August 1979, Vol. 42, No. 8:621-700

August, 1979
Vol. 42, No. 8
Pages 621-700
CODEN: JFPRDR 42(8):621-700 (1979)
ISSN: 0362-028X

Journal of Food Protection®

An International Journal Concerned With An Adequate Food Supply That Is Safe, Wholesome, Nutritious, and Palatable

Published Monthly by the International Association of Milk, Food, and Environmental Sanitarians, Inc., Ames, Iowa, U.S.A.

WHY NSF *DE-LISTS* SOME MANUFACTURERS

When the manufacturer of a product covered by an NSF standard applies to the Foundation for recognition of his products—and eligibility to use the NSF seal—he enters into a contract. This agreement provides that:

Each product which is to bear the seal must fulfill the requirements of an applicable NSF standard.

Each such product must undergo scientific inspection to determine that it fully adheres to the requirements of the standard.

Each manufacturer agrees to unannounced inspections at manufacturing plants by NSF representatives. These representatives may take samples, chosen at random, from the production line for on-the-spot inspection. Or they may send these random samples to the NSF laboratory in Ann Arbor to determine if the sample products are up to standard.

The manufacturer and the product must fulfill *all* of these requirements or, with regret, we de-list them. This process is only fair to the manufacturers who comply, and to consumers or users who rely on the NSF seal.

NSF

National Sanitation Foundation—an independent non profit, non governmental organization dedicated to environmental quality. Offices and laboratories: NSF Building, Ann Arbor, Mich. 48105 (313)-769-8010

One name, two great ideas for better sanitation:

Transflow®

Transflow® tubing

Dairymen across the country rely on Transflow raw milk tubing to protect the wholesomeness and flavor of their product. That's because Transflow tubing is specially designed for the rigid sanitation requirements of the dairy industry.

Transflow tubing cleans quickly and thoroughly. Its inner bore — smoother than rubber tubing and stainless steel pipe — leaves no place for butterfat or milkstone to accumulate. And because Transflow tubing is clear, you can **see** that it's clean and residue-free.

Insist on genuine Transflow M-34R tubing. It complies with FDA and 3-A standards, and is available at local dairy suppliers everywhere.

Transflow® paneling

The Transflow paneling system is fast gaining a reputation as the premier wall and ceiling covering for dairy operations.

The system — panels, moldings and connectors — installs easily with no special tools. Transflow panels are built tough to withstand repeated cleaning, yet stay bright and new-looking for years. They inhibit mold growth, require little maintenance, and never need painting. Like Transflow tubing, Transflow paneling meets FDA and 3-A standards.

Don't accept substitutes — choose Transflow paneling for your operation. When Transflow paneling goes up, maintenance goes down.

33-154

Transflow Hot-Line:
1-800-321-9634

Call toll-free for more information on Transflow paneling and the location of your nearest dealer. Ohio call collect 216-630-9230.

NORTON PLASTICS AND SYNTHETICS DIVISION
P.O. BOX 350 AKRON, OHIO 44309 TEL: (216) 630-9230

IAMFES
Sustaining Members

Alpha Chemical Services, Inc.
P.O. Box 431
Stoughton, MA 02072

Babson Bros. Co.
2100 S. York Road
Oak Brook, Illinois 60101

Bio-Lab
P.O. Box 1489
Decatur, GA 30031

Blue-White Industries and Mullarkey Assoc.
12604 S. Kroll Drive
Alsip, IL 60658

Borden, Inc.
Dairy & Services Div.
165 N. Washington, Ave.
Columbus, Ohio 43216

Darigold
635 Elliott Ave. W.
Seattle, WA 98109

De Laval Separator Co.
350 Dutchess Turnpike
Poughkeepsie, NY 12602

Difco Laboratories
P.O. Box 1058-A
Detroit, MI 48232

Diversey Chemicals
1855 S. Mt. Prospect Rd.
Des Plaines, IL 60018

Eastern Crown, Inc.
P.O. Box 216
Vernon, N.Y. 13476

G. B. Fermentation, Inc.
One North Broadway
Des Plaines, IL 60016

H. B. Fuller Co.
Monarch Chemicals Div.
390 Jackson St. NE
Minneapolis, MN 55421

Klenzade Division
Economics Laboratory, Inc.
Osborn Building
St. Paul, MN 55102

Maddelena's Inc.
139 Lakeville St.
Petaluma, California 94952

Maryland & Virginia Milk Producers Assn., Inc.
P.O. Box 9154 Rosslyn Station
Arlington, Virginia 22209

Nasco International
901 Janesville Ave.
Fort Atkinson, Wisconsin 53538

Norton Co.
P.O. Box 350
Akron, Ohio 44309

Seiberling Associates, Inc.
441 Clark St.
So. Beloit, Illinois 61080

Single Service Institute
1025 Connecticut Ave., NW
Suite 1015
Washington, DC 20036

Valley of Virginia
41 W. Washington St.
Harrisonburg, VA 22801

West Agro-Chemical, Inc.
P.O. Box 1386
Shawnee Mission, Kansas 66222

Zero Manufacturing Co.
811 Duncan Ave.
Washington, MO 63090

Journal of Food Protection

ISSN:0362-028X

Official Publication
International Association of Milk, Food, and Environmental Sanitarians, Inc., Reg. U.S. Pat. Off.

Vol. 42 August 1979 No. 8

Research Papers

General Interest Papers

Field Topics

*Asterisk designates person to whom inquiries regarding this paper should be addressed.

The Journal of Food Protection is issued monthly beginning with the January number. Each volume comprises 12 numbers. Published by the International Association of Milk, Food and Environmental Sanitarians, Inc. with executive offices of the Association, 413 Kellogg Ave., P.O. Box 701, Ames, Ia. 50010. Printed by Heuss Printing and Signs, Inc., 911 Second St., Ames, Iowa 50010.
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.

Manuscripts: Correspondence regarding manuscripts and other reading material should be addressed to Dr. Elmer H. Marth, Dept. of Food Science, University of Wisconsin, Madison, Wis. 53706.

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

Orders for Reprints: All orders for reprints should be sent to IAMFES, Inc., P.O. Box 701, Ames, IA 50010. *Note:* Single reprints are *not* available from the above address; address requests to principal author.

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

Subscription Rates: $32.00 per volume, one volume per year, January through December. Single copies $3.00 each.

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

Membership Dues: Membership in the Association is available to individuals only. Dues are $21.00 per calendar year and include subscription to the JOURNAL OF FOOD PROTECTION. Student membership is $5.00 per year with certification.

Claims: Notice of failure to receive copies must be reported within 30 days domestic, 90 days foreign.

All correspondence regarding changes of address and dues should be sent to IAMFES, In., P.O. Box 701, Ames, IA 50010. (515) 232-6699.

Journal of Food Protection Vol. 42, No. 8, Pages 624-630 (August, 1979)

Fate of Enteropathogens Inoculated onto Chopped Ham[1]

M. E. STILES* and L.-K. NG

Faculty of Home Economics, The University of Alberta, Edmonton, Alberta T6G 2M8, Canada

(Received for publication August 29, 1978)

ABSTRACT

A survey of 36 pairs of new (< 10 days from manufacture) and old (pull date) samples of vacuum-packaged, sliced chopped ham were analyzed for total microbial load, specific pathogen count and pH. Results indicated a wide range of microbial loads, absence of pathogenic bacteria at the levels tested, and presumptive group D streptococci generally < 100/g except for 22% of new samples and 44% of old samples, which had presumptive group D counts > 100 but $< 10^6$/g. The survey results also indicated marked differences in pH between products from different manufacturers. Product from two manufacturers was selected for inoculation studies. Chopped ham sandwiches were inoculated with a mixture of five enteropathogenic bacteria and held at 30, 21 and 4 C for up to 24 h. *Bacillus cereus, Escherichia coli, Salmonella typhimurium* and *Staphylococcus aureus*, but not *Clostridium perfringens*, grew in low competition product under the severely abusive holding temperature of 30 C in < 24 h, at 21 C in > 24 h. Product from one manufacturer inhibited the gram negative pathogens. Results indicated that chopped ham in sandwiches required almost unrealistic mishandling to develop a food poisoning potential by enterotoxigenic bacteria, but infective pathogens survived well.

Chopped ham may be classified as an intermediate product between an integral meat such as ham and an emulsion type product such as bologna (*14*). In fact, it is comminuted ham suspended in an emulsion base. It is prepared as a nonfermented sausage type product, which is cooked, cured and smoked during manufacture. It is commonly sold as a sliced, vacuum-packaged luncheon meat that might be used in sandwiches. Slicing is an important source of contamination of luncheon meats (*14,15*). Furthermore, like all of these meats, chopped ham is a perishable product, and bacterial growth is expected during storage (*2,14*). Under proper conditions of handling and storage, lactic acid bacteria should predominate in the bacterial population within 2 weeks (*1,2,14*).

Contaminating bacteria, especially at the time of sandwich preparation in the foodservice industry or by the consumer, could include pathogenic bacteria. In a study of the microbial quality of retail samples of bologna (*18*) and ham (Steele and Stiles, unpublished data) it was found that pathogen levels were low, generally below the minimum detectable levels of conventional plating methods. However, other studies have shown the presence of potentially pathogenic *Staphylococcus aureus* in vacuum-packaged luncheon meats (*5*), and the presence of other pathogens has been reported (*3*).

Although processed meats combine several inhibitory agents such as nitrite, pH, salt, microbial competition, and low oxygen concentration, Paradis and Stiles (*19*) showed that several pathogenic bacteria could grow on bologna in sandwiches under abusive temperature conditions. The objectives of this study were to determine the quality of vacuum-packaged, sliced chopped ham offered for sale in the retail marketplace, and to select chopped ham product for use in an inoculation study to determine the food poisoning potential of enteropathogenic bacteria inoculated onto the product, under conditions simulating consumer contamination during sandwich making, and during subsequent abusive and ideal holding of the sandwiches.

MATERIALS AND METHODS

Survey study

Vacuum-packaged, sliced chopped ham representing the product of four manufacturers available from four retail stores was purchased for analysis in the laboratory. It was intended to select "new" (< 10 days from manufacture) and "old" (> 21 days from manufacture) samples in the marketplace, but it was too difficult to obtain old product. As a result, paired samples of new product were purchased, one analyzed immediately, the other stored at 4 C to manufacturer's pull date before analysis.

Sample preparation consisted of cutting an 11-g wedge aseptically through all slices in the package, and homogenizing it in 99 ml of sterile, 0.1% peptone water in a Waring Blendor jar for 2 min. All bacteriological tests, except *Salmonella*, were carried out on this sample. *Salmonella* was determined by homogenizing a 25-g wedge in 150 ml of sterile nutrient broth (Difco), for non-selective enrichment.

Bacteriological analyses were carried out by plating appropriate dilutions of the homogenized sample in duplicate onto Plate Count agar (Difco) incubated at 35 C for 48 h (Aerobic Plate Count, AP35), 21 C for 72 h (Total Plate Count, AP21) and at 4 C for 10 days (Psychrotroph Count, PSY); KF Streptococcus agar (Difco) for presumptive group D streptococci; APT agar (Difco) incubated at 30 C for 48 h for lactic acid bacteria (*4,6*); LBS agar (*20*) for lactobacilli; and STAA agar (*7*), which was subsequently flooded with 2-3 ml of 0.1% p-phenylene-diamine monohydrochloride (Sigma Chemicals), to eliminate oxidase-positive colonies from the *Microbacterium thermosphactum* (STAA) count.

Potential pathogens were monitored as follows: *Bacillus cereus* on Mannitol Egg Yolk Phenol Red Polymyxin (MYP) agar (*16*); *Clostridium perfringens* on Egg Yolk Tryptose-Sulfite-Cycloserine (TSC) agar (*8*) incubated anaerobically in a H_2/CO_2 atmosphere (using BBL anaerobic jars and "gas-pak" cartridges); coliforms and *Escherichia coli* using a 3-tube modification (*21*) of the Canadian Health Protection Branch (HPB) acceptable method (*9*); *Salmonella* by non-selective preenrichment, followed by selective enrichment in Selenite Cystine broth (Difco) and streaking on Brilliant Green and Bismuth Sulfite agars (Difco), suspicious colonies were confirmed by

[1]*Supported by funds from Health and Welfare Canada, Research Grants Program.*

biochemical tests and serological typing; *Staphylococcus aureus* on Baird-Parker medium (BP) prepared according to Holbrook et al. (*12*).

Samples were incubated at 35 C for 24 and 48 h, except where indicated above. Similarly, STAA plates were incubated at 21 C for 72 h, and APT and MYP at 30 C for 24 and 48 h. Presumptive *E. coli* were determined by transferring from gas-positive Lauryl Tryptose tubes to EC medium (Difco) and incubating at 45 ± 0.01 C for 24 and 48 h.

Inoculation study

Freshly sliced, but not packaged, chopped ham was obtained from two manufacturers and taken to the laboratory and vacuum-packaged in aluminum-nylon-polypropylene pouches (Cryovac Division, Grace Chemicals, Mississauga, Canada) under 26 lb./in.^{2} vacuum. Packaged product was stored at 4 C for 24 h and 30 days before use in sandwich making experiments.

At the time of sandwich making, slices of chopped ham were contaminated with a mixture of five enteropathogenic bacteria: *B. cereus* B4AC, *C. perfringens* 8239-H, *E. coli* 7A, *S. aureus* S-6 (all obtained from Dr. Hauschild, Health Protection Branch, Health and Welfare Canada, Ottawa) and *S. typhimurium* ATCC 13311. *C. perfringens* was grown in Cooked Meat medium (Difco) at 35 C for 24 h, all other pathogens were grown aerobically in Tryptic Soy broth (Difco) at 35 C. All cultures were diluted with 0.1% sterile peptone water to achieve required inoculum concentrations, and then mixed to give the final inoculum. Uninoculated (control) samples were also made into sandwiches.

Similar procedures to those described for the survey study were followed, except that the whole slice of chopped ham was homogenized in 99 ml of sterile, 0.1% peptone water, and the appropriate correction made to obtain counts per g. *Salmonella* was determined on this sample by plating on Brilliant Green agar (BGA) (Difco), and carrying out the enrichment process on an aliquot of homogenate, if the *Salmonella* count was below 100/slice. *E. coli* was counted on Violet Red Bile (VRBA) agar (Difco) according to the HPB method described above. If the control sample contained bile-precipitating colonies on VRBA, the presumptive *E. coli* count was determined for the inoculated sample by difference between the most probable numbers in EC at 45 ± 0.01 C.

Counts were confirmed as follows: *B. cereus* and *C. perfringens* by gram stain, differential spore stain and catalase test; *E. coli* by gas production on EC medium at 45 ± 0.01 C; *S. typhimurium* by biochemical tests on TSI slants and serological screening with Salmonella antisera; and *S. aureus* by coagulase test using EDTA coagulase plasma (Difco) as described in the HPB method (*10*). In the inoculation study, confirmation was inferred by absence of typical colonies on plates from the control sandwiches.

pH

pH was determined throughout the studies using a single probe electrode (Fisher Scientific Co., Cat. No. 13-639-90) between the chopped ham slices. Readings were taken at five different points and the mean pH recorded.

Analyses

Analyses of Variance and Duncan's Multiple Range tests were carried out using a computerized statistical package (*17*).

RESULTS

A total of 36 paired samples of vacuum-packaged, sliced chopped ham (9 samples from each of four different manufacturers) were purchased and tested as described. Profiles of the pooled data for saprophytic and indicator organism counts are shown in Table 1. Potential pathogen counts are not shown because no detectable counts were observed for *B. cereus, C. perfringens* and *S. aureus*-i.e. counts < 100/g, and *Salmonella* was absent in 25 g.

Total and lactic acid bacteria counts were as expected for a cooked, vacuum-packaged processed meat, approximately 10^3/g in the new product, and 10^7/g in the old product. Lactobacilli and *M. thermosphactum* were less predictable, especially in the old product, where counts ranged from < 100 to 10^8/g. Presumptive group D streptococci were generally < 100/g, but some samples increased to 10^4 to 10^5/g during storage to pull date.

The pH of the retail packages of chopped ham is given in Table 2. Only one of the new samples had pH < 6.0, whereas 15 samples of pull date product had pH < 6.0. An analysis of variance of manufacturer by pH drop revealed a significant difference ($p = 0.001$) between manufacturers, which was shown (Duncan's Multiple Range test, 5% level) to be due to product from manufacturer C having a significantly larger pH drop than product from the other manufacturers. Mean pH drop for manufacturer A was 0.21; B, 0.22; C, 0.75; and D, 0.15.

TABLE 1. *Profile of saprophytic and indicator organism counts for 36 paired samples (new and pull date) of vacuum-packaged, sliced chopped ham purchased from retail stores.*

Plate count and age of product	Number of samples with counts/g in the range:					$\log_{10}$ geometric mean count
	$<10^2$	10^2-$<10^4$	10^4-$<10^6$	10^6-$<10^8$	$>10^8$	
Total count 35 C						
New	11	15	5	5	—	3.19
Pull date	—	2	3	20	11	7.24
Total count 21 C						
New	7	17	9	3	—	3.39
Pull date	—	1	1	12	22	7.90
Psychrotroph count						
New	12	12	10	2	—	2.91
Pull date	3	2	2	21	8	6.76
Lactic Acid bacteria						
New	13	14	5	3	1	2.99
Pull date	—	1	1	16	18	7.68
Lactobacilli						
New	29	5	1	1	—	2.99
Pull date	7	5	5	11	8	7.68
M. thermosphactum						
New	25	6	5	—	—	1.84
Pull date	17	4	9	5	1	3.48
Group D streptococci						
New	28	7	1	—	—	0.79
Pull date	20	11	5	—	—	1.57

The correlation between pH drop and APT count (lactic acid bacteria) was poor. However, for individual manufacturer's product, manufacturer D had a correlation coefficient (r) of 0.82 ($p = 0.007$), manufacturer B, $r = 0.65$ ($p = 0.056$) and for manufacturers A and C, $r = -0.15$ and -0.48, respectively.

A factorial analysis of manufacturer × age of product × saprophytic counts (AP35, AP21, PSY and APT) indicated significant differences between manufacturers ($p = 0.001$), age of product ($p = 0.001$) and type of count ($p = 0.001$). Interaction effects were also significant ($p = 0.022$) between manufacturer and type of count. This was attributable to PSY counts for manufacturers A and B. Because of the interaction effects, one-way analyses of variance and Duncan's Multiple Range tests were carried out for the different saprophytic counts on new and old chopped ham. The results are shown in Table 3.

Significant differences were observed between manufacturers for new product. In almost all instances, manufacturer B had the lowest saprophytic counts, and new product counts on manufacturer C's product were significantly different from manufacturer B's product. Based on the differences in pH drop and total counts, product from manufacturers B and C was selected for use in subsequent inoculation studies.

The pH values of three batches of product used in the inoculation study are shown in Table 4. Initial pH for new product from manufacturer C was >6.0, for old product it was <6.0. In contrast, for Manufacturer B, pH of new and old product was >6.0; in some instances old product pH was slightly greater than new product pH. The pH changes for the inoculation study were in accord with the survey results. The mean moisture content for product C was 69.6% (range 68.1 - 71.2) and for product B was 59.4% (range 58.1 - 61.5). Hence the products used in the inoculation study were markedly different.

Changes in the APT count during the inoculation studies are shown in Fig. 1. Using the APT count at time 0 h as the reference, increases were measured by recording the ratio of the number of colonies recovered during the experiment (N_R)/the initial number of colonies at time 0 h (N_0). Within 24 h at 4 C little or no change in

TABLE 2. *The pH profile of chopped ham samples for new and pull date product.*

Product	pH value						
	< 5.25	5.25 - 5.49	5.50 - 5.74	5.75 - 5.99	6.00 - 6.24	6.25 - 6.49	>6.50
All manufacturers							
New product	—	—	—	1	6	24	5
Pull date product	2	2	2	9	11	9	1
New product							
Manufacturer							
A	—	—	—	1	3	5	—
B	—	—	—	—	1	7	1
C	—	—	—	—	1	8	—
D	—	—	—	—	1	4	4
Pull date product							
Manufacturer							
A	1	—	1	1	4	2	—
B	—	—	—	3	4	2	—
C	1	2	1	5	—	—	—
D	—	—	—	—	3	5	1

TABLE 3. *One-way analysis of variance and Duncan's multiple range test (5% level) for differences in saprophytic counts between manufacturers for new and old chopped ham.*

Count	Product	Probability ratio	Duncan's Test (5% level) Order of Means (lowest to highest)[a]			
AP35	New	0.0589	B 2.27	D 2.87	A 3.07	C 4.56
	Old	0.0894	B 6.23	A 7.24	D 7.68	C 7.80
AP21	New	0.0502	B 2.71	A 3.08	D 3.18	C 4.59
	Old	0.3023	B 7.47	A 7.60	D 8.07	C 8.47
PSY	New	0.0007	B 1.64	A 2.20	D 3.17	C 4.61
	Old	0.0857	A 5.35	C 6.64	B 7.21	D 7.85
APT	New	0.0047	B 1.30	A 3.09	D 3.14	C 4.42
	Old	0.2387	B 7.04	A 7.51	D 7.84	C 8.33

[a]Underlined means indicate no significant difference between them at the 5% level of probability.

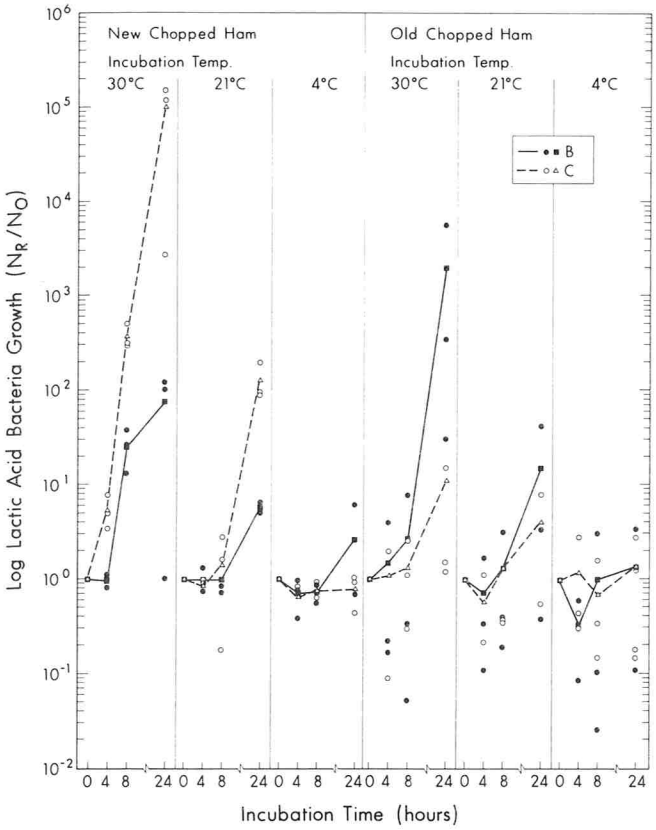


Figure 1. *Change in lactic acid bacteria counts in new and old chopped ham from 2 manufacturers B and C held at 30, 21 and 4 C up to 24 h.*
N_R Number of organisms recovered at specified time intervals;
N_O Number of organisms recovered at time 0 h.

APT count was observed, but at 21 and 30 C increases in APT count were considerable. In new chopped ham held at 30 C, the APT count for product C increased 10^5-fold, compared to a 100-fold increase for product B. In contrast, in old chopped ham, the APT count for product B increased 1,000-fold, compared to a 10-fold increase for product C. Such differences were not observed for AP35 or AP21 counts in new product, yet in old product both AP35 and AP21 counts increased dramatically at 30 C (1,000-fold), and slightly (up to 10-fold) at 21 C.

Changes in pathogen numbers were calculated using initial number of pathogens inoculated (N_I) and numbers recovered (N_R) at each test time. Differences between numbers of pathogens inoculated and the initial counts determined on chopped ham slices (0 h), indicated that most pathogens were 1/10th to 1/100th of the numbers inoculated, suggesting death or injury of 90 to 99% of the inoculated cells.

Changes for *B. cereus* are shown in Fig. 2. In new product at 30 C, *B. cereus* increased 10^4-fold and 100-fold on C and B product, respectively; but at 21 C for 24 h, a 100-fold increase occurred only on product C. In contrast, on old chopped ham at 30 C, *B. cereus* increased 1,000-fold on product B but decreased on product C.

C. perfringens generally failed to grow on new product. The viable count of *C. perfringens* decreased and subsequently recovered to initial levels of inoculation. On old product, the count of *C. perfringens* decreased during holding at the different incubation temperatures.

Changes in *E. coli* on new product are shown in Fig. 3. *E. coli* grew 1,000-fold on new chopped ham from manufacturer C at 30 and 21 C, but failed to grow on manufacturer B's product. Changes in *S. typhimurium* on new product are also shown in Fig. 3. *S. typhimurium* was also inhibited on manufacturer B's product. On old product from both manufacturers these gram-negative pathogens survived but failed to grow.

Changes in *S. aureus* inoculated onto new and old chopped ham are shown in Fig. 4. On new product, incubated at 30 C, 10^4-fold increases in *S. aureus* occurred. Similarly, on old product from manufacturer B, *S. aureus* increased 10^5-fold.

TABLE 4. *pH of control samples of chopped ham used in the inoculation study.*

Product	Batch 1		Batch 2		Batch 3	
	New	Old	New	Old	New	Old
Manufacturer B						
Initial	6.25	6.02	6.13	6.19	6.15	6.25
8-h incubation						
30 C	6.50	5.75	6.32	6.30	6.22	6.10
21 C	6.45	6.20	6.22	6.15	6.22	6.30
4 C	6.45	6.05	6.09	6.15	6.18	6.20
24-h incubation						
30 C	6.30	5.48	6.29	5.95	6.20	5.90
21 C	6.32	5.59	6.28	6.05	6.28	6.20
4 C	6.20	5.90	6.12	6.15	6.18	6.30
Manufacturer C						
Initial	6.50	5.39	6.12	5.50	6.25	5.75
8-h incubation						
30 C	6.50	5.60	6.30	5.50	6.45	5.52
21 C	6.45	5.59	6.23	5.70	6.38	5.78
4 C	6.45	5.50	6.12	5.70	6.25	5.75
24-h incubation						
30 C	5.90	5.53	5.80	5.50	5.70	5.39
21 C	6.40	5.53	6.12	5.50	6.30	5.50
4 C	6.30	5.55	6.20	5.52	6.30	5.52

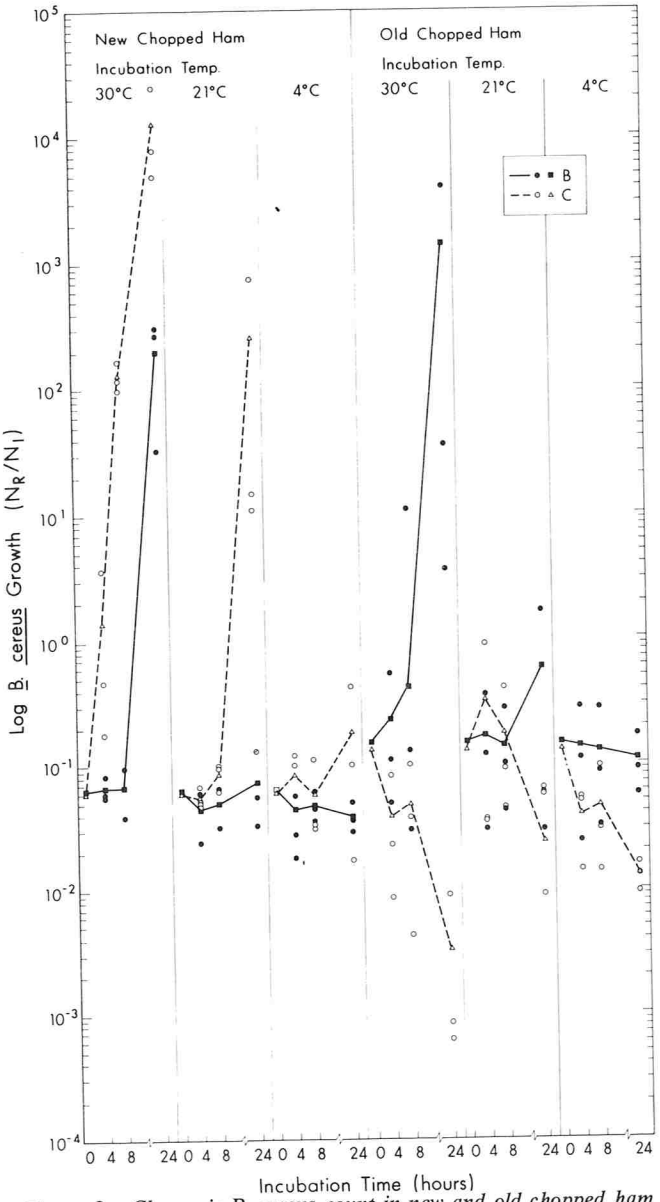


Figure 2. *Change in* B. cereus *count in new and old chopped ham from 2 manufacturers B and C held at 30, 21 and 4 C up to 24 h.*
N_R Number of organisms recovered at specified time intervals;
N_I Number of organisms inoculated onto samples.

DISCUSSION AND CONCLUSIONS

Only nine paired samples of chopped ham samples from four manufacturers were analyzed in the survey study, which limits the conclusions that can be drawn from these data. However, the survey of retail samples of chopped ham from four manufacturers indicated that the microbial quality of this product was comparable with that reported for bologna (*18*). Initial counts of 10^2 to 10^3 organisms/g were in accordance with other literature reports for sliced luncheon meats (*2,11,15*). Similarly, saprophytic counts of 10^7 to 10^8/g at pull date (old samples) were to be expected. However, not all samples had saprophytic counts that achieved maximum population, notably product from manufacturer B, which made this product markedly different from others included in the study.

The pH also represented a marked difference between

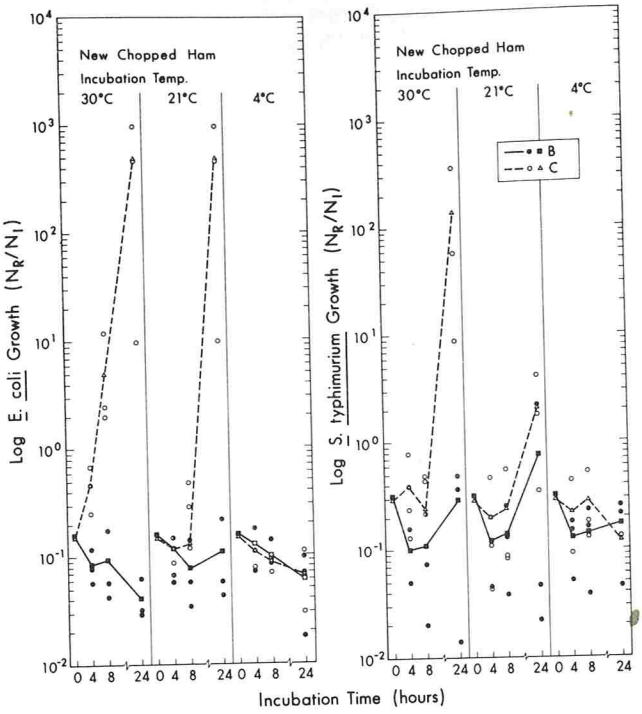


Figure 3. *Change in* E. coli *and* S. typhimurium *counts in new chopped ham from 2 manufacturers B and C held at 30, 21 and 4 C up to 24 h.*
N_R Number of organisms recovered at specified time intervals;
N_I Number of organisms inoculated onto samples.

manufacturers' product, singling out product from manufacturer C, which had a significantly different pH drop between new and pull date product. Correlation of lactic acid bacteria count with pH drop was generally poor, which cannot be readily explained, but might be attributed to lower carbohydrate contents of ham compared to comminuted meats (*14*), and/or the buffering capacity of the product (*13*) from manufacturer D. Neither *M. thermosphactum* nor lactobacilli grew to represent significant proportions of the microbial flora of these chopped hams, confirming observations on bologna (*18*).

Pathogenic bacteria in the chopped ham samples were not detectable at the lower limits of the selective tests, suggesting safe products entering the marketplace. The only indicator or potentially pathogenic organisms detected were presumptive group D streptococci on KF agar, and they failed to predominate in the population even at pull date. However, the inoculation studies indicated that initial counts of potential pathogens must be low, since counts < 100/g, under abusive conditions of temperature storage, could permit pathogen growth to potentially hazardous levels.

The competitive flora present in product B differed from that in product C, so that even after storage of product B to pull date, only low or intermediate competition occurred in this product. This was confirmed in an associated study (Stiles and Ng, unpublished data) in which chopped ham was contaminated to simulate manufacturer contamination. The incomplete growth of the saprophytic flora was shown by

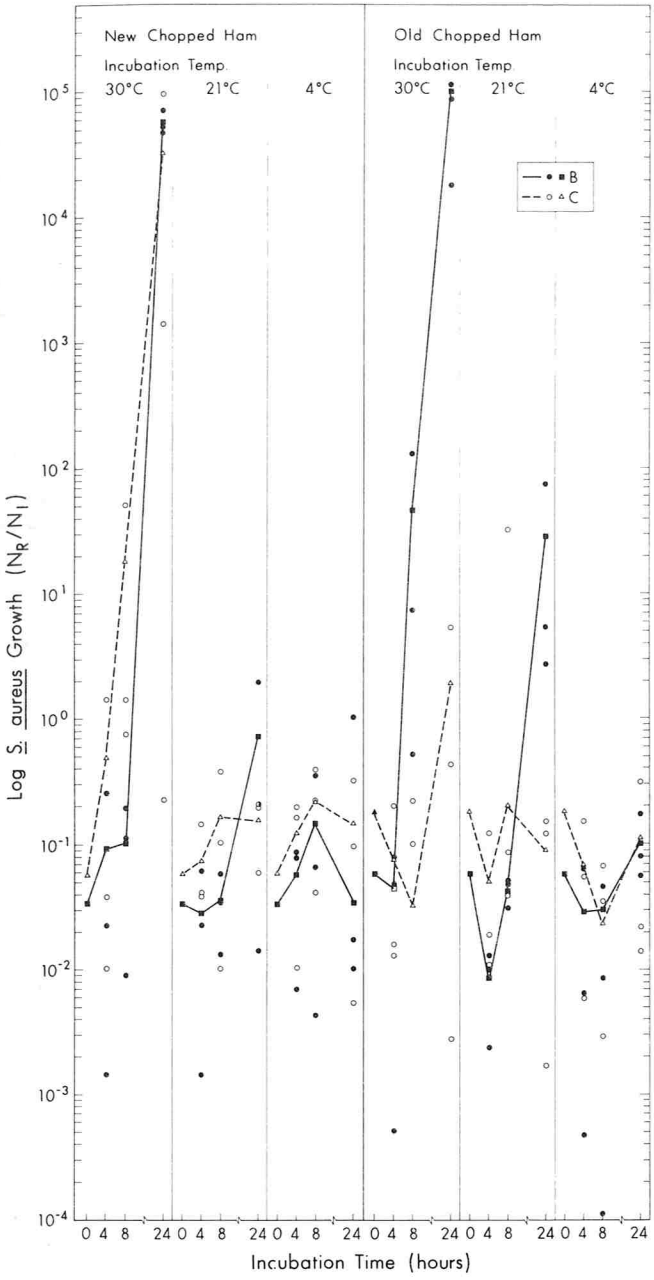


Figure 4. *Change in* S. aureus *counts in new and old chopped ham from 2 manufacturers B and C held at 30, 21 and 4 C up to 24 h.*
N_R Number of organisms recovered at specified time intervals;
N_I Number of organsms inoculated onto samples.

counts $< 10^8$/g at pull date, and growth of the saprophytic population during incubation of sandwiches at 30 and 21 C. This could have been influenced by pH differences between the manufacturers' product, or the marked difference in moisture contents. For pathogen growth, old product from manufacturer B was similar to new product from manufacturers B and C.

