at 5 C.

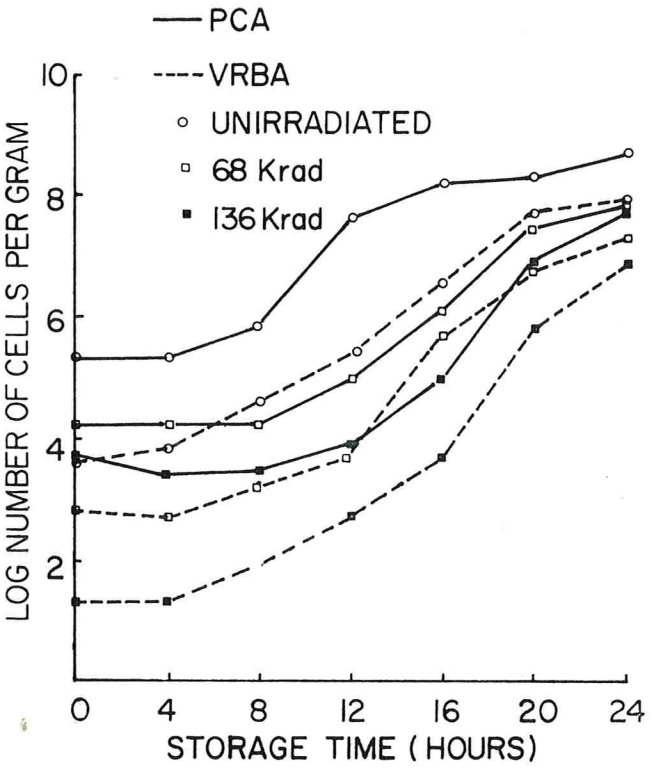

Figure 8. Comparative growth of *E. coli* and other members of the microflora in raw and in radurized ground beef at 25 C.

coliform count. Certain of the natural coliform contaminants were able to grow at 5 C in unirradiated samples, but no growth was observed in six separate experiments with radurized samples by the end of the 6-day test period. Average results are given in Fig. 9. The coliform count of radurized samples decreased slightly during storage at 5 C.

Coliforms in raw and radurized ground beef from the supermarket were characterized. The distribution of isolates is shown in Tables 1 and 2. The *Aerobacter* isolates are reported with the tacit assumption that *Klebsiella pneumoniae,* if present, would not contribute significantly to the numbers. The intermediates included those coliform bacteria not conforming to the traditional patterns of *Aerobacter* and *Escherichia* for indole, methyl red, Voges-Proskauer, and citrate tests. In the raw product *E. coli* constituted 70-77% of the coliforms. *Escherichia coli* was the most resistant to radiation of the three groups. In the raw samples multiplication of coliforms at 5 C was attributed to the intermediates. At 25 C *E. coli* grew most rapidly of the three groups.

DISCUSSION

Fresh ground beef constitutes one of the most

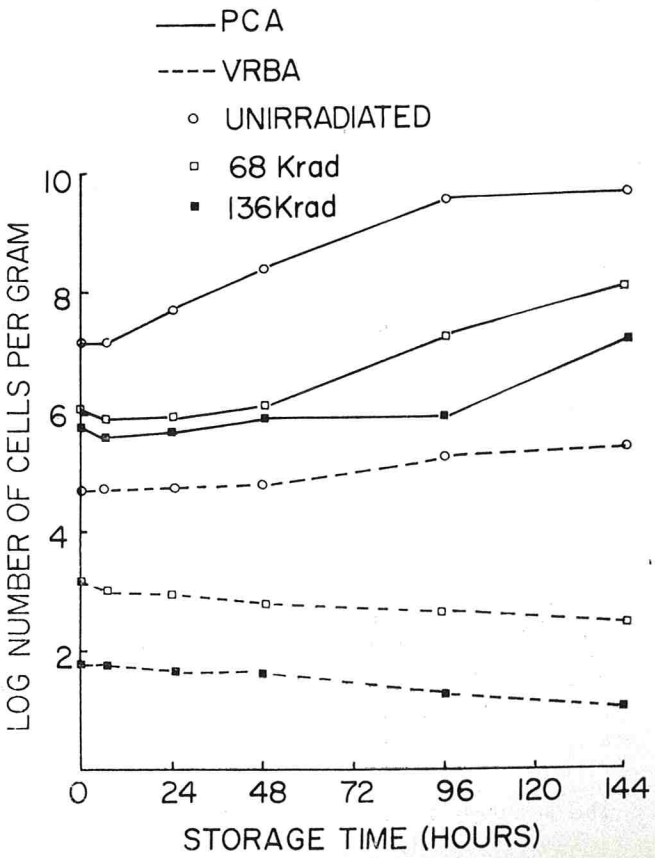

Figure 9. The fate of inherent coliform contaminants in raw and radurized ground beef at 5 C.

challenging of meat products for quality assurance and public health protection. Common reports of retail products with counts of well over 10^6 organisms per gram *(4, 9, 10, 15)* denote microbial contamination that should be reduced or eliminated. Contamination at this level indicates poor quality and potential hazards that persist or increase at common storage conditions of 5 C (Fig. 1, 2, 3, 7, 8). There is a marked increase in hazard with chance mishandling such as leaving the product at room temperature (Fig. 4, 5, 6, 9).

Our data indicate that radurization of ground beef reduced the incidence of salmonellae and coliform bacteria. It would be logical to expect that other members of the *Enterobacteriaceae* would be similarly affected.

Radurization of ground beef with dose levels used in this work reduced the total microflora and increased the lag in subsequent growth. Though our experiments were designed to emphasize the fate of the inoculated bacteria, it was apparent that the residual flora from the natural contaminants was predominant in subsequent growth at 5 C. A study of the nature and fate of surviving members of the microflora will be the subject of a later communication. Comparative growth of salmonellae, coliforms, and other members of the microflora arising from inherent contamination was similar at 25 C in raw and in radurized ground beef.

While the general pattern of growth was similar for the previously mentioned groups of bacteria, changes were apparent in the nature of the coliforms. At 5 C the inherent coliform contaminants grew in ground beef, which is in agreement with the work of Rey et al. *(12)*. The coliforms surviving radurization, however, did not grow. The growth in unirradiated samples was attributed to intermediates of *Escherichia-Aerobacter*.

Radurization may be used directly to eliminate contamination by destruction of microorganisms. An indirect and greater benefit, however, may be through shelf-life extension, which would allow central processing and packaging thereby reducing retail store contamination.

While radurization of red meat is not an accepted commercial process by United States regulatory agencies, because of the lack of proven safety of the process, ultimate use may be favored by public health protection. These potential benefits to society may offset the hazards, if any, from radurized products.

ACKNOWLEDGMENTS

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LOCATING LEAKS IN EGG PASTEURIZERS

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ABSTRACT

The U. S. Department of Agriculture tests for leaks in egg pasteurizers indicate only whether a leak is or is not present. Three tests for locating leaks in plate heat exchangers, after leaks have been indicated, have been evaluated from laboratory and plant trials. For an entire regeneration or cooling section a test using diazo paper and ammonia gas is feasible. Individual plates removed from the press can be tested by using diazo paper and ammonium hydroxide. A penetrant dye and developer test will confirm the exact location of a hole after its possible location has been reduced to a small area.

A method for detection of leaks in the heat transfer surfaces of the regeneration section of an egg pasteurizer was proposed in 1969 *(2)*. In cooperative work with the Poultry Division of Consumer and Marketing Service, USDA, this method was tried in commercial pasteurizers; necessary modifications were made; the test method was expanded to include the cooling section, and a detailed set of instructions and an equipment list *(1)* for the test were prepared. The test is now being conducted by the Poultry Di vision in all egg pasteurizing plants.

The object of the test is to detect holes in the heat transfer surfaces so that defective plates can be eliminated. The usual procedure is to pressurize the pasteurized-product-flow systems through the regeneration and cooling sections with a fluorocarbon refrigerant gas such as R-12. The air on the opposite side of the heat transfer surface from these systems is then checked with a halogen detector for the presence of the fluorocarbon to see if leakage has occurred.

The test does not give the specific location of a leak. When positive tests occurred the pasteurizing company was informed. In some instances with plate-type pasteurizers the company has replaced only plates showing worn areas, but commonly all plates in the section were replaced. On several occasions, faulty plates have been replaced with used plates whose history was unknown. There was no assurance that the replacements were free of holes unless another test was done.

In one instance with a tube type pasteurizer individual tubes were isolated and fluorocarbon tested to confirm leakage. In plate pasteurizers it is possible by repeated fluorocarbon testing, after changing or removal of plates, to narrow down the location of leaks, but this procedure is impractical for plant usage. Visual inspection of plates over a strong light in a dark room failed to show any holes in two instances where a positive test had been found. Locating holes in the plates may reveal important information on their origin and may prevent the use of defective plates in egg pasteurizers and reduce replacement costs.

This paper describes the results of laboratory and plant work on methods to locate holes in plates. These methods are intended for use after a positive fluorocarbon test. It is believed that they are roughly equivalent in sensitivity to the fluorocarbon test for indicating small holes.

PENETRANT DYE AND DEVELOPER TEST

Some work on locating leaks was done in the laboratory with a commercially available penetrant dye and developer that is used to show flaws in welds. (Spotcheck, Magnaflux Corp., Chicago, Ill.)[1]. Stainless steel disks in which holes had been made for use in development of the fluorocarbon tests were used. The dye was sprayed on one side of the disk and allowed a few minutes to dry; the developer was then sprayed on the other side. With proper application very small holes with an equivalent diameter of 0.0001 inch could be detected by the dye passing through the holes and spreading out in the developer to give small pinpoints of color.

This method was tried on a small commercial plate pasteurizer that gave a positive fluorocarbon test of the regeneration section. Plates were removed from the press and the gaskets masked to keep off the dye and solvents. Opposite sides of several plates were sprayed with the dye and developer. No holes were found; a later test showed that the hole was in a plate that was not tested. The dye and developer were removed from the plates by a variety of methods, none of which was easy and effective. The steps required to carry out this method of testing, especially the cleaning of the plates, were too time consuming to be practical if a large number of plates were to be tested. In later work this method was found to be an excellent way to show the exact location of a hole after its location had been narrowed down to a small area on one or two plates.

DIAZO PAPER AND AMMONIA TESTS

Diazo paper is available from several companies (see the

[1]Reference to a company or product name does not imply approval or recommendation of the product by the U. S. Department of Agriculture to the exclusion of others that may be suitable.

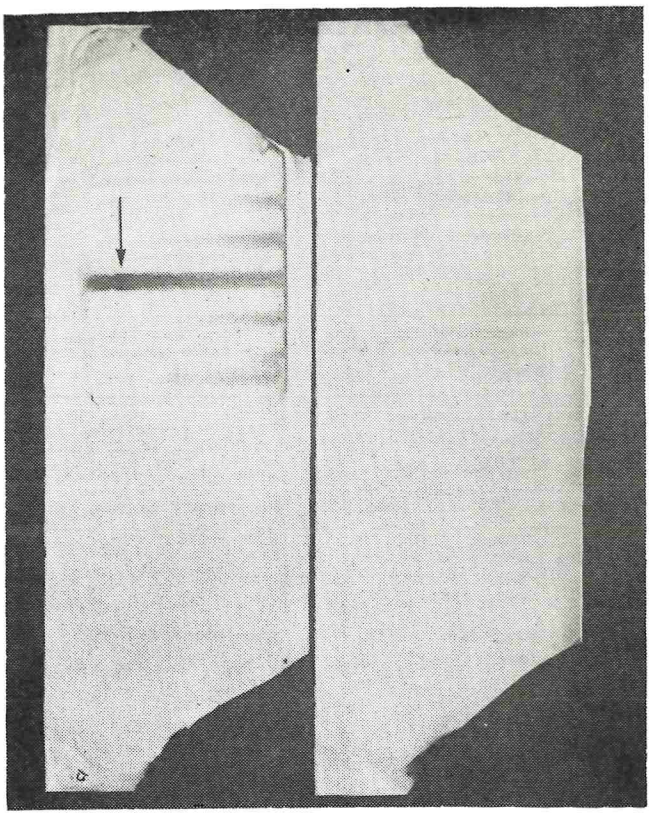

Figure 1. Two sets of diazo paper from test of regeneration section. On the left the arrow indicates the location of the hole through which the ammonia flowed to give the colored area. The right set of papers was between plates without holes. The colored areas at the ends of the diazo paper were outside the plate gaskets and were caused by ammonia leaking past the gaskets. The corners were cut off the papers so that the ports carrying ammonia would not be blocked. On many pasteurizers the corners to be cut off would be diagonally opposite instead of on the same side.

yellow pages in the telephone directories) for use in blueprinting. It is not expensive; the cost was less than 5 cents per plate for the tests described below. Ammonia turned the papers used in this test an intense blue. In laboratory tests, sufficient ammonia gas came through a hole of 0.0001 inch equivalent diameter, when held tightly over a bottle of 28% ammonium hydroxide, to make a small blue dot on diazo paper that was in contact for a few seconds with the opposite side of the hole.

Section test

This test method has been used on the regeneration sections of two commercial pasteurizers. After a positive fluorocarbon test, the fluorocarbon cylinder was replaced with a small ammonia cylinder so that the pasteurized-product side of the section could be charged with ammonia. The raw product side was then "papered." Different types of paper were used on the two pasteurizers, Ozalid 208S and 200 SE. The latter is more independent of humidity and temperature, but both worked very well. A set of two sheets of paper with the active sides out was used in each flow space between plates where non-pasteurized product flowed. The sheets extended beyond all of the gasket that retained raw product in the plates but did not cover any of the corner gaskets around the two ports which were to carry ammonia. The other two ports were left covered by the paper to restrict the movement of any ammonia that leaked through. This arrangement gives active diazo paper facing all the active heat transfer surface through which leaks may cause contamination of pasteurized product. The presses were closed tightly and charged with ammonia. Venting of the air was necessary to permit the ammonia to get through the entire regeneration sections. Care was necessary in using ammonia so that objectionable concentrations in the work areas did not occur from venting or from opening the press or lines used to charge the press.

The first test was done with the ammonia pressure at 5 psi for 5 min. After venting and blowing the ammonia from the press it was opened for examination of the paper. Too much of the paper was colored for inexperienced observers to locate the hole. Over half of two sheets and nearly half of two others were deeply colored.

These four sheets were replaced and the test repeated at 3 psi for 10 sec. Two sheets then showed a few square inches of intense color and some lightly colored areas. On examination of the plate area facing the intensely colored paper a small corroded spot was found. By use of the penetrant dye and developer test it was confirmed that this corrosion had caused a very small hole through the plate.

The test of the second pasteurizer was also run at 3 psi for 10 sec. Two sets of paper were found to have colored areas. On one set an intense blue spot in a well colored 1 inch × 10 inch area on one sheet marked the location of a hole (Fig. 1). The hole was confirmed by the penetrant dye and developer test. The other sheet of the set had a corresponding but lighter colored area. Apparently some ammonia had

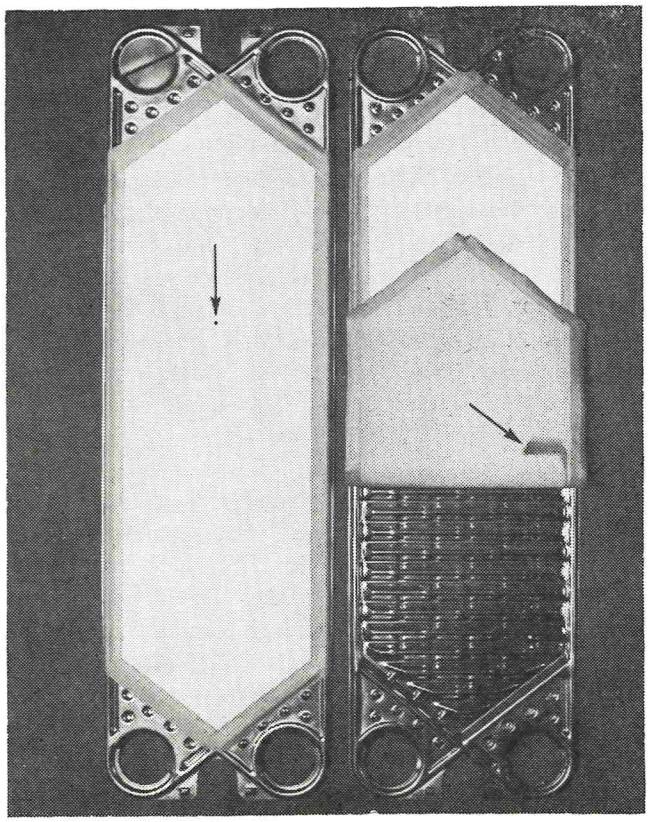

Figure 2. Diazo paper taped over the active heat transfer area of single plates. The arrow on the left paper indicates the hole punched for applying vacuum. On the right the paper has been rolled back to show the colored area caused by the ammonia that came through the hole in the plate.

permeated through both sheets of paper and reacted with the diazo coating on the second sheet. The two plates corresponding to the other set of papers were removed from the press, critically examined, and tested by the method described later for individual plates. No holes were found in either of these plates. It is not certain how this set of diazo papers was exposed to ammonia. The gasket sealing the space in which this set was located was loose on the plate and in poor condition. There was outward leakage of ammonia past the gaskets of several plates. Some of this ammonia may have gotten back in during the test or it may have been held between the main gasket and the port gasket and gotten in when the press was opened. The only inconvenience of this false indication was the necessity of checking two plates individually.

After the plate with the hole was replaced, a fluorocarbon test on the regeneration section indicated that there were no more leaks.

Individual plate test

After tests on the first pasteurizer, the value of an easy method for testing individual plates was apparent. In the laboratory several methods were tried on the defective plate from that pasteurizer. The following is the best of these methods; it was used to test some individual plates from the second pasteurizer.

A sheet of diazo paper was cut 1/8 inch to 1/4 inch smaller on all sides than the active heat transfer area of the plate to be tested and sealed to one side of the plate with masking tape with the active face inward (Fig. 2). Cutting the paper undersize was found to improve the chances of a good seal by the masking tape. A small hole was punched in the paper with a pencil point. About 20 ml of 28-30% ammonium hydroxide was poured on another plate in a band 1 or 2 inches in from the edges. The plate to be tested, with the paper upward, was placed on the plate with the ammonium hydroxide. Vacuum was applied to the small hole in the paper by means of the vacuum cleaner and hose from the fluorocarbon testing kit. The seal of the masking tape was checked for tightness and vacuum was applied for another minute. If there was a hole in the plate, ammonia gas would be drawn through into the space between the plate and the paper. The paper was removed and examined for colored areas indicating holes. On some tests there was blue color along the edge of the sheet from ammonia entering under the masking tape where there were wrinkles in the tape or paper. If the paper was removed with care and was not colored it could be used for tests on other plates. The ammonium hydroxide on the bottom plate lost ammonia rapidly and was replenished after 10 min.

DEFECTIVE PLATES

Positive fluorocarbon tests have been obtained on one or more units of all major equipment brands. Some of the units had regasketed plates obtained on an exchange program in which used plates regasketed by a supplier are sent to the pasteurizing company, which then returns its worn plates including those with worn gaskets. The acceptable ones of these plates are regasketed and sent to the next egg pasteurizing company that requests that type of plate. It is uncertain how many times plates are regasketed but eventually some may develop holes through the heat exchange surface. Some, especially those with very small holes, may not have been eliminated by the supplier who did the regasketing.

The two pasteurizers on which the leak location work was done happened to be of the same brand and used the same size plates. They were the first units of this brand that were found with a hole in the plates. The first pasteurizer was 6 years old with 21 plates of 316 stainless steel in the regeneration section. They had not been involved in any exchange program when new gaskets were needed. All of the plates appeared to be in good condition with only a small amount of wear at the points where the plates contacted each other. The hole that was found was not at a contact point. It showed near the bottom gasket as a very small corroded area on one side and pinholed through to the other side where it was almost invisible. It was too small for any significant quantity of egg product to have flowed through. The plant had only recently come under Federal inspection. No previous fluorocarbon test had been run so it is unknown how long the hole existed. The plant has had an excellent record of producing Salmonella-free products with low total counts since it came under Federal inspection. The defective plate was replaced with a new 316 stainless steel plate.

