

August 1975
Vol. 38, No. 8
Pages 441-500
JMFTA 38(8): 441-500 (1975)

Journal of
Milk and Food
Technology



Official
Publication

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July 4th, 1975

Remember INDEPENDENCE DAY in 1776!

At Lexington and Concord, someone asked the British General, "who fired the first shot"? He said, "what does it matter, now"? The AMERICAN REVOLUTION was off and running towards individual "independence and freedom" for our country!

DECLARATION of INDEPENDENCE, CONSTITUTIONAL REPUBLIC and BILL of RIGHTS for "we the people" followed, whose lawful freedoms are threatened more than ever, today, by "industrial voluntary standards" depletion of America's remaining assets, and ugly "rip offs" of individual's rights by "uniformity and conformity" none laws.

With no Thomas Paine and his 89 page "common sense" to hot foot National and Individual lawful freedom; we as an independent Manufacturer of COMMERCIAL REFRIGERATORS, patented Easy Swing DOORS and ECONO-COVER night cover invention for "open supermarket freezer and refrigerator cases", with a patented new building facility "fireproofed" and upgraded items and new concepts in "display refrigeration" technology coming, many tell us that our, sometimes satirical, ELIASON "Lawful Competitive Free Enterprise" NEWSLETTER could have had much to do with our direct communications and survival.

Many NAS, IAMFES, NEHA and STATE public health sanitarians have urged us since we began EXHIBITING at annual NEHA conferences, some STATE seminars and advertising in the NEHA Journal, to develop the "MANUFACTURERS warranty/guarantee of Sanitary, Safety and Performance SPECIFICATIONS plan" that we published and copyrighted for FOOD RETAILERS and PUBLIC HEALTH SANITARIANS to use for MANUFACTURERS who would make a commitment, that their FOOD, SANITATION, HEALTH and SAFETY related products would fulfill the LAW, as well as valid PUBLIC HEALTH requirements made available through competitive upgrading.

From June 27 through July 2, we enjoyed the direct communication with delegates to the NEHA Annual Conference, in Minneapolis. Our EXHIBIT BOOTHS 1 and 2, were busily engaged in discussions on TRUTH IN SANITATION, SANITARY SPECS and VISIBLE CLEANLINESS that we advertise in the NEHA and other trade journals. Uncensored and open dialogue on all aspects of the scene, with much input towards the "viable warranty/guarantee plan" developments, were enjoyed in the Radisson Hotel's ELIASON Parlor Suite 1174.

On August 10 - 13, we hope to meet more delegates to the IAMFES "international" Annual Conference, at meetings and the ELIASON Parlor Suite "open forum", at the Royal York Hotel, in Toronto, Ontario, Canada. We also plan to bring our messages and learn a lot from IAMFES members, through ads in the IAMFES Milk Journal, whose two articles on "sanitarian's professional accountability and the common sense of sanitation performance requirements" were timely and appropriate in this frequently anticompetitive world.

Though we've EXHIBITED at industry trade shows since 1963 and at annual Sanitary Environmental national and some state conferences since 1969, with 20 years background in FOOD STORES, WAREHOUSES, RESTAURANTS and FARM MILK COOLING innovative upgrading, it is now time for more regional facility, product and services expansion, based on our belief that "best for the purpose high quality, long life modern designs, performance and safety in nutritive perishable foods" can move ahead sensibly by better direct communications between individual industry firms, users of equipment and products and municipal, county and state public health. Yes, we are honorably commercial, but "armed with the lawful prerogatives provided by our pioneer forefathers", may we all make it with FREEDOM, way beyond next May Day and the 4th of July 1776 celebration, in our American republics, where "viable longevity is our heritage difference" between just LUCK and our TALENTS!

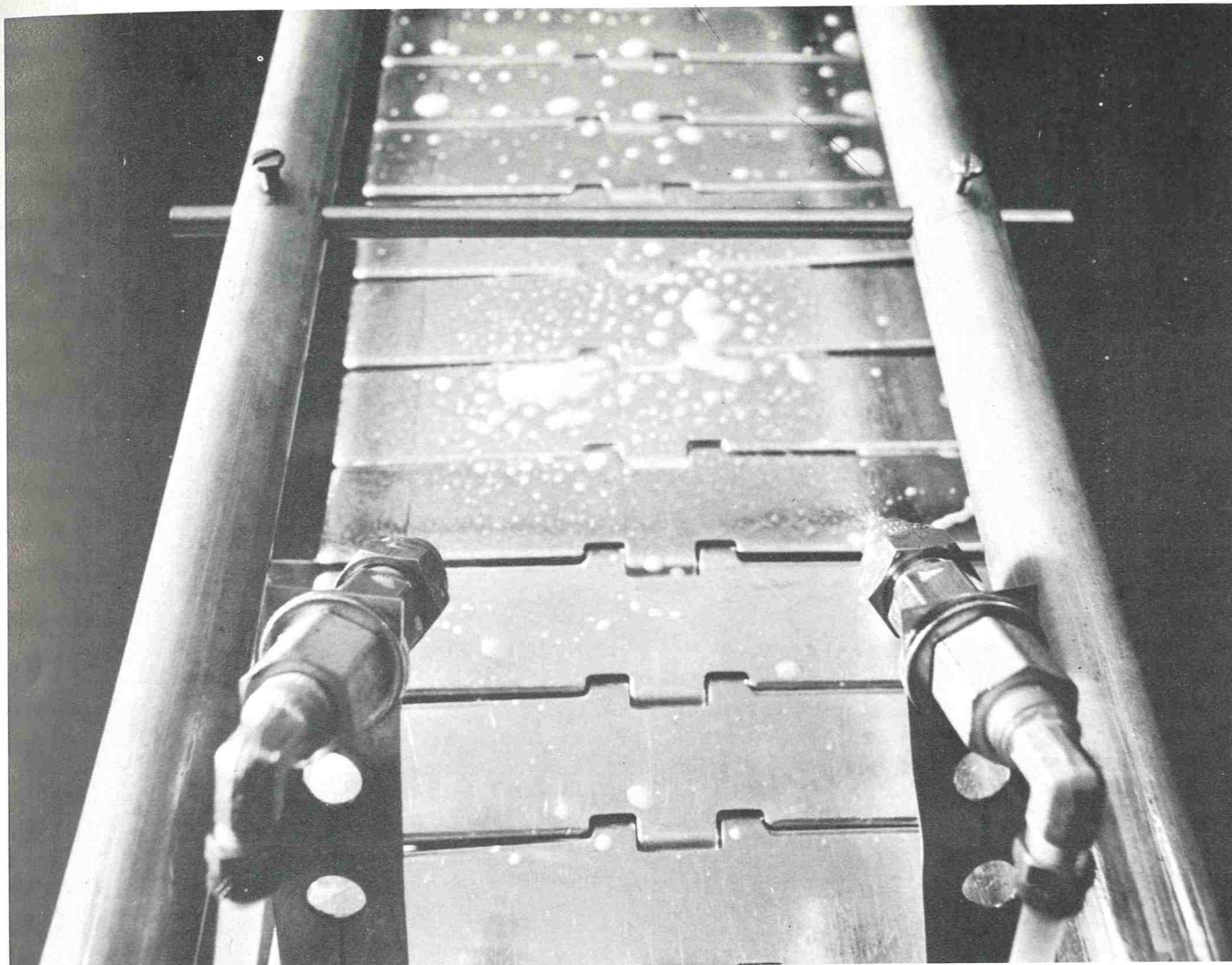
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Carl Eliason, President

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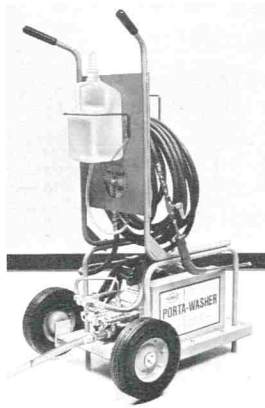
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2nd Class postage paid at Ames, Ia. 50010.
Editorial Offices: Dr. Elmer H. Marth, Dept. of Food Science, University of Wisconsin, Madison, Wis. 53706. Earl O. Wright, P.O. Box 701, Ames, Ia. 50010.

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1 yr. Vol. 38, 1975-----\$16.00
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Growth and Preservation Parameters for Preparation of a Mixed Species Culture Concentrate for Cheese Manufacture¹

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(Received for publication September 4, 1974)

ABSTRACT

The production of a frozen concentrated cheese starter by batch culture fermentation of a mixed species cheese starter culture consisting of *Streptococcus cremoris* and *Streptococcus diacetilactis* was investigated. A response surface analysis to optimize growth conditions was made using 20% NaOH or 20% NH₄OH as neutralizers and pH and temperature as variables. The yield of cell mass was about two-fold higher using NH₄OH as the neutralizer. The optimal growth conditions for the latter were pH 6.1 to 6.5 and 24 to 27 C. Optimal balance of species of one *S. diacetilactis* per six *S. cremoris* cells was observed from a growth pH of 6.1 to 6.4 and temperatures of 23 to 26 C using NH₄OH. A pH of 6.2 to 6.5 and temperature of 25 to 27 C yielded cells of highest acid-producing ability when NH₄OH was the neutralizer. Cultures grown at 25 C using NH₄OH were also more resistant to freezing than NaOH neutralized cultures. Glycerol was the only cryoprotective agent examined which protected the culture during freezing and frozen storage. Lactose and malic acid appeared to have a destructive effect on the starter concentrate during frozen storage. Conditions of growth and type of cryoprotective agent affected each species individually. Gouda cheese prepared from a frozen concentrate was equivalent in quality to that prepared from a conventional bulk starter culture.

In spite of many scientific and technological advances, the daily production of bulk starter in the cheese factory remains a cumbersome and time consuming process which is never entirely free from the risk of failure due either to the activity of bacteriophage or the presence of inhibitors in the starter milk. The replacement of bulk starter by concentrated starter cultures produced at a central plant and used immediately or preserved for later use would be desirable and has been the objective of current dairy research and development. During the last several years one of the major considerations was whether to use continuous or batch culture techniques for producing cell biomass.

Batch culture methods for producing concentrated starter cultures have been extensively investigated by many workers and in some instances put into commercial production (16). The main parameters of batch culture that have the greatest influence on increasing the cell biomass and obtaining a highly biological active culture are: (a) composition of the growth medium (2, 3, 12, 24),

(b) pH during growth and type of alkali used as the neutralizer (4, 18, 21), (c) temperature during growth (11, 15, 27), (d) time of fermentation (16, 26), and (e) method of harvesting the cells (4, 16, 25). However, it appears that only Gilliland (11) and Anderson & Leesment (1) have examined the growth relations on mixed strain starters concentrates as affected by the above parameters.

Several methods of preserving starter bacteria have been described and it has been established that freezing and storing the concentrates in liquid nitrogen (-196 C) is the best method for maintaining the viability and biological activity of the cells (6, 10, 13, 16, 18, 20, 25). The problems, however, that arise from the use of liquid nitrogen are many, i.e., the mass freezing of concentrates is not very effective due to small volumes that can be frozen at once and the high cost of the method, as well as the necessity of special equipment. It seems that the use of a method of freezing and storing the concentrated starters at temperatures between -10 to -30 C, which can be obtained easily by commercial freezers, should be a main research objective. In order to provide better viability and activity during freezing and frozen storage at subzero temperatures several compounds are used as cryoprotective agents (10, 14, 16, 17, 25). In all cases, neutralization of the lactic culture concentrates before freezing appears to be essential.

This paper describes the effects of growth conditions and type of alkali used as neutralizer on the total cell yield, balance of species, and activity of a mixed species starter culture used for Gouda cheese manufacture as well as the ability of the concentrated culture to withstand freezing and frozen storage at -10 and -30 C. The effect of several cryoprotective agents on the viability of the culture after frozen storage and on each component species, as well as on the activity of the starter upon freezing and frozen storage at -10 and -30 C was examined. Gouda cheese was made by using the frozen concentrates and was compared to cheese prepared from a normal bulk starter culture.

MATERIALS AND METHODS

Cultures

The mixed cheese starter culture was obtained from the collection maintained in the Department of Food Science and Nutrition,

¹Scientific Journal Series Paper No. 8820 Minnesota Agricultural Experiment Station, St. Paul, Minnesota 55108.

²This work was taken from a thesis submitted by J. D. Efstathiou to the faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Master of Science.

University of Minnesota. The culture, designated N cheese starter culture, originated from the Netherlands and has been propagated in our laboratory for many years. The N culture was grown in 11% reconstituted nonfat dry milk and maintained by incubating at 21 C until coagulation (18 h) and stored at 4 C between transfers.

Media

In order to isolate and identify the acid and aroma producers in the N mixed cheese starter, the medium of Galesloot et al. (9) was used routinely. The differential agar medium of Reddy et al. (19) also was used. Total count was estimated by means of Elliker's agar (8).

Growth studies

A complete factorial experiment was performed using a response surface design and analysis (7). The levels of pH and temperature that would give the maximum population, and the best component species balance, as well as the highest activity were estimated. The total number of trials was 10 and the levels of the factors were: pH 5.5, 20 C; pH 7.0, 20 C; pH 5.5, 30 C; pH 7.0, 30 C; pH 5.5, 25 C; pH 7.0, 25 C; pH 6.25, 20 C; pH 6.25, 30 C; pH 6.25, 25 C; and pH 6.25, 25 C. The experiments were performed twice using 20% NaOH or 20% NH₄OH as neutralizer, in order to find which would provide the highest population, activity and species balance under similar growth conditions. The growth medium was 11% nonfat dry milk. The milk was steamed for 60 min and cooled immediately. Agitation control was used (150 rpm) during growth. Cells were harvested after 18 h which corresponded to the late log to early stationary phase as confirmed by growth curve. Growth studies were performed using a microfermentor (New Brunswick, N.B., N.J.) with automatic pH, temperature and stirring speed control. The jar of the microfermentor was sterilized separately from the growth medium. The volume of medium used for each trial was 4L and the inoculum was 1% (v/v). Samples were drawn after 18 h and viability (total count, species balance) as well as activity determined. Activity was measured by means of a Beckman pH meter and Beckman automatic titrimeter, using 0.111 N NaOH and an end point of pH 8.5. The samples for activity determinations were obtained from 100 ml of 11% nonfat dry milk. The milk was inoculated with 1% (v/v) of cultures to be tested and incubated for 6 h at 32 C. Ten ml samples were used and the activity was measured by subtracting the titratable acidity of the milk from that of the test culture. Nutrient agar was used for the estimation of contaminants developed during the batch fermentation. All tests were performed in triplicate. At the end of the growth period, the temperature of the medium was dropped to 0 C and sodium citrate was added to a final concentration of 2%. The pH was then adjusted when necessary to 6.9 and the medium was allowed to stand for 10 min. The cells were harvested and concentrated by using a Sorval centrifuge (refrigerated) at 8,900 × g for 30 min. The cell mass was washed three successive times with 0.05 M phosphate buffer, pH 6.8, to clean the cells from residual milk which might contain undesirable fermentation end products. The cell mass was then resuspended in 11% nonfat dry milk at a rate of 3% of the original volume. Uniform resuspension was accomplished by using a Waring blender and blending the mixture (cells + milk + cryoprotective agent, if added) for 1.5 min. At this point count, species' balance as well as activity were determined by diluting the concentrates to their original volume (1/33). The balance of strains was the same since viability and activity showed a 4 to 5% decline compared to the culture before concentration. The concentrates were frozen and stored at -10 or -30 C in screw cap tubes (10 ml) or screw cap plastic bottles (125 ml). Cultures were periodically examined for their viability, strain balance and activity. The cultures were thawed quickly in a water bath (45 C), diluted to their original volume (1/33) and were plated. Dilution blanks contained 0.11% nonfat dry milk. The effects of several compounds as cryoprotective agents were examined, namely; 5% yeast extract, 7.5% lactose, 10% sucrose, 2% L-malic acid, 1% L-glutamic acid 0.06 M, pH 7.0, 10% glycerol and 5% N-Z amine NAK (percentages refer to the final concentration of the compound in the cell suspensions). The cultures were examined as above and controls (without cryoprotective agents) were run at the same time.

Cheese making trials

Gouda cheese was made from the same lot of milk by using the

frozen concentrates and conventional starter. Quantities of 125 ml of frozen concentrated starter cultures were thawed quickly in a 45 C water bath and 4 liters of 11% nonfat dry milk were inoculated and allowed to stand at room temperature for 1 h to give the organisms the opportunity to partially recover from frozen storage. The conventional starter before inoculation had a titratable acidity of 1.05 to 1.10% expressed as % lactic acid, and the frozen concentrate had an acidity of 0.25 to 0.45. The entire amount of frozen concentrate (125 ml) was used for vat inoculation (450 lbs of milk) and a 1% inoculum (v/v) from the conventional starter. Organoleptic evaluation of the cheese was made after one month and at one month intervals for up to four months.

RESULTS AND DISCUSSION

By using the differential agar media of Galesloot et al (9) and Reddy et al. (19), it was found that *S. cremoris* and *S. diacetilactis* were the component species of the N starter cultures. The total population was about 5.0×10^8 CFU/ml, and the ratio of component species (aroma/acid producers) was about 1/7 during the exponential to early stationary phase of growth.

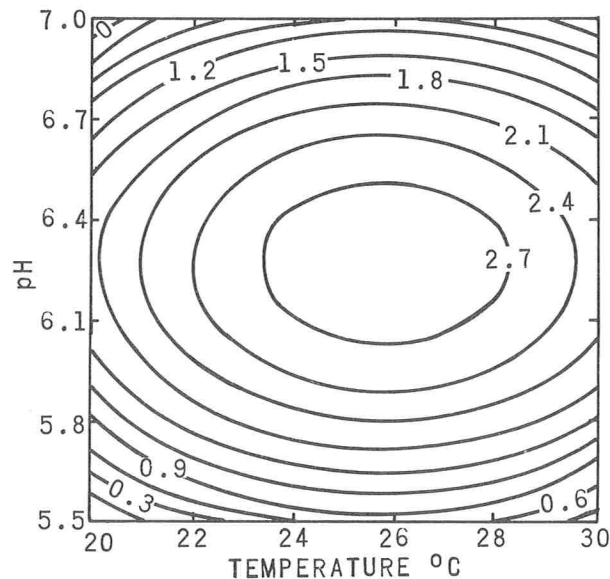


Figure 1. Response surface indicating yields of cell mass of N starter culture as influenced by the pH and temperature of growth, 20% NaOH was used as the neutralizer. Numbers identifying contour lines are yields of cell mass, e.g. a pH of 6.0 to 6.5 and temperature from 23.5 to 28.0 C is predicted to give a yield of at least 2.7×10^9 CFU/ml.

Figures 1 and 2 show that the highest viable count was obtained by using a pH between 6.10 and 6.45 and a temperature between 24.0 and 28.0 C, as well as pH from 6.0 to 6.50 and temperature from 23.5 to 28.0 C when 20% NH₄OH or 20% NaOH was used as neutralizer, respectively. From Fig. 4 it appears that the best strain balance (1/7) was obtained by using a variety of pH and temperature conditions, i.e., pH range 5.80 to 6.80 and 26.0 to 29.0 C as well as pH 5.80 to 6.80 and 20.0 to 23.0 C or pH 6.4 to 6.8 and 20.0 to 29.0 C as well as pH 6.1 to 5.8 and 20.0 to 29.0 C. The highest cell yield was also obtained at this range of pH and temperature. The best strain balance obtained using 20% NaOH as neutralizer was 1/8 which was obtained at pH 6.0 to 6.6 and 23.5 to 26.5 C (Fig. 3). The conditions necessary to

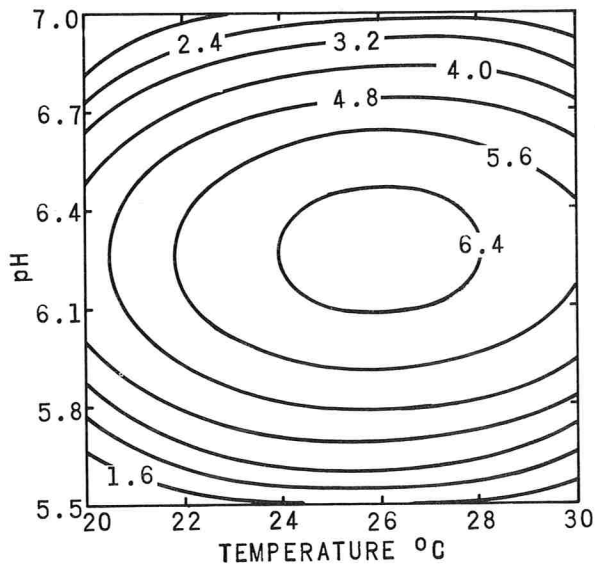


Figure 2. Response surface indicating yields of cell mass of *N* starter culture as influenced by the pH and temperature of growth, 20% NH_4OH was used as the neutralizer. Numbers identifying contour lines are yields of cell mass, e.g., pH of 6.10 to 6.45 and temperature from 24.0 to 28.0 C is predicted to yield at least 6.4×10^9 CFU/ml.

obtain the highest cell mass were also in this range. By conducting similar response surface analysis for the observed activities of the cultures, by using inocula having equal viable counts we found that when 20% NaOH was used as the neutralizer highest activity was obtained at pH 5.90 to 6.60 and 22.5 to 28.0 C, or at pH 6.25 to 6.45 and 25.0 to 27.0 C when 20% NH_4OH was used as the neutralizer (Fig. 5, 6). The optimal conditions necessary for growth using 20% NaOH as the neutralizer were a pH between 6.0 and 6.50 and a temperature between 23.5 to 26.5 C, or pH from 6.1 to 6.45 and a

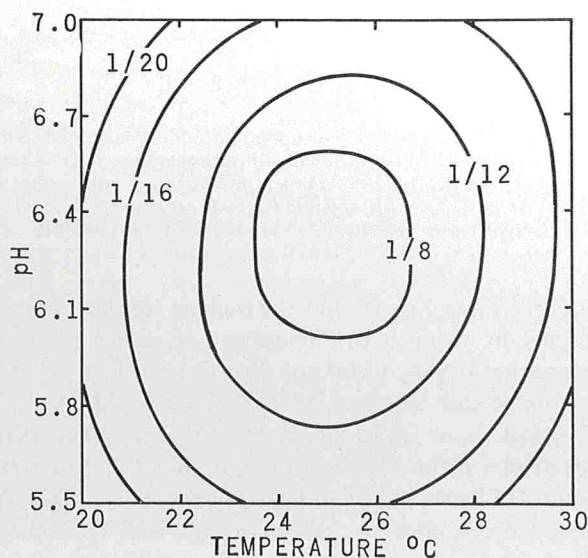


Figure 3. Response surface indicating balance of species in the *N* starter culture (*S. diacetylactis*/*S. cremoris*) as influenced by the pH and temperature of growth, 20% NaOH was used as the neutralizer. Numbers identifying contour lines are the species balance rates, e.g., pH of 5.75 to 6.80 and temperature from 23.5 to 26.5 C is predicted to give a strain balance of at least 1/12.

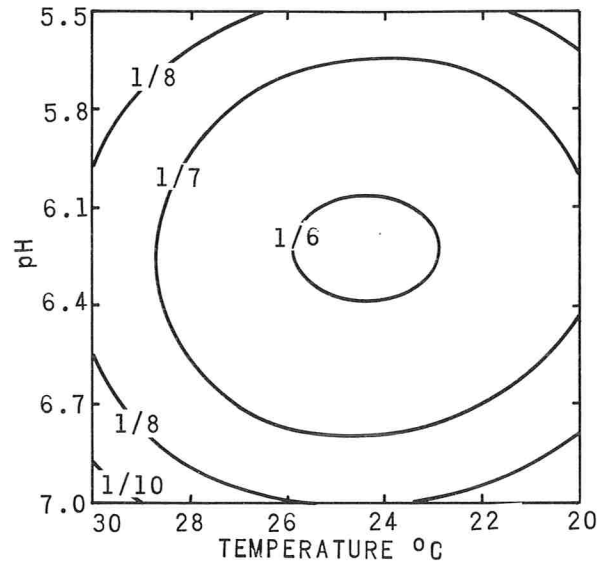


Figure 4. Response surface indicating balance of species of the *N* starter culture (*S. diacetylactis*/*S. cremoris*) as influenced by the pH and temperature of growth, 20% NH_4OH was used as the neutralizer. Numbers identifying contour lines are the species balance rates, e.g., a pH of 6.1 to 6.4 and temperatures from 23.0 to 26.0 C is predicted to give a strain balance of at least 1/6.

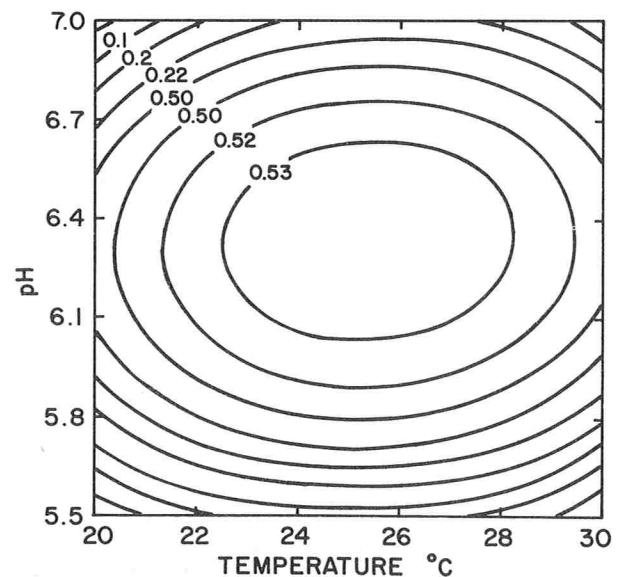


Figure 5. Response surface indicating activities of the *N* starter culture as influenced by the pH and temperature of growth, 20% NaOH was used as the neutralizer. Numbers identifying contour lines are the obtained culture activities, e.g., a pH of 5.90 and 6.60 and temperature from 22.5 to 28.0 C is predicted to give a culture having an activity of at least 0.53.

temperature from 24.5 to 27.0 C when 20% NH_4OH was used as the neutralizer. The use of NH_4OH gave a two-fold increase in cell yield, which agrees with results of other investigators (4, 11, 18, 22, 25). Concentrates prepared using NH_4OH were also found to have higher acid producing ability than those prepared with NaOH.