C. perfringens failed to grow on chopped ham under conditions of this study. *B. cereus* and *S. aureus* grew on new product B and C, and old product B, but only under severely abusive conditions. The ability of *B. cereus* and *S. aureus* to grow on old product from manufacturer B, might be due to the lower competition and lower lactic acid bacteria count of this product. However, with *S. aureus*, the ratio of *S. aureus* to lactic acid bacteria was such that the inhibitory effects suggested by Kao and Frazier (*13*) should have influenced *S. aureus* growth. However, in this product, the lactic acid bacteria failed to reduce pH and group D streptococci did not grow. Despite the lack of inhibition of these gram-positive pathogens in old product from manufacturer B, the gram-negative pathogens, *E. coli* and *S. typhimurium*, failed to grow on product B, even on new product from B held at 30 C up to 24 h. This inhibitory effect of product B was not explained by these data, or other reports, but may account in part for the slower growth of saprophytic flora in product B.

These data indicate that general rules for growth or inhibition of pathogenic bacteria in chopped ham cannot be drawn. New product, or product with limited saprophytic flora, would generally allow food poisoning bacteria to grow under conditions of severe temperature abuse, such as storage at 30 C for < 24 h or at 21 C for > 24 h. Proper handling with emphasis on temperature control would appear to be more significant to product safety than limiting total microbial load. However, infective pathogens such as *E. coli* and *S. typhimurium* generally survived well on chopped ham, emphasizing the need to avoid contamination with these organisms.

REFERENCES

1. Allen, J. R., and E. M. Foster. 1960. Spoilage of vacuum-packed sliced processed meats during refrigerated storage. Food Res. 25:19-25.
2. Alm, F., I. Ericksen, and N. Molin. 1961. The effect of vacuum packaging on some sliced processed meat products as judged by organoleptic and bacteriologic analysis. Food Technol. 15:199-203.
3. Bartl, V. 1973. Semi-preserved foods: general microbiology and food poisoning. pp. 89-106. In B. C. Hobbs and J. H. B. Christian (eds.) The Microbiological Safety of Food. Academic Press, N.Y.
4. Deibel, R. H., C. F. Niven, Jr., and G. D. Wilson. 1961. Microbiology of meat curing III. Some microbiological and related technological aspects in the manufacture of fermented sausages. Appl. Microbiol. 9:156-161.
5. Duitschaever, C. L. 1977. Bacteriological evaluation of some luncheon meats in the Canadian retail market. J. Food Prot. 40: 382-384.
6. Evans, J. B., and C. F. Niven, Jr. 1951. Nutrition of the heterofermentative lactobacilli that cause greening of cured meat products. J. Bacteriol. 62:599-603.
7. Gardner, G. A. 1966. A selective medium for the enumeration of *Microbacterium thermosphactum* in meat and meat products. J. Appl. Bacteriol. 29:455-460.
8. Harmon, S. M., D. A. Kautter, and J. T. Peeler. 1971. Improved medium for enumeration of *Clostridium perfringens*. Appl. Microbiol. 22:688-692.
9. Health Protection Branch. 1974. Determination of coliforms and faecal coliforms in foods. Acceptable Method MFA-19. May, 1974. Bureau of Microbial Hazards, Health Protection Branch, Ottawa, Canada.
10. Health Protection Branch. 1974. Determination of coagulase-positive staphylococci in foods. Acceptable Method MFA. Oct., 1974. Bureau of Microbial Hazards, Health Protection Branch, Ottawa, Canada.
11. Hill, W. M., J. Resume, and J. C. Wilcox. 1976. Total plate count and sensory evaluation as measures of luncheon meat shelf life. J. Milk Food Technol. 39:759-762.

12. Holbrook, R., J. M. Anderson, and A. C. Baird-Parker. 1969. The performance of a stable version of Baird-Parker's Medium for isolating *Staphylococcus aureus*. J. Appl. Bacteriol. 32:187-192.
13. Kao, C. T., and W. C. Frazier. 1966. Effect of lactic acid bacteria on growth of *Staphylococcus aureus*. Appl. Microbiol. 14:251-255.
14. Kempton, A. G., and S. R. Bobier. 1970. Bacterial growth in refrigerated, vacuum-packed luncheon meats. Can. J. Microbiol. 16:287-297.
15. Mol, J. H. H., J. E. A. Hietbrink, H. W. M. Mollen, and J. van Tinteren. 1971. Observations on the microflora of vacuum packed sliced cooked meat products. J. Appl. Bacteriol. 34:377-397.
16. Mossel, D. A. A., M. J. Koopman, and E. Jongerius. 1967. Enumeration of *Bacillus cereus* in foods. Appl. Microbiol. 15: 650-653.
17. Nie, N. H., C. H. Hull, J. G. Jenkins, K. Steinbrenner, and D. H. Bent. 1975. Statistical package for the social sciences. 2nd ed. McGraw-Hill Book Co., N.Y.
18. Paradis, D. C., and M. E. Stiles. 1978. A study of microbial quality of vacuum packaged, sliced bologna. J. Food Prot. 41:811-815.
19. Paradis, D. C., and M. E. Stiles. 1978. Food poisoning potential of pathogens inoculated onto bologna in sandwiches. J. Food Prot. 41:953-956.
20. Rogosa, M., J. A. Mitchell, and R. F. Wiseman. 1951. A selective medium for the isolation and enumeration of oral and fecal lactobacilli. J. Bacteriol. 62:132-133.
21. Thatcher, F. S., and D. S. Clark. 1968. Microorganisms in foods: Their significance and methods of enumeration. University of Toronto Press, Toronto.

Coming Events, *con't from p. 698*

Sept. 23-29--XV INTERNATIONAL CONGRESS OF REFRIGERATION. Venice, Italy. Contact: XV International Congress of Refrigeration, American Express Co. S.A.I., Conventions Service Italy, Piazza Mignanelli, 4, 00187-Rome, Italy.

Sept. 26-27--SOUTH DAKOTA STATE DAIRY CONVENTION. Downtown Holiday Inn, Sioux Falls, South Dakota 57100. Contact: Shirley W. Seas, Secretary, Dairy Science Department, South Dakota State University, Brookings, South Dakota 57007, 605-688-5420.

Sept. 27-28--NSF SEMINARS, Boston, Ma. See Feb. 11-12, 1980, NSF entry. Contact: Education Service, National Sanitation Foundation, P.O. Box 1468, Ann Arbor, MI 48106, 313-769-8010.

Sept. 28--SYMPOSIUM ON THE PRACTICAL APPLICATIONS OF MICROWAVE ENERGY. Kansas State University Union, KSU, Manhattan, KS 66506. Contact: D. Y. C. Fung, Chairman, or F. E. Cunningham, Co-Chairman, Call Hall, KSU, Manhattan, KS 66506, 913-532-5654.

Oct. 1-3--ADVANCED FOOD PLANT SANITATION COURSE. New Orleans, LA. Sponsored by American Institute of Baking, 1213 Bakers Way, Manhattan, KS 66502. Contact: Darrell Brensing, AIB, 913-537-4750.

Oct. 8-9--NSF SEMINARS, Nashville, TN. See Feb, 11-12, 1980, NSF entry. Contact: Education Service, National Sanitation Foundation, P.O Box 1468, Ann Arbor, MI 48106, 313-769-8010.

Oct. 9-11--FOOD SAFETY AND QUALITY ASSURANCE IN FOOD SERVICES, Seminar sponsored by Capsule Laboratories. Radisson Hotel, St. Paul, MN. Contact: Darrell Bigalke, Capsule Laboratories, 840 Sibley Memorial Highway, St. Paul, MN 55118, 612-457-4926.

Oct. 14-17--24th ANNUAL ATLANTIC FISHERIES TECHNOLOGISTS CONFERENCE. Danvers, MA. Contact: Fred J. King, 1979 AFTC Secretary, Gloucester Laboratory, Northeast Fisheries Center, National Marine Fisheries Center, National Marine Fisheries Service, Emerson Ave., Gloucester, MA 01930, 617-281-3600, ext. 296.

Oct. 17--IOWA MILK AND FOOD SANITARIANS, Sheraton Inn, Cedar Rapids, IA. Contact: Hale Hansen, State Health Dept., Lucas Office Bldg., Des Moines, IA 50319.

Oct. 17-18--NEBRASKA DAIRY INDUSTRIES ASSOCIATION, 25th Annual Convention. Holiday Inn, 72nd and Grover Streets, Omaha, NB. Contact: T. A. Evans, Exec. Secretary, 116 Filley Hall, East Campus, UN-L, Lincoln, NB, 68583.

Oct. 20-25--COMBINED CONFERENCES, EXPOSITIONS: National Environmental Sanitation & Maintenance Educational Conferences, Expositions of Environmental Management Association, The Green Industry Division, Food Sanitation Institute, Health Care Facilities Subsidiary, and Building Service Manager's Institute. Caribbean Gulf Resort Hotel, Clearwater Beach, FL. Contact, for any of the five conferences: EMA, 1701 Drew St., Clearwater Beach, FL, 33515, 813-446-1674.

Oct. 22-23--CALIFORNIA ASSOCIATION OF DAIRY AND MILK SANITARIANS, Annual Meeting. Hyatt House, San Jose, CA. Contact: Pat Dolan, Dairy Foods Consultants, 4009 Cayente Way, Sacramento, CA 95825.

Nov. 3-6--1979 AMERICAN MEAT INSTITUTE CONVENTION. McCormick Place and The Conrad Hilton, Chicago. Contact: Judi Winslow, American Meat Institute, P.O. Box 3556, Washington, D.C. 20007, 703-841-2431.

Nov. 5-6--NSF SEMINARS, Reno NV. See Feb. 11-12, 1980, NSF entry. Contact: Education Service, National Sanitation Foundation, P.O. Box 1468, Ann Arbor, MI 48106, 313-769-8010.

Nov. 11-15--FOOD AND DAIRY EXPO '79. McCormick Place, Chicago, IL. Contact: Wes Dibbern, Dairy and Food Industries Supply Assoc., 5530 Wisconsin Ave., Suite 1050, Washington, D.C. 20015, 301-652-4420.

Nov. 27-29--INTERNATIONAL CONFERENCE ON UHT PROCESSING AND ASEPTIC PACKAGING OF MILK AND MILK PRODUCTS. North Carolina State University, Raleigh, NC 27650. Contact: W. M. Roberts, Dept. of Food Science, NCSU, Raleigh, NC, 27650.

Dec. 3-4--NSF SEMINARS, Orlando, FL. See Feb. 11-12, 1980, NSF entry. Contact: Education Service, National Sanitation Foundation, P.O. Box 1468, Ann Arbor, MI 48106, 313-769-8010.

Dec. 3-8, 10-15--DAIRY DAYS. Sponsored by University of Nebraska-Lincoln Institute of Agriculture and Natural Resources, Nebraska State Dept. of Agriculture, and Nebraska milk marketing outlets. Cooperating organizations are Nebraska Veterinary Medical Association and Dairy Women of Nebraska. Two programs, one week apart, will be held in the following locations: Columbus, NB---Dec. 3, 10; Beemer, NB---4, 11; Hartington, NB---5, 12; O'Neill, NB---6, 13; Ravenna, NB---7, 14; Beatrice, NB---8, 15. Contact: Nebraska Dairy Women, plant fieldmen, or county extension agents for advance registration.

Jan. 14-15--NSF SEMINARS, Los Angeles, CA. See Feb. 11-12 NSF entry. Contact: Education Service, National Sanitation Foundation, P.O. Box 1468, Ann Arbor, MI 48106, 313-769-8010.

Feb. 11-12--NSF SEMINARS, Kansas City, MO. First day seminar covers sanitation aspects of design and construction of foodservice equipment. Second seminar covers specific facility plan preparation and review. Participants may attend either one or both seminars. Contact: Education Service, National Sanitation Foundation, P.O. Box 1468, Ann Arbor, MI 48106, 313-769-8010.

Mar. 26, 1980--ONTARIO FOOD PROTECTION ASSOCIATION, Annual Meeting. Holiday Inn, 970 Dixon Road, Toronto.

Journal of Food Protection Vol. 42, No. 8, Pages 631-637 (August, 1979)

Factors Influencing Tenacity of Dried Milk Films Exposed to High Humidity [1,2]

R. C. MABESA[3], R. T. MARSHALL[3]* and M. E. ANDERSON[4]

Department of Food Science and Nutrition, University of Missouri-Columbia, Columbia, Missouri 65211 and U.S. Department of Agriculture, Science and Education Administration, Agricultural Research, Columbia, Missouri 65211

(Received for publication October 13, 1978)

ABSTRACT

Rinsability of milk films on stainless steel was impaired by exposure to 100% relative humidity (RH). Rinsability was determined by automated Lowry protein tests of detergent used to remove films. Residue of milk films was 1% of the initial soil load when dried on stainless steel plates without humidification, but was 6.35% of the initial load after drying (30 min), humidification (15 min) and redrying (30 min) all at 37 C. Three successive exposures to 100% RH for 7.5 min at 37 C, each followed by 30 min of drying, yielded a residue of nearly 30% of the initial soil load. Exposure at 37 C produced the maximum amount of residue on plates. Experimental temperatures ranged from 0 to 75 C. Temperature of milk applied to plates was of little importance. Raw milk formed more tenacious film than skim milk or major components of milk. Milk produced during colder months yielded less soluble films than milk produced during warmer months. Lowering of milk pH to 5.7, adding soluble calcium, and aging milk at 0 C increased residues. Chelation of soluble calcium with EDTA or dissociation of milk protein with sodium dodecyl sulfate decreased soil residue levels. Exposure of instantized nonfat dry milk to the high humidity treatment decreased its solubility more than tenfold.

High relative humidity (RH) may cause formation of tenacious residue on stainless steel. Such a residue was observed when plates were left standing overnight under humid conditions in an experimental spray unit (*2*). The tenacious film that had formed on these plates resisted spray-rinsing with water at 35 C for 30 sec.

The role of high RH in increasing the tenacity of milk films has not been explained, but the role of moisture in development of less washable fatty soil in fabrics has been reported (*18*). The latter was attributed to possible polymerization of unsaturated oils. Aging effects on tristearin have also been observed on glass (*4*) and on stainless steel (*9*). The former report attributed aging to removal of a layer of moisture from the surface of glass and the latter to the transition of the fast-removed species to a slowly removed one.

High humidity exists in milk processing equipment such as pipelines, tanks, pasteurizers and separators. If such conditions cause buildup of tenacious soil, energy and detergent requirements will be increased. The efficacy of halogenated sanitizers may also be affected. Thus cleaning and sanitizing can be impaired.

The objectives of this research were to quantitate the effects of high RH on the tenacity of milk films and to determine effects of the following variables on film tenacity: time, temperature and number of exposures to high RH, age of milk, fractions of milk, pH, season of the year, and selected additives. Protein was chosen as the indicator of tenacious soil because it is an important and representative constituent of milk soil and is likely to be denatured in high humidity. Tenacious soil was that milk film which resisted rinsing with water and was removed by washing with a specially formulated detergent.

MATERIALS AND METHODS

Stainless steel plates

Plates used in the study were made of 16-gauge stainless steel, type 304, with a No. 4 finish. They measured 10 × 10 cm and 1.5-mm thick. Each had a hole, 2.5 mm in diameter, centered 5 mm from one edge.

Milk

Raw grade A milk was obtained fresh for each replication and stored at 0 ± 1 C until ready for use.

Soiling procedure

Clean plates and glass slides were immersed in cold (5-7 C) milk for 60 sec and then stacked vertically and separated in the incubator to air-dry (10-20% RH) at 37 C for 30 min.

Detergent solution

The detergent solution contained 3.75 g/l of a powdered formulation of the following components:

Compound	*g/100 g*
Sodium tripolyphosphate (purified, granular)	35
Sodium metasilicate (technical, granular)	25
Sodium hydroxide (electrolytic pellets, anhydrous)	17
Sodium carbonate (Certified, ACS, anhydrous)	20
Triton CF-10	3

Fresh detergent solution was prepared daily with distilled water. This formulation possessed enough detergency to remove practically all protein. In preliminary experiments, no protein was detected in fresh detergent solution used to rewash plates.

Washing plates — vessels and procedure

Washing vessels were made of translucent plastic 3-mm thick and were glued together with silicone caulk. Internal dimensions were 3-cm width, 11-cm length and 15-cm height. With slotted plastic at the sides, each held five stainless steel plates, 4 to 5 mm apart, in a vertical plane. Two 1-cm blocks at the bottom raised the plates to allow a magnetic

[1] *Contribution from the University of Missouri Experiment Station, Journal Series No. 8184.*
[2] *Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.*
[3] *Department of Food Science and Nutrition.*
[4] *U.S. Department of Agriculture.*

stirring bar to rotate (300 rpm). For ease of placement and removal, plates were hooked by wires to a bar.

Rinsing and washing were accomplished in the vessel at 25 ± 2 C by submerging five plates in 350 ml of distilled water and detergent solution, respectively, and stirring for 15 min. After plates were rinsed, they were flushed twice with distilled water to remove solubilized protein. Rinse solution was discarded unless specified, but protein content of detergent solutions was determined with each set of plates.

Determining concentrations of protein

A standard curve was prepared with pooled instantized nonfat dry milk (NDM) dissolved in detergent solution. The NDM had an average Kjeldahl nitrogen content of 5.59% (35.66% protein) as determined in triplicate tests (*5*). NDM was carefully weighed, transferred to 1000-ml volumetric flasks, dissolved in detergent solution, and made to volume. Solutions contained 5, 10, 25, 50, 100, 150, 200, 250, 300, and 350 μg of protein/ml. Absorbances were obtained by the automated Lowry test (*3,13*), for each concentration. Average values from two replicate experiments were plotted as a standard curve, which was then used to determine protein concentrations in detergent solutions used to wash plates. The amount of protein removed per unit area (μg/cm²) was calculated from the concentration of protein, the volume of detergent, and the area of the plates.

High humidity chamber

The high humidity chamber was a thermostatically regulated incubator with a circulating fan. High humidity (100%) was produced with a vaporizer and monitored with a portable psychrometer.

Design and analysis of experiments

Experiments were duplicated. Each treatment involved 75 soiled stainless steel plates which were randomly divided into groups of 25 and handled as follows unless specified otherwise:

Unrinsed controls. Plates in subgroups of five were washed in detergent solution immediately after they were soiled.

Unexposed. Soiled plates were incubated at 10 to 20% RH and 37 C for 112.5 min. Subgroups of five plates were then rinsed and washed.

Exposed. Soiled plates were exposed to 100% RH at 37 C for 7.5 min, then dried at 10 to 20% RH and 37 C for 30 min, except in the experiment on "Temperature of exposure." Plates were exposed to these conditions three successive times, for a total of 112.5 min, except in the experiment on "Single exposure--various times." Subgroups of five were then rinsed and washed.

Tenacious milk soil was designated as that film which resisted rinsing and was collected by washing rinsed plates with detergent solution. Quantities of protein recovered in detergent solutions used to wash each subgroup of five plates were averaged. The percentage of protein residue on treated plates was then calculated as follows:

$$\%\,\text{Residue} = \frac{\overline{X}_t}{\overline{X}_c} \times 100$$

Where: $\overline{X}_c$ = mean μg of protein/cm² applied to control plates.
$\overline{X}_t$ = mean μg of protein/cm² remaining on treated plates.

Percentages were subjected to analyses of variance (*16*) to reveal treatment effects. Means were differentiated by Duncan's New Multiple Range Test (*11*).

Treatments

Single exposure--various times. Soiled plates were subjected once to 100% RH for 3.75, 7.5, 15, or 30 min, then dried at 37 C for 30 min.

Triple exposure--various times. Soiled plates were exposed three successive times to 100% RH and drying for times specified in the preceding experiment.

Temperature of exposure. Soiled plates were three times exposed to 100% RH at seven temperatures; namely 0, 10, 20, 37, 50, 63, and 75 ± 1 C. After humidification, plates were dried at 37 C for 30 min. Two vaporizers were used to saturate air in the 63- and 75-C incubators. A small fan was used in all incubators to promote circulation. Unexposed plates were incubated at the same respective temperatures and about 10% RH.

Combined and individual effects of high humidity and drying. The 75 soiled plates used in each replication of this experiment were randomly divided as follows: (*a*) Combined exposure to 100% RH and drying. Plates were exposed three times to 100% RH for 7.5 min at 37 C and dried 30 min at 37 C after each exposure. (*b*) Exposure to 100% RH only. Plates were exposed to 100% RH continuously for 22.5 min (7.5 min × three exposures) at 37 C, then dried for 30 min at 37 C. (*c*) Drying only. Plates were continuously dried for 90 min at 37 C.

Additives. The following were added to milk before plates were soiled: calcium chloride (dihydrate, crystals, Certified ACS)-0.05, 0.1, 0.2, and 0.4 M; sodium dodecyl sulfate (SDS) (powder) - 0.1, 0.2, 0.4, 0.8, and 1%; ethylenediamine tetraacetic acid (EDTA) (Certified ACS) - 0.2, 0.4, 0.8, and 1.6 g/l (w/v). Each solution was stirred for 15 min and cooled to 5-7 C before plates were soiled.

pH. The pH of 1 l of milk at about 1 C was either decreased to 5.7 with 0.1 N HCl or increased to 7.7 with 0.1 N NaOH. The pH meter used for measurements was equipped with a combination electrode. Solutions were allowed to equilibrate 30 min before plates were soiled.

Age of milk. Sixteen liters of fresh commingled milk were stored at 5-7 C. Commencing on the day of milk collection, and every second day thereafter for 16 days, 1 l of milk was used to soil plates.

Temperature of milk. Plates were soiled in aliquots of commingled milk adjusted to 0, 10, 20, and 40 C (± 1 C).

Components of milk. From commingled milk 1 l was used to soil plates and 6 l were separated with a cream separator. The resulting skim milk and cream were used to soil plates. The remaining skim milk was cooled to 5 C and acidified to pH 4.6 with 0.1 N HCl. The precipitated casein was collected on cheese cloth. Fine particles were collected on Whatman No. 40 filter paper in a Büchner funnel. The precipitate was washed three times in excess distilled water to remove whey. The casein was resuspended in a volume of NaOH solution, at pH 11.0, sufficient to equal the original volume of skim milk. Both the casein and the whey were adjusted to pH 6.7, then cooled to 5-7 C. After the plates were soiled, casein was reprecipitated by acidification to pH 4.6. The curd was filtered out, washed as before and drained. Reprecipitated casein was then added back to whey, and the pH was adjusted to 11.0 for resolubilization. Before this recombined skim milk was used to soil plates, its pH was adjusted to 6.7, and its temperature was adjusted to 5-7 C. The cream was then added to the recombined skim milk to produce recombined whole milk, which was stirred vigorously with a magnetic stirrer for 30 min in an ice bath. The pH was adjusted to 6.7 and plates were soiled immediately.

Nonfat dry milk (NDM) versus raw milk. Raw milk and reconstituted instant NDM (10%) were each used to soil 75 plates.

Solubility index of exposed instant nonfat dry milk. In each of five replications 10 g of instant NDM were weighed into each of two large petri dishes (140 mm × 20 mm). The sample in one dish was exposed three times to 100% RH with drying, as previously; the other was incubated at 37 C for 112.5 min. Contents of each dish were mixed after each cycle of the high humidity/drying treatment. Dishes were sealed with masking tape and kept at room temperature until samples were used. Solubility indexes were determined according to the method of the American Dry Milk Institute(*1*). Briefly, samples were stirred in water under specified conditions and particles which failed to dissolve were centrifuged out. Insoluble material was quantitated in graduated conical centrifuge tubes.

Seasonal variations. Data obtained in analyses of fresh raw milk during the cold and warm months were compiled for comparisons.

RESULTS AND DISCUSSION

Single exposure--various times

Milk dried onto stainless steel plates became more tenacious when exposed to high humidity (Table 1). Less than 1% of the initial soil load on unexposed plates remained after rinsing, and the amount of residue did not change with the length of time they were held dry. Even after exposure of plates to high humidity for only 3.75 min, the residue on them was nearly three times as

much as that on unrinsed controls. Residue increased by four times when exposure time was doubled (7.5 min) and by nearly seven times when exposure time was quadrupled (15 min). Doubling time of exposure again (30 min) failed to increase residue recovered.

Triple exposure--various times

Exposure of films to high humidity three times, followed immediately by drying, further enhanced formation of tenacious films (Table 2). Each exposure duration yielded significantly different results. Maximum residue, nearly 30% of the initial residue, occurred when plates were exposed for 7.5 min. This quantity was more than five times greater than the maximum quantity recovered after a single-exposure (6.25% - 15 min).

Individual effects of high humidity versus drying

Data in Table 3 reveal that both high humidity and drying are required to form much tenacious film. On continuous exposure to high RH without drying, only 2.31% of the total soil remained after rinsing. After continuous drying, only 3.25% of the original soil remained. Although significant differences occurred between treatment means, the difference between the combined high humidity/drying treatment and either single factor was substantially greater than the difference between the means of the two individual factors. Summed residues from individual treatments were less than 25% of the tenacious residue obtained when they acted together. The high humidity treatment yielded slightly lower residue than the drying treatment because of the washing effect of prolonged exposure to the moisture-laden atmosphere.

Drying may have altered the surface properties of casein micelles such that aggregation was favored upon humidification. When dry film is moistened with water, fluidity is somewhat restored due to mobility of hydrated polypeptide chains. Thus their reactive groups may form adhesive and cohesive bonds, both of which types are mostly hydrogen bonds. Withdrawal of moisture on drying decreases hydrogen bonding. However, compaction of molecules increases as the film is concentrated. The proximity of proteins to each other probably favored Van der Wall's interaction and electrostatic attraction, particularly between carboxylate residues and calcium, leading to formation of salt bridges. These forces may also have contributed to protein aggregation. In addition, there were probably effects of denaturation, as induced by lactose crystallization, and repellency afforded by lipids.

TABLE 3. *Mean quantities of protein recovered from rinsed plates exposed to high humidity, to drying, and to combined high humidity and drying.*[1]

		Treatment		
		High humidity only	Drying only	High humidity and drying
Treated[2]	μg/cm²	0.81	1.12	8.68
	%of unrinsed	2.31^{c}	3.25^{b}	23.84^{a}
Unrinsed[3]	μg/cm²	35.11^{z}	34.48^{z}	36.41^{z}

[1]n = 10 sets of 5 plates each.
[2,3]Where superscripts differ, means are significantly different ($P \leq 0.05$), Duncan (*11*).

Temperature of exposure

On unrinsed control plates, the amount of soil deposited increased with temperature from 31.5 to 34.3 μg/cm². The quantity of residue on unexposed plates was unaffected by temperature of incubation through 37 C (Fig. 1), but significant increases in residue occurred as temperature was raised to 50, 63 and 75 C. Considerably more soil was left on surfaces of exposed samples than unexposed samples, except at 75 C. Humidification caused a sharp rise in soil residues above 20 C. Quantities of residue peaked near 37 C then declined and leveled off between 50 and 60 C. A small, though significant, rise in soil residues was observed at 75 C. Thus protein became insoluble in high humidity at about 37 C, and the solubility of unexposed films decreased at high temperatures.

TABLE 1. *Mean quantities of protein recovered as affected by time of exposure of milk films to high humidity in a single treatment.*[1]

		Duration of exposure to 100% RH (min)			
Treatment		3.75	7.5	15.0	30.0
Unexposed[2]	μg/cm²	0.35	0.35	0.35	0.34
	%of unrinsed	0.96^{d}	0.96^{d}	0.93^{d}	0.91^{d}
Exposed[2]	μg/cm²	0.98	1.61	2.38	2.21
	%of unrinsed	2.67^{c}	4.42^{b}	6.35^{a}	5.92^{a}
Unrinsed[3]	μg/cm²	36.65^{z}	36.40^{z}	37.49^{z}	37.31^{z}

[1]n = 10 sets of 5 plates each.
[2,3]Where superscripts differ, means are significantly different ($P < 0.05$), Duncan (*11*).

TABLE 2. *Mean quantities of protein recovered as affected by exposure of milk films to high humidity in three successive treatments.*[1]

		Duration of exposure to 100% RH (min)			
Treatment		3.75	7.5	15.0	30.0
Unexposed[2]	μg/cm²	0.34	0.34	0.35	0.34
	%of unrinsed	0.98^{e}	0.96^{e}	0.96^{e}	0.98^{e}
Exposed[2]	μg/cm²	3.36	10.50	6.13	4.34
	%of unrinsed	9.69^{d}	29.70^{a}	16.74^{b}	12.46^{c}
Unrinsed[3]	μg/cm²	34.69^{y}	35.35^{zy}	36.61^{z}	34.83^{y}

[1]n = 10 sets of 5 plates each.
[2,3]Where superscripts differ, means are significantly different ($P < 0.05$), Duncan (*11*).

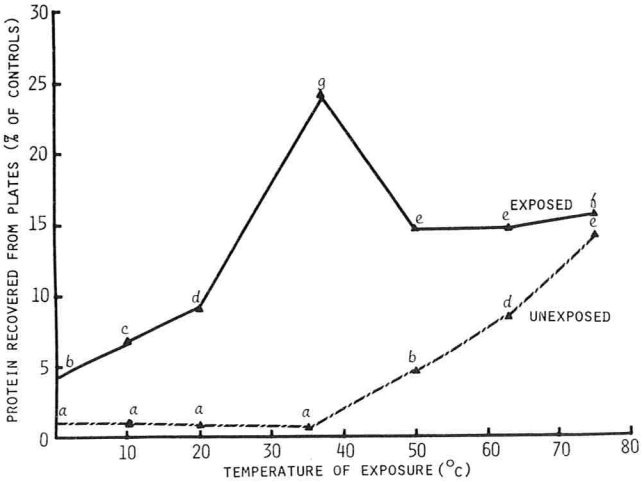


Figure 1. *Protein recovered from rinsed plates treated at various temperatures, expressed as percentages of the initial protein. Points with different letters* (a-g) *differed significantly in protein recovered* ($P < 0.05$).

Effects of additives on formation of tenacious films

Calcium chloride. Amounts of tenacious residue on unexposed, exposed and unrinsed control plates increased with additions of $CaCl_2$ (Table 4). The amount of residue on exposed plates paralleled that obtained for unexposed plates, but was 15% to 25% higher at concentrations of $CaCl_2$ below 0.4 M. When 0.4 M $CaCl_2$ was added, only small quantities of soil on both exposed and unexposed plates were rinsed off. Thus, regardless of humidity, soil became quite tenacious when high quantities of calcium were added. This experiment confirms the importance of calcium in formation of tenacious films and emphasizes the value of soft water in cleaning dairy equipment. Residual calcium on equipment would not only enhance formation of milkstone but also increase tenacity of proteinacious milk films upon exposure to high humidity.

Sodium dodecyl sulfate (SDS). SDS was added to milk to dissociate protein. Addition of 0.1% (equivalent to 0.003 M) SDS significantly ($P < 0.05$) decreased the amount of residual soil, but increasing the concentration had little additional effect (Table 5). This detergent practically cancelled the effects of high humidity, bringing soil levels on exposed plates after rinsing near those on unexposed plates. Thus, little tenacious film was formed if protein was dissociated, even though there was a pronounced increase in amounts of soil deposited on plates on dipping.

SDS interacts with proteins and causes dissociation of high-molecular weight aggregates into monomers (*14*). Cheeseman (*10*) suggested that the detergent binds with hydrophobic regions of casein to form complexes. When complexes form, the insolubilizing effect of high humidity on protein becomes insignificant. Reduced size of the casein plus its presumed complexation with SDS increased rinsability of the milk film.

SDS lowered the surface tension of milk such that it wet plate surfaces better than did milk without SDS. This improvement in wettability increased with detergent concentration in milk at least up to 1%, as exhibited by the increasing soil load on control plates.

Ethylenediamine tetraacetic acid (EDTA). Unexposed samples did not react significantly to EDTA in concentrations up to 800 mg/l, but residue increased considerably when 1600 mg/l were added (Fig. 2). Residue on exposed plates was lowered significantly by 200 to 800 mg/l of EDTA, but more than three times as much was left on exposed plates with 1600 mg/l as on exposed plates without EDTA and unexposed plates with equal EDTA.

We had already observed that added soluble calcium increased tenacity of residue. Total calcium in milk is estimated to be 30 mM (1200 mg Ca^{++}/l) of which about 20 mM is colloidal, 10 mM is soluble and 3 mM is

TABLE 4. *Mean quantities of protein recovered from plates soiled with milk containing added calcium chloride ($CaCl_2$).*[1]

Concentration $CaCl_2$ in milk (molar)	Treatment				
	Unexposed[2]		Exposed[2]		Unrinsed[3]
	μg/cm²	% of unrinsed	μg/cm²	% of unrinsed	μg/cm²
0	0.35	1.22[h]	2.28	7.97[g]	28.60[v]
0.05	12.18	28.25[f]	17.96	41.65[e]	43.12[w]
0.10	18.80	29.13[f]	29.72	46.05[d]	64.54[x]
0.20	66.26	65.51[c]	92.40	91.35[b]	101.15[y]
0.40	116.00	100.00[a]	116.00	100.00[a]	116.00[z]

[1]n = 10 sets of 5 plates each.
[2,3]Where superscripts differ, means are significantly different ($P < 0.05$), Duncan (*11*).

TABLE 5. *Mean quantities of protein recovered from plates soiled with milk containing varying amounts of sodium dodecyl sulfate (SDS).*[1]

Concentration of SDS in milk (%)	Treatment				
	Unexposed[2]		Exposed[2]		Unrinsed[3]
	μg/cm²	% of unrinsed	μg/cm²	% of unrinsed	μg/cm²
0	0.35	1.25[b]	1.72	6.12[a]	28.11[x]
0.1	0.35	1.12[bc]	0.35	1.12[bc]	31.36[y]
0.2	0.34	0.86[d]	0.35	0.89[d]	39.45[z]
0.4	0.34	1.04[c]	0.42	1.28[b]	32.73[y]
0.8	0.34	0.83[d]	0.39	0.95[cd]	41.13[z]
1.0	0.34	0.82[d]	0.35	0.85[d]	41.41[z]

[1]n = 10 sets of 5 plates each.
[2,3]Where superscripts differ, means are significantly different ($P < 0.05$), Duncan (*11*).