The second pasteurizer was 5 years old with 37 plates in the regeneration section that were originally of 316 stainless steel. Shortly before the test was done the section had been restreamed by the addition of several used plates from another pasteurizer, and some regasketing had been done that may have resulted in exchange of plates. The plate with the hole was found to be made of 304 stainless steel and had the word "loan" written on with an etching tool. The hole was at a contact point and other points showed appreciable wear. The history of this plate is quite uncertain. It was replaced with a used plate.

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STERILIZED DAIRY AND ANALOGUE PRODUCTS[1]

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ABSTRACT

The paper discusses changes taking place in processing, packaging, and distribution of sterilized dairy products. The origins and purposes of present governmental controls are reviewed in the light of these changes. The impact of substitutes for dairy products is discussed, and the need for a new approach to regulating dairy products in a manner which would reflect the changes taking place in the industry is stressed.

In the beginning there was raw milk and cream
and people died from disease borne by dairy products
And along came pasteurization
and the children stayed healthy.
But the mothers complained because the cream soured;
and they turned to false Gods.
And along came sterilized dairy products
and the mothers were happy again.
But still there was no peace in the land,
for people could not agree
upon what was meant by "sterilized."
And so it came to pass that people were confused
and flocked in greater numbers to worship the substitutes.
And their children never knew
the goodness of cream.

Let us hope that a statement like this will not find its way into the time capsule for future anthropologists to ponder which dairy products may have been meant. That we are in a state of confusion in the dairy industry with regard to sterilized dairy products is probably an understatement.

ITEM

The same, identical 30% butterfat product, marketed in different parts of the U. S. has to be labeled as follows to comply with State regulations:

1. Sterilized Whipping Cream with Stabilizer and Emulsifier
2. Sterilized Medium Whipping Cream with Stabilizers and Emulsifiers
3. Sterilized Cream Whipping
4. Sterilized Cream for Whipping
5. Sterilized Cream for Whipping with Stabilizer and Emulsifier
6. Sterilized Medium Cream for Whipping with Stabilizers and Emulsifiers
7. Grade A Sterilized Whipping Cream with Stabilizers and Emulsifiers
8. Grade A Sterilized Cream for Whipping
9. Grade A Pasteurized Whipping Cream with Stabilizers and Emulsifiers
10. Grade A Pasteurized Medium Whipping Cream with Stabilizers and Emulsifiers

This is just applicable to 30% cream. Of course some of these labels still have to meet higher butterfat standards.

ITEM

In order to sell this one product nationally, the manufacturing plant has to be under the direct inspection and supervision of:

1. The Federal Food and Drug Administration
2. The U. S. Public Health Service
3. The State in which the plant is located
4. At Least 5 States plus the District of Columbia
5. At least one major municipality

ITEM

Product going into some States has to be manufactured on production lines reserved exclusively for Grade A dairy products.

ITEM

In order to sell such a product, licenses are required from most States and numerous municipalities.

Now let's contrast this to Cool Whip, a pre-whipped frozen substitute whipping cream.

- Same label can be used in every State of the Union
- The manufacturer is responsible only for "good manufacturing practices" under FDA regulations.
- He is only under the inspection of the Federal FDA and that of his State.
- He is free from any licensing restrictions.
- The product can be manufactured on any type of equipment the manufacturer chooses.

Furthermore, it can be formulated in any way desired as long as the ingredients are approved by the FDA for use in foods. In this way the manufacturer is able to design a product which matches exactly consumer preferences.

Contrast this with the handicaps encountered by the dairy technologist who merely wants to update products by improving them functionally so that the housewife is satisfied.

- The law tells him how much butterfat he has to use!
- The state law tells him the quantity of stabilizers and emulsifiers he may use!
- The law prevents him from adding fats which may be either of nutritional or functional benefit!
- The law tells him what process to use and what

[1]Presented at the 57th Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, Inc., Cedar Rapids, Iowa, August 17-20, 1970.

kind of equipment he needs!

Small wonder, then, that today's technologists like to work with synthetics instead of basic dairy products. Let me give you three more examples:

1. Under today's market conditions, and those often found in the kitchen, it is desirable to freeze foods, especially luxury items. Studies have shown that freezability is one of the desirable features of whipping cream analogues. Now, it would be relatively easy to build some freeze resistence into whipping cream, but it is illegal to do so.
2. The dairy industry is trying to find new markets for butterfat. There is a surplus of butterfat and hence a crying need for innovations. But innovation is hampered by regulations. Let me explain: Butterfat is a mixed glyceride composed of a variety of fatty acids. Some of these fatty acids are saturated, some unsaturated, and some even polyunsaturated. At various times of the year and, depending on the feed, there are variances in the composition of the fat. Winter butter is harder and sometimes nearly white, and summer butter is softer and yellow. So butter is far from being a uniform compound. By physical separation, i.e. by melting, fractional crystallization, followed by centrifugal separation of the higher melting fraction, we can make a soft spreading butter which is also lower in saturated fats than ordinary butter. Only we cannot call it butter. The other fraction, the harder fats, are ideally suited for the baking industry where it is desirable for making Danish butter pastry. But that fraction also cannot be called butter either. So the pastry cannot legally be called "all butter pastry." In other words, even though butter is not a uniform compound, the industry is unable to adjust its composition despite great demand for the modified products. While we are stockpiling butter, the vegetable fat industry is tailormaking its products for the consumer and industrial markets, and it is taking an ever increasing share of the total market for fats.
3. The best known example, though, I saved for last. It is, of course, the Filled Milk Act. Technologists are going to great lengths, and expense, to simulate through chemistry the totality of functions and nutritional attributes naturally inherent in non-fat milk solids. Great quantities of casein or sodium caseinate are brought into this country to provide some of these functions. Non-fat milk solids could easily provide some of these functions, and probably at a lower price. Even though repeal of the Filled Milk Act would undoubtedly increase the price for solids because of the laws of supply and demand, I am sure that the food industry would find it advantageous to switch to filled products. I must stress, though, that repeal of the Act alone would not suffice without accompanying legislation which would allow the use of any approved additives similar to what is now permissable with dairy analogues.

How did we get into this dilemma, and more importantly, how can we get out of it? I believe it is important to examine the original rationale behind the legislation which has become so restrictive. It began around the turn of the centry with studies of how disease may be spread through milk. By 1924 the Public Health Service developed a model regulation for voluntary adoption. The individual States also adopted dairy legislation, some patterning themselves after the Pasteurized Milk Ordinance, others going their own way, and a similar development took place at the local level.

The step from safeguarding the public's health to setting up trade barriers is small, often only one of interpretation. However, discrimination must have taken place to warrant the following statement in the Introduction to the 1965 Revision of the Ordinance:

> "The model ordinance discourages the use of public health regulations to establish unwarranted trade barriers against the acceptance of high quality milk from other milk sheds."

An integral part of the Grade "A" program from its very inception has been the upgrading of sanitation at the farm level. This was necessary because the final product quality reaching the consumer is much dependent on what quality raw product reaches the processing plant. At the farm level, this meant investment of capital. To compensate the farmer for his extra outlays, Grade "A" milk was sold at a premium. The problem with this system was that gradually the upgraded product became the standard.

According to USDA, 27 States have now attained a *one* grade status, namely Grade "A". We have reached a point where all milk in these States is produced and processed under Grade "A" conditions. And of course this milk commands a Grade "A" price, which was the incentive for the farmer to upgrade his operation in the first place. This is where he began to make the transition from public health to economics.

As long as this farm milk is bottled and sold as fluid milk, there is little argument as to what price the farmer should be paid. He upgraded his operation to give the consumer the best milk possible. Therefore, the consumer should pay a fair price so that farmer, processor, and distributor can survive. And as long as there is no lower priced alternative for milk, consumers will continue to buy fresh milk at almost any reasonable price.

But what happens when for reasons of oversupply, more Grade "A" milk is produced by farmers than consumers want to buy? We then fractionate the milk into cream and skim milk and at this point economics and public health get all mixed up. Often skim milk or cream are converted to cultured dairy products which, because of their acidity present an entirely different picture from the standpoint of public health than does fluid milk. Cream goes into half and half, table cream, and whipping creams, all of which are non-essentials in consumer's eyes as

compared to fresh milk. And non-essentials are not purchased regularly as is milk, so a slower turnover at the store level makes it mandatory to provide longer shelf life for these products. In other words, ordinary pasteurization just is not enough for these specialty dairy products! Not because the public's health is endangered, but because 1 to 2 week's shelf life is insufficient, in most instances, to assure that housewives always receive sweet and wholesome products.

What is needed, therefore, is additional heat treatment beyond pasteurization which would assure total destruction of all viable organisms—"Sterilized" products, in other words. This means additional investment for equipment by the processor. Normally such added cost is passed on to the consumer. However, with non-essentials, where substitutes are available to consumers and where dietary causes prompt many to cut back on the intake of high fat products, any increase in the sales price usually results in reduced purchases. So, like it or not, the prices received by farmers for his milk must reflect its ultimate usage, irrespective of the grade.

This thinking is not really new, since the Milk Marketing Orders have used the concept of "Classes of Utilization" for a long time. What is new is the recognition by some farm groups that many specialty dairy products, heretofore classed as "fluid milk products" should be priced as Class II utilization irrespective of grade.

This is a big step forward, but only one of many needed. More importantly, there must be an unshackling of industry to allow it to develop products which can compete. I would like to quote from a speech recently given by the Assistant Secretary of Agriculture, Mr. Richard E. Lyng before the International Association of Milk Control Agencies.

> "Many of our food laws are duplicative and inconsistent. They create unnecessary costs by local, State, and Federal agencies. Milk controls were among our earliest food laws, and they were originally set up to protect local citizens from milk produced at nearby dairies. Much has changed, and is changing, in the movement of dairy products. As these changes take place, we must be increasingly alert to the need for eliminating duplication, inconsistencies, and unnecessary costs in regulation and control operations."

With this in mind, I would like to offer some suggestions for your consideration:

1. Let us recognize the intrinsic difference between pasteurized milk and all dairy products derived from it.
2. Let us understand that today's consumer does not concern himself with the why's, but the how's. Not why a product costs more, but how much more it costs. Not why a dairy product does not whip, freeze, or curdle, but how easy the substitutes can be used.
3. "Truth in labeling" is a household term these days. To consumer this is equivalent to "understandable labeling." When the same product has to be labeled ten different ways to comply with local regulations the whole industry gets a black eye.
4. Most of all we have to understand that most dairy products today have to travel through long distribution channels which put them into Interstate Commerce. As the Secretary of Agriculture pointed out, regulations and control should reflect this. They do not, at present.

I hope that these remarks are not interpreted to mean the abolition of controls. What I am asking for is an updating of these controls and regulations. Like the external skelton of many animals which has to be shed to allow for growth and the development of a new and larger skelton, new legislation has to be developed to accomodate growth. To carry the analogy further, the animal is vulnerable to predators during this time of adjustment and some do not survive, but without it the species would surely die.

For decades now milk has ceased to be a public health hazard, thanks to the effective work of the dairy and food sanitarians. What we need today is a market orientation at the regulatory level. Our primary concern should be whether present regulations, other than those directly concerned with health, hinder or help the sale of dairy products. I am speaking of product standards, labeling, and pricing.

- Standards which allow approved optional ingredients to make products fit consumer needs.
- Labeling which is informative while at the same time putting the product in the best light. For instance requiring identification of additives on the front panel confuses consumers and detracts from the product image.
- Pricing which is competitive.

This kind of orientation, together with a full understanding that we are all a part of *one* national dairy industry would put us into the race for the consumer's dollar, using a Mustang instead of an Edsel.

MECHANISM AND DETECTION OF MICROBIAL SPOILAGE IN MEATS AT LOW TEMPERATURES: A STATUS REPORT[1, 2]

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ABSTRACT

After 80 years of research, we do not yet know the precise mechanisms by which meats, poultry, and seafoods undergo microbial spoilage at low temperatures. While over 40 different methods have been proposed for detecting incipient spoilage during this time, the lack of general agreement on the validity of any of these methods reflects in large part our lack of agreement on a precise definition of microbial spoilage. Several of the more recently proposed methods for detecting spoilage are discussed in terms of their contribution to our knowledge of spoilage mechanisms along with a brief review of historical beginnings.

It is well established that fresh meats undergo microbial spoilage unless steps are taken to prevent this action. While such meats incubated at room temperatures will spoil faster than those held at refrigerator temperatures between 5-7 C, spoilage of meats at low-temperatures occurs but at a much slower rate. For the past 10 years, we have been attempting to understand the precise mechanisms by which meats such as ground beef undergo microbial spoilage at refrigerator temperatures. While our efforts have been aimed primarily at fresh beef, we have also looked at poultry and shrimp.

Our findings to date may be summarized as follows. *(a)* The heterogenous flora of fresh ground beef becomes quite homogeneous when the meat is allowed to undergo spoilage at between 5-7 C. This fact was established years ago by numerous investigators *(1, 3, 16)*. *(b)* Beef allowed to undergo spoilage at these temperatures contains essentially the same quantity of protein as fresh beef, that is, spoilage is not accompanied by any significant protein breakdown or proteolysis. *(c)* The most striking change that occurs when fresh beef undergoes microbial spoilage at low temperatures is an increase in the hydration capacity of its proteins. *(d)* The spoilage of poultry and shrimp occurs essentially in the same manner as fresh beef with the principal difference being a faster rate in the latter products.

[1]Contribution no. 295 from the Department of Biology, College of Liberal Arts.

[2]Presented in part at the 71st Annual Meeting of the American Society for Microbiology, Minneapolis, Minn., May 2-7, 1971.

MICROBIAL ECOLOGY OF MEAT SPOILAGE

Microbial spoilage of meats is perhaps best viewed from the standpoint of microbial ecology. The microflora of such products seeks to perpetuate itself and during this process, conversion of organic matter to inorganic is effected. When one examines the ecologic parameters of microbial growth in or on refrigerated meats, the temperature of incubation is found to be the most important factor which controls growth and growth activity. When microorganisms grow on substrates consisting of simple and complex nutrient constituents, the simple constituents are attacked preferential to the more complex ones. With fresh beef, the soluble nonprotein-N constituents such as free amino acids, nucleotides, etc., are utilized preferential to beef proteins (Table 1). The generally low carbohydrate content of beef (1% or less) places spoilage organisms in the position of utilizing free amino acids in part as sources of energy. Production of ammonia, hydrogen sulfide, indole, skatole, and amines by spoilage organisms in meats is the result of amino acid utilization per the following:

FREE AMINO ACIDS $\longrightarrow$ H_2S, NH_3, INDOLE, ETC.

TABLE 1. GENERAL CHEMICAL COMPOSITION OF FRESH GROUND BEEF, AND THE EFFECT OF THE LOW-TEMPERATURE SPOILAGE FLORA ON CONSTITUENTS

General chem. comp. (Lawrie, 18)		Spoilage flora effects
Water	75.5%	
Protein	18.0%	
Myofibrillar	7.5%	Not utilized; hydrated
Sarcoplasmic	6.0%	Some utilized
Mitochondrial, etc.	2.0%	Not utilized
Fat	3.0%	Slowly oxidized
Soluble non-prot. subs.	3.5%	
Nitrogenous	1.57%	Utilized readily
Carbohydrate	1.18%	Utilized readily
Inorganic	0.63%	Utilized
Others	0.10%	Some utilized

With the exhaustion of the amino acid supply, nonprotein-N compounds such as nucleotides *(15)* and low molecular weight proteins such as those of the sarcoplasm *(9, 14)* are apparently next attacked by the spoilage flora. The structural proteins of beef

remain essentially intact for several weeks after incipient spoilage is noted in refrigerated ground beef *(7, 31)*. There is neither a loss of trichloroacetic acid-precipitable protein during this time (Table 2), nor is there a reduction in the antigenicity of these proteins *(20)*.

Refrigerator temperatures exert themselves most significantly in preventing growth of all but a few genera of organisms normally found in fresh meats (Table 3). Unless meats have been treated with antibiotics or other inhibitors, bacteria invariably outgrow any molds and yeasts that may be present.

While some strains of the genera *Pseudomonas* and *Achromobacter* that grow at refrigerator temperatures are often able to degrade gelatin, these organisms generally lack the ability to degrade natural proteins such as casein, purified myofibrillar proteins, etc. Not only has this been found for beef proteins but also for seafoods *(19)* as well as for 0.6 *M* KCl extracts of porcine muscle *(2)*.

In addition to the parameters of incubation temperature and nutrient content, the generally low pH of fresh meats prevents growth of some and slows the growth rate of most all spoilage bacteria. In spite of the generally low Eh potential of meats, aerobic and facultatively anaerobic organisms invariably dominate the spoilage flora. The faster growth rate of bacteria establishes these organisms as the dominant part of the flora at the expense of yeasts and molds except in certain special instances where the growth of bacteria is not favored.

Table 2. Effect of the low-temperature spoilage flora on the protein content of beef-round muscles

Days held	ERV	pH	% Total N	% Total Prot.
0	49	5.75	3.37	21.06
3	40	6.30	3.48	21.75
5	41	6.10	3.25	20.31
7[a]	28	7.00	—	—
10	8	7.40	3.44	21.50
18	0	8.10	3.51	21.94

[a]First sign of detectable spoilage.

Table 3. The principal genera of microorganisms in fresh and spoiled ground beef

Fresh		Spoiled
Pseudomonas	*Thamnidium*	Largely -
Achromobacter	*Mucor*	*Pseudomonas*
Proteus	*Rhizopus*	*Achromobacter*
Micrococcus	*Cladosporium*	*Acinetobacter*
Flavobacterium	*Penicillium*	Occasionally -
Alcaligenes	*Sporotrichum*	*Streptococcus*
Streptococcus		*Alcaligenes*
Lactobacillus	*Candida*	*Aeromonas*
Bacillus	*Torulopsis*	*Proteus*
Sarcina	*Rhodotorula*	
Escherichia		
Klebsiella		
Microbacterium		
Aeromonas		
Acinetobacter		

Spoilage Mechanisms

Early studies on the mechanism by which meats undergo microbial spoilage were all carried out at temperatures above the refrigerator range. One of the first reports of this type is that of Mace (*circa* 1900, cited by Tissier and Martelly) who observed that meat spoils in essentially two phases—a flat odor produced by aerobic sporeformers in the first phase followed by gram-negative bacteria and a clearly putrid odor during the second phase. Tissier and Martelly *(29)* confirmed the work of Mace in 1902. The role of bacteria in meat spoilage was emphasized later by Ottolenghi *(21)*, Weinzirl *(32)*, and others. While Eber was apparently the first to propose ammonia detection as a means of assessing the spoilage of meats in 1892 *(30)*, chemical changes that occur when meats undergo microbial spoilage were apparently not studied further until the work of Ottolenghi in 1913 *(21)*, Falk et al. in 1919 *(4)*, Tillmans et al. *(28)*, Schmidt *(23)*, and others. By 1940, over 30 methods for detecting meat spoilage had been described most all of which provided some clues as to spoilage mechanisms.

While the work of Kirsch et al. *(16)* showed that low temperature-spoiled beef contained only a small number of bacteria in contrast to fresh beef, the actual role played by these organisms in bringing about spoilage is not yet fully understood. Until about 10 years ago, the view was widespread that meat spoilage bacteria proceeded immediately to break down meat proteins to free amino acids. This view, in great part, was based on the fact that most spoilage bacteria can degrade nutrient gelatin; but it is a very poor substrate on which to determine the proteolytic capacities of any organism.