Several workers (3, 5, 18) reported that growth of lactic streptococci at pH 6.0 to 6.5 favors production of maximum cell numbers. However, these investigators worked with single strain starter cultures and did not

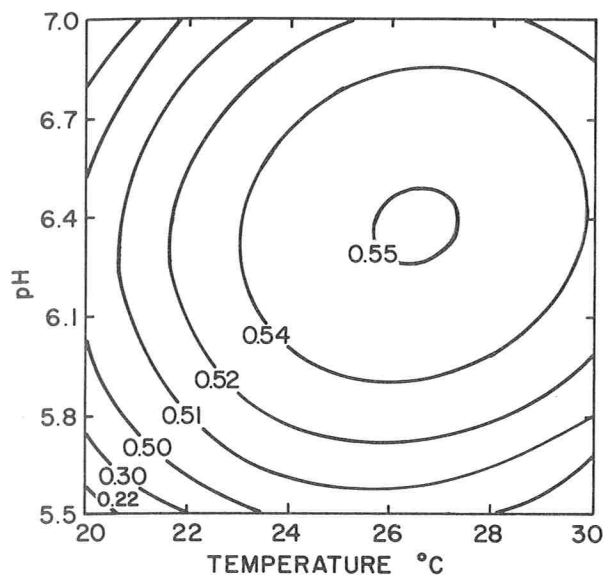


Figure 6. Response surface indicating activities of the *N* starter culture as influenced by the pH and temperature of growth, 20% NH_4OH was used as the neutralizer. Numbers identifying contour lines are the obtained culture activities, e.g., a pH of 6.25 to 6.45 and temperature from 25.5 to 27.0 C is predicted to give a culture having an activity of at least 0.55.

examine the effect of pH on species balance. In this study, pH and temperature appeared to affect the balance of strains but temperature appeared to be the predominant factor. It is known that every organism has its own requirements of pH and temperature for optimal growth. In the case of lactic streptococci, the optimum pH range appears to be more narrow than that for temperature. According to our results, a temperature of about 25 C was favorable not only for obtaining maximum population but also for good species balance. These results are in agreement with other investigators (2, 3, 15, 18). From our data it is obvious that the combined effects of pH, temperature and type of neutralizer during growth should be taken into consideration and not each variable individually. In order to confirm the computer obtained plots (Fig. 1-6), pH and temperature growth levels were selected randomly and the mixed starter culture was grown as described before. The total viable counts, the ratio of the component species and the biological activity were determined. The range of the obtained data was $\pm 5\%$ of the levels corresponding to the plots.

In preparing the starter concentrates the method of harvesting the cells was important. When Na-citrate was used and the pH of the medium was adjusted to 6.9, recoveries of cell yield up to 96% were obtained, compared to 75% without Na-citrate treatment. The adjustment of pH was necessary only when cultures were grown at pH 6.24 or less. These results agree with others (4, 11, 25).

This study showed that the most significant single factor influencing the survival of starter culture organisms was the temperature of freezing and frozen storage. In general, the highest recovery was obtained

when cultures were frozen and stored at -30 C in 11% nonfat dry milk with or without additives.

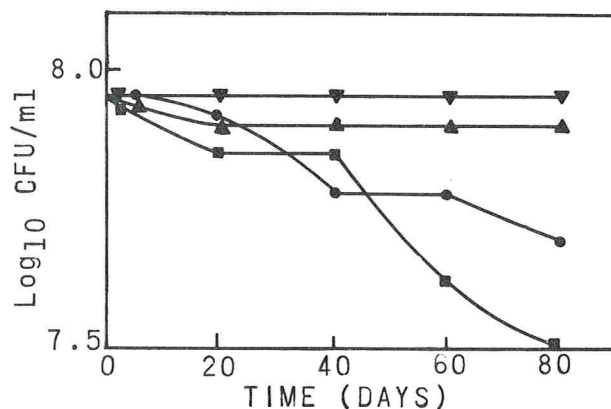


Figure 7. Effect of glycerol used as a cryoprotective agent on the ability of the *N* starter to withstand freezing and frozen storage at -10 and -30 C respectively. Cultures were grown at pH 6.25 and 25 C with 20% NaOH used as the neutralizer. Control (●—●) and with 10% glycerol added (▲—▲) cultures were frozen and stored at -10 C . Control (■—■) and with 10% glycerol added (▼—▼) cultures were frozen and stored at -30 C .

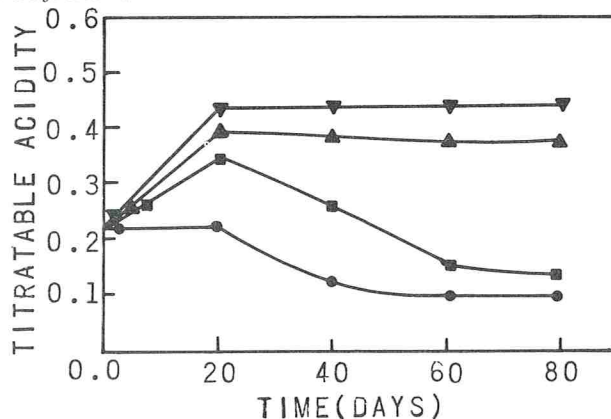


Figure 8. Effect of glycerol used as a cryoprotective agent on the ability of the *N* starter to maintain its biological activity upon freezing and frozen storage at -10 and -30 C respectively. Cultures were grown at pH 6.25 and 25 C with 20% NaOH used as the neutralizer. Control (●—●) and with 10% glycerol added (▲—▲) cultures were frozen and stored at -10 C . Control (■—■) and with 10% glycerol added (▼—▼) cultures were frozen and stored at -30 C .

Glycerol was the most advantageous in preserving the viability and biological activity of the *N* starter (Fig. 7, 8). This effect may have existed because of the $-\text{OH}$ groups which stabilized the conformation of the cellular constituents (17). Glycerol enhanced rather than just protected the biological activity of the frozen culture (Fig. 8). Gibson et al. (10) found that glycerol did have a protective effect on *S. lactis* and *S. cremoris* when these cultures were stored at -23.3 C , but retarded acid development during incubation after thawing.

Data not presented here show that only glycerol provided protection for *S. diacetylactis* and lactose, sucrose and L-malic acid did not provide protection to this aroma producer. L-glutamic acid and N-Z Amine NAK appeared to be equally protective for both species. Yeast extract appeared to affect equally both strains

when cultures were grown at pH 5.5 and 30 C with 20% NaOH as the neutralizer, but provided better protection to *S. cremoris* when 20% NH₄OH was used as the neutralizer. Both component organisms appeared to be more resistant to freezing and frozen storage when grown at 25 C with NH₄OH used as the neutralizer. When NaOH was used as the neutralizer the resistance of *S. diacetilactis* seemed to be influenced more (depending on the growth conditions) than that of *S. cremoris*. The results indicate that careful selection of the proper cryoprotective agent as well as strain selection (11, 23) should be the main consideration in preparing frozen starter concentrates.

Cheese prepared by using the concentrated frozen N culture stored at -10 to -30 C for as long as 150 days was evaluated after one month and at one-month intervals for up to four months for taste and flavor. During the cheese making process the frozen starter culture exhibited a delay in acid production, but the final pH of the cheese was the same as that of the control. The cheese prepared by using the frozen starter concentrates was equal or better in quality than that prepared by using conventional N starter culture. The cheese also did not develop any unusual organoleptic characteristics during storage (up to 3 months). However, the most striking property of the cheese was that it did not develop bitterness which was present in the control cheese.

The results suggest that appropriate growth conditions must be chosen to obtain a high cell yield with high biological activity and optimum species balance able to withstand freezing and frozen storage. Between the two sub-zero temperatures employed, -30 C was the better storage temperature. Of the cryoprotective agents examined, only glycerol was found to be protective to both cell viability and biological activity. However, some of these compounds appeared to individually affect the viability of the species or their acid producing ability. Further investigations on how freezing and frozen storage influence the membrane system and biological activity of each species are required.

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Behavior of Selected Food-Borne Pathogens in Raw Ground Beef

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(Received for publication May 2, 1975)

ABSTRACT

Raw ground beef was inoculated with five strains each of *Escherichia coli*, enterococci, salmonellae, staphylococci, *Bacillus cereus*, and *Clostridium perfringens*. Changes in population levels of these organisms, psychrotrophs, and total aerobic flora as these were influenced by temperature and packaging film were recorded. Among the organisms inoculated, only *E. coli*, salmonellae, and the enterococci were able to grow and then only at the highest test temperature (12.5 C). As expected, the packaging film did not influence the behavior of any of the test organisms. These results and the fact that a cooking step is involved demonstrate why ground beef is very rarely involved as a vehicle in bacterial food poisoning. This study indicates that there is no reason to expect protection of public health to evolve from bacteriological standards which limit numbers of non-pathogenic organisms.

Many questions are being raised about formulation, preservation, and microbiological safety of various foods. Many, if not most, of these questions are valid and the answers to them will either allow us to breathe easier or to modify what we have been doing to minimize hazards that might exist. This desirable goal can only be attained by supplying scientifically derived answers to scientifically relevant questions. Unfortunately, in our zeal to make things better, often legislation is drafted that is not based on scientific knowledge and time, effort, and money are wasted while the problems still exist.

The microbiological content of our raw meat supply has received attention recently (1, 5, 6). At least one state (Oregon) has adopted legislation that imposes microbiological standards for fresh meats. Several other states currently are considering similar legislation. The purpose of this study was to measure the behavior of food-borne pathogens in raw ground beef stored under a variety of conditions simulating commercial or consumer practice and to see if this behavior bears any relationship to the aerobic plate count of the product.

MATERIALS AND METHODS

Organisms

Ground beef was inoculated with: *Salmonella typhimurium*, *S. illinois*, *S. infantis*, *S. london*, and *S. tennessee*, five strains of *Escherichia coli*, five strains of *Bacillus cereus*, five enterotoxigenic strains of *Clostridium perfringens*, five strains of enterococci (three strains of *Streptococcus faecalis* and two strains of *S. faecium*) and five strains of *Staphylococcus aureus* chosen to include producers of A, B, C, D and E enterotoxins. Cultures were maintained on appropriate laboratory media until needed.

Preparation of test cultures

Salmonellae, *E. coli*, *S. aureus*, and *B. cereus* were grown in trypticase soy broth. *Clostridium perfringens* was grown in thioglycolate broth and the enterococci in APT broth. Each culture was decimally diluted and plated to enumerate the organisms present so that these standardized cultures could be diluted and pooled to achieve a predetermined level for inoculation into the meat. The levels varied for each organism and were chosen with the following considerations; (a) that the level exceeded the base line level of that species already present in the frozen product and (b) that the selected level be such that enumeration would be facilitated.

Meat

The meat employed in this study was ground beef, produced commercially, and supplied in the frozen state as one-quarter pound patties by Otto Meat Products, Inc., West Chicago, Illinois.

Inoculation, packaging and incubation

The beef patties were thawed and placed in a Hobart mixer (model D-300). The mixing, inoculation, and packaging were done in a cold room at 2 C. The mixture of bacterial cultures was added to the meat at a rate of 60 ml to 100 lb. The inoculum was added dropwise to the meat mass and mixing was continued for an additional 15 min.

After mixing, 200-g portions of meat were removed and wrapped in cellophane or saran. The wrapped meat was then stored at the desired test temperature (i.e. 1, 4.5, 7, and 12.5 ± 0.5 C).

Enumeration procedures

At selected intervals, four samples representing the specific temperature-packaging film combination were removed from storage. Twenty-five grams from each package was composited to make a 100-g test sample. This test sample was homogenized in a blender (Waring 5011) in 900 ml 0.1% peptone water. Aliquots of the homogenate were then taken for quantitation of the specific test organisms as follows:

(a) *Salmonellae*. Salmonellae were enumerated by the 3-tube most probable number (MPN) procedure employing nutrient broth pre-enrichment (24 h, 37 C) enrichment in selenite-cystine broth (24 hr 37 C) followed by streaking on brilliant green sulfa agar (24 hr 37 C). The lowest dilution employed was 10 ml of the homogenate (representing 1 g of meat). Suspicious colonies appearing on the agar medium were confirmed by picking to lysine iron agar and by a positive reaction in polyvalent H agglutination tests.

(b) *E. coli*. *E. coli* were enumerated by the 3-tube MPN procedure. Aliquots of the relevant dilutions were inoculated directly into EC broth for incubation of 45.5 C. Tubes showing gas after 24 h were streaked onto EMB agar for production of typical *E. coli* colonies. It was previously determined that each of the test strains employed would form gas in EC broth at 45.5 C.

(c) *Enterococci*. Enterococci were quantitated by employing pour plates of KF agar incubated at 37 C for 48 h.

(d) *Staphylococci*. *S. aureus* was enumerated by surface plating on Baird-Parker agar which was then incubated at 37 C for 24-30 h. Colonies (usually five) evidencing the typical *S. aureus* reaction on this medium were selected from the countable plate and tested for coagulase production by the tube method using rabbit plasma containing EDTA.

(e) *B. cereus*. *B. cereus* were enumerated by surface plating on KG agar (3) and incubating at 32 C for 24 h.

(f) *C. perfringens*. Pour plates of tryptose-sulfite-cycloserine agar from which the egg yolk was omitted as described by Hauschild and Hilsheimer (2) were employed. Incubation was in Gas Pak (BBL) under H₂ and CO₂ (Gaspak, BBL) at 37 C for 24 h.

(g) Total aerobic plate count. Plate count agar pour plates were incubated at 25 C for 72 h.

(h) psychrotrophs. Psychrotrophs were enumerated in pour plates of plate count agar which were incubated at 4.5 C for 10 days.

Each test organism was enumerated without regard to the identity of the strain or serotype that persisted longest or proliferated to the greatest extent. Similarly, total aerobic plate counts and psychrotroph counts were made with regard to numbers only and identification of predominant species or groups was not undertaken.

RESULTS AND DISCUSSION

The microbiological profile obtained from five separate samples of the uninoculated ground beef is summarized in Table 1. This products was good quality

TABLE 1. *Microbial flora of ground beef employed in this study*

Organism	No./g ¹	Range (per g)
Total aerobic count	230,000	144,000-320,000
Psychrotrophs	87,000	34,000-112,000
Salmonellae	<0.03	—
<i>S. aureus</i>	3,200	2,100-4,000
<i>B. cereus</i>	100	100
<i>E. coli</i>	155	75-240
<i>C. perfringens</i>	100	100
Enterococci	4,400	3,900-5,200

¹Average of 5 separate samples.

beef and contained a microbial flora representative of what would be expected under good manufacturing processes.

The temperatures of incubation were chosen with regard to simulating what might be encountered in the distribution chain. The range includes what might be described as ideal (1 C) and what would be significant abuse (12.5 C) yet still be termed refrigeration. It is realized that this approach can be only an approximation at best since in practice one would expect the meat to undergo cyclic variations in temperature.

Similarly, saran and cellophane were chosen to represent 'anaerobic' and 'aerobic' packaging, respectively. The packaging material used for raw ground beef had little or no influence on the behavior of organisms of public health significance during the shelf life of the product. This is not surprising since with the exception of *C. perfringens* all of the test cultures are facultative anaerobes. Changes in the population of test organisms, psychrotrophs, and total aerobic flora as these are influenced by film wrap and temperature are shown in Tables 2a-h. Although to our knowledge, this approach of monitoring the behavior of various food-borne pathogens in raw ground beef as a function of temperature and type of wrap has not been undertaken before, the data obtained are not surprising and probably would be predictable by knowledgeable microbiologists.

TABLE 2. *Influence of storage temperature and wrapping film on the survival and growth (number per gram) of selected bacteria in raw ground beef*

TABLE 2A. SALMONELLAE		1		4.5		7		12.5	
Temperature of Storage (°C):									
Type of film wrap ¹ :	C	S	C	S	C	S	C	S	
Time of storage (days)									
0		430	430		210			210	
1			210	150	150	150	430	150	
2			460	210	150	150	2100	1500	
3	430	150	240	460	210	150	24000	11000	
4							24000	24000	
5	240	23	460	240	460	240	93000	93000	
7	93	460	460	93					
10	460	460							
14	93	23							

TABLE 2B. *C. PERFRINGENS*

0		1,000	1,500		1,000		1,100
1			<100	100	<100	<100	100
2			100	100	<100	<100	150
3	150	140			170	180	25
4							<10
5	100	100	15	30	10	40	5
7	65	85	15	15			
10	45	75					
14	15	10					

TABLE 2E. ENTEROCOCCI

0	16,000		17,000		13,000		16,000
1			16,000	15,000	14,000	17,000	16,000
2			20,000	17,000	15,000	19,000	22,000
3	21,000	17,000	21,000	16,000	23,000	20,000	14,000
4							21,000
5	20,000	25,000	12,000	34,000	12,000	24,000	6,500
7	19,000	18,000	16,000	24,000			6,300
10	20,000	51,000					
14	18,000	12,000					

TABLE 2D. *B. CEREBUS*

0	900		1000			1100		1000
1			450	350	700	650	500	400
2			400	550	500	450	550	100
3	350	450	150	100	350	300	100	100
4							100	100
5	100	150	150	100	100	100	100	100
7	100	100	100	100				
10	100	100						
14	100	100						

TABLE 2E. ENTEROCOCCI

0		39000		34000	51000		57000	
1			29000	47000	35000	40000	29000	41000
2			45000	28000	35000	42000	190000	180000
3	45000	33000	38000	29000	45000	20000	700000	580000
4							1,100,000	1,100,000
5	47000	40000	30000	34000	38000	45000	4,100,000	2,100,000
7	44000	55000	41000	47000				
10	50000	51000						
14	42000	39000						

TABLE 2F. *E. COLI*

0	75			43	93			93
1			240	430	43	93	430	93
2			240	43	93	43	2,400	240
3	93	43	240	43	150	23	24,000	4,600
4							>24,000	11,000
5	43	23	43	43	240	75	46,000	240,000
7	23	43	23	75				
10	23	23						
14	43	23						

TABLE 2G. PSYCHROTROPHS

0	101,000			91,000		92,000	66,000	
1			83,000	92,000	130,000	120,000	220,000	180,000
2			310,000	230,000	170,000	170,000	2,100,000	2,500,000
3	110,000	160,000	1,300,000	920,000	1,400,000	450,000	50,000,000	54,000,000
4							320,000,000	190,000,000
5	460,000	350,000	380,000,000	97,000,000	140,000,000	57,000,000	620,000,000	1,200,000,000
7	4,000,000	3,000,000	2,600,000,000	850,000,000				
10	150,000,000	57,000,000						
14	620,000,000	660,000,000						

TABLE 2H. AEROBIC PLATE COUNT

0	350,000		170,000			200,000	210,000	
1			340,000	250,000	220,000	440,000	540,000	500,000
2			420,000	340,000	360,000	320,000	30,000,000	23,000,000
3	200,000	240,000	1,600,000	560,000	2,200,000	650,000	450,000,000	460,000,000
4							520,000,000	280,000,000
5	660,000	310,000	360,000,000	85,000,000	90,000,000	43,000,000	630,000,000	1,200,000,000
7	2,700,000	2,000,000	1,200,000,000	900,000				
10	150,000,000	50,000,000						
14	490,000,000	500,000,000						

¹C = cellophane
S = saran

Of all the pathogens employed in this study, only salmonellae were able to proliferate and only at the 12.5 C storage temperature. It is true that a product containing 93,000 or even 24,000 salmonellae/g would constitute a potential public health hazard. However, there are several mollifying considerations which must be borne in mind before signaling an alarm at these findings. First, the growth of salmonellae in this product is a function of the initial population not only of salmonellae but also of the normal meat flora. The number of salmonellae inoculated into the meat in this study is very much higher than one would expect to occur in naturally contaminated raw ground beef. Investigation has shown that the frequency of salmonella contamination in raw ground beef is very low

(4, 6) and it is reasonable to expect that contaminated beef would contain less than 1 salmonella/g. Thus the initial level employed in this study is at least 100 times higher than would likely be encountered in practice. Moreover, the microbiological quality of the meat used in this study was very high resulting in an unrealistically low normal flora to salmonellae ratio. Even with tipping the balance in favor of salmonellae in this fashion, these pathogens were only able to attain a level of 1500-2100/g after 2 days. At 3 days the number of salmonellae had increased significantly but the normal flora numbered in the hundreds of millions (Table 2h) and the ground beef was organoleptically unacceptable. It is extremely unlikely that anyone would eat the meat raw with the odor of spoilage as evident as it was.

The second consideration is that with the exception of a few specialty items or ethnic dishes consumption of raw ground beef in this country is not a common occurrence. Cooking the ground beef would certainly reduce the number, if not completely eliminate viable salmonellae, thus eliminating any public health hazard.

This combination of circumstances, i.e. necessity for a high initial load of salmonellae in the presence of a low level of normal meat micro-organisms, protracted temperature abuse, and consumption of raw meat in an incipient state of spoilage make it highly unlikely that raw ground beef would ever be a significant source of human salmonellosis. This tenet is reinforced by the paucity of documented cases of human salmonellosis definitively ascribed to ingestion of raw beef.

Of the other test organisms, only *E. coli* and the enterococci evidenced appreciable increase in number and then only under conditions of refrigerator abuse (i.e. 12.5 C). The evidence linking enterococci to human food poisoning is tenuous and there is some question whether this organism is truly pathogenic.

E. coli behaved in much the same fashion as salmonellae and although some strains of this species are enteropathogenic for humans the conditions necessary for food poisoning due to *E. coli* in raw meat are as unlikely as they are for salmonellae.

The three gram positive pathogens, *S. aureus*, *B. cereus*, and *C. perfringens* were unable to proliferate in the ground beef as it was stored in this project. This likely is due to various factors, e.g. (a) temperature of storage was below minimum growth temperature for the species (b) inability to compete with the normal flora of the meat, or (c) sensitivity of vegetative cell form to low temperature resulting in a loss of viability (most pertinent to *C. perfringens*).

This study has shown that food poisoning microorganisms are unable to compete effectively with the natural flora of raw ground beef over a wide range of refrigeration temperatures. This fact has several ramifications. First, it demonstrates why bacterial food poisoning is so rarely attributed to raw ground beef. Second, and almost equally important, it negates the

feasability of protecting the public health by establishing microbiological standards for raw ground beef. There is no evidence in the scientific literature nor was any generated in this study to suggest a correlation between the total aerobic plate count or *E. coli* levels in raw ground beef and the potential of that material to cause food poisoning.

The promulgation of microbiological standards limiting nonpathogens in raw meat products in the desire to protect public health is an exercise in futility that quite likely may result in either unenforceable legislation or considerable economic loss and wastage of a valuable source of protein.

NOTE ADDED IN PROOF

During preparation of this manuscript, an article appeared in *Morbidity and Mortality Weekly Reports* (Vol. 24, No. 27, Pages 229-230) which summarizes the available epidemiologic data on the involvement of ground beef in food-borne illness.

ACKNOWLEDGMENT

Research supported by the College of Agricultural and Life Sciences, University of Wisconsin, Madison. The authors wish to acknowledge the generosity of Dr. R. H. Deibel, Dr. C. Duncan, and Dr. M. S. Bergdoll who supplied the cultures of enterococci, *C. perfringens* and staphylococci, respectively.

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Comparative Validity of Members of the Total Coliform and Fecal Coliform Groups for Indicating the Presence of *Salmonella* in the Eastern Oyster, *Crassostrea virginica*

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(Received for publication December 11, 1974)

ABSTRACT

During a 24-month survey, 539 samples each of the Eastern oyster, *Crassostrea virginica*, and the overlying water were collected to determine the relation of most probable number (MPN) of the total and fecal coliform groups in shellfish and water to the presence of *Salmonella* in the shellfish themselves. Occurrence of *Salmonella* in the shellfish more closely paralleled a progressive increase in the fecal coliform MPN as compared to the total coliform MPN in the water and shellfish meat. The percentage of *Salmonella*-positive shellfish samples was somewhat higher in oysters harvested from waters conforming to the present bacteriological approved growing area standard of ≤ 70 total coliforms per 100 ml water as compared to these same waters meeting a recently proposed fecal coliform standard of ≤ 14 organisms per 100 ml. In no instance was *Salmonella* detected in oysters from growing areas officially approved for harvesting on the basis of both a bacteriological and sanitary survey. Of a variety of enrichment broths and plating media used for recovery of *Salmonella* from oysters, direct enrichment in tetrathionate broth with added brilliant green followed by streaking on bismuth sulfite agar was the most productive combination of media for recovering a large variety of *Salmonella* serotypes.

The routine analysis of shellfish-growing waters for the presence of pathogens remains an impractical task for state and federal officials charged with guaranteeing the quality of shellfish. The concept of utilizing coliforms to infer the presence of pathogens is based on work of Excherich (5) who, in 1885, identified *Bacillus coli* as being part of the natural intestinal microflora in warm-blooded animals. During the 90 years that followed, published reports appeared both supporting (9, 10, 13) and criticizing (6, 7, 11) the use of coliforms to infer the presence of such pathogens as *Salmonella*, *Shigella*, *Vibrio*, *Pasteurella*, *Leptospira*, and enteroviruses in fresh waters.

The National Shellfish Sanitation Program (NSSP), a cooperative organization of representatives from the

shellfish-producing states, private industry, and the Food and Drug Administration (FDA), has traditionally utilized the total coliform group to assess the bacteriological quality and safety of shellfish-growing waters. At the 1974 NSSP National Workshop (Proceedings of the Eighth NSSP Workshop, *in press*) a proposal was made to substitute a fecal coliform standard for the total coliform standard. Anticipating such a proposal, it was decided to determine the comparative validity of members of the total coliform and fecal coliform groups for indicating the presence of *Salmonella* in shellfish. Results of this survey are detailed in this paper.

MATERIAL AND METHODS

Sampling areas and collection of samples

The Eastern oyster, *Crassostrea virginica*, was chosen for this study because more is known and has been published about the bacteriology and physiology of this species of shellfish (8), it is the most economically important shellfish species in this country, and it is readily available. Water and shellfish samples were collected by the Northeast Technical Services Unit (NETSU) located at Davisville, Rhode Island, and the Gulf Coast Technical Services Unit (GCTSU) located at Dauphin Island, Alabama, both of which are field facilities of FDA's Shellfish Sanitation Branch. Each field unit routinely collected up to eight shellfish samples monthly from an area approved for harvesting and a similar number of samples from an adjacent, conditionally approved, restricted, or prohibited area. These sampling areas were all classified in accordance with the specification of the NSSP Manual of Operations (17). From each shellfish sampling site, a sample of surface water immediately overlying the shellfish bed was collected in sterile glass containers and determinations of salinity (conductometric method), temperature, and content of total coliforms and fecal coliforms were made. Water samples were iced at or below 10 C during transportation to the laboratory in accordance with American Public Health Association (APHA) recommendations (1). In no instance did more than 30 h elapse between collection and initiation of analysis of the water samples.

Rather than restricting the survey to a limited number of sampling sites, it was decided that sampling from a varied and extensive geographical area would be more compatible with the objectives of this study. NETSU was responsible for collecting samples from estuaries in Rhode Island, Connecticut, New York, New Jersey, and Maryland, whereas GCTSU collected samples from South Carolina, Florida, Alabama, Mississippi, and Louisiana. Water depth over the actual sampling areas ranged from 1 to 40 ft. Salinities ranged from <1.0 to 30.0 parts per thousand, and water temperatures ranged from 0.8 to 18.8 C during November-April and from 16.6 to 30.5 C during May-October. The survey was initiated on July 1, 1972, and ended on June 30, 1974.

From each sampling site, enough shellstock was collected to yield a sample of 500 g of shellfish meat and liquor. Additionally, a surface water sample of 150 to 200 ml was collected at each sampling site.

Coliform analysis

Before being shucked, the shellstock was scrubbed thoroughly to remove loose material and debris from the shell surface. Shellfish meats were tared into sterile 1-pint polyethylene jars. To effect an even distribution of the analytical work load, all water samples and those shellfish samples from approved growing areas were analyzed by the field units, and all shellfish samples from non-approved areas were sent to the Division of Microbiology laboratories in Washington, D.C. Polyethylene jars of shellfish samples were placed in insulated mailing containers (Polyfoam Packers Corp., Chicago, Ill.), packed with Polar Ice refrigerant (Divajex, Santa Ana, Calif.), and mailed air express. Preliminary experiments demonstrated the capacity of this system to maintain the shellfish at 4 to 7 C for 24 h.