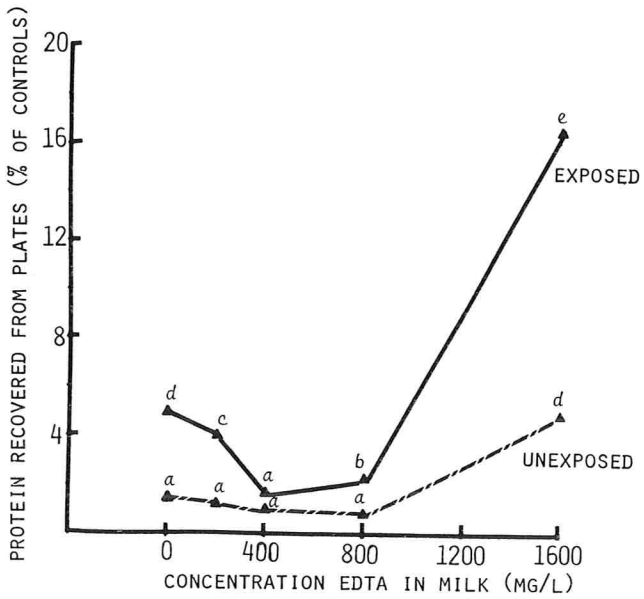


Figure 2. *Percentages of protein recovered from exposed and unexposed plates soiled with milk containing varying amounts of ethylenediamine tetraacetic acid (EDTA). Points with different letters* (a-e) *differed significantly in protein recovered (P < 0.05).*

ionized (*15*). EDTA binds Ca^{++} on a 1:1 basis (*19*). As ionized and soluble calcium were progressively bound with 200 to 400 mg/l of EDTA, the reaction to high humidity decreased. However, quantities of EDTA sufficient to bind, as well, colloidal calcium (800 mg Ca^{++}/l) increased the response to high humidity. Addition of 1600 mg/l caused the protein to become much more sensitive to high humidity, and quantities of residue approached the large amount normally deposited when there was no additive. The mechanism of this latter effect is unexplained.

pH

More tenacious residue formed in high RH with acidic (pH 5.7) and alkaline (pH 7.7) milks than in milk of pH 6.7, the normal pH (Table 6). Higher residue was obtained with pH 5.7 than with pH 6.7 and pH 7.7. The effect of low pH was probably related to decreases in hydration and in the colloidal calcium of the micelles.

Adjusting pH from 6.7 to 7.7 could have caused increasing adhesiveness of caseinate. Viscosity increases as micelles swell when the pH of milk is raised (*7*). Also, more calcium bridges may have been formed because of the increasing availability of nonprotonated protein species ($H_2NCHRCOO^-$) as pH was increased. Caution must be observed in the interpretation of these results because differences between treatment means, though statistically significant, were small.

TABLE 6. *Mean quantities of protein recovered from plates soiled with milk of varying pH.*[1]

Treatment		pH of milk 5.7	6.7	7.7
Unexposed[2]	μg/cm²	0.34	0.34	0.34
	%of unrinsed	0.97d	0.94d	1.02d
Exposed[2]	μg/cm²	2.45	1.79	1.89
	%of unrinsed	6.92a	4.95c	5.60b
Unrinsed[3]	μg/cm²	35.42z	36.05z	33.73y

[1]n = 10 sets of 5 plates each.
[2,3]Where superscripts differ, means are significantly different (P ≤ 0.05), Duncan (*11*).

Age of milk

Generally, as milk aged, soil deposition on dipped unrinsed plates increased, and the film became more susceptible to effects of high humidity. There was no significant change in the percentage of the initial protein left on unexposed plates as milk aged up to 14 days (Fig. 3). The significant ($P < 0.05$) increase observed after 16 days indicated that deteriorated milk was more tenacious even without exposure to high humidity. The amount of soil that remained on unexposed plates at 16 days, 10.68% of initial soil, approximated that from 4- or 6-day old milk on plates exposed to high humidity.

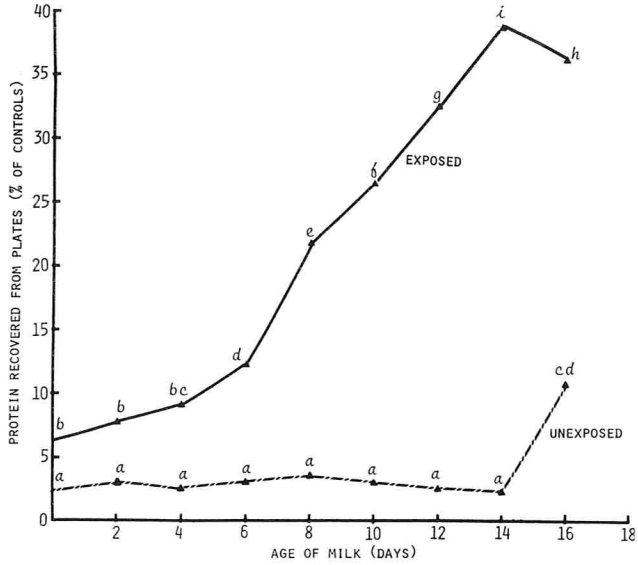


Figure 3. *Percentages of protein recovered from exposed and unexposed plates soiled with milk of increasing age. Points with different letters* (a-i) *were significantly different (P < 0.05).*

With exposed samples, age of milk became important on the 6th day, when a sharp rise in residue was observed (Fig. 3). Increases continued until the 14th day, but a slight decrease was noted on the 16th day.

Although pH was not measured, slight precipitation of protein was observed on the 14th day; therefore, developed acidity may have increased the sensitivity of protein to high RH. The slight decrease in residue on exposed plates at 16 days suggests partial proteolysis, hence higher protein solubility. However, unexposed samples exhibited a sharp rise in soil level at 16 days, indicating that the solubility of proteins in the milk had decreased. Proteolytic effect was not as evident in unexposed samples because protein coagulation predominated over proteolysis. Increasing adherence of residue to stainless steel as milk aged was observed by Berridge and Scurlock (*8*), who suggested that developed acidity was the likely cause.

Temperature of milk

Although differences between treatments were small, milk deposited at 10 C rinsed less readily ($P < 0.05$) than

that deposited at 0, 20 or 40 C, and protein recovered was 9.24% vs. 8.09, 8.27 and 7.42% of unrinsed controls, respectively. Quantities of protein deposited initially on plates were significantly higher ($P < 0.05$) with each successive increase in temperature, ranging from 19.5 to 33 $\mu g/cm^2$. Therefore, actual quantities left on plates after rinsing were highest on plates exposed to milk at 40 C (1.58 $\mu g/cm^2$ at 0 C vs 2.45 $\mu g/cm^2$ at 40 C). Increasing temperature decreased viscosity which probably allowed quicker formation of adsorption films (*17*).

Reaction of separated components of milk

Generally, as components were removed from milk, less protein remained on exposed plates after rinsing (Fig. 4). More than 12% of the soil remained on plates when whole milk was used, but this amount decreased to a low of 1.5% with casein solution. When separated components were recombined as with casein plus whey (recombined skim milk) and skim milk plus cream (recombined whole milk), the amount of soil left on plates increased to 8.82% and 7.21%, respectively. Although these levels were still considerably lower than the 12.55% initially found for whole milk, the trend toward greater tenacity of soil as components were recombined was clear. There was no difference in the amount of soil remaining on exposed and unexposed plates when whey was used for soiling, and little difference was observed in the experiment with casein solution. Calcium was limited in the casein because it had been acid-precipitated, and calcium is necessary for development of stickiness. Calcium was largely in the whey, but whey lacked casein, which was later shown to be involved in formation of tenacious film.

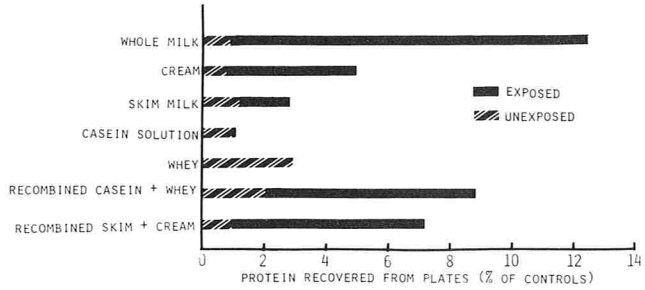


Figure 4. *Percentages of protein recovered from exposed and unexposed plates soiled with milk and various milk components.*

Fat contributed to formation of tenacious film. Less residue was recovered from exposed and rinsed plates soiled with skim milk than with cream, despite the lesser amount of protein and lactose in cream than in skim milk. The influence of fat was also evident in comparisons of amounts of residue on rinsed, exposed plates soiled with whole milk and skim milk. Fat, being hydrophobic, protects the film surface against rinsing. However, phospholipids are highly surface active and may also have played a role by affecting deposition.

Limited decreases in response to high humidity observed in recombined skim and whole milks were expected because there was up to 10% dilution during isolation, separation and recombination. However, the significantly higher levels of soil obtained from plates soiled with recombined casein and whey than from plates soiled with skim milk was difficult to explain. The only likely explanation was related to the significantly thinner initial film when recombined skim milk was used (24.6 $\mu g/cm^2$) than when skim milk (43.5 $\mu g/cm^2$) was used. With the thinner film, more efficient moisture transfer would have been achieved. It is hypothesized that moisture penetration of thicker films is hindered, thereby decreasing the aggregative insolubilizing effect of high humidity.

Reaction of nonfat dry milk and raw milk to high humidity

More than 27% of the initial soil remained on plates exposed to high RH when raw milk was used to soil plates, but less than 11% was left from NDM. Both had practically the same soil load initially, 34.13 $\mu g/cm^2$ for NDM and 33.57 $\mu g/cm^2$ for raw milk.

The larger residue of whole milk than of NDM can be partly attributed to the hydrophobic nature of fat. However, fat in the whole milk did not cause formation of tenacious milk film without exposure to high humidity. This was evidenced by the small amount of residue on all unexposed plates soiled with each type of milk. Thus the conclusion is reinforced that proteins and salts are largely responsible for adhesion of milk upon exposure to high humidity.

Denaturation of whey protein and insolubilization of some calcium during heat-processing of NDM could have impaired its response to high humidity.

Solubility of instant nonfat dry milk exposed to high humidity

The solubility index of instant NDM increased from 0.35 to 5.74 ml (n = 5) on exposure to high humidity. The considerable decrease in solubility caused by exposure of NDM samples to high humidity indicated extensive changes in the casein. Hall and Hedrick (*12*) attributed the insolubility of NDM to the inability of the casein, when denatured, to form a stable dispersion when recombined with water.

Dry film was present near the top of each tube containing exposed samples (B, Fig. 5) and absent in tubes with unexposed milk (A, Fig. 5). This film failed to rinse from tubes without vigorous brushing.

Seasonal variation

Season of the year apparently affects formation of tenacious residue on plates exposed to high humidity. Representative data obtained during winter and summer months are listed in Table 7. About three times as much residue appeared on exposed plates during the cold months of November through February than appeared from May through August. This might have been related to higher protein expected in milk during the cold months than during the warm months (*6*). However, quantities of protein on unrinsed control plates were as high in May and June as in the winter months. This result strongly suggests that the important factor was

TABLE 7. *Mean percentages of protein remaining on unexposed and exposed plates and amount (μg/cm²) of protein deposited on unrinsed plates during winter and summer months (n = 5).*

Treatment	Winter				Summer			
	Nov	Dec	Jan	Feb	May	Jun	Jul	Aug
Unexposed	0.96[1]	1.01	1.04	1.01	2.96	0.94	1.60	1.22
Exposed	29.70[1]	27.64	23.98	12.55	7.57	4.95	9.24	7.97
Unrinsed	35.35[2]	33.57	32.69	34.58	32.10	36.05	21.21	28.60

[1]Percentage of respective control.
[2]In μg/cm².

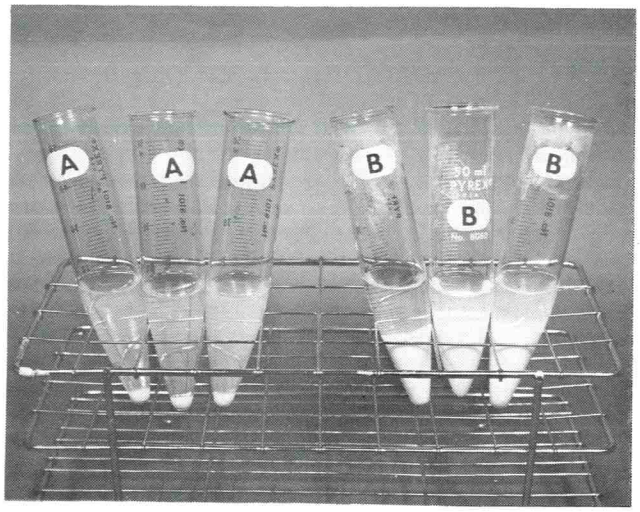


Figure 5. *Effect of exposure to high humidity on solubility index of instant nonfat dry milk (A - unexposed, B - exposed).*

sensitivity of casein to humidity, and not quantity of casein in milk.

SUMMARY

The following factors influenced tenacity of films on stainless steel surfaces upon exposure to high humidity:

1. *Time and temperature.* Optimal conditions were 37 C for 7.5 min, and three successive exposures were more effective than one.

2. *Combination of humidity and drying.* Exposure to 100% RH followed by drying was necessary to form sticky films.

3. *Concentration of calcium.* Addition of calcium enhanced response to high humidity. Binding of serum calcium with EDTA decreased amount of tenacious residue formed. Addition of enough EDTA to bind most of the calcium produced tenacious residue.

4. *State of protein.* Dissociation of milk protein with SDS rendered it insensitive to the humidification treatment.

5. *pH of milk at soiling.* The tendency of milk to react to humidity was greater at pH 5.7 than at 6.7 or 7.7.

6. *Age of milk.* Reactivity to high humidity increased with age up to 2 weeks.

7. *Soiling temperature.* As a percentage of initial soil load, residue after rinsing was affected little by temperature of milk applied.

8. *Type of milk.* Whole milk yielded more tenacious soil than skim milk or 10% NDM.

9. *Season of the year.* More tenacious residue formed during the winter than during the summer.

REFERENCES

1. American Dry Milk Institute. 1971. Standards for grades of dry milks including methods of analysis. Bulletin 916, American Dry Milk Institute, Inc., Chicago, Ill. p. 53.
2. Anderson, M. E., J. R. Fischer, R. T. Marshall, D. B. Brooker, and T. F. Webb. 1972. Comparative effectiveness of various acids in removing calcium from milk films on stainless steel. J. Milk Food Technol. 35:325-327.
3. Anderson, M. E., and R. T. Marshall. 1975. An automated continuous protein analyzer: modification of the Lowry method. J. Food Sci. 40:728-731.
4. Anderson, R. M., J. J. Satanek, and J. C. Harris. 1960. Removal of fatty soil from glass. Electrolyte detergent-builder effect. J. Am. Oil Chem. Soc. 37:119-121.
5. AOAC. 1975. Official methods of analysis, 12th ed. Association of Official Analytical Chemists, Washington, D.C.
6. Barnard, S. E., and G. L. Hargrove. 1975. Protein content in raw and fluid milk products. J. Milk Food Technol. 38:380-382.
7. Beeby, R., and K. Kumetat. 1959. Viscosity changes in concentrated skim milk treated with alkali, urea and calcium-complexing agents. I. The importance of the casein micelle. J. Dairy Res. 26:248-257.
8. Berridge, N. J., and P. G. Scurlock. 1976. Adhesion of coagulating protein (casein) to various surfaces. J. Dairy Sci. 59:1401-1408.
9. Bourne, M. C., and W. G. Jennings. 1961. Some physicochemical relationships in cleaning hard surfaces. Food Technol. 15:495-499.
10. Cheeseman, G. C. 1968. A preliminary study by gel filtration and ultracentrifugation of the interaction of bovine milk caseins with detergents. J. Dairy Res. 35:439-446.
11. Duncan, D. B. 1955. Multiple range and multiple F test. Biometrics 11:1-42.
12. Hall, C. W., and T. I. Hedrick. 1971. Drying of milk and milk products, 2nd ed. AVI Publishing Co., Inc., Westport, Conn. p. 338.
13. Huang, Y. W., R. T. Marshall, M. E. Anderson, and C. Charoen. 1976. Automated modified Lowry method for protein analysis of milk. J. Food Sci. 41:1219-1221.
14. Lehninger, A. L. 1975. Biochemistry. Worth Publishers, Inc., New York, NY. p. 1104.
15. Parry, Jr., R. M. 1974. Milk coagulation and protein denaturation. p. 603 to 661. *In* B. H. Webb, A. H. Anderson, and J. A. Alford (eds.) Fundamentals of dairy chemistry. Avi Publishing Co., Inc., Westport, Conn.
16. Snedecor, G. W., and W. G. Cochran. 1967. Statistical methods, 6th ed. Iowa State University Press, Ames, Iowa. p. 593.
17. Sommer, H. H. 1952. Market milk and related products, 3rd ed. Published by the author. Madison, Wis. pp. 672-676.
18. Utermohlen, W. P., Jr., and E. L. Wallace. 1947. Detergency studies. Textile Res. J. 17:670-688.
19. Wharton, D. C., and R. E. McCarty. 1972. Experiments and methods in biochemistry. MacMillan Publishing Co., Inc., New York, NY. p. 350.

Journal of Food Protection Vol. 42, No. 8, Pages 638-644 (August, 1979)

ICMSF Methods Studies. XI. Collaborative/Comparative Studies on Determination of Coliforms Using the Most Probable Number Procedure[1]

J. H. SILLIKER[2], D. A. GABIS[2]* and A. MAY[3]

Silliker Laboratories, 1304 Halsted Street, Chicago Heights, Illinois 60411 and The Pillsbury Company, Minneapolis, Minnesota

(Received for publication October 30, 1978)

ABSTRACT

Results of two international collaborative studies on the MPN technique for determination of coliforms in foods are reported. Three methods involving use of different presumptive and confirmatory media were compared. Results of one collaborative study conducted among 15 laboratories using eight different types of inoculated foods showed differences among the laboratories as great as 3.3 log units. The greatest difference between confirmatory tests using different media was 0.5 log units. Results of the other collaborative study conducted among five laboratories using three types of naturally contaminated foods showed differences among the laboratories as great as 1.4 log units. The greatest difference between tests using different media was 0.2 log unit. Both studies showed that the 95% confidence limit for a single value reported by a given laboratory was ± 1 log unit or ± 0.45 log unit for a mean of five values. The second study showed that a major source of variation within laboratories was between replicate aliquots. The findings are discussed in terms of their significance with respect to the monitoring of microbiological specifications for food.

A principal aim of the International Commission on Microbiological Specifications for Foods (ICMSF) is evaluation of microbiological methods. The first book (*4*) by ICMSF is a collection of internationally recognized methods for determination of particular microorganisms. The Commission is evaluating these methods through comparative and collaborative testing programs so that the "best" method may be selected when more than one is given for a particular microorganism.

This communication reports the results of two collaborative studies on determination of coliforms using the most probable number (MPN) procedure. Thatcher and Clark (*4*) included three methods for determination of coliforms: (*a*) presumptive determination using Lauryl Sulfate Tryptose Broth (LST) with confirmation of gas-positive tubes in Brilliant Green Lactose Bile Broth 2% (BGLB) or by streaking on Eosin Methylene-Blue (EMB) or Endo (E) agar plates (*1*), (*b*) determination of coliforms using MacConkey Broth (MAC) with no confirmation procedure, and (*c*) presumptive determination by inoculation of BGLB, with confirmation accomplished by streaking Violet-Red Bile Agar (VRB) or E agar.

[1]*International Commission on Microbiological Specifications for Foods (ICMSF) of the International Association of Microbiological Associations.*
[2]*Silliker Laboratories.*
[3]*The Pillsbury Company.*

MATERIALS AND METHODS

Collaborative I

Cultures. Three cultures of typical *Enterobacter aerogenes* were freshly isolated from raw milk, pasta and egg albumen. Three cultures of typical *Escherichia coli* were freshly isolated from pecans, raw milk and ground beef. The cultures were isolated using the methods of Thatcher and Clark (*4*), namely, Method 1 for enumeration of coliforms, Method 1 for determination of coliform organisms of fecal origin, and identification tests for coliform organisms. The cultures were carried in Brain Heart Infusion Broth (Difco, Detroit, MI) with incubation at 35 C and were transferred daily until used for inoculation.

Samples. Commercially available pasteurized frozen whole eggs, pasteurized vanilla-flavored ice cream, frozen peas, ground beef, meat meal, dried egg albumen, coconut and non-fat dry milk (NDM) were inoculated with the cultures of *E. aerogenes* and *E. coli*.

Inoculation of frozen products. The six broth cultures were mixed together in equal proportions. Fifty milliliters of thawed ice cream and 50 ml of thawed eggs were inoculated with 0.5 ml of the culture blend. A mixture of 50 g of ground beef and 50 ml of Nutrient Broth (Difco) was inoculated with 0.5 ml of the culture blend. A mixture of 50 g of frozen peas and 50 ml of Nutrient Broth was inoculated with 0.5 ml of the culture mix. The inoculated products were incubated at 35 C for 24 h. After allowing the *E. aerogenes-E. coli* cultures to grow in the products, the bulk sample material was inoculated. To produce the "high" level of contamination, 100 ml of a 1:10 dilution of the artificially contaminated eggs, ice cream, peas or ground beef were added to approximately 4,500 g of each product, respectively. The inocula were added slowly with mixing to facilitate homogenous distribution of the contamination throughout each product. The frozen eggs and ice cream were mixed for 1 h and the frozen peas 0.5 h in a Patterson-Kelley twin-shell tumble mixer with an intensifier bar (Model LB-S-16, Patterson-Kelley, Stroudsburg, PA). The inoculated ground beef was mixed by passing it three times through a meat grinder with a 3-mm hole size plate. The meat was mixed 10 min by hand between each grinding. The low level of contamination was effected for these products by preparing a 1:10 dilution of the above "high" level product with uninoculated material.

Both levels of inoculated product were then packaged in approximately 50-g amounts in sterile polyethylene bags and stored at - 20 C until shipped to the collaborating laboratories.

Inoculation of low moisture samples. The same six cultures used for the frozen foods were used for inoculating the low-moisture foods. Preparation of meat meal involved incubating 50 g of meat meal in 50 ml of Nutrient Broth with 0.5 ml of the mixed *E. aerogenes-E. coli* cultures and incubating for 24 h at 35 C.

Approximately 4,500 g of meat meal were inoculated with 100 ml of a 1:10 dilution of the meat meal-Nutrient Broth culture to give a high level of contamination. The inoculum was added very slowly with simultaneous hand-mixing to promote even distribution of the contamination and to avoid lumping. The contaminated meat meal then was mixed for 1 h in the Patterson-Kelley blender. Meat meal with the "high" level of contamination was then diluted (1:10) with uninoculated material to obtain the "low" level. The "low" level material was mixed for 1 h in the Patterson-Kelley blender. The "high" and

"low" level meat meal was then packaged (50 g) in sterile polyethylene bags and held for shipment at 4-6 C.

The "high" and "low" levels of contamination of desiccated coconut were prepared essentially as the meat meal except that the coconut was blended for 2.5 h in the Patterson-Kelley blender. Packaging and storage were the same as those for the meat meal.

Dried egg albumen and non-fat dry milk (NDM) were prepared in the same fashion. Fifty grams of egg albumen and 50 g of NDM were mixed with 200 ml of distilled water and inoculated with the *E. aerogenes-E. coli* cultures described above. These were incubated 24 h at 35 C. These inocula were prepared for mixing into the dry uninoculated product by mixing 100 ml of the NDM culture or egg albumen culture with 100 ml of corn oil plus 2% (v/v) Tergitol Anionic-7 (Union Carbide, Chicago, IL). The entire mixture was then added to the product in a drop-wise fashion to approximately 4,500 g of each product. This produced the "high" level. Dilution (1:10) of the "high" level food with uninoculated material yielded the "low" level. The mixing, storing and shipping procedures were the same as for the meat meal samples.

Before shipment, samples were analyzed to determine the dilution range to be used. Instructions for analysis were sent to each collaborating laboratory. The samples were shipped by air freight, and each laboratory began the analyses within 48 h of shipment.

Duplicate subsamples were sent to each of 14-15 laboratories in Europe and North and South America. In each laboratory two analysts proceeded independently to analyze the samples. Frozen samples were shipped with dry-ice and were received at the collaborating laboratories in a frozen state.

All media were purchased from Difco. Each laboratory was provided with the media, the same lot of medium of each type being used in each of the laboratories.

The presumptive media for each of the three procedures were prepared according to the manufacturer's directions, using 10 ml of double strength medium when the inoculum consisted of 10 ml of the primary dilution and 10 ml of single strength medium for inocula of 1 ml or 0.1 ml. Three-tube MPN determinations were carried out in each of the three presumptive broths. The presumptive tubes contained Durham tubes for detection of gas.

Each analyst weighed 10 g of sample into a sterile blender to which was added 90 ml of phosphate buffered dilution water, following which the sample was blended 2 min at high speed as prescribed in Thatcher and Clark (*4*).

The presumptive tubes were examined for gas after 24 and 48 h incubation at 35 ± 1 C. Tubes showing gas were subcultured for confirmation of coliforms. Gas-positive LST broth tubes (Method 1) were confirmed by transferring a loopful into BGLB and streaking onto EMB and E agar plates. The confirmatory broth medium was examined for gas production and the two agar media for the presence of coliform colonies after 24 h of incubation at 35 C. Though Method 2 does not require confirmation of gas-positive tubes, each tube showing gas, either at 24 or 48 h, was streaked onto EMB agar which was incubated 24 h at 35 C and then examined for coliform colonies. The gas-positive BGLB presumptive tubes (Method 3) were streaked onto VRB and E agar plates which were incubated at 35 C for 24 h and examined for presence of coliform colonies.

On the basis of the pattern of gas-positive tubes, MPN values were computed on each of the samples.

Collaborative II

Naturally contaminated samples of peanut butter, dried buttermilk and dried egg albumen were obtained. These were thoroughly mixed. Peanut butter was mixed by raising the temperature of approximately 4,500 g of product to 40 C and mixing for 30 min with a portable cake mixer. The dried egg albumen and dried buttermilk were mixed in the Patterson-Kelley blender for 1 h.

The samples were distributed to five laboratories by air freight. Each laboratory analyzed the samples using the same three presumptive media as were employed in Collaborative I, namely LST, MAC and BGLB. Gas-positive tubes developing after 24 or 48 h of incubation at 35 C were streaked onto EMB agar for confirmation. Two analysts in each of the five laboratories analyzed triplicate aliquots of three subsamples from each master sample sent to the laboratory, i.e. each technician analyzed a total of nine subsamples in each of the three presumptive media. Further, all presumptive tubes failing to show gas after 48 h of incubation were streaked onto EMB agar in an effort to determine whether anaerogenic coliforms were being overlooked and/or whether individual laboratories were encountering difficulty in detecting gas production.

RESULTS

Collaborative I

The mean logarithms of MPN values for each laboratory are summarized in Table 1. Each MPN value represents the results obtained from presumptive and confirmed tests for all three methods for a particular food and level of inoculation. Laboratory means not connected by a line were found to be significantly different at the 5% level. The smallest range between laboratories was 0.86 log unit for ice cream and the largest range of 3.36 log units was for non-fat dry milk inoculated at the high level. For each and every sample, regardless of the level of contamination, there were significant differences among the laboratories. The magnitude of the differences among laboratories varied from food to food. With regard to level of contamination, no pattern was observed indicating that one level of contamination produced more variation among laboratories than the other.

In general, the differences between methods were appreciably smaller than the differences between laboratories. Table 2 gives the mean log of the MPN values for both the presumptive and confirmed tests for each method. The largest range between methods was 0.93 log unit as compared to 3.36 log units between laboratories.

Table 3 presents results of the analysis of variance (*3*). The levels of contamination, laboratories and tests (presumptive and confirmed) were examined for significant sources of variation. The level by laboratories, level by tests, laboratory by test and levels by laboratories by test interactions were also studied.

In every instance where data were available, there was significant variation due to the level of contamination. This is what one would expect to find, since the high-level samples received 10 times the inoculum received by the low level. But the inspection of the raw data did not in many cases indicate a clear difference.

There was significant variation between laboratories for all food products examined, as was apparent from Table 1. There was a significant variation between the presumptive and confirmed tests with frozen eggs, ice cream, frozen ground beef and meat meal. This would indicate that these samples constituted a problem with false-positive presumptives. This is of interest, since the same cultures were inoculated into all foods, suggesting that the food itself or its indigenous microflora affected the results.

The levels-by-laboratory interaction was significant for coliforms in the ground beef, meat meal, egg albumen,

TABLE 1. *Collaborative I: Comparison of laboratory means derived from analysis of inoculated samples of frozen and dried foods.*

Frozen peas - only one level: (95% confidence interval for a single log value = ± 1.19)														
Laboratory:	8	16	3	10	12	1	5	15	6	2	14	11	7	13
$\overline{X}$[a]	3.02	2.88	2.71	2.61	2.57	2.52	2.46	2.45	2.44	2.40	2.11	2.06	1.37	0.6
b														
Frozen eggs - only one level: (95% confidence interval for a single log value = ± 1.27)														
Laboratory:	13	7	2	12	5	16	10	8	15	11	14	3	1	6
$\overline{X}$	3.87	3.51	2.7	2.65	2.51	2.48	2.45	2.43	2.03	1.89	1.84	1.70	1.54	1.42
Ice cream - only one level; (95% confidence interval for a single log value = ± 0.80)														
Laboratory:	13	12	2	6	11	16	8	5	15	7	1	10	3	14
$\overline{X}$	3.78	3.74	3.69	3.60	3.60	3.60	3.59	3.48	3.45	3.48	3.40	3.26	2.96	2.92
Frozen ground beef - high level of contamination: (95% confidence interval for a single log value = ± 0.98)														
Laboratory:	16	7	12	5	11	14	2	8	10	3	13	6	1	15
$\overline{X}$	4.38	4.26	4.24	4.13	4.0	3.93	3.92	3.78	3.78	3.63	3.53	3.42	3.33	3.30
Frozen ground beef - low level of contamination:														
Laboratory:	13	7	5	14	16	11	10	12	3	6	2	15	8	1
$\overline{X}$	3.66	3.07	2.77	2.67	2.64	2.63	2.62	2.49	2.49	2.34	2.21	2.19	2.17	1.84
Meat meal - high level of contamination: (95% confidence interval for a single log value = ± 1.03)														
Laboratory:	4	7	12	9	3	14	10	6	8	2	11	5	1	16	13
$\overline{X}$	2.12	1.39	1.36	1.34	1.29	1.28	1.28	1.22	1.17	1.17	1.12	.98	.62	.58	.54
Meat meal - low level of contamination:														
Laboratory	10	8	7	9	3	11	2	4	6	5	13	12	1	14	16
$\overline{X}$	.57	.51	.40	.34	.25	.22	.21	.14	.11	.098	.024	- .019	- .072	- .256	- .319
Egg albumen - high level of contamination: (95% confidence interval for a single log value = ± 1.03)														
Laboratory:	2	1	3	6	4	7	5	11	9	8	10	16	12	13	14
$\overline{X}$	3.04	3.04	2.94	2.93	2.87	2.87	2.81	2.79	2.60	2.32	2.29	2.18	1.97	.492	- .068
Egg albumen - low level of contamination:														
Laboratory:	8	2	1	4	6	7	16	11	3	5	10	9	12	14	13
$\overline{X}$	2.89	1.74	1.65	1.64	1.54	1.52	1.51	1.44	1.36	1.23	1.0	.789	.543	.002	- .135
Coconut - high level of contamination: (95% confidence interval for a single log value = ± 1.02)														
Laboratory:	4	7	10	11	6	5	3	2	8	1	9	12	13	14
$\overline{X}$	2.84	2.79	2.68	2.65	2.64	2.55	2.48	2.46	2.40	2.31	1.99	1.79	1.54	1.25
Coconut - low level of contamination:														
Laboratory:	7	8	4	2	5	11	10	3	12	9	13	6	14	1
$\overline{X}$	1.48	1.33	1.31	1.06	.91	.83	.79	.69	.56	.55	.31	.28	.24	.11
Non-fat dry milk - high level of contamination: (95% confidence interval for a single log value = ± 1.32)														
Laboratory:	5	8	7	4	2	1	10	12	11	9	6	3	14	13
$\overline{X}$	3.57	3.10	2.76	2.54	2.49	2.44	2.44	2.41	2.31	2.26	2.18	2.10	.91	.21
Non-fat dry milk - low level of contamination:														
Laboratory:	4	5	3	11	1	7	8	2	12	6	10	9	14	13
$\overline{X}$	2.32	2.20	2.00	1.96	1.65	1.19	1.06	1.06	.71	.53	.26	- .012	- .29	- .51

[a]Means represent $\log_{10}$ of results by laboratory, combining values from LST, MAC and BGLB.
[b]Means not connected by a line are significantly different at the 5% level.

coconut and non-fat dried milk samples, indicating that the technique of the laboratory was affected by the level of contamination.

Collaborative II

Table 4 shows the comparison of the logarithms of the laboratory means for each sample and each type of test, viz. presumptive and confirmed. Also, where no significant differences between the types of tests were detected, the means across the three tests are given. Means not connected by a line are significantly different at the 5% level. Thus, on the presumptive test for peanut butter, Laboratory 1 was significantly lower than the other laboratories. Furthermore, Laboratories 1 and 5 were significantly lower than the others when the confirmed test for gas-positives was considered. Also, on

TABLE 2. *Collaborative I: Comparison of means for test methods derived from analysis of inoculated samples of frozen and dried foods.*[a]

Frozen peas	*Log*	Frozen eggs	*Log*
MAC-P	2.42	LST-P	2.75
BGLB-P	2.41	MAC-P	2.65
BGLB-E	2.37	MHC-EMB	2.52
BGLB-VRB	2.34	LST-EMB	2.40
MAC-EMB	2.32	LST-BGLB	2.36
LST-E	2.27	LST-E	2.32
LST-P	2.26	BGLB-P	2.22
LST-EMB	2.16	BGLB-VRB	2.00
LST-BGLB	2.10	BGLB-E	1.99

Ice cream	*Log*	Frozen ground beef (Both levels)	*Log*
MAC-P	3.77	MAC-P	3.41
LST-P	3.70	LST-P	3.25
BGLB-P	3.60	BGLB-P	3.23
MAC-EMB	3.57	MAC-EMB	3.20
LST-EMB	3.48	BGLB-VRB	3.19
LST-BGLB	3.44	LST-E	3.17
BGLB-VRB	3.37	BGLB-E	3.14
LST-E	3.19	LST-EMB	3.11
BGLB-E	3.05	LST-BGLB	3.06

Meat meal (High level)	*Log*	Meat meal (Low level)	*Log*
LST-P	1.33	LST-P	.74
MAC-P	1.30	MAC-P	.46
BGLB-P	1.21	BGLB-P	.35
LST-E	1.15	LST-E	.07
MAC-EMB	1.15	MAC-EMB	.05
LST-BGLB	1.09	LST-EMB	.02
BGLB-E	1.09	LST-BGLB	.01
LST-EMB	1.09	BGLB-VRB	-.18
BGLB-VRB	1.06	BGLB-E	-.19

Egg albumen (Both levels)	*Log*	Coconut (Both levels)	*Log*
BGLB-P	1.84	LST-P	1.63
LST-P	1.81	LST-BGLB	1.63
BGLB-E	1.81	LST-E	1.61
BGLB-VRB	1.81	LST-EMB	1.60
LST-BGLB	1.79	BGLB-P	1.53
LST-EMB	1.79	MAC-P	1.48
LST-E	1.79	BGLB-VRB	1.47
MAC-P	1.78	BGLB-E	1.43
MAC-EMB	1.73	MAC-EMB	1.38

Non-fat dry milk (Both levels)	*Log*
BGLB-P	1.70
MAC-P	1.69
MAC-EMB	1.67
LST-P	1.63
LST-EMB	1.62
LST-BGLB	1.62
LST-E	1.61
BGLB-VRB	1.61
BGLB-EMB	1.59

[a]Key: LST-P (Method 1 - presumptive).
LST-BGLB (Method 1 - confirmed).
LST-EMB (Method 1 - confirmed).
LST-E (Method 1 - confirmed).
BGLB-P (Method 2 - presumptive).
BGLB-E (Method 2 - confirmed).
BGLB-VRB (Method 2 - confirmed).
MAC-P (Method 3 - presumptive).
MAC-EMB (Method 3 - confirmed).

the confirmed test including gas-negatives, Laboratory 5 was significantly lower than all the others, and Laboratory 1 was significantly lower than Laboratories 2 and 3. Note that Laboratory 5 had the highest presumptive mean but was lowest on the confirmed test. In regard to precision in the analysis of peanut butter,

TABLE 3. *Collaborative I: Summary of the Analyses of Variance of MPN results.*

I. Sources of variation tested for significance:

A. Levels of inoculation
B. Laboratories
C. Tests - between presumptive and confirmed
D. Levels × laboratories
E. Levels × tests
F. Laboratories × tests
G. Levels × laboratories × tests

II. Significant sources of variation:

A. Frozen peas (only one level)
Presumptive/confirmed coliforms
Laboratories***
B. Frozen eggs (only one level)
Presumptive/confirmed
Laboratories**
Tests**
C. Ice cream (only one level)
Presumptive/confirmed coliforms
Laboratories**
Tests**
D. Frozen ground beef
Presumptive/confirmed coliforms
Levels**
Laboratories**
Tests**
Levels × laboratories**
Laboratories × tests*
E. Meat Meal
Presumptive/confirmed coliforms
Levels**
Laboratories*
Tests*
Levels × laboratories*
Levels × tests*
F. Egg albumen
Presumptive/confirmed coliforms
Levels**
Laboratories**
Levels × laboratories*
G. Coconut
Presumptive/confirmed coliforms
Levels**
Laboratories**
Levels × laboratories*
Levels × tests*
H. Non-fat dry milk
Presumptive/confirmed coliforms
Levels**
Laboratories**
Levels × laboratories**

*Significant at the 5% level
**Significant at the 1% level
***Significant at the 0.1% level

the 95% confidence limit for a single log value was ± 0.88 log unit. The position of Laboratory 5 in the confirmed test rank indicates a lack of experience in interpreting the colonies on the confirmatory agar, as can be seen for both the peanut butter and buttermilk.