From research carried out in this laboratory, the most dramatic change that takes place when fresh beef undergoes spoilage at low temperatures is the increase in hydration capacity. This phenomenon was first revealed by use of the extract-release volume (ERV) phenomenon *(11, 12)*. By this technique, fresh beef, pork, poultry, and certain seafoods release large volumes of aqueous extract (possess low hydration capacities) while little or no extract is released after frank microbial spoilage has set in (increased hydration). The change in hydration capacity is somewhat linear from freshness to spoilage, thus allowing for a cut-off point between freshness and spoilage depending upon how the latter is defined.

TABLE 4. SUMMARY COMPARISON OF FRESH AND LOW-TEMPERATURE SPOILED GROUND BEEF

Property	Fresh	Spoiled
1. pH	5.6-5.8	Above 8.0
2. Odor	Fresh	Unpleasant
3. Tactile response	Nontacky; wet	Tacky
4. State of hydration	Low	High
5. No. orgs./g	Generally low	High, ca. 10^8-10^9
6. General type of flora	Mixed; many types	Homogeneous; few types
7. Level of free amino acids	Generally high	Low
8. Level of nucleotides	Generally high	Low
9. Level of amino sugar complexes	Generally low	Considerably higher
10. State of proteins	Low degree of hydration	Highly hydrated

While they do not respond in the same way, the phenomena of water-holding capacity (WHC) employing a modified filter paper press method *(13)*, meat swelling, and viscosity of meat homogenates all reflect changes in hydration capacity as meats undergo spoilage *(24, 27)*.

While the precise mechanism by which spoiling meat proteins become hydrated is not yet fully understood, the following represents what appears to be at least part of the mechanism: *(a)* increased pH caused by microbial production of alkalizing substances; *(b)* microbial alterations of metal ion balance on the surfaces of protein molecules; *(c)* loosening of native protein structure by spoilage flora and/or tissue cathepsins resulting in the release of more water-binding sites; and *(d)* production of amino-sugar complexes by the spoilage flora.

With respect to pH, its increase normally accompanies low temperature meat spoilage. Its increase is known to cause increases in water-holding capacity of fresh meats even in the absence of microbial activity. The spoilage flora is known to alter the metal ion balance which exists in and on skeletal muscles. By adding an excess of Ca^{++} or Mg^{++} to fresh beef, WHC can be shown to increase sharply. In the absence of complete proteolysis during spoilage, there is apparently a loosening of native protein structures by microbial actions and/or beef cathepsins resulting in the freeing of water-binding sites. Weak proteolytic activity has been shown to bring about an increase in muscle hydration *(11)* while complete protein breakdown would result in completely soluble products.

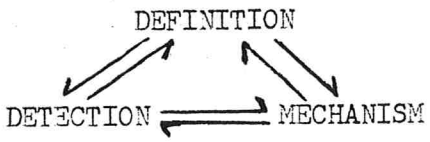
The fourth possible mechanism listed above consists of the production of amino-sugar complexes by the spoilage flora. We have shown that amino-sugars such as glucosamine will cause an increase in WHC of fresh beef without changes in pH. This has been shown for both hexosamines and pentosamines. We have also shown that amino sugar complexes increase in spoiling meats in approximately the same proportion as numbers of spoilage organisms when meats are allowed to undergo spoilage in the conventional manner *(25)* It seems reasonable that a state of hydration of skeletal muscle proteins is necessary before microorganisms can degrade these complex molecules. While experimental proof is yet lacking, it is possible that amino-sugar complex formation is favored by release of ammonia by the spoilage flora during its utilization of free amino acids as sources of energy. This might, indeed, represent one of the primary functions of amino-sugar formation under conditions such as those that exist when meats undergo low-temperature spoilage. Glucosamine or amino sugars are not produced by the flora which develops on fresh meats which are wrapped in gas-impermeable plastic bags and incubated at around 5 C *(25)*. This attests to the importance of growth of the normal spoilage flora in the production of amino-sugar complexes and further renders total count data more meaningless as a reflection of the state of spoilage.

Table 4 presents a summary of most of the changes known to occur when fresh beef undergoes spoilage at low temperatures. While low-temperature spoiled or putrid beef is low in nucleotides and free amino acids, it has large numbers of gram-negative bacteria which in itself makes the product undesirable for human consumption. In terms of the structural proteins, however, spoiled or putrid beef is not unlike fresh beef in containing high levels of these components. They differ from those in fresh beef by their state of increased hydration.

Since it is extremely difficult, if not impossible, to produce in fresh, sterile meats all of the features characteristic of that which spoils normally by a mixed flora, it seems highly desirable that attempts be made to elucidate the role played by each member of the spoilage flora alone and in association with other spoilers. Achievement of this goal will aid immensely in solving the riddle of how meats undergo microbial spoilage.

DETECTION AND MEASUREMENT

Our lack of general agreement upon any single method to detect or measure meat spoilage largely results from our lack of agreement on a definition of spoilage. While any potential method must be able to respond to and reflect human quality attributes, the method should also be based on a change or changes in meat quality attributes so it allows for flexibility in terms of personal tastes. It is well known that while a given sample of meat may be considered spoiled and inedible by one person, the same sample may be considered desirable by yet another person. This reflects, among other things, our lack of precision as to what constitutes spoiled meat. Also, this may be the main reason why we, first, have and continue to have described so many different techniques of detecting spoilage, and second, why we are unable to find general agreement on the useful value of any of the more than 40 methods described over the past 80 years. Further, there are certain obvious relationships between meat spoilage and how this spoilage is defined and detected or measured. These may be depicted in a cyclical menner per the following in order to bring out how dependent one factor is upon another:



A sound definition of meat spoilage seems vital to the proper elucidation of mechanisms and development of methods of detection.

While spoilage detection methods should more properly follow from our elucidation of the meat spoilage process, methods for detecting and measuring spoilage in meats date back at least 80 years in spite of the fact that we do not yet know the precise mechanisms involved. The early investigators were concerned with both the problems of sanitary quality and degree of freshness or state of spoilage. The use of total bacterial numbers and indicator organisms in meats as indices of sanitary quality is well established. Use of total numbers as well as fractional counts of aerobes and anaerobes as a means of determining the degree of spoilage of meats, however, is of questionable value. With respect to specific spoilage phenomena such as off-odor and slime development on meat surfaces, total numbers have been shown to be of value *(1)*.

In regard to use of methods that measure breakdown products of bacterial action such as hydrogen sulfide, ammonia, etc., these methods tend to be undesirable because of their general dependence on the presence and activity of organisms capable of producing the desired compound or reaction. The general variability that exists between the flora and composition of meats from different carcasses and between portions from the same carcass tend to render somewhat meaningless any chemical method that measures the presence and concentration of only one substance. The pros and cons of some metabolic by-products as indicators of meat quality were recently reviewed by Fields et al. *(6)*. Methods such as pH measurement tend to reflect what is already obvious and generally do not allow for projections in terms of storage life. While pH changes are partly reflected in the titrimetric method of assessing microbial spoilage, this method was found recently to be of promise for beef although previous workers found it to be rather unsatisfactory for certain seafoods *(26)*.

With fish and seafoods, the reduction of trimethylamine-oxide (TMAO) to trimethylamine (TMA) has received a lot of attention as a means of estimating sea-fish freshness. By this method, the fish flora produces enzymes which effect the reduction of TMAO to TMA and the speed and quantity of change reflects the general bacterial load of the product. What complicates this method, however, is the fact that not only do certain pelagic fish such as tuna contain muscle enzymes able to reduce TMAO to TMA, but the gut portion of some fish species contain enzymes that will also reduce TMAO. The volatile reducing substances (VRS) method of Lang et al. *(17)* appears to be of value for seafoods perhaps because several compounds are detected and measured rather than only one. The problem of freshness tests for seafoods has been reviewed more completely by Farber *(5)*, for seafoods and meats by Pearson *(22)* and Herbert et al. *(8)*, and for meats by Turner *(30)* and Ingram and Dainty *(10)*.

The use of ERV as a means of detecting spoilage in red meats and poultry was reported from this laboratory in 1964 and for shrimp in 1971 *(27)*. Measurement of WHC as a means to determine microbial quality was described in 1965 *(13)*, whereas meat swelling and viscosity were described in 1969 *(24)*. As stated above, all of these methods measure changes in the hydration capacity of meat muscles as they undergo microbial spoilage. The changes are not known to be dependent on presence and activity of any one given organism nor on the response of only one muscle protein. As previously stated, they all reflect on the apparent mechanism by which microorganisms bring about meat spoilage at low temperatures, but whether the phenomenon that is measured is the appropriate undesirable change that occurs when fresh meats become spoiled is not clear at this time. The most urgent problem that confronts us in

this whole area is the one of identifying what it is that we seek to avoid in refusing to accept spoiled meats as desirable products.

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RECRUITING PERSONNEL FOR A DYNAMIC INDUSTRY IN THE SOARING SEVENTIES[1]

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Abstract

The dairy industry and regulatory agencies are suffering from a severe manpower shortage. Recent surveys show that enrollment in agricultural colleges is up, but that the dairy science and regulatory-oriented curricula are not drawing their share of the new student crop.

Suggestions for rectifying these manpower shortages include: changing the image of agriculture in general; involving regulatory and dairy industry people in a one to one career guidance situation with potential recruits; increasing incomes and inducements for dairy- and regulatory-related professions; and establishing innovative training programs and curricula to interest a wider segment of our young people in dairy foods processing and regulatory professions.

"After 5 years of hard work at the Massachusetts Institute of Technology, David Ernst, 26, will get his Ph.D. in August and emerge as one of the best trained young physicists in America. Unfortunately, that may not be enough to assure him job security in his field. When Ernst recently sought a post at Ohio's Heidelberg College, which was looking for a physics teacher to enlarge its 4-man department, he might have expected little trouble in landing it. But this year, despite his impeccable credentials, Heidelberg turned him down. There were 361 inquiries for the job" *(2)*.

The *Time* article goes on to mention that the entire country has only 3000 good academic research or teaching posts in physics. Meantime, graduate schools are turning out 300 new Ph.D.'s in physics each year. And still students swarm to get training in this field. MIT's Physics Professor Lee Gradzins tells new students that high-energy physics is "very exciting, but don't attempt to make a living at it."

Contrast this job situation with the one that exists in agriculture. According to a fairly recent report *(1)*, there are two jobs waiting for every graduate of an agricultural college. There will be even more openings in business to process food for the growing population and to provide supplies, credit, and management services for farmers. Many job-placement directors and hosts of dairy processors state that there now are at least 3 jobs available for every graduate specializing in the dairy processing field. A similar manpower shortage exists in the regulatory agencies.

[1]Presented at the 57th Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, Inc., Cedar Rapids, Iowa, August 17-20, 1970.

Changed Image

The question begs — why aren't the dairy-food science departments getting their fair share of increasing numbers of agriculture college enrollees?

Like it or not, the term dairy science has less than a desirable image in the minds of potential recruits. Unfortunately, the hayseed farmer caricature is brought to mind to "sophisticated" high-schoolers and collegians when the word "dairy" is mentioned. One student, when writing an essay ("Changing the Image of Agriculture") for a General Agriculture course at the University of Kentucky stated, "For the last hundred years, the image of the farmer in the minds of most Americans has been typified by the painting, 'American Gothic.' This painting portrays the farmer as a man in bib-overalls, a calico shirt, a wornout straw hat, and holding a pitchfork. His wife is portrayed as a thin, gaunt woman, old before her time from hard work and too many children. This picture also leaves the impression that the farmer and his family are out of touch with everything."

This erroneous image stems from the fact that our industry and some University Dairy-Food Science Departments haven't communicated effectively. Very few vocation seekers realize that dairy science is more than farms and ruminants, but that it includes quality control, plant management, and sales activities, to name a few. For too long, we've defaulted to the public and let them think in terms of the cow culture. Granted, catering to the cow may be essential for obtaining students which are interested in the production phases of dairying, but the bovine-based appeal can be detrimental when attempting to motivate "urbanites" to seek a career in the dairy foods processing industry.

The sanitarian's image is not much better. Regulatory personnel have been termed "panhandlers" and "privy sniffers" by ignorant segments of the general populace.

Regretfully, we have done precious little towards correcting the erroneous image surrounding dairy and regulatory vocations, and have failed to communicate the true facts about these challenging vocations to the general public. Information which should be in the hands of the average citizen ends up in production, technology, and regulatory journals, but it

goes no farther than these internal sources. Most of the information on agriculture and related vocations issued by the universities, which could change ag's image for the better, ends up on the "farm page" of the newspapers. The "farm page" gets extremely limited readership.

It is suggested that dairy foods plant executives and regulatory personnel make themselves available to give informative talks at high school career days and civic club meetings, stressing the fact that dairy and regulatory vocations require competency in business, chemistry, bacteriology, and engineering backgrounds. These representatives would do well to establish close friendships with high school guidance counselors. A broadened and better image of our industry could conceivably result, which in turn, might help alleviate our manpower shortage.

Industry leaders have also suggested that the International Association of Milk, Food, and Environmental Sanitarians establish an "action" committee for public relations purposes, that it designate representatives to work with state high school counselor associations, and that it publish some career information bulletins dealing with sanitary engineering and dairy and food science for distribution to counselors and libraries. Contrary to what most believe, guidance counselors are earnestly seeking for more information about agriculture, dairy science, and environmental sanitation careers.

Changing our image will be most helpful in alleviating the manpower shortage. But, that's not enough.

Increased Involvement

The most effective recruiting is done on a one to one basis. If every person affiliated with the dairy industry and regulatory agencies became genuinely and personally involved with a potential prospect for these fields of endeavor, and effectively and challengingly presented our case, our manpower shortage could be extensively rectified. A campus religious group called the "Navigators" has as its theme, the ministry of multiplication, or each one teach one. They are doing an effective job in reaching collegians for Christ. However, solving the manpower crisis will also require more cash.

Increased Incomes and Inducements

Today's ag college graduate with a B.S. degree can expect to be hired at an initial salary of $8200 per year. The inflationary trends are pushing the wage scales up at a spiraling rate. It is readily admitted that some of these graduates may not be worth the $8200. But those that are will apply themselves and will expect to be rewarded at a level commensurate with their output. Needless to say, many of those top producing ag graduates soon fall into listlessness because they aren't rewarded monetarily for superior accomplishments. Their incentives are "killed" because they are underpaid.

The dairy industry and regulatory agencies have not distinguished themselves as pace-setters in adequate monetary rewards for superior accomplishment and in initial inducements for hiring purposes.

Take health departments for instance. Low salaries are identified by 90% of state and local health departments as interfering with recruitment according to Walker *(3)*. The author says there is a direct correlation between the percentage of vacancies and the relative salary of the positions compared to income in the professions outside the health department. In other words, where health agency's salaries are comparable to those for similar employment in other agencies or to income from private practice, there is little difficulty in keeping positions filled. When there is a salary deficiency, there are continuing vacancies.

Other inducements for feeding our industry and health agencies "top young blood" are also needed. The lack of opportunity for upward mobility remains a stumbling block for many public health professionals and dairy industry personnel. For example, studies have shown that potential candidates for employment in community health programs soon lose interest in a system which limits the opportunity for promotion to the top. The opportunity to advance, to conduct research, to participate in shaping of programs, to tackle difficult problems are necessary ingredients for job satisfaction.

The same situation applies to the dairy processing industry. Trained individuals should be given jobs which are challenging. Requiring a dairy food scientist to work on a bottle washer for two years to "learn the business" is like asking fully equipped skin divers to pull bath tub plugs.

Ironically, even some of the well-paid, challenged employees depart the dairy foods industry because of the existing manpower shortage itself. They grew old before their time because they were overworked since there was a lack of qualified help. How many of our young, innovative people are going to stay at a position which requires 16 hr a day, on-the-job participation? This is not an exception. Many are promised co-workers who can share the responsibilty, but never get them. Disenchantment with the job and the dairy industry inevitably results.

Other situations have caused downright bitterness towards our industry. Processing plants have been shut down with two weeks' notice to good employees

and field personnel who have put years into the company. No job alternatives are offered and bitter men trained in dairying carry their grudge into the labor market. Such practices should cease.

Then, too, there are narrow-minded plant managers and regulatory directors who won't send their employees to any educational events. They won't allow their personnel to intermingle and exchange ideas with "competitors" for fear some of their company or agency secrets will be given away or their employees might be "stolen." Monastic thinking along these lines is detrimental to the entire dairy and regulatory fields and inhibits our recruiting efforts. On the other hand, some industry and regulatory personnel fail to apply the excellent information presented at educational conferences and meetings. To some, conference time is vacation time—educational activities take second place to entertainment. Small wonder that some of the dairy companies and regulatory agencies are cutting down on their travel expenses for employees who wish to attend educational meetings.

A better image, increased incomes and inducements, and greater involvement will certainly help us in our recruiting activities. So will innovation.

Innovation

New methods for training sanitarians need to be developed. Means of getting people interested in public health must be initiated. Walker *(3)* suggests some positive approaches which the health departments can take:

(*a*) Provide public health summer employment for health profession students. Cleveland is presently giving this experience to 10 such students. New York has given experience for 16 years to a total of over 400 "health-oriented" students. Nine per cent have chosen careers in public health.

(*b*) Training programs designed to provide the transition from basic professional education to public health and sanitation specialist will help solve the manpower shortage in the public health field.

(*c*) Conduct continuous in-service training of subprofessionals (sanitarian aides with at least a high school education), and encourage them to get college level training with tuition paid by the health department.

The universities are changing their dairy curricula to meet changing times. They also are innovating. The day of university department mergers is with us. Dairy sections are being absorbed into food and animal sciences programs. People whose training has been strictly in dairy products are becoming fewer and fewer, but food technologists are on the increase.

Industry—university cooperative programs are being expanded. Relevant environmental health programs are being initiated. There is a need for more.

All of us can be innovators. The only restriction or limitation on what can be done to bring in more manpower is our imagination.

The manpower crisis in the dairy industry and regulatory agencies can and must be corrected. A changed image, greater involvement, more income and inducements, and innovations would be steps in the right direction.

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UNIVERSITY OF MARYLAND 1972-'73 DAIRY CONFERENCE AND SHORT COURSES

November 15, 1972—University of Maryland 28th Annual Dairy Technology Conference, Adult Education Center, College Park, Maryland.

February 5 - February 14, 1973—University of Maryland 23rd Annual Ice Cream Short Course, Department of Dairy Science, Center of Animal Sciences, College Park, Maryland.

February 15, 1973—University of Maryland 23rd Annual Ice Cream Conference, Adult Education Center, College Park, Maryland.

March 14, 1973—University of Maryland Annual Cultured Products Symposium, Adult Education Center, College Park, Maryland.

May 15, 1973—University of Maryland Soft Serve Ice Cream Conference, Adult Education Center, College Park, Maryland.

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THE MILK INDUSTRY IN MEXICO[1]

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ABSTRACT

General information pertaining to the milk industry in Mexico and its economic importance in relation to national economy is presented. In addition, a study of milk marketing and analysis of the principle existing problems for the increase of milk production are discussed, along with the solutions believed to be most adequate. Among the selected solutions the most favored the author found to be: control of the importation of dried skimmed milk, revision of the present milk code, attractive prices to producers, lowering cost of production, public promotion of milk and dairy products for increased consumption, and reorganization of the dairy industry to incorporate the most advanced technology emphasizing lower production cost and increased returns for the dairymen.

The dairy industry arrived in Mexico from Europe along with the first five cows brought from Spain by the conqueror, Hernán Cortés, when he landed in Veracruz in 1519. From then on, this type of livestock has proliferated to the extent that in 1969 there were 24,876,236 head of cattle in Mexico.

It is estimated that of this total 4,960,000 are dairy cows (exclusively females over 3 years old) of which only 744,000 are special dairy breeds (85-90% are Holstein Friesian while the remainder are Swiss, Jersey, Guernsey, etc.). It is interesting to note, that of this quantity 15 to 20% are mechanically milked.

There are two classes of milk consumed in Mexico. These are cow's milk and goat's milk. The former accounts for 94% of the total milk consumption, whereas the latter only 6%.