Two hundred grams of the shellfish sample were used for determining the total coliform and fecal coliform content and the remaining 300 g were reserved for the analysis of *Salmonella*. The most probable number (MPN) technique was used to quantitate levels of total coliforms and fecal coliforms in the shellfish meats and waters. Dilutions of water samples were directly inoculated into lauryl sulfate tryptose (LST) broth (Difco) and 200-g oyster samples were each blended in a high-speed blender with an equal weight of phosphate buffer dilution water for 60-120 sec at approximately 14,000 rev/min. Appropriate dilutions were made of the oyster homogenate and inoculated into LST broth. Tubes of LST broth, inoculated with dilutions of the water or shellfish samples, were incubated at 35 ± 0.5 C and at 24- and 48-h intervals. Sterile applicator sticks were used to make transfers from gassing tubes of LST broth to tubes containing brilliant green lactose bile (BGLB) broth (Difco). Cultures producing gas in BGLB broth after 48 h of incubation at 35 C were classified as total coliforms. Fecal coliform confirmation was made by transferring all tubes of LST broth positive for gas after 24 to 18 h of incubation at 35 ± 0.5 C to EC medium. The EC medium was incubated in a water bath at 44.5 ± 0.2 C for 24 h. All cultures producing gas in EC medium under these conditions were classified as fecal coliforms.

Salmonella analysis

Both the lactose pre-enrichment and direct selective enrichment procedures of the *Bacteriological Analytical Manual* (BAM) (18) were used in the *Salmonella* analysis of the oysters. The 300-g reserves of the shellfish samples were divided into three 100-g portions, each of which was blended in a high-speed blender for 60-120 sec at 14,000 rev/min with 150 ml of 35 C-tempered lactose, selenite cystine (SC), or tetrathionate (TT) broth containing 10 mg of brilliant green dye per liter. The homogenates were poured into flasks containing the re-

mainder (750 ml) of the 900 ml of the respective broths. After adjusting the pH of the broth mixtures to 6.8 ± 0.2, flasks were incubated in a walk-in incubator at 35 C. After 24 h of incubation, 100 ml of lactose pre-enrichment mixture was subcultured to flasks containing 900 ml of fresh, 35 C-tempered SC or TT broth. After 24 and 48 h of incubation, the contents of the flasks containing the selective enrichment broths were streaked with a 3-mm loop to plates of brilliant green, bismuth sulfite (BS) (Difco), and *Salmonella-Shigella* agar. All plates were incubated and examined after a 24-h incubation period. The BS agar plates were examined after 24 h, but were incubated an additional 24 h at which time they were re-examined. When present, at least two colonies suspicious for *Salmonella* were picked per plate in tandem to triple sugar iron agar and lysine iron agar. Cultures giving reactions typical for *Salmonella* were screened biochemically (4). The somatic and serological grouping, as described by Edwards (3), was followed by definitive serotyping (4).

RESULTS AND DISCUSSION

The relationship between the levels of total coliforms and fecal coliforms in water to the presence of *Salmonella* in the shellfish harvested from these waters was established (Table 1). The present growing-area

TABLE 1. Correlation of *Salmonella* in oysters to the total coliform and fecal coliform densities of the overlying waters

Indicator organism	Indicator organism density in overlying waters per 100 ml	Number of oyster samples examined	<i>Salmonella</i> -Positive	
			Number	Percent
Total coliform	0-70	199	15	7.5
	71-200	105	13	12.4
	201-1,000	144	15	10.4
	>1,000	91	17	18.7
	Total	539	60	
	Overall average			11.1
Fecal coliform	0-14	255	6	2.4
	15-70	118	6	5.1
	71-200	59	13	22.0
	201-1,000	66	18	27.3
	>1,000	41	17	41.5
	Total	539	60	
	Overall average			11.1

standard allows the harvesting of shellfish from waters having a total coliform MPN of <70 organisms per 100 ml of water (17). The proposed standard would allow <14 fecal coliform organisms per 100 ml of approved shellfish-growing water (Proceedings of the Eighth NSSP Workshop, *in press*). Except for these two categories, the categories of water MPN in Table 1 are arbitrary. In the shellfish harvested from waters within allowable limits of total coliform and fecal coliform levels, 7.5 and 2.4%, respectively, of the samples contained *Salmonella*. There was no level of fecal coliforms in the water below which *Salmonella* could not be recovered from the shellfish meats. One *Salmonella*-positive shellfish sample was harvested from water having a total coliform and fecal coliform MPN of 11 and <1.8, respectively. However, all *Salmonella*-positive shellfish samples were obtained from waters which did not meet approved growing-area criteria. Waters which may be intermittently polluted will at times meet the bacteriological standards but fail to meet the sanitary survey criteria needed to be

classified as an approved shellfish-growing area. Demonstration of increased recovery of *Salmonella* from shellfish harvested from waters with a progressive increase in total coliform MPN was not apparent. With the fecal coliform group, however, there was an increase in the number of *Salmonella*-positive oyster samples with a progressive increase in the MPN of the overlying waters.

In addition to a growing-area standard, a market quality standard is used to indicate the degree of process and handling abuse of the product after harvesting. Shellfish having a fecal coliform MNP of ≤ 230 per 100 g and a total plate count not in excess of 500,000 organisms/g of shellfish meat are considered to be of acceptable quality at the wholesale market (17). With the exception of this category of fecal coliform MPN, the range of MPN categories shown in Table 2 is arbitrary.

TABLE 2. Correlation of *Salmonella* in oysters to the total coliform and fecal coliform densities in oysters

Indicator organism	Indicator organism density in oyster meats per 100 g	Number of oyster samples examined	Salmonella-Positive	
			Number	Percent
Total coliform	0-100	57	0	0
	101-1,000	119	1	0.8
	1,001-10,000	121	5	4.1
	10,001-100,000	113	26	23.0
	>100,000	129	28	21.7
	Total	539	60	
	Overall average			11.1
Fecal coliform	0-230	268	5	1.9
	231-1,000	84	10	11.9
	1,001-10,000	97	19	19.6
	>10,000	90	26	28.9
	Total	539	60	
		Overall average		

The isolation of *Salmonella* from the shellfish meat was not consistent with the level of total coliforms in the shellfish meat. However, a direct relationship was demonstrated in the shellfish meats between the presence of *Salmonella* and levels of fecal coliforms.

The relative efficacy of the enrichment broths and plating media in the recovery of *Salmonella* from oysters is shown in Table 3. Most of the procedures in the BAM

TABLE 3. *Salmonella*-positive oyster samples listed by pre-enrichment, selective enrichment, and plating media

Pre-enrichment and selective enrichment media	Number of samples positive			Total
	Brilliant green	Bismuth sulfite	<i>Salmonella-Shigella</i>	
Lactose pre-enriched, selenite cystine	11 (18.3) ^a	15 (25.0)	7 (11.7)	33
Lactose pre-enriched, tetrathionate	21 (35.0)	39 (65.0)	18 (30.0)	78
Selenite cystine	18 (30.0)	21 (35.0)	9 (15.0)	48
Tetrathionate	29 (48.3)	50 (83.3)	21 (35.0)	100
Total	79	125	55	259

^aNumbers in parentheses percentage of the total number of 60 *Salmonella*-positive oyster samples.

(18) used in the analysis of foods for the presence of *Salmonella* require pre-enrichment of the sample in lactose broth, a practice based on the finding of North

(15). Hence, the practice of pre-enriching most processed foods in the analysis for *Salmonella* has been accepted as a standard procedure by FDA. In a raw product such as oysters, lactose-fermenting organisms may be expected to successfully compete and over-grow the *Salmonella* organisms in certain instances. To maximize the possibility of *Salmonella* recovery from oysters, both the lactose pre-enrichment and the direct selective enrichment procedures were used. The most productive combination of enrichment broths and plating media was direct enrichment in TT broth followed by streaking on plates of BS agar. Of the 60 samples positive for *Salmonella*, 50 (83.3%) were positive by this combination.

TABLE 4. Relative frequency of isolation of *Salmonella* serotypes from oysters

Serotype	Somatic group	Number of samples positive for this serotype
<i>S. derby</i>	B	21
<i>S. infantis</i>	C ₁	19
<i>S. newport</i>	C ₂	12
<i>S. hartford</i>	C ₁	4
<i>S. heidelberg</i>	B	3
<i>S. manhattan</i>	C ₂	3
<i>S. binza</i>	E ₂	2
<i>S. montevideo</i>	C ₁	2
<i>S. muenchen</i>	C ₂	2
<i>S. typhimurium</i>	B	2
<i>S. worthington</i>	G	2
<i>S. gaminara</i>	I	1
<i>S. give</i>	E ₁	1
<i>S. kentucky</i>	C ₂	1
<i>S. mississippi</i>	G	1
<i>S. rubislaw</i>	F	1
<i>S. sachsenwald</i>	R	1

Table 4 shows the relative frequency of isolation of 17 *Salmonella* serotypes from oysters. Although a large variety of serotypes was isolated, *Salmonella derby*, *Salmonella infantis*, and *Salmonella newport* accounted for most of the isolates. No member of the closely-related genus, *Arizona*, was isolated.

Salmonella was chosen as representative of pathogenic flora since the methodology for its detection is established and relatively simple. Other pathogens such as *Vibrio parahaemolyticus* and enteroviruses may constitute a part of the pathogen load in shellfish and should, therefore, lead to caution in the interpretation of data from this study. Results of this survey demonstrate the greater validity of the fecal coliform group as compared to the total coliform group for indicating the presence of 17 serotypes of *Salmonella* in the Eastern oyster, *Crassostrea virginica*. The percentage of *Salmonella*-positive shellfish samples was somewhat higher in oysters harvested from waters conforming with the present approved growing-area standard of ≤ 70 organisms per 100 ml of water as compared to these same waters meeting the recently proposed fecal coliform standard of ≤ 14 organisms per 100 ml (Table 1). Furthermore, a more evident correlation was seen between the presence of *Salmonella* in shellfish and the levels of fecal coliform in these shellfish compared to the

levels of total coliforms (Table 2). The findings of this study would support the use of the fecal coliform standard for judging the safety of shellfish-growing waters. The continued introduction of improved techniques for detecting bacterial pathogens (2, 12, 14, 16) as well as development of practical methods for detecting viral pathogens in sea water may ultimately make the routine search for the specific pathogens themselves a distinct possibility.

ACKNOWLEDGMENTS

The authors thank Captain Jim Verber, Chief, Northeast Technical Services Unit, and Dr. Al Story, Chief, Gulf Coast Technical Services Unit, and their staffs for collection of samples. Appreciation is expressed to Aida Romero and Paul Poelma, Division of Microbiology, for serotyping of *Salmonella* isolates. Special thanks also are expressed to Dan Hunt, Shellfish Sanitation Branch, for helpful suggestions and for co-ordination of the activities of the field units.

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Improvements on the Agar Plate Method to Determine Lysozyme¹

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(Received for publication November 7, 1974)

ABSTRACT

Through systematic investigation of several variables the agar plate assay for lysozyme was modified so that concentrations as low as 5 µg egg white lysozyme per 100 ml of sample could be determined in 3 h. Plates were stained with 0.5 g buffalo black in 100 ml 7% acetic acid followed by destaining in 7% acetic acid until satisfactory contrast between clear zones of lysis and surrounding seeded agarose was obtained. Standard egg white lysozyme solutions remained stable for at least 7 days at -5 C. The best lyso-plate method included 1.0 g agarose with 0.1 g NaCl in 100 ml of a pH 7.0 phosphate buffer seeded with 0.020 g *Micrococcus lysodeikticus* and incubation at 47 C for 3 h.

Lysozymes (E.C.3.2.1.17) are protein molecules with molecular weights of 15,000 to 30,000. They are able to split the bonds between N-acetyl muramic acid and N-acetyl-glucosamine of bacterial cell walls (10). They are particularly active against *Micrococcus lysodeikticus* because of its loose cell wall structure. Because of their bactericidal properties, lysozymes have been suggested for use as a preservative in the food industry. In nature lysozymes are found in several organs, tissues, and in secretions of animals and even in plants. Hen egg white lysozyme (EWL) is presently the most important commercially available lysozyme.

Akashi (1) found that cured ground beef was preserved more effectively with the combination of 3% of NaCl, 12.5 ppm of NaNO₂, and 50 or 200 ppm of lysozyme than either by lysozyme alone or by the salts alone. In later work with Vienna sausage Akashi (2) found that dipping them in 0.05% of lysozyme gave better results than using other chemical preservatives.

A lysozyme was used by Pulay and Krasz (15, 16) to prevent blowing of Trappist and Ovar cheeses by butyric acid bacteria. They found that addition of 0.2% (v/v) of egg white or 0.001% (w/w) of lysozyme to pasteurized cheese milk prevented the blowing during ripening by inhibiting *Clostridium* spp. The addition had no effect on the growth of lactic acid and coliform bacteria.

Lysozyme, along with ovalbumin and ovomucin, has been used to preserve dried milk components for pediatric use (11). Sawada et al. (18) used 0.05-0.10 mg of

lysozyme/ml of sterilized milk to promote growth of *Lactobacillus bifidus* and to assure the absence of other bacteria in the intestinal tract of infants who were given this milk. Ferlazzo et al. (7) found that at lysozyme concentrations above 0.012% in cow milk the total bacterial count and coliform count were reduced.

Yajima et al. (23) suggested that lysozyme be used to preserve sake. Wines have also been preserved by addition of lysozyme or its salts (5).

Sauter and Peterson (17) found that eggs with a high lysozyme content maintained better quality during storage than did eggs with a low lysozyme content.

Before lysozymes can be used to a greater extent and advantage in food preservation, a suitable method must be available to assay the food lysozyme concentration. For most food systems spectrophotometry (4, 13, 19) would be impractical, since a clear solution is required. A decolorizing process might destroy the lysozyme being measured. The available lyso-plate methods (3, 12), although easily done, take 12-18 h and are not sensitive enough for relatively low lysozyme concentrations.

This study was undertaken to develop an improved lyso-plate method with which the lysozyme content of a fluid preparation can be determined in 3 h with reproducible results.

EXPERIMENTAL

The standard lysozyme used was 3× crystallized, dialyzed, and lyophilized egg white lysozyme with an activity of 21,000 units/mg (Sigma Chemical Co., St. Louis, Mo.). One unit of activity produces a OD₄₅₀ of 0.001 per minute at pH 6.24 and 25 C in a 2.6-ml suspension of *M. lysodeikticus* and a light path of 1 cm. Sigma was chosen as the supplier because its lysozyme had previously been found to be pure and homogeneous by acrylamide gel electrophoresis and acrylamide isoelectric focusing. Also, the activity of other Sigma lots had been shown to stay relatively constant for up to 2 years. Lysozyme from other suppliers lost activity rather rapidly, most probably due to impurities. Fresh standards were prepared daily. Various concentrations were obtained by dissolving the lysozyme in glass-distilled water and making serial dilutions. The standards were stable up to a week when kept at -5 C. The dried *M. lysodeikticus* cells were also obtained from Sigma Chemical Co.

Agar (Difco) and a purified version thereof, agarose, were obtained from Fisher Scientific Co., Pittsburgh, Pa.

A pH 7.0 buffer was prepared by dissolving 9.365 g of dibasic potassium phosphate and 4.216 g of monobasic potassium phosphate in glass-distilled water and bringing the final volume to 1000 ml. The pH 6.3 buffer was a 0.006 M buffer made with 9.030 g of KH₂PO₄ per 1000 ml of distilled water.

¹Authorized for publication as Paper No. 4665 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

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One or 1.5 g of agar or agarose with or without 0.1 g of NaCl was added to 100 ml of buffer along with 0.02 or 0.03 g of *M. lysodeikticus*. The mixture was heated to 90 C on a hot plate and stirred magnetically so that there was no appearance of bubbles in the solution. NaCl was added to increase the sensitivity of the test organism to lysozyme (6, 8, 9, 22). Stirring was continued until the temperature reached 65-70 C. Then the agar was poured into a 2-quart oblong Pyrex glass baking dish to a depth of 2mm (100 ml of agar mixture). When a layer of

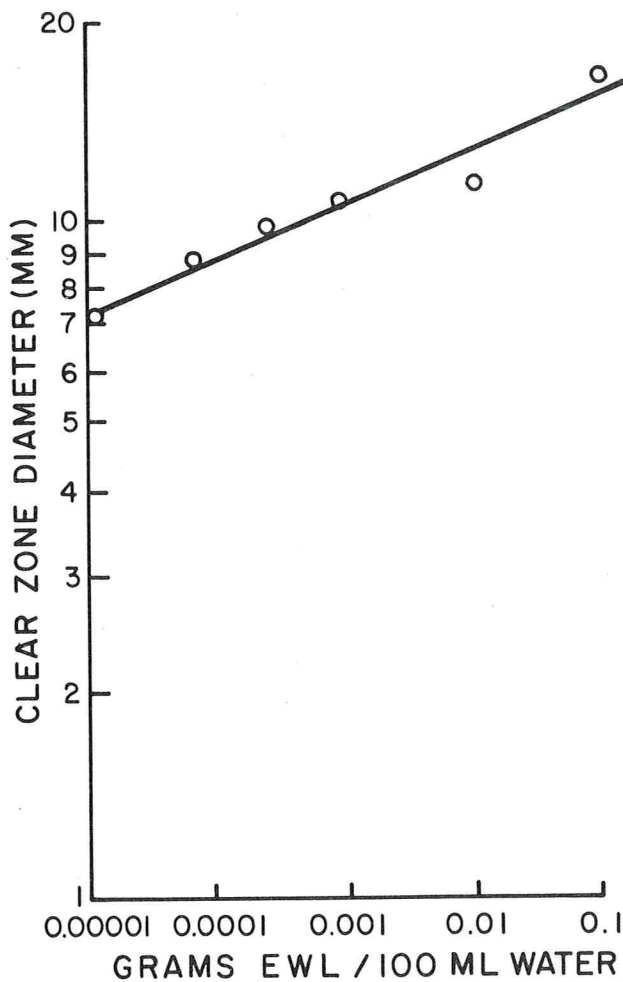


Figure 1. Standard curve for lysozyme concentrations produced with agarose as the supporting medium.

TABLE 1. Diameters (in mm) of zones on agar plates under various conditions

Grams of agar	pH	Temp. (C)	Concentration (g EWL/100 ml)					
			Without NaCl			With NaCl		
			0.05	0.005	0.0005	0.05	0.005	0.0005
1.0	7.0	47	11.1	8.3	7.1	12.7	9.1	7.1
		RT	9.7	7.4	6.0	10.9	8.8	6.9
		5	8.5	7.0	—	9.4	7.1	—
	6.3	47	8.3	8.0	—	9.1	8.0	6.0
		RT	—	—	—	7.0	6.9	—
		5	—	—	—	—	—	—
1.5	7.0	47	11.9	9.3	7.0	11.1	8.7	7.5
		RT	9.2	8.1	—	9.9	7.7	6.0
		5	7.0	—	—	7.5	—	—
	6.3	47	8.5	7.4	—	8.9	8.0	6.1
		RT	—	—	—	8.0	6.5	—
		5	—	—	—	—	—	—

microscope slides was used in the dish, a 2-mm depth required 150 ml. Slides were used in staining experiments.

After solidification, holes or wells were punched into the agar with the small end of a transfer pipette. These wells were 1 mm in diameter and were about 2 cm apart allowing 24 assays to be made per dish. Lysozyme standards or samples were placed onto the wells with a micro-syringe. A droplet was never larger than 4 mm in diameter and rapidly diffused into the agar medium surrounding the well. The dish was then covered with foil and incubated at 5 C, 47 C, or room temperature (RT). Agar plates were used as soon as possible after preparation.

After 3 h clear zones, formed by lysing action of lysozyme on bacterial cells in the agar, were measured with a pair of dividers and a ruler (in millimeters). When necessary, indistinct zones were made clearer by soaking for 12-18 h in 0.1 N NaOH.

For photographic purposes, agar slides were stained with 0.5 g of buffalo black (Allied Chemical Co.) in 100 ml of 7% (v/v) acetic acid and then destained in 7% (v/v) acetic acid until enough contrast was apparent.

Standard curves were produced by plotting the diameter of the clear zones (logarithmic scale) against EWL (arithmetic scale). The direct proportionality of clear zone diameter logarithm to lysozyme concentration is shown in Fig. 1.

The objective of this study was to systematically experiment with various conditions to develop an agar plate procedure for the determination of relatively low lysozyme concentrations in 3 h, thus making it suitable for work with low-level lysozyme applications or residual testing, and also with bovine milk which contains lysozyme at a concentration about three orders lower than in human milk.

RESULTS AND DISCUSSION

Tables 1 and 2 indicate the lytic response of *M. lysodeikticus* to the following variables: 0.05, 0.005, and 0.0005 g of EWL/100 ml; agar vs. agarose; buffer pH 7.0 vs. pH 6.3; incubation temperatures of 47 C, RT, and 5 C; and addition of 0.1 g NaCl.

In analyzing the data, standard deviations of the variables were determined and evaluated for homogeneity by the test of Steel and Torrie (21). Student's t-test for heterogeneous variations (14) was used to find the best test. With the t-test, the crude standard deviations were adjusted according to Sheppard's method (20) by which an adjustment of $-1/12$ of the interval between groups is necessary to obtain a more accurate deviation when the raw data are grouped.

In looking at the combinations with agar (Table 1), it was evident that certain ones could be eliminated from

TABLE 2. Diameters (in mm) of zones on agarose plates under various conditions

Grams of agarose	pH	Temp. (C)	Concentration (g EWL/100 ml)					
			Without NaCl			With NaCl		
			0.05	0.005	0.0005	0.05	0.005	0.0005
1.0	7.0	47	15.9	11.0	8.1	16.5	12.2	8.9
		RT	12.5	9.5	7.0	12.5	9.8	7.1
		5	10.1	8.1	—	10.3	8.2	6.0
	6.3	47	12.6	9.7	7.8	14.6	10.6	8.1
		RT	11.4	8.6	—	11.9	9.8	7.9
		5	8.3	—	—	8.5	6.4	—
1.5	7.0	47	15.2	10.9	6.3	16.6	12.0	6.8
		RT	12.7	9.4	7.4	13.2	9.8	7.8
		5	7.5	—	—	8.1	6.1	—
	6.3	47	11.6	9.2	—	13.4	10.1	7.1
		RT	9.4	7.0	—	10.8	8.0	7.0
		5	—	—	—	—	—	—

the statistical analysis. The 0.066 M phosphate buffer (pH 6.3) plates gave either no zones or no zones were apparent with 0.0005 g of EWL/100 ml. The combination of 1.0 g of agar and pH 6.3 buffer gave no zones in 3 h; even after 20 h the zones were only very indistinct.

In determining whether NaCl is necessary for agarose plates, data were again analysed statistically. On plates containing 1.5 g of agarose the addition of 0.1 g of NaCl gave significantly larger zones ($t = 5.29$) than appeared on those plates without it when both contained 0.05 g of EWL/100 ml; when this was 0.005 or 0.0005 g of EWL/100 ml, values were $t = 3.81$ and $t = 3.15$, respectively.

Zones on plates containing 1.0 g of agarose and 0.1 g of NaCl were not significantly larger than were zones on plates without the salt when concentrations of EWL were 0.05 g ($t = 0.92$) and 0.0005 g ($t = 1.40$)/100 ml. Zones obtained when 0.005 g of EWL was used were significantly larger for each concentration of NaCl, hence it was concluded to be effective for increasing lysozyme activity.

Incubation of plates at 47 C gave significantly larger zones, except for zones obtained with 0.0005 g EWL/100 ml and 1.5 g of agarose in plates incubated at room temperature; in this instance, however, zones were not as clear as those on plates incubated at 47 C.

From these results it was concluded that the best lyso-plate method tested included 1.0 g of agarose with 0.1 g of NaCl in 100 ml of a pH 7.0 phosphate buffer seeded with 0.020 g of *M. lysodeikticus* and incubation at 47 C for 3 h. This type of agarose plate gave significantly larger zones than did plates with either 1.0 or 1.5 g of agar.

If a permanent photographic record of the assay is desired, microscopic slides may be used as the agarose carrier. The clear zones of an unstained slide are not recognized by photography or xerography for lack of contrast.

The slides were stained with 0.5 g of buffalo black per 100 ml of 7% (v/v) acetic acid. Immersion for about 5

min proved most satisfactory. Destaining of the clear zones was accomplished by first immersing the slide in 7% acetic acid for about 5 min to remove excess dye, then by again exposing the slide to 7% acetic acid in a 400-ml beaker with gentle stirring (magnetic) until satisfactory contrast was developed. Figure 2 is an example of such a

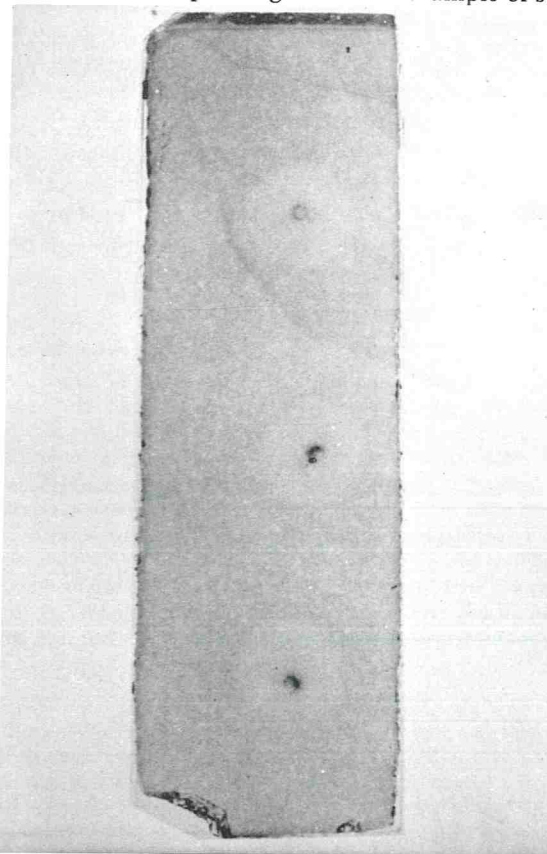


Figure 2. Example of a stained agar lyso-plate showing clear zones of lysis.

slide. To withstand this procedure and for sufficient gel strength, 1.5 g of agarose (or agar)/100 ml buffer was necessary.

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The Influence of Temperature on Growth, Sporulation, and Heat Resistance of Spores of Six Strains of *Clostridium perfringens*

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(Received for publication October 30, 1974)

ABSTRACT

The influence of temperature on growth, sporulation, and heat resistance of six strains of *Clostridium perfringens* was examined. The range of temperatures for growth and sporulation varied between strains and was influenced by the culture medium. Optimum growth occurred in Thioglycolate medium between 30 and 40 C for all strains. Optimum sporulation in Ellner's medium occurred in the 37 to 40 C range for all strains. The maximum number of spores produced varied with the strain. Heat resistance of the spores varied between strains but was not modified by temperature of incubation.

Enteropathogenicity of *Clostridium perfringens* has been associated with sporulating ability of a given strain and with heat resistance of the spores (5, 8, 16, 20). Heat resistant spores and poor sporulating ability have been attributed to food poisoning strains isolated in Europe and Asia, but food poisoning strains isolated from foods in the United States vary in respect to these characteristics (16, 21). Nishida et al. (23) have observed that strains of *C. perfringens* isolated from soil and feces that produce many spores are weakly heat resistant, whereas strains producing highly resistant spores sporulate poorly.