A comparison among the laboratory means from the buttermilk indicates that no significant differences were observed between laboratories, even though a range of 0.693 log unit was observed. On the confirmed tests including gas-negatives and -positives, Laboratory 5 was again significantly lower than all other laboratories. Precision for the buttermilk samples at the 95% confidence limit was ± 1.03 log units.

Comparison of laboratory means for egg albumen shows that Laboratory 2 was significantly lower than the others for the presumptive test and that Laboratory 5

TABLE 4. *Collaborative II: Comparisons among the logs of the laboratory means for each sample and each type of test.*[a]

I. Peanut Butter

A. Presumptive test

Laboratory:	1	2	3	4	5
$\overline{X}$	1.123	1.478	1.670	1.702	1.758

B. Confirmed test - gas positives

Laboratory:	5	1	4	2	3
$\overline{X}$	.566	1.006	1.407	1.408	1.495

C. Confirmed - all tubes

Laboratory:	5	1	4	3	2
$\overline{X}$	.566	1.187	1.412	1.519	1.611

D. 95% Confidence interval for a single log value = ± 0.88 log unit.

II. Buttermilk

A. Presumptive

Laboratory:	2	1	5	4	3
$\overline{X}$	.446	.737	.759	.935	1.139

B. Confirmed - gas positives

Laboratory:	5	2	1	4	3
$\overline{X}$	- .287	.249	.493	.809	1.139

C. Confirmed - all tubes

Laboratory:	5	1	2	4	3
$\overline{X}$	- .285	.762	.753	.839	1.146

D. 95% confidence interval for a single log value = ± 1.03 log units.

III. Egg albumen

A. Presumptive and both confirmed tests[b]

Laboratory:	2	4	5	3	1
$\overline{X}$	.724	.917	.991	1.177	1.180

B. Media:

	BGLB	LST	MAC
	.918	.958	1.117

C. 95% confidence interval for a single log value = ± 0.87 log unit.

[a]Means not connected by a line are significantly different.
[b]There were no significant differences between presumptive and confirmed results; $\overline{X}$ represents value for all methods and all tests.

was significantly lower than 1 or 3. There was no significant difference between the presumptive and confirmed test for egg albumen samples. The 95% confidence limit for a single log value was ± 0.87.

While there were no significant differences among the three media for peanut butter and buttermilk, egg albumen enriched in MAC yielded significantly lower values on the presumptive tests than LST or BGLB, indicating a greater selectivity of MAC in this test, since as previously indicated the three confirmed tests showed no significant differences.

The confirmation of gas-negative tubes did not yield significantly higher results than confirmation of gas-positive tubes alone.

Despite more careful experimental design than in Collaborative Test I, including more replication and more extensive mixing of the samples, the observed 95% confidence intervals for a single log value reported from the two studies are comparable. For example, the 95% confidence interval for a log value for egg albumen in Collaborative II was ± 0.87 as compared to ± 1.03 for Collaborative I. On the other hand, more replication was done by each laboratory for Collaborative II and hence, a smaller difference between laboratory means was required for significance. For example, for egg albumen in Collaborative II a difference between laboratory means of 0.19 was required to be significant at the 5% level, while in Collaborative I a difference of 0.60 was required to be significant. Thus, of the two studies, Collaborative II can be said to be more sensitive to detecting differences between laboratories. The sources of variation and their significance for this test series are summarized in Table 5. Variation due to laboratories and tests was significant as well as the laboratory-by-media interaction for peanut butter. Media were significant sources of variation with egg albumen, as has been discussed above.

The partition of experimental error was done on the ANOVA basis among replicate aliquots, subsamples and analysts. These results are seen in Table 6. For all three foods, error due to replicate aliquots of the product was greater than either the subsample or analyst error. This was true despite the thorough mixing of the sample. For example, with peanut butter 96% of the error came from replicate aliquots.

DISCUSSION

The lack of precision of MPN estimates of bacterial densities is recognized, although those who interpret results often assume precision that does not exist. MPN tables in *Standard Methods for the Examination of Water and Wastewater* (2) show 95% confidence limits for 5- and 3-tube MPN determinations. The values are based upon the fact that the logarithms of MPN

TABLE 5. *Collaborative II: Summary of Analyses of Variance of MPN results.*

I. Sources of variation studied
- A. Laboratories (L)
- B. Media (M)
- C. Tests - presumptive and confirmed (T)
- D. L × M
- E. L × T
- F. M × T
- G. L × M × T
- H. Analysts
- I. Subsamples
- J. Replicates

II. Significant sources of variation
- A. Peanut Butter
  1. Laboratories***
  2. Tests***
  3. L × T***
- B. Buttermilk
  1. Laboratories***
  2. Tests**
  3. L × T**
- C. Egg albumen
  1. Laboratories***
  2. Media***

**Significant at the 1% level
***Significant at the 0.1% level

TABLE 6. *Collaborative II: Percent contribution of source of error.*[a]

Sample type	Replicate aliquots	Subsamples	Analysts
Peanut butter	96.0	2.0	2.0
Buttermilk	62.0	11.0	27.0
Egg albumen	78.0	17.0	5.0

[a]Summary showing percent contribution of source of experimental error within individual laboratories in ICMSF Coliform MPN collaborative study on naturally contaminated low moisture food samples.

estimates for a 10-tube multiple dilution test at a given bacterial density are approximately normally distributed with a mean close to the logarithm of the given bacterial density and with a standard deviation which does not vary greatly with the bacterial density (*3*). It has been verified that the 3-tube and 5-tube MPN estimates are approximately logarithmically normal, and the values for the 95% confidence limits are based on the logarithmically normal assumption. Woodward (*5*) indicates that the 95% confidence limit for a single determination, using three tubes at each dilution, covers a range of approximately 1.27 log cycles.

The design of Collaborative Study II permitted calculation of the 95% confidence intervals for a single log value. The values for peanut butter, buttermilk and egg albumen were respectively ± 0.88, ± 1.03 and ± 0.87, indicating ranges of 1.76, 2.06 and 1.74. Obviously the 95% confidence intervals for these three products are considerably broader than would be expected on the basis of the 95% confidence intervals given in the standard MPN tables. The average range for the three products was 1.85 and the average 95% confidence interval for a single log value would be ± 0.925.

These findings are of considerable importance relative to microbiological specifications for foods, involving the use of the MPN procedure. Let us consider a specification of no greater than 10 coliforms per gram using a 3-tube MPN procedure. Based upon the MPN tables, if the true level of coliforms in the sample were 10, then one would be 95% confident on the basis of a single determination that the logarithm of the observed MPN would be no more than ± 0.535 i.e., the log of the observed value would be below 1.535. Stated differently, one is 95% confident that the observed value would be no greater than 34. Now, considering the results from Collaborative Study II wherein the average 95% confidence interval for a single log value was ± 0.925, a much broader range is expected, with the upper limit being 60. Thus in administering a specification of no greater than 10 coliforms per gram, based on published confidence limits, one should accept product showing coliform levels as high as 34 per gram, but the observed variability would suggest that one ought to accept product with almost twice that level. Precision can be improved by analyzing replicate aliquots. In general, the 95% confidence limit for a single value reported by a given laboratory is ± 1 log unit and the corresponding 95% confidence limit for a mean of five replicates would be ± 0.45 log unit.

Unfortunately, microbiological specifications for foods seldom take into account the limitations of the analytical procedures required to monitor the specifications. In the future, it would be desirable if microbiological specifications carried with them a statement of the precision of the analytical method employed to monitor them. This would serve as a guide to those responsible for administering such specifications.

The 95% confidence intervals for a single determination calculated from Collaborative Studies I and II were comparable. However, comparison of Tables 1 and 4 indicate that the range of the mean values of the laboratories in Collaborative I was considerably greater than in Collaborative II. This may reflect more thorough mixing of the samples used in Collaborative II as well as increased replication. Furthermore, in Collaborative I the samples were artificially inoculated, whereas in Collaborative II the three samples were naturally contaminated with coliforms. Artificial inoculation may have confounded the problem of mixing.

Significant variation between laboratories was noted in both collaborative studies. The data summarized in Table 6 indicate that with respect to variation within a given laboratory, variation between replicate aliquots is overwhelmingly the most important source of error. Since variation attributed to subsamples was relatively small, one can assume that exhaustive mixing before preparation of subsamples for distribution to the collaborating laboratories was effective in reducing subsample variation. Such thorough mixing is virtually never done in routine preparation of samples for analysis. Though we have not conducted specific studies on the variation in replicate aliquots of unmixed samples, it seems logical that in practice the variation would be even greater than was observed in the thoroughly mixed samples analyzed in Collaborative II.

Accordingly, if exhaustive mixing of a "captive" sample results in a 95% confidence interval in the range of ± 1 log unit for an individual result, then the variability inherent in obtaining a representative sample from the field would be expected to be even greater. Thus two problems of considerable magnitude confound administration of microbiological specifications for foods. The first relates to the difficulty in obtaining a sample which is truly representative of the lot in question and the second relates to the uneven distribution of microorganisms within the sample unit presented to the laboratory for analysis. The 95% confidence limits appearing in MPN tables do not contemplate either of these problems. It is doubtful that the precision observed in Collaborative II is ever achieved in the routine analysis of food products.

The results of the two collaborative studies indicate that differences between the three methods are relatively small when compared to the differences between laboratories using the same methods and following a common protocol. Consequently, at this time there is no basis for selecting any one of the three procedures published in Thatcher and Clark (*4*) as the "best" method.

ACKNOWLEDGMENTS

We thank ARS-ERRC of USDA for direct financial assistance. We also thank Drs. D. S. Clark, G. Mocquot, J. C. Olson, Jr., H. Pivnick and Dr. M. van Schothorst for helpful review and criticism of this manuscript.

We thank the following collaborators and their staffs for their considerable contribution: C. Comminazzini, Provincial Laboratory of Hygiene and Prophylaxis, Novara, Italy; O. Emberger, Charles University of Prague, Prague, Czechoslovakia; R. Ferguson, The Pillsbury Company, Minneapolis, Minnesota; R. W. Hill, Health and Welfare Canada, Toronto, Ontario; B. C. Hobbs, Food Hygiene Reference Laboratory, London, England; E. H. Kampelmacher, Rijks Institut voor de Volksgezondheid, Bilthoven, The Netherlands; A. G. Kitchell, Agricultural Research Council, Meat Research Institute, Langford, England; H. V. Leininger, U. S. Food and Drug Administration, Minneapolis, Minnesota; A. Loit, Health and Welfare Canada, Montreal, Quebec; H. Lundbeck, National Bacteriological Laboratory, Stockholm, Sweden; W. R. Mason, General Foods, Ltd., Toronto, Ontario; G. Mocquot, Central Station for Dairy Research and Technology of Animal Products, CNRZ, Jouy-en-Josas, France; C. F. Niven, Jr., Del Monte Corporation, Walnut Creek, California; L. Ormay, Institute of Nutrition, Budapest, Hungary; M. A. Rata, Universidad Nacional, Mayor de San Marcos, Lima, Peru; B. Simonsen, Danish Meat Products Laboratory, Copenhagen, Denmark and E. Todd, Health and Welfare Canada, Ottawa, Ontario.

Finally, we thank the following food companies and agencies for providing general financial support to ICMSF and its methods-testing program:

Amatil Ltd., (Australia); American Can Co., (U.S.A.); Arnott's Biscuits Ltd., (Australia); Bacon and Meat Manufacturers' Assoc., (U.K.); Beatrice Foods Co., (U.S.A.); Beecham Group Ltd., (U.K.); Brooke-Bond Oxo Ltd., (U. K.); Brown and Polson Ltd., (U.K.); Burns Food Ltd., (Canada); Cadbury Schweppes Ltd., (Australia); Campbell Soup Co. Ltd., (Canada); Canada Packers Ltd., Carlo Erba Institute for Therapeutic Research, (Italy); Central Alberta Dairy Pool, (Canada); Centro Studi sull' Alimentazione Gino Alfonso Sada, (Italy); Christie Brown and Co. Ltd., (Canada); Coca-Cola Co., (U.S.A.); C.P.C. International Inc., (U.S.A.); C.S.R. Ltd., (Australia); Del Monte Corp., (U.S.A.); Difco Laboratories (U.S.A.); Distillers Co. Ltd., (U.K.); Findus Ltd., (Sweden); Geinoca Food Service Ltd., (Canada); General Foods Corporation, (U.S.A.); General Foods Ltd., (Canada); Gerber Products Co., (U.S.A.); H. J. Heinz Co. Ltd., (U.K.); Horne and Pitfield Foods Ltd., (Canada); Infant Formula Council, (U.S.A.); International Union of Biological Societies; I.T.T. Continental Baking Co., (U.S.A.); J. Sainsbury Ltd., (U.K.); Jannock Corp. Ltd., (Canada); John Labatt Ltd., (Canada); Kellogg/Salada Ltd., (Canada); Kraft Foods Ltd., (Australia); Langnese-Iglo GmbH, (B.R.D.); Maizena Gesellschaft mbH, (B.R.D.); Maple Leaf Mills Ltd., (Canada); Marks and Spencer Ltd., (U.K.); Mars Ltd., (U.K.); McCormick and Co. Inc., (U.S.A.); Milk Marketing Board, (U.K.); National Fisheries Institute Inc., (U.S.A.); New Zealand Manufacturers' Federation; Plasmon Ltd. (Italy); R.H.M. Research Ltd., (U.K.); R.J.R. Foods Inc., (U.S.A.); Reckitt and Colman Ltd., (U.K.); Ross Laboratories, (U.S.A.); Spillers Ltd., (U.K.); Standard Brands Ltd., (Canada); Swift Canadian Co. Ltd., (Canada); Tate and Lyle Refineries Ltd., (U.K.); Terme de Crodo, (Italy); Tesco Stores Ltd., (U.K.); The Borden Co. Ltd., (Canada); The J. Lyons Groups of Companies, (U.K.); The Pillsbury Co., (U.S.A.); The Quaker Oats Co. Ltd., (Canada); The Quaker Oats Co., (U.S.A.); Thomas J. Lipton, Inc., (U.S.A.); Unigate Australia Ltd.; Unilever Ltd., (U.K.); Wattie Industries Ltd., (N.Z.); World Health Organization.

REFERENCES

1. American Public Health Association. 1966. Recommended methods for the microbiological examination of foods. Edited by J. M. Scharf. Washington, D.C. pp. 131-138.
2. American Public Health Association. 1971. Standard methods for the examination of water and wastewater. 13th ed. Washington, D.C. pp. 673-676.
3. Eisenhart, C., and P. W. Wilson. 1943. Statistical methods and control in bacteriology. Bacteriol. Rev. 7:57-137.
4. Thatcher, F. S., and D. S. Clark (eds.) 1968. Microorganisms in foods: their significance and methods of enumeration. University of Toronto Press, Toronto, Ontario. pp. 59-82.
5. Woodward, R. L. 1957. How probable is the Most Probable Number? J. Amer. Water Works Assoc. 49:1060-1068.

Journal of Food Protection Vol. 42, No.8, Pages 645-650 (August, 1979)

Soil Retention and Bacterial Harborage on Simulated Pewter Dinnerware

CLARENCE A. JOHNSON* and DARLENE A. LEWIS

Capsule Testing Laboratories, Division of Economics Laboratory, Inc., Osborn Building, St. Paul, Minnesota 55102

(Received for publication November 8, 1978)

ABSTRACT

Soil retention and harborage of *Staphylococcus aureus* ATCC 25923 were investigated comparing stainless steel, plastic dinnerware, sand-cast aluminum dinnerware with two different surface finishes and aluminum dinnerware after prolonged restaurant use. The test consisted of ten repetitions of soiling with inoculated food soil, air drying, then washing at either 160 F or 130 F and rinsing at 180 F before analysis. Samples were analyzed by swabbing one-half of the surface with swabs in 0.1 N HCl with subsequent atomic absorption spectroscopy to detect residual calcium left from food soil, and swabbing the other half with swabs in buffered distilled water and Triton-X 100 followed by pour-plating to recover *S. aureus* harbored on the surface. Statistical analysis of the data indicated that if detergent and dishmachine manufacturers' recommendations are carefully carried out, no significant difference in soil retention or bacterial harborage could be revealed between the various test materials or between wash solution temperatures.

Reports implicating contaminated dishware as potential fomites in transmitting disease date back to the early 20th century. Glassware has been implicated in reports by Kupchik and Katz (*8*) and Lynch and Cummings (*9*) dating back to 1907 and 1919, respectively. More recently, the Food and Drug Administration (FDA), at the request of the U.S. Comptroller General, surveyed 185 randomly selected restaurants in the United States. In a report released in 1975 (*4*), the agency found that 90% of the restaurants surveyed were "unsanitary"[1] and that 54% had inadequate facilities for washing and sanitizing equipment and utensils. The agency also suggested that "equipment and utensils that are not thoroughly cleaned, sanitized and maintained in good repair can harbor accumulations of food and other residues that support harmful bacterial growth which may be transmitted to customers and employees". In 1972, Jopke et al. (*7*) reported finding high total numbers of bacteria on hospital tableware and concluded that the contamination immediately upon washing could be correlated to low wash-water temperature during wash and rinse cycles. They also pointed out, however, that most significant contamination resulted from storage and handling after machine washing. Finally, they note that the 30-colonies-per-area-tested criterion established in the American Hospital Association's Food Service Manual for Health Care Institutions seems an easily attainable standard; however, unnecessary handling, improper storage and inadequate dishwashing practices increase the risk of contamination and reduce sanitation effectiveness. All the results clearly indicate the need to assess potential public health hazards in evaluating tableware sanitation.

Several reports (*3,9,12,15*) refer to the microbial harboring effects of alkaline-earth type films produced by detergent, water hardness constituents and soil interactions; however, few quantitative data are available which clearly indicate this concern. Schneider et al. (*16*) used a model system to investigate the harboring effect of calcium and magnesium films on *Bacillus subtilis* spores. They noted that a direct relationship existed between calcium deposition (above a threshold of 3 μg Ca^{++}/cm^2) and *B. subtilis* spore harborage. Another study conducted by Maxcy (*12*) indicated that residual soil to a level of less than 1 μg/ml of volume of milk equipment (pipelines, fillers and the like) was sufficient to support bacterial growth on even well-cleaned surfaces that were not visually soiled. Heinz et al. (*6*) also noted calcium salts were a major component of milk soils, and acid recovery of quantities as small as $\geq$ 0.11 mg/100 cm^2 was indicative of a soiled surface.

This study was designed to determine potential public health significance of the possibility of bacterial harborage and soil retention on sand-cast aluminum tableware commonly used as simulated pewter in foodservice establishments. To achieve this end, the material was evaluated as follows: (*a*) cleaning and comparing to a stainless steel standard, (*b*) cleaning and comparing to new, unused tableware and similar tableware after prolonged use in a normal restaurant operation, (*c*) evaluating the effect of surface finish in relation to cleaning and sanitation effectiveness, (*d*) comparison of sanitation of plastic reusable dinner plates and the aluminum product, and (*e*) to determine the effect of wash solution temperature on soil-film buildup and microbial load.

MATERIALS AND METHODS

Test materials

Five test-material variables were used in this investigation. Ten aluminum dinner plates were chosen randomly from a production warehouse in Syracuse, New York. This product was finished by peening with granite at a density of 85 lb./ft.^{3} This finish is designated as N-1 for test purposes (Table 1). An additional 10 samples were finished by a modification of surface peening to work-harden the surface and close small pits resulting from the sand cast. Steel at a bulk density of 300 lb./ft^3 was used in the modified peening procedure. This surface finish is designated N-2.

Comparable pieces of sand-cast aluminum dinnerware were selected from a Washington, D.C. restaurant after prolonged use. Ten of these samples were collected and were used to determine the effect of

[1]*Note that "unsanitary" in this reference meant more than unclean tableware; included were rodent or insect infestation, poor housekeeping, etc.*

TABLE 1. *Test material code designations.*

Code	Key
SS	Stainless steel reference blank (304 Blend, No. 4 finish)
N-1	Aluminum dinner plate — in-line surface finish
N-2	Aluminum dinner plate — experimental surface peening
U	Used aluminum dinner plate
Plx	Plastic NSF[a] approved dinner plate

[a]NSF = National Sanitation Foundation.

prolonged normal use on bacterial harborage and soil retention. These samples were finished with the N-1 method and were designated as U to denote their used condition. The eating surface on all aluminum dinnerware was 40 in.² (260 cm²).

Ten reusable plastic dinner plates commercially available to the foodservice industry, each with a 40-in.² (260 cm²) face were designated as Plx for test purposes. Ten stainless steel blanks (304 Blend, No. 4 Finish) were used as reference for cleanability and bacterial harborage. These stainless steel blanks had an area of 54 in² (348 cm²); however, only 40 in.² (260 cm²) were used in the test protocol.

Each test material was coded (example: N-1 numbers 1-10, N-2 numbers 1-10, etc.) and three samples of each series were randomly selected using a table of random numbers for each individual dishmachine rack (see below for soil/wash procedure). This random selection gave each dishmachine rack a total of 15 samples (three from each series of test materials) to be subjected to 10 soil/wash cycles before analysis. After each series of 10 soil/wash cycles and subsequent analysis, samples were returned to the test variable series and another rack of 15 samples was again randomly selected from all 10 samples in the series.

Dishmachine specifications

A Hobart Model 8 batch-type dishmachine with a mercury-actuated, dial-type thermometer was used for the test. This machine is classified as a door-type, single stationary rack design. One complete cycle consisted of a 45-sec wash at either 160 F (71 C) or 130 F (55 C), 5-sec pause, and 12-sec rinse at 180 F (82 C). Racks used for this machine held 16 pieces of dinnerware; however, only 15 pieces were tested in each rack. The location of the vacant space was not considered significant.

Water and detergent formulation

Water used in this test was Economics Laboratory well-water at 16 grains hardness (as $CaCO_3$). A standard, commercially available detergent developed for use on aluminum pewter-type products was employed. The level of detergent use was 0.2%, manufacturer's recommended use level for water of this hardness. Detergent and/or water hardness conditions were not evaluated in this test; consequently, the detergent levels established by the manufacturers were not varied. The detergent formulation used in this analysis was 30% tripolyphosphate (granular) (added to chelate calcium and magnesium ions), 1% D-500 (a proprietary defoaming agent), 15% sodium carbonate (to add alkalinity), 4% CBD-56 (an organic chlorine source added as a destaining agent), and 50% sodium metasilicate (anhydrous) (to add alkalinity and protect metal surfaces).

Food soil

Research has indicated that food-soil and soil-film buildup is probably a function of calcium and magnesium salt interaction with protein and lipoprotein moieties of food (*14*). In this study, then, food soil was developed to simulate commonly encountered restaurant waste and provide sources of calcium and magnesium ions, protein, lipoprotein, animal fat, and carbohydrates. To achieve this end, equal weights of the following ingredients were mixed, warmed to 100 F (38 C), and held in a liquid state at 100 F (38 C) until used: (*a*) pasteurized process cheese, (*b*) prepared liquid beef gravy (canned), and (*c*) animal lard.

All products used to prepare soil are commercially available. Stock soil was prepared fresh each day. From the stock, 75 g were weighed into a beaker for inoculation with test culture (see below). Following inoculation and thorough mixing, 5-g aliquots were deposited on each test sample and spread to an even film with the aid of a sanitized rubber policeman. Soil was allowed to air dry for 15 to 20 min at 80 F (27 C) before test articles were loaded on racks for washing.

Test organism and inoculum

The test organism was *Staphylococcus aureus* ATCC #25923. The stock culture was prepared from a dehydrated paper disc inoculated with test organisms (Bacto Disc, Difco). These were rehydrated in 100 ml of Tryptic Soy Broth (TSB) (Gibco Catalog No. M49800). This culture was grown 24 h at 35 C, then transferred by loop to 100 ml of sterile TSB. After 24 h of incubation at 35 C, the broth culture was enumerated by pour-plating appropriate dilutions with Tryptic Soy Agar (TSA) (Gibco Catalog No. M49600). The remaining broth culture was used as the test inoculum. Tryptic Soy Agar plates were incubated 24 h at 35 C for enumeration as colony forming units (CFU) per ml of test culture. *S. aureus* test culture was added to the soil at a concentration of 0.1 ml per gram, yielding 0.5 ml of culture per dinner plate soiled (i.e. 0.1 ml of culture per gram soil × 5 g soil per dinner plate). This concentration was sufficient to provide each plate with a soiled inoculum of 10^6 to 10^7 organisms.

Test procedures and analysis

From each set of test materials, three samples were randomly selected for each replicate. A replicate consisted of a 15-sample dish rack repeatedly soiled (with inoculated soil), dried and washed 10 times before analysis. This 10-soil-wash cycle was instituted to allow buildup of a soil matrix necessary for harboring bacterial contaminants. Additionally, a positive control sample was included on the tenth cycle of each replicate. This control was a stainless steel blank selected at random from the seven remaining samples in the series. The control was soiled, dried and then analyzed using the procedures described below to provide background data for calcium and bacterial levels of soiled products. Between each of the 10 wash cycles, the dishmachine was drained and recharged with fresh water and detergent. Each replicate was at either 160 or 130-F wash temperature; however, the 180-F rinse was used throughout. Six replicates (consisting of 10 repeated soil-dry-wash cycles) were examined, producing 60 washes and 90 samples at each wash solution temperature.

After 10 cycles, the test materials were immediately brought to the laboratory for analysis. Calcium residues were determined by swabbing one-half of the plate face (130 cm²) with a disposable swab submersed in 5 ml of 0.1 N HCl. Swabs were taken covering the entire 130-cm² area in each of three directions, rinsing the swab head in the solution between direction changes. The HCl-solution was analyzed for calcium residues in a Perkin-Elmer 403 double beam atomic absorption spectrometer using a procedure described in *Standard Methods for the Examination of Water and Wastewater* (*1*). Atomic absorption values in ppm were converted to $\mu g\ Ca^{++}$ per unit area by the following calculation:

$$\mu g\ Ca^{++} \text{ per } 130\ cm^2 = Ca^{++}\ \mu g/ml \text{ (or ppm)} \times 5\ ml$$

Background levels of calcium were determined on each test material by thorough washing with phosphoric acid to remove residual film that may have been present followed by the acid swab procedure and atomic absorption analysis. In addition, the effect of water-hardness film-deposition was assessed by thorough acid washing of 15 samples randomly selected from all test materials, machine washing through 10 consecutive wash cycles with 0.2% detergent added (no soil on materials), then analyzing for residual calcium using the outlined procedure.

Microbiological analysis was done on one-half of each plate face (130 cm² opposite that used for calcium determination) following the 10-wash sequence. Plates were tested by swabbing with a sterile disposable swab covering the entire 130-cm² area in each of three directions. Before swabbing and between direction changes, the swab was rinsed in 5 ml of sterile distilled water containing a 0.1% phosphate buffer and 0.2% Triton-X 100 added as a surfactant to help remove soil and dislodge organisms from the surface of the plate. Upon completion of the swabbing procedure, the swabs were placed in the tube containing the buffered distilled water, shaken on a vortex shaker,

and the entire 5-ml amount was plated using a pour-plate procedure. Organisms were grown in TSA and incubated 24 h at 35 C before enumerating. Positive controls, soiled on the tenth wash, were allowed to dry, then swabbed for calcium and bacteria in the soiled state. Swabs were analyzed according to the above procedures for calcium determination and total microbiological count except that appropriate dilutions of the 5-ml swab transfer media were plated to get the microbiological estimates.

Statistical analysis of results

A non-parametric 'sign' test was used to analyze the microbiological data. The sign test was performed for all possible combinations of two plate types (i.e. Plx versus U, SS versus N-1, etc.) to determine a comparison of microbiological cleanliness due to type of test material. In addition, the data were evaluated by this test to reveal differences due to temperature of wash solution.

Calcium residuals were analyzed by calculating arithmetic means for calcium residues on all test materials in a dishmachine rack and comparing these results to 95% confidence limits about the means established for controls (background controls on acid washed surfaces and controls used to determine the effect of water hardness on calcium film deposition).

RESULTS

Cleanliness determined by bacterial analysis

A 10^8 to 10^9 CFU-per ml culture of *S. aureus* was added to prepared food soil sufficient to inoculate each test article with 10^6 to 10^7 CFU per 130 cm². To establish a soil matrix, test articles were repeatedly soiled, dried and machine-washed 10 times before analysis, with dishmachine wash temperatures of 160 or 130 F and sanitizing rinse of 180 F. A summary of results of six trials at the 160-F wash solution temperature (representing 60 washes and 90 samples) is given in Table 2. Results of these tests indicate that washing and sanitizing under these conditions will yield a reduction in total microbial load from an initial 7.5×10^6 inoculum to 10^{-1} to 10^0 CFU per 130 cm² (6 to 7 log cycle reductions). Sixty-seven percent (10 of 15 samples of test material) averaged less than 1 CFU per utensil. Additionally, averages of all test materials indicated that all surfaces could be consistently cleaned bacteriologically to a level of less than 10 CFU per 130 cm². Ranges determined for all test materials were small (less than 1 to 60 CFU per 130 cm²), indicating that variability in bacterial cleanliness within test groups (i.e. SS versus SS, N-1 versus N-1, etc.) was slight. Even at the maximum level of contamination (60 CFU per 130 cm²), all six sets of test materials produced results well within established guidelines of less than 12.5 CFU per inch² (approximately 2 CFU per cm²) (*5*).

TABLE 2. *Effect of washing at 160 F on recovery of* S. aureus *after ten soil/wash cycles.*

Material code	6-Trial average CFU/130 cm²[a] (arithmatic mean)	6-Trial average CFU/130 cm² (log)	Test mat'l. average CFU/130 cm²	Range CFU/ 130 cm²
SS	< 1	< 0		
SS	0.67	− 0.17	0.3	< 1 - 2
SS	0.17	− 0.77		
Plx	1.67	0.22		
Plx	0.17	− 0.77	0.8	< 1 - 8
Plx	0.17	− 0.77		
N-1	0.67	− 0.17		
N-1	12.17	1.08	5.0	< 1 - 60
N-1	2.17	0.33		
N-2	1.00	0		
N-2	0.50	− 0.30	0.6	< 1 - 3
N-2	0.17	− 0.77		
U	4.50	0.65		
U	0.60	0.22	1.7	< 1 - 23
U	0.17	− 0.77		
Positive control:	7.5×10^6	6.88	$2.3 \times 10^6 - 2.0 \times 10^7$	

[a]To be significant from a public health standpoint, ⩾ 12.5 CFU per in.² (approximately 2.0/cm²) is necessary (*5*).

Statistical evaluation of the results are displayed in Table 3. Results of bacterial recovery were found to be non-normal, even after conversion to $\log_{10}$. Data showed a strong skewing effect on the low end (log less than 0 was routinely found in all test materials), making it impossible to analyze with common comparisons of means, such as the Student's T-test. For this reason, the non-parametric sign test was chosen for statistical analysis of all bacterial results. In this evaluation, bacterial counts for any two particular types of plates obtained under similar test conditions were paired. A "positive" difference means that the second-named plate type in the comparison gave the higher bacterial count. A "negative" difference means that the first-named plate type gave the higher count. Zero-difference pairs are discarded in this test. A significant difference (at $a = 0.10$) requires that the smaller of the two numbers (positive and/or negative) be less than or equal to the critical number (table value). None of the comparisons in Table 3 meet this criterion; consequently, no results are

TABLE 3. *Paired comparisons of residual bacterial counts on plates via the "sign test": Comparison within wash temperatures of 130 and 160 F.*[a]

Comparison	130 F			160 F		
	+	−	Critical No.	+	−	Critical No.
SS versus P	6	3	1	4	4	1
N-1	6	3	1	7	3	1
N-2	7	4	2	5	3	1
U	3	3	0	8	4	2
P versus N-1	5	5	1	6	4	1
N-2	5	5	1	4	3	0
U	3	7	1	7	4	2
N-1 versus N-2	7	5	2	4	7	2
U	4	4	1	5	5	1
N-2 versus U	3	7	1	6	6	2

[a]To be significant at $a = 0.10$, the smallest of the two numbers (positive or negative) must be ⩽ the critical number.

significantly different. We can say from this evaluation that no statistically significant differences were found between any of the plate-type pairs; subsequently, variation in the bacterial cleanliness of these test materials was not indicated in this experiment.

Decreasing the wash solution temperature to 130 F had a slight effect on bacterial retention. A summary of results, shown in Table 4, indicates a reduction in bacterial load from an average inoculum of 3.4×10^7 to less than 1 to 100 CFU per 130 cm², a reduction of 5 to 7 log cycles. One sample in the N-1 series had a bacterial count of 118 CFU per 130 cm² — the highest recovered at either wash solution temperature. The count of 118 CFU per 130 cm² (or approximately 0.9 per cm²) remains well within the established guidelines of approximately 2.0 per cm². The range of results at this wash solution temperature was somewhat wider than that noted for the 160-F wash temperature; however, the less than 1 to 118 CFU per 130 cm² overall range does not exhibit wide variability within or between test materials.