According to data revealed by the National Census, the value of milk production in the country was approximately 356 million dollars in 1960. This places the industry in the most important position in the rural economy ahead of corn production which had a value of 327 million dollars, meat (including beef, goat, and mutton) with a value of 231 million dollars and wheat with a value of 80 million dollars.

The Gross National Product (GNP) was 36,450 million dollars in 1969[2]. The livestock industry accounted for 4.9% of this total of which 35.9% was from milk production. From the above figures we can deduce that milk production accounted for 1.76% of the GNP.

It is estimated that by 1969 the production of cow's milk reached 1,216 million gallons (4,562 million liters) while that of goat's milk was 56 million gallons (210 million liters). Since the milk industry's share of the GNP was 1.76%, the industry is one of the most important in Mexico. Nevertheless, because of the industry's dispersal throughout the country, there is little incentive given on the part of the authorities for promotion of the industry.

MARKETING

It is estimated that the 1969 production was distributed through the channels listed in Table 1.

MILK PRODUCTION

There is a large shortage of milk in Mexico. We should reflect for a moment on these questions: Why, in a country which has always supplied its internal demand for milk, is there an increasing shortage which obliges the government to import increasing quantities of non-fat dry milk and butterfat? Are the efforts of the cattle raisers sufficient to permit the industry to keep pace with the country's demographic and economic growth? Has the interest of potential investors been sufficiently aroused to promote investment in the dairy industry?

Unfortunately, the answers to the above questions and to many others which could be posed in this respect are all negative. The forecast made by Mexican and foreign technicians who have studied the situation is that in the next few years there will be chronic shortages of milk which will cause a further increase in imports. The efforts of persons who have traditionally been in charge of the industry are not enough. There are no new important investment flows being channeled into this line of activity.

We may ask, then, what is occurring in the dairy industry? In our estimation, incentives, understanding, and equitable treatment for the milk producer are lacking. Improving the herds, business consolidation, and production of a better hygienic quality of milk do not suffice. The industry's costs continue to rise and it must subsidize other important industrial sectors. The agricultural and transport industries, packaging, concentrated feeds, and all the other elements which play a role in milk production are all being affected by a constant rise in costs. Protec-

[1]Presented at the 58th Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, Inc., San Diego, California, August 16-19, 1971.
[2]Banco Nacional de Mexico estimates

tionist measures affect all these industries preventing cattlemen from importing equipment and machinery which would help detain this trend of rising costs at prices below those on the internal market.

The above-mentioned factors have made it necessary for the Government (under the auspices of the CONASUPO) to import considerable amounts of non-fat dry milk to supply the more needy economic classes with products such as filled milk. Many industries also require powdered milk which, in many instances, cannot be substituted by fluid milk.

These imports, which are increasingly on the rise, reflect two things:

(a) On the one hand, the increase in consumption is, in part, the result of the stratospheric population increase (3.6%). However, this increase is not in proportion to the increase in per capita consumption, which is known to be quite low. In Mexico City, where per capita consumption is the highest in the country, there was a daily per capita consumption of 236 ml of natural milk and 79 ml of filled milk made with imported dry milk, giving a total of 315 ml daily per capita consumption.

The availibility of milk per inhabitant, per year, is on the decline in Mexico according to statistics from the FAO.

With this we agree, but according to that international organization milk availability stands at 156 g per day, per capita. This is much less than the actual production of milk and according to our consumption figures, in 1969 availability was 290 g per day, per capita.

(b) Domestic milk production is in relative decline as well as the breeding of dairy cattle. According to statistics from the 1930 national census, there were 218 cows for every 1,000 inhabitants while in 1960 there were only 198. This represents a 10% drop. It is quite probable that the 1970 census will reveal even more astonishing figures since the rural population is known to be decreasing. In 1930, 66% of the population was rural while in 1960 the rural population accounted for only 49% of the total. It is expected that the 1970 census shows that only 39.5% of the population is rural.

WHAT ARE THE STEPS TO BE TAKEN TO END THE CHRONIC DEFICIT IN MILK PRODUCTION AND HOW CAN PRODUCTION BE INCREASED?

(a) Improve the quality of the livestock.

(b) Improve the feeding techniques of the cattle and protect them against plagues and diseases.

(c) Promote an increase in agricultural and livestock productivity. Nevertheless, this has its limits because of the prices which these products command on the market which results in a relatively low economic utilization capacity of capital. Agricultural and livestock productivity in Mexico have not reached their maximum potential. Furthermore Mexico is far from fulfilling its potential or even nearing the superior levels of efficiency attained in some of the more highly developed countries.

(d) Foment and promote production on the *ejidos* (small properties given to farmers under the agrarian reform, usually with very low productivity), large extensions of property and small properties, stimulating the development of the new milk producing areas.

(e) Promote the establishment of receiving stations or farm cooperatives or mixed enterprises composed of farmers in combination with private investors whereby the producer could deliver his milk directly without having to go through middlemen and for which he would receive a guaranteed price which would never be below the minimum cost of his product.

(f) Set up new industrial centers for milk processing.

(g) Prices should be in accordance with costs and there should be no more than a fair percentage of profit. These prices should have a sliding scale to make the necessary adjustments to compensate for changes in costs since the idea is not to take advantage of changes in a speculative way, but rather to keep pace with the growth of the national economy.

It is necessary to remove, on a nation-wide basis, all price controls on milk so that according to supply and demand, the market will find its own level. If aid and support are given to milk producers, production will rise to such an extent that the price will drop and eventually find its natural market level.

It is often argued that raising the price of milk will reduce its availability to the majority of consumers. Actually when it can be clearly seen that salary increases do not proportionately increase the demand for milk, this situation indicates that there is a definite relationship between salary increases and increases in the money spent by the public on superfluous items.

Everyone agrees that we have reached a point in our economic growth from which we cannot advance unless we raise the level of the peasant and small farmer by taking measures to permit them to attain a greater purchasing power. The simplest way to do this is by livestock raising which not only helps

TABLE 1. TOTAL PRODUCTION AND AMOUNTS DELIVERED FOR PROCESSING INTO DAIRY PRODUCTS.

ESTIMATED PRODUCTION OF MILK IN MEXICO IN 1969	4772[a]	4562.6 (Cow's milk)	CONSUMPTION AS FLUID MILK 3698.3(77.5%*)	PASTEURIZED	922.9(19.33)
				RAW	277.4(58.16)
		210 (Goat's milk)	LARGE COMPANIES		369.6(8.16)
			Evaporated milk, whole	146.9(3.07)	
			Dried milk, whole	97.2(2.04)	
			Dietetic milks[b]	75.1(1.57)	
			Condensed milk	52.3(1.09)	
			Dried milk, skim	18.1(0.38)	
			INDUSTRIAL MANUFACTURING 1073.7(22.5%)		
			OTHER INDUSTRIES		684.1(14.33)
			Cheese	445.9(9.36)	
			Butter	139.7(2.93)	
			Cream	70.1(1.46)	
			Other products[c]	27.4(0.58)	

[a]All figures represent millions of liters.
[b]Figure estimated taking as average 70% milk content.
[c]Burnt milk confections, cottage cheese, yogurt, and ice-cream.
*The figures in parenthesis represent the percentage of the total production.

to tie the farmer to the land and thereby avoid the *bracero* (migrant farm labor) system, but also gives him a higher income than that obtained from many traditional crops.

In the states where the ecology permits it, there is no doubt that the quickest way for the small farmer to obtain immediate income is through the breeding of dairy cattle. Since this type of farmer is not usually prepared for the care of this special type of livestock, the private sector should provide its collaboration and cooperation in giving technical assistance to the small farmer.

It is urgent to create the necessary social and economic climate in Mexico so that the country can meet its internal demand for milk and end imports which bleed the nation and impoverish the rural classes.

The increase in domestic milk production should go hand in hand with a gradual prohibition of imports of powdered milk. This measure would constitute an authentic incentive to the domestic cattle industry.

As pointed out before, a policy giving incentives to cattle raising, or to the rural producers who form the backbone of the nation, will stimulate these producers and will lead to self-sufficiency in the supply of domestic needs through domestic production.

The prices should be remunerative, and to achieve this, cattle-raisers should join together to work more efficiently and try to reduce production, processing, and distribution costs.

One of the solutions to these problems which will be put into practice shortly is the measure taken by the Asociacion de Ganaderos Productores de Leche Pura, S.A. de C.V. This organization has joined together to process and distribute the milk its members produce through a well-managed firm which uses up-to-date techniques from the technological and commercial points of view.

It is considered that this type of modern enterprise is necessary to keep down costs and to present to the public a uniform product of the highest quality.

This association is installing a central processing plant on the outskirts of Mexico City which would have the following main objectives:

(a) Pasteurize members' milk in volume to keep down processing costs.

(b) Increase the daily production of each member in the immediate future by eliminating many of the problems with which he is presently faced such as planting crops, breeding cattle, milking cows, and processing and distributing milk.

(c) When the dairyman is concerned only with producing milk and no longer, as at present, has to worry about the complex problems of processing and marketing (problems, with which in most instances he is not familiar), he can then concentrate all his efforts on milk production.

(d) Ultimately establish as a by-product of the organization units aimed at the industrialization of the milk. These units would work at preparing balanced rations for cattle, med-

icines, etc., as well as dealing directly with sales representatives of farm implements, tractors, trucks, tires, etc., in favor of the members, as well as setting up a centralized distribution system for the products sold by the organization.

(e) After consultation with the relevant authorities, set up an intensive consumer education program to diffuse the norms of better nutrition through hygienic products.

(c) Bring into the group other dairymen in the Mexico City area or from other milk-producing areas who produce, or have the capacity for producing, milk, meeting the hygienic standards set up by the Ministry of Public Health and by the organization itself, no matter what volume they produce.

The advantages of this plan are the following: *(a)* Absolute security, proven by the competent authorities and the consumers, that the organization's milk will always be of the highest quality. *(b)* More and better health education for dairymen as well as for consumers. *(c)* More and less complicated facilities to enable health effective control at a lower cost. *(d)* As previously mentioned, a modern economic organization based on rationality and productivity, can act in such a way as to avoid rising costs by buying in volume, reducing costs on certain items as may be revealed by productivity studies, etc.

HEALTH REGULATIONS

The regulations concerning milk and dairy products presently in force in the country are obsolete. They are out of line with the real situation in the rural areas as well as in the industry itself. Most of these regulations are bad copies of foreign industry codes.

Were this antiquated piece of legislation faithfully adhered to, it would be totally inapplicable for 100% of the country's producers of milk and other dairy products.

It is an urgent matter to adequately regulate the dairy industry so as to build it up on a sound basis. However, it should be feasible to comply with the reglations of a new code so as not to place producers on a permanent basis, in a shaky legal position.

THE DEMAND

Another highly important problem is the necessity to create a greater demand through health education campaigns jointly sponsored by the producers and health authorities. It is argued that the needy classes cannot afford the price of milk. However, it is these same needy classes which consume a higher percentage of soft drinks than any sector in the world. Likewise, although there exists chronic malnutrition, a great part of the meager salaries of these groups of people goes toward buying beer and other alcoholic beverages produced by companies, which have at their disposal, fabulous sums of capital to saturate the advertising media with publicity aimed at creating a necessity for these products in the mind of the consumer.

DIMENSIONS OF INSANITARY CONDITIONS IN THE FOOD MANUFACTURING INDUSTRY FOOD AND DRUG ADMINISTRATION DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

This is the title of a Digest published by the Comptroller General's Office, giving an excellent review of the General Accounting Office's (GAO) report to Congress on the insanitary conditions found in a sampling of food manufacturing and processing plants selected at random in six FDA districts including 21 states.

This Digest ought to be considered required reading by managements of food plants and food warehouses all over the country to give them a better understanding of what to expect in the future by way of increased inspectional activity of Food & Drug.

This publication can be obtained free by writing the American Sanitation Institute, P. O. Box 9502, St. Louis, Mo. 63161.

WHY THE REVIEW WAS MADE

The Food and Drug Administration (FDA) is required, by law, to provide assurance that food products shipped across State borders—which includes most of the foods purchased by the American people—are processed under sanitary conditions and are safe, pure, and wholesome to eat.

(Continued on Page 481)

BETTER UTILIZATION OF FISHERY PRODUCTS THROUGH IMPROVED AND NEW HANDLING AND PROCESSING CONCEPTS[1]

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ABSTRACT

Some of the recent developments of our laboratory include: *(a)* CO_2 in refrigerated seawater (RSW) as a preservative; *(b)* separation of crabmeat from shell by centrifuge; and *(c)* separation of meat from skin and bones by machine.

(a) Addition of CO_2 to the conventional RSW system, which is used aboard the vessel and at processing plants ashore, results in a system with the advantage of inhibition of microbial growth when compared to the original system. The CO_2 in RSW system has been shown to be effective in maintaining the quality of salmon, rockfish, halibut, and shrimp, at higher levels than the conventional RSW system or ice.

(b) Through use of a method involving centrifugation, it has been demonstrated that up to 15-20% of crab waste can be recovered as crabmeat. Separation of crabmeat from shell by a machine specifically designed for this purpose is being evaluated currently.

(c) By machine separation, it has been shown that yields of edible flesh up to 49% and 46% can be achieved for Pacific hake and silvergray rockfish, respectively. Of the many possible uses of machine-separated flesh, our laboratory has demonstrated that this raw material can be used to prepare products such as spreads and modified protein and protein isolates.

Commercial fishing in the United States is still largely a "hunting" activity. Thus, there is only limited control over, for example, the timing and the volume of a fish catch and there is no control over the genetic or environmental factors that might affect the quality of the product. In the future, there will most probably be a gradual change from the present type of fishery to aquaculture—an activity in which many of the present uncontrolled factors will be under some greater level of control.

In the meantime, until there is a significant aquaculture industry, there are many innovations which can be made in our present commercial fishery practices that will result in improved utilization of fishery products. For example, more effective coolants would be of value in handling a "glut" of fish or shellfish aboard the vessel; mechanical handling of the product at the processing plant would be a great aid in maintaining processing schedules during periods when large volumes of product are being landed; since we cannot control the types of fish which are available, we must seek the best ways to utilize the less desirable species in an efficient manner.

In consideration of the above, the following are a number of the recent developments of our laboratory: *(a)* CO_2 in refrigerated seawater (RSW) as a preservative; *(b)* separation of crabmeat from shell by centrifuge; and *(c)* separation of meat from skin and bones by machine and the demonstration of new uses of the minced fish flesh.

CARBON DIOXIDE IN RSW AS A PRESERVATIVE

For a number of years, RSW has been used as a medium for chilling whole fish, both aboard the fishing vessel and ashore. Although the RSW cooling system offers the advantages of *(a)* rapid cooling, *(b)* adaptability to materials handling techniques, and *(c)* holding temperatures as low as 29 F, there is a gradual increase in the bacterial population in the coolant. The effectiveness of gaseous CO_2 in controlling the growth of microflora on meat products led us to test the potential usefulness of CO_2 in RSW as a preservative.

In a typical test of this cooling system, whole fish were placed in a tank in a 1:1 ratio by weight of fish to 3% brine. The contents of the tank were maintained at approximately 30 F and CO_2 was continuously metered into the brine. Rockfish and chum salmon were held in this system and compared to similar samples held in RSW without CO_2. It was shown that storage of these species in brine treated with CO_2 increased their storage life by at least 1 week. The CO_2 inhibited bacterial growth and retarded the rate at which the fish decreased in quality *(1)*.

Pacific pink shrimp is an abundant species that is commercially fished from California to Alaska. These shrimp are normally iced aboard the fishing vessel and transported to the processing plant. Since shrimp are susceptible to bacterial spoilage, pink shrimp cannot be safely kept in ice for more than 3 or 4 days. Because of the storage problems aboard the vessel and the great potential of this fishery, tests were carried out to determine the effectiveness

[1]Presented at the 58th Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, San Diego, California, August 15-19, 1971.

of CO_2 in RSW as a coolant for shrimp *(3)*.

In these tests, shrimp of known history (in terms of quality) were loaded at either a 1:1 or 1:2 (shrimp:brine weight) ratio into a tank of 3.2% brine that had been precooled to 31 F and had been saturated with CO_2. Results of these tests showed that shrimp stored in RSW modified with CO_2 maintained their color, developed a desirable chewy texture, and had a good flavor. Bacterial counts remained at the initial level throughout the storage period. The color stability of the shrimp held in this system apparently results from inactivity of the polyphenoloxidase enzymes. Oxygen, which is required in the reaction catalyzed by these enzymes, is probably excluded by the CO_2. Our tests indicate that the use of CO_2 in the RSW system aboard the fishing vessel can increase the keeping time of shrimp roughly from 2-3 days to 6-7 days.

Separation of Crabmeat from Shell by Centrifuge

Both the commercial picking of cooked Dungeness crab and the flotation procedure for separating picked crabmeat from shell and tendon are hand operations. Although numerous attempts have been made to develop mechanical crabmeat extractors or "shakers" that would eliminate much of the hand labor, such devices have not come into general use. The mechanical removal of crabmeat from the crab offers great potential benefits in terms of the production of a uniform, high quality product.

This is a report of some tests made to evaluate the use of a centrifuge for mechanizing some of the Dungeness crab processing operations *(4)*. These tests were made on *(a)* commercially picked crabmeat (which still contained shell fragments and tendon), *(b)* crab shell scrap (the shell remaining after the removal of meat by hand), and *(c)* chopped crab body and leg sections using an industrial, solid-bowl centrifuge which was designed to separate continuously heavy particulate matter from a slurry.

(a) The results of this test indicated that pieces of shell and tendon can be removed rapidly and efficiently from hand-picked crabmeat. Little or no meat was carried over with the shell or tendon, and aside from the pieces of tendon that were attached to meat, no shell or tendon remained in the meat.

(b) Dungeness crab shell scrap (chopped into pieces ranging in size from about 1/8 to 1/4 inch and fed into the centrifuge in saturated brine) was separated into meat-free shell and shell-free meat. With three different lots of scrap used, the meat recovered ranged from 14% to 20% of the weight of the scrap. This represents approximately 15% of the weight of the meat removed by hand picking. This meat obtained from the shell scrap was of small particle size, darker in color, and flakier than pieces of muscle from which shell and tendon were removed by centrifugation. The meat separated from shell scrap could be valuable alone, but it appeared that its greatest value was as an ingredient for blending with larger, more fibrous pieces of meat for use in manufactured products such as crab cakes.

(c) In separate experiments, Dungeness crab body and leg sections, blue crab claws, and snow crab legs and bodies were chopped and then fed into the centrifuge in a saturated brine slurry. In all instances the meat was separated from shell and tendon and the shell was free from meat. Yields from blue crab claws, snow crab bodies, and snow crab legs were 31%, 52%, and 29%, respectively.

Since completion of this work, a centrifuge has been developed by the Bird Machine Company[1] for the specific purpose of separating crabmeat from shell and tendon. Initial trials with this machine are now being made in crab processing plants.

Separation of Meat from Skin and Bones by Machine and the Demonstration of Uses of the Minced Fish Flesh

Meeting the expanding demand for fishery products will require utilization of presently non-commercial species and industrial species as sources of food. Use of these resources for food is dependent, among other things, on the economical recovery of edible flesh from these fish. Also, utilization of these resources will require development of foods that are new and unique in appearance and texture.

Reported here are the results of a study made with a laboratory model Japanese fish deboner. The effectiveness of this machine was demonstrated for separation of flesh from skin and bones of some species of fish which are currently underutilized. Because the mechanically removed flesh is minced as a result of passing through the 1/8-inch orifices of the separator, some end uses have been demonstrated for this flesh product.

Flesh separator results

Essentially, flesh is removed from headed-and-gutted fish as they pass between a continuous rubber belt and a stainless steel perforated drum. The pressure, applied by the belt on the fish, forces the flesh through the perforations of the drum while the skin and bones pass to the "waste" discharge chute.