Ingestion of large numbers of cells of certain strains of this organism has produced enteritis in man and in laboratory animals (7, 9, 17, 19). Also, large numbers of cells injected into ligated loops of the ileum of rabbits result in accumulation of fluid (10). The factor that triggers the enteric symptoms is produced after ingestion of large numbers of the vegetative forms and during subsequent sporulation in the intestine (18). From these observations, it seems that multiplication of vegetative cells, sporulation, and the heat resistance of spores play important roles in the epidemiology of food poisoning caused by *C. perfringens*.

Temperature plays an essential role in bacterial growth and in endospore formation. The suggestion has been made that heat resistance of spores of *C. perfringens* is a genetically controlled characteristic (6, 10); others have reported modification of this characteristic as being induced by the nature of the sporulation medium (14, 22). Cooking temperature will kill vegetative cells of this organism but may cause heat

activation of any spores present in a food. During cooling of large quantities of cooked food, as may be necessary in institutional kitchens, and during subsequent heating before serving, food is exposed to a gradient of temperatures conducive to rapid multiplication of heat activated spores. Strains of *C. perfringens* that sporulate with production of heat resistant spores and that are capable of growth over a wide range of temperatures would be of major concern as a source of food poisoning.

The objectives of the present investigation were to study the influence of temperatures below, within, and above the optimum temperature range reported for *C. perfringens* on: (a) numbers of vegetative cells produced, (b) numbers of spores produced, and (c) thermal resistance of spores produced.

MATERIALS AND METHODS

Bacterial cultures

Six strains of *C. perfringens* were examined for the influence of temperature of incubation on production of vegetative cells, production of spores, and heat resistance of the spores. One strain, designated as A23, was obtained as Hobb's strain serotype 2, NCTC 8238, and was originally isolated from a food poisoning outbreak in England. Strain FDI was obtained from the Food and Drug Administration Laboratories, Kansas City, Missouri; it also was associated with a food poisoning outbreak. The other four strains were isolated and identified in our laboratories. Strains E1 and E2 were isolated from chicken liver, strain E15 from calves liver, and E19 from beef liver. Stock cultures of these organisms were maintained in Cooked Meat Medium (Difco) stored at room temperature.

Determination of effect of temperature on growth

Stock cultures were heated at 80 C for 20 min and then cooled rapidly by immersion in ice water; this treatment destroyed vegetative cells and activated spores. One milliliter of this heated culture was transferred to Thioglycolate Broth containing glucose (Difco); spores were not formed in this medium. Vegetative growth was determined for each strain at 5, 15, 22, 30, 40, 45, and 50 C. Cell counts were made by using the medium recommended by Walker and Rey (26). This medium consists of the basal medium for SPS agar (1) with the substitution of 400 µg of D-cycloserine/ml of medium for polymyxin and sulfadiazine. The antibiotic was not used after pure cultures were obtained. The pouch method of Bladel and Greenberg (3) was used to maintain anaerobic conditions. The pouches were incubated at 37 C for 18 to 24 h before counts were made.

Determination of sporulation

Stock cultures were heat-shocked and transfers made to fluid

Thioglycolate Medium by following the procedure described under determination of growth. This preparation was incubated at 37 C for 6 h to promote multiplication of vegetative cells. Sporulation broth was inoculated from this culture of vegetative cells at the level of 0.1 ml of inoculum to 10 ml of sporulation broth.

The sporulation medium of Ellner (11), which promotes high spore yields, was used for this part of the investigation. Temperatures tested for sporulation were 27, 32, 37, 40, 45, and 50 C. Numbers of spores were determined periodically at each temperature. The spore count was made by heat shocking the sporulating cell suspensions at 80 C for 20 min, plating in pouches, and incubating in the same manner as described for determination of growth. Colony counts after 24 h of incubation were recorded as the spore count.

Determination of heat resistance of spores

Ellner's medium has been described as producing atypical morphology and heat resistance of spores of *C. perfringens* (7, 16, 18); therefore, cooked meat medium was used for production of spores when heat resistance was investigated. Preparation of the inoculum and inoculation of the Cooked Meat Medium (CMM) were the same as described previously. Cultures were incubated for 3 days at 32 C, 24 to 30 h at 37 and 40 C, and 16 to 18 h at 45 C and 50 C. These incubation periods corresponded in most instances to maximal and constant levels of numbers of spores. Sporulation at 27 C was not determined in CMM because only one strain produced spores in Ellner's medium at this temperature. Total viable count, viable spore count, and heat resistance of the spores were recorded for each sample.

Survival time was used for comparisons of heat resistance. Cultures in Cooked Meat Medium were heated to 95 C in a water bath and then held at this temperature while numbers of viable spores were determined periodically. The number of minutes required to obtain one or fewer spores/ml was recorded as survival time.

Statistical analysis

All spore and cell counts were converted to logarithms for statistical analyses and represented the average of three replications. Data for the effect of temperature on number of spores that developed in Ellner's medium were analyzed by analysis of variance. The experimental design was a cross classification between strains and temperatures; their interaction was used as the error term for these two sources of variation. Time was considered a subplot on a split-plot arrangement. The error mean was used for testing time and its interactions, but with a conservative number of degrees of freedom as recommended by Greenhouse and Geisser (13) because a positive autocorrelation is expected when time is used as a classification with this type of design.

Results for influence of temperature on heat resistance of spores were evaluated by covariance analysis. The survival time was adjusted for numbers of spores; this adjustment was made to balance any effect that differences in concentration of cells or spores could have on survival of spores to heat.

RESULTS AND DISCUSSION

Effect of temperature on growth of vegetative cells

None of the strains initiated growth at 5 C; indeed, a slight decrease in total numbers became evident during the 6-day incubation period. Growth patterns at all temperatures except 5 C are shown in Fig. 1. Four strains, E1, E2, A23, and FD1, did not grow at 15 C; strains E15 and E19 increased in numbers by one or two log cycles during 3 days of incubation. Other workers also have observed the failure of *C. perfringens* to grow in the temperature range of 5 to 15 C (2, 6, 15). At 22 C,

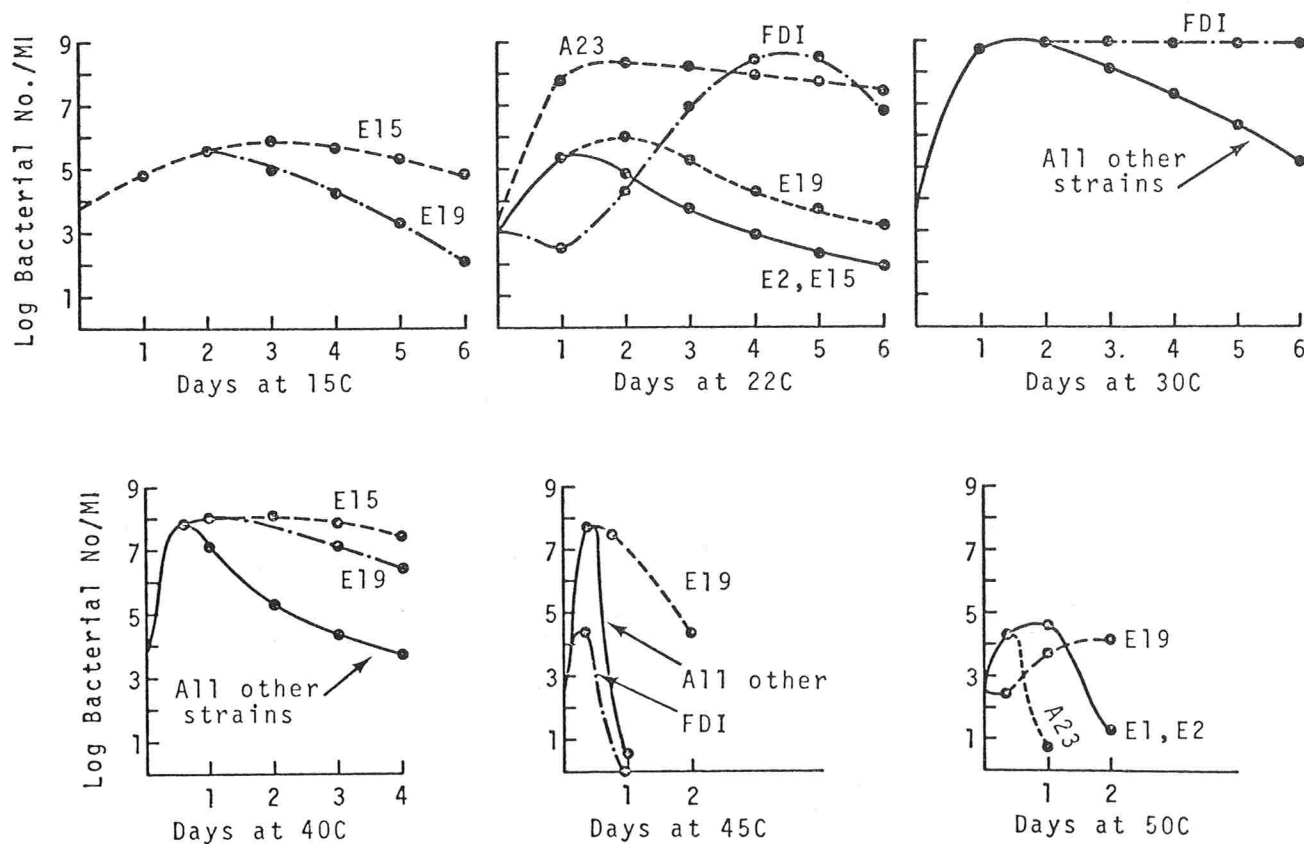


Figure 1. Influence of temperature of incubation on growth of six strains of *Clostridium perfringens* in Thioglycolate broth.

only strain E1 was unable to grow. The other strains varied in level of growth; strains A23 and FD1 approached a maximum level of 10^8 cells/ml of medium; the remaining strains reached a level of 10^5 cells/ml after 24 to 48 h.

All cultures except FD1 multiplied and reached maximum numbers rapidly at 45 C, followed by a rapid decrease in numbers. Strain FD1 increased in numbers about $1\frac{1}{2}$ log cycles, the others by about 5 cycles during the initial 8 h. After 24 h at 45 C, viable cells were recovered only from cultures of strain E9. At 50 C, growth was even more restricted and variable than at 45 C. Two strains, E1 and FD1, were incapable of growth at this temperature, no viable cells were detected after 8 h. Other strains showed an initial increase in numbers, but died off within 24 h except strain E19, which still persisted after 48 h.

All strains multiplied rapidly at 30 and 40 C, with the highest numbers of cells developing at 30 C. Strain FD1 maintained a constant number of cells over the 6-day incubation period; at 30 C, the other strains showed a decrease in numbers after 48 h. At 40 C, the tendency toward a decrease in total numbers after 24 to 48 h was evident in all cultures.

Maximum cell numbers and minimum generation time may be used as criteria for determining optimum growth conditions. Smith (24) observed that none of the five strains of *C. perfringens* that he studied grew appreciably at 15 C. All five strains, however, grew well over a range of 20 to 50 C with maximal growth between 30 and 45 C. Above 45 C, the generation time increased sharply for two strains and little or no growth was observed at 55 C for any of the strains. Breed et al. (4) list the optimum growth temperature for this organism as being between 35 and 37 C. The minimum generation times and maximum cells numbers for the six strains observed in

our work occurred in the broad range of 30 to 40 C. Below 30 C or above 40 C, considerable variation occurred among the six strains as to growth rate, maximum cell numbers, and lowest or highest temperature for initiation of growth. Thus, an optimum temperature range for growth of *C. perfringens* seems to be characteristic, but the extreme temperatures at which growth will occur, which is important for foods, are evidently characteristic of the strain.

Effect of temperature on sporulation

All strains formed spores between the temperatures of 32 and 40 C. Strain A23 was the only one to form spores at a temperature (27 C) below this range. At temperatures above 40 C, strain E19 formed spores at 45 C, and strain FD1 at both 45 C and 50 C (Fig. 2). An

TABLE 1. Analysis of variance on numbers of spores produced in Ellner's medium at 32, 37 and 40 C

Source of variation	Degrees of freedom (D.F.)	Mean square
Strain (A)	5	49.38**
Temperature (B)	2	39.75**
(A) × (B) Error a	10	4.11
Conservative D. F.		
Time (C)	1	201.68*
(A) × (C)	5	14.0*
(B) × (C)	2	6.36
(A) × (B) × (C)	10	7.76
Residual error b	45	2.45

*Significant at P = 0.01

**Significant at P = 0.05

analysis of variance (Table 1) indicated a significant difference in sporulation of the six strains in the 32 C to 40 C range. Data below 32 C and above 40 C were not included to make a weighted test for all six strains. An inspection of Fig. 2 indicates major differences in sporulation at 32 C. An evaluation of strain differences

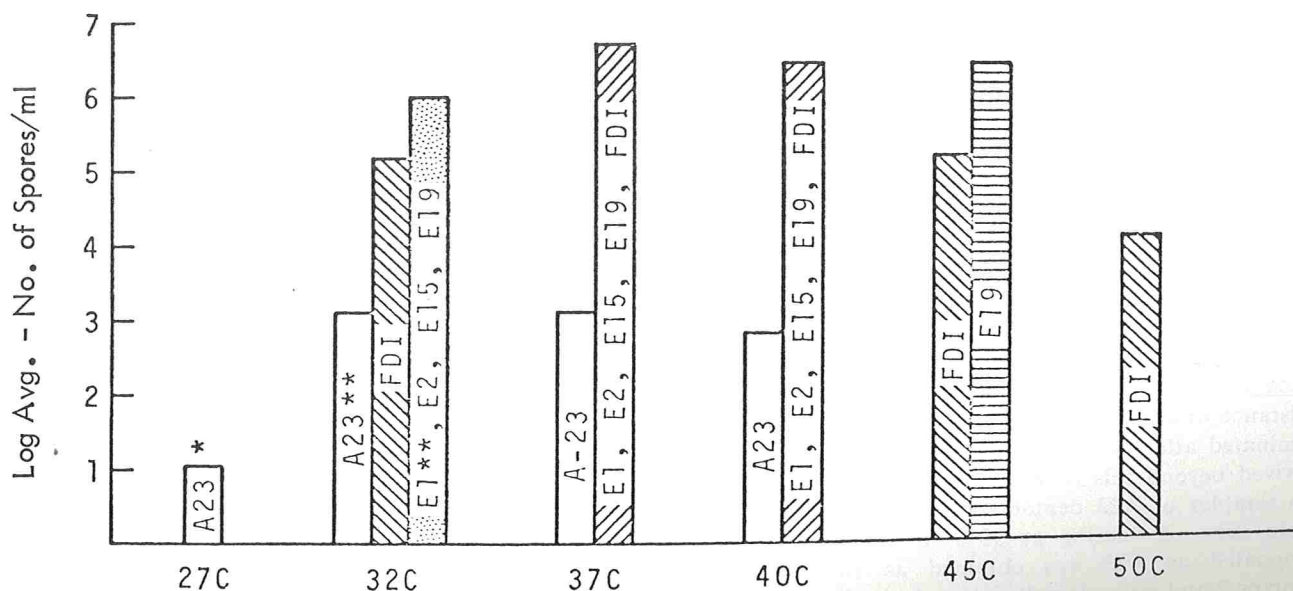


Figure 2. Influence of temperature of incubation on sporulation of six strains of *C. perfringens* in Ellner's medium. *Sporulation occurred after 96 h of incubation. **Sporulation occurred after 46 h of incubation. In all other instances sporulation occurred within 24 h.

at 37 and 40 C by the multiple range test indicated that the low spore yield of A23 was the only significant difference among strains at these two temperatures.

In most instances, spores were produced within 24 h; strains E1 and A23, however, produced spores only after 46 h of incubation at 32 C, and strain A23 produced spores only after 96 h of incubation at 27 C. Two strains sporulated at 45 C; FD1 reached maximum numbers of spores within 8 h of incubation; E19 required 24 h. At 50 C, strain FD1 was the only one capable of spore formation; the maximum number of spores was formed in less than 24 h.

According to analysis of variance, temperature of incubation has a significant effect on numbers of spores produced. At 37 and 40 C, spore yields are almost identical and higher than at any other temperature. The ability to sporulate below 32 C or above 40 C varied with the strain. A comparison of the results presented in Fig. 1 and 2 shows that the temperature for sporulation is more restricted than that for vegetative growth. That strain FD1 was able to grow at higher temperatures in Ellner's medium than in Thioglycolate medium while the opposite was true for strain A23 emphasizes the relationship between strain and growth medium and the temperature range for growth or sporulation.

Effect of incubation temperature on heat resistance of spores

Several reports exist indicating that the temperature of cultivation modifies heat resistance of spores (12, 25). In our studies, a comparison was made of the thermal resistances of spores of *C. perfringens* produced at different temperatures. An analysis of variance showed that temperature of cultivation had no significant influence on the resistance of spores of the six strains tested (Table 2). There were, however, significant differ-

TABLE 2. Analysis for differences on survival of spores to heating at 95 C

Source of variation	Degrees of freedom	Mean Square
Strain (A)	5	1,465.16**
Temperature (B)	3	66.50
(A) × (B)	15	45.72
Error	40	25.72
No. of cells	1	0.01
No. of spores	1	2.02
Error	38	27.02

ences between strains and survival times. Strain A23, which consistently sporulated poorly, had the highest resistance of all strains tested. Our survival tests were terminated after heating at 95 C for 45 min, and A23 survived beyond this time. Additional tests made with two samples of A23 demonstrated that spores of this strain survived heating at 95 C for 3 h. The strain designated as A23 was obtained as Hobb's strain serotype 2 and was originally isolated as a heat resistant strain. On the other hand, other strains that produced

significantly larger numbers of spores had survival times at 95 C of less than 10 min to approximately 35 min, depending on the strain.

The analysis of covariance showed that, for a given strain, only a limited amount of variability in survival times was attributable to variations in concentrations of spores and viable spores. The variability in numbers of spores ranged from 10^4 to 10^5 spores/ml of cooked meat medium except for A23, which yielded a level of 10^2 spores/ml. The number of spores/ml was always higher in Ellner's medium than in CMM (Figure 2, Table 3).

TABLE 3. Effect of sporulation temperature on heat resistance of spores of *C. perfringens* produced in Cooked Meat Medium (CMM)

Strain	No. spores/ml CMM	Sporulation temperature (min. survival at 95 C)			
		32 C	37 C	40 C	45 C
E1	1×10^4	9	8	8	— ¹
E2	1×10^4	10	18	12	— ¹
E15	1×10^5	36	36	28	— ¹
E19	1×10^5	17	17	16	10
FD1	1×10^5	10	12	9	11
A23	1×10^2	>45	>45	>45	>45

¹No spores formed in CMM at this temperature.

Collee et al. (5) also compared survival of two strains of *C. perfringens* at levels of 10^4 and 10^5 spores/ml and found no relationship between thermal resistance and concentration of spores. The heat resistance varied mainly with the strain, and resistance was not appreciably changed by the temperature at which the spore was formed.

In summary, the temperature range for sporulation of six strains of *C. perfringens* was more restricted than the temperature range for growth. The optimum temperatures for vegetative growth and for sporulation were approximately the same; but the minimum and maximum temperatures for growth and for sporulation varied with the strain and changed for some strains with changes in the culture medium. The temperature at which spores were formed had no pronounced influence on heat resistance; thermal resistance of spores did vary among strains, however.

ACKNOWLEDGMENT

Journal Paper No. J-8018 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa. Project No. 1896. This investigation was supported in part by U.S. Public Health Service Grant No. FD00444.

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Fungi in Foods

VI. A Comparison of Media to Enumerate Yeasts and Molds¹

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ABSTRACT

Five media, with added antibiotics, were compared for their ability to recover yeasts and molds from 31 foods. Overall, no significant difference in recovery was found among Mycophil, Plate Count, Malt, or Potato dextrose agars but all were superior to Sugar Free agar. On the basis of recovery of organisms, availability, ease of management, and cost, Plate Count agar with antibiotics is recommended for routine enumeration of yeasts and molds in foods.

One of the major problems faced by a food laboratory is selection of media for routine microbiological analyses of foods. Under most conditions it is neither feasible nor economical to employ a multiplicity of media, particularly when analyzing for the less important groups of organisms. However, when only one medium or method is used, every attempt should be made to ensure that maximum recovery of the organisms under investigation will result. Several factors are involved in the performance of a medium (8), such as pH, time and temperature of incubation, nutrient availability, lot variation, inhibitors present, and influence of food components.

Although a number of studies have been done on the usefulness of different media for yeast and mold counts (4, 5, 7, 9), Potato Dextrose agar is still widely used (1, 3). To evaluate the efficiency of recovery of yeasts and molds from a variety of foods, five media were tested for

¹Florida Agricultural Experiment Stations Journal Series No. 5702.

recovery of fungi from 31 fresh samples of meat, cheese, and vegetables. Three of these are commonly employed media, one is a general purpose medium and one was a medium used with some success during preliminary studies.

MATERIAL AND METHODS

Food samples were obtained from retail outlets in the Gainesville, Florida area. Dilutions were prepared by blending 50 g of sample with 450 ml phosphate buffer for 2 min (1). Additional dilutions were prepared as needed. Series of duplicate plates were poured with Plate Count agar (Difco), Malt agar (Difco), Mycophil agar (BBL), Sugar Free agar (BBL), and Potato Dextrose agar (Difco). The pH of the media were not adjusted and following sterilization 100 mg each of chloramphenicol and chlortetracycline were added per liter (6). Incubation was at 22 C for 5 days. Any suspect colonies were gram-stained to confirm their identity as fungi.

Data were subjected to analysis of variance and Duncan's multiple range test, following which the media were ranked according to the nonparametric test developed by Friedman (2).

RESULTS AND DISCUSSION

Preliminary experiments had indicated that while differences existed in recoveries between media, the differences were generally not marked and often difficult to interpret due to the many fold differences in counts between the food products analyzed. Therefore, collection and analysis of data were organized with this observation in mind.

TABLE 1. *Effect of medium¹ on recovery of yeasts and molds from various foods.*

Sample	Organisms per gram				
	A ²	E	C	D	B
Celery	63,000	53,000	46,000	46,000	43,000
Carrots	50,000	45,000	44,000	42,000	41,000
Radishes	78,000	72,000	61,000	43,000	6,500
Cole Slaw	100,000	90,000	62,000	56,000	54,000
Okra	2,100,000	1,800,000	1,600,000	1,600,000	1,500,000
Asparagus	1,800,000	1,800,000	1,700,000	1,600,000	720,000
Green beans	180,000	170,000	160,000	140,000	110,000
Tomatoes	16,000	16,000	15,000	15,000	13,000

TABLE 1. (Continued)

Bell pepper	<u>C</u> 3,500	<u>B</u> 3,300	<u>E</u> 3,000	<u>A</u> 3,000	<u>D</u> 2,100
Cauliflower	<u>E</u> 15,000	<u>C</u> 12,000	<u>D</u> 11,000	<u>A</u> 9,200	<u>B</u> 5,000
Colby cheese	<u>A</u> 98,000,000	<u>E</u> 88,000,000	<u>D</u> 75,000,000	<u>C</u> 71,000,000	<u>B</u> 62,000,000
Swiss cheese	<u>C</u> 3,000,000	<u>E</u> 3,000,000	<u>B</u> 2,800,000	<u>A</u> 2,600,000	<u>D</u> 100,000
Mozzarella cheese	<u>D</u> 32,000	<u>A</u> 31,000	<u>E</u> 28,000	<u>C</u> 27,000	<u>B</u> 26,000
Munster cheese	<u>A</u> 580,000	<u>D</u> 530,000	<u>C</u> 440,000	<u>E</u> 440,000	<u>B</u> 380,000
Edam cheese	<u>D</u> 37,000	<u>E</u> 34,000	<u>A</u> 24,000	<u>B</u> 15,000	<u>C</u> 13,000
Cheddar cheese I	<u>B</u> 15,000	<u>C</u> 13,000	<u>A</u> 12,000	<u>E</u> 12,000	<u>D</u> 9,000
Cheddar cheese II	<u>C</u> 13,000	<u>A</u> 13,000	<u>B</u> 13,000	<u>D</u> 11,000	<u>E</u> 10,000
Cheese spread	<u>B</u> 13,000	<u>E</u> 12,000	<u>D</u> 12,000	<u>C</u> 11,000	<u>A</u> 11,000
Onion flavored cheese	<u>E</u> 15,000	<u>A</u> 14,000	<u>C</u> 14,000	<u>B</u> 12,000	<u>D</u> 11,000
Gouda cheese	<u>E</u> 1,800	<u>C</u> 1,800	<u>A</u> 1,600	<u>B</u> 1,200	<u>D</u> 750
Brick cheese	<u>A</u> 98,000	<u>E</u> 88,000	<u>D</u> 75,000	<u>C</u> 71,000	<u>B</u> 62,000
Lamb patties	<u>E</u> 3,100	<u>C</u> 2,900	<u>D</u> 2,900	<u>A</u> 2,500	<u>B</u> 2,500
Pork patties	<u>B</u> 480,000	<u>C</u> 480,000	<u>A</u> 440,000	<u>E</u> 380,000	<u>D</u> 270,000
Lamb chops	<u>D</u> 820,000	<u>A</u> 800,000	<u>E</u> 640,000	<u>C</u> 560,000	<u>B</u> 370,000
Ham steak	<u>B</u> 57,000	<u>C</u> 56,000	<u>E</u> 53,000	<u>A</u> 50,000	<u>D</u> 28,000
Beef steak	<u>B</u> 140,000	<u>A</u> 120,000	<u>C</u> 110,000	<u>D</u> 99,000	<u>E</u> 94,000
Breaded veal	<u>C</u> 4,700	<u>B</u> 4,000	<u>A</u> 3,800	<u>D</u> 3,600	<u>E</u> 3,500
Sausage	<u>B</u> 850	<u>C</u> 800	<u>E</u> 750	<u>A</u> 600	<u>D</u> 350
Chicken	<u>B</u> 38,000	<u>E</u> 37,000	<u>C</u> 36,000	<u>D</u> 35,000	<u>A</u> 34,000
Pork ribs	<u>C</u> 2,200	<u>B</u> 2,000	<u>E</u> 2,000	<u>A</u> 1,800	<u>D</u> 1,800
Ground beef	<u>E</u> 10,000	<u>A</u> 9,000	<u>D</u> 8,000	<u>C</u> 8,000	<u>B</u> 7,000

¹APlate Count Agar, ^bPotato Dextrose, ^CMalt Agar, ^DSugar Free Agar, and ^EMycophil Agar

²Recoveries underscored by same line are not significantly different at the .05 level.

Table 1 lists the samples analyzed and reports the counts obtained. When the media were ranked from 1 to 5 for highest to lowest recoveries from each sample, this procedure ignored the fact that many apparent differences between recoveries were or were not significant. The method of considering only significant differences, which was adopted, seemed a more legitimate method of weighing the relative efficiencies of the five media. Using this basis of evaluation, rank sums

for Mycophil agar, Plate Count agar, Malt agar, and Potato agar were not different, but all four were superior to Sugar Free agar ($P < .01$).

In preliminary studies Sugar Free agar worked particularly well with cheese samples and therefore was included in this study. However, when compared to the other media for all food samples, it ranked last in recovery of fungi. Potato Dextrose agar did not do as well as expected; possibly this is due to its limited nutrient

composition. Plate Count agar is the only one of the five media tested which is supplemented with an additional source of nutrients (yeast extract). The other media contain a single source of nutrients from potato, soy, malt, or proteins.