TABLE 4. *Effect of washing at 130 F on recovery of* S. aureus *after ten soil/wash cycles.*

Material code	6-Trial average CFU/130 cm² (arithmatic mean)	6-Trial average CFU/130 cm² (log)	Test mat'l. average CFU/130 cm²	Range CFU/ 130 cm²
SS	2.17	0.34		
SS	0.50	−0.30	2.5	< 1 - 10
SS	0.67	−0.17		
Plx	6.33	0.80		
Plx	1.00	0	3.3	< 1 - 33
Plx	2.67	0.43		
N-1	0.33	−0.48		
N-1	21.80	1.34	7.6	< 1 - 118
N-1	0.50	−0.30		
N-2	3.80	0.58		
N-2	1.17	0.06	2.5	< 10 - 11
N-2	2.67	0.43		
U	17.17	1.23		
U	< 1	< 0	6.3	< 1 - 95
U	1.83	0.26		
Positive control:	3.4×10^7	7.53	1.9×10^7 — 4.5×10^7	

[a]To be significant from a public health standpoint, ⩾ 12.5 CFU per in.² (approximately 2.0/cm²) is necessary (5).

Because of the skewing effect with either arithmetic or log means, the sign test was again the analysis of choice in evaluation of these results. Again, no significant difference in bacterial retention between test materials was evident (Table 3). Using this same test statistic to compare test materials between wash solution temperatures (Table 5), it was noted that no significant difference existed. These results point out that: (*a*) no difference in bacterial retention existed between various surfaces at a given wash temperature, and (*b*) lowering the wash temperature did not produce significant bacterial retention when all other parameters (such as detergent concentration and wash cycle time) were constant and performed correctly.

TABLE 5. *Paired comparison of residual bacterial counts on plates via the "sign test": Comparison between wash temperatures of 130 and 160 F.*

Comparison	Positive differences	Negative differences	Critical number[a] for $\alpha = 0.10$
SS versus Plastic	10	7	4
SS versus N-1	13	6	5
SS versus N-2	12	7	5
SS versus U	11	7	5
Plastic versus N-1	11	9	5
Plastic versus N-2	9	8	4
Plastic versus U	10	11	6
N-1 versus N-2	11	12	7
N-1 versus U	9	9	5
N-2 versus U	9	13	6

[a]To be significant at $\alpha = 0.10$, the smallest of the two numbers (positive or negative) must be ⩽ the critical number.

Soil retention

Calcium film deposition has been shown to be an important harborage for microorganisms on food contact surfaces (*3,10,12,15,16*). Table 6 indicates the degree of calcium retention on test materials. Soiling, as evidenced by positive soiled controls, yielded an average calcium ion concentration (both 130 and 160-F wash solution temperatures) of 331.5 μg per 130 cm² (range 151.1 to 596.5 μg per 130 cm²). Calcium residues following 10 soil/wash cycles averaged 52.6 μg per 130 cm² at 160-F wash temperature, and 53.0 μg per 130 cm² at 130 F — an average reduction of 278.5 to 278.9 μg per 130 cm² at 160 and 130-F wash temperatures, respectively. Additionally, the background (negative) control revealed that an average of 43.5 μg of calcium per 130 cm² was inherent in the test materials, and a control analyzed to determine the effect of water hardness on film deposition had an average residual calcium level of 60.5 μg per 130 cm² — an average retention of 17 μg per 130 cm².

TABLE 6. *Calcium determination after ten soil/wash cycles.*

Wash temperature	Rack number	Soiled control (μg/130 cm²)	X Calcium residual (μg/130 cm²)
160 F	1	151.1	46.5
	2	290.5	58.5
	3	219.0	43.0
	4	596.5	46.0
	5	211.0	61.0
	6	377.0	60.5
130 F	1	429.5	55.9
	2	447.5	49.0
	3	186.0	52.0
	4	552.5	56.0
	5	152.3	55.5
	6	363.5	51.0
		($\bar{X}$ = 331.5)	95% 14 df CI about $\bar{X}$ (μg/130 cm²)
Background control[a]		43.5	36.0 - 51.0
Water-film control[b]		60.5	55.0 - 66.5

[a]Random samples washed in phosphoric acid to remove residual film, then analyzed for calcium using the acid-swab atomic absorption technique.

[b]Random samples washed in phosphoric acid, then submitted to ten wash cycles (detergent added to wash solution, but no soil on plate) before analysis.

The average calcium retention for all 15 samples in each test rack was calculated and compared to the 95% confidence intervals about the means of background and water-film controls. Table 6 indicates that average calcium levels for all test samples were within or below the confidence intervals about the means for the water-film control (95% $CI_{14\ df} = 55.0$ to 66.5 μg per 130 cm²). Additionally, 5 of 12 sets of samples were within the confidence intervals established for background levels of calcium, indicating a calcium-film retention less than would be expected for washing in water and detergent alone with no soil added to the test material.

DISCUSSION

It is important to note that the concern over public health associated with tableware is well documented (*2,3,4,7,8*). Many reports note that improper handling, storage and use of dinnerware may contribute a significant number of microbiological contaminants even though proper dishwashing may have left the material in an acceptable sanitary condition (*4,6,7*). The mechanism of soil retention and its effect on microbial harborage remains speculative. Peart (*14*) revealed that soil adhesion to china was affected by lipoprotein present in the soil system and the availability of calcium and magnesium ions. The ions, she speculated, act as bridges between protein moieties and may also act on the lipid to form a soap-like structure. This same bridging effect may explain the low calcium noted by these experiments. The average calcium levels for test materials often fell below the levels expected due to the water hardness. It is possible that calcium complexes established in the food soil are responsible for keeping residual levels of calcium on the material surface low by washing them off as the surface soil is removed.

Recovery of calcium residues from food contact surfaces has been established as a good indicator of surface cleanliness (*6,12,16*); however, methods of recovery are in question. Some workers have used radioactive isotopes to label soil (*10,13*); however, Maxcy (*12*) points out that low levels of soil residue may not be accurately detected by these means. The acid-swab recovery method developed by Heinz et al. (*6*) appears to be the most reliable method of recovery presently available, being able to detect levels of residual calcium as low as 0.07 mg per 100 cm². Schneider et al. (*16*) used this procedure successfully to show that bacterial harborage may begin at a calcium residue level of ⩾ 3 μg per cm² above base line. Additionally, they noted that an increase in calcium level on the surface of their test materials was directly correlated with harborage of *B. subtilis* spores. Ridenour and Armbruster (*15*) noted that film buildup and organic soil contamination had a direct effect on bacterial removal. They showed that for the six organisms tested, *Micrococcus aureus* (present nomenclature *S. aureus*) was the most difficult to remove and was also most easily harbored by residual soil on all surfaces tested. Results in these experiments substantiate the sensitivity of the calcium recovery techniques based on acid removal and atomic absorption analysis with levels as low as 30 μg calcium per 130 cm² reported (0.23 μg/cm²). The wash/rinse sequence employed in this investigation, however, was sufficient to reduce bacterial numbers and calcium residues to such an extent that the effects of film on bacterial harborage may only be speculated. *S. aureus* was chosen as the test organism in these experiments due to its public health significance in causing foodborne disease (*2*) and because of its strong adherence to surfaces of commonly employed dinnerware (*15*). Spore-forming organisms may show greater adherence and harboring effects in the spore state (*16*); however, these organisms were not chosen because aerobic spore-forming organisms have not been implicated in foodborne disease transmission via contaminated tableware, and anaerobic spores do not outgrow under strongly aerobic conditions and consequently would not present a problem in disease transmission. Methods of recovery used in these experiments were proven to be most effective when compared to rinsing techniques or swabbing with buffered water alone. In addition, incubating plates for 24 or 48 h did not reveal significant differences in recovery of heat-stressed cells after rinsing in the dishmachine; consequently, the 24-h incubation was employed.

Data presented in this report suggest that all test materials may be adequately cleaned at both 130 and 160 F using wash conditions recommended by detergent and dishmachine manufacturers. These results, therefore, concur with those of other investigators (*4,7,8*) in suggesting that the public health concern is primarily due to human factors in inadequate dishmachine operation, handling of utensils after washing and improper storage before use.

ACKNOWLEDGMENT

The authors wish to express appreciation to the Economics Laboratory Computer Science Department for statistical design and analysis, and to Countryware Corp., Syracuse, NY for supplying test materials and assistance.

REFERENCES

1. American Public Health Association. 1975. Standard methods for the examination of water and wastewater, 14th ed. Section 301 A II, Determination of calcium by direct aspiration into an air-acetylene flame. APHA, Washington, D.C.
2. Center for Disease Control. 1977. Foodborne and waterborne disease outbreaks. Annual Summary, 1976. U.S. Dept. HEW, PHS, Atlanta, Georgia.
3. Cox, W. C. 1937. The use of dishwashing machines: pasteurization of eating utensils. Presented to lab. section, APHA, 60th Annual Meeting, Washington, D.C.
4. Comptroller General of the U.S. 1975. Federal support for restaurant sanitation found largely inadequate. Report to Congress, 36 pp.
5. Department of Health, Education, and Welfare. 1974. Foodservice Sanitation. Fed. Reg., 39(191):35438-35499.
6. Heinz, J. V., R. T. Marshall, and M. E. Anderson. 1967. Determining cleanliness of milk contact surfaces by analyzing for calcium residual: preliminary studies. J. Milk Food Technol. 30:337-339.
7. Jopke, W. H., S. D. Sorenson, D. R. Hass, and A. C. Donovan. 1972. Microbial contamination on hospital tableware. Hosp. Prog.

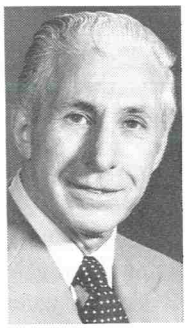
53(6):30-33.
8. Kupchik, G. J., and J. Katz. 1977. Potential health hazards associated with reusable foodservice utensils. J. Food Protect. 40:132-134.
9. Lynch, C., and J. G. Cummings. 1919. Distribution of influenza by indirect contact — hands and eating utensils. Am. J. Public Health 9:25-38.
10. Martin, A. T., C. H. Shiflett, and J. C. Wilson. 1952. Use of P^{32}, Sr^{89}, and CA^{45} in mechanical dishwashing studies. Minn. Acad. Sci., Proc. 20:28-31.
11. Maxcy, R. B. 1975. Fate of bacteria exposed to washing and drying on stainless steel. J. Milk Food Technol. 38:192-194.
12. Maxcy, R. B. 1966. The relationship between residual soil and microbial growth after circulation cleaning. Food Technol. 23:123-126.
13. National Sanitation Foundation. 1970. Standard No. 36 for dinnerware. NSF, Ann Arbor, Michigan.
14. Peart, M. V. 1969. Effects of selected factors on the adhesion and disintegration of a complex food soil. Ph.D. Thesis, Purdue University, Lafayette, Indiana.
15. Ridenour, G. M., and E. H. Armbruster. 1953. Bacterial cleanability of various types of eating surfaces. Am. J. Public Health 43:138-149.
16. Schneider, P. M., F. F. Busta, and C. R. McDuff. 1978. Microbial harboring characteristics of dishmachine-filmed glassware. J. Food Protect. 41:800-805.

IFT Award Winners Announced

Award winners at the Institute of Food Technologists' annual meeting in June included Dr. Theodore P. Labuza, Dr. Robert H. Cotton, Dr. F. Jack Francis, and Dr. John Hawthorn. Armour Research Center of Armour & Co. and Grumman Allied Industries jointly won the Industrial Achievement Award.


Dr. Labuza


Dr. Cotton


Dr. Francis


Dr. Hawthorn

Dr. Labuza, professor of food science at the University of Minnesota, was presented the 1979 Wm. V. Cruess award. The award honors a person who has achieved excellence in teaching food science and technology and consists of a bronze medal and a $1000 honorarium.

Prior to joining the faculty of Massachusetts Institute of Technology, Dr. Labuza received his degrees in food engineering from the same university. He joined the University of Minnesota faculty in 1971.

He has been a visiting lecturer in nutrition at the Mayo Clinic, Rochester, MN, and has served as a visiting professor at the Universite des Sciences et Techniquies, Montpellier, France.

During his tenure at Minnesota, he has developed and taught 10 food science and nutrition courses, introductory to graduate level. Dr. Labuza has written over 120 research articles and four books on nutrition and in 1972 he received IFT's Samuel Cate Prescott Award as outstanding young researcher. He is an IAMFES member.

The recipient of the 1979 Babcock-Hart Award was Dr. Robert H. Cotton, chief food scientist at ITT Corp. The award recognizes a person for contributions to food technology which have improved public health through nutritious food. It consists of a plaque and $1000 honorarium.

Dr. Cotton received his education in chemistry, chemical engineering, and nutrition at Bowdoin College, MIT, and Pennsylvania State University, respectively.

He has worked for the National Research Corp., Holly Sugar Corp., and Huron Milling Co. He was named director of research for ITT Continental Baking Corp. in 1958, elected a vice president in 1965, and was named to his present position in 1975. He has served as a panel member of the White House Conference on Food, Nutrition and Health and as an advisor on foods and containers to the armed forces. He served as the first director general of Fundacion Chile, a center for applied research and technology transfer, jointly funded by Chile and ITT Corp.

The Nicholas Appert Award was presented to Dr. F. Jack Francis, professor of food science at the University of Massachusetts. The Appert Award is presented to honor a person for excellence in and contributions to the field of food technology. It consists of a bronze medal and a $1000 honorarium.

Dr. Francis received his bachelor's and master's degrees from the University of Toronto and joined the faculty there before moving to the faculty of the University of Guelph in 1950. Dr. Francis joined the University of Massachusetts faculty in 1954 upon receiving his doctorate there in food technology. He has remained there since, being named to the Nicholas Appert Professorship in 1964 and head of the department of food science and nutrition in 1971. His research emphasis in recent years has concentrated on pigments, particularly degradation, and on color and quality of foods.

He has published more than 225 papers and articles and has authored or co-authored five books. Dr. Francis was elected an IFT Fellow in 1975 and is currently chairman of IFT's Expert Panel on Food Safety and Nutrition and president-elect of IFT.

Dr. John Hawthorn, professor and head of the department of food science and nutrition at the University of Strathclyde, Glasgow, Scotland, was presented the 1979 IFT International Award. The award

con't p. 665

Journal of Food Protection Vol. 42, No. 8, Pages 651-653 (August, 1979)

Physical, Chemical and Palatability Traits of Electrically Stimulated, Hot-boned, Vacuum-packaged Beef

S. C. SEIDEMAN, G. C. SMITH*, T. R. DUTSON and Z. L. CARPENTER

Meats and Muscle Biology Section, Department of Animal Science, Texas Agricultural Experiment Station, College Station, Texas 77843

(Received for publication November 20, 1978)

ABSTRACT

The right sides of six beef carcasses were electrically stimulated, whereas the left sides were not so treated. Sections of longissimus and semimembranosus muscles were removed from electrically stimulated sides at 1 h postmortem (hot-boned) and from sides which were not electrically stimulated at 24 h postmortem (conventionally boned); these muscles were vacuum-packaged immediately after removal and aged for 2 weeks at 1 ± 1 C. After aging, steaks were removed and used for determinations of palatability, cooking loss, pH, sarcomere length and protein solubility. Further evaluation included purification and electrophoresis of myofibrillar proteins. Longissimus muscles from electrically stimulated, hot-boned beef had lower ($P < .05$) juiciness ratings than those from conventionally handled sides; semimembranosus muscles from electrically stimulated, hot-boned sides sustained lower ($P < .05$) weight losses during storage but higher ($P < .05$) cooking losses than muscles from conventionally handled sides. Hot-boning of electrically stimulated beef had no effect ($P > .05$) on tenderness, flavor desirability, pH, sarcomere length, amount of non-protein nitrogen or percentage of soluble myofibrillar proteins; slight alterations were noted in the electrophoretic banding patterns, on SDS gels, of myofibrillar proteins from electrically stimulated, hot-boned sides. Hot-boned beef was as palatable as conventionally handled beef if sides were electrically stimulated on the kill floor before hot-boning and if beef from both treatments was vacuum-packaged and aged for 2 weeks at 1 ± 1 C.

Consumer studies (*2*) have shown that tenderness is the most important palatability factor determining the acceptance of beef. Physical restraint and high temperature conditioning improve tenderness by preventing cold-shortening and/or by enhancing lysosomal enzyme activity but have attendant problems - altered carcass shape and prolonged chilling periods - which have deterred industry application (*17*). Electrical stimulation accelerates postmortem pH decline, hastens onset of rigor mortis and improves tenderness (*3, 7*).

Hot-boning lessens the weight of product per animal to be chilled (*9*), but release of normal physical-anatomical restraints on muscles and increased rate of muscle cooling (because of lesser mass) allows or enhances cold-shortening and results in beef that is substantially less tender than conventionally handled beef (*9*). Beef muscle excised before onset of rigor mortis shortens by as much as 50% of its original length upon exposure to 0 C, resulting in a substantial decrease in tenderness (*4*). Several researchers (*3, 5, 7*) have suggested that electrical stimulation could be used to prevent cold-shortening or to increase the rate of conditioning of carcasses. Deleterious effects of hot-boning on tenderness might also be partially negated by aging in vacuum packages (*13*).

The purpose of this study was to characterize physical, chemical and palatability traits of electrically stimulated, hot-boned, vacuum-packaged beef.

EXPERIMENTAL

Preparation of samples

Six steers (370 kg live weight, U.S. Good grade) were conventionally slaughtered. At 30 to 40 min post-exsanguination, the right side of each carcass was electrically stimulated (ES) with 25 impulses of 0.5 to 1 sec duration using an "Electro-Sting" (Model 160-ESS) stunning unit which delivered 440 volts (AC), 5 amp and 50-60 cycles per second. At 45 to 60 min post-exsanguination, sections of longissimus and semimembranosus muscles were hot-boned from each electrically stimulated side, weighed and vacuum-packaged. A chamber-type vacuum-packaging machine (Conofresh 6000) was used with film bags with the following characteristics: oxygen transmission rate of 0.41 to 0.75 cc/100 in^2/24 h/75 F/50% RH; moisture vapor transmission rate of 0.18 to 0.20 g/100 in^2/24 h/100 F/70% RH. Muscles were vacuum-packaged at maximum capacity of the vacuum-packaging machine, with a chamber vacuum of 29.4 inches of Hg. The left side of each carcass was placed in a 1 to 3 C cooler immediately after splitting; after 24 h, sections of boneless longissimus and semimembranosus muscles were removed from each control side, weighed and vacuum packaged. After a 2-week aging period in vacuum packages, each muscle section was unwrapped and reweighed to determine weight loss during storage, then cut into three 2.5-cm steaks. Two steaks were used for cooking and palatability evaluation while the third steak was used for sarcomere length, pH, protein solubility and myofibrillar protein determinations. Steaks were double-wrapped in polyethylene-coated paper, frozen and stored at - 34 C for approximately 3 weeks before histological, chemical or organoleptic analyses.

Cooking traits and palatability evaluation

Two steaks from each longissimus muscle and two steaks from each semimembranosus muscle were thawed (24 h at 2 C) and broiled on Farberware Open-Hearth Broilers to an internal temperature of 70 C (monitored by the use of copper-constantan thermocouples). The percentage cooking loss was calculated by use of weights obtained before and after cooking. One cooked steak from each muscle was cut into 1.5 × 1.5 × 1.5 cm samples and random samples were presented to an eight-member trained sensory panel for evaluation of juiciness (8 = extremely juicy, 1 = extremely dry), muscle fiber tenderness and overall tenderness (8 = extremely tender, 1 = extremely tough), organoleptically detectable connective tissue (8 = none, 1 = abundant), flavor desirability and overall palatability (8 = extremely desirable, 1 = extremely undesirable). The second cooked steak from each muscle was used to obtain four 1.27-cm cores for shear force determinations by use of the Warner-Bratzler shear machine.

Sarcomere length and pH

A 3-g sample of longissimus muscle from each side was placed in 30 ml of 0.25 M sucrose and blended at low speed for 30 sec in a Virtis

homogenizer. Sarcomere length was measured on the homogenate by use of Zeiss W-4 phase contrast microscope equipped with a Timbrell Coulter Shearicon particle counter/size analyzer. The mean sarcomere length was determined by measuring groups of four sarcomeres from each of 30 myofibrils. The pH of each longissimus muscle sample was obtained from the same homogenate used for sarcomere length determinations.

Protein solubility

Percentages of non-protein nitrogen (NPN), sarcoplasmic protein and myofibrillar protein were determined (*12*) on longissimus muscle samples. Protein contents of the sarcoplasmic and myofibrillar fractions were determined by the biuret procedure (*10*). Total protein and NPN were measured by a micro-kjeldahl procedure (*6*).

Myofibrillar protein determination and quantitation

Myofibrils from longissimus muscles were purified (*16*) and subsequently subjected to electrophoretic separation on 7.5% SDS-polyacrylamide gels (*23*). After electrophoresis and destaining, the gels were scanned with a Photovolt-Densicord densitometer equipped with an integraph model 49 integrator. The area of each peak was used to calculate the relative percentage of each myofibrillar protein in the gel.

Statistical analysis

The data were analyzed by use of analysis of variance (*21*).

RESULTS

Mean values for storage loss, cooking characteristics and palatability traits for muscle sections are given in Table 1. Except for lower ($P < .05$) juiciness ratings for longissimus muscles and decreased ($P < .05$) storage loss and increased ($P < .05$) cooking loss for semimembranosus muscles, electrical stimulation followed by hot-boning had no significant effect on storage, cooking or palatability traits when compared to samples of the same muscle from conventionally handled sides.

Mean values for pH, sarcomere length, non-protein nitrogen and protein solubility of longissimus muscles are shown in Table 2. Electrical stimulation followed by hot-boning, as compared to conventional handling, had no significant effect on pH, sarcomere length, amount of non-protein nitrogen or myofibrillar protein solubility. The percentage of sarcoplasmic proteins soluble in 0.03 M KPO_4 from muscles of electrically stimulated, hot-boned sides was higher ($P < .05$) than that from muscles of conventionally handled sides.

TABLE 2. *Mean values for pH, sarcomere length and percentages of protein from longissimus muscles.*

Trait	Treatment: Not electrically stimulated, conventionally boned	Treatment: Electrically stimulated, hot-boned
pH value	5.36[d]	5.45[d]
Sarcomere length (μm)	1.74[d]	1.74[d]
Non-protein nitrogen (%)[a]	4.11[d]	4.24[d]
Sarcoplasmic protein (%)[b]	23.32[e]	27.42[d]
Insoluble protein (%)[b]	49.71[d]	51.03[d]
Myofibrillar protein (%)[b]	26.97[d]	21.55[d]
Myosin (%)[c]	55.39[d]	58.17[d]
M-protein (%)[c]	10.56[d]	8.96[e]
C-protein (%)[c]	4.25[d]	3.29[e]
a-actinin (%)[c]	4.90[d]	1.96[e]
Actin (%)[c]	21.80[d]	24.61[d]
Troponin-T and *a*-tropomyosin (%)[c]	0.66[d]	0.87[d]
Myosin, light chain 1 (%)[c]	2.44[d]	2.14[d]

[a]Non-protein nitrogen as a percentage of total nitrogen.
[b]Percentage of total muscle protein.
[c]Percentage of myofibrillar protein.
[de]Means in the same row bearing a common superscript letter do not differ ($P > 0.05$).

The relative percentages of myofibrillar proteins on SDS gels from myofibrils of longissimus muscles are also shown in Table 2. There were higher ($P < .05$) percentages of protein in the M-protein, C-protein and *a*-actinin regions of gels from myofibrils of longissimus muscles from conventionally handled sides when compared to samples from sides which had been electrically stimulated and hot-boned.

DISCUSSION

The potential advantage of hot-boning is removal of bone and excess fat before chilling, thereby reducing the amount of material to be chilled (*9*). Since electrical stimulation promotes rapid onset of rigor mortis, the period between slaughter and preparation of retail cuts could be minimized by combining electrical stimulation and hot-boning. Commercial use of electrical stimulation would not disrupt product flow through a plant, requires little time and does not detract from carcass appearance (*18*).

Electrical stimulation increases tenderness (*18,19*); the most plausible explanations for such tenderization are

TABLE 1. *Mean values for storage loss and certain cooking and palatability traits for longissimus and semimembranosus muscles.*

Trait	Longissimus: Not electrically stimulated, conventionally boned	Longissimus: Electrically stimulated, hot-boned	Semimembranosus: Not electrically stimulated, conventionally boned	Semimembranosus: Electrically stimulated, hot-boned
Storage loss (%)	0.69[a]	0.57[a]	1.97[a]	0.05[b]
Cooking loss (%)	28.12[a]	28.98[a]	29.82[b]	36.08[a]
Shear value (kg)[c]	5.52[a]	4.68[a]	5.05[a]	4.44[a]
Juiciness rating[d]	5.2[a]	4.3[b]	5.0[a]	4.4[a]
Muscle fiber tenderness rating[e]	4.7[a]	4.9[a]	5.1[a]	4.8[a]
Amount of connective tissue rating[f]	5.7[a]	5.8[a]	5.2[a]	5.4[a]
Overall tenderness rating[e]	4.8[a]	4.8[a]	4.6[a]	4.5[a]
Flavor desirability rating[g]	5.3[a]	5.1[a]	5.1[a]	5.2[a]
Overall palatability rating[g]	4.8[a]	4.7[a]	4.8[a]	4.5[a]

[ab]Means for the same trait and for the same muscle bearing a common superscript letter do not differ ($P > .05$).
[c]Means based on eight 1.27 cm diameter cores from each of six steaks.
[d]Means based on an 8-point scale (8 = extremely juicy; 1 = extremely dry).
[e]Means based on an 8-point scale (8 = extremely tender; 1 = extremely tough).
[f]Organoleptically-detectable connective tissue scored on the basis of an 8-point scale (8 = none; 1 = abundant).
[g]Means based on an 8-point scale (8 = extremely desirable; 1 = extremely undesirable).

physical disruption of myofibrils (*19*), earlier release/enhanced activity of lysosomal enzymes (*8*) and earlier rigor onset/reduced cold-shortening of myofibrils (*3,7*).

In other research (*20*), the muscle pH resulting from low-voltage electrical stimulation was lower than that from non-electrically stimulated sides at 1, 4 and 24 h postmortem. Another study (*1*) suggests that although electrical stimulation promotes pH decline, it has little effect on ultimate pH. Our study also suggests that electrical stimulation did not affect final pH. When pH declines rapidly while muscle temperature is still high, increased protein denaturation, decreased water-holding capacity (*22*) and earlier release of lysosomal enzymes (*8,14,15*) are possible. Data of the present study further suggest that there could be some degradation or release of M-protein, C-protein and *a*-actinin from the myofibrils of electrically stimulated, hot-boned muscle.

Once pH goes below 6.0 to 6.2, which it does rather rapidly in electrically stimulated carcasses, the tendency for the muscle to cold-shorten is reduced (*11*). Yet, at least one study (*19*) reported no significant differences in sarcomere lengths of muscles from electrically stimulated sides as compared to muscles from sides that were not electrically stimulated. Data of the present study agree with the latter study (*19*) in this regard; there was no significant difference in sarcomere length between muscles from conventionally handled and electrically stimulated sides. The increase in tenderness resulting from electrical stimulation may be effected by physical damage of muscle fibers rather than by prevention of cold-shortening (*19*).

In addition to tenderness improvement, the rapid decline in pH due to electrical stimulation may cause differences in other palatability and cooking attributes. Electrical stimulation decreased juiciness ratings and increased cooking losses as compared to control (untreated) beef in one study (*19*); very similar results were evident for longissimus and semimembranosus muscles, respectively, in the present study. Increased flavor desirability ratings due to electrical stimulation have been reported (*7,18,19*); flavor desirability ratings were not affected by electrical stimulation in the present study.

CONCLUSION

Based on the results of the present study, hot-boned beef can be as palatable as conventionally handled beef if sides are electrically stimulated on the kill floor before hot-boning and if beef from both treatments is vacuum-packaged and aged for 2 weeks at 1 ± 1 C.

ACKNOWLEDGMENTS

T.A. 14435, Texas Agricultural Experiment Station. This study was partially supported by The King Ranch, Inc., Kingsville, TX; The Natural Fibers and Food Protein Commission of Texas, Renner, TX; and Texas Cattle Feeders Association, Amarillo, TX.

REFERENCES

1. Adams, K. L. 1977. Effects of electrical stimulation and high temperature pre-rigor conditioning on myofibrillar proteins of bovine muscle. M.S. Thesis. Texas A&M University, College Station, Texas.
2. Bratzler, L. J. 1971. Palatability characteristics of meat: Palatability factors and evaluation. In J.F. Price and B. S. Schweigert (eds.) The science of meat and meat products (2nd ed.). W. H. Freeman and Co., San Francisco, CA.
3. Carse, W. A. 1973. Meat quality and the acceleration of postmortem glycolysis by electrical stimulation. J. Food Technol. 8:163-166.
4. Cassens, R. G. 1977. Muscle biochemistry: The importance of myofiber type. Food Technol. 31:76-81.
5. Chrystall, B. B., and C. J. Hagyard. 1976. Electrical stimulation and lamb tenderness. New Zealand J. Agri. Res. 19:7-11.
6. Conway, E. J. 1958. Micro-diffusion analysis and volumetric error. The MacMillan Co., New York, NY.
7. Davey, C. L., K. V. Gilbert, and W. A. Carse. 1976. Carcass electrical stimulation to prevent cold-shortening toughness in beef. New Zealand J. Agri. Res. 19:13-18.
8. Dutson, T. R., G. C. Smith, and Z. L. Carpenter. 1979. Lysosomal enzyme distribution in electrically stimulated ovine muscle. J. Food Sci. (In press)
9. Falk, S. N., R. L. Henrickson, and R. D. Morrison. 1975. Effect of boning beef carcasses prior to chilling on meat tenderness. J. Food Sci. 40:1075-1079.
10. Gornall, A. G., C. T. Bardawill, and M. M. David. 1949. Determination of serum proteins by means of the biuret procedure. J. Biol. Chem. 177:751-766.
11. Harris, P. V. 1975. Meat chilling. CSIRO. Food Res. Q. 35:49-56.
12. Helander, E. 1957. On quantitative muscle protein determination. Acta. Physiol. Scand 41(141):5-99.
13. Minks, D., and W. C. Stringer. 1972. The influence of aging beef in vacuum. J. Food Sci. 37:736-738.
14. Moeller, P. W., P. A. Fields, T. R. Dutson, W. A. Landmann, and Z. L. Carpenter. 1976. Effect of high temperature conditioning on subcellular distribution and levels of lysosomal enzymes. J. Food Sci. 41:216-217.
15. Moeller, P. W., P. A. Fields, T. R. Dutson, W. A. Landmann, and Z. L. Carpenter. 1977. High temperature effects on lysosomal enzyme distribution and fragmentation of bovine muscle. J. Food Sci. 42:510-512.
16. Rome, E. 1972. Relaxation of glycerinated muscle. Low angle x-ray diffusion studies. J. Molecular Biol. 65:331-337.
17. Savell, J. W. 1978. Effect of electrical stimulation on quality-indicating, palatability and histological characteristics of beef. Ph.D. dissertation, Texas A&M University, College Station.
18. Savell, J. W., G. C. Smith, T. R. Dutson, Z. L. Carpenter, and D. A. Suter. 1977. Effect of electrical stimulation on palatability of beef, lamb and goat meat. J. Food Sci. 42:702-705.
19. Savell, J. W., T. R. Dutson, G. C. Smith, and Z. L. Carpenter. 1978. Structural changes in electrically stimulated beef muscle. J. Food Sci. 43:1606-1607.
20. Shaw, F. D., and D. J. Walker. 1977. Effect of low voltage stimulation of beef carcasses on muscle pH. J. Food Sci. 42: 1140-1141.
21. Steel, R. G. D., and J. H. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill Book Co., New York, NY.
22. Tarrant, P. V. 1977. The effect of hot-boning on glycolysis in beef muscle. J. Sci. Food Agri. 28:927-930.
23. Weber, K., and M. Osborn. 1969. The reliability of molecular weight determination by dodecyl sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem. 244:4406-4412.

Journal of Food Protection Vol. 42, No. 8, Pages 654-655 (August, 1979)

Evaluation of Millipore Cartridge-type Water Treatment Systems for Microbiological Applications

BARBARA L. GREEN and WARREN LITSKY*

Department of Environmental Sciences, University of Massachusetts, Amherst, Massachusetts 01003

(Received for publication January 15, 1979)

ABSTRACT

Two different cartridge-type water treatment systems were evaluated for use in the microbiology laboratory, employing the Distilled Water Suitability Test (DWST). The Milli-Q system fed by distilled water and the Milli-R/Q system fed by tap water both produced water acceptable for microbiological use as measured by the DWST. Concentrations of ionic species (as measured by conductivity) in Milli-Q and Milli-R/Q products were significantly lower than in the double distilled control water.

It has long been recognized that water used for preparation of culture media, reagents and dilution blanks must be free from inhibitory substances or excessive nutrients that adversely affect microbiological procedures. Such biological toxicity or nutritive releases can be measured by a suitability test (*1*) that compares growth responses of *Enterobacter aerogenes* in a minimal growth medium prepared with the test water to that employing a double distilled water control.

Recently, many laboratory stills have been replaced by deionization columns and cartridge-type water treatment systems as sources of non-toxic, high-purity, reagent-grade water. Because of the ease in handling, convenience and energy-saving characteristics of these cartridge systems, their conversions in many instances have been made and put in line without adequate evaluation. The present study was designed to evaluate the suitability of water from two different cartridge-type systems when double distilled water was used as the standard, as stipulated in the Distilled Water Suitability Test (DWST, *1*).

The first treatment system, the Milli-Q (Millipore Corporation, Bedford, Massachusetts) has been widely accepted as a replacement for the laboratory distillation apparatus in production of reagent grade water. Pretreatment, which usually consists of reverse osmosis, distillation, or single-bed deionization, is recommended with the Milli-Q.

The second treatment system, the Milli-R/Q, introduced recently by Millipore Corporation, is a small 20 liters/day system with pretreatment built in. A schematic of the system is shown in Fig. 1. The system consists of a 5 μ prefilter, A, followed by a spiral-wound type reverse osmosis membrane cartridge, B. The concentrate from the reverse osmosis cartridge goes to drain and the permeate is treated with activated carbon and mixed bed deionization resin in a polishing cartridge, C. The final product is filtered by a 0.45 μ final filter, D, before going into a 20-liter storage tank, E. The tank is vented to the atmosphere via a CO_2 trap, F, and is equipped with a level control valve, G. The system operates on line pressure (40-100 psig), and the only power requirement is to operate the meg-o-lite, H, which has two lights--green light indicative of stored water resistivity greater than 1 megohm-cm and amber light indicative of resistivity less than 1 megohm-cm.