Yields, based on whole fish, for northern anchovy, English sole, Pacific hake (Puget Sound), Pacific herring, lingcod, silvergray rockfish, starry flounder,

[1]Trade names referred to in this publication do not imply endorsement of commercial products.

and Pacific cod were 61.6%, 60.2%, 49.0%, 66.6%, 47.0%, 46.5%, 42.9%, and 37.8%, respectively. In comparison, the yield of flesh using conventional filleting techniques ranged from 25% to 30% *(2)*.

The potential of the flesh-separating machine for increasing yield, lowering costs, and mechanizing the whole process of flesh separation is high. This development can lead into new concepts on the total utilization of our fishing resource.

Potential uses of minced fish flesh

Our broad interest is to demonstrate that minced flesh which is produced by machine separation can be utilized efficiently in a number of food products.

Spreads. The spread is an example of the use of minced flesh in a snack or specialty product. Essentially, this spread is made up of the whole edible flesh plus flavors and colors. The minced flesh is processed mechanically and enzymically to give the physical form which results in textural smoothness and spreadability. This material, which is neutral white in appearance and relatively bland in flavor, may be readily modified by incorporating ingredients such as olives, caraway, pimiento, pepperoni, etc., to give a broad line of products for the retail and/or institutional trade.

Tests have shown that the heat-pasteurized spread has a storage life in sealed containers of 2 months at 33 F and, after opening, a storage life of about 3 weeks at 35-40 F (M. Patashnik, *personal communication*).

Modified protein. The usefulness of a protein for incorporation into a food is largely determined by its functional properties. Controlled alteration of functional properties can be accomplished by enzymic or chemical modification of the protein. In our work, we have demonstrated that a chemically modified protein (which has interesting properties) can be produced from minced flesh. Essentially, the preparation of this modified protein involves removal of water-soluble protein from the minced flesh; salt solubilization of the myofibrillar protein; and subsequent chemical reaction of this protein with succinic anhydride. The succinylated protein resulting from this reaction has the following gross properties: *(a)* water solubility in the pH range 5.5-11; *(b)* not heat-coagulable by heating at neutral pH, *(c)* dried material readily rehydrates, and *(d)* it forms a gel at a concentration of 1-2%.

This succinylated myofibrillar protein is but one example of the apparently many possible new products that can be made through the modification of protein from fish.

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DIMENSIONS OF INSANITARY CONDITIONS

(Continued from Page 478)

The General Accounting Office (GAO) wanted to know whether FDA was able to provide this assurance.

FDA describes the food industry in the United States as comprising some 60,000 establishments whose output results in about $110 *billion* in purchases by consumers each year.

FDA's inventory of establishments subject to inspection includes about 32,000 food manufacturing and processing plants. FDA inspects such plants to determine whether their products meet requirements of the Food, Drug, and Cosmetic Act (FD & C Act). FDA's inventory includes also about 28,000 establishments of other types, such as storage facilities and repacking and relabeling plants. It excludes restaurants, retail stores, and meat and poultry slaughtering and processing plants.

To assess sanitary conditions in the food manufacturing industry, GAO requested FDA to inspect 97 food manufacturing and processing plants selected at random from about 4,550 food manufacturing and processing plants in six FDA districts including 21 States. (See pp. 19 and 20).

GAO auditors accompanied FDA inspectors on their inspections of 95 of the plants.

The 97 plants had annual sales of about $443 million. They manufactured or processed bakery products, candy, fish, flour, carbonated beverages, cheese, ice cream, fruits, vegetables, popcorn, chips, sugar, jams and jellies, macaroni, pizzas, spices, etc.

This report has two basic purposes: (1) to show the dimensions of insanitary conditions in the food manufacturing industry and (2) to suggest ways to improve the FDA's management of the program which is intended to ensure compliance by the industry with standards of sanitation required by the FD&C Act. Conditions believed to exist in the industry have been projected through the use of statistical sampling techniques. Therefore it would not be

(Continued on Page 488)

BEHAVIOR OF *SALMONELLA TYPHIMURIUM* IN SKIMMILK DURING FERMENTATION BY LACTIC ACID BACTERIA[1]

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Abstract

Skimmilk was inoculated with *Salmonella typhimurium* (approximately 10^3/ml) and with *Streptococcus cremoris*, *Streptococcus lactis*, mixtures of *S. cremoris* and *S. lactis*, *Streptococcus diacetilactis*, *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, mixtures of *S. thermophilus* and *L. bulgaricus*, a mixture of *L. helveticus* and *S. thermophilus*, and *Leuconostoc citrovorum*. Inocula of lactic acid bacteria ranged from 0.25 to 5.0% and incubation temperatures from 21 to 42 C. *Streptococcus cremoris*, *S. lactis*, and mixtures of the two repressed growth but did not inactivate *S. typhimurium* during 18 hr of incubation at 21 or 30 C when the lactic inoculum was 0.25%. An increase in inoculum to 1% resulted in inactivation of *S. typhimurium* at 30 C by some of the mixed cultures. Both *S. diacetilactis* and *L. citrovorum* were less inhibitory to *S. typhimurium* than were *S. cremoris* or *S. lactis*. When added at the 1% level, *Streptococcus thermophilus* was more detrimental to *S. typhimurium* at 42 C than was *L. bulgaricus*. Mixtures of these two lactic acid bacteria, when added at levels of 1.0 and 5.0%, caused virtually complete inactivation of *S. typhimurium* during the interval between 8 and 18 hr of incubation at 42 C.

Lactic acid bacteria are widely used to produce a variety of cultured dairy foods with desirable flavor, texture, or other characteristics. An additional benefit which may result from use of these bacteria, is the inhibition and/or inactivation of certain microorganisms able to cause food-borne illness.

Recently Goepfert et al. *(7)*, Park et al. *(17)*, and Hargrove et al. *(9)* demonstrated that salmonellae could grow in milk and cheese curd during the early stages of the fermentation involved in Cheddar cheese manufacture and that the salmonellae survived for some time in the finished cheese. Additionally, Park et al. *(17)* reported that replacement of the normal lactic culture with one which produced acid slowly resulted in enhanced growth of *Salmonella typhimurium* during cheesemaking and in extended survival of the bacterium in cheese during ripening. Hargrove et al. *(9)* attributed survival of salmonellae in Cheddar cheese to several factors including the amount and kind of starter used. Behavior of salmonellae in some other fermented dairy foods has been discussed by Marth *(13)*.

That lactic acid bacteria produce acid and possibly other substances which can influence growth and/or survival of other bacteria is well recognized *(2, 4, 8, 12, 14, 18, 24, 26)*. Some investigators have reported that lactic acid bacteria are detrimental to salmonellae. Vedamuthu et al. *(25)* found that *Streptococcus diacetilactis* inhibited growth of *Salmonella typhi* on agar plates and Sorrells and Speck *(21)* observed inhibition of several salmonellae by culture filtrates of *Leuconostoc citrovorum*. Inhibition of salmonellae by *Lactobacillus acidophilus (8)*, *Lactobacillus bulgaricus (3, 20)*, and *L. bulgaricus* plus *Streptococcus thermophilus (23)* also was reported.

Although several investigators have indicated that lactic acid bacteria are detrimental to salmonellae, many of the investigations were conducted using artificial media or conditions substantially different from those which prevail in natural milk fermentations. Hence, a series of experiments were conducted to determine the behavior of *S. typhimurium* in skimmilk which was inoculated with those lactic acid bacteria commonly used to manufacture several different cultured dairy foods. Furthermore, sizes of inocula and temperatures of incubation were those that might be employed in commercial practices. Results of the experiments are described in this paper.

Materials and Methods

Cultures

Cultures used in these tests, their abbreviations, and their sources are listed in Table 1. The strain of *L. citrovorum* was chosen because it produced a substantial amount of biacetyl and related compounds in acidified milk as evidenced by results of the creatine test *(6)*.

Preparation of cultured skimmilks

Prescription bottles (8-oz, screw-cap) were filled with 156 ml of skimmilk and then were autoclaved for 15 min at 121 C. Milks then were held overnight at 21, 30, or 42 C. Tempered milks were inoculated with 0.25, 1.0, or 5.0% of 24-hr old active milk cultures of lactic acid bacteria. Sufficient of a 24-hr old broth culture of *S. typhimurium* was added to provide approximately 10^3 salmonellae per milliliter of skimmilk. Inoculated skimmilks were then incubated for 18 hr at 21, 30, or 42 C. Samples were taken at 2- or 3-hr intervals and were tested for numbers of salmonellae and lactic acid bacteria and for pH. Experiments were done in duplicate and results reported are average values.

Tests were also conducted with acidified milk cultures of *L. citrovorum*. In this instance, skimmilk at 30 C was

[1]Research supported by the College of Agricultural and Life Sciences of the University of Wisconsin, Madison.

TABLE 1. CULTURES USED, THEIR ABBREVIATION, AND THEIR SOURCE.

Culture	Abbreviation	Source
L. bulgaricus	LB5	Marschall Div., Miles Lab.
L. bulgaricus	LB7	Marschall Div., Miles Lab.
L. citrovorum	20	Dept. of Food Technol., Iowa State U.
S. cremoris	C1	Dept. of Food Science, U. of Wis.
S. cremoris	US3	Dept. of Food Science, U. of Wis.
S. diacetilactis	34	Dept. of Animal Science, Purdue U.
S. lactis	C6	Dept. of Food Science, U. of Wis.
S. lactis	C10	Dept. of Food Science, U. of Wis.
S. thermophilus	ST[a]	Marschall Div., Miles Lab.
S. thermophilus	ST4[b]	Marschall Div., Miles Lab.
S. typhimurium	—	Dept. of Bacteriology, U. of Wis.
L. helveticus + *S. thermophilus*	LBST4[b]	Marschall Div., Miles Lab.
S. cremoris + *S. lactis*	MQ3[c]	Marschall Div., Miles Lab.
S. cremoris + *S. lactis*	VT3[c]	Marschall Div., Miles Lab.
S. cremoris + *S. lactis*	R1[d]	Marschall Div., Miles Lab.
S. cremoris + *S. lactis*	LD[d]	Marschall Div., Miles Lab.

[a]suggested for Swiss cheese manufacture
[b]Suggested for yogurt manufacture
[c]Suggested for Cheddar cheese manufacture
[d]Suggested for cottage cheese manufacture

inoculated with 0.25% of a 24-hr old culture of *L. citrovorum* and with enough of a 24-hr old culture of *S. typhimurium* to provide approximately 10^3 salmonellae per milliliter. The skimmilk thus inoculated was incubated for 15 hr at 30 C after which the pH was adjusted to 4.5, 5.0, and 5.5 with sterile 50% citric acid. The acidified cultures were held at 30 C for 50 hr and were sampled at 10-hr intervals during this time. These experiments also were done in duplicate and reported results are average values.

Enumeration of salmonellae and lactic acid bacteria

Plate count procedures as described below and 11-ml samples were used to enumerate salmonellae and lactic acid bacteria. Plate count agar (Difco) and incubation at 42 C for 48 hr served to enumerate salmonellae except when lactobacilli, *S. thermophilus*, or *L. citrovorum* were studied since these lactic acid bacteria also formed colonies at 42 C. In those instances, brilliant green agar (Difco) and incubation at 42 C for 48 hr were used to enumerate salmonellae.

When coagulated milks were tested for salmonellae, enough sterile 40% NaOH was added aseptically to the sterile buffered water blank before use so the final pH value of the sample-diluent mixture approximated 7.0. Preliminary trials indicated that recovery of salmonellae was markedly improved when the acid of the cultured milks was neutralized in the initial water blank.

Numbers of lactobacilli and of *S. thermophilus* were determined with APT agar (Difco) and incubation at 42 C for 48 hr. Plate count agar and incubation at 30 C served to enumerate the other lactic acid bacteria except *L. citrovorum* which required 120 hr of incubation before colonies were readily visible. Numbers obtained by these tests represented the total of both salmonellae and lactic acid bacteria. Hence, the numbers of salmonellae were subtracted from this "total count" to give the number of lactic acid bacteria.

Measurement of pH

The pH of skimmilks was measured with a Beckman pH meter equipped with a glass electrode.

RESULTS AND DISCUSSION

Streptococcus cremoris and Streptococcus lactis

Two cultures each of *S. cremoris* and *S. lactis* and four commercial cultures made up of these two organ-

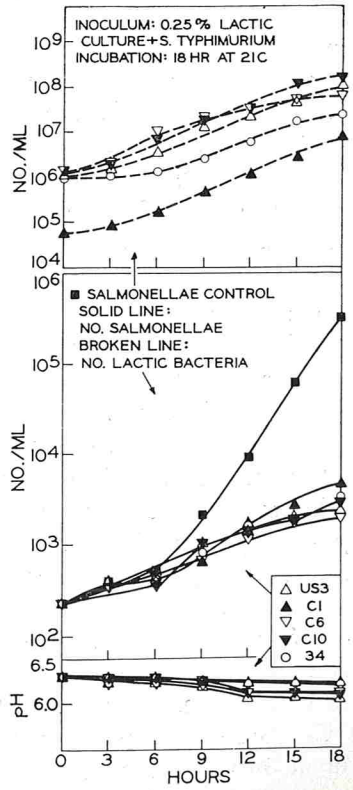

Figure 1. Changes in pH, in numbers of *Salmonella typhimurium*, and in numbers of *Streptococcus cremoris* (US3, C1), *Streptococcus lactis* (C6, C10), and *Streptococcus diacetilactis* (34) during incubation of skimmilk at 21 C.

isms were tested for their effect on growth of S. *typhimurium* in skimmilk. When pure cultures were tested, an inoculum of 0.25% and incubation temperatures of 21 C (Fig. 1) and of 30 C (Fig. 2) were used. Two levels (0.25 and 1.0%) of inoculum and incubation at 21 or 30 C were used in studies with mixed cultures (Fig. 3, 4, 5). When pure cultures of S. *cremoris* (C1 and US3) and S. *lactis* (C6 and C10) were incubated at 21 C (Fig. 1), all the cultures markedly repressed growth of S. *typhimurium*. The salmonellae increased in number by more than three logs when grown alone but only by one log or less when grown together with these lactic streptococci. This repression does not appear to be attributable to acid production alone since the decrease in pH during incubation was minimal because of the low inoculum and low incubation temperature. There was no evidence during the incubation that growth of salmonellae was stopped or that the organisms were being inactivated.

Incubation at 30 C instead of 21 C yielded the data shown in Fig. 2. In this instance, growth of S. *typhimurium* not only was repressed but three (2 S. *lactis* and 1 S. *cremoris*) of the lactic cultures did not permit growth of the *Salmonella* after 9 hr of incubation. Stabilization of the *Salmonella* population coincided with a marked drop in pH of the cultured milks. A lower inoculum of S. *cremoris* C1 and slower acid production by this culture resulted in less repression of the *Salmonella* during the 18-hr incubation period. The *Salmonella* grew more rapidly during the early hours of incubation at 30 C (Fig. 2) than at 21 C (Fig. 1) and hence somewhat greater populations were attained at 30 C than at 21 C before the lactic acid bacteria exerted their inhibitory influence.

Results obtained with commercial cultures that were mixtures of S. *lactis* and S. *cremoris* are recorded in Fig. 3, 4, and 5. With an inoculum of 0.25% lactic culture and incubation at 21 C, growth of S. *typhimurium* was less than one log in magnitude during the 18-hr incubation. Repression of the *Salmonella* by the mixed lactic culture at 21 C was somewhat greater than by the pure cultures at the same temperature (Fig. 1). Increasing the temperature to 30 C (Fig. 4) resulted in more rapid growth of the *Salmonella* during the first 9 hr of incubation and hence there were more salmonellae in the cultured milk after 18 hr at 30 C than after 18 hr at 21 C. Growth of the *Salmonella* stopped after 12-15 hr at 30 C when three of the four commercial cultures were used. Cessation of growth again coincided with a marked drop in pH, as happened when pure cultures were used (Fig. 2).

Behavior of S. *typhimurium* was somewhat dif-

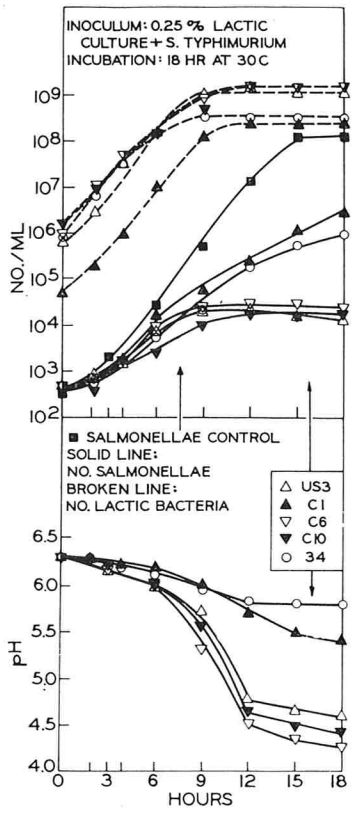

Figure 2. Changes in pH, in numbers of *Salmonella typhimurium*, and in numbers of *Streptococcus cremoris* (US3, C1) *Streptococcus lactis* (C6, C10), and *Streptococcus diacetilactis* (34) during incubation of skimmilk at 30 C.

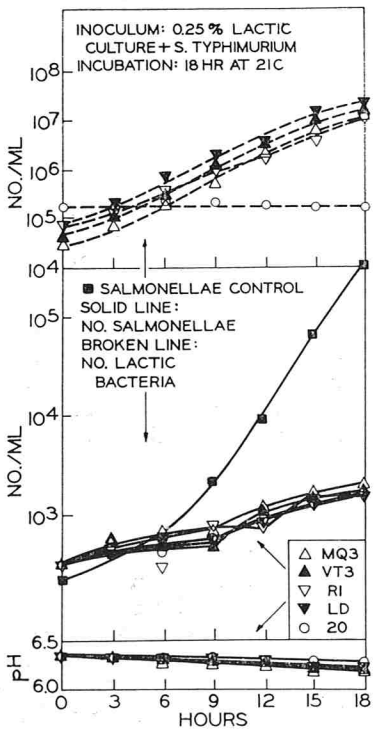

Figure 3. Changes in pH, in numbers of *Salmonella typhimurium*, and in numbers of lactic streptococci (mixture of *Streptococcus cremoris* and *Streptococcus lactis*, MQ3, VT3, R1, and LD) and *Leuconostoc citrovorum* (20) during incubation of skimmilk at 21 C.

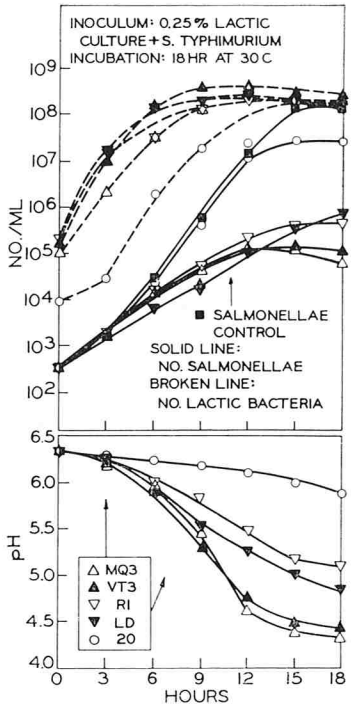

Figure 4. Changes in pH, in numbers of *Salmonella typhimurium*, and in numbers of lactic streptococci (mixture of *Streptococcus cremoris* and *Streptococcus lactis*, MQ3, VT3, R1, and LD) and *Leuconostoc citrovorum* (20) during incubation of skimmilk at 30 C.

ferent when a 1.0% inoculum (instead of 0.25%) was used (Fig. 5). The *Salmonella* grew rapidly during the first 6 hr of incubation and more slowly for the next 3 to 9 hr, depending on the lactic culture that was present. Growth of the *Salmonella* was inhibited most rapidly by the two lactic cultures (MQ3 and C6) that produced acid most vigorously. The number of viable salmonellae actually began to decrease during the later stages of the fermentation carried out by three of the four lactic cultures. This loss of viability undoubtedly is related to exposure of *S. typhimurium* to higher concentrations of acid for a longer time than occurred in any of the other experiments with these lactic acid bacteria.