This study indicates that Plate count agar with antibiotics, although not ranking first, is a workable medium for routine yeast and mold counts from foods. It is inexpensive, available in most laboratories, and its use results in good recovery of fungi from foods.

ACKNOWLEDGMENTS

Appreciation is expressed to Drs. R. C. Littell and F. W. Knapp for help in the statistical analyses.

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Survival of Enteric Viruses on Fresh Vegetables

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(Received for publication January 8, 1975)

ABSTRACT

Survival of enteric viruses under several environmental conditions was studied to determine whether contaminated vegetables pose a health hazard. Five test viruses stored in water or phosphate-buffered saline survived without significant loss for 36 days at 4 C. Virus decay followed a first-order reaction during room-temperature storage or evaporation at 4 C. Virus decay was more rapid on glass than on vegetables; presence of feces delayed decay on the vegetable surface. Examination of 14 vegetable samples over a three-month period showed no naturally-occurring viruses.

Several outbreaks of hepatitis have been attributed to consumption of raw vegetables (4, 5, 9). Watercress from a polluted stream was responsible for 129 cases in a colony of farmers in Tennessee, U.S.A. in 1971 (5). Sandwiches containing raw vegetables may have been responsible for 156 cases among shore-based personnel of the Mediterranean Fleet of the U.S. Navy while at the Port of Naples, Italy in 1959 (9). Lettuce, in a salad, was implicated as the vehicle responsible for infecting 222 students and staff from a college in South Carolina, U.S.A., in 1952 (4). The source of contamination in the latter two outbreaks was unknown. Enteroviruses have been isolated from vegetables, soil, and waste water used to irrigate fields (1, 2). Clearly, raw vegetables if contaminated, may be a health hazard.

Vegetables may become contaminated with human waste at any stage between growth of the vegetable and its consumption. This laboratory is studying some factors which influence survival of viruses as surface contaminants of vegetables. An earlier report (7) showed that coxsackievirus B5 survived well on lettuce when kept moist, but viability was rapidly lost if storage conditions were dry. In the current report we describe survival of five representative enteric viruses under several environmental conditions when they are introduced as contaminants of raw vegetables. The findings may help to evaluate the potential hazards of such contamination.

MATERIALS AND METHODS

Viruses

Coxsackievirus type B5 was obtained from Microbiological Associates, Inc., Bethesda, Md., poliovirus type 1 (Sabin), echovirus type 7, reovirus type 1, and adenovirus type 7a from the Bureau of Viral Diseases, Laboratory Centre for Disease Control, Health Protection Branch, Health and Welfare Canada, Ottawa. Coxsackievirus and poliovirus were grown and assayed in HEp-2 cells, echovirus in Vero cells, reovirus and adenovirus in primary African green monkey kidney cells. Dilutions were made in water or dilute feces as indicated. Dilute

feces was prepared by mixing 1 g of human adult feces in 100 ml of water, centrifuging at 1000 × g for 15 min and using the supernatant fluid as the diluent.

Cell cultures

HEp-2 cells from Microbiological Associates, Inc., and Vero cells from the American Type Culture Collection, Rockville, Md., were propagated in medium 199 containing 10% fetal bovine serum. Stock cell cultures were grown in Roux bottles as monolayers at 36 C. Primary African green monkey kidney cells obtained from Connaught Medical Research Laboratories, Toronto, Canada, as suspensions, were seeded into Roux bottles and incubated at 36 C for 6 days. The medium was then replaced with medium 199 containing 2% fetal bovine serum, and cultures were incubated for an additional 2 to 8 days before use.

Virus assay

Monolayers were prepared from stock cultures in 60 × 15 mm plastic dishes, 24 h before assay. The fluid was poured off and each dish received 2.5 ml of virus-containing sample. Cultures were shaken for 2 h at room temperature (about 22 C), the sample poured off, an agar overlay applied (6) and cultures were stored at 36 C in a humidified, 5% CO₂ atmosphere. Enterovirus plaques were counted after 3 days, reovirus and adenovirus plaques after 10 days.

Storage of virus in water and phosphate-buffered saline

Survival of the five test viruses was determined as follows. Two samples of 40 ml of water and 50 ml of phosphate-buffered saline (PBS) in screw-capped bottles were inoculated with 2000 plaque-forming units (PFU) of virus in 0.05 ml of water. After storage at 4 C or room temperature, duplicate aliquots of 2 ml of the water samples and 2.5 ml of the PBS samples were removed at 0 time, at day 1, and every 3-7 days up to 36 days. Before assay, water samples were made compatible with cells by addition of 0.5 ml of a mixture at pH 7.2 of equal volumes of 10 × medium 199 and fetal bovine serum. PBS samples were used directly. Plaque counts were averaged and expressed as percent of the count at zero-time. Differences greater than 25% were considered significant. (The percentage error of the assay system has been found to vary from 2 to 25%, with a mean of 9%.)

Vegetable preparation

Vegetables were purchased at local grocery stores and washed thoroughly in tap water. Celery leafstalks, lettuce leaves, and carrots were cut into 2-4 cm pieces. Green peppers, tomatoes, and radishes were used whole. Samples were air-dried briefly under laminar flow to evaporate surface moisture, then placed in open Pyrex dishes.

Inoculation and assay of virus on vegetable and glass surfaces

A 0.05-ml drop of water or dilute feces containing 400 PFU of virus was placed on a vegetable sample, a Pyrex surface, in screw-capped bottles containing 10 ml of PBS for virus control or an infusion of 8 ml of water and 1 cm³ of vegetable. In some instances about 0.1 g of feces was added first to the surface of glass or vegetables, and the virus inoculum mixed with a platinum wire. Samples were stored at 4 C for 1 to several days. Vegetable samples were left uncovered or enclosed in polyethylene bags containing a dish of water to maintain humidity. To determine the effect of a nitrogen atmosphere, samples were placed in a polyethylene bag with a dish of drierite, evacuated, then filled with nitrogen or air, three times.

Virus was removed by washing a vegetable sample or glass surface

with 10 ml of PBS containing 1% fetal bovine serum of pH 8 (6). The wash was repipetted 10 times over the inoculum area and centrifuged at $15,000 \times g$ for 30 min to remove bacteria. (Preliminary experiments had indicated that there were no significant differences in counts between portions of wash samples centrifuged and not centrifuged.) Triplicate aliquots of 2.5 ml of wash or virus control were assayed on appropriate monolayers. Triplicate aliquots of 2 ml of vegetable infusion were mixed with 0.5 ml of equal volumes of $10 \times$ medium 199 and fetal bovine serum before assay. Results were recorded as percent of the corresponding virus control maintained under similar conditions.

Test for naturally-occurring viruses in vegetables

At the time vegetables were collected for the tests above, additional samples were examined for the presence of naturally-occurring viruses. The four outer leaves of a head of lettuce were shaken for 3 min in 300 ml of PBS containing 1% fetal bovine serum, pH 8. Leafstalks of celery, groups of 6 carrots, 20 radishes, 3 green peppers, and 3 tomatoes were similarly washed. Washes were concentrated to 15 ml with a type PM30 ultrafiltration membrane fitted in a Diaflo Model 402 ultra-filtration cell (Amicon Corp., Lexington, Mass.). Concentrates were centrifuged at $15,000 \times g$ for 30 min to remove bacteria. Aliquots of 2.5 ml were assayed on monolayers of HEp-2, Vero, and African green monkey kidney cells. Monolayers of each received an agar or 5-ml liquid overlay of the appropriate growth medium. A blind pass was made from each liquid sample.

RESULTS

Survival of enteric viruses in water and PBS

The five test viruses showed no significant drop in titer within the 36-day storage at 4 C in water or PBS; all counts were at least 78% of the counts at zero time. At room temperature the number of plaque-forming units decreased progressively with time (Fig. 1) in essentially a first-order reaction. After 5 days, recoveries were less than 50% in all instances, except echovirus in PBS.

In all subsequent experiments on vegetables which were performed at 4 C, control samples (in PBS) showed no significant decrease in virus titer from zero time to sampling times up to 8 days.

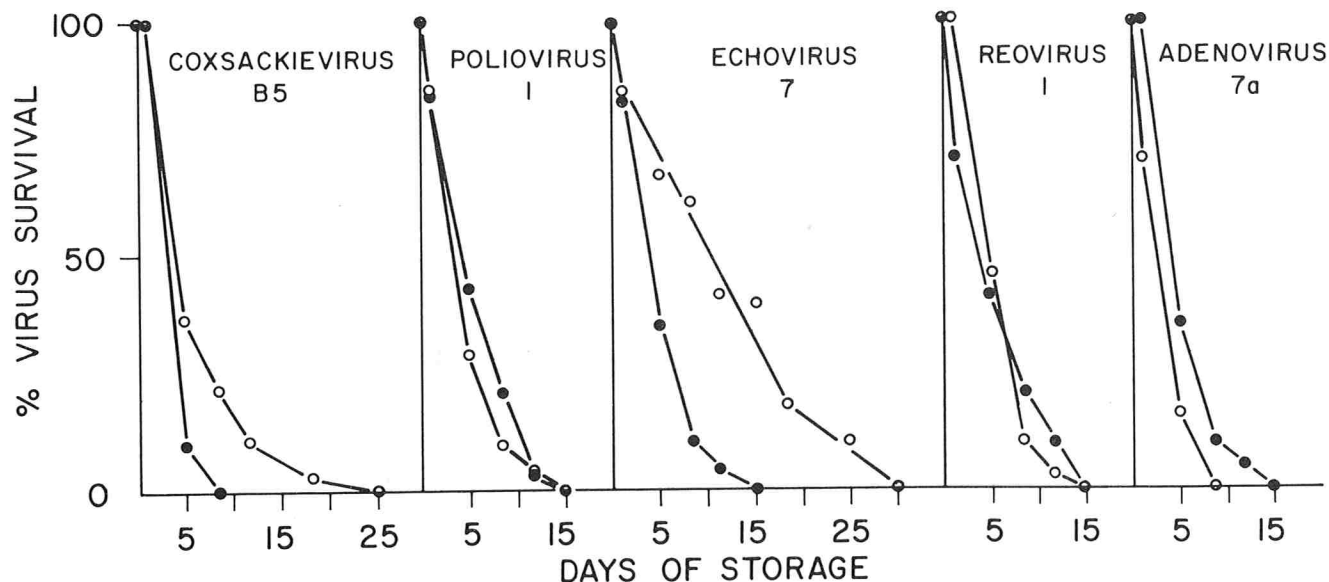


Figure 1. Survival of enteric viruses at room temperature in water, closed circles, and in PBS, open circles. Results of duplicate samples were averaged. Variation between duplicate samples was <15%.

Effect of drying on viruses

In two separate experiments it was found that on a glass surface each test virus, in a one-drop water inoculum, survived without loss for 1 and 4 days at 4 C in a humid atmosphere; the inoculum drops were visible at the time of assay. No virus (<1%) was recovered from any of the open dishes after 1 day; no inoculum drops were visible.

Tests with coxsackievirus, using duplicate samples in dry air and nitrogen, showed that neither a nitrogen atmosphere nor the presence of feces prevented virus inactivation on a glass surface in 1 day. Some virus survived on lettuce especially in contact with feces (Table 1).

TABLE 1. Percent survival of coxsackievirus B5 on glass or lettuce with and without feces after storage at 4 C for 1 day in dry air or nitrogen

Virus inoculum with	Storage			
	In air		In nitrogen	
	On glass	On lettuce	On glass	On lettuce
No feces	<1 ^a	20 ^b	<1	24
Dilute feces	<1	36	<1	22
Feces	<1	60	<1	48

^aNo plaques were found.

^bAn average of 2 samples. Variation between samples was <17%.

Survival of viruses on vegetables

In an early experiment it was found that coxsackievirus survived without loss of titer on covered pieces of celery for at least 8 days at 4 C; the virus inoculum drops did not evaporate. Virus on uncovered samples showed a progressive decrease in viability with time to no recovery (<1%) at day 5 (Fig. 2). Celery was turgid and moist at the inoculum site after 1 day, turgid but dry after 2 days, and limp after 5 days. To test the

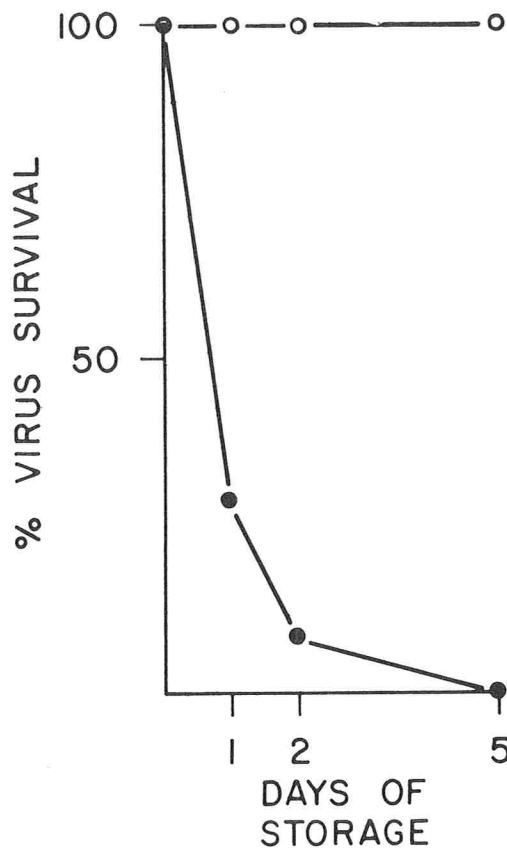


Figure 2. Survival of coxsackievirus B5 at 4 C on celery in a humid atmosphere, open circles, and exposed to air, closed circles. Single samples were assayed.

effect of rapid evaporation, the coxsackievirus inoculum of four samples was dried under laminar airflow for 20 min at room temperature. Immediate assay showed an average virus recovery of 12% (6-20%); no significant virus loss occurred in the four covered control samples.

In a subsequent experiment the five test viruses were compared for survival after 1, 4, and 8 days at 4 C on covered or air-exposed pieces of lettuce and celery. A similar experiment compared coxsackievirus, reovirus, and adenovirus on green peppers and coxsackievirus on tomatoes. (Single or duplicate samples were used for each condition.) Quantitative recoveries of all viruses were made from vegetable samples kept moist enough to prevent inoculum drying after 8 days of storage. Recoveries from uncovered samples after 1 day varied <1-31%; after 4 days no recoveries were made. The inoculum on tomatoes and green peppers evaporated within 1 day; some moisture was still present on lettuce and celery pieces at this time.

To test the effect of feces on decay, coxsackievirus or poliovirus in water or dilute feces was placed on the surface of radishes or carrot pieces. Virus in water was also mixed with feces on the surface of some samples. As with the other vegetables, the presence of moisture assured virus survival. The virus inoculum on uncovered samples evaporated rapidly and virus titer decreased with time. Feces delayed these effects (Table 2).

TABLE 2. Percent survival of coxsackievirus B5 on single samples of radish or carrot, with and without feces, after storage at 4 C for 1 and 5 days

Virus inoculum with	Storage			
	On radish		On carrot	
	1 day	5 days	1 day	5 days
No feces	9	<1	9	<1
Dilute feces	18	5	14	4
Feces	24	12	21	7

Effect of vegetable infusions on viruses

No significant drop in titer occurred in any of the conditions tested, i.e., the five test viruses stored with infusions of lettuce, celery, or green pepper for 1, 4, and 8 days and coxsackievirus and poliovirus with radish and carrot for 1 and 5 days and tomato for 1 day. These vegetables contained no apparent viral inhibitors.

Naturally-occurring viruses on vegetables

Between May and July, 1974, six heads of lettuce, two celeries, two lots each of tomatoes and green peppers, and one bunch each of carrots and radishes were tested for the presence of naturally-occurring viruses. None were found.

DISCUSSION

Storage temperature and moisture are important factors influencing the survival of enteric viruses. Bagdasar'yan studied the survival of several enteroviruses on vegetables; survival was dependent on storage temperature; refrigeration supported the viability of viruses longer than room temperature (2). Most of our experiments with vegetables were conducted at 4 C to eliminate the rapid virus decay (loss of virus infectivity) which occurred in water and PBS at room temperature. Virus inactivation was even more rapid with drying; both types of decay followed a first-order reaction. Changes which occurred were not simply related to oxygen exposure since drying in a nitrogen atmosphere resulted in similar virus losses. Virus decay on vegetable surfaces was less rapid than on glass, presumably because of transpiration moisture.

Of the vegetables tested, lettuce and celery were best suited for virus survival, because of their relatively moist surfaces. Indeed, leafy vegetables have been implicated in disease (4, 5). Evaporation was rapid from the shiny surface of tomatoes and green peppers. These vegetables, commonly stored uncovered, retain turgidity for several days; little transpiration occurs. Carrots and radishes, when left uncovered, dried rapidly to become visibly limp. Mixtures of virus and feces extended the period of virus viability on lettuce, carrots, and radishes.

It is unfortunate that all of the virus on vegetable surfaces is not removed with water. Previous results (7) showed a 33-51% recovery of coxsackievirus B5 from lettuce with a water wash at pH 7-8. Similar results were found in later studies with celery (unpublished results). Any residual virus may act as a source of infection.

However, during growth and marketing, vegetables are normally subjected to periods of surface drying and this, according to our results, adversely affects virus survival. In addition, other workers have implicated solar radiation in virus destruction on vegetables (8). Such factors contribute in the assurance that a reasonably high degree of safety prevails when vegetables reach the consumer market. This is substantiated by our results in which we found no virus in random sampling of vegetables in our area. As a special safeguard, washed vegetables may be towel- or air-dried and stored for several hours before consumption.

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Inactivation of Poliovirus 1 and Coxsackievirus B-2 in Broiled Hamburgers

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(Received for publication April 4, 1975)

ABSTRACT

Thermal stability of two enteroviruses, poliovirus 1 and coxsackievirus B-2, inoculated into ground beef patties was investigated using household broiling procedures. Internal temperatures during cooking were monitored by thermocouples placed in the centers of patties. The appearance of the centers of hamburgers correlated with the temperatures reached: red-pink-rare, 60 C (140 F); pink-brown-medium, 71 C (160 F); and brown-well-done, 76.7 C (170 F). Cooked and uncooked virus-inoculated patties were assayed for viral plaque forming units produced in Vero monkey kidney cell cultures. No viruses were detected in patties cooked to 60 C (140 F) and held at room temperature for 3 min. However, virus was recovered from 8 of 24 patties cooked to 60 C (140 F) and immediately cooled to 23 C (74 F). No viruses were detected in patties heated to 71 C (160 F) or 76.7 C (170 F) internal temperatures. Results indicate that the cooking time and temperatures used to prepare rare hamburgers wherein the center meat remains red may not be sufficient to inactivate viruses that might be present in the sample especially if the hamburger is consumed or cooled within 3 min of cooking. When frozen or partially defrosted patties are cooked, extensive external cooking can occur with little or no visible change in the coloration of the center meat.

Over 1×10^{10} kg (22 billion lb) of beef were consumed in the United States in 1973. Approximately half of this beef was consumed in the form of ground beef or hamburger—an average of about 454 g (1 lb) per week for each resident in the United States. There are no Federal microbiological standards for ground beef, and State and local regulations vary from being relatively strict to nonexistent. The quality of the market product basically depends on the sanitary control used by the processor and the market outlets, and on the effectiveness of the local health agencies (1).

In 1969 we reported finding viruses in 3 out of 12 market samples of ground beef (8). The isolates were identified as poliovirus 1, 2, and 3, and echovirus 6; the number of viruses recovered varied from 1 to 195 viral plaque forming units (PFU)/5-g sample. Therefore, a 113 g ($\frac{1}{4}$ lb) hamburger could contain from 23 to 4,400 PFU of virus.

Because of these findings, we conducted a study to determine if household cooking processes inactivated viruses. Ground beef patties were inoculated with either poliovirus 1 or coxsackievirus B-2 and were broiled in a small household cooking unit. The directions followed

were obtained from cookbooks commonly used in the home (2, 3). Processing temperatures of 60 C (140 F), 71 C (160 F), and 76.7 C (170 F) were used for rare, medium, and well-done hamburgers (7).

MATERIALS AND METHODS

Virus

Stock cultures of poliovirus 1 (ATCC VR 59) and coxsackievirus B-2 (ATCC VR 29) were propagated in Vero cell cultures (ATCC CCL 81). Leibovitz medium (L-15) containing 2% heat-inactivated fetal bovine serum was used to propagate virus in cell monolayers (6). Virus pools were prepared and used for the standard inocula.

Media

The diluents, suspending media, and growth media for the cells and the viral plaque assay were the same as those previously published (9). Eagle minimal essential medium (MEM), with Hanks balanced salts containing high concentrations of antibiotics, was used to prepare the meat slurries in these studies (4).

Methods

The glass wool filtration method (11) was utilized for recovery of virus from inoculated raw and cooked ground beef patties. Two-hundred milliliters of fluid diluent were added to the beef sample (a 113-g patty), the pH was adjusted to 8.5, and the slurry was shaken for 15 min. The pH was readjusted to 8.5, and the slurry was poured over glass wool. Twenty milliliters of the filtrate were assayed in 1-ml portions added to 45 cm² monolayer Vero cell cultures in 177.4 ml (6-oz) bottles. The cell cultures were overlaid with agar medium and incubated at 36 C (97 F). Virus plaques were counted and marked daily for 2 weeks.

Cooking procedure

Ground beef, 25% fat content, was purchased in a frozen lot of preformed patties. Individual patties (113-g) were thawed and placed in a plastic bag, and 3×10^5 PFU of poliovirus 1 or 3×10^4 PFU of coxsackievirus B-2 were added. The sample was kneaded for 2 min to evenly distribute the virus, and patties were reformed using a commercial hamburger form to produce individual units of 9 cm (3.5-in.) diameter and 1.3 cm (0.5-in.) thickness. Starting at the circumference of each ground beef unit, a copper-constantan thermocouple was inserted radially to a depth of 3.8 cm (1.5 in.). After cooking, the meat shrank to a diameter of 7.6 cm (3 in.), positioning the thermocouple at the geometric center of the patty. The patties were placed on a rack in a small commercial broiler that had been preheated to a temperature of 274 C (525 F). The hamburgers were placed 7.6 cm (3 in.) from the heating element and cooked in the following manner:

- | | |
|---------|---|
| Rare: | Broiled for 3.5 min, then turned and broiled until the internal temperature reached 60 C (140 F). (Total avg. cooking time: 7.9 min). |
| Medium: | Broiled for 4 min, then turned and broiled until the internal temperature reached 71 C (160 F). (Total avg. cooking time: 10.3 min). |

Well-done: Broiled for 5 min, then turned and broiled until the internal temperature reached 76.7 C (170 F). (Total avg. cooking time: 11.1 min).

After removal from the broiler, the hamburgers were left at room temperature for 3 min and then analyzed for viral content. The 3-min period was the time estimated between the end of the cooking process and the actual eating of the hamburger in the home. A number of samples were immediately disrupted into 200 ml of cold [5 C (40 F)] diluent and processed to determine whether viral inactivation was complete after the broiling process or dependent on the continuing heat treatment during the 3-min holding period.

Some consumers and commercial establishments prepare individual hamburger units and freeze them until use. The frozen or partially thawed raw patty is put directly onto the grill or into the broiler and cooked. A number of hamburgers were prepared in this manner, and the internal temperature was monitored.

RESULTS AND DISCUSSION

Several uninoculated hamburgers were cooked and examined organoleptically. The color at the center of each patty was the most useful characteristic in subjectively determining "doneness" of the meat. The rare patties were red to pink in the center, the medium were pink to brown, and the well-done were brown.

Survival of poliovirus 1 and coxsackievirus B-2 in the 81 heated samples was seen only in the rare patties [60 C (140 F)] that were cooled to 23 C (74 F) within 15 sec by disrupting them into cold [5 C (40 F)] liquid medium; 1.4% and 1.7% of the total poliovirus 1 and coxsackievirus B-2 inoculum were recovered, respectively, from 4 of 6 and 4 of 18 cooled patties. The ranges of recoveries from these patties were 0.06% to 2.9% for poliovirus 1 and 0.01% to 9.9% for coxsackievirus B-2. The percentages of recoveries were based on the number of viral PFU recovered from uncooked positive control patties and these controls yielded an average recovery of 54% of the seed virus inputs from 26 patties. The recovery is similar to that reported in a detailed paper on this method (11). No viruses were detected in 18 uncooled patties or from nine patties cooked to 71 C (160 F) and cooled. In actual practice, such a cooling procedure would not be used; however, its effect could be duplicated by consumers who start eating the hamburger immediately upon being served. The data indicate that hamburgers should reach an internal temperature higher than 60 C (140 F) if a margin of safety is desired. The most practical indicator of the desired temperature is the color; a hamburger should be brown to pinkish brown on the inside. The 39 medium and well-done patties yielded no detectable virus. All patties held for 3 min at room temperature [21 C (70 F)] after cooking essentially maintained their internal temperatures [i.e., this temperature dropped 3 C (5.4 F) or less].

Frozen [-11.1 C (12 F)] patties containing thermocouples were broiled for the same number of minutes required to bring the unfrozen [10 C (50 F)] patties to internal temperatures of 60 C (140 F), 71 C (160 F), and 76.7 C (170 F). The temperatures actually achieved were 18.3 C (65 F), 37.8 C (100 F), and 51.7 C (125 F) with subsequent temperature drops as observed in the patties

cooked from the unfrozen state. All centers of the cooked-frozen patties were red although exteriors appeared well-done. High-temperature searing of hamburgers produces a similar product. The average time required to cook the frozen patties to the rare, medium, and well-done stages was 14, 17, and 18 min, respectively.

Thermal destruction curves of other viruses in milk and milk products have been shown to be virtually asymptotic in configuration at temperatures below 60 C (140 F). Viral destruction rates at temperatures above 60 C (140 F) approached a first-order reaction (10). It is probable that similar viral destruction rates occur during the cooking of ground beef and that the viruses surviving at 60 C (140 F) are from the second portion or "tail" of such a curve. Cooking the meat to internal temperatures above 60 C (140 F) enhances the probability of viral destruction.

The amount of virus in foods is also important. Studies on heat inactivation of Sabin type 1 poliovirus at concentrations of approximately 1×10^7 pfu/gm in ground beef showed some virus survival in meat held at 80 C (176 F) for 5 min (5).

The incidence of viral contamination in foods is only partially known, and the thermostability of viruses in foods can differ. However, the inactivation of two enteroviruses, poliovirus 1 and coxsackievirus B-2, in ground beef patties broiled to internal temperatures above 60 C (140 F) indicates that heating food in this manner could be an effective barrier to consumption of such infectious agents. Proper cooking of hamburgers should be coupled with long-established procedures of good sanitation, such as the use of healthy animals as a food source and scrupulous cleanliness in all phases of food handling.

ACKNOWLEDGMENT

This research was supported in part by an interagency agreement AMXRED-73-184 with the U.S. Army Natick Development Center.

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Specialized Items Increase, Total Production Steady, Reports 1974 "Milk Facts"

These and other pertinent economic figures are reported in "Milk Facts," the annual publication of the Milk Industry Foundation. Included in the booklet is information on milk production, processing, distribution, consumption, nutrition and marketing. The Foundation is the national association of dairy processor and distributor companies.