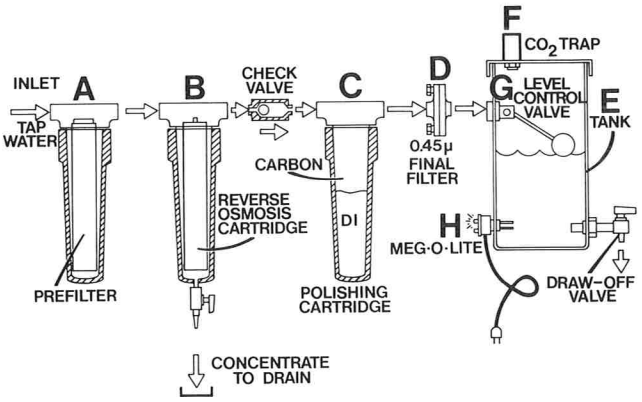


Figure 1. *Flow schematic of Milli-R/Q system.*

MATERIALS AND METHODS

The DWST was performed in strict accordance with *Standard Methods* (*1*). *Enterobacter aerogenes* (ATCC 13048) was used as the test organism. Due to previous reports regarding impurities (*1,3-5*), all chemicals used to prepare the stock solutions were Fisher "certified" reagents with the exception of the potassium dihydrogen phosphate (Mallinckrodt, analytical reagent grade).

The source of all test waters was the Amherst municipal water supply which services the University. A physical/chemical analysis of this source water is presented in Table 1.

The DWST was performed on the test waters from the two systems which were set up as follows: (*a*) the source water was distilled (Loughborough glass still, Bellco Glass Inc., Vineland, New Jersey), stored in a carboy and fed to the Milli-Q system, and (*b*) the source water was fed directly to the Milli-R/Q system without any pretreatment. Milli-Q cartridges were in normal use for 5 months and the Milli-R/Q cartridges for 4 months before suitability testing.

The water used as the control was collected from the glass still and redistilled in a bench-type glass distillation apparatus immediately before performing the tests. Conductivity, which is a widely used measure of concentration of ionic species (*2,3*), was monitored with an EP meter (Myron L. Co.) for 4 months before and during performance of these tests. Measured ranges are presented in Table 2.

TABLE 1. *Analysis of University of Massachusetts tap water.*[a]

Constituent	Concentration (mg/l except as noted)
Turbidity	2.0 Turbidity units
Sediment	2
Color	15 Color units
Odor	1 Ep
pH	5.8
Alkalinity	3 as Ca CO_3
Hardness	7 as $CaCO_3$
Calcium (Ca)	1.9
Magnesium (Mg)	0.5
Sodium (Na)	3.4
Potassium (K)	0.6
Iron (Fe)	0.25
Manganese (Mn)	0.06
Silica (SiO_2)	16.0
Sulfate (SO_4)	9
Chloride (Cl)	6
Conductivity	41 micromhos/cm
Nitrogen (Ammonia)	0.09
Nitrogen (NO_3)	0.1
Nitrogen (NO_2)	0.001
Copper (Cu)	0.02

[a]Source of analysis: Commonwealth of Massachusetts, Dept. of Environmental Quality Engineering, 2/14/78.

TABLE 2. *Conductivity range of water samples.*

Treatment	Specific conductance (micromhos/cm)
Milli-Q	0.13-0.15
Milli-R/Q	0.3-1.6[a]
Redistilled glass (Control)	0.9-1.0
Untreated tap water	47-50

[a]For four months before the tests, conductivity of Milli-R/Q water ranged from 0.3 to 0.5 micromhos/cm. The value of 1.6 micromhos/cm was observed on the day of the last test when polishing cartridge was near exhaustion.

RESULTS AND DISCUSSION

Duplicate Suitability Tests were performed concurrently from single samples of each of the two test waters for 3 consecutive weeks. Bacterial numbers in each flask were determined and ratios of test water to control water calculated. Any ratio between 0.8 and 3.0 indicates no toxic substances present and the water is considered "suitable." When the ratio exceeds 1.2, growth-stimulating substances are present, but because of the extreme sensitivity of the test, ratios up to 3.0 have little practical significance. Values below 0.8 are positive indication of biological toxicity (*1*). As shown in Tables 3 and 4, ratios in this study ranged from 0.8 to 1.7 for the Milli-Q and from 0.9 to 1.6 for the Milli-R/Q system. These values are indicative of excellent water quality.

TABLE 3. *Milli-Q water suitability test results.*

	No. of Bacteria ($\times 10^4$/ml)		
Date	Test water	Redistilled control water	Test water to control ratio
3/1/78	170	100	1.7
	130	101	1.3
3/7/78	47	52	0.9
	80	81	1.0
3/14/78	155	106	1.5
	102	135	0.8

TABLE 4. *Milli-R/Q water suitability test results.*

	No. of bacteria ($\times 10^4$/ml)		
Date	Test water	Redistilled control water	Test water to control ratio
3/1/78	154	100	1.6
	151	101	1.5
3/7/78	49	52	1.0
	84	81	1.0
3/14/78	108	106	1.0
	126	135	0.9

The Milli-Q system fed by distilled water and the Milli-R/Q system fed by tap water both produced water suitable by the DWST using double distilled water as the control, as prescribed in the *Standard Methods* procedure. Concentrations of ionic species (as measured by conductivity) in Milli-Q and Milli-R/Q products were found to be significantly lower than double distilled water, most likely because carry-over of impurities is a problem inherent to distillation.

Thus, it was concluded that, when properly installed and maintained, the Milli-Q system with adequate pretreatment and the tap-water-fed Milli-R/Q system can be expected to yield water suitable for microbiological analyses, as judged by the DWST.

REFERENCES

1. American Public Health Association. 1975. Standard methods for the examination of water and wastewater, 14th ed. American Public Health Assocation, Inc., Washington, D.C.
2. Fisher, S. 1967. Analysis of high quality water. Industrial Water Engineering. Nov:17-20.
3. Geldreich, E. E., and H. F. Clark. 1965. Distilled water suitability for microbiological applications. J. Milk Food Technol. 28:351-355.
4. Geldreich, E. E. 1975. Handbook for evaluating water bacteriology laboratories. EPA-670/9-75, U.S. Environmental Protection Agency, Cincinnati, Ohio.
5. Jensen, J. P., and W. J. Hausler. 1976. Contribution of KH_2PO_4 to toxicity in phosphate buffered dilution water systems. J. Milk Food Technol. 39:852-853.

Journal of Food Protection Vol. 42, No. 8, Pages 656-657 (August, 1979)

A Research Note

Effect of Potassium Sorbate on the Microbiological Quality of Butter

AJAY KAUL*, JASJIT SINGH and R. K. KUILA

Department of Dairy Bacteriology, National Dairy Research Institute, Karnal, Haryana, India

(Received for publication January 5, 1979)

ABSTRACT

Butter samples treated with potassium sorbate and sodium chloride were analyzed weekly for bacteriological qualities after storage at different temperatures. Addition of 0.1% potassium sorbate and incorporation of 2% sodium chloride plus 0.1 percent potassium sorbate resulted in inhibition of mold growth in all the samples at the end of 4 weeks at -18 C and 5 C. However the effect of potassium sorbate alone was less pronounced, irrespective of storage temperature. As regards coliform count in control butter, it increased rapidly at 22 C, but decreased when butter was stored at 5 C or -18 C. Addition of potassium sorbate to butter samples stored at -18 C reduced the coliform count to zero after 4 weeks, while butter with added potassium sorbate and sodium chloride showed a zero count after 1 week, indicating a cumulative inhibitory effect.

Enzyme activity in microorganisms causes development of hydrolytic rancidity and other defects in butter. Spoilage of butter is generally due to mold growth, which is a rather widespread and serious problem in India. During the present investigation emphasis was given to the mold and coliform count of butter. These organisms are responsible for deterioration of butter in terms of appearance and other physical characteristics. Such problems occur in the summer and especially when transportation is done to far off places. To prevent such degradation, addition of potassium sorbate was tried to prevent mold growth and survival of coliforms in butter during transportation and storage. Some workers (*4-6*) have reported the inhibitory action of potassium sorbate on growth of molds and bacteria in different dairy products. The present communication reports on the influence of potassium sorbate on the microflora of butter at storage temperature.

MATERIALS AND METHODS

Butter was manufactured from pasteurized cream (40% fat) at the experimental dairy, National Dairy Research Institute, Karnal and was divided into three equal parts, A, B and C. 'A' was kept as control, to 'B' 0.1% potassium sorbate and 2% sodium chloride were added, while 'C' received only 0.1% potassium sorbate. Working of 'B' and 'C' was done with wooden paddles and the final moisture was adjusted to 17.5%.

Preparation of samples

With the aid of a sterile spatula, a well-mixed portion of the butter was transferred to a sterile test tube (20 × 10 cms). It was kept in a water bath at 45 C for 15 min before analysis. Eleven milliliters of the warm (maintained at 45 C) butter samples in the test tube were slowly transferred to 99 ml of phosphate buffer solution in a milk dilution bottle. The bottle was shaked well for 25 times to obtain a final fine suspension without lumps. Suitable dilutions of the samples were prepared and 1.0 ml of each dilution was plated for mold (*1*) and coliform (*3*) count.

Packaging and storage of butter

Butter samples of 100 g were wrapped with vegetable parchment paper and eight samples from each lot were kept at three different temperatures, -18 C, 5 C and 22 C for storage and time course studies.

RESULTS AND DISCUSSION

The results on the effect of storage temperature and addition of potassium sorbate on the mold and coliform counts are depicted in the figures. In control butter stored at 22 C, the mold count continued to increase with storage time, but there was a decrease at 5 C and -18 C (Fig. 1). Addition of 0.1% potassium sorbate and incorporation of 2% sodium chloride plus 0.1 percent potassium sorbate resulted in inhibition of mold growth in all the butter samples at the end of 4 weeks at -18 C and 5 C. However, the effect of potassium sorbate alone was less pronounced, irrespective of storage temperature. These observations are comparable to earlier results (*7*), indicating that butter packed in paper treated with brine plus 0.5% potassium sorbate was free from mold growth and that packed in paper treated with brine alone showed considerable mold growth.

Since the coliform bacteria are an index of insanitary condition during the manufacture of butter, it was deemed worth while to explore the effect of storage time and preservative on these bacteria. The coliform count in control butter increased rapidly at 22 C but decreased in butter stored at 5 C and -18 C after 6 weeks (Fig. 2). Addition of potassium sorbate to butter samples stored at -18 C reduced the coliform count to zero after 4 weeks. Butter with added potassium sorbate and sodium chloride showed a zero count after 1 week, indicating a cumulative inhibiting effect. These results are in conformity with earlier observations (*2*) that potassium sorbate was effective in controlling psychrotrophs, coliforms, yeasts and molds.

In view of lack of available information on influence of preservatives on spoilage and deterioration of butter due

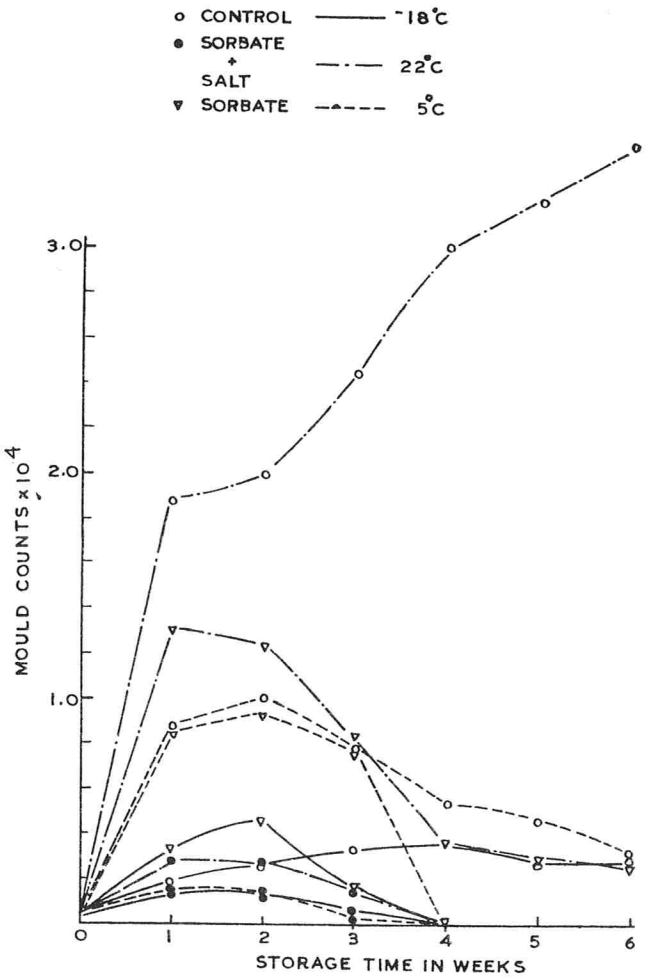


Figure 1. *Effect of storage temperature and potassium sorbate on mold counts of butter.*

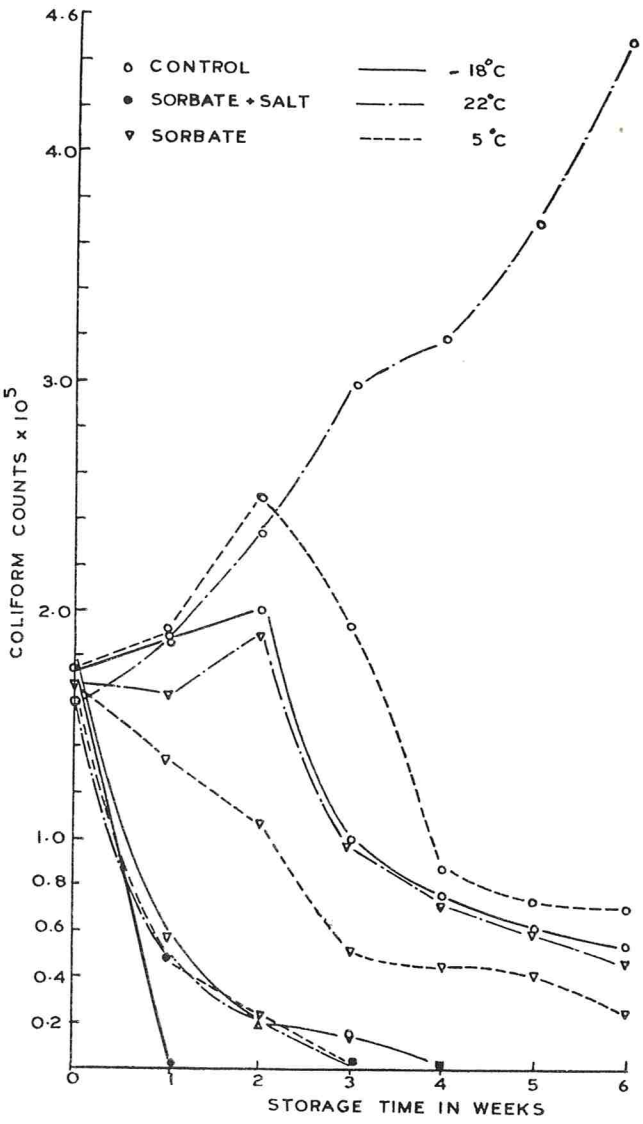


Figure 2. *Effect of storage temperature and potassium sorbate on coliform counts in butter.*

to the microbial activity under tropical conditions, the current report on effect of potassium sorbate on microbiological quality of butter should be viewed with considerable interest.

REFERENCES

1. American Public Health Assoc. 1972. Standard methods for the examination of dairy products. 13th ed. Am. Pub. Health Assoc. Washington, D.C.
2. Chakraborty, B. K., and T. Kristofferson. 1963. Evaluation of methods for improving the keeping quality of creamed cottage cheese. J. Dairy Sci. 46:603.
3. Chalmers, C. H. 1962. Bacteria in relation to milk supply. 4th ed. Edward Arnold Publishers Ltd., London.
4. Gale, G. R., C. D. Kendall, and A. S. Bernhiem. 1964. Antimicrobial activity of thiobenzoates. Proc. Soc. Exp. Biol. Med. 115:191.
5. Jha, Y. K. S. Singh, and S. Singh. 1976. Effect of antioxidants and microbial substances on keeping quality of Khoa. Indian J. Dairy Sci. 30:11.
6. Mustafa, H. H., and E. B. Collins. 1969. Effect of selected food additives on growth of *Pseudomonas fragi*. J. Dairy Sci. 52:3.
7. Olsson, I., and S. Johnson. 1959. Sorbate-treated wrapping paper as protection against mould growth in butter. Dairy Sci. Abst. 22:464.

Journal of Food Protection Vol. 42, No. 8, Pages 658-659 (August, 1979)

A Research Note

Fluorescence in Candies Caused by Methyl Anthranilate, a Flavoring Agent

MASATAKE TOYODA, YOSHIO ITO and MASAHIRO IWAIDA*

National Institute of Hygienic Sciences, Osaka Branch, Hoenzaka, Higashi-ku, Osaka, 540, Japan

(Received for publication January 29, 1979)

ABSTRACT

A fluorescent substance was found in imported fruit candy. In the elution test of the fluorescent substance, it reacted in the same manner as fluorescent dyes. Further pursuit revealed that the fluorescence was derived from the grape flavor preparation added during manufacture and the fluorescence spectra of the candy and the grape flavor were quite coincident with that of methyl anthranilate. Methyl anthranilate contents in the candy and grape flavor were determined gas chromatographically to be 40.0 and 33,000 ppm, respectively, and it was calculated that the addition level of grape flavor to the candy was about 0.12%.

It is prohibited in Japan to add fluorescent dyes to whiten foods and quantities of foods imported to Japan are rejected because they show fluorescence. There are several kinds of food constituents, however, that have their own fluorescence. Riboflavin, its esters and Maillard reaction products are well-known examples of fluorescent substances. Besides, it is prescribed in the Japanese Standards of Food Additives (*3*) that methyl anthranilate and methyl N-methylanthranilate used for preparation of flavoring agents have characterstic bluish violet fluorescence. In carrying out the official elution test for fluorescent dyes (*2*), these flavoring substances react in the same manner as do fluorescent dyes. It was reported in 1977 by Williams and Slavin (*4*) that less than 0.5 μg of methyl anthranilate per ml in grape beverages can be determined by use of high-performance liquid chromatograph with a fluorescence detector. In June 1978, imported fruit candy was noted to have a unique fluorescence, and the origin of this fluorescence was pursued by fluorometry and gas liquid chromatography.

MATERIALS AND METHODS

Materials

Fruit candy imported from Brazil and an artificial grape flavor preparation that had been used in the manufacture were used as test samples.

Reagents

Reference standards of methyl anthranilate and methyl N-methylanthranilate were supplied by Wako Pure Chemical Ind. Ltd., Osaka. All other reagents used were of analytical grade.

Elution of fluorescence substances

The elution test was carried out according to the official method of Japan (*2*), its details being compiled in the Sanitary Inspection Guide (*1*). The procedure is as follows:

"Take ca. 10 g of candy, crush into pieces, add 100 ml of dilute ammonia alkaline solution and keep at room temperature for 30 min, shaking occasionally. Centrifuge and use the clear supernatant liquid as the test solution. Take 50 ml of the test solution, add a few drops of dilute hydrochloric acid to make the pH of the supernatant liquid acidic. Next, dip 5 × 5 cm of wet absorbent gauze of Pharmacopoeia grade and heat for 30 min on a boiling water bath. Afterwards, take out the piece of gauze, wash well with water, wring and then put it under UV irradiation from UV lamps having the major wavelengths of 366 and 253.7 nm."

Fluorometry

A Hitachi fluorospectrophotometer MPF-2 was used. Fluorescence of the grape flavor was measured in acetone solution at the concentration of 10 mg/ml. As for testing the candy, 10 g was dissolved in 150 ml of warm water, extracted twice with each 200 and 150 ml of ether, the combined ether layer was concentrated in vacuo to almost dryness and then made to 4 ml with acetone and subjected to fluorometry.

Conditions for gas chromatography

A Yanaco G-80 gas chromatograph (Yanagimoto Mfg. Co. Ltd., Kyoto) with flame ionization detecting system was used. It was equipped with glass tubes of ∅ 3 mm × 1.5 m in length packed with 5% DEGS + 1%H_3PO_4/Chromosorb W 60-80. Operating conditions were the following: column temp.: 150 C, injection port temp.: 230 C, carrier gas: N_2 (17 ml/min), and sensitivity and range: 10^{-1}, 1/64.

RESULTS AND DISCUSSION

The fruit candy showed a purple fluorescence under irradiation by the shorter wavelength of ultraviolet rays. Strong fluorescence of the same color was observed under irradiation in the artificial grape flavor preparation used for manufacture of the candy.

The fluorescent substance acted in the same manner as fluorescent dye in the elution test of fluorescent substance being absorbed on gauze tissues.

It is prescribed in the Japanese Standards of Food Additives (*3*) that both methyl anthranilate and methyl N-methylanthranilate have bluish-violet fluorescence and the presence of either or both of these two substances in the candy and grape flavor was examined. From the results on authentic samples, the fluorescence of methyl anthranilate and methyl N-methylanthranilate was characterized as shown in Table 1.

TABLE 1. *Fluorescence characteristics of anthranilates.*[a]

Substance	Excitation wavelength (nm)	Maximal emission spectrum (nm)
Methyl anthranilate	252	393
Methyl N-methyl anthranilate	258	413

[a]Note: Measured at the concentration of 1 mg/ml acetone

The fluorescence spectra of candy, methyl anthranilate and grape flavor are demonstrated in Fig. 1. The fluorescence spectrum of grape flavor was quite coincident with that of methyl anthranilate, indicating that the fluorescent substance in the candy was derived from the added grape flavor and that the fluorescence of grape flavor was due to the presence in it of methyl anthranilate.

Next, a quantitative determination was carried out gas chromatographically. Aliquots of solutions prepared for fluorescence determination were injected into a gas chromatograph. As is shown in Fig. 2, the retention time of methyl anthranilate was about 5.7 min under the operation condition, while the peak of methyl N-methyl anthranilate had been confirmed to appear earlier than that of methyl anthranilate, the retention time being around 3.5 min. Methyl anthranilate at 3.30% was present in the grape flavor preparation, while the fruit candy contained 40.0 ppm of methyl anthranilate; from the results it was calculated that the addition level of grape flavor preparation as one of the raw materials was about 0.12%.

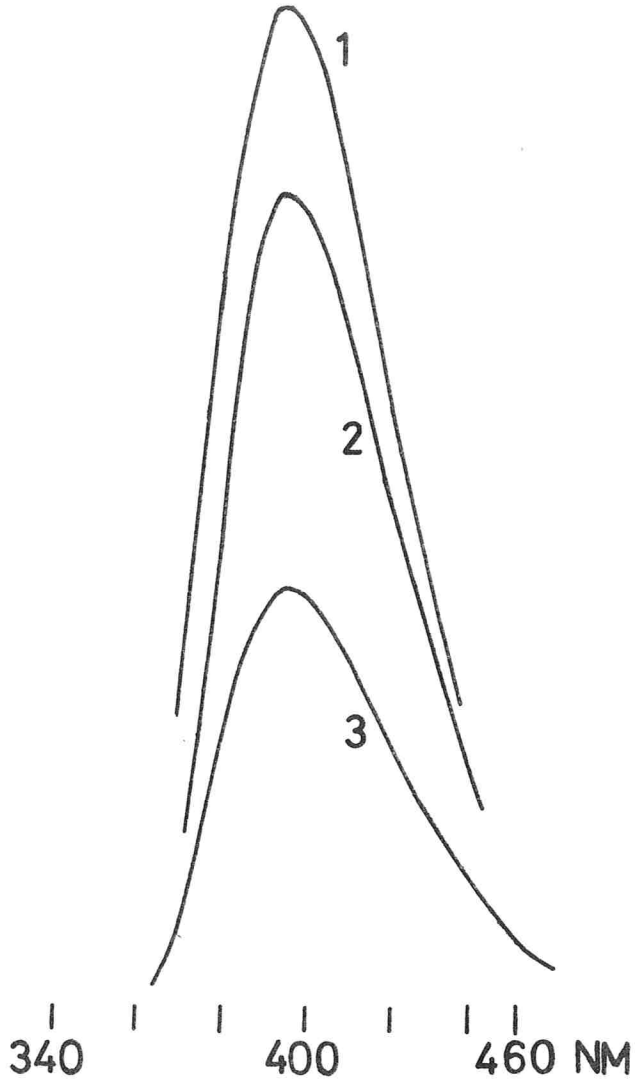


Figure 1. *Fluorescence scans of methyl anthranilate, candy and grape flavor, 1: candy, 2: methyl anthranilate, 3: grape flavor.*

Since methyl anthranilate and methyl N-methyl anthranilate are widely used for preparation of fruit flavors (melon, orange, lemon and grape), it is often encountered that candies and confectioneries show fluorescence under UV irradiation. Moreover, these two are absorbed on gauze tissues in the elution test of fluorescent substances, acting in the same manner as fluorescent dyes. Hence there is a danger of misjudging them as fluorescent dyes.

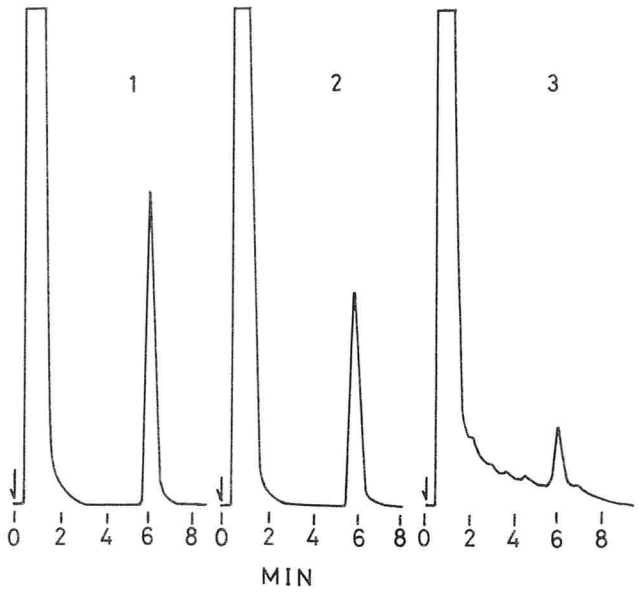


Figure 2. *Gas liquid chromatograms of methyl anthranilate, candy and grape flavor. 1: methyl anthranilate, 2: grape flavor, 3: candy.*

REFERENCES

1. Environmental Sanitation Bureau of the Ministry of Health and Welfare. 1973. Fluorescent dyes in foods. Sanitary Inspection Guide, vol. 1: pp. 475-488. Japan Food Hygiene Association.
2. Head of the Food Hygienic Section of the Ministry of Health and Welfare. 1971, May 8th. On the inspection method of container-package containing fluorescent substance. Communication No. 244.
3. Ministry of Health and Welfare. 1974. The Japanese standards of food additives, 3rd ed. The Japanese Union of Food Additives Association. pp. 319, 325.
4. Williams, A. T. R., and W. Slavin. 1977. Determination of methyl anthranilate in grape beverages by high-pressure liquid chromatography and fluorescence. J. Agric. Food Chem. 25:756-759.

Journal of Food Protection Vol. 42, No. 8, Pages 660-661 (August, 1979)

A Research Note

Recovering Low Levels of Various *Salmonella* Serotypes from Deep-frozen Broiler Carcasses by Direct Enrichment

N. A. COX* and A. J. MERCURI

Microbiology Research Unit, United States Department of Agriculture, Science and Education Administration, Federal Research, Richard B. Russell Research Center, Athens, Georgia 30604

(Received for publication November 22, 1978)

ABSTRACT

Two hundred and forty broiler carcass halves were each inoculated with either 14 or 180 cells of *Salmonella typhimurium*. Each carcass half was then placed in a plastic bag, blast-frozen (-40 C) for 6 h, and stored at -23 C. After 1, 7 and 30 days of frozen storage, 80 of these samples were removed and allowed to thaw; then each carcass-half was shaken in its bag with 150 ml of added sterile water. Lactose broth was used to preenrich 40 of these rinse-fluid samples and selenite cystine broth was used for direct enrichment of the remaining 40 samples. *S. typhimurium* was successfully recovered from all 240 samples. Other serotypes successfully recovered by direct enrichment on similarly frozen carcass-halves stored for 30 days were *Salmonella california, Salmonella derby, Salmonella heidelberg, Salmonella montevideo, Salmonella newport* and *Salmonella senftenberg*. These data suggest that a preenrichment medium such as lactose broth may not be necessary for detection of salmonella on frozen broiler carcasses.

There have been several studies on the effect of freezing on bacterial cells. Ulrich and Halvorson (*13*) reported that the greatest destruction of viable cells occurred in the first 24 h of freezing. Van Eseltine et al. (*15*) observed that rapid freezing or freezing at temperatures below -24 C did not result in a reduction of bacterial numbers. According to Thomason et al. (*12*), preenrichment in lactose broth as suggested by North (*8*) has become the method for isolating salmonellae from dried and frozen foods in the United States. Silliker and Gabis (*10*) preenriched frozen raw meat samples in lactose broth for detection of salmonellae. The U.S. Department of Agriculture in the APHIS *Microbiology Laboratory Guidebook* (*14*) recommends that lactose broth be used for preenriching raw meat and products that have been subjected to freezing, drying or chemical treatment. Recently, it was suggested that buffered peptone water be used for preenrichment of sublethally injured salmonellae in frozen meat samples (*3*). We could find no evidence in the literature, however, to indicate that use of a preenrichment medium for detecting salmonellae in frozen poultry is actually necessary. A number of researchers working with unfrozen broiler carcasses have included the preenrichment step (*2,4,5*), perhaps in the belief that the bacteria on the carcass were subjected to considerable stress during commercial processing. In a recent study (*1*) with unfrozen broiler carcasses, however, we found that preenrichment with lactose broth did not increase the number of salmonella-positive samples when compared to direct enrichment with selenite cystine broth. This study was undertaken to determine if enrichment with selenite cystine, without a preenrichment step, could also be used for recovery of salmonella from broiler carcasses that had been blast frozen and stored at -23 C for up to 30 days.

MATERIALS AND METHODS

Experiment 1

Each of 120 freshly processed broiler carcasses was cut in half along the longitudinal axis. The carcass-halves in one group were each inoculated with a low level (about 14 cells) and those in the second group with a high level (about 180 cells) of a strain of *Salmonella typhimurium* resistant to 100 ppm of nalidixic acid. The inoculum (0.1 ml) was pipetted onto each carcass-half and thoroughly rubbed into the skin using a sterile bent glass rod. After inoculation, each carcass-half was placed in a plastic bag that was closed with a twist tie, then blast frozen (-40 C) for 6 h, and stored at -23 C. After 1, 7 and 30 days, 80 of the 240 halves were removed from storage and allowed to thaw completely; then each was vigorously shaken for 1 min in its bag with 150 ml of added sterile water. Concentrated (10×) lactose broth (Difco) was added to 40 of these rinse fluid samples to yield a single-strength preenrichment medium. Concentrated (10×) selenite cystine broth (Difco) was added to the remaining 40 samples to yield a single-strength direct enrichment medium. All 80 samples were then incubated for 24 h at 37 C. From the lactose samples, 10-ml portions were each aseptically transferred to 90 ml of selenite cystine broth and incubated for 24 h at 37 C. Three loopfuls (3-mm loop) of each of the 80 samples incubated for 24 h at 37 C in selenite cystine were then streaked onto MacConkey agar (Difco) plates containing 100 ppm of nalidixic acid. The plates were also incubated for 24 h at 37 C. Colonies appearing on these plates were picked and serologically confirmed to be our marker organism.

Experiment 2

After observing the results of Experiment 1, we conducted a second experiment in which low levels (about 16-42 cells) of six additional strains of salmonellae resistant to nalidixic acid (*Salmonella california, Salmonella derby, Salmonella heidelberg, Salmonella montevideo, Salmonella newport,* and *Salmonella senftenberg*) were each inoculated onto 40 broiler carcass-halves. These 240 (6 strains × 40 carcasses) halves were each placed in a plastic bag, blast frozen (-40 C) for 6 h, and stored at -23 C for 30 days. Following this, they were removed, thawed, and sampled using the identical procedure described in Experiment 1.

RESULTS AND DISCUSSION

In Experiment 1, we recovered the inoculated *S.*

typhimurium from all 240 broiler carcass halves regardless of inoculum level, length of frozen storage and/or medium used. The 24 h enrichment with selenite cystine was as effective as the 48-h preenrichment (lactose)-enrichment procedure in detecting the marker organism. Both methods resulted in 100% detection (20 positive recoveries from 20 samples).

For the second experiment we used only an inoculum level of approximately 30 cells because a number of published reports have indicated that levels of salmonellae on salmonella-positive carcasses are extremely low, e.g., 1 to 30/carcass (*11*), an average of 17/100 g of skin (*7*), and less than 100/100 g of skin (*6*). With *S. california, S. derby, S. heidelberg, S. montevideo, S. newport* and *S. senftenberg,* the single enrichment and the preenrichment followed by enrichment methods yielded the same number of positive detections (Table 1).

TABLE 1. *Recovery of six* Salmonella *serotypes from frozen broiler carcasses after 30 days of storage (-23 C) with lactose and selenite cystine broth.*

Salmonella serotype	Inoculum level (No. of cells)	Medium used[a]	
		Lactose	Selenite cystine
S. california	16	20/20	20/20
S. derby	42	20/20	20/20
S. heidelberg	32	20/20	20/20
S. montevideo	24	20/20	19/20
S. newport	32	20/20	20/20
S. senftenberg	38	19/20	20/20

[a]Number of positive recoveries/number of samples.

The results of this study strongly suggest that a preenrichment, or recovery, medium such as lactose may not be necessary for detecting the presence of low levels of salmonellae on frozen broiler carcasses. Under our experimental conditions, the stress of freezing and thawing, frozen storage, or a combination of these, was apparently not severe enough to warrant preenrichment. Ray and Speck (*9*) showed greater recovery of the nonlethally injured cells of frozen *S. anatum* NF3 with selenite cystine broth than with tetrathionate broth. Our use of selenite cystine broth in this study may partly explain our success in recovering salmonellae from frozen carcasses. A number of compounds both inside and outside the cells, e.g., amino acids, peptones, milk proteins, etc., act as cryoprotective agents, protecting many bacterial cells against death and injury from freezing (*9*). The presence of many of these substances in the skin of the broiler carcass may also partly explain our results.

Preenrichment is more laborious and takes longer (up to 24 additional hours) than direct enrichment. Therefore, since preenrichment does not increase the number of salmonella-positive samples, and in this study it certainly did not, then it should be omitted as part of the recommended sampling procedure. We realize that this procedural modification may not be readily accepted by some researchers because preenrichment has been considered imperative for recovery of salmonella from frozen foods for many years. We feel that the results of this study warrant the further investigation of direct enrichment procedures for recovering salmonellae from broiler carcasses stored for long periods, as well as from other frozen foods. Since the extent to which a cell will be damaged by freezing can be affected by the physiological state of the cell as well as by the surrounding environment, direct enrichment for recovering naturally occurring salmonellae on frozen broiler carcasses should be investigated.