Streptococcus diacetilactis and Leuconostoc citrovorum

Streptococcus diacetilactis (34) repressed growth of *S. typhimurium* as effectively as did *S. lactis* and *S. cremoris* at 21 C (Fig. 1). At 30 C (Fig. 2), growth of *S. typhimurium* in the presence of *S. diacetilactis* exceeded that which occurred when the other lactic cultures except *S. cremoris* C1 were present. Results presented in Fig. 1 and 2 were obtained with an inoculum of 0.25%. When this was increased to 1.0%, results reported in Fig. 5 were obtained. Again, growth of *S. typhimurium* was repressed by *S. diacetilactis* but less than that obtained with mixed cultures of *S. lactis* and *S. cremoris*.

Data obtained when *L. citrovorum* (20) and *S. typhimurium* grew together appear in Fig. 3 and 4. At 21 C, *S. typhimurium* was effectively repressed even though *L. citrovorum* exhibited little or no growth during the 18-hr incubation period. The strain of *L. citrovorum* used in these tests was somewhat unusual in that it grew poorly or not at all at 21 C but did well at 42 C. At 30 C, *L. citrovorum* grew well but had only minimal inhibitory effects on *S. typhimurium* and they were not evident until after 12 hr of incubation.

A separate experiment was conducted to determine if *L. citrovorum* could cause changes in numbers of *S. typhimurium* during extended incubation after milk was acidified with citric acid as might be done if the organism were used to produce a flavorful dressing for cottage cheese. Data from the test (Fig. 6) suggest that this strain of *L. citrovorum* had little, if any, effect on viability of *S. typhimurium* during the holding period. To be sure, there was no further growth of *S. typhimurium* during this time but that also was accomplished by use of citric acid alone.

Lactobacilli and Streptococcus thermophilus

Data on the behavior of *S. typhimurium* in skimmilk cultured at 42 C with *L. bulgaricus* (LB5, LB7)

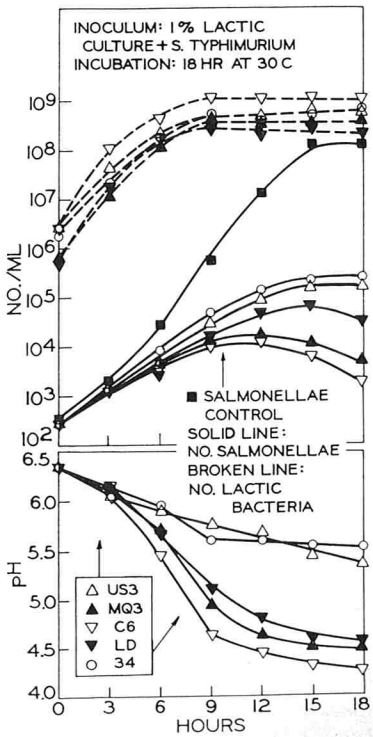

Figure 5. Changes in pH, in numbers of *Salmonella typhimurium*, and in lactic acid bacteria when skimmilk received a 1% inoculum of lactics and was held at 30 C; lactic cultures identified in titles of Fig. 2 and 3.

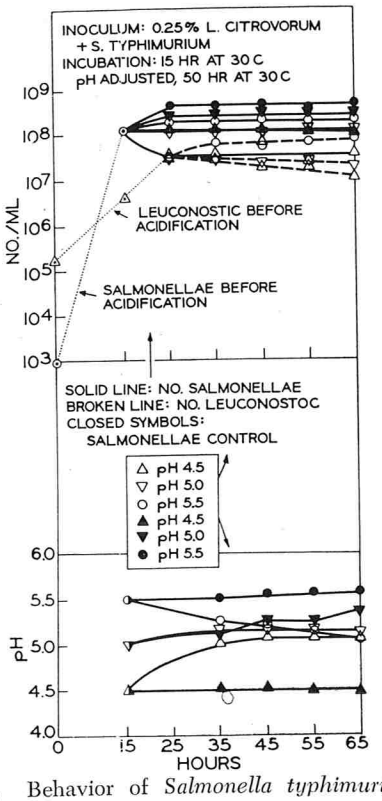

Figure 6. Behavior of *Salmonella typhimurium* in skimmilks cultured with *Leuconostoc citrovorum*, acidified with citric acid, and incubated further at 30 C.

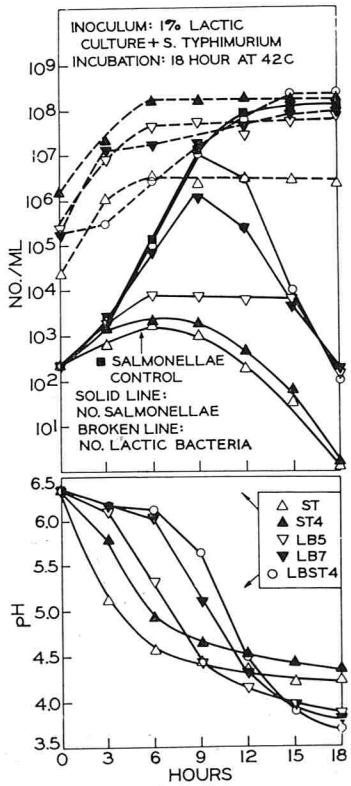

Figure 7. Changes in pH, numbers of *Salmonella typhimurium*, and numbers of lactic acid bacteria, when *Streptococcus thermophilus* (ST, ST4), *Lactobacillus bulgaricus* (LB5, LB7), and a mixture of *Lactobacillus helveticus* and *Streptococcus thermophilus* (LBST4) were added to skimmilk and then incubated at 42 C.

or *S. thermophilus* (ST, ST4) are presented in Fig. 7. Results differ markedly from those observed when the other lactic acid bacteria were evaluated. When *S. thermophilus* was present, the *Salmonella* grew to a limited extent during the first 6 hr of incubation and then numbers began to decline so that few if any viable salmonellae remained after 18 hr of incubation. The onset of inactivation generally coincided with a reduction in pH of the skimmilk to approximately 5.0 or below.

Pure cultures of *Lactobacillus bulgaricus* yielded somewhat different results in that *S. typhimurium* attained a higher population during the early stages of growth than when *S. thermophilus* was present. Undoubtedly, the slower growth of the lactobacillus as evidenced by the change in pH, in part, accounts for the difference. Attainment in the skimmilk of a pH value of 4.5 to 5.0 again was accompanied by a loss in viability of salmonellae. The mixed culture composed of *Lactobacillus helveticus* and *S. thermophilus* (LBST4) was less effective than were cultures of *L. bulgaricus* or *S. thermophilus* in repressing initial growth of *S. typhimurium*. There was, however, a marked loss of viability by the *Salmonella* after 9 hr of incubation even though the pH at this point still exceeded 5.5 This suggests that factors other than pH may contribute to demise of salmonellae when these lactic acid bacteria are present. When the inoculum of the mixed culture was increased to 5% (Fig. 8), *S. typhimurium* failed to grow, even during the early stages of incubation. In fact, salmonellae present began to lose viability after 9 hr and were completely inactivated (within the limits of testing procedures) after 12 hr of incubation.

Additional tests were made with mixed cultures of *L. bulgaricus* and *S. thermophilus* and results appear in Fig. 8. When 5% inocula of *L. bulgaricus* plus *S. thermophilus* or of *L. helveticus* plus *S. thermophilus* were used, *S. typhimurium* failed to grow and additionally, was inactivated within 8 to 12 hr of incubation. Use of a 1% inoculum composed of *L. bulgaricus* LB7 and *S. thermophilus* ST4 allowed some initial growth of *S. typhimurium* but this was followed by inactivation so no detectable salmonellae were present in 18 hr. The other mixture of these two lactic acid bacteria, when added at the 1% level, affected *S. typhimurium* in a manner similar to that observed when a 5% inoculum was used.

It is clearly evident from these data that the lactic acid bacteria commonly used in dairy fermentations are detrimental to growth of salmonellae and sometimes can inactivate them during the course of the fermentation. Inhibition or inactivation of salmonellae by lactic acid bacteria is affected by the incuba-

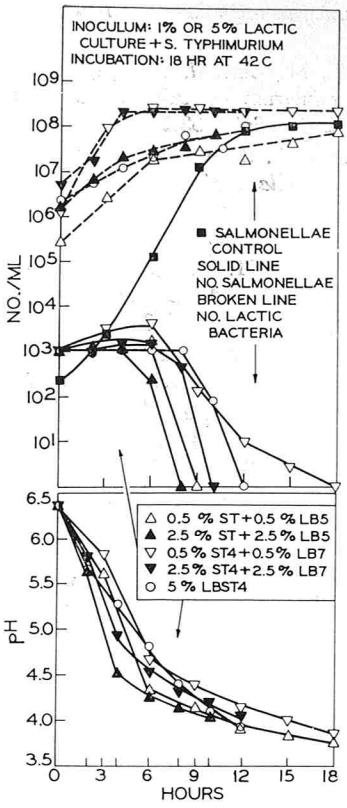

Figure 8. Changes in pH, numbers of *Salmonella typhimurium*, and numbers of lactic acid bacteria when mixtures of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* and of *Streptococcus thermophilus* and *Lactobacillus helveticus* were added to skimmilk and then incubated at 42 C. Lactic cultures identified in title of Fig. 7.

tion temperature, size of inoculum, and kind of lactic acid bacterium.

Suppression of growth by the lactic streptococci was clearly evident at 21 C even though there was only a slight shift in pH during the course of the experiment. An increase of the temperature to 30 C resulted in a higher population of salmonellae, at least in 18 hr, than at 21 C before growth was halted by action of the lactic culture. Increasing the inoculum from 0.25 to 1.0% had no appreciable effect on growth of S. *typhimurium* during the early part of the incubation period, but did result in some inactivation of the *Salmonella* before the 18-hr incubation was completed. Inactivation probably resulted because the salmonellae were exposed to a higher concentration of lactic acid for a longer time than when a lower inoculum was used.

Streptococcus thermophilus, at 42 C, initially inhibited growth of S. *typhimurium* and then caused inactivation of the cells which were present. Although *L. bulgaricus* was less detrimental to S. *typhimurium* than was S. *thermophilus*, when these two lactic acid bacteria were combined their activity against the *Salmonella* appeared greater than when either was used alone.

The heterofermentative S. *diacetilactis* and *L. citrovorum* were less active against S. *typhimurium* than were the other lactic acid bacteria. From a practical viewpoint, it is more important that the homofermentative lactic acid bacteria be detrimental to salmonellae since those lactic acid organisms are predominant during the initial stages of the dairy fermentations.

Although these data do not provide all of the answers on ways in which the lactic acid bacteria inhibit or inactivate salmonellae, they do suggest that more than acid production alone is involved. Acid, however, is probably the single most important factor which causes demise of the salmonellae. According to Subramanian and Marth *(22)*, acids also may retard growth of salmonellae. Other investigators have attributed inhibitory properties of lactic acid bacteria to production of antibiotics *(1, 10, 16, 20)*, biacetyl *(9, 11, 15, 19)*, acetic acid *(18, 21)*, and hydrogen peroxide *(5)*.

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DIMENSIONS OF INSANITARY CONDITIONS

(Continued from Page 481)

equitable to single out by name the 97 plants visited from the 4,550 plants which formed the basis for the statistical projection. Accordingly the plants have not been identified in the report.

FINDINGS AND CONCLUSIONS

Overall findings

During the past 3 years, FDA inspections have indicated that sanitary conditions in the food industry in the United States are deteriorating. FDA did not know how extensive these insanitary conditions were and therefore could not provide the assurance of consumer protection required by the law.

A serious problem of insanitary conditions exists in the food manufacturing industry. Several actions must be taken by FDA to alleviate these conditions.

Existing conditions

Of the 97 plants included in the sample, 39, or about 40 percent, were operating under insanitary conditions. Of these, 23, or about 24 percent, were operating under serious insanitary conditions having potential for causing, or having already caused, product contamination.

Photographs of conditions at some plants, taken during FDA-GAO inspections, and detailed descriptions of some of the inspection results, will be found in chapter 2.

On the basis of the sample, GAO estimated that 1,800, or about 40 percent of the 4,550 plants were operating under insanitary conditions, including 1,000, or about 24 percent, operating under serious insanitary conditions.

FDA officials advised GAO that conditions at plants located in the 21 States would, in their opinion, be representative of conditions at plants nationwide.

Inspection manpower

FDA has not had the money or manpower to identify promptly all the food plants operating under insanitary conditions. During the last 3 years, FDA has sharply reduced its sanitation inspection coverage of food plants in an attempt to cope with more critical problems, such as microbiological contamination and drug hazards.

FDA has a management improvement program under way to develop a system for improving the effectiveness of its field operations. (See p. 31.)

Although it has a responsibility under the FD&C Act, FDA generally does not inspect restaurants and other retail food stores but relies instead on State and local officials for this regulation. (See p. 25.)

According to officials of the Department of Health, Education, and Welfare (HEW), the President, HEW, and FDA have recognized the need to increase and improve the inspection capability of FDA to make an effective impact upon present insanitary conditions of the food manufacturing industry.

Enforcement

In several instances of insanitary conditions found during plant inspections, GAO noted a need for more timely and aggresive enforcement action by FDA. In 14 of 111 enforcement actions reviewed, or

(Continued on Page 495)

SURVIVAL OF *SALMONELLA TYPHIMURIUM* IN REFRIGERATED CULTURED MILKS[1]

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Abstract

Cultured skimmilks containing *Salmonella typhimurium* were prepared at 21, 30, or 42 C using different species of lactic acid bacteria (0.25, 1.0, and 5% inoculum) either singly or in combinations. Several commercial cultures also were used. Cultured skimmilks were stored at 11 C and tested at 3-day intervals for numbers of viable salmonellae and lactic acid bacteria and for pH. Survival of *S. typhimurium* varied from 6 to 9 days and from 3 to 6 days in milks cultured with 0.25% *Streptococcus lactis* at 21 and 30 C, respectively. Increasing the inoculum to 1% with incubation at 30 C yielded a product no more detrimental to *S. typhimurium* than when the lower inoculum was used at 30 C. Survival of *S. typhimurium* always exceeded 9 days when *S. cremoris* was used to make cultured skimmilks. Products made with commercial mixed cultures composed of *S. lactis* and *S. cremoris* allowed *S. typhimurium* to survive for periods intermediate between the extremes observed when pure cultures were used. Skimmilks cultured with *Streptococcus diacetilactis* and *Leuconostoc citrovorum,* even when skimmilks cultured with the latter organism were acidified with citric acid and incubated further, were essentially without effect on survival of *S. typhimurium* during refrigerated storage. Use of *Streptococcus thermophilus* (1% culture, 42 C incubation) yielded cultured skimmilks that were most detrimental to survival of salmonellae, whereas skimmilks fermented with *Lactobacillus bulgaricus* permitted survival of low numbers of salmonellae beyond 9 days. Milks cultured at 42 C with a 5% inoculum of *S. thermophilus* mixed with *L. bulgaricus* or *Lactobacillus helveticus* were free of viable salmonellae before the incubation was complete. Salmonellae grown in skimmilk at 21 C without a lactic culture were more resistant to inactivation during refrigerated storage than was *S. typhimurium* grown at 30 or 42 C.

Recent concern with salmonellae in manufactured dairy products was prompted by the discovery, in 1966, of *Salmonella newbrunswick* in certain lots of nonfat dry milk *(12).* The incidence of salmonellosis in the U. S. attributable to manufactured milk products remains low although in the past illness caused by *Salmonella typhimurium* or *Salmonella typhi* resulted from consumption of several different kinds of cheese that were contaminated with these bacteria. Three U. S. outbreaks of food-borne disease (the causative organisms were not given) involving 106 cases were attributed to buttermilk made from raw milk *(5, 6).* However, outbreaks of salmonellosis in this country have not been associated with such cultured foods as buttermilk, acidophilus milk, Bulgarian buttermilk, or yogurt *(12, 28).*

The fate of salmonellae in cultured milks has been studied and, in general, investigators agree that such foods provide an environment which is unfavorable for survival of these bacteria *(12).* However, the reports disagree on how long salmonellae survive in cultured milks. Survival of a few hours to many weeks have been observed *(1, 12, 24, 26, 28)* but an adequate explanation for this variability is lacking.

Park and Marth *(17)* conducted a series of experiments to determine the behavior of *S. typhimurium* when grown together with different lactic acid bacteria. These experiments yielded a series of cultured skimmilks which contained salmonellae. Hence it was possible to study the fate of salmonellae in these products during refrigerated storage, and, perhaps, to provide an explanation for the great variability in results observed by others who conducted similar experiments. Results of the study are reported in this paper.

Materials and Methods

Bacterial cultures

Cultures used in these experiments were described earlier by Park and Marth *(17).*

Preparation of cultured skimmilks

Cultured skimmilks were prepared as described by Park and Marth *(17)* and stored at 11 C for 9 days. Stored products were examined at 3-day intervals for numbers of viable salmonellae and lactic acid bacteria and for pH, as measured with a Beckman pH meter equipped with a glass electrode.

Enumeration of salmonellae and lactic acid bacteria

Methods described by Park and Marth *(17)* were used to enumerate salmonellae and lactic acid bacteria present in cultured skimmilks, except that brilliant green agar (Difco, EBL) was used to determine numbers of salmonellae. This was done to insure that salmonellae could be enumerated since they were present in small numbers amongst a flora which consisted of overwhelming numbers of lactic acid bacteria.

Before sampling cultured skimmilks, sufficient sterile 40% NaOH was added aseptically to 99 ml of sterile phosphate buffered dilution water so the pH of the product-dilution water mixture approximated 7.0. Earlier observations *(17)* indicated that more viable salmonellae were recovered from cultured skimmilks when such neutralization was practiced.

[1]Research supported by the College of Agricultural and Life Sciences, University of Wisconsin, Madison.

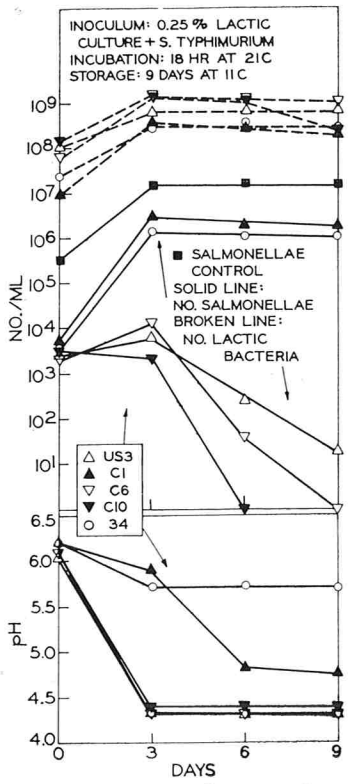

Figure 1. Changes in pH and numbers of *Salmonella typhimurium* and lactic acid bacteria in cultured skimmilks stored at 11 C after they were prepared at 21 C with a 0.25% inoculum *Streptococcus lactis* (C6, C10), *Streptococcus crecoccus cremoris* (US3, C1), or *Streptococcus diacetilactis* (34).

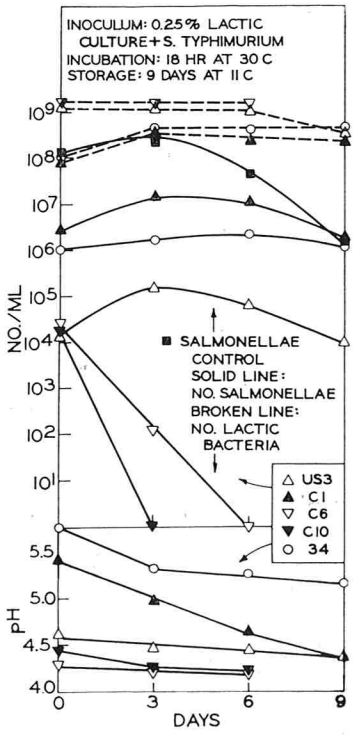

Figure 2. Changes in pH and in numbers of *Salmonella typhimurium* and lactic acid bacteria in cultured skimmilks stored at 11 C after they were prepared at 30 C with a 0.25% inoculum *Streptococcus lactis* (C6, C10) *Streptococcus cremoris* (US3, C1), or *Streptococcus diacetilactis* (34).