Some additional highlights of "Milk Facts" are that:

- * Milk production in 1973 reached 115.4 billion pounds, approximately the same as the prior year.
- * Output per cow rose two percent upsetting the two percent drop in milk cows.
- * Dairy farmer cash receipts were up 16% over the previous year, totaling \$9.1 billion for milk sold to processors.
- * The "real" price of milk rose in 1974, but was still lower than in the 1950's and early 1960's.
- * Sales of fluid milk total 26.7 billion quarts, down two percent from 1973.
- * Lowfat and skim milk sales were up six and one-half percent and now account for thirty percent of total fluid milk sales.
- * Whole milk sales were down five and one-half percent, cottage cheese down eleven percent but yogurt was up eight percent.
- * Also up were cheese (five percent), ice cream (one percent), and butter (six percent).

"Milk Facts" contains several new features this year, one being a report on consumer profile and their attitude toward yogurt and cottage cheese. Reported for instance,

was that nearly two thirds of all U.S. families bought cottage cheese during the six months period surveyed; the pint is the most popular cottage cheese size and the large urban areas show the greatest interest in cottage cheese.

Yogurt is also popular in the larger cities and strawberry is its preferred flavor. Females are greater consumers of yogurt, particularly in the 17-19 years of age bracket. Yogurt, considered a particularly good food product by persons who are weight conscious, is considered good at lunch or as a snack between meals.

Another feature of the book is a pre bicentennial section on the "History of the Dairy Industry" which recounts the beginning of the dairy industry in the U.S. from 1611 through current times.

The thirty-two page booklet of the Foundation contains interesting data on how milk moves from the dairy farm to the ultimate consumer in the home, how the industry is regulated for product wholesomeness, purity, and the basic price paid to farmers, and how milk and milk products are used all over the world. Data are presented by individual states, regions and nationally, and a segment is devoted to the various nutritional elements in fluid milk.

Copies of "Milk Facts" are available from MIF for members at 8c per copy up to 1,000 or 7c per copy for orders of 1,000 or more. Nonmembers may secure copies at 11c per copy. Orders should go to the Milk Industry Foundation, 910 17th Street, N.W., Washington, D.C. 20006.

Environmental Factors Affecting Survival and Growth of *Vibrio parahaemolyticus*. A Review

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(Received for publication January 13, 1975)

ABSTRACT

Effects of temperature, salinity, water activity, and pH on survival and growth of *Vibrio parahaemolyticus* are reviewed. Maximum and minimum tolerances to these environmental factors are summarized, with special emphasis on conditions which exert stress or lethal effects on the organism. Behavior of *V. parahaemolyticus* as influenced by the interaction of environmental parameters is discussed.

Since the early 1960's *Vibrio parahaemolyticus* has been documented as the etiologic agent responsible for causing hundreds of foodborne disease outbreaks each year in Japan. Not until 1969, when *V. parahaemolyticus* was suspected as the cause of a foodborne disease outbreak in the state of Washington, did public health laboratories in the United States generate an increased interest concerning the organism's potential to cause illness. A great deal of research data has been published relating to the morphological and physiological characteristics of *V. parahaemolyticus* since it was first isolated in Japan in the early 1950's. This paper presents an update on effects of the environment on survival and growth of *V. parahaemolyticus*. Data presented are by no means comprehensive; however, sufficient information is given to provide the epidemiologist and public health sanitarian with a reasonable picture of the tolerance of *V. parahaemolyticus* to interacting environmental stresses. Such information would be of value during the examination of foodborne illnesses suspect as having been caused by *V. parahaemolyticus*.

HABITAT

Being halophilic in nature, it is not surprising that *V. parahaemolyticus* is found in estuarine and in brackish water. The organism has also been isolated from inland salt springs (19). Suitable growth substrates for *V. parahaemolyticus*, however, are not limited to shellfish, fish, and plankton. *V. parahaemolyticus* has been demonstrated to grow on extracts of radish, cabbage, lettuce, cucumber, pumpkin, and potato (38). The organism appears to be associated with habitats high in organic nutrient content. Limited data indicate that in marine environments which receive animal wastes, the total counts of *V. parahaemolyticus* and related vibrios

are generally higher than in water low in organic material (4). This relationship has not been thoroughly documented.

The organism has been isolated from coastal waters from around the world. Geographic distribution of *V. parahaemolyticus* in water habitats seems to be dictated for the most part by water temperature. Its presence in higher populations is correlated with increased water temperature during the summer months (8, 12, 23, 27, 28, 34, 37, 47, 48). Seasonal occurrence of illness caused by *V. parahaemolyticus* is sometimes referred to as "summer diarrhea."

OUTBREAKS OF GASTROENTERITIS

Fourteen common-source gastroenteritis outbreaks attributed to *V. parahaemolyticus* originating from coastal waters of the United States were reported to the National Center for Disease Control from 1969-1973 (8). Eight outbreaks were confirmed, while six were not. Maryland, which reported the first laboratory-confirmed outbreaks in 1971, accounted for six of the 14 reported, while eight other states reported one each. All outbreaks except one occurred between June and October, and all were attributed to the consumption of seafood.

TEMPERATURE FOR GROWTH AND SURVIVAL

Optimum temperatures for growth of *V. parahaemolyticus* range from 35 to 37 C. Lowest temperatures reported for growth range from 3 to 13 C and are highly dependent upon the growth substrate and habitat under study. Vanderzant and Nickelson (43) reported a sharp decrease in viable *V. parahaemolyticus* in inoculated whole peeled deveined shrimp during the first 2 days of storage at 3, 7, 10, and -18 C. During the next 6 days of storage, counts remained about the same. The same authors reported a slight increase in the population of *V. parahaemolyticus* in homogenized shrimp during the first 12 h of storage at 3, 7, and 10 C followed by decreases between 4 and 8 days. Lowest survival was noted in samples stored at 3 and -18 C.

The lowest temperature reported for growth of *V. parahaemolyticus* in laboratory media is 5 C (5). This

lower temperature limit for growth is markedly affected by pH. Of six strains tested, the lowest pH permitting growth at 5 C was pH 7.3. One strain would not grow at 5 C in media below pH 7.7. Jackson (13) reported minimum growth temperatures of 9.5 to 10.5 C in laboratory media maintained in a stationary position during incubation. Stirring of a laboratory medium (Trypticase soy broth plus 3% sodium chloride, pH 7.2) resulted in some cultures growing at 7 and 8 C. These data are in close agreement with the earlier report (5) using a similar medium which indicated that pH 7.1 to 7.7 was minimal for growth of *V. parahaemolyticus* at 9 C.

Thomson and Thacker (40) showed that *V. parahaemolyticus* can multiply to dangerous levels when oysters are stored at temperatures greater than 8 C. Multiplication occurred at 10 and 12 C and loss of viability was noted during storage at -20, 0, and 4 C. Liston et al. (25), in a summary of the growth characteristics of vibrios, listed *V. parahaemolyticus* as having a minimum growth temperature range of 5 to 8 C. The organism may survive for long periods on the surface of sea fish held at 5 and 8 C (34).

V. parahaemolyticus on whole fish and shellfish is, however, susceptible to the lethal effects of chilling and freezing, with chilling generally yielding the higher lethality (24). Asakawa (1) reported a greater rate of decline of viable *V. parahaemolyticus* in laboratory media held at -10 C than at -20 C. Survival of *V. parahaemolyticus* in raw tuna was higher at storage temperatures of -10 and -20 C than at 0 C. Johnson and Liston (15) tested the survival of *V. parahaemolyticus* on marine fish and shellfish held under refrigerated and frozen conditions. In fish fillets, viable *V. parahaemolyticus* were detected after 15 days at 1 C and 60 days at -15 and -30 C. Viable organisms were recovered from crab meat stored at 1, -15, and -30 C for 30 days. The organism could be detected in inoculated oysters after 40 days of storage at 1 C and 130 days at -15 and -30 C. Loss in viability was shown to occur at 11, 8, and 5 C. Johnson et al. (16) demonstrated that *V. parahaemolyticus* could survive at least 3 weeks at 4 C with little or no apparent decrease in population. Matches et al. (26) reported that holding inoculated English sole homogenate at 0.6 C resulted in higher lethality to the bacterium than storage at -18 or -34 C. Significant differences in survival have been noted for one of three *V. parahaemolyticus* strains in Trypticase soy broth held at 5, -5, and -18 ± 1 C (10). Survival at -5 C was less than at 5 and -18 C. Fish homogenate tended to stabilize *V. parahaemolyticus*, regardless of the test temperature. Temmyo (38) showed complete lethality of *V. parahaemolyticus* in saurel and mackerel at -10 and -16 C for 4 days.

V. parahaemolyticus can be isolated relatively easily from oysters and marine sediment samples, even when they are at temperatures below 10 C; however, it can be isolated less readily from sea water and not at all when the water temperature falls below 13 C (29). Kaneko and

Colwell (18) found very large numbers of *V. parahaemolyticus* in and on zooplankton, particularly when the water temperature rose above 14 C. They theorized that the organism survived in plankton and sediments during the winter months, increasing in numbers in the water during the summer.

V. parahaemolyticus has been isolated from breaded frozen shrimp and oysters (14, 25) and frozen tuna, Cuttlefish, and cod fish (38). Mishandling of these items in the market place or in the home could result in *V. parahaemolyticus* growth and subsequent increased potential for human foodborne infection. These data point up the fact that, although *V. parahaemolyticus* is sensitive to refrigeration and frozen conditions, such conditions should not be relied upon for the destruction of the organism.

Maximum growth temperatures for *V. parahaemolyticus* range from 42 to 44 C (25). Temperatures vary among strains and are affected by culturing conditions. However, some strains examined by investigators were observed to grow at 42 C (30, 32, 41, 42), 43 C (14, 15, 17, 24, 29, 31, 34), and 44 C (13).

INACTIVATION BY HEAT

Although *V. parahaemolyticus* is sensitive to heat, a wide range of rates for thermal inactivation has been reported. Susceptibility of the organism to inactivation at elevated temperatures is greatly dependent upon cultural procedures used to grow the organism as well as the type of menstruum in which the organism is placed during the heat treatment. Vanderzant and Nickelson (43) were not able to recover viable *V. parahaemolyticus* from shrimp homogenate initially containing 500 cells/ml after the homogenate was heated for 15 min at 60 or 80 C or 5 min at 100 C. However, when the initial population was 2×10^6 /ml, *V. parahaemolyticus* could be recovered from samples heated for 15 min at 60 or 80 C. No survivors were noted in the homogenates heated for 1 or 5 min at 100 C. Others have reported killing times of 30 min at 60 C (25) and 15 min at 60 C (34). Temmyo (38) reported that *V. parahaemolyticus* could be killed if heated in peptone solution for 10 min at 55 C or for 5 min at 60 C. He also tested susceptibility of the organism to heating conditions simulating those which might be found in processing plants, restaurants, or homes. Saurel, octopus, and cuttlefish were inoculated with *V. parahaemolyticus*, covered with flour, and fried in salad oil for about 2 min at 180 C. No survivors were detected. Saurel, yellow tail, and flying fish were contaminated with *V. parahaemolyticus* and grilled over a gas gridiron, an electric fish roaster, and in an electric oven. No *V. parahaemolyticus* were detected on samples judged as underdone or well done.

Goldmintz (12) and Beuchat (5) noted that *V. parahaemolyticus* inactivation at elevated temperatures was related to pH of the heating menstruum. Both authors reported that highest resistance to heat was at pH 7.0. Sensitivity increased as the pH became acidic or alkaline.

Thermal death time curves for several strains of *V. parahaemolyticus* have been noted to follow diphasic patterns. Baab and Johnson (2) reported fast, followed by slower rates of inactivation of *V. parahaemolyticus* from continuous cultures when the organism was heated at 49 C. Goldmintz (12) showed the same diphasic inactivation curve phenomenon when cells were heated at 48 and 55 C. We have also noted two-slope inactivation curves for *V. parahaemolyticus* heated at 47 C in buffer containing elevated levels of sodium chloride (7).

The presence of sodium chloride in heating menstrua appears to have a protective effect against inactivation. A crab meat-salt broth containing 1.5×10^6 cells/ml was heated at 48 and 55 C and this resulted in approximately 2.5 and 4-log reductions, respectively, of viable *V. parahaemolyticus* (12). Complete elimination of viable cells was achieved when the broth was heated for 10 min at 65 C. Covert and Woodburn (10) showed that sodium chloride, at concentrations ranging to 12% in Trypticase soy broth, afforded protection against lethality at 48 C. *V. parahaemolyticus* was less sensitive to inactivation at 48 C when fish homogenate was used as the heating medium. Addition of sodium chloride to the fish homogenate resulted in even greater heat resistance. Salt concentration in recovery media has also been shown to influence the resuscitation of thermally stressed cells (44).

Growth temperature affects the heat resistance of *V. parahaemolyticus*, as does the sodium chloride content of the growth medium (7). Cells grown at 21 C were more sensitive to heating at 47 C than were cells grown at 29 C. Cells cultured at 29 C were, in turn, more sensitive to heat than cells grown at 37 C. Cells grown in Tryptic soy broth containing 3.0 or 7.4% sodium chloride displayed more resistance to heat than those grown in broth containing 0.5% salt. The same authors reported a relationship between the fatty acid content of *V. parahaemolyticus* and the growth temperature and heat sensitivity of the organism.

TOLERANCE OF SODIUM CHLORIDE

In addition to interacting effects of sodium chloride on survival of *V. parahaemolyticus* in heated and refrigerated or frozen media, there are other effects exerted by sodium chloride at normal growing temperatures. The organism requires sodium chloride for growth and will multiply in substrates containing as little as 0.5% of salt. In fact hemolysin production, associated with the infectious nature of *V. parahaemolyticus*, was shown to be increased considerably in brain-heart infusion containing 0.5% sodium chloride (46). This was in contrast to the observation that 3% (approximately 0.5 M) sodium chloride was required for luxuriant growth of the bacterium. The 3% level is generally accepted as an optimal concentration for growth of *V. parahaemolyticus*. Less agreement is noted, however, on the organism's maximum sodium chloride tolerance. One criterion for

identifying *V. parahaemolyticus* from *V. alginolyticus* and some other vibrios is the inability of *V. parahaemolyticus* to grow well in Trypticase broth containing 10% sodium chloride (3). The inability to grow in appropriate nutrient media containing 10% sodium chloride has been reported by many researchers (11, 14, 18, 21, 29, 31, 32, 34, 35, 45). One author states that the maximum sodium chloride concentration tolerated by *V. parahaemolyticus* is 8% (22). On the other hand, numerous reports show that some *V. parahaemolyticus* strains can tolerate and grow in media containing 10% sodium chloride (6, 9, 17, 20, 25, 36, 41, 42, 48). These apparent discrepancies are related to other media constituents and pH, incubation temperatures, and variability among strains of *V. parahaemolyticus*. It has been demonstrated that halotolerant marine vibrios, including *V. parahaemolyticus*, are not homogeneous with respect to their sodium ion requirement and its substitution with other monovalent cations (33). The authors suggest that *Vibrio* biotypes exist which have intermediate properties between *V. parahaemolyticus* and *V. alginolyticus*.

Tolerance of *V. parahaemolyticus* to low and high sodium chloride concentrations is related to osmotic as well as ionic sensitivity. The bacterium is readily inactivated in distilled water, 90% death occurring within 0.9 to 4.4 min exposure (21). Sensitivity is dependent upon cultural age. Resistance to inactivation increases as the organism passes through log, stationary, and negative log phases of growth. Temmyo (38) also reported a rapid killing of *V. parahaemolyticus* in distilled water.

Inhibition of growth in substrates containing low or high sodium chloride levels results in part from unfavorable water activity (a_w). Beuchat (6) reported that the maximum generation time for *V. parahaemolyticus* was observed in Tryptic soy broth containing 2.94% sodium chloride which corresponded to an a_w of 0.992. Media containing lower or higher concentrations of sodium chloride resulted in higher or lower a_w , respectively, and slower generation times. Minimum a_w for growth in Tryptic soy broth with added sodium chloride was 0.948 at 29 C. Other solutes were added to Tryptic soy broth containing 2.9% salt to achieve reductions in a_w and establish minimal a_w for growth. Minimal a_w for growth of *V. parahaemolyticus* was 0.937 when glycerol was used as the test solute, 0.945 for potassium chloride, 0.957 for sucrose, 0.983 for glucose, and 0.986 for propylene oxide. It appears that minimal a_w for growth depends upon the solute used to control a_w .

Water activity tolerance of several strains of vibrios was investigated as a possible diagnostic tool (32). Based on optimal growth responses in 1% peptone water containing 0 to 12% sodium chloride (a_w 0.9988 to 0.9268), it was suggested that a_w data could be useful for identification of vibrios and for assessing favorable a_w ranges for their growth in foods.

DRYING AND REACTION OF SUBSTRATE

V. parahaemolyticus is very sensitive to drying (39). Rapid death of the bacterium occurred when inoculated membrane filters were placed in a container with silica gel and when an inoculated chopping board was allowed to dry (38).

The optimum pH range for growth of *V. parahaemolyticus* is 7.6 to 8.6. Growth has been reported in media at pH 5.0 to 11.0 (30, 41). Two of six strains tested for minimum pH tolerance in our laboratory grew at pH 4.8 (5). Shrimp homogenate adjusted to pH 4.0 or lower was reported to be highly lethal to *V. parahaemolyticus* (43). The latter authors showed a sharp drop in viable cells at pH 5.0, with no survivors detectable after 15 min. Viable population in homogenates adjusted at pH 6.0 to 10 remained about the same for 2 h.

SUMMARY

In summary, the response of *V. parahaemolyticus* to environmental conditions is dependent upon complex interactions between many chemical and physical forces. The organism is very sensitive to both heat and cold, generally displaying more resistance to freezing than chilling. Its resistance to potentially lethal extremes in temperature is greatly affected by the chemical makeup of the medium in which the organism is treated. *V. parahaemolyticus* is sensitive to desiccation and grows best in a medium containing sufficient sodium chloride (about 3%) to reduce the a_w to about 0.992. It prefers an alkaline pH for growth, but is most resistant to heat at pH 7.0.

It is not surprising that outbreaks of foodborne illness attributable to *V. parahaemolyticus* in the United States have occurred due to consumption of seafoods which were contaminated after cooking or steaming. The organism would not be expected to withstand the thermal treatment normally administered during these cooking procedures. Investigations have, in fact, suggested that the cooked food products implicated in U.S. outbreaks were contaminated with raw products while being transported or stored and were subjected to time-temperature conditions adequate to support growth of *V. parahaemolyticus*. It is important that the sanitarian be aware of the environmental optima and limits for growth and survival of *V. parahaemolyticus* to assess the organism's likelihood of being the etiologic agent responsible for foodborne disease outbreaks.

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Use of Counts After Preliminary Incubation to Improve Raw Milk Quality for a Denver Plant

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(Received for publication January 2, 1975)

ABSTRACT

A Denver processing plant was dissatisfied with limited ability of Standard Plate Counts to reflect production conditions on farms. They adopted Preliminary Incubation (PI) at 13 C (55 F) for 18-20 h and reduced the maximum permissible counts in their Milk Quality Rating Program. As a result the percentage of samples with counts >100,000/ml dropped from 23.9 in April 1973 to 0.5 in September 1974. High PI counts on tanker loads were traced to contamination from farm bulk tank outlets. This contamination was not revealed in counts without PI.

There is increasing recognition that the main purpose in doing bacteriological tests on raw milk is to give the consumer some assurance that it has been produced under acceptable conditions. Hartley et al. (6) have shown that the Psychrotrophic Bacteria Count (PBC) best indicates such conditions. However, incubation at 7 C (45 F) for 10 days (1) is generally considered impracticable for routine testing; a faster method of detecting these organisms is badly needed. For this purpose Preliminary Incubation (PI) of samples at 13 C (55 F) for 18 h has proven useful (7). Under these conditions, gram-negative rods, which comprise the bulk of the psychrotrophs (10), outgrow other types. As these enter the milk from neglected cows or equipment, a high count following PI strongly suggests appreciable contamination from these sources. Some psychrotrophs fail to show up on the Standard Plate count (SPC) at 32 C., this temperature being too high for them (6, 8). With the prospect of a lower incubation temperature and longer time being adopted in the next edition of *Standard Methods for the Examination of Dairy Products*, the SPC should be a more useful procedure, especially after PI.

Despite its theoretical advantages, published results of survey-type studies (6, 9, 12) have generally shown little or no advantage in using PI. However, a number of people engaged in raw milk quality improvement have been favorably impressed with its ability to indicate where cooling has been substituted for cleaning and sanitizing (2, 3, 4, 5, 16). Convincing evidence of its value is found in the results obtained by a fluid milk processing plant in Denver, Colorado. Since 1955 they had operated a Milk Quality Rating Program; the maximum raw milk plate count requirements per milliliter for the top three categories were 15,000 for Category [1], 20,000 for [2] and 24,000 for [3]. After 20 years of experience it became apparent that something

better was needed, as some producers were getting top ratings even though their production practices left much to be desired.

In the hope that PI would help, a 6-month trial was conducted. This satisfied them that it gave a much better indication of production practices, so the Milk Quality Rating Program was revised to incorporate the PI Raw Plate Count to replace the standard Raw Plate Count previously in use. At the same time the acceptable count limits were lowered to [1] 8,000, [2] 16,000 and [3] 24,000/ml for the top three categories. Since counts after PI usually *average* four times higher than before PI [and may be as high as 130 times (7)], these standards are more rigorous than any I know of. To meet them no producer dare cut any corners.

With the cooperation of the fieldman, this program was put into effect on March 1, 1973. During the preceding 6 months, 45 of 110 producers had shown high enough PI counts to exclude them from the top three categories. These 45 were then subjected to weekly testing for 2 months, while field calls were made on many of them to help them improve their PI counts.

The effect of the new program is graphically illustrated in Table 1, which shows the percentages of semi-monthly

TABLE 1. *Distribution of PI counts, 1973-4*

Number of samples	Month	8,000/ml or less (%)	24,000/ml or less (%)	Over 100,000/ml (%)	Percent producers qualifying
<i>1973</i>					
232	Jan.	32.9	59.0	16.7	52
236	Feb.	20.4	54.4	21.7	58
233 ^a	Mar.	38.2	65.5	15.0	27
234	April	30.3	49.1	23.9	19
228	May	45.6	65.5	17.7	25
227	June	52.9	72.7	11.9	35
226	July	59.3	80.5	3.1	31
227	Aug.	63.0	82.8	7.9	40
229	Sept.	68.1	89.5	5.2	61
221	Oct.	61.5	82.3	2.3	59
210	Nov.	53.8	89.0	2.9	72
213	Dec.	58.7	81.2	2.3	69
<i>1974</i>					
209	Jan.	61.7	85.6	4.3	60
210	Feb.	55.0	79.9	7.1	59
211	Mar.	67.3	90.0	3.8	65
205	April	55.1	76.6	5.8	57
205	May	66.9	86.3	2.0	56
204	June	65.7	84.8	2.0	62
201	July	69.1	87.1	2.0	60
203	Aug.	64.0	87.2	1.5	65
202	Sept.	79.7	94.1	0.5	69

^aStarted new Milk Quality Rating Program, employing PI counts.

samples falling into the several categories. Of particular interest is the sharp drop in the percentage of samples with PI counts >100,000/ml from 23.9 in April to 2.3 in October, then on down to 0.5 in September 1974. If Grade-A milk is allowed up to 100,000/ml *without PI*, one wonders how milk of the quality indicated in this table should be designated!

It is also noteworthy that the percentage of producers qualifying for quality rating with the much stiffer standards after March 1, 1973 was actually higher for most months after the change was made. This shows that standards as rigorous as these can be met by most producers, because with few exceptions the fieldman was able to find the cause of a high PI count. And there has been a noticeable improvement in production practices.

Another interesting finding of the usefulness of PI was made. PI counts on tanker loads of milk frequently exceeded the average of counts from producers' samples from that load. This indicated contamination after milk had left the farm bulk tanks *which was not reflected by the count before PI*. One hauler noted that in many instances the tank outlet contained some milk. Usually this resulted because the producer had drawn off milk from the outlet for household use. To determine whether this condition was responsible for the higher PI counts from tanker loads, counts were made for 11 loads, then for the next 10 loads the hauler washed and sanitized the outlet of each vat before emptying it. As data in Table 2

TABLE 2. *Effect of washing and sanitizing bulk tank outlets*

Before correcting bad conditions		After correcting	
11 Loads ^a		10 Loads ^a	
	Route 206		
SPC	SPC-PI	SPC	SPC-PI
24,000	260,000	36,000	74,000
	Route 207		
25,000	280,000	41,000	140,000

^aCounts represent arithmetic averages.

show, this procedure sharply reduced the spread between counts before and after PI, indicating a much smaller contamination with psychrotrophs. This is of great importance where milk is held in storage at the plant for several days before processing (13).

Although pasteurization, with rare exceptions, destroys psychrotrophs, many of these possess heat-resistant enzymes which continue their attack on

protein and/or fat after pasteurization and at normal storage temperatures (13, 14). Thus there is every reason to believe that, other things being equal, raw milk containing many psychrotrophs will, even after processing, possess inferior shelf-life (14).

Results presented here show that PI before plating markedly increased the effectiveness of the plate count on raw milk in indicating those farms where sanitary conditions were sub-standard. This led to a definite improvement in the bacteriological quality of the supply, which should be reflected in the finished product.

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Oregon's Experience With Microbiological Standards for Meat

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(Received for publication June 6, 1975)

ABSTRACT

Oregon is the first state to establish microbiological standards for meat products at the retail level. Using data from sampling results of a 1972 survey of meat products sold at retail, microbiological standards for meat were adopted at a public hearing effective May 1, 1973. The standards as adopted set a limit of 5 million aerobic plate count (APC) per gram for fresh or frozen meats (including ground and whole cuts) and 1 million organisms (APC) for processed (cooked) meats. The limit for *Escherichia coli* is 50 per gram for fresh or frozen meat products and 10 per gram for processed meats. The APC provision does not apply to meat food products that have been fermented or inoculated. Prepackaged samples are obtained from retail display cases on a scheduled basis and shipped to the department laboratory for analysis. Enforcement policy is to take penalty action on the third successive standard violation of the same product. On 1830 samples in 1974, there were three APC and eight *E. coli* enforcement actions taken on fresh ground meat products and none on processed meats. Less than 1% of all samples collected resulted in ultimate enforcement action. Microbiological results to date indicate a decline in the number exceeding standards. Improvement in sanitary conditions of retail meat markets is evidenced by a decrease in the number of items checked for violation of the sanitation standards. Bacterial standards are proving to be a useful tool for meat market operators to relate clean meat handling practices and proper temperature control to compliance with the standards.

Establishment of bacterial standards for meat products by the state of Oregon didn't just happen. They came into being as the result of a series of events.

The development of the standards in Oregon started with the city of Portland Health Department. It initiated a sampling program of meat for bacterial analysis in the late 1950's. Results led to promulgation of a city ordinance establishing a 10,000,000 per gram aerobic plate count (APC) standard for meat products. It was a limited program as to sampling and enforcement.

The State Department of Agriculture meat sampling program started when in 1966 it moved into a new building having a meat laboratory. An unofficial sampling program was carried on for some time in connection with limited retail meat market inspection.

The experience gained from this sampling and inspection led the department to establish by regulation in 1971 an APC level of 10,000,000 per gram and an *Escherichia coli* standard of 50 per gram for fresh ground meat products as a meat quality improvement and a meat market sanitation inspection tool. The enforcement of the standards was slowed down by the Federal/State Meat Inspections Program which shifted

the emphasis away from retail market sampling and inspection to the processing plants. This situation prevailed until 1972 when the state dropped out of the federal program and the department reactivated its sampling and inspection of retail markets.