REFERENCES

1. Cox, N. A., A. J. Mercuri, D. A. Tanner, M. O. Carson, J. E. Thomson, and J. S. Bailey. 1978. Effectiveness of sampling methods for *Salmonella* detection on processed broilers. J. Food Prot. 41: 341-343.
2. Dougherty, T. J. 1974. *Salmonella* contamination in a commercial poultry (broiler) processing operation. Poultry Sci. 53:814-821.
3. Edel, W., and E. H. Kampelmacher. 1973. Comparative studies on the isolation of "sub-lethally injured" salmonellae in nine European laboratories. Bull. W.H.O. 48:167-174.
4. Gabis, D. A., and J. H. Silliker. 1974. ICMSF Method Studies. II. Comparison of analytical schemes for detection of *Salmonella* in high-moisture foods. Can. J. Microbiol. 20:663-669.
5. Hall, H. E., D. F. Brown, and R. Angelotti. 1964. The quantification of salmonellae in foods by using the lactose pre-enrichment method of North. J. Milk Food Technol. 27:235-240.
6. Mulder, R. W. A. W., S. Notermans, and E. H. Kampelmacher. 1977. Inactivation of *Salmonella* on chilled and deep frozen broiler carcasses by irradiation. J. Appl. Bacteriol. 42:179-185.
7. Notermans, S., M. Van Schothorst, F. M. Van Leusden, and E. H. Kampelmacher. 1975. Onderzoekingen over het kwantitatief voorkomen van salmonellae bij diepvrieskuikens. Tijdschrift voor Diergeneeskunde 100:648-653.
8. North, W. R., Jr. 1961. Lactose pre-enrichment method for isolation of *Salmonella* from dried egg albumen. Its use in a survey of commercially produced albumen. Appl. Microbiol. 9:188-195.
9. Ray, B., and M. L. Speck. 1973. Freeze-injury in bacteria. CRC Crit. Rev. Clin. Lab. Sci. 4:161-213.
10. Silliker, J. H., and D. A. Gabis. 1974. ICMSF methods studies. V. The influence of selective enrichment media and incubation temperatures on the detection of salmonellae in raw frozen meats. Can. J. Microbiol. 20:813-816.
11. Surkiewicz, B. F., R. W. Johnston, A. B. Moran, and G. W. Krumm. 1969. A bacteriological survey of chicken eviscerating plants. Food Technol. 23:80-85.
12. Thomason, B. M., D. J. Dodd, and W. B. Cherry. 1977. Increased recovery of salmonellae from environmental samples enriched with buffered peptone water. Appl. Environ. Microbiol. 34:270-273.
13. Ulrich, J. A., and H. O. Halvorson. 1947. Changes in foods during storage in frozen conditions. Minn. Univ. Hormel Inst. Ann. Rept., 48:48-52.
14. USDA. 1974. Microbiology laboratory guidebook. Animal, Plant and Health Inspection Service, Washington, D.C.
15. Van Eseltine, W. P., L. F. Nellis, F. A. Lee, and G. J. Hucker. 1948. Effect of rate of freezing on bacterial content of frozen vegetables. Food Res. 13:271-280.

Journal of Food Protection Vol. 42, No. 8, Pages 662-663 (August, 1979)

A Research Note

Incidence of *Salmonella* in Pork and Poultry Products

C. L. DUITSCHAEVER* and CAROLE BUTEAU

Department of Food Science, University of Guelph, Guelph, Ontario, N1G 2W1, Canada

(Received for publication November 24, 1978)

ABSTRACT

A total of 223 retail samples consisting of pork sausage (105), ground pork (25), pork chops (50), barbecued pork (33), turkey sausage (3) and chicken pieces (7) was analyzed and revealed the presence of *Salmonella* sp. in 16.14% of the samples. Of the 10 different serotypes isolated, *Salmonella agona* was predominant.

Salmonellosis remains one of the major foodborne health hazards and meat and meat products play an important role as a reservoir and in dissemination of salmonellae (*3-7, 9-14*). A survey of pork meat products and comminuted turkey meat carried out in the U.S.A. revealed the presence of *Salmonella* sp. in 28% of pork sausage samples (*12*) and in 28% of fresh (*6*) and in 38% of frozen turkey meat samples (*7*). Fresh turkey sausage is a relatively new product and not widely available in the retail market in Canada. Only a few samples could be obtained at the time of this study. Because of the present concern and impending legislation regarding the safety of barbecued meats in Canada (*8*) samples of Chinese-style barbecued pork were included in this survey. This limited and preliminary study was commissioned by the Canadian Broadcasting Corporation and reports on the incidence of *Salmonella* in pork meat products and a few samples of poultry products purchased at random in retail stores.

MATERIALS AND METHODS

A total of 223 samples (Table 1) was analyzed. The samples, in approximately 500-g quantities, were purchased at random, simulating consumer practice, from retail outlets in the Toronto area over a period of 22 weeks during the summer of 1978. The samples were transported to the laboratory in an insulated ice chest and analyzed within 2-4 h after purchase. The experimental procedure consisted of homogenizing, where applicable, 50 g of sample in a Waring blender with 450 g of lactose broth. For pork chops, barbecued pork and chicken samples, the pieces were placed in a sterile plastic bag, 500 ml of lactose broth was added and the contents were vigorously shaken for 1 min. The homogenate and the rinse plus contents were then incubated at 41 ± 1 C for 24 h (*4,11*, lactose pre-enrichment). The rationale for using 41 C for pre-enrichment was that elevated temperature suppresses many competing gram-negative bacteria and aids in dispersal of fat in the food sample. After incubation, 10 ml were transferred to each of 100 ml tetrathionate-novobiocin and selenite-cystine broth for enrichment at 37 C for 24 h. A loopful (0.01 ml) of each of these broths was streaked onto salmonella-shigella, bismuth-sulfite and xylose-lactose-desoxycholate agar plates which were incubated for 18-24 h at 37 C and checked for suspect colonies. Negative plates were reincubated for 24 h. At least two suspect colonies were picked from each plate, purified on MacConkey agar (18-24 h at 37 C) and transferred onto triple-sugar-iron (TSI), lysine-iron-agar (LIA) slants and into malonate broth. Isolates showing reactions typical of salmonellae were examined for further biochemical identification using the microscreening system (API 20, *1*). Cultures, presumptively positive for salmonellae, were confirmed by serology using *Salmonella* "O" antiserum (Difco) and by serotyping.

TABLE 1. *Incidence of* Salmonella *in retail meat samples.*

Product	No. of samples	No. of samples containing salmonellae	Percent
Sausage (pork)	105	15	14
Sausage (turkey)	3	3	100
Ground pork	25	5	20
Pork chops	50	7	14
Cut-up chicken	7	5	71
Barbecued back pork	33	1	3
Total	223	36	16.14

RESULTS AND DISCUSSION

The incidence of contamination by salmonellae in each type of product is given in Table 1. The extent of contamination in 180 fresh pork meat samples was 15%. The overall incidence was 16.14% for all 223 samples. Other workers have reported percentages of contamination by *Salmonella* of pork meat products varying from 21.5% (*14*), 22.8% (*10*), 23% (*5*), 28% (*12*) to 38% (*13*). The results of the present study show that a significant portion of fresh pork meat products available in the retail market in Canada is contaminated with salmonellae. Although only a few samples of raw chicken pieces were included in this survey, the recovery of salmonellae from 5 of 7 samples suggests that poultry remain a potentially dangerous reservoir of these pathogens (*4*). There were also only three samples of turkey sausage analyzed for salmonellae. Therefore, no results of any real meaning could be obtained. But the fact that these three samples were positive suggests that turkey sausage is a product with a high degree of contamination with *Salmonella*.

The serotypes of salmonellae isolated are shown in Table 2. *Salmonella agona* was isolated most frequently and from pork meat only. Isolation of that serotype from human sources has been increasing since 1970 (*2*).

Results of this limited study indicate that there is a serious problem with *Salmonella* contamination in fresh

TABLE 2. *Serotypes of* Salmonella *isolated from retail meat samples.*

Serotype	Type of product						Total isolates
	Sausage (pork)	Sausage (turkey)	Ground pork	Pork chops	Chicken pieces	Grilled pork	
S. agona	4		4	2		1	11
S. anatum	1						1
S. derby	1						1
S. hadar		1		1			2
S. infantis	4						4
S. london				3			3
S. saint-paul	2	2			1		5
S. schwarzengrund	1						1
S. thomasville			1				1
S. typhimurium	3[a]			1	4		8
	16	3	5	7	5	1	37

[a]One sample contained both, *S. agona* and *S. typhimurium*.

pork and comminuted turkey meat products sold at the retail level in Canada. These preliminary results also support the desirability to gather additional national data to assess the extent of the contamination. The presence of *Salmonella* in ready-to-eat Chinese-style barbecued pork emphasizes the need for adequate legislation regarding temperatures of storage of this product in retail stores.

ACKNOWLEDGMENTS

This research was supported in part by the Canadian Broadcasting Corporation (Toronto) and by the Ontario Ministry of Agriculture and Food. We appreciate the assistance of the Laboratory Services Branch, Ministry of Health, Toronto, Ontario in the serotyping of the isolates.

REFERENCES

1. Analytab Products Inc., 919 Third Avenue, New York, N.Y. 10022.
2. Anon. 1975. Salmonella surveillance reports. Annual summary. Center for Disease Control, Atlanta, Ga., U.S.A.
3. Childers, A. B., E. E. Keahey, and P. G. Vincent. 1973. Sources of salmonellae contamination of meat slaughtering procedures. II. J. Milk Food Technol. 36:635-638.
4. Duitschaever, C. L. 1977. Incidence of *Salmonella* in raw cut-up chicken. J. Food Prot. 40:191-192.
5. Galton, M. M., W. D. Lowery, and A. V. Hardy. 1954. *Salmonella* in fresh and smoked pork sausages. J. Infect. Dis. 95:232-235.
6. Guthertz, L. S., J. T. Fruin, D. Spicer, and J. L. Fowler. 1976. Microbiology of fresh comminuted turkey meat. J. Milk Food Technol. 39:823-829.
7. Guthertz, L. S., J. T. Fruin, R. L. Ukolur, and J. L. Fowler. 1977. Microbial quality of frozen comminuted turkey meat. J. Food Sci. 42:1344-1347.
8. Health and Welfare Canada. 1978. News release, 85, June 2.
9. Ladiges, W. C., and J. F. Foster. 1974. Incidence of *Salmonella* in beef and chicken. J. Milk Food Technol. 37:213-214.
10. Roberts, D., K. Boag, and M. L. M. Hall, and C. R. Shipp. 1975. The isolation of salmonellas from British pork sausages and sausage meat. J. Hyg., Camb. 75:173-184.
11. Seligman, R., and H. Frank-Blum. 1974. Microbial quality of barbecued chicken from commercial rotisseries. J. Milk Food Technol. 37:473-476.
12. Surkiewicz, B. F., R. W. Johnston, R. P. Elliott, and E. R. Simmons. 1972. Bacteriological survey of fresh pork sausage produced at establishments under federal inspection. Appl. Microbiol. 23:515-520.
13. Weissman, M. A., and J. A. Carpenter. 1969. Incidence of salmonellae in meat and meat products. Appl. Microbiol. 17:899-902.
14. Swaminathan, B., M. A. B. Link, and J. C. Ayres. 1978. Incidence of salmonellae in raw meat and poultry samples in retail stores. J. Food Prot. 41:518-520.

1979 Joint Meeting of ADMI/WPI Held in Chicago

A. H. Kaemmer, President of the American Dry Milk Institute, and Pete Frigo, President of the Whey Products Institute, joined in welcoming members and guests to the joint Annual Meeting of the two organizations in Chicago, April 25-27, 1979. This was the 8th Annual Meeting for WPI and the 54th Annual Meeting for ADMI. Registration for the meeting exceeded 500, the largest participation since the joint Annual Meetings of the two Institutes were first held in 1972.

Kaemmer's opening remarks touched briefly on pending federal regulations concerning dry milk manufacturers, the need to conserve energy, and other matters of industry interest. Frigo noted with encouragement the pending initiation of whey industry programs in Washington, the establishment of specific areas of primary Institute activity, and he urged member participation in research activities involving current energy related proposals.

H. F. Paul, Jr., Business Manager for the American Dry Milk Institute and the Whey Products Institute, reported production and sales statistics for 1978. Total nonfat dry milk production for 1978 was 928.8 million pounds, a decrease of 177 million pounds, or 16% under 1977.

Paul continued with a report of production and sales trends of whey products during 1978, as compiled from a Whey Products Institute survey of cooperating whey processors, estimated to include 80% of total domestic sales. Sales (on a whey solids basis) as reported in the survey were: sweet-type dry whey, 386.0 million pounds; modified whey, 42.3 million pounds; condensed whey, 69.6 million pounds; lactose, 93.4 million pounds. Over 409 million pounds of whey solids were reported sold in animal feed products.

Journal of Food Protection Vol. 42, No. 8, Pages 664-665 (August, 1979)

A Research Note

Antibacterial Activity of Yogurt Starter in Cow and Buffalo Milk

JASJIT SINGH, ADARSH KHANNA and HARISH CHANDER

Division of Dairy Bacteriology, National Dairy Research Institute, Karnal - 132001, India

(Received for publication November 28, 1978)

ABSTRACT

A comparative assessment of the antibacterial activity of pure and mixed-strain cultures of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* against *Escherichia coli, Staphylococcus aureus, Pseudomonas fragi* and *Micrococcus flavus* was made using cow and buffalo milk. A culture filtrate from *S. thermophilus* exhibited no antagonistic action against any of the test organisms, irrespective of the type of milk used and the period of incubation. Culture filtrates from *L. bulgaricus* and the mixed culture caused pronounced inhibition of all the test organisms used. Antibacterial activity of yogurt culture was greater in buffalo than cow milk.

Although the antibacterial activity of lactic acid bacteria in cow's milk has been investigated, the research work on the role of these cultures in milk of different species like buffalo milk has started gathering momentum only recently. In India, both cow and buffalo milk are used for production of fermented milk products. Dutta et al. (*3*) and Singh and Ranganathan (*5*) noted considerable variations in regard to the biochemical performance by lactic acid bacteria grown in milk of different species. The inhibitory action which has been shown for growing as well as cell-free culture filtrates of lactic acid bacteria has been attributed to the influence of pH, lactic acid, hydrogen peroxide and unidentified antibacterial substances (*2,6-8*).

The present study was undertaken with a view to show the antibacterial activity of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in cow and buffalo milk.

MATERIALS AND METHODS

Pure and mixed strains of *S. thermophilus* (Hst) and *L. bulgaricus* (RTS) were inoculated into cow and buffalo milk at the 1% level. In case of mixed-strain inoculum, 0.5% of each culture was added before incubation at 37 C for 16 and 24 h. The coagulated milk was centrifuged at 3000 rpm for 15 min. and clear supernatant whey (presumed to contain antibacterial substances) was sterilized by Seitz filtration. The supernatant liquid was used for testing inhibitory action with the agar cup (modified well) assay technique (*6*) against strains of *Staphylococcus aureus, Escherichia coli, Pseudomonas fragi* and *Micrococcus flavus* associated with the spoilage of yogurt and other milk preparations. Plates were filled with about 15 ml of the medium seeded with 0.1 ml of the culture of the test organism. After the agar had solidified, four to five wells of uniform size (6 mm dia.) were made on the agar surface and 0.02-ml amounts of the culture filtrate were introduced into each well. The plates were incubated at 37 C for 24 h and examined for formation of clear zones of inhibition of growth around the wells.

RESULTS AND DISCUSSION

The results presented in Table 1 on the comparative assessment of antibacterial activity of pure and mixed strain cultures of *S. thermophilus* and *L. bulgaricus* against *E. coli, S. aureus, P. fragi* and *M. flavus* indicate that culture filtrate from *S. thermophilus* exhibited no antagonistic action against any of the test organisms, irrespective of the period of incubation and the type of milk used. These findings are in conformity with those of Akopyan et al. (*1*) who reported no inhibitory activity of *S. thermophilus* against *E. coli*. The earlier report of

TABLE 1. *Estimation of antibacterial activity of cell-free filtrates from pure and mixed strain cultures of* Streptococcus thermophilus *and* Lactobacillus bulgaricus *in cow and buffalo milk.*[a]

Test organism	Period of incubation (h)	Cell-free filtrates					
		S. thermophilus		*L. bulgaricus*		*S. thermophilus* + *L. bulgaricus*	
		CM[b]	BM[c]	CM	BM	CM	BM
		(Diameter of zone of inhibition in mm)[d]					
Staphylococcus aureus	16	0	0	11.78	14.32	15.59	19.04
	24	0	0	13.75	16.58	16.52	21.05
Escherichia coli	16	0	0	12.24	16.86	14.32	24.48
	24	0	0	16.85	19.65	18.25	27.35
Pseudomonas fragi	16	0	0	11.78	13.05	12.70	14.32
	24	0	0	13.25	17.52	17.50	19.25
Micrococcus flavus	16	0	0	24.48	19.04	27.02	29.56
	24	0	0	25.65	22.56	29.58	30.05

[a]Agar well method used; results of representative trials.
[b]CM = Cow milk.
[c]BM = Buffalo milk.
[d]Excluding diameter of well = 6 mm (0.02 ml of each cell-free filtrate introduced into wells on seeded agar plates).

Rasid and Mitic (*4*) regarding the inhibitory activity of *S thermophilus* is in contradiction to the current observations and it may be due to differences in the nature of strains or the type of suspending medium.

Culture filtrate from *L. bulgaricus* caused pronounced inhibition of all the test organisms used. Further, it was interesting to note that the mixed culture showed greater inhibition as compared to *L. bulgaricus* alone. These observations emphasized the probable role of *S. thermophilus* in stimulation of the antibacterial activity of *L. bulgaricus* in mixed culture. As regards the type of milk used, the results show greater antagonistic action of yogurt cultures in buffalo than cow milk.

In view of the lack of available information on the inhibitory action of yogurt cultures, the current report on the antibacterial activity of mixed culture of *S. thermophilus* and *L. bulgaricus* in buffalo milk should be of interest.

REFERENCES

1. Akopyan, L. G., and F. G. Sarukhanyan. 1974. Antagonistic properties of lactic streptococci. Biologicheskii Zhurnal Armenii 27:17-22. (Cited in Dairy Sci. Abst. 1975, 37, 779).
2. Dahiya, R. S., and M. L. Speck. 1968. Hydrogen peroxide formation by lactobacilli and its effect on *Staphylococcus aureus*. J. Dairy Sci. 51:1568-1572.
3. Dutta, S. M., R. K. Kuila, B. Ranganathan, and H. Laxminarayana. 1971. A comparative study of the activity of starter cultures in different types of milk. Milchwissenschaft 26:158-161.
4. Rasic, J., and S. Mitic. 1963. Study of the antibacterial activity of yogurt cultures. Le Lait 43:489-499.
5. Singh, J., and B. Ranganathan. 1978. A comparison of the activity of *Lactobacillus bulgaricus* and one of its mutants in different types of milk. J. Dairy Res. 45:123-125.
6. Singh, J., and H. Laxminarayana. 1973. Antibacterial activity of lactobacilli. Indian J. Dairy Sci. 26:135-136.
7. Shahani, K. M., J. R. Vakil, and A. Kilara. 1976. Natural antibiotic activity of *Lactobacillus acidophilus* and *bulgaricus*. Cultured Dairy Products J. 11:14-17.
8. Spillmann, H., Z. Puhan, and M. Banhegyi. 1978. Antimicrobial activity of thermophilic lactobacilli. Milchwissenschaft 33:148-153.

IFT Award Winners, *con't from p. 650*

recognizes an individual's efforts to promote the international exchange of ideas and understanding in the field of food technology. It includes an inscribed silver salver and a $1000 honorarium.

Dr. Hawthorn received his degrees from the Glasgow University and the University of Glasgow. His work has included studying the movement of radioactive materials through the food chain, part of the special problems created in Scotland in the late 1950's following radioactive fallout from weapons testing. He helped convert the Scottish School of Bakery of Glasgow's Royal College of Science & Technology into a modern dept. of food science. He was appointed to the first chair of food science in the United Kingdom, in that department. Dr. Hawthorn helped establish the British Institute of Food Science and Technology and the International Union of Food Science and Technology. He has worked closely with the departments of nutrition at the Universities of nutrition at the Universities of Ghana and Ife and served as advisor to the Universities of Singapore and Malaysia.

Jointly awarded the 1979 Food Technology Industrial Achievement Award were Armour Research Center of Armour & Co. and Grumman Allied Industries, a subsidiary of Grumman Corp. They were recognized for their development and manufacture of a hypobaric system for the transportation and warehousing of fresh fruits, vegetables, and meats

The award is presented to recognize an outstanding food process or application which represents a significant advance, successfully applied to a commercial operation.

The award is in the form of bronze plaques presented to each of the organizations involved in the development.

The new system, the Dormovac System, extends the transportation and storage life of perishable commodities by up to six times. It combines low pressure with high humidity and low temperatures to provide a controlled environment. Undesirable gases released by the commodity are continuously flushed away.

The hypothesis of "dormancy under vacuum" was first developed for fresh fruit in 1962 by Dr. Stanley Burg, then of the University of Miami Medical School. He approached Grumman Allied Industries to build a commercial prototype.

The Armour Research Center purchased the first Dormavac prototype for determing its use with fresh meats. Fresh pork was first shipped to Hawaii in 1977 and lamb and beef were shipped to Iran in 1978.

NRA Offers Sanitation Manual

The National Restaurant Association's "Sanitation Operations Manual," is a sanitation guide designed primarily for the restaurant owner or operator, to help him meet the public health responsibilities of a food service operation. Many practicing sanitarians may also find this a useful working tool.

Information covered in the manual includes food care, personnel, equipment, facilities, the local ordinance, program development, microbiology, foodborne diseases, and public health information.

The manual costs $20.00 and may be ordered from NRA at the following address:
Educational Materials Center
National Restaurant Association
Suite 2600
One IBM Plaza
Chicago, IL 60611
312-787-2525

Journal of Food Protection Vol. 42, No. 8, Pages 666-667 (August, 1979)

Changes in Soluble Nitrogen, pH and Lactic Acid During Ripening of Chabichou-type Cheese

A. F. WOLFSCHOON[1]* and M. MANSUR FURTADO

Instituto de Laticinios Candido Tostes, Departamento de Tecnologia de Alimentos, Empresa de Pesquisa Agropecuaria de Minas Gerais, 36100 Juiz de Fora, Minas Gerais, Brazil

(Received for publication November 29, 1978)

ABSTRACT

Twenty-eight samples of Chabichou-type cheese made from goat's milk in Juiz de Fora, Brazil, were tested for soluble and total nitrogen, titratable acidity (expressed as percent lactic acid), and pH during a 30-day period. Soluble N amounted to 45% of total N after 30 days, and pH and lactic acid were increased and decreased, respectively, from 5.20 to 6.82 and from 0.83 to 0.05%. The composition of 14 cheese samples was determined between the first and fourth day after production. The minimal and maximal values were as follows: 20-25% fat, 16-20% protein, 1.6-2.3% soluble protein, 51-53% moisture, 1.4-2.2% NaCl, 0.7-0.9% lactic acid and 5.2-5.3 pH.

During cheesemaking and cheese ripening a gradual protein breakdown takes place; this is a complex process in which the enzymes from rennet, from starter bacteria and from milk itself play an important role (*2,9*). In mold-ripened cheese, the phenomena occurring during cheese ripening, partially caused by the proteolytic enzymes from specific molds, contribute to development of the organoleptic characteristics of the cheese.

In ripened cheeses with surface mold of the *Penicillium roqueforti* type, the proteolytic activity of this microorganism causes a breakdown of as_1- and β-casein (*3*) and an increase in pH 4.6-soluble N to about 50% of the total N (*5*). Formation of amino acids and other nitrogenated compounds also results in an increase in pH during cheese ripening; the relation between pH and ripening days has been studied for Camembert cheese by Lenoir (*7*).

The Chabichou cheese has been produced in the Départments Vienne and Deux-Sévres as well as in Poitiers, in France for many years. Experiments were conducted at the Dairy Institute Candido Tostes (*4*) to adapt the French technique of Chabichou cheese-making to the conditions in Brazil. This cheese was produced from goat's milk and the *Penicillium glaucum* (or *roqueforti*) was used as the lipolytic and proteolytic agent during the ripening. An active proteolysis that lead to appearance of significant amounts of soluble N during ripening of the cheese was observed. Changes in pH and in lactic acid content were followed as long as the ripening took place. The composition (minimal and maximal values) of cheese was also determined. The present paper reports the results of such determinations.

[1]*Present address: Institut für Milchwissenschaft der Technischen Universität München, 8050 Freising-Weihenstephan. W. Germany.*

EXPERIMENTAL PROCEDURES

Cheese were manufactured on different dates from goat's milk according to the previously described technique (*4*). Cheese samples were taken from the ripening chamber 1,4 (n = 14), 7 (n = 3), 14 (n = 4), 21 (n = 3) and 30 (n = 4) days after production and were analysed for total and soluble N, lactic acid and pH. Between the first and fourth day (after production) 14 cheeses were analysed to determine the fat, protein, moisture and salt content, as well as the above-mentioned parameters. Each time a cheese not previously investigated was used; the samples analysed were not always from the same batch of cheese. All determinations were made in duplicate.

Total and soluble N were determined according to the method described by Kosikowski (*6*) but using 1 g of cheese and only 50 mg of the diluted sample to perform the Kjeldahl determination with a micro-Kjeldahl kit (AOAC 47.021; 1975). Fat was determined using 3 g of cheese, a Van-Gulik butyrometer, 10 ml of H_2SO_4 (d = 1.820), 5 ml of warm water and 1 ml of amyl alcohol (d = 0.815) for each determination. Determination of moisture and sodium chloride content was according to the method described by Kosikowski (*6*). The titratable acidity was measured (using 0.1 N NaOH) and the titration value of the sample was assumed to be lactic acid (*8*). The pH was determined according to Kosikowski (*6*) using a Radiometer potentiometer Model THM 26 (Denmark).

RESULTS AND DISCUSSION

Liberation of soluble nitrogen

Liberation of soluble N proceeded continuously throughout the ripening of the cheese. Soluble N increased from about 10% (first days) to about 26% of total N after 2 weeks (normal ripening period for this cheese), and up to 36% of total N at the end of 3 weeks. After 30 days, such N amounted to 45% of total N. High values for pH 4.6-soluble N were reported for Danish cheese (*5*). Levels of about 55% were found in inoculated cheeses produced aseptically and with *P. roqueforti* and *Penicillium caseicolum* (*3*). Proteolysis was evident on the surface of the Chabichou-type cheese and proceeded progressively to the center during ripening; this occurred because the *P. glaucum* was sprayed on the cheese surface where it grew. Due to its endo- and exopeptidase activity, an increase in soluble N was brought about and in a centripetal way. The changes in concentrations of soluble N with time of ripening of the Chabichou type cheese are shown in Fig. 1.

pH, lactic acid content and cheese ripening

Changes in pH paralleled the proteolytic activity in the cheese because the amino acids, peptides, peptones and amines formed during the protein breakdown contribute

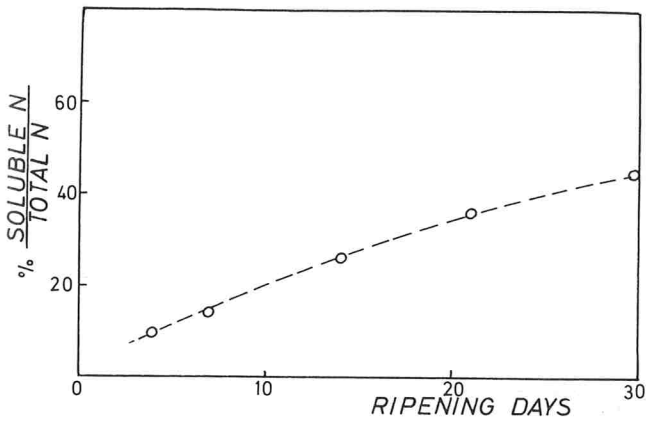


Figure 1. *Changes in concentrations of soluble nitrogen with time of ripening.*

to changes in basicity of the cheese. One day after the cheese was produced, the pH of the cheese was near 5.20, and after 7 days it increased by 0.4 pH-unit (for the same period soluble N increased approximately 4%). After 14 days, the pH was about 5.81, and after 21 days, it was slightly over 6, whereas at the end of 30 days it reached 6.82. This is shown in Fig. 2. Desmazeaud and coworkers (*3*) reported that in cheeses containing *Penicillium* molds the pH increased after 12 days of ripening, and after 27 days the pH stayed constant at about 7.

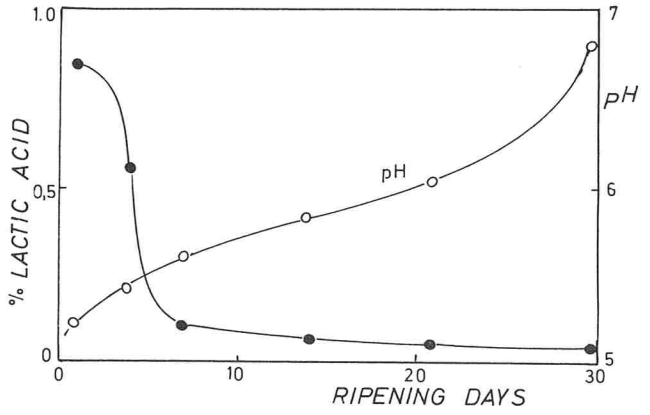


Figure 2. *Changes in pH and percent lactic acid during ripening of the Chabichou type cheese.*

On the other hand, the pH increased in the same way as the lactic acid content decreased. After cheesemaking, the percentage of lactic acid in the Chabichou cheese was about 0.85%, and after 4 days it decreased to 0.55%. After 1 week, the value decreased to about 0.10%, and at the end of 30 days it was less than 0.05% (See Fig. 2). The breakdown of sugars during Camembert ripening was studied by Berner (*1*). He showed that sugar breakdown proceeded more slowly in the interior of the cheese than in the rind, the velocity of breakdown depending on the number of microorganisms in the internal and external parts of the cheese. The decrease of lactic acid during ripening of Chabichou-type cheese probably reflects the amount metabolized by the mold. It was also observed that pH increased more rapidly in cheese rind than in the center; however, not enough data were obtained to report a mean value.

Finally, Table 1 gives the minimal and maximal values experimentally determined (n = 14) for the composition of the cheese.

TABLE 1. *Composition of Chabichou-type cheese.*[a]

Component	Minimal	Maximal
Fat (%)	20.0	25.0
Total protein (%)	16.0	20.0
Soluble protein (%)	1.6	2.3
Moisture (%)	51.0	53.0
Fat in dry matter (%)	42.0	51.2
Salt (NaCl) (%)	1.4	2.2
Lactic acid (%)	0.7	0.9
pH	5.2	5.3

[a]14 cheese samples analysed within 1 and 4 days after production.

REFERENCES

1. Berner, G. 1971. Milchsäureabbau während der Camembert-Reifung. Milchwissenschaft 26:685.
2. Desmazeaud, M. J., and J. C. Gripon. 1977. General mechanism of protein breakdown during cheese ripening. Milchwissenschaft 32:731.
3. Desmazeaud, M. J., J. C. Gripon, D. Le Bars, and J. L. Bergere. 1976. Etude du role des microorganismes et des enzymes au cours de la maturation des fromages. III. Influence des microorganismes. Le Lait 56:379.
4. Mansur Furtado, M. and A. F. Wolfschoon. 1979. Fabricacao de queijo tipo Chabichou. II. Adaptacao da tecnologia. Rev. Inst. Lat. Candido Tostes (In press).
5. Ismail, A. A., and K. Hansen. 1972. Accumulation of free amino acids during cheese ripening of some types of Danish cheese. Milchwissenschaft 27:556.
6. Kosikowski, F. 1977. Cheese and fermented milk foods. 2nd ed. Edwards Bros. Inc., Ann Arbor, Michigan, USA.
7. Lenoir, J. 1963. Note sur la degradation des protides au cours de la maturation du Camembert. Le Lait 43:154.
8. Ramos, Córdova, M. 1976. Manual de análisis de leche y lacticínios. 2nd ed. Bern, despacho 201, Mexico, D.F.
9. Visser, F. M. W., and A. E. A. Groot-Mostert. 1977. Contribution of enzymes from rennet, starter bacteria and milk to proteolysis and flavor development in Gouda cheese. 4. Protein breakdown. Netherlands Milk Dairy J. 31:247.

Journal of Food Protection Vol. 42, No. 8, Pages 668-674 (August, 1979)

Measurement of Water Activity in Foods: A Review

B. A. PRIOR

Department of Microbiology, University of the Orange Free State, P.O. Box 339, Bloemfontein 9300, South Africa

(Received for publication November 27, 1978)

ABSTRACT

The large number of methods and instruments reported in the literature to measure water activity in foods are reviewed. The methods are based on the colligative properties of solutions and water activity can be determined by: (*a*) measurement of the freezing point depression of a liquid or (*b*) measurement of the equilibrium relative humidity of a solid or liquid and conversion of these measurements to water activity. The methods are divided into those requiring simple laboratory apparatus and those requiring specialized apparatus. Of the methods requiring only simple laboratory apparatus, the water sorption isotherm method is reported to have the best precision and accuracy. Disadvantages of these methods are their limited range of measurement and long equilibration times necessary before measurement. The primary advantages of methods requiring specialized apparatus are that the water activity of the sample can be more rapidly determined, fewer manipulative steps are necessary and measurements can be made over a wider range than using simple equipment. The electric hygrometer, dew point hygrometer and vapor pressure manometer are reported to give good precision and accuracy. Some methods are unsuitable to test foods containing volatiles or excessive numbers of microorganisms.

Control of water in foods is one of man's oldest means of preserving food. This method of preservation restricts the availability of water for microbial growth and biochemical reactions. Methods to control liquid water include removal of water by drying, solidifying water by freezing and addition of electrolytes such as NaCl or non-electrolytes such as sucrose. When solutes are added to water or water is removed, changes in the colligative properties occur. For example, the vapor pressure and freezing point decrease and boiling point and osmotic pressure increase. These changes result from a decrease in activity of the solvent water.

Microbial growth and biochemical reactions are determined by the degree of availability of the water in the food and this is commonly expressed as the water activity (a_W). Water activity is defined as the ratio of the equilibrium vapor pressure of the sample (P) to the equilibrium vapor pressure of pure water (P_O) at the same temperature (*37*). Thus water activity $= \frac{P}{P_O}$ and values range between 0 and 1. While a_W is temperature dependent, it varies only slightly over the range of temperature values that permit microbial growth (*18*). However, instruments used to measure a_W must often be corrected for temperature.

Since Scott (*37*) published his classic review on the water relations of food-spoilage microorganisms, this concept of a_W has received wide acceptance among food scientists. However, its use has been limited by a lack of reliable methods of determination (*34*). The purpose of this review is to compare the variety of methods available to measure a_W.

OTHER UNITS OF MEASUREMENT RELATED TO WATER ACTIVITY

Water relations are also commonly measured in terms of equilibrium relative humidity and osmotic pressure and often similar instruments are used to measure these terms and a_W. Equilibrium relative humidity (ERH) is numerically equal to a_W but is expressed as a percentage and is always 100 times larger than the a_W value. The term "a_W" is generally preferred to ERH by food scientists since a_W defines the activity of water in solids and liquids, while ERH refers to the surrounding atmosphere (*37*).

The term "osmotic pressure" is often used by botanists in understanding water relations of plants and is inversely related to a_W by the equation:

$$\text{osmotic pressure} = \frac{-RT}{\overline{V} \ln a_W}$$

where $\overline{V}$ is the partial molal volume of water
R, the gas constant and
T is the absolute temperature (*4*).