Eleven milliliters of cultured skimmilk were then added to the dilution water and appropriate dilutions were made. When undiluted products were to be plated, 10 ml of a 1:10 dilution were divided equally among 5 petri dishes. Sufficient brilliant green agar was then added to insure solidification of the agar-sample mixture and to insure that the medium was not unduly diluted. Plates to enumerate salmonellae were incubated for 48 hr at 42 C and then were counted. Typical "salmonella-like" colonies were confirmed by inoculating into triple sugar iron agar.

Results and Discussion

Streptococcus lactis and Streptococcus cremoris

Cultured skimmilks were made using a 0.25% inoculum of *S. lactis* or *S. cremoris* and incubation at 21 C (Fig. 1) and 30 C (Fig. 2). Additional cultured skimmilks were made with commercial cultures, which consisted of mixtures of *S. lactis* and *S. cremoris*. These cultures were added at a level of 0.25% and incubation was at 21 C (Fig. 3) and 30 C (Fig. 4). Additionally, some cultured skimmilks were made at 30 C with a 1.0% inoculum of several pure and mixed cultures of these lactic acid bacteria (Fig. 5).

The pH of all cultured skimmilks produced at 21 C with 0.25% inoculum of either *S. lactis* or *S. cremoris* exceeded 6.0 when the skimmilks were refrigerated. There was a rapid drop in the pH of three cultured skimmilks during the first 3 days of storage and only a slow drop in pH of the other cultured skimmilk during the 9-day storage period. Inactivation of salmonellae in three of the cultured skimmilks, two produced by *S. lactis* (C6 and C10) and one produced by *S. cremoris* (US3), accompanied the sharp decline in pH. Even though the pH of these three cultured skimmilks was similar (<4.5), there was variability in survival of *S. typhimurium* in the three products. No viable salmonellae could be recovered from one product after 6 days and from another after 9 days, whereas inactivation of salmonellae was about 97% complete in the third product after 9 days. Differences in rate of inactivation observed in these products suggest that lactic acid is not the only factor which governs survival of salmonellae in cultured skimmilks.

One culture of *S. cremoris* (C1) produced acid slowly during refrigerated storage. In this instance, *S. typhimurium* actually grew in the cultured skimmilk during the first 3 days of storage and then remained constant in number even though the pH of the product after 6 days was below 5.0.

Incubation at 30 C instead of 21 C (Fig. 2) resulted in cultured skimmilks with a lower pH value at the time they were refrigerated. Although the initial number of *S. typhimurium* was higher than in products made at 21 C, inactivation was more rapid in cultured skimmilks produced with the two cultures

of *S. lactis*. Behavior of *S. typhimurium* was markedly different during storage of cultured skimmilks made with *S. cremoris* at 30 C than at 21 C. The *Salmonella* grew in both skimmilks during the first 3 days of storage even though the pH of one was near 4.5. Although there was evidence for some loss of viability, high populations of *S. typhimurium* remained in both products after 9 days of storage.

The pH of skimmilks cultured at 21 C with a 0.25% inoculum of commercial mixed lactic cultures (Fig. 3) was similar to pH values obtained when pure cultures were used (Fig. 1). In general, demise of *S. typhimurium* did not appear to be as rapid as when pure cultures of *S. lactis* were used even though pH values were similar. Viable salmonellae had nearly disappeared, after 9 days, from only one of the skimmilks cultured at 21 C with commercial cultures. One of the mixed cultures (R1) produced acid rather slowly although the population of the culture was adequate. Growth of *S. typhimurium* was evident in the cultured skimmilk made with this culture.

Incubation at 30 C of skimmilks cultured with commercial lactic cultures resulted in a pH value of approximately 5.0 or below in all products when they were refrigerated. The rate at which *S. typhimurium* was inactivated in three of the products (made with culture MQ3, VT3, and LD) was comparable, although from 10^2 to nearly 10^5 salmonellae persisted in the cultured skimmilks after 9 days of storage. In contrast, viability of *S. typhimurium* was unaffected in skimmilk cultured with R1, even though the pH was near 5.0 during the entire storage period.

It also must be pointed out that viability of *S. typhimurium* grown in skimmilk at 30 C without lactic culture and then refrigerated declined during refrigerated storage (Fig. 2, 4, and 5), whereas viability was unaffected during storage when growth was at 21 C (Fig. 1 and 3). The loss of viability of *S. typhimurium* grown at 42 C and then refrigerated was even greater than that noted when the culture incubated at 30 C was refrigerated (Fig. 7).

Use of a 1.0% inoculum and 30 C incubation (Fig. 5) produced a cultured milk which yielded results somewhat different from those obtained from a product made with a 0.25% inoculum. One culture each of *S. lactis* and *S. cremoris* and two commercial cultures were tested under these conditions. Inactivation of *S. typhimurium* was complete or nearly so in one product (*S. lactis* C6) after 6 days and in two others (*S. lactis* + *S. cremoris*, MQ3 and LD) after 9 days of storage. As before, *S. cremoris* US3 produced acid less actively than the other cultures and the cultured skimmilk made with it was essentially without effect on *S. typhimurium*.

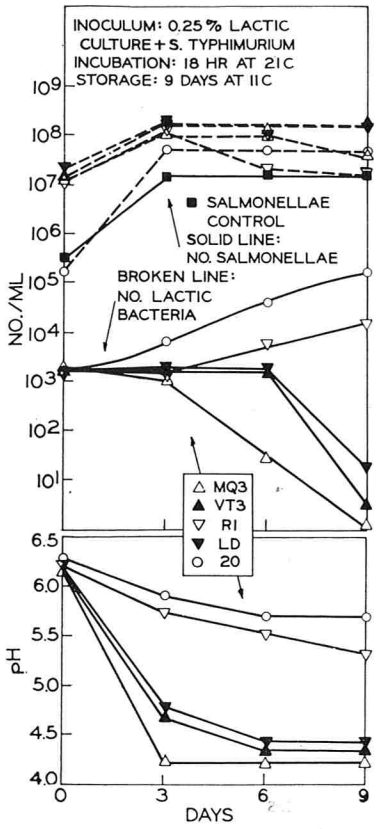

Figure 3. Canges in pH and in numbers of *Salmonella typhimurium* and lactic acid bacteria in cultured skimmilks stored at 11 C after they were prepared at 21 C with an inoculum of 0.25% of commercial mixed cultures composed of *Streptococcus lactis* and *Streptococcus cremoris* (MQ3, VT3, R1, LD), or with *Leuconostoc citrovorum* (20).

Streptococcus diacetilactis and Leuconostoc citrovorum

Both of the heterofermentative lactic streptococci, *S. diacetilactis* (34) and *L. citrovorum* (20), were used to produce cultured skimmilks which contained *S. typhimurium*. The *Salmonella* grew during the first 3 days of refrigerated storage in skimmilk cultured at 21 C with a 0.25% inoculum of *S. diacetilactis*. No inactivation of *S. typhimurium* in the cultured skimmilk was evident during the remaining 6 days of storage (Fig. 1). Results obtained when skimmilk was cultured at 30 C and then refrigerated differed only in that growth of *S. typhimurium* was not evident during refrigerated storage (Fig. 2). Use of a 1% inoculum of *S. diacetilactis* and incubation at 30 C yielded a cultured skimmilk which had no effect on viability of *S. typhimurium* during refrigerated storage (Fig. 5).

Cultured skimmilks produced at either 21 (Fig. 3) or 30 C (Fig. 4) with a 0.25% inoculum of *L. citrovorum* were not detrimental to *S. typhimurium* during refrigerated storage. In fact, the *Salmonella* grew in both products during storage. A second

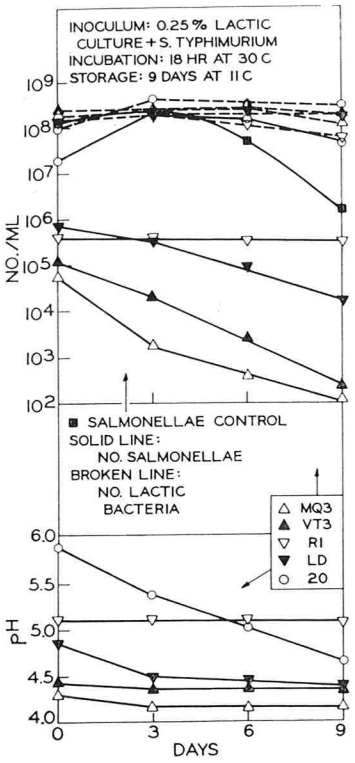

Figure 4. Changes in pH and in numbers of *Salmonella typhimurium* and lactic acid bacteria in cultured skimmilks stored at 11 C after they were prepared at 30 C with an inoculum of 0.25% of commercial mixed cultures composed of *Streptococcus lactis* and *Streptococcus cremoris* (MQ3, VT3, R1, LD) or with *Leuconostoc citrovorum* (20).

experiment was conducted in which *L. citrovorum* and S. *typhimurium* grew in skimmilk for 15 hr at 30 C, the skimmilk was then acidified with citric acid to pH values of 4.5, 5.0, and 5.5, and the acidified skimmilks were held at 30 C for an additional 50 hr to permit development of flavor compounds by *L. citrovorum*. Skimmilks were then refrigerated. Results of the experiment appear in Fig. 6. It is clearly evident that survival of S. *typhimurium* was unaffected either by skimmilks cultured with *L. citrovorum* or by uncultured skimmilks at pH values of 4.5 to 5.5.

Streptococcus thermophilus and lactobacilli

Cultured skimmilks were prepared at 42 C using a 1% inoculum of S. *thermophilus* (ST, ST4), *L. bulgaricus* (LB5, LB7), or mixture of S. *thermophilus* and *L. helveticus* (LBST4). Survival of S. *typhimurium* during refrigerated storage of these products is detailed in Fig. 7. It is immediately apparent that skimmilks cultured with S. *thermophilus* or a mixture of S. *thermophilus* and *L. helveticus* were more detrimental to survival of S. *typhimurium* than were those cultured with *L. bulgaricus*. This occurred even though the pH values of skimmilks cultured with *L. bulgaricus* were markedly lower than those of skimmilks fermented with S. *thermophilus*. Skimmilks cultured in whole or in part with S. *thermophilus* were free from viable salmonellae after 3 or 6 days of storage, whereas approximately 90% of the viable S. *typhimurium* cells were inactivated after 9 days in skimmilks prepared with *L. bulgaricus*.

It should be mentioned that additional cultured skimmilks were made at 42 C using either a 1% or 5% inoculum composed of 50:50 mixtures of S. *thermophilus* and *L. bulgaricus* or with a 5% inoculum of the commercially prepared mixture of S. *thermophilus* and *L. helveticus*. The skimmilks also were inoculated with S. *typhimurium* at the onset of incubation. Viable salmonellae were not detectable in any of these products at the conclusion of the incubation and hence they were not subjected to testing during refrigerated storage.

DISCUSSION

Results of this investigation indicate that survival (or inactivation) of salmonellae in cultured skimmilks is related to the species of lactic acid bacterium used to prepare the product, the strain of such a species, the level of inoculum used, the incubation

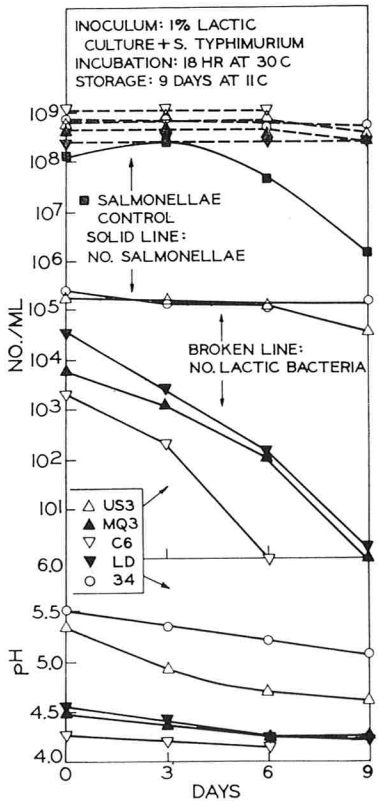

Figure 5. Changes in pH and in numbers of *Salmonella tyhpimurium* and lactic acid bacteria in cultured skimmilks stored at 11 C after they were prepared at 30 C with a 1.0% inoculum of *Streptococcus cremoris* (US3), commercially prepared mixtures of the two (MQ3, LD), *Streptococcus lactis* (C6) or *Streptococcus diacetilactis* (34).

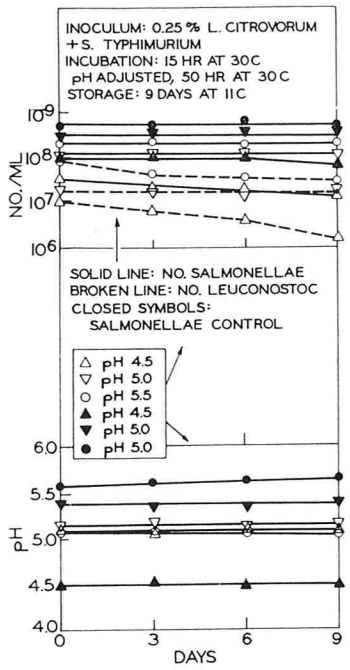

Figure 6. Changes in pH and in numbers of *Salmonella typhimurium* and *Leuconostoc citrovorum* in cultured skimmilks stored at 11 C after they were prepared with a 0.25% inoculum of *L. citrovorum*, incubation at 30 C for 15 hr, acidification with citric acid to pH 4.5, 5.0, and 5.5, followed by an additional 50 hr at 30 C.

temperature at which the fermentation was carried out, and the amount and speed of acid production. To be sure, acid formation, in part, is related to the other factors but they influenced survival of S. *typhimurium* beyond that attributable to acid production. A few examples will be given to illustrate this point. First, cultured skimmilks made with S. *thermophilus* were more detrimental to S. *typhimurium* than were those made with S. *lactis* and they, in turn, were more detrimental than were cultured skimmilks produced by S. *cremoris* even though the pH values of the products often were similar.

Second, variation in survival of S. *typhimurium* existed when skimmilks were cultured with different strains of the same lactic acid bacterium. Such variation was noted between strains of S. *lactis* and S. *thermophilus* even though the pH values of products made with the different strains were similar. Variation also existed when strains of S. *cremoris* were used but, in this instance, there also were substantial differences in the pH of the cultured skimmilks.

Third, skimmilks cultured with a larger (1.0% versus 0.25%) inoculum were more detrimental to survival of S. *typhimurium* even though the pH of the products in both instances approximated 4.5. This was particularly true when commercial mixed cultures were used.

Fourth, inactivation of S. *typhimurium* was generally more rapid in skimmilks cultured at 30 C rather than at 21 C. Undoubtedly, the amount of acid and the rate at which it was produced were related to inactivation of salmonellae in cultured skimmilks made at the higher temperature. However, the first three factors just discussed also remained operative in products made at the higher temperature.

Fifth, that the amount of acid present in the cultured skimmilk as reflected by its pH, affected survival of S. *typhimurium* in the product is most clearly evident in milks cultured at 21 C.

Although numerous investigators have reported that salmonellae are inactivated in cultured milks, few of them agree on how long it takes for this to happen. Data reported in this paper help to explain why such variable results were reported in the past. In experiments by other investigators, more often than not, salmonellae were introduced into the acid product after it was manufactured rather than at the onset of culturing as was done in these studies. The shock to the organism caused by sudden replacement of a near-neutral with an acid environment could be expected to have variable effects on survival, depending on the condition of the salmonellae when used as inoculum. Furthermore, cultures and manufacturing processes used to manufacture cultured milks often were unknown or unspecified. Results of these

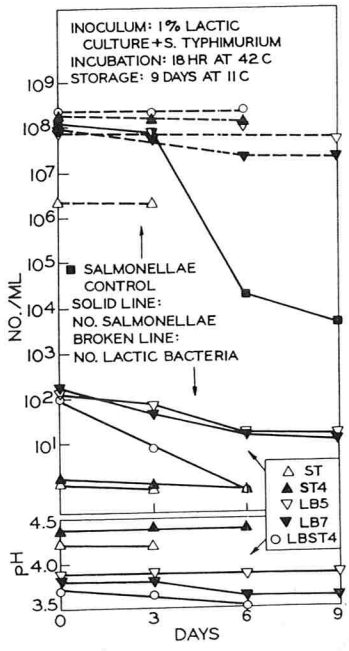

Figure 7. Changes in pH and in numbers of *Salmonella typhimurium* and lactic acid bacteria in cultured skimmilks stored at 11 C after they were prepared at 42 C with a 1.0% inoculum of *Streptococcus thermophilus* (ST, ST4), *Lactobacillus bulgaricus* (LB5, LB7), or a mixture of S. *thermophilus* and *Lactobacillus helveticus* (LBST4).

tests clearly indicate that both are important factors which can affect survival of salmonellae in the finished product.

Data obtained in this study suggest that the heterofermentative lactic streptococci have essentially no detrimental effect on S. *typhimurium*. This was surprising, especially when other investigators have reported that these bacteria are antagonistic to salmonellae *(23, 27)*, other gram-negative bacteria *(13, 14)*, and even *Staphylococcus aureus (13)*. Furthermore, several workers *(9, 11, 16, 19)* have reported that biacetyl (produced by these bacteria) has antibacterial properties, particularly at pH values below 5.0 *(19)*. Many of the experiments conducted by other workers utilized artificial media rather than food systems and this, in part, may account for some of the differences between their and our results. A noteworthy exception to this is the work of Mather and Babel *(14)* in which creamed cottage cheese was used. Another cause of differences may be the cultures used in the various studies. We used only one strain each of S. *diacetilactis* and *L. citrovorum*. It is possible that other strains would be more detrimental to salmonellae than the one which was tested. Marth and Hussong *(13)* found strain differences when they studied the ability of *L. citrovorum* to inhibit some gram-negative bacteria which can cause spoilage of cottage cheese. Results from this study are similar to those of Hargrove et al. *(9)* who found that *L. citrovorum* in cheese caused salmonellae to survive longer than they did in control cheeses.

The question about variable survival of salmonellae in the presence of different strains of the lactic acid bacterium is sharpened by these results but remains unanswered. Production of inhibitory substances has been reported for a variety of lactic acid bacteria *(4, 8, 10, 18, 21, 25)*. It is possible that some of these substances, if present, can act in concert with the acid and cause more rapid demise of salmonellae than can be produced by acid alone. This remains to be demonstrated by the appropriate experimental evidence. Claims have been made that both S. *thermophilus* and *L. bulgaricus* produce antibacterial agents effective against salmonellae *(2, 25)* although other investigators *(9, 20)* found *L. bulgaricus* was not detrimental to salmonellae. Additional evidence for the presence of anti-salmonellae substances other than acid comes indirectly from the work of Chung and Goepfert *(3)*. These workers found that some salmonellae could grow in laboratory media acidified to pH 4.4 with lactic acid. In contrast, in these tests salmonellae were regularly inactivated in cultured skimmilks with similar pH values. Hence, it is likely that acid was only one of several factors in cultured milks which combined to inactivate the salmonellae.