About the same time, the city of Portland discontinued its municipal milk and food establishment inspection programs which placed the Division of Food and Dairy of the Department of Agriculture in the unique position of being the only agency in the state responsible for inspection of all milk, food, and retail meat and food establishments. I believe Oregon is the only state where this situation exists. It has a great advantage over fragmented state and local inspection programs that exist in most states by being able to achieve a more efficient, effective statewide uniform inspection, sampling, and enforcement program.

Support from consumer organizations also played an important part in getting legislative approval of the stepped up inspection of retail meat and food markets. At the same time, local headlines were being made in the state by the Oregon Students Interest Research Group (the first college-level consumer organization established by Ralph Nader). It used data from the city of Portland report on bacterial load analysis of luncheon meat products and issued a news release stating that "a potentially serious public health consequence exists because high bacteria counts have been discovered in ready-to-eat luncheon meats." Other consumer groups joined in with the student group demanding that something be done about the problem.

To counter and answer these concerns, the Department of Agriculture began a special investigative microbiological survey of prepackaged luncheon meats in April, 1972 to determine the quality of these processed meats at the retail level in Oregon. The state was divided into five sampling areas, taking into consideration population as well as location to major distribution points. Two hundred and fifty samples were taken from the retail meat case with a market basket-type approach with the inspector instructed to select products from the meat case in much the same manner as a housewife would. The samples were taken from three major chain outlets and three independent stores within each marketing area. The entire spectrum of prepackaged luncheon meats were divided into ten major categories

and a specified number in each category were sampled.

The department sought the counsel of a committee of knowledgeable scientists to assist with the survey, evaluate the analytical results of the luncheon meat samples surveyed including a review of fresh meat sampling records, and formulate reasonable bacterial standards for meat food products.

RESULTS OF THE SURVEY

The survey committee issued the following finding and conclusions in September 1972:

- (a) "A comparative report of the total aerobic plate count indicates that over a third of the products sampled, both the fresh and processed meat products, were in excess of ten million colonies per gram. However, on the other hand, 43% of the fresh meats and 63% of the processed meats had less than one million organisms per gram.
- (b) "As can be noted from the data, no difference was detected in the quality of the product in relationship to the market area nor in the type of store, that is, chain store versus independent. However, during the months of warmer temperatures, higher counts were obtained.
- (c) "The data suggest the possibility of improper handling, rotation of stock, storage, refrigeration, and transportation of meat products exists in Oregon and could very likely be contributing factors for high bacteria counts. There was, however, no indication of high bacterial counts in processed meats at the point of manufacture.
- (d) "In summary, the data show that majority of the products offered for sale to consumers in Oregon are within acceptable bacterial levels. They also indicate that reasonable standards can be achieved by the industry. Unfortunately, excessive counts encountered point up the need for suitable standards and adequate enforcement. In simple terms, it means that the processed product the consumer may buy at a retail store is likely to be either relatively free from or loaded with bacteria."

The committee concluded its report by recommending that:

- (a) Total bacterial count (APC) should not exceed 5 million organisms per gram for fresh meat (including ground and whole cuts) and 1 million organisms per gram for processed meats.
- (b) The organism *E. coli* should not exceed the most probable number (MPN) or 50 per gram for fresh meats or 10 per gram for processed meats.

PUBLIC HEARING

These bacterial levels as recommended by the committee were submitted to a public hearing February 27, 1973, by the Department.

The preponderance of testimony and evidence received was in support of the proposed standards as being in the interest of consumer health protection and improvement

of meat product quality. Although there were many representatives of meat processors and retailers in attendance at the hearing, no adverse testimony was received. The following proposed standards were adopted and they became effective May 1, 1973:

- (1) Meat food products, whether fresh, frozen, prepared or otherwise manufactured, shall be deemed to be adulterated as defined in ORS Chapters 616 and 619 if:
 - (a) The bacterial level exceeds 5 million organisms per gram in fresh or frozen meat food products (including ground, chipped, fabricated, and whole cuts thereof), or exceeds 1 million organisms per gram in meat food products which have been cooked, smoked, or otherwise heat-treated.
 - (b) The most probable number (MPN) of *E. coli* organisms exceed 50 per gram in fresh or frozen meat food products (including ground, chopped, fabricated, and whole cuts thereof), or exceeds 10 per gram in meat food products which have been cooked, smoked, or otherwise heat-treated.
- (2) The APC provisions shall not apply to meat food products which have been fermented or inoculated as a procedure of preparation or manufacture.
- (3) The microbial examinations and procedures utilized to determine compliance with the standards shall be those recognized and accepted by the United States Department of Agriculture and published in the Microbiology Laboratory Guidebook of the United States Department of Agriculture, Consumer and Marketing Service, Technical Services Division, and shall also include standard procedures for the confirmation of *E. coli* organisms when they are determined to be present.

INSPECTION AND SAMPLING

Upon adoption of the revised meat bacterial standards including processed meat products, the department proceeded to organize the retail meat market inspection and sampling program. An educational program was developed for the sanitarians to acquaint the retail meat market and food store management with the new standards which preceded their enforcement.

Compliance is usually in direct ratio to the degree of enforcement of a standard. The department's enforcement policy is to try to obtain compliance by persuasion coupled with an educational program as to the purpose of the standard. Violation warning notices with inspections are used to try to determine cause of the violation. Penalty action is taken only where these administrative actions fail to obtain compliance.

The enforcement policy on bacterial standard violations is a warning notice on the first two violations on the same product and penalty action on the third successive violation. An inspection is usually made of the meat market between each of the first two violations.

We collect approximately 200 samples per month. The sample pick up for each sanitarian is scheduled out of

the head office by the Sanitarian Supervisor for the 2400 retail markets. Each sanitarian picks up 10-30 samples averaging five per store, depending on assigned markets, with about 70% of the samples collected being fresh meat and 30% processed meats.

Scheduled samples are analyzed for aerobic plate count, *E. coli*, *Salmonella*, coagulase positive *Staphylococcus aureus*, and *Clostridium perfringens*. The policy on samples in violation of the APC or *E. coli* standard is to resample the product within 60 days of the previous sampling. The same resampling policy also applies to products containing pathogens. This means the meat market operator will have time after he receives the laboratory results on the prior sample to take corrective action before the next resampling occurs.

COURT ACTION

Where three successive APC or *E. coli* violations have occurred on the same product, a request is routed to the Department enforcement section to implement penalty action. It receives the case file, makes an investigation, and if it determines that the sampling and analytical procedures are in order, it will file charges with the district attorney in the county where the violation occurred. This results in a misdemeanor charge which is a criminal complaint court action under the Oregon Criminal Code. Less than 1% of samples analyzed actually results in ultimate enforcement action. Our sampling and enforcement program did not fully get underway until October 1973. During this time, the department filed its first two court complaints on violations of the APC standards. Both were on prepackaged sliced ham. Eleven court complaints were filed in 1974; Three on APC violations and eight on *E. coli*—All on fresh ground meat products.

Maintaining sample integrity and chain of evidence is essential to sustain violation charges. We have had to carefully outline the sampling procedures. The sanitarian picks up prepackaged meats out of the display case the same as a customer would. He pays for the sample at the checkstand, gets a receipt, marks an identification number on the sample and fills out the sample laboratory form, puts the sample immediately on dry ice in an insulated sample case, seals the case, and ships it to the department laboratory by bus line. On the second resample, one person in the laboratory is detailed to break the seal, log in the sample, and supervise the analytical procedure all the way through to the final reading and logging of the counts. In this way, there will be only one laboratory person responsible for the receiving, analysis, and reporting of the results. He then will be the only laboratory witness that will be required to testify at a trial. Just about every aspect of our sampling, analytical, and result recording procedures have been subject to court review. Cases have been lost on technicalities. But so far, there has been no successful challenge to the analytical methodology.

LACK OF KNOWLEDGE

A major problem in achieving compliance with the bacterial standards is the lack of knowledge about meat microbiology by the retail market operators. We recognized this problem at the outset of implementing the inspection and enforcement program. We used our consumer officer to develop educational material such as a pamphlet on bacteria in meat for our sanitarians to hand out to the retail market operators.

We related the sampling results to the sanitary condition of their markets and the keeping quality of the meat. This approach has gotten their attention and we have obtained significant improvements in the sanitary condition of the markets. They have been able to see a direct relation between cleanliness and storage time and temperature and the quality of meat. We have gotten sawdust out of the shops and now have clean floors. We acquainted them with cleansing agents and sanitizers. The response has been good. They are having less waste, longer shelf life, and better consumer acceptance of products.

Another factor that has played a significant part in getting compliance with the bacterial standards is the 1973 legislative passage of the open date labeling law for perishable foods including meat products. Fresh meats are labeled with the pack date and processed meats with the pull date. This has made for a much better inventory control of meats being offered for sale resulting in fresher products in the display case.

Another assist is the cooperation of the Oregon State University Food Science and Technology Department Extension Specialists who have developed a meat sanitation training seminar for retail meat market operators. They also have been doing some important temperature profile studies of retail meat and food refrigerated display cases. As the result of their temperature findings, serious deficiencies have been uncovered which have been of help to the department in its inspection work and to the retail market operator in keeping the products properly refrigerated. Temperature abuse of meat products at the retail level is, without question, one of the principal contributing factors to high counts.

Our consumer officer's *Consumer Protection Newsletter*, published every other month, includes the list of meat markets violating the bacterial standards. This is an aid to enforcement. But the primary aim will continue to be to use the newsletter as a means to bring about a better awareness of consumer concerns on the part of the meat industry, while at the same time informing the consumer of problems industry has in satisfying those concerns.

While the department recognizes the public health aspect of the presence of pathogens in meat products, I want to again emphasize that the department's primary reason for establishing bacterial standards, was to use them as a tool for improving the sanitation of meat handling practices in retail markets, and to enhance the

quality of meat being offered for sale to the consumer, with public health protection being an expected additional benefit.

RESULTS TO DATE

In answer to the question, what do the bacterial findings to date show?—let me preface my remarks by stating that we do not have sufficient data to draw positive conclusions on the results obtained at this time.

However, the data obtained so far do indicate some interesting trends by comparing the 1972 six-month survey findings with the analytical results for the 12-month enforcement period ending October 1974 and the three-month period terminating April 1975.

With processed meat products, the 1972 survey revealed that 37% of the samples exceeded an APC of one million per gram. Whereas 34% and 21% violated the standard for the respective 12- and 3-month enforcement periods.

The fresh meat APC findings for these same periods also showed a significant improvement in meeting the 5 million per gram standard. The counts exceeding the standard decreased from the survey finding of 43% to 11 and 10% for the respective 12- and 3-month enforcement periods.

One significant finding with regard to the microbiological content of processed meat products was that no samples were found that exceeded the *E. coli* standards of 10 organisms per gram for either of the 12- or 3-month enforcement periods covered in this report. These findings are indicative of the good condition of these products as they come out of the processing plants.

The same is not true concerning *E. coli* levels in fresh meat products, as evidenced by the number of penalty actions taken to date for violation of the 50 per gram standard. The findings do show, however, a significant indication of compliance improvement. There is a decline from 16% of the samples exceeding the standard for the 12-month period as compared to 9% in violation for the 1975 3-month summary.

An analysis of the records as to the number of meat samples with three consecutive violations of the bacterial standard which results in enforcement action, indicates the degree of ability by meat markets to comply with the standards. With processed meats, less than 1% (0.9%) had three consecutive APC violation of the one million per gram standard for the 12-month summary and none for the 1975 3-month record period. For fresh meat products, there was a slight increase for the same periods, going from 0.0 to 0.2% of the samples having three consecutive APC violations.

With regard to products having three consecutive *E. coli* violations, there were none for processed meats. Whereas the number for fresh meat products declined from 0.7% of all samples analyzed for the 12-month record period to 0.2% in the 1975 3-month study.

These findings to date, which indicate a significant downward trend in number of samples in violation of the standards, reflect the improved meat handling practices in the retail markets as evidenced by the decrease in number of items being checked in the inspection reports as being in violation of the sanitation standards.

Enforcement of the open date labeling law is having a decided effect on compliance with the bacterial standards, especially in the case of processed meats. Meat sanitation programs instituted by the chain stores are having a significant effect as are also the handling practices.

With regard to pathogens in meat products samples during the 12-month enforcement period, of 1830 fresh meat samples, 143 or 8% had a staphylococcus count of over 1000 per gram; 35 or 2% of the samples indicated the presence of *Salmonella*, and 368 or 20% of the samples were positive on test for *C. perfringens*.

With processed meats, only 4 of 454 or 0.8% of the samples contained coagulase positive staphylococcus organisms. Fifteen or 3% were *C. perfringens* positive and no *Salmonella* organisms were detected in any of the samples.

Salmonella positive meat products are resampled until a negative test is obtained. Fresh pork products account for most of the *Salmonella* positive findings. Meat products with a coagulase positive staphylococcus count of over 1000 per gram and any sample containing *C. perfringens* over 10 per gram are also resampled. Presence of these organisms in a sample are noted on the laboratory report going to the meat market. This is followed by sanitation inspection of the market before resampling. In most instances, the counts are brought within the arbitrarily established number by this procedure.

AOAC analytical procedures are used for detection of *Salmonella*, Staphylococci, and *C. perfringens* and all samples are incubated at 35 C.

A 1964 report by a National Academy of Science subcommittee on food microbiology of the Food Protection Committee in a review of the public health hazards which result from microbiological contamination of food, recommended that bacterial standards should accomplish what they purport to do, i.e., reduce public health hazard; be technically feasible, i.e. attainable under conditions of good manufacturing practices; and be administratively feasible.

The Department is of the opinion, based on our experience to date in the enforcement of Oregon meat bacterial standards, that this recommended basic criterion for their establishment has been achieved.

ACKNOWLEDGMENT

This paper was presented at the annual meeting of the Food Research Institute, University of Wisconsin, Madison, May 22-23, 1975. The paper appeared originally in the *Association of Food and Drug Officials Quarterly Bulletin*, Vol. 39, No. 3.

A Retailer's Experience with the Oregon Bacterial Standards for Meat

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(Received for publication June 5, 1975)

ABSTRACT

The Oregon retail food industry's experience with bacterial regulations on meats serves as the basis for questioning: (a) the effectiveness of such an approach in reducing public health hazards; (b) the technical feasibility of such standards in relation to current practices of the entire meat industry (including packer, breaker, and distributor); and (c) the feasibility of administering such a regulation in an effective compliance program. As recounted herein, specific compliance programs instituted by Safeway Stores, Inc. have reduced the percentage of lots of ground beef exceeding Oregon *Escherichia coli* standards from 50% to about 30%. Oregon arrest and conviction records seem to indicate the industry is not currently able to meet the state's standards consistently and that the courts may not be entirely convinced of the validity and/or significance of the charges. Inability of current procedures to inform store managers of *E. coli* counts before sale of product, presence of *E. coli* on carcasses at time of receipt at breaking plants, and the inherent variability of the MPN technique are cited as weaknesses of the enforcement program.

Basic principles for setting microbiological criteria on foods have been suggested in the 1964 report of the Food Protection Committee of the Food and Nutrition Board of the National Academy of Sciences—National Research Council on p. 39 of its Publication 1195 wherein it is proposed, "Microbiological criteria should: (a) accomplish what they purport to do, i.e., reduce public health hazards; (b) be technically feasible, i.e., attainable under conditions of good commercial practice; and (c) be administratively feasible." Perhaps the technical and administrative feasibility of such bacterial standards on meats may best be evaluated on the basis of the following case history of the efforts of one retail food chain to meet bacterial standards established by the Oregon State Department of Agriculture. While the validity of the use of such standards for regulatory purposes was questioned, it was hoped that costs of monitoring and controlling bacterial counts on raw meats might be off-set by longer shelf-life of product and improved consumer acceptability.

MEAT DISTRIBUTION SYSTEM

Safeway's meat distribution system in Oregon entails the purchase (usually from packers within Oregon and adjacent states) of quarters of U.S.D.A. choice beef or of cows and also of fresh (unfrozen) boneless beef from local packers and/or boners. These raw ingredients are shipped to a central breaking plant where quarters are

broken into primal and sub-primal cuts for wrapping and shipment to a store. Final breakdown to retail cuts is done at the retail store just before packaging for display in the retail display counters. Perhaps three-fourths of the Safeway ground beef distributed in Oregon is ground under controlled conditions in the meats warehouse where it is packaged into 20-lb. "keeper cases" for shipment to stores. At the stores the product is re-packaged in retail packages. Regular ground beef is re-packaged directly from keeper cases to retail packages whereas lean ground beef is passed once through the store grinder before packaging for retail. Store trim is ground at the stores and packaged separately from warehouse product.

It is felt that a higher level of bacterial control should be possible with centrally ground product than can be achieved in a distribution system entailing delivery of the quarters of beef to each local store where supervision over sanitation and temperature control would be subject to greater store-to-store variation.

EXPERIENCE WITH STANDARDS

The following section of this discussion relates to some of Safeway Stores' experiences and observations relative to the Oregon State Department of Agriculture Regulation CH 603.28-400 defining adulteration of meat as defined in ORS Chapters 616 and 619, effective 5-1-73. The standards in Table 1 were applied to meats

TABLE 1. Oregon bacterial standards for meats at the retail store

Type of meat product	No./g considered violative	
	Aerobic plate count	<i>E. coli</i> (MPN)
Fresh	>5,000,000	>50
Processed	>1,000,000	>10

offered for retail sale in Oregon in May, 1973. The compliance program is based upon issuance of a criminal citation to the store manager following the third successive violative sample of any given product. By April, 1974 our Portland Retail Division recognized the need for technical assistance. In May, 1974 Safeway Stores, Inc. established a corporate level Quality Assurance Department with the immediate assignment of learning how the corporation could meet these and similar bacterial standards on meats. Construction of a

laboratory for microbiological monitoring of meat for our Portland Division Stores, commenced in June, was completed in September. One of the Dairy Division's best Quality Control Supervisors, whose qualifications included the M.S. degree in Meat Microbiology from the University of Nebraska, was transferred to the Portland Division as the company's first Retail Division Quality Assurance Supervisor. To assist him with the bacteriological monitoring two Food Technology graduates, one from Washington State University and the other from Oregon State University were hired.

Since the end of September this laboratory has been engaged primarily in monitoring incoming carcasses and boneless beef at our meats warehouse for presence of *Escherichia coli*, monitoring effectiveness of cleaning and sanitization of equipment in the warehouse, monitoring bacterial levels on finished ground beef and sausage products, and doing comparative analyses on duplicates of those samples picked up by state inspectors from our stores. In addition, the Quality Assurance Supervisor (together with the meat buyer) has: (a) visited all of the Division's regular beef suppliers and is working with them towards reduction of bacterial loads on carcasses and boneless beef, (b) been working towards improved temperature controls during shipping (as well as in the meat warehouse and in carcasses received), (c) visited most of our stores that have been warned of two successive violative samples of a given product, surveying store conditions and discussing steps that can be taken at store level to minimize bacterial contamination and growth, (d) held a Sanitation Seminar for all meat warehouse personnel, and (e) assisted with a Sanitation Seminar presented in each district for all Meat Department Managers, Store Managers, District Meat Merchandisers, and District Managers.

Sanitation has become a major concern of all people in any way involved in handling meat in this Division including those Division and District staff, Distribution Center personnel, and Retail Store people who have an effect on the sanitation of our meat.

Regardless of the work with packers, the improvement in Good Manufacturing Practices at the Meat Warehouse, and the attention to temperatures and product freshness, probably a third of the lots of ground beef processed in our Meats Warehouse already exceed the Oregon standard for *E. coli* at the time they are packaged before being shipped to the retail stores. Even though we feel our sanitation and care of the product are well above average for the industry, we are not yet able to meet the Oregon standards routinely. Thus, our current emphasis is in working with suppliers towards reduction of bacterial loads on carcasses and in further control of bacterial buildup at the meats warehouse level.

LEGAL ACTION

Through March, 1975, four Safeway store managers have been charged with the offering for sale of adulterated meat, all due to high *E. coli* counts. One

manager is on a 2-year probation resulting from his guilty plea. The probation is contingent upon his not again offering adulterated meat for sale within the state of Oregon. The case against the second manager was dismissed when the State failed to have one of its analysts available for questioning by the defense attorney. A "not guilty" verdict was decreed in the third case because the prosecutor had failed to prove "reckless negligence" on the part of the store manager. The judge commented that the constitutionality of the regulation might need to be tested in court. The fourth store manager is still awaiting scheduling of the trial date.

During the eighteen-month period from the end of August, 1973 to the end of February, 1975, the state of Oregon filed charges against 24 store managers throughout the industry. At the time of the summarization five cases had not been disposed of, the state prevailed in 10 of the cases, and the defendant was not found guilty in the other nine cases. Thus, it appears that the industry is not in position at the present time to meet the specific standards established by the State and also that the State may be experiencing difficulty convincing the courts of the validity and/or significance of its charges.

THE ESCHERICHIA COLI PROBLEM

All four charges against Safeway personnel have resulted from excessive *E. coli* counts in ground beef. The MPN *E. coli* determination normally requires about six days to obtain confirmed results. This perishable product should be sold in less than six days. Therefore a store manager cannot know the *E. coli* count of his product before he sells it. Furthermore, were he to have it analyzed and hold up sale until the analytical results were available, the counts would then be entirely different (and more likely to be violative) by the time the product was offered for sale. Thus the store manager has no way to ascertain that meat he is offering for sale does or does not meet standards either before or at the time it is offered for sale.

While it is possible for *E. coli* to be introduced onto (or into) the meat at store level, it is far more likely that the *E. coli* was already present on the meat at the time of its receipt at the store, a situation over which the store manager would have very little, if any, control. Thus, it seems patently unfair to hold a store manager criminally liable for conditions which are completely beyond his control.

In bacterial monitoring of beef carcasses for *E. coli* we find that isolation rates have ranged from about 10% to 40% on individual shipments from various packers. Thus, it is apparent that although regulatory monitoring for *E. coli* begins with the retailer, many carcasses are already contaminated by the time they are received at the breaking plant.

Due to the degree of variability inherent within the MPN *E. coli* procedure, this test is satisfactory for qualitative determination of the presence of *E. coli* but

not for the quantitative determination of actual numbers present. Thus the 95% confidence limits for an MPN index of 64 in water samples would include actual numbers of organisms ranging all the way from 15 to 380 with a probability of 2½% of the samples yielding this 64 index when their actual numbers of *E. coli* were even less than 15.

The value to the consuming public of the bacterial standards approach to regulation of foods at the retail store level may be questioned on the grounds that this approach: (a) will not increase the protection of the consumer against health hazards, (b) will not indicate the level of sanitation of the environment to which the food has been subjected, (c) will not indicate either actual or potential spoilage of the food, and (d) can indicate only a

falsely-assumed aesthetic property of the food. Certainly we feel that the Oregon retail food industry's experience with similar regulations on meats warrants questioning (a) the effectiveness of such an approach in accomplishing any of the afore-mentioned objectives, (b) the technical feasibility of such standards in relation to current practices of the entire meat industry (including packer, breaker, and distributor), and (c) the feasibility of administering such a regulation in an effective compliance program.

ACKNOWLEDGMENT

This paper was presented at the Annual Meeting of the Food Research Institute, University of Wisconsin, Madison, Wisconsin, May 22-23, 1975.

News and Events

Food and Feed Lab Courses Scheduled

Two laboratory short courses for food and feed technologists and scientists in the food and feed industries will be held in September at the University of Minnesota, St. Paul. The two courses run on consecutive weeks, and any person registering for both will receive a registration fee reduction.

Microbiological Examination of Food, September 15-19, will cover laboratory procedures and safeguards; media preparation; isolation and identification of salmonellae, *staphylococcus aureus*, and *clostridium perfringens* from food; yeasts and molds; total numbers of microorganisms; foodborne diseases; indicator organisms, and interpretation of results. Registration fee is \$150.

Mycotoxin Analysis of Food and Feed, September 22-26 will include analysis of mycotoxin with laboratory exercises, review of fungi capable of producing mycotoxins,

lectures on yeast and thermophilic fungi in food and sessions on the importance and impact of mycotoxins in industry, symptoms of mycotoxins in animals and environmental factors of mycotoxin production. Registration fee is \$185.

The fee for anyone registering for both sessions is \$300. Fees cover supplies, instructional materials and a mycotoxin analysis kit. Participants may bring a sample for analysis to each course.

Contact the Office of Special Programs, 405 Coffey Hall, University of Minnesota, St. Paul 55108, for further information. The courses are sponsored by the University's Departments of Food Science and Nutrition and Plant Pathology in cooperation with the Agricultural Extension Service.

Marketing to Highlight ACDPI Session

More than two hundred cultured products processors, allied tradesmen, regulatory personnel, and university staff are anticipated to attend the 1975 American Cultured Dairy Products Institute Annual Meeting, according to Secretary Dr. C. Bronson Lane. Stouffer's Inn, Louisville, Kentucky will host the session, scheduled for September 17-18.

A half-day will be devoted entirely to effective marketing and merchandising of cultured products. Speakers for this session include: Dr. G. Quackenbush, UDIA, Chicago; Barry Pfouts, D'Arcy-MacManus and Masius Advertising Agency, Chicago; M. J. Adamson, Superior Dairies, Austin, Texas; Dr. B. R. Weinstein, Milk Proteins, Inc., Troy, Michigan; Jerry Schultz, Fairmont Foods, Kansas City, Missouri; and Marion Jones, G. P. Gundlach and Co., Cincinnati, Ohio.

The meeting will also feature a "mini-clinic" on troubleshooting cultured product quality problems.

Consultants Neil Angevine, Dr. H. C. Olson, Erik Lundstedt along with Harry Daume, Marschall Div.-Miles Labs, and Dale Appel, The Kroger Company, Livonia, Michigan, will participate in this event.

Some "crystall-ball gazing" on cultures, fruits and flavorings, packages, plants, manufacturing equipment and products is also on tap for attendees. Some of the "prognosticators" are: Dr. George Muck, Dean Foods Co.; Luther Elkins, The Southland Corp.; Dr. Robert Sellars, Chr. Hansen's Lab.; Don Crowell, International Paper Co.; Walter Woods, DaSi Industries; Paul Ramsey, Ramsey Labs.; Dr. V. H. Nielsen, Iowa State University; John F. Speer, Jr., MIF/IAICM; George Much, Anderson Bros. Mfg. Co.

Additional information and/or advance registration materials can be obtained from ACDPI headquarters, 910-17th Street, N.W., Washington, D.C. 20006.

Dairy Research, Inc. Award Goes to Thomas Richardson



Thomas Richardson

Dr. Thomas Richardson, recipient of the 1975 Dairy Research, Inc. Award, was born in Fort Lupton, Colorado on December 4, 1931. He was graduated from high school at Fredrick, Colorado in 1949. After a year or so as a manual laborer, he elected for additional academic training.

He received the B.S. degree in Pharmacy from the University of Colorado at Boulder in 1954. Richardson received the M.S. degree in Veterinary Science in 1956, and the Ph.D. degree in Biochemistry in 1959, both from the University of Wisconsin, Madison.

After two years as a post-doctoral fellow with Professor A. L. Tappel, Department of Food Science and Technology, at the University of California at Davis, Dr. Richardson returned to the University of Wisconsin in 1962 as an Assistant Professor of Food Science. He became an Associate Professor in 1965 and Professor in 1970, the position he now holds.