Osmotic pressure can be expressed in terms of atmospheres, bars or ergs cm^{-3} (*8*). Biologists have divided osmotic pressure into solute osmotic pressure and matrix effects resulting from water-solid interactions at the surfaces of the colloids (*29*). Evidently food scientists have not felt this necessary in understanding water in foods.

METHODS OF MEASURING WATER ACTIVITY

The measurement of a_W is based on the colligative properties of solutions and may be measured by a number of means.

1. Measurement of the freezing point depression of a solution and conversion to a_W.
2. Measurement of the ERH of a solid or liquid. This may be determined by two means:
   (*a*) A quantity of the substance to be measured is enclosed with a small quantity of gas (usually air) and the relative humidity or vapor pressure of this gas is measured once equilibrium is reached.
   (*b*) A sample of the substance is placed in a gas at known temperature and relative humidity and the moisture absorbed or lost by the sample is determined.

In comparing these methods, the accuracy should be distinguished from the precision of a method and this has been done where possible. Often these terms have been confused in the articles under review. Precision is defined here as the reproducibility of the method when it is repeated on a homogeneous sample under controlled conditions and is represented as standard deviation (S.D.) or preferably coefficient of variation (C.V.). Accuracy refers to the degree of agreement between the a_w value measured by the test method and the true a_w value. While a method may have a high precision, there can be a significant error between the true value and the measured value. On the other hand, a method may be accurate but lack precision because of low instrument sensitivity or other factors beyond control of the analyst.

MEASUREMENT OF THE FREEZING POINT DEPRESSION

This method is most suitable for determination of a_w in solutions in the upper a_w range (> 0.8) and has been used under experimental conditions by some authors (*14,16,25,40*). The freezing point must be measured with a thermometer (either a calibrated mercury or electronic thermometer) sensitive to 0.1 C so that an a_w of three decimal places can be calculated. Usually the sample is cooled in an alcohol bath below 0 C and freezing is induced by addition of an ice crystal to the supercooled solution.

The freezing point determination of a_w is based on Raoult's Law which states that depression of the freezing point of a solution is directly related to the lowering of the vapor pressure above the solution compared to that above pure water at the same temperature and pressure. Thus a_w is also depressed (*4*). The vapor pressure of the solution is determined from the freezing point depression by referring to standard tables such as in the *Handbook of Chemistry and Physics* (*47*) and dividing by the vapor pressure of pure water to give a_w.

A second method is based on a form of Raoult's Law which states that vapor pressure of the solvent in a solution (P) divided by the vapor pressure of the pure solvent (P_o) is equal to the mole fraction of the solvent in solution (N) (*40*).

$$\text{Thus, } \frac{P}{P_o} = N = \frac{n_1}{n_1 + n_2} = a_w$$

where n_1 = number of moles of solvent in the medium
n_2 = number of moles of solute (effective)

n_2 can be determined from the freezing point depression using the following equation:

$$n_2 = \frac{G\,\Delta T_f}{1000\,K_f}$$

where G = grams of solvent used in preparation
ΔT_f = freezing point depression in °C
K_f = molal freezing point depression constant (1.86 for water).

The precision of the method does not appear to have been previously reported but Kang et al. (*16*) found the average experimental error to be ± 0.002 a_w unit.

MEASUREMENT OF THE ERH OF A SOLID OR LIQUID

Methods where the ERH of the sample are measured can be conveniently divided into those requiring simple laboratory apparatus and those requiring specialized instruments.

Methods requiring simple laboratory apparatus

Table 1 lists various methods for determination of a_w using simple laboratory apparatus. The salt-impregnated filter paper method of Kvaale and Dalhoff (*19*) is based on the fact that a salt will not dissolve unless the surrounding humidity level rises to a point which is equal to the saturation moisture content of the salt. Filter paper strips are dipped into various saturated salt solutions, dried and affixed inside the upper lid of a petri dish while the sample is placed in the lower dish. The petri dish is sealed and allowed to equilibrate for 20 h at 20 C. If the papers absorb moisture, the a_w of the sample is above that of the salt in the paper. Thus the a_w lies between the wet paper of the highest a_w and the dry paper of the lowest a_w. Limitations are the range of a_w measurement and an accuracy dependent upon the choice of salts for preparing the filter papers. The authors reported an accuracy of 0.005 a_w. The a_w of bacon has been determined by this method (*19*).

Solomon (*39*) developed a simple method for

TABLE 1. *Comparison of methods to determine a_w using simple laboratory equipment.*

Method	Range of a_w measurement	Precision	Accuracy (a_w unit)	Equilibration time (h)	Reference
Salt impregnated filter paper	0.9 -1.0	N.S.[a]	0.005	20	*19*
Humidity-induced color changes	0.3 -1.0	0.05-0.15[b]	N.S.	2	*39*
Water sorption isotherm (protein)	0.79-0.99	0.003-0.012[b]	N.S.	24	*10*
Water sorption isotherm (micro-crystalline cellulose)	0.85-0.98	1.1%-2.5%[c]	0.002-0.02	24	*21,22,44*
Graphical interpolation	0-0.989	8.6%[c] 0.2%-2.5%[c]	N.S.	1-24	*23,24,31,33*

[a]Not stated in publication.
[b]95% confidence limits in a_w unit.
[c]Coefficient of variation.

measurement of the a_w of grain based on color changes of cobalt thiocyanate with ERH. Paper is impregnated with the salt and equilibrated in the atmosphere of the sample. The paper is immediately mounted on white opal glass in oil and matched with standards of known a_w. The method was used over a wide a_w range (Table 1) and only 2 h of equilibration is necessary. Between 0.7 and 1.0 a_w, the 95% confidence limits of the method were ± 0.05 a_w unit. However, the confidence limits dropped to ± 0.15 a_w unit for measurements between 0.3 and 0.7 a_w.

A method was recently developed for measurement of a_w using a water sorption isotherm standard (Table 1) (*10,44*). The particular sample is placed in a desiccator containing a known weight of dried protein (*10*) or microcrystalline cellulose (*44*), evacuated and allowed to equilibrate. The amount of water taken up by the protein or cellulose is dependent upon the original a_w of the sample and the a_w is read off a standard water sorption isotherm. Labuza et al. (*21*) reported that microcrystalline cellulose was more stable as absorbant than the protein. The method has successfully been used to measure a wide range of meat, dairy, dessert, bakery and pet products (*10,33,38,44*). These methods are not suitable to determine the a_w of protein solutions or items that may foam and the presence of glycols in foods may also interfere (*21*).

With the graphical interpolation method of Landrock and Proctor (*23*), samples are placed in atmospheres of known relative humidities (controlled by saturated salt solutions or sulfuric acid) and allowed to equilibrate. A sample with an a_w below that of the test atmosphere will absorb moisture while the opposite occurs to samples with an a_w above the test atmosphere. The change in weight due to loss or gain of moisture is plotted against relative humidity, and the relative humidity at which no change occurs is estimated by interpolation. This value is simply converted to a_w by division by 100.

The a_w of the sample may be determined within an equilibration time of 1 h (*23*), but Leistner and Rödel (*24*) recommend 24 h at 25 C. However, spoilage of high a_w products held at this temperature and for this time can occur. The method is most suitable for determining the a_w of food products below 0.90 (*19,23*) although it has successfully been used to measure values up to 0.989 (*5*). The a_w of powdered potatoes, coffee, dried chocolate syrup, soda crackers, gelatin dessert (*23*), cheese, luncheon meat (*33*) and various meat products (*5*) has been measured. The precision of the method tends to be poor. Landrock and Proctor (*23*) reported a coefficient of variation of 8.6% for the determination of the a_w of gelatin dessert while Prior et al. (*33*) reported 2.5% for bread. Precision was much better for testing cheese and luncheon meat (*33*). An estimate of the a_w of the sample is necessary (within 0.2 a_w unit) before accurate and precise measurements can be made and this may lengthen the determination time. Errors can also occur due to loss of weight during manipulation of the food. The cumbersome nature of the method is an added disadvantage.

Water sorption isotherms (curves relating a_w to the moisture content of foods at a constant temperature) are commonly used in laboratories for the study of moisture in foods. By determining the moisture content of the food, the a_w of the sample is read off the isotherm. However, compared to the previously discussed methods, water sorption isotherms have a number of disadvantages: (*a*) variations in the composition of the food sample from that used to construct the original isotherm can result in an incorrect a_w value (*4*) and (*b*) many hygroscopic solid foods exhibit hysteresis in their water sorption isotherms (*20*). Further details are provided by Gál (*11*) and Loncin et al. (*26*).

Methods requiring specialized apparatus

The dew point method. The sample is placed in a chamber containing a mirror, sample holder and a means of detecting condensate on the mirror and allowed to equilibrate with the surrounding air space. The a_w is determined by cooling the mirror until droplets of water vapor form on the mirror at which point the temperature is measured. This is the dew point temperature and is directly related to the a_w of the sample (*2*). The a_w can also be obtained from the vapor pressure in the chamber at dew point (*7*), but Anagnostopoulos (*2*) reported that temperature measurement is usually easier. The mirror can be cooled by Peltier cooling (*2,24*) and by using coolants such as petroleum (*3*) or acetone (*7*). The dew formed may be observed visually or with a photo-electric cell (*4*).

A_w values between 0 (*7*) and 1 (*3,24*) have been measured with the dew point apparatus (Table 2). The precision (S.D.) of the method is reported by Northolt (*31*) to be 0.003 a_w unit while the accuracy varies between 0.003 and 0.005 a_w unit (Table 2). An equilibration time between 2 and 3 h is recommended before the a_w of solid food can be measured (*24*). However, the equilibration time before the a_w of liquid is measured can be as short as 10 min (*7*). An additional advantage is that determinations can be made over a wide range of temperatures of equilibration.

Most authors (*2,7,31*) have constructed their own apparatus for dew point measurement. However, Rödel and Leistner (*35*) adapted a commercial dew point hygrometer (E G and G, Waltham, MA; Model 880) to measure a_w.

The a_w of foods such as syrups (*7*), solid bakery products (*7*), wheat, sorghums, kernels, groundnut meal, coffee beans, blackseed pepper (*3*) and meats (*24*) have been measured using this technique.

Electronic hygrometers. These instruments are widely used to measure the a_w of foods in spite of their expense. Their popularity is based mainly on precision, accuracy and convenience and there are a number of different types of instruments available commercially. The instrument consists of a sensor containing a hygroscopic

material, usually LiCl, a sample chamber and a potentiometer. The conductivity of the hygroscopic material in the sensor changes according to the relative humidity in the chamber above the sample (*24,27,42*). The Sina-equihygroscope (Nova Sina, Zürich, Switzerland; marketed in U.S.A. by Beckman Instruments, Inc., Cedar Grove, N.J.) is based on this principle. The instrument is able to measure a_w between 0.02 and 0.99 by changing the sensor (Table 2). A precision (C.V.) of 0.27% (*15*) to 0.53% (*42*) has been reported while the accuracy varies between 0.002 (*43*) and 0.02 a_w unit (*21*). The accuracy of the instrument is dependent on calibration against saturated salt solutions and use of standard calibration curves (*24*). Equilibration times of 30 min (*21*) to 24 h (*15*) have been recommended for measurement of the a_w of food. Long equilibration times pose the danger that the a_w of the sample may change as a result of microbial growth.

The Hygrodynamics hygrometer (American Instrument Co., Silver Spring, MD) is similar to the Sina instrument and measures a_w values between 0.05 and 0.99 using various sensors. Precision (C.V.) between 3.6 and 4.8% (*21*) and accuracies between 0.005 (*6*) and 0.11 (*21*) have been reported.

An electric hygrometer (Phys- Chemical Research Corp., New York) used by Hägerdal and Löfqvist (*13*) to measure the a_w of food proteins is based on the change of resistance of sulfonated polystyrene in the sensor with change in relative humidity. A_w values between 0.11 and 0.92 can be measured. The maximum error of the instrument was 0.016 a_w unit while 1 h was required for equilibration. An advantage of the instrument is the small sample size (0.3 - 0.8 g) necessary for a measurement.

Some workers have reported that hygrometers are inaccurate at a_w values above 0.90 (*10,44*) and the sensors lose accuracy with age (*11,13,44*). They are also subject to errors due to absorption of volatiles such as glycerol (*42,44*). Troller (*42*), on the other hand, found the precision of the Sina-equihygroscope to improve with greater a_w values. He found contamination to be transitory depending on the degree and duration of exposure. Sensor accuracy recovered within 1 or 2 wk at ambient relative humidity and temperature. Labuza et al. (*21*) removed contaminants such as water, propylene glycol and glycerol by keeping the sensor in an evacuated desiccator. The manufacturers of the Sina-equihygroscope also supply filters that will screen the sensor from volatile compounds.

Electric hygrometers have been used to measure the a_w of a wide range of foods including meats, fermented sausage (*24*), cheese, bread, intermediate moisture foods, dry soup mix (*21*), fruit jelly and chocolate syrup (*42*).

Hair hygrometers. These instruments are extensively used for routine measurement of the a_w of meat products in food inspection laboratories in Germany (*24, 36*). The measurement of a_w is based on the change of the length of a hair with change of relative humidity in an enclosed chamber (*31*). The instrument manufactured by Lufft Metallbarometerfabriek (Stuttgart, Germany; marketed in U.S.A. by Abbeon Cal Inc., Santa Barbara, CA) has been used by Leistner's group to measure the a_w of meats in the range of 0.85 to 1.00, although the manufacturers suggest that a_w values down to 0.4 can be measured. The instrument should be calibrated at least weekly (*36*) and preferably just before use at the same temperature as for the sample. About 3 h of equilibration at constant temperature is necessary before reading (*24*), although Labuza et al. (*22*) suggested that a longer period is necessary at high a_w values. The manufacturer recommends equilibration at 20 C. If this temperature is not convenient, the a_w may be corrected for temperature, although they are most accurate when kept at constant temperature in an incubator. Rödel et al. (*36*) reported a precision (C.V.) between 0.26 and 0.36% for two sets of nine determinations of sausage, although Labuza et al. (*22*) found the instrument to be less precise (C.V. = 2.18%) when measuring the a_w of Parmesan cheese of 0.73 a_w. Measurements in most instances are within 2%

TABLE 2. *Comparison of methods to determine a_w using specialized apparatus.*

Method or instrument	Range of a_w measurement	Precision	Accuracy (a_w unit)	Equilibrium time	Reference
EG and G dew point hygrometer	0.72 - 1.00	N.S.[a]	0.003	2-3 h	*23,35*
Laboratory made dew point hygrometers	0.75 - 0.99	N.S.	0.003	15 min	*2*
	0.5 - 1.00	N.S.	0.003 - 0.005	N.S.	*3*
	—	0.003[b]	0.005	N.S.	*31*
Hygrodynamics hygrometer	0.05 - 0.99	3.6% - 4.8%[d]	0.005 - 0.11	1-24 h	*6,9,21,32,41,44*
Sina equihygroscope	0.02 - 0.99	0.27% - 0.53%[d]	0.002 - 0.02	0.5 - 24 h	*15,21,31,42,43,45*
Phys-chemical hygrometer	0.11 - 0.92	N.S.	0.016	1 h	*13*
Lufft hair hygrometer	0.4 - 1.0	0.26% - 2.18%[d]	0.02	3 h	*22,36*
Wescor pyschrometer	0.935 - 1.0	0.18% - 0.35%[d]	N.S.	1 h	*33*
Vapor pressure manometer	0 - 0.9	0.62% - 1.20%[d]	0.005[c]	1 h	*17,20,21,22,44*

[a]Not stated.
[b]Standard deviation in a_w unit.
[c]At 0.85 a_w.
[d]Coefficient of variation.

of the value of the Sina-equihygroscope when meat samples are tested (*36*). Labuza (*20*) reported that hair hygrometers are most accurate between 0.3 and 0.8 a_w. Presence of volatile glycols during long equilibration periods may damage the sensitivity of the instrument (*22*).

Psychrometers. The water relations of plant material are often measured using psychrometers (*8,46*). Prior et al. (*33*) have used a psychrometer (Wescor Inc., Logan, Utah) to measure the a_w of solutions and foods such as bread, cheese and meat. The instrument consists of a chamber containing a thermocouple. The sample is placed in the chamber and allowed to equilibrate for at least 10 min (for liquids) or 1 h (for foods). The water vapor is cooled by Peltier cooling and water vapor condenses on the thermocouple. The rate of evaporation from the thermocouple into the vapor state is proportional to the psychrometer reading and the a_w is determined from a standard curve prepared against standards of known a_w. While the thermocouple of the psychrometer may become contaminated with repeated use, it is easily cleaned.

While the range of the Wescor psychrometer is limited to a_w values between 0.935 and 1.0 (*33*), other instruments such as the electric hygrometer are reported to be inaccurate in this range (*10,44*) and thus the psychrometer can complement other instruments for measurement of moist foods. The Wescor psychrometer has a precision (C.V.) between 0.18 and 0.35% a_w unit when used to test foods (*33*).

Vapor pressure manometers. Manometers are used to determine a_w by measuring the vapor pressure directly above foods (*1,17,44*). The sample is placed in a flask connected to a manometer and evacuated so that minimum moisture is lost from the sample. The system is kept at constant temperature and the sample is allowed to equilibrate for approximately 1 h (*20*). The vapor pressure is measured. The evaporated water is then removed and the vapor pressure of the remaining gases and volatiles is measured. The a_w of the food is obtained from the difference between the two readings divided by the vapor pressure of pure water at the same temperature (*21*).

The method is effective over an a_w range between 0 and 0.9 (*17,44*). At a_w values above 0.9, the method is inaccurate because of temperature control problems (*44*). At an a_w value of 0.85, the accuracy is ± 0.005 (*1*) but above 0.85, the accuracy falls to ± 0.02 (*44*). At a_w values below 0.9, short equilibration time and poor temperature control can also lead to inaccurate results (*22*). A precision of 1.20% (C.V.) has been reported for measurement of saturated Li_2SO_4 solutions (*21*), 0.62% for Parmesan cheese and 1.02% for dog food (*22*).

Labuza's group has used the method to measure the a_w of foods such as pet foods, bread, pancake batter, cheese, soup mix and egg products (*21,38*). The method cannot be used for fermented foods or samples supporting microbial growth because of gas evolution. The presence of volatiles in the food may contribute to the vapor pressure and give erroneous results. However, foods containing glycerol and propylene glycol have been measured successfully (*21*).

Other instruments. While the osmometer is commonly used to determine osmotic pressure, Mozumber et al. (*28*) used a vapor pressure osmometer (Wescan Instruments Inc., Santa Clara, CA, Model 232) to measure the a_w of experimental solutions with a_w values greater than 0.9674. These instruments are generally limited to determining a_w in the upper range and their expense detracts from their wide use as a_w meters.

Norrish (*30*) constructed an instrument to determine the a_w of confectionery syrups based on the change of resistance of a ceramic pellet (doped and fired titanium dioxide) with a change in relative humidity. The pellet was placed in a measuring cell containing the sample and equilibrated at 25 C for 1 h. The instrument was used between 0.5 and 1.0 a_w. At a_w values between 0.9 and 0.95, an accuracy of ± 0.001 a_w was observed but fell to ± 0.02 a_w between 0.5 and 0.55 a_w.

A pressure cell was developed by Gur-Arieh et al. (*12*) to measure the a_w of flour with a high moisture content. The flour sample was allowed to equilibrate for 36 h or longer with water under pressure separated from the sample by a porous membrane. After equilibration at constant temperature the water content of the sample was analyzed. The thermodynamic relationship $\Delta P = -\frac{RT}{\overline{V}} \ln a_w$, enabled calculation of the a_w from the pressure applied (ΔP) and the moisture content of sample after equilibration. The authors reported a range of 0.67 to 0.96 a_w for the apparatus.

CONCLUSIONS

This paper has attempted to review the available information on the measurement of a_w in foods. Comparison of the methods is complicated by research workers often failing to distinguish between the precision and accuracy of a method and also using different statistical methods for determination of these parameters. Use of saturated salt solutions to check accuracy is often unreliable as different a_w values have sometimes been reported for the same salt in the literature (*21,31*).

The freezing point method and methods summarized in Table 1 have the advantage that simple laboratory equipment may be used and the cost of the determination is kept to a minimum. Most of these methods are used to measure a_w of a wide range of foods in the upper range (> 0.7 a_w). The best precision and accuracy has been reported for the water-sorption isotherm methods (*10,44*). With exception of the humidity-induced color change method (*39*), all the methods require a long equilibration time before measurement and this can sometimes result in interference by microbial growth during equilibration. Labuza et al. (*21*) prevented microbial growth during equilibration by addition of potassium sorbate to the

food being tested, but whether the salt significantly reduced the a_w of the food, was not mentioned. The filter paper method (*19*) can be done at lower temperatures to prevent microbial growth.

The principal advantages of methods requiring specialized apparatus (Table 2) are that the a_w of the sample can be more rapidly determined, the reading is often given directly in ERH or a_w, and fewer manipulative steps are necessary than when using simple equipment. Some of the instruments (Hygrodynamics and Sina hygrometers) can measure the a_w of foods over the full range. In a controlled comparative study of various instruments, Labuza et al. (*21*) recommended the vapor pressure manometer as it gave better precision and accuracy than the Hygrodynamics and Sina hygrometers when used to measure a wide range of foods and saturated salt solutions.

In a subsequent comparative study, Labuza et al. (*22*) found that the water sorption isotherm procedure and the Lufft hair hygrometer gave similar a_w readings when used to test foods and standards and were reasonably accurate. The vapor pressure manometer, on the other hand, gave a significantly lower a_w but was more precise than the other two methods.

Other factors which affect the accuracy and precision of these methods are control of equilibration temperature, frequent calibration of instruments and training of technicians to carry out determinations (*22*). While a number of instruments have temperature-compensating devices so that measurements may be made at room temperature, better results are generally obtained by equilibration at constant temperature.

ACKNOWLEDGMENT

The author thanks V. R. Smith and D. P. Theron for reviewing the manuscript.

REFERENCES

1. Acott, K. M., and T. P. Labuza. 1975. Inhibition of *Aspergillus niger* in an intermediate moisture food system. J. Food Sci. 40:137-139.
2. Anagnostopoulos, G. D. 1973. Water activity in biological systems: a dew-point method for its determination. J. Gen. Microbiol. 77: 233-235.
3. Ayerst, G. 1965. Determination of the water activity of some hygroscopic food materials by a dew point method. J. Sci. Food Agric. 16:71-78.
4. Ayerst, G. 1965. Water activity - Its measurement and significance in biology. Int. Biodeterioration Bull. 1:13-26.
5. Bem, Z., and L. Leistner. 1970. Bestimmung der Wasseraktivität von Fleisch und Fleischwaren mit der Methode von Landrock und Proctor. Die Fleischwirtschaft 50:1412-1414.
6. Beuchat, L. R. 1974. Combined effects of water activity, solute and temperature on the growth of *Vibrio parahaemolyticus*. Appl. Microbiol. 27:1075-1080.
7. Bogsanyi, D. J., and D. G. Weeden. 1968. A simple apparatus for water pressure-moisture studies by a dew point method. Chem. Ind. 23:741-742.
8. Boyer, J. S. 1969. Measurement of the water status of plants. Ann. Rev. Plant Physiol. 20:351-364.
9. Chordash, R. A., and N. N. Potter. 1972. Effects of dehydration through the intermediate moisture range on water activity, microbial growth and texture of selected foods. J. Milk Food Technol. 35:395-398.
10. Fett, H. M. 1973. Water activity determination in foods in the range 0.80 to 0.99. J. Food Sci. 38:1097-1098.
11. Gál, S. 1975. Recent advances in techniques for the determination of sorption isotherms. pp. 139-154. In R. B. Duckworth (ed.) Water relations of foods. Academic Press, London.
12. Gur-Arieh, C., A. I. Nelson, M. P. Steinberg, and L. S. Wei. 1965. Water activity of flour at high moisture contents as measured with a pressure membrane cell. J. Food Sci. 30:188-191.
13. Hägerdal, B., and B. Löfqvist. 1973. Screening method based on electric hygrometer for obtaining water sorption isotherms. J. Agric. Food Chem. 21:445-451.
14. Juven, B. J., and R. Gertshovhi. 1976. The effect of salting on the microbiology of poultry meat. J. Milk Food Technol. 39:13-17.
15. Karan-Djurdjić, S., and L. Leistner. 1970. Messung der Wasseraktivität von Fleisch und Fleischwaren mit dem SINA-Gerät. Die Fleischwirtschaft 50:1104-1106.
16. Kang, C. K., M. Woodburn, A. Pagenkopf, and R. Cheney. 1969. Growth, sporulation and germination of *Clostridium perfringens* in media of controlled water activity. Appl. Microbiol. 18:798-805.
17. Karel, M., and J. T. R. Nickerson. 1964. Effects of relative humidity, air and vacuum on browning of dehydrated orange juice. Food Technol. 18:1214-1218.
18. Krispen, K., and W. Rödel. 1976. Bedeutung der Temperatur für den a_w-Wert von Fleisch und Fleischerzeugnissen. Die Fleischwirtschaft 56:709-714.
19. Kvaale, O., and E. Dalhoff. 1963. Determination of the equilibrium relative humidity of foods. Food Technol. 17:659-661.
20. Labuza, T. P. 1975. Interpretation of sorption data in relation to the state of constituent water. pp. 155-172. In R. B. Duckworth (ed.) Water relations of foods. Academic Press, London.
21. Labuza, T. P., K. Acott, S. R. Tatini, R. Y. Lee, J. Flink, and W. McCall. 1976. Water activity determination: a collaborative study of different methods. J. Food Sci. 41:910-917.
22. Labuza, T. P., L. N. Kreisman, C. A. Heinz and P. P. Lewicki. 1977. Evaluation of the Abbeon cup analyzer compared to the VPM and Fett-Vos methods for water activity measurement. J. Food Process. Preservation 1:31-41.
23. Landrock, A. H., and B. E. Proctor. 1951. A new graphical interpolation method for obtaining humidity equilibria data, with special reference to its role in food packaging studies. Food Technol. 5:332-337.
24. Leistner, L., and W. Rödel. 1975. The significance of water activity for micro-organisms in meats. pp. 309-323. In R. B. Duckworth (ed.) Water relations of foods. Academic Press, London.
25. Limsong, S., and W. C. Frazier. 1966. Adaptation of *Pseudomonas fluorescens* to low levels of water activity produced by different solutes. Appl. Microbiol. 14:899-901.
26. Loncin, M., J. J. Bimbenet, and J. Lenges. 1968. Influence of the activity of water on the spoilage of foodstuffs. J. Food Technol. 3:131-142.
27. Mossel, D. A. A., and H. J. L. van Kuijk. 1955. A new and simple technique for the direct determination of the equilibrium relative humidity of foods. Food Res. 20:415-423.
28. Mozumber, B. K.G., N. E. Caroselli, and L. S. Albert. 1970. Influence of water activity, temperature and their interaction on germination of *Verticillium albo-atrum* conidia. Plant Physiol. 46:347-349.
29. Nobel, P. S. 1974. Introduction to biophysical plant physiology. W. H. Freeman and Co., San Francisco. 488 p.
30. Norrish, R. S. 1966. An equation for the activity coefficients and equilibrium relative humidity of water in confectionery syrups. J. Food Technol. 1:25-39.
31. Northolt, M. D. 1972. De microbiologische betekenis en het meten van de wateractiviteit (a_w) in voedingsmiddelen. Voedingsmiddelentechnologie 3:151-156.
32. Plitman, M., Y. Park, R. Gomez, and A. J. Sinskey. 1973. Viability of *Staphylococcus aureus* in intermediate moisture meats. J. Food Sci. 38:1004-1008.
33. Prior, B. A., C. Casaleggio, and H. J. J. van Vuuren. 1977. Psychrometric determination of water activity in the high a_w range. J. Food

Prot. 40:537-539.
34. Quinn, F. C. 1976. The quality of water - the "other" raw material. Paper Trade Journal, 4 Sept. :40-44.
35. Rödel, W., and L. Leistner. 1972. Messung der Wasseraktivität (a_w - Wert) von Fleisch und Fleischwaren mit einem Taupunkt-Hygrometer. Die Fleischwirtschaft 52:1461-1462.
36. Rödel, W., H. Ponert, and L. Leistner. 1975. Verbesserter a_w-Wert Messer zur Bestimmung der Wasseraktivität (a_w-Wert) von Fleisch und Fleischwaren. Die Fleischwirtschaft 55:557-558.
37. Scott, W. J. 1957. Water relations of food spoilage microorganisms. Adv. Food Res. 7:83-127.
38. Sloan, A. E., and T. P. Labuza. 1976. Prediction of water activity lowering ability of food humectants at high a_w. J. Food Sci. 41: 532-535.
39. Solomon, M. E. 1957. Estimation of humidity with colbalt thiocyanate papers and permanent colour standards. Bull. Ent. Res. 48:489-506.
40. Strong, D. H., E. M. Foster, and C. L. Duncan. 1970. Influence of water activity on the growth of *Clostridium perfringens*. Appl. Microbiol. 19:980-987.
41. Troller, J. A. 1971. Effect of water activity on enterotoxin B production and growth of *Staphylococcus aureus*. Appl. Microbiol. 21:435-439.
42. Troller, J. A. 1977. Statistical analysis of a_w measurement obtained with the Sina scope. J. Food Sci. 42:86-90.
43. Troller, J. A., and J. V. Stinson. 1975. Influence of water activity on growth and enterotoxin formation by *Staphylococcus aureus* in foods. J. Food Sci. 40:802-804.
44. Vos, P. T., and T. P. Labuza. 1974. Technique for measurement of water activity in the high a_w range. J. Agric. Food Chem. 22: 326-327.
45. Vrchlabský, J., and L. Leistner. 1970. Erprobung eines Sina-Gerätes mit eingebautem Schreiber zur Messung der Wasseraktivität von Fleisch und Fleischwaren. Die Fleischwirtschaft 50: 1106-1107.
46. Weibe, H. H., G. S. Campbell, W. H. Gardner, S. L. Rawlins, J. W. Cary, and R. W. Brown. 1971. Measurement of plant and soil water status. Bulletin 484. Utah Agric. Expt. Sta. 71 pp.
47. Wolf, A. V., M. G. Brown, and P. G. Prentiss. 1976. Concentrative properties of aqueous solutions: conversion tables. pp. D218-D267. In R. C. West (ed.) Handbook of chemistry and physics (57th ed.), C.R.C. Press, Cleveland, Ohio.

Mycoplasma Mastitis Alert

by D. E. Jasper, DVM, Ph.D, School of Veterinary Medicine, University of California, Davis 95616

Mycoplasma are small microbes intermediate in character between viruses and bacteria and several species cause mastitis.

Mastitis due to mycoplasma should be suspected if the following occurs:

1. An increase in severe mastitis cases that resist treatment but cows are not sick.
2. Mastitis cases which typically involve more than one quarter (often all four quarters) in the same cow.
3. Marked loss of production in affected cows. Some may simply just dry up.

Any of the above can occur occasionally with mastitis due to other causes but a pattern of cases like these suggests that mycoplasma may be the cause. Some infected cows do not show many signs of mastitis and may be a source of spread in the herd. Such carrier cows may also be purchased unknowingly and spread infection in a previously clean herd.

Diagnosis

Whenever mycoplasma mastitis is suspected, milk samples should be collected and submitted through a veterinarian for laboratory culture with a special request for a mycoplasma culture.

Prevention

Since some herds have become infected through purchase in infected carrier cows, it is a good practice to check all purchased cows for all mastitis bacteria, including mycoplasma, before putting the cows in the regular herd. A culture of bulk tank milk from the herd of origin may reveal the mycoplasmas are present in that herd. Never milk fresh cows in a hospital barn or string where cows with mastitis are milked. Always use very careful hygiene practices when treating cows for any kind of mastitis since careless treatment can spread mycoplasma and other serious forms of mastitis.

Control

Treatment is not usually effective. Control must therefore be accomplished by segregation and/or culling of infected cows since the major means of spread appears to be from infected cows to clean cows during milking.

Selection of specific procedures for a given herd should be made in consultation with the herd veterinarian who is familiar with circumstances at the dairy.

Current Guidelines for Elimination of Mycoplasma Mastitis from Dairy Herds

1. Culture all cows, or all cows in infected strings, using composite samples (one sample includes milk from all four quarters). Appropriate samples of bulk tank milk may help to classify strings as infected or not infected.

2. Remove all cows with positive mycoplasma milk cultures from the main milking strings of the herd. The following alternatives may be considered:

   A. Market infected cows for slaughter. This is the recommendation of choice for most severe clinical infections or for herds with only a few infected cows.

   B. Segregate infected cows. Those that recover can be milked but should not be returned to herd strings until two or more negative tests have been obtained.

   C. Dry infected cows and resample at least two times after freshening. Remove cows positive at that time.

   D. Cows without obvious mastitis and yielding only small numbers of organisms should be removed from the main milking strings until two consecutive re-examinations show them to be negative strings. Do not mingle these with clinical cows or with negative strings.

3. Monitor the herd weekly by sampling the tank milk after each string is milked once each week until four negative tests have been ob-

con't p. 678

Journal of Food Protection Vol. 42, No. 8, Pages 675-678 (August, 1979)

Quality Standards For Retail Meats

GAIL C. HOLLAND

Meat Packers Council of Canada, 5233 Dundas Street, W., Islington, Ontario M9B 1A6, Canada

(Received for publication November 24, 1978)

ABSTRACT

To be of public benefit a quality standard for meat at retail must do as it purports to do — i.e. to reduce the public hazard and/or prevent consumer deception. In addition, it must be technically workable for both the industry and the administrative branches of regulatory agencies. With processed meats and ground beef, it has not been demonstrated that they present a potential hazard. In addition, if a health hazard were demonstrated, it would not be reduced by the use of microbiological quality standards such as Aerobic Plate Count or number of *Escherichia coli*. Use of the bacterial criterion Aerobic Plate Count of 10^7/g, in place of organoleptic standards could reduce the retail shelf-life of processed meats by 20-66%. An Aerobic Plate Count, 10^7/g, could remove in excess of 33% of ground beef sold at retail level. In both instances a substantial quantity of wholesome meat would be prematurely removed from the retail market. Chemical standards such as protein, fat, moisture, cereal, are relatively static and do not substantially change over the retail shelf-life of meat. However the bacterial population in meat demonstrates a dynamic growth pattern. Hence the Aerobic Plate Count may indicate product age, but not necessarily product deterioration or potential health hazard.

E. coli, although an intestinal bacterium, is capable of growing outside the host intestine, thrive on a variety of substrates and thrive for long periods. Thus the presence of *E. coli* cannot be correlated with the extent of initial fecal contamination, nor with the presence of pathogens. A program of increased awareness of personal hygiene, temperature control, stock rotation, elimination of areas for potential cross-contamination and a regular sanitation program throughout the meat cycle will guarantee the microbiological quality of meats at retail.

Quality standards for meats at retail may be categorized as sensory attributes and chemical and microbiological criteria. Sensory quality standards may include appearance — such as color, size, shape; kinesthetics — such as texture, mouthfeel, consistency-viscosity; and flavor senses, i.e. taste and smell. The purpose of sensory standards is not so much consumer protection as it is a means for processors to determine consumer preference and to tailor a product to satisfy this preference. Sensory attributes, which generally are buyer-seller specifications, are ultimately valuable purchasing tools of the consumer