Finally, it should be mentioned that cultured milks afford the consumer substantial protection against ingesting viable forms of certain food-borne pathogens. This claim can be made for few other foods because most of them can be abused in some manner so they become hazardous. It is virtually impossible to do the same with cultured milks unless they are diluted with other substances. Even if cultured milks were inadvertently contaminated with low numbers of salmonellae at the time of manufacture, these bacteria probably would not be viable when the product reached the consumer. Additionally, if the consumer contaminated the product with salmonellae, the cultured milk would be free of viable salmonellae after a few days unless an overwhelming number of contaminants were added. Other reports by Goel et al. *(7)* and by Minor and Marth *(15)* indicate that cultured milks also are unfavorable for survival of *Escherichia coli, Enterobacter aerogenes*, and *Staphylococcus aureus*.

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DIMENSIONS OF INSANITARY CONDITIONS

(Continued from Page 488)

13 percent, the action to correct the problem was inadequate for a variety of reasons. (See p. 35.)

Although judgment is involved in selecting the approiate actions in each case, criteria or guidelines are needed to assist the FDA districts in making these decisions, particularly for repeated violators.

Causes of conditions

Although responsibility for sanitation rests with the food manufacturers, GAO believes that factors contributing to the poor sanitation conditions in the industry are (1) FDA's limitation in resources to make and (2) lack of timely and aggressive enforcement actions by FDA when poor sanitation conditions are found.

During fiscal year 1972 FDA plans to inspect about 9,400 food establishments and has 210 inspectors to do the job. The planned number of inspections clearly is inadequate to detect all insanitary establishments.

FDA's inventory of food manufacturers for planning inspections and measuring the scope of its plant inspection responsibility was not complete or accurate. For six FDA districts, 22 percent of the plants listed were out of busines, 8 percent were misclassified as food manufacturers, and 6 percent were not an FDA inspection responsibility.

FDA officials told GAO that there are food plants in existence which may not be on its inventory because, in the absence of plant registration requirements, FDA does not have an effective means of identifying all food plants subject to the FD&C Act. (See p. 19.)

More effective use of consumer complaints, an accurate inventory of food plants subject to inspection, and data indicating the effectiveness of inspections and regulatory actions could contribute to improving sanitary conditions of the food manufacturing industry.

FDA should (1) notify violators officially of sanitation standards violated, (2) request a prompt reply, and (3) monitor cases to promote corrective action. Without these actions, plants may continue to disregard sanitation standards, making reinspections necessary to determine whether corrective actions have been taken. (See p. 40.)

Providing in the law for civil penalties (fines) for violations of the FD&C Act would allow FDA more flexibility in enforcing sanitation standards. (See p. 40.)

Consumer complaints

FDA is devising a computerized system to record consumer complaints to identify industry and product problem areas. The output of the system, in GAO's opinion, should be used also to monitor the disposition of such complaints.

Insanitary products that had reached the consumers might have gone undetected by FDA for some time had not the consumer complained.

RECOMMENDATIONS OR SUGGESTIONS

GAO recommends that the Secretary, HEW, direct FDA, to:

—Periodically select and inspect a representative

(Continued on Page 498)

A COMPARISON OF THREE STAINING METHODS FOR DIRECT MICROSCOPIC COUNTING OF BACTERIA IN MILK

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(Received for publication March 27, 1972)

ABSTRACT

The Levowitz-Weber (LW) stain was compared collaboratively with two new stains, the pH 4 toluidine blue (pH 4 TBO), and the periodic acid-bisulfite-toluidine blue (PST), for direct microscopic counting of bacteria in nonfat dry milk (NDM). Mean counts for 10 samples (18 determinations each) were for the PST, pH 4 TBO, and LW stains, respectively, 89.1, 65.1, and 64.8 million/g. The mean count for the PST stain was significantly higher than the other two at the 5% level. The higher counts with the PST stain were as expected since the PST stain is designed to improve staining of heat plasmolyzed cells such as are found in NDM. Interlaboratory variation in counts was high. This variation was independent of the stain used or the laboratory preparing the stained smear. More work is needed on techniques for direct microscopic counting of bacteria to reduce interlaboratory variability.

Following extensive investigations of methods to stain bacteria in milk for direct microscopic counting, two new staining procedures were proposed by the author *(1, 2, 3)*. Studies in our laboratory *(4)* showed these methods to be equal or superior to the Levowitz-Weber (LW) stain recommended in the 12th edition of *Standard Methods (6)*. Therefore, in connection with the preparation of the 13th edition of *Standard Methods* a collaborative study was undertaken to compare the two new proposed methods with LW stain.

Two new methods are *(a)* a short procedure involving a dip in 0.1% toluidine blue buffered to pH 4, and *(b)* a long procedure considered to be particularly advantageous for staining of cells plasmolyzed by heat treatment. This procedure involves fixation in alcohol-acetic acid and successive treatments with periodic acid, bisulfite, and pH 4 toluidine blue.

Nine laboratories, familiar with staining techniques for the direct microscopic count (DMC), participated in the collaborative study.

PROCEDURE

Ten samples of nonfat dry milk (NDM) covering a range of bacterial counts were selected for the study. Seven of the samples were furnished by J. L. Dizikes, U. S. Department of Agriculture, Consumer and Marketing Service Laboratory, Chicago, and three were commercial instant nonfat dry milks purchased at a local supermarket. Each collaborator was sent prestained smears of each of the 10 samples prepared by each of the three staining methods. Each collaborator also was furnished a sample from each lot of NDM with instructions for preparing films and staining the smears. The NDM samples sent out were coded differently from the prestained smears. The collaborators were asked to return their stained smears to us together with results and any comments.

Preparation and staining of smears

Instructions to collaborators were as follows:

A. *Reagents*—Highest available purity chemicals should be used.
   1. *Fixative*—3 parts absolute ethanol, 1 part glacial acetic acid. Prepared fresh daily.
   2. *Periodic acid*—0.5 g in 100 ml water.
   3. *Na or K metabisulfite*—5 g in 100 ml water. Store in tightly stoppered container. Keeps indefinitely.
   4. *pH 4 Toluidine blue*—Dissolve 0.1 g toluidine blue (certified stain), 1.291 g citric acid monohydrate, and 1.093 g disodium phosphate (anhydrous) in 100 ml water. Keeps indefinitely.

B. *Procedure*
   1. Prepare smears according to *Standard Methods.*
   2. Place smear in xylene for 1 min; remove and dry (forced air is helpful for drying in this and subsequent steps). Defatting is unnecessary with nonfat milk.

   pH 4 TBO Stain
   3. Immerse or flood slide for 2 min in pH 4 toluidine blue; rinse lightly with tap water and dry.

   Periodic acid-bisulfite-toluidine blue (PST)
   3. Place slide in fresh fixative for 30 min; remove and dry
   4. Place slide in periodic acid for 5 min; remove; dip briefly in absolute ethanol and dry.
   5. Place slide in bisulfite solution for 5 min; remove and rinse thoroughly with water.
   6. Place slide in pH 4 toluidine blue for 5 min; rinse lightly with running tap water and dry.

   LW Procedure; smears were prepared and stained according to *Standard Methods (6).*

RESULTS AND CONCLUSIONS

Participating laboratories generally reported no difficulty in preparing and staining smears by each of the three methods. One laboratory reported problems with sloughing of smears by the PST procedure, and one reported sloughing of smears using the LW procedure. Examination of smears stained by participating laboratories and returned to us confirmed that smears were stained satisfactorily by all three methods.

The results summarized in Table 1 show substantial interlaboratory variation in counts regardless of the staining method. Statistical analysis of the data

TABLE 1. MEAN VALUES FOR DIRECT MICROSCOPIC COUNTS OF 10 NDM SAMPLES[1]

Sample	Stain: PST — Mean of 18 detmns.	PST — Standard deviation	pH 4 TBO — Mean of 18 detmns.	pH 4 TBO — Standard deviation	LW — Mean of 18 detmns.	LW — Standard deviation
	($\times 10^6$/g)					
1	18.8	14.2	17.6	15.9	13.9	15.3
2	55.1	34.2	29.3	29.6	30.1	20.4
3	52.6	31.3	42.6	28.5	39.4	22.6
4	120.8	55.6	94.3	61.9	88.1	38.8
5	167.9	54.6	118.2	47.8	132.2	41.8
6	116.9	40.5	88.5	36.8	92.9	26.7
7	191.3	56.6	149.3	49.3	152.2	39.3
8	85.4	41.8	44.9	34.3	44.4	37.1
9	46.2	28.9	32.2	24.4	29.2	20.6
10	43.2	26.8	34.2	33.2	25.3	20.2
Mean all samples	89.8[2]		65.1		64.8	

[1]Combined data from 9 laboratories for smears prestained and stained by participating laboratories.
[2]Significantly different from the other two means at the 5% level.

showed that the results are not significantly affected by the source of the stained smear (prepared in our laboratory or the participating laboratory) despite visible differences among the collaborators in methods of preparing smears. This further confirms that participating laboratories were able to prepare and stain smears satisfactorily by all three methods using the instructions provided. It also shows that the method of preparing and staining the smears is not a significant cause of interlaboratory variation in results.

When all the data for all samples (Table 1) were analyzed, counts obtained using the PST stain (89.1 million/g) were significantly higher than those obtained by the pH4 TBO (65.1 million/g) or the LW (64.8 million/g) stains. Table 1 also shows that the average count for each sample was highest using the PST stain. The pH4 TBO and LW stains are both essentially nucleic acid stains and therefore would be expected to give similar results as was found. The PST stain, in addition, stains polysaccharides and should therefore improve staining of plasmolyzed cells in heat treated products where nucleic acids have leached out of the cells. The results confirm this prediction.

One laboratory used a strip counting procedure rather than counts of individual fields. The counts generally were higher than counts by other laboratories. Statistical analyses made with and without the data from this laboratory gave the same conclusions, so these data were left in the final analysis.

Schultze et al. *(5)* found that with direct microscopic counting of somatic cells, individual counter biases could be an important source of error. Minor variations in procedures, which were theoretically equivalent to one another, in some instances strongly affected the counting performance of individual technicians. They therefore recommend that any quantitative microscopic procedure be specified in detail and that a single reference method should be selected. They further recommend that procedural variations should not be permitted unless first evaluated in a collaborative study. These comments should certainly apply to direct microscopic counting of bacteria since bacteria are far more difficult than somatic cells to locate and recognize under the microscope.

Since the method of preparing and staining smears is not a significant source of interlaboratory variation, the observed variation must result from the counting technique itself. Dabbah and Moats *(4)* discuss the expected variations in DMCs from purely random effects. The skill of the analyst in locating and identifying bacteria in the microscopic field also is undoubtedly an important factor.

SUMMARY

(a) Of the three stains tested on NDM, the Levowitz-Weber and the pH 4 toluidine blue gave identical counts. The periodic acid-bisulfite-toluidine blue stain gave significantly higher counts. *(b)* Substantial interlaboratory variation occurred. This variation was independent of the staining method used and the source of the smears (our laboratory or the participating laboratory). Factors in the counting technique are therefore responsible for the observed interlaboratory variations.

ACKNOWLEDGMENTS

The author thanks the participating laboratories for their

cooperation in this study and Mr. Roy Heatwole, U. S. Department of Agriculture, Agricultural Research Service, Biometrical Services, for statistical analysis of the data.

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DIMENSIONS OF INSANITARY CONDITIONS

(Continued from Page 495

number of food plants to assess industrywide conditions and report its assessments to the Congress.

—Periodically evaluate the accuracy of the inventory of food plants to be inspected so that FDA will know the scope of its responsibilities and resources needed for sanitation inspections. FDA should provide this data to the Congress for the same reason.

—Establish milestones for implementing its management improvement program for using statistical techniques to identify problem areas, allocate resources, and measure the effectiveness of its regulatory actions.

—Monitor the implementation of the improvement program and advise appropriate congressional committees periodically on the progress being made in, as well as the various levels of resources needed for, implementing the program; and develop an interim plan of action, pending the completion of this program, for consideration by the Congress.

—Establish criteria for the districts to use in determining (1) when more aggresive action should be taken against plants that violate good manufacturing practice regulations and (2) what type of action should be taken.

—Take a stronger enforcement posture against those plants that show continuing flagrant disregard of the FD&C Act.

—Issue written notices in all cases of plants not complying with the FD&C Act and request written responses on actions taken or planned to correct the violations and to ensure continued compliance.

—Obtain feedback on the disposition of all cases referred to State or other regulatory bodies for corrective action.

—Implement a uniform system for recording consumer complaints to monitor the disposition of complaints at the local level and to provide headquarters' officials with a means of identifying industry and product problems affecting more than one district.

AGENCY ACTIONS AND UNRESOLVED ISSUES

GAO submitted a draft of this report to the Secretary, HEW, for comment. The views of FDA and HEW were discussed with GAO and included in the report. HEW concurred in GAO's recommendations and advised that a number of corrective actions had been or would be taken. (See pp. 17, 22, 32, 40, and 44.)

MATTERS FOR CONSIDERATION BY THE CONGRESS

In the light of the insanitary conditions shown to exist in the food manufacturing industry, the Congress should, upon receipt of a more accurate inventory of food plants under FDA's jurisdiction and an interim plan of action, considere the adequacy of FDA's inspectional coverage of food plants with the resources available under its current appropriation.

The Congress should also be aware that FDA relies almost entirely on State and local governments for inspectional coverage of some 500,000 restaurants and retail food stores that receive or ship products interstate. Inspections of these establishments by FDA to the extent necessary to judge whether such reliance is justified, would require the use of inspection resources.

To attain additional flexibility for enforcing the FD&C Act, the Congress should consider amending the law to provide for civil penalties when food sanitation standards are violated.

ASSOCIATION AFFAIRS

TESTING AND LABELING HIGHLIGHT 1972 DAIRY FIELDMEN'S CONFERENCE

Sidney E. Barnard
Extension Dairy Specialist
Pennsylvania State University

Mr. Glen Witte, Milk Industry Foundation, outlined the nutritional labeling of foods as proposed by the Food and Drug Administration. There is little

doubt that this will soon be a reality. More than 275 persons participated in the two day conference held at the Keller Conference Center at the Pennsylvania State University on June 6 and 7, 1972.

Dr. Fred Kroger, Food Scientist at Penn State, predicted that protein testing will be adopted when nutritional labeling is required. He reviewed present research involving protein testing of Milk and indicated that values were somewhat lower than previously accepted.

Universal sampling is being widely adopted by cooperatives in the northeast. Sidney Barnard, Extension Dairy Specialist at Penn State, outlined sampling and handling procedures, advantages, and demonstrated some of the sampling containers and cases now used.

George Fouse, Chief of the Division of Milk Sanitation, Pennsylvania Department of Agriculture, discussed the proposed changes in the standards of identity for dairy products which are expected to be adopted in Pennsylvania this summer. He and Dr. Charles Livak, Director of Quality Control, Penn Dairies, Inc. also reviewed a survey of current regulations for abnormal milk and antibiotics testing 11 northeastern states. Attempts are being made to have uniform regulations.

Interpretions of the USPHS Milk Ordinance and code were reviewed by George W. Hansen, Jr. Milk and Food Consultant from Region III. He stressed rigid enforcement of present regulations, including that covering buried well casings.

Three topics related to pollution control. Pesticides and PCB regulations and control were outlined by Delbert Bierlein, Pesticides coordinator at Penn State. Pollution of water, air and foods was reviewed by George B. Wolff. Roger Grout, Agricultural Engineer at Penn State outlined manure stocking procedures and the problems associated with it.

Two presentations were made on dairy barn design and construction. Rodney O. Martin, Director of Farm Systems Research for Agway outlined the factors influencing the design of milk production systems. The reorganization and remoldeling of the Penn State dairy production operation were outlined by Dr. Earl Kesler. The latest in milking and cooling systems is included with a milking parlor.

Dr. David Kradel and Dr. Robert Eberhart of the Veterinary Science Department at Penn State discussed an animal ecology research project and the latest in mastitis control.

Jack Kooker, Chairman of the Milk Market Referendum Committe presented the final proposal for promotion of dairy products to be financed by farmer funds, if aproved in a referendum. This would complement the federal order program in southeastern Pennsylvania.

William Johnstone, Extension Agricultural Economist at Penn State reviewed milk marketing in Pennsylvania. He expressed doubt for the retention of retail price control.

Dates of June 12-13 were set for the 1973 Dairy Fieldmen's conference.

FLORIDA ASSOCIATION OF MILK, FOOD AND ENVIRONMENTAL SANITARIANS

Dave Fry presented Sanitarians Annual Award by John Miller, (left) President at annual meeting.

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Dairy authorities speak out on better cow milking.

The importance of individual quarter milking

George D. Marx / University of Minnesota / Crookston

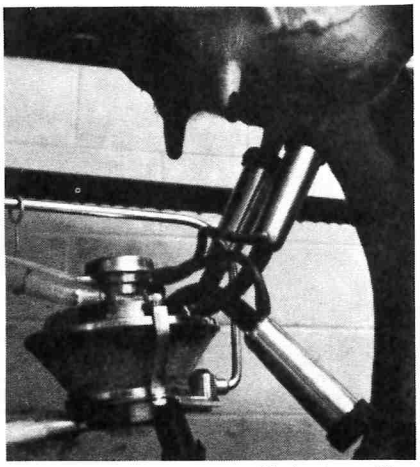
Characteristics of individual quarters of a cow's udder were studied using special equipment for milking 60 cows at the University of Minnesota, Northwest Experiment Station, Crookston. Sixteen of these cows were milked with a quarter milking machine at every milking during the entire lactation.

Factors such as milking time, machine stripping, amount and percent of complementary milk, incidence of mastitis and treatment, loss of milk on quarters with mastitis, bacterial organisms and abnormal physical condition of the udder and teat were studied.

The average milk yield of each quarter in percentage of total yield of the entire udder was 20.4% for the RF, 30.2 for the RR, 30.7 for the LR and 18.7 for the LF quarter. Similar percentages were obtained when comparing morning and evening milkings. Rear quarters produced 61% of the total milk and front quarters 39% for the total lactation; however, during the first and second month of lactation, rear quarters produced 58% and front quarters 42% of the total production and, by the ninth and tenth month of lactation, rear quarters were producing 66.5% and front quarters only 33.5% of the milk. Average daily production was 48.2 pounds.

Average milking time including machine stripping for the RF was 3.86 minutes, RR 4.42, LR 4.57, and LF 4.04 minutes. There were distinct differences between the milking rate of the higher-yielding rear and lower-yielding front quarters. Rear quarters produced an average of 0.54 pounds more milk per minute than front quarters, took 9% longer to milk and took 36% longer to machine strip.

Front quarters milk out faster than rear quarters, so dairymen should be prepared to take the machine off those quarters when they are milked out to avoid mastitis. Most herds have just as much mastitis on the front quarters as rear quarters, despite the lower amount of milk produced and less stress on front quarters. This high incidence of mastitis is likely caused by over-milking.



Complementary milk is the milk remaining in the cow's udder after normal milking is completed and can be obtained by giving oxytocin injections. Monthly checks of the cows in the experiment showed the percent complementary milk on the front two quarters averaged 17.8% and rear quarters averaged 14.4%. Cows with a high percentage of complementary milk will decrease in milk production faster during the lactation cycle than those with a low percentage. Individual quarter milking is likely to keep complementary milk to a minimum and still avoid the hazards of over-milking individual quarters.

MILKING PROCEDURE

Quarter milking is important but only one part of proper milking procedure. The program used at the Northwest Experiment Station at the University of Minnesota is keeping mastitis under control. The herd of 60 cows once had mastitis infections in 30% of the quarters but this has been reduced to below 1% at the present time, using the following type of milking management:

1. The strip cup is used at every milking to detect clinical mastitis.
2. Routine use of the CMT test to detect subclinical mastitis.
3. Organisms causing mastitis are identified by laboratory culturing of milk samples on every quarter four times a year.
4. Milking equipment is maintained and kept in good repair. Our policy has been to follow the manufacturer's recommendations.
5. A teat dip is used immediately following each milking.
6. We treat all persistent infections during the dry period.
7. Continual attention is given to proper milking procedures.

The ultimate goal for this research project and our general recommendations are to help dairymen get top production and a respectable margin of profit from dairying.