Richardson has published approximately 80 scholarly, scientific papers. The areas of research that are pertinent to this award deal with fatty acids in food products, properties of interesterified milkfat, whipping characteristics of high-fat milk powders, structure of the casein micelle, studies of the gross milk-fat globule and its interphase materials, commercial utilization of whey in polyurethane foams, and immobilization of enzymes and use of these enzymes in dairy and other food products. Results of some of Dr. Richardson's research have been reported in the *Journal of Milk and Food Technology*.

Dr. Richardson received the Dairy Research, Inc. Award at the 70th Annual Meeting of the American Dairy Science Association. The meeting was held at Kansas State University, Manhattan.

Fumigation Seminar Planned in Madison

Pest controllers can update their knowledge of the basic principles and practices of safe, effective and legal fumigation procedures at a seminar-symposium to be held November 18-20 in Madison.

Advance registration is required by November 1. Enrollment will be limited on a first-come basis, according to W. L. Gojmerac, University of Wisconsin-Extension insect specialist.

Fumigation is a highly technical and specialized way to control insects in food processing and handling plants, shipping containers and storage areas. It's directly influenced by environmental and occupational safety regulations, according to Michael Shinkle, President, Environmental Management Services (EMS), Inc., Kenosha, Wis.

EMS is co-sponsoring the symposium along with the University of Wisconsin-Extension.

Besides pest controllers, sanitarians and fumigators, Gojmerac says manufacturers, formulators and suppliers as well as government workers and university scientists can benefit from this symposium.

University researchers and U.S. Department of Agriculture (USDA) scientists will discuss insect life cycles, habits and latest research findings. Industry representatives will review and describe the chemistry of common fumigants like methyl bromide and EDB.

In addition, Gojmerac says, experienced fumigators will outline basic fumigation practices and government officials will explain new and pending regulations affecting fumigation.

Individuals will have opportunities to meet informally to discuss mutual problems. Fumigation application, safety and monitoring equipment will be on display.

Program participants include Howard D. Nelson and Wendell E. Burkholder, USDA Stored Product Insect Research Laboratory; Rudolph G. Strong, University of California, Riverside; Paul Van Der Schaaf, Dow Chemical Co.; John V. Osmun, Purdue University; Donald Wilbur, Jr., Industrial Fumigants; and Ray Liscombe, Phostoxin Corp.

Each person attending will receive a certificate of participation. Participants may also apply for one unit of UW-Extension continuing education credit.

Seminar fee is \$100 per person. This includes several meals, instructional materials and speaker expenses. Lodging is extra.

For more information, contact W. L. Gojmerac in the Entomology Department, 237 Russell Laboratories, University of Wisconsin, Madison, Wisconsin 53706 (608/262-1762).

E. H. Marth Receives the Pfizer Award



Dr. Elmer H. Marth

Dr. Elmer H. Marth, winner of the 1975 Pfizer Inc. Award was born in 1927 on a dairy farm near the small town of Jackson, Wisconsin. He grew up on this farm in southeastern Wisconsin and continued to spend summers there while he completed his undergraduate education as a bacteriology major at the University of Wisconsin. He continued his education at the University of Wisconsin, receiving the M.S. degree in 1952 and the Ph.D. degree in 1954, both in bacteriology. In the same year Marth joined the staff at the University of Wisconsin as a project associate and the following year became an instructor of bacteriology.

In 1957, he left his academic position and joined the Research and Development Division of Kraftco

Corporation, where Dr. Marth later became Associate Manager of the Microbiology Laboratory. After nine years of outstanding work in the dairy industry, Marth returned to the University of Wisconsin where he is now a Professor of Food Science and Bacteriology and is affiliated with the Food Research Institute.

Dr. Marth has demonstrated the unique ability to identify problems of significance to the dairy industry and to obtain solutions that have won wide recognition. In 1964, he was issued a patent covering a process to improve cottage cheese yields by recovering 70% of the whey proteins in the curd. The heat treatments involved in this process resulted in a product with 6 to 8 weeks of shelf-life without the use of preservatives. Marth has contributed much to basic knowledge in dairy microbiology. In 1966, he reported that various species of *Penicillium* can metabolize potassium sorbate, a chemical used in the cheese industry to prevent surface growth of mold, to 1, 3 pentadiene which has a hydrocarbon-like odor and taste. His extensive research in the areas of propagation and nutritional requirements of bacteria used in dairy cultures has led to appreciable decreases in generation times of culture organisms. He has directed considerable research effort toward inhibiting the production of highly toxic aflatoxins in cheese by lowering storage temperatures, which precludes the growth of toxin-producing molds. He is also a widely respected and frequently consulted authority on salmonellae and staphylococcal growth in dairy foods and other food commodities.

Dr. Marth belongs to many professional organizations and honorary societies. He is a prolific writer, having authored or coauthored over 200 publications in 10 different domestic and foreign scientific journals. Many of his papers have appeared in the *Journal of Milk and Food Technology*.

Marth also is the Editor of the *Journal of Milk and Food Technology* and has contributed sections or chapters to numerous textbooks.

Dr. Marth received the Pfizer Award at the 70th Annual Meeting of the American Dairy Science Association. The meeting was held at Kansas State University, Manhattan.

Milk Processors Workshop

A workshop for milk processing personnel has been scheduled for October 27-November 6, 1975, at The Pennsylvania State University. It is conducted by the staff of the Food Science Department.

Subjects to be covered will include composition and properties and processing of fluid milk products, soft ice cream, buttermilk, sour cream and cottage cheese. Quality control procedures will be taught including basic fat tests, cryoscopy and flavor evaluation. Other subjects

will include labeling requirements, basic dairy arithmetic and cleaning and sanitizing of dairy equipment. The workshop will be directed to dairy plant processing personnel.

For additional information and a copy of the program brochure contact:

Agricultural Conference Coordinator
410 J. O. Keller Building
University Park, Pennsylvania 16802

R. T. Marshall Receives the Milk Industry Foundation Teaching Award



Dr. R. T. Marshall

Dr. R. T. Marshall, the recipient of the Milk Industry Foundation Teaching Award in dairy manufacturing for 1975 has been characterized by his former students as a teacher having an enthusiasm for the dairy industry and the ability to communicate that enthusiasm and to motivate students. He is described as a teacher who presents his knowledge of current concepts and practices in a manner that is understood and appreciated by his students.

Dr. Marshall has complete responsibility for teaching two courses in Dairy Technology as well as a dairy products judging course and shares the teaching duties in five other courses. He has advised approximately 150 B.S. students, 43 M.S. candidates, and 17 Ph.D. candidates.

He has served on a student tutoring committee of Gamma Sigma Delta, the honor society of Agriculture and received an award of merit for outstanding service to Agriculture from that organization in 1968. In 1974 Marshall promoted and coordinated the establishment of an ice cream and milk institute scholarship of \$500.00 to be awarded annually to a student in dairy technology. He also serves as the chairman of the scholarship committee of his state association of milk and food sanitarians and coordinates their annual scholarship awards.

The recipient has coached a dairy products evaluation

team since 1960 and in 1973 his team placed first in the national contest. Marshall also has been active in the Milk Quality and Dairy Foods Judging contests held by the Future Farmers of America. He conducts judging clinics and supervised the preparation of a slide and cassette presentation as a training aid for such contests. He is superintendent of the State FFA contest in Milk Quality and Dairy Foods and is principal advisor to the National FFA for the Milk Quality and Dairy Foods contests. Dr. Marshall was awarded the Honorary State Farmers Degree by the Future Farmers of America in 1972.

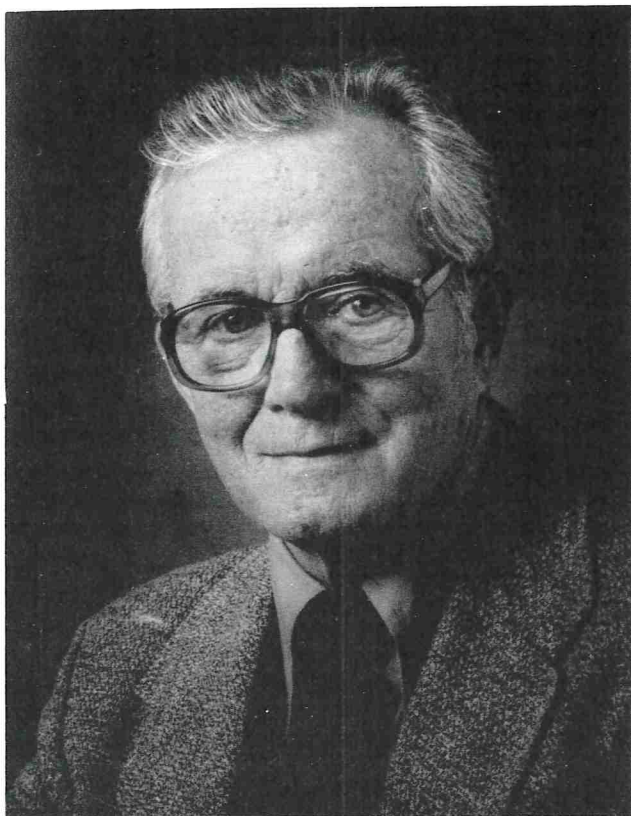
As a member of the American Dairy Science Association he has served as chairman of the Dairy Products Evaluation Committee and has held numerous offices in the industry and business section and the dairy foods division of the association. He is serving on the editorial board of the *Journal of Milk and Food Technology* which is published by the International Association of Milk, Food and Environmental Sanitarians as well as serving as a representative of that group to the American Public Health Association's Intersociety Council on Standard Methods for the Examination of Dairy Products. In addition he is a member of American Society for Microbiology, the Institute of Food Technologists, and the National Mastitis Council.

Marshall has contributed to the 12th and 13th editions of *Standard Methods for the Examination of Dairy Products* and is involved with the planning, organizing, and editing of the 14th edition. He also is co-author of a book *The Science of Providing Milk for Man* published this year by McGraw-Hill and Company.

The 1975 Award recipient was born in Missouri. After receiving the B.S. degree in Dairy Manufacturing in 1954 from the University of Missouri at Columbia, he served as a pilot in the U.S. Air Force from 1954-1957. He then returned to the University of Missouri for graduate work, receiving the M.S. degree in dairy manufacturing in 1958 and the Ph.D. degree in dairy manufacturing in 1960. He has served on the faculty at the University of Missouri since 1960 and presently is Professor of Food Science and Nutrition.

Dr. Marshall received the award at the 70th Annual Meeting of the American Dairy Science Association. The meeting was held at Kansas State University, Manhattan.

Walter F. Snyder Award



Charles L. Senn

The recipient of the Walter F. Snyder Award for 1975 was Charles L. Senn of Los Angeles.

The award is made possible by National Sanitation Foundation (NSF). He is a native of Wisconsin and earned his bachelor's degree at the University of Wisconsin. He began his career as head of a work party for the state road commission and progressed through various professional assignments to the position of sanitary engineer and chief of sanitary inspection for the City of Milwaukee.

From 1943 to 1964 he served as environmental health director for the City of Los Angeles, a post which he won in a national civil service examination.

Professor Senn is a lecturer at the School of Medicine, University of Southern California. He teaches public health engineering and environmental management at UCLA where he earned his master of science degree.

He is the author of more than 100 published papers, a diplomate of two professional societies, a member of Chi Epsilon, and an elected member of Delta Omega and Sigma Xi. He is serving with the expert panel on environmental health for the World Health Organization. He is also a fellow of the American Public Health Association and a member of its governing council.

Horticulture Department Chairman Named at Maryland

Dr. Bernard A. ("Pete") Twigg, well-known in the fruit and vegetable food-processing industry, has been named horticulture department chairman for the College of Agriculture at the University of Maryland in College Park.

The appointment became effective July 1 (1975), according to an announcement by Dr. Francis C. Stark, Jr., provost for the university's Division of Agricultural and Life Sciences. Dr. Twigg has been acting department chairman since July 1, 1974, succeeding Dr. Stark when he moved into the provost's position.

A western Maryland native, Dr. Twigg has been affiliated with the University of Maryland for 27 years, first as an undergraduate and graduate student and later as an Extension fruit and vegetable processing specialist.

He has co-authored two volumes of a book on *Quality Control for the Food Industry* with Dr. Amihud Kramer, professor of horticulture at the College Park campus. The two University of Maryland faculty members have also written three chapters or sections on quality control in two volumes of another professional book, *The Freezing Preservation of Foods*.

In addition, Dr. Twigg was editor of *Ingredient Technology for Product Development*, a manual published in 1974 by the Institute of Food Technologists at Chicago, Ill. He also serves on the editorial board for the *Journal of Milk and Food Technology*, and he is a member of the research paper review committee for the *Journal of Texture Studies*.

All told, the horticulture department administrator has been author or co-author of more than 95 publications or other creative efforts.

Professional activities have included serving as an officer or committee chairman for the Maryland and District of Columbia sections of the Institute of Food Technologists, the national IFT and the American Society for Horticultural Science.

Last year he was chairman for the national IFT short course, and this year he gave a two-hour presentation on quality assurance during the 1975 short course. The two-day event followed the thirty-fifth annual meeting of the Institute of Food Technologists, held during the week of June 8 at the Conrad Hilton hotel in Chicago.

Dr. Twigg holds membership in several honorary societies. He is listed in *American Men of Science*, as well as *Who's Who in the East* and *Contemporary Authors of America*.

Known widely throughout the Mid-Atlantic food-processing industry by his nickname, "Pete" Twigg grew up on a mountain farm near Oldtown (Allegany county). He now lives with his wife and children in College Park (Prince Georges county).

Association Affairs

AFFILIATES OF International Assn. of Milk, Food and Environmental Sanitarians

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Pennsylvania Dairy Fieldmen's Conference



Left to Right: Gerald Shick being presented the Sanitarian of the Year Award by President Henry Wingert.

Gerald Shick received the Sanitarian of the Year Award at the 33rd Annual Pennsylvania Dairy Fieldmen's Conference. He is a sanitarian with Keystone-Harmony Dairy in Pittsburgh and has been secretary-treasurer of the Pennsylvania Dairy Sanitarians Association for the past four years.

More than 250 persons participated in the conference at the Pennsylvania State University on June 9-11, 1975. Four Pennsylvania Dairy Sanitarian Association members received certificates of service and life membership. They were Golden Davis, John Ginck, Howard Johnston, and Willard Walton.

Twenty persons presented talks and participated in the panel on herd health. Nearly all of the topics and speakers were suggested by the Pennsylvania Dairy Sanitarians Association. Earl Wright, Executive



Awarded Certificates of Service and Life Memberships, left to right - John Blyholder receiving award for Willard Walton, Richard Weaver receiving award for Golden Davis, Howard Johnston and John Ginck.

Secretary of IAMFES spoke on the role of the association.

Other speakers from industry, regulatory and education discussed economics, uniform farm guidelines, milk pricing, promotion, waste disposal, large herd management, water conditions and detergents, freezing points, sampling, PMO revision, and IMS conference, new equipment, and Pennsylvania regulations.

George Mansell was elected President of the Pennsylvania Dairy Sanitarians Association and Don Breiner, President-Elect. The Vice-President is Ray Ackerman and the Secretary-Treasurer, Alfred Gottfreid. The next meeting of the association will be September 26, 1975.

The 1976 Pennsylvania Dairy Fieldmen's Conference will be held June 7-9, at the Keller Conference Center at the Pennsylvania State University in State College, Pennsylvania.

SANITARIAN'S CITATION AWARD of the Missouri Association of Milk and Food Sanitarians 1974-75



Mr. Gerald Burns (left), outgoing President of the Missouri Association of Milk, Food and Environmental Sanitarians, presenting to Mr. Vernon Cupps (right), Chief of Milk Control, St. Louis City Health Department, the 1975 Missouri Affiliate's Sanitarian's Award. Also enclosed is resume of Mr. Cupps and his contribution to the Grade "A" Milk Sanitation Program for the St. Louis area.



The 1975-76 Affiliate officers, left to right, Gerald Burns, outgoing President, Chief of Milk Control, Kansas City Health Department; Mike Sanford, President, Director of Columbia City Health Department; James Jameson, First Vice-President, Supervisor of Milk Control, St. Louis County Health Department; and Erwin P. Gadd, Secretary-Treasurer, Missouri Division of Health. Chester Edwards, Second Vice-President, Sanitarian of Platte County Health Department, was not available for photograph.

Baird, Viets, Potts, Crownover, Dromgold, Sanford, Kennedy and Copenhaver are names we have honored over the past 8 years as distinguished sanitarians. Now another name is added to the list and his record is also exposed both to give him due recognition and to inspire others.

His career in milk sanitation is approaching 30 years and throughout it he has served the citizens of this state most admirably. Effects of his work have, of course, reached well beyond boundaries of our state. Until 3 years ago he worked primarily in dairy farm sanitation with 17 years in supervisory positions. At times he has supervised inspection of as many as 1,000 grade A dairy farms.

Pipeline milkers and bulk tanks came into use during our recipients career, and he contributed much to technology of their use. He has a keen ability to analyze problems and to match cause with effect. He knows well and frequently uses resources that contribute to getting the job done.

Presently, the awardee is Administrative Chief of Milk Control in a market that supplies about 2 million pounds of grade A milk and milk products to consumers per day. His department has a record of being rated above 90% on sanitation ratings throughout his administration.

The awardee is a member of the Missouri Mastitis Council to which he contributes meaningfully with a wealth of experience with milk and dairy cows.

In this association he served as a member of the program planning committee for several years, was president in 1957, and has been a member for 28 years.

Our hats are off to you Vernon R. Cupps. Your associates nominated you because of their admiration for your record and the selection committee heartily agreed that you deserve the award. Sincere congratulations.

Ontario Sanitarian of the Year

The Ontario Milk and Food Sanitarians held their annual meeting on March 26, 1975 in Etobicoke, Ontario at which time Donald J. Wood received the **SANITARIAN OF THE YEAR AWARD**. Mr. Wood died a few days after he was nominated therefore the award was made posthumously to Mrs. Wood.



Left to Right: E. H. Smith, Brenda Trask, Mrs. Donald J. Wood and J. L. Baker.

Mr. Wood supported the Ontario Milk and Food Sanitarians in every way possible. His support of the idea behind the Sanitarians and his continuing diligence in promoting ideas concerned with hygiene and sanitary control of food products should have been sufficient to warrant his recommendation for this award. However, he was not satisfied to maintain the status quo. He was always looking for improvements in existing quality control techniques and questioning the capability of existing products and techniques to satisfy the needs of the food producers and food processors.

Report of the 3-A Sanitary Standards Symbol Administrative Council, 1973-1974

Trustees on the Council are as follows: K. G. Weckel, Chairman; W. S. Clark, Jr., Vice Chairman; E. O. Wright, Secretary-treasurer; F. E. Uetz, Ass't. secretary-treasurer; P. K. Girton, Member; D. G. Colony, Member; P. J. Dolan, Member; and D. C. Cleveland, Member.

Two meetings of the Symbol Council were held during the period, August 1, 1973-July 31, 1974. One was held October 10 and 11 at Millville, Pennsylvania, and the other May 9 and 10 at Denver, Colorado. Complaints registered by sanitarians and others were dealt with and actions were recommended to overcome these problems.

The executive office of the Council was moved from Iowa State University, Ames, Iowa to the Executive Office Building at 413 Kellogg Avenue, Ames, Iowa. The office is shared jointly with IAMFES. The Secretary-Treasurer of the Council is also the Executive Secretary of IAMFES.

A new hyphenated serial numbering system for identifying 3-A Sanitary Standards has been developed. Equipment manufacturers should now be using the applicable number along with the 3-A Symbol on newly manufactured equipment. By the new system, sanitarians and fieldmen may check the equipment against the proper standard.

During the year six new authorizations were issued and six relinquished; authorizations effective were 167. The comparative numbers of authorizations covering each type of equipment are found in Table 1.

TABLE 1. 3-A Symbol Council Authorizations

	Sanitary standards serial numbers	Authorizations in effect on July 31, '73	Authorizations in effect on July 31, '74
Storage tanks	01-04	15	15
Pumps	02-03	18	18
Homogenizers & pumps	04-03	5	6
Auto milk tanks	05-11	18	16
Fittings (Piping)	08-09	24	22
Fittings (Thermo)	09-00	3	3
Filters	10-01	1	1
Heat exchange (Plate)	11-03	7	7
Heat exchange (Tube)	12-04	8	8
Farm milk tanks	13-01	17	16
Leak protector valves	14-00	4	4
Evaporators	16-04	5	6
Fillers & sealers	17-00	5	4
Ice cream freezers	19-00	2	2
Silo-type tanks	22-01	9	9
Package fillers	23-00	4	5
Batch pasteurizers	24-00	5	5
Batch processors	25-00	6	6
Dry milk sifters	26-00	7	7
Flow meters	28-00	4	5
Air eliminators	29-00	0	1
Farm milk storage tanks	30-00	0	1
TOTAL		167	167

Through the cooperation of sanitarians, fieldmen, and the excellent cooperation of the industry and the 3-A Sanitary Standards Committee, the Symbol Council has had a very successful year.

Respectfully submitted,
K. G. Weckel
 Chairman
 3-A Symbol Council

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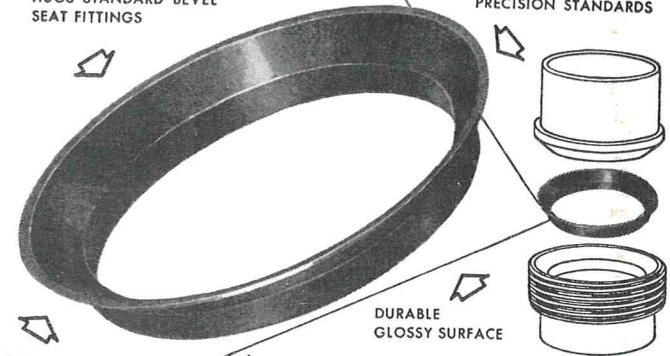
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SELF-CENTERING
SNAP-TITE
Gaskets

* MADE FROM
TEFLON

SIZES 1" - 1½"
2" - 2½" - 3"

"The Sophisticated Gasket"
THE IDEAL UNION SEAL FOR
BOTH VACUUM AND
PRESSURE LINES

Gasket Color . . .
slightly off-white

SNAP-TITE self-centering gaskets of TEFLON are designed for all
standard bevel seat sanitary fittings. They SNAP into place provid-
ing self-alignment and ease of assembly and disassembly.

HAYNES SNAP-TITES of TEFLON are unaffected by cleaning solu-
tions, steam and solvents. They will not embrittle at temperatures
as low as minus 200° F. and are impervious to heat up to 500° F.

FOR A FITTING GASKET THAT WILL OUT-PERFORM ALL OTHERS...

Specify . . . **HAYNES SNAP-TITES of TEFLON**

• TEFLON ACCEPTED SAFE FOR USE ON FOOD & PROCESSING
EQUIPMENT BY U. S. FOOD AND DRUG ADMINISTRATION

* Gaskets made of DuPont TEFLON® TFE-FLUOROCARBON RESINS

THE HAYNES MANUFACTURING COMPANY
4180 LORAIN AVENUE • CLEVELAND, OHIO 44113

A HEAVY DUTY SANITARY LUBRICANT



Available in both
SPRAY AND TUBE

All Lubri-Film ingredients are
approved additives and can be
safely utilized as a lubricant for
food processing equipment when
used in compliance with existing
food additive regulations.

ESPECIALLY DEVELOPED FOR LUBRICATION OF FOOD
PROCESSING AND PACKAGING EQUIPMENT

For Use in Dairies — Ice Cream Plants — Breweries —
Beverage Plants — Bakeries — Canneries — Packing Plants

SANITARY • NON TOXIC • ODORLESS • TASTELESS

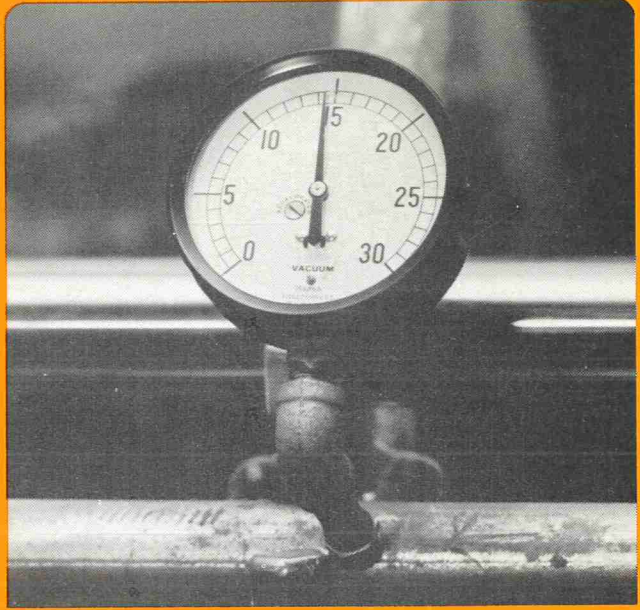
SPRAY — PACKED 6 — 16 OZ. CANS PER CARTON

TUBES — PACKED 12 — 4 OZ. TUBES PER CARTON

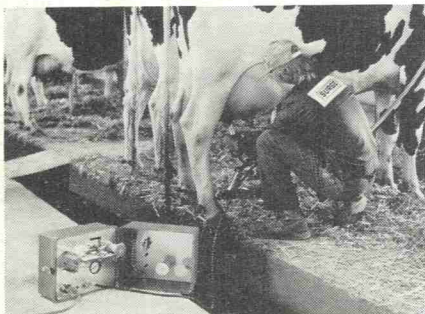
THE HAYNES MANUFACTURING CO.
CLEVELAND, OHIO 44113



The place to measure vacuum pump performance is here...



not here.



A free levograph test from your Surge dealer evaluates your vacuum system's efficiency in minutes.

Of all the equipment you own, none is more important than your vacuum pump, because the efficiency of your entire milking system depends on it.

You can feed the right feed; cull your cows carefully; and follow all the latest herd management practices, only to find you're being robbed by a worn or faulty vacuum pump.

Right now, your Surge dealer is offering you a valuable, money-saving opportunity. At no cost or obligation to

you, he will completely test your entire vacuum system using the latest vacuum system diagnostic equipment.

Your pump may appear to be in good running order, but there's a good chance it's costing you money month-after-month. Since vacuum pumps wear out gradually, many dairymen never realize how inefficient their system has become until the installation of a new pump produces a noticeable increase in milk production.

Surge features help produce more milk, protect herd health.

To maintain a constant 12"-13" of vacuum at the teat end, you need a pump that's built to last, and has a high reserve capacity. A unit like the famous Surge Alamo vacuum pump, with features like:

1. **Continuous flood-type lubrication** that assures a better seal between rotor vanes and cylinder wall, providing maximum air-moving capacity.
2. **An exclusive oil reclaimer system** that reclaims, filters, and recirculates up to 95% of the oil from the pump exhaust.
3. **Filtered air intake** to prevent dirt or other foreign matter from reaching the rotor.

4. **Large oil reserve** (1½ gallons) reduces the chance of pump failure or injury due to a depleted oil supply.

5. **Oil level and temperature gauges** tell you at a glance if proper oil temperatures and levels are being maintained.

Contact your Surge dealer today. He'll thoroughly test your present system at no cost, no obligation. Should you need a new pump, he'll recommend the Surge Alamo model that's right for your operation, your future.

"You're a step ahead with Surge"



For more information see your Surge Dealer or write to Babson Bros. Co. 2100 S. York Road, Oak Brook, Illinois 60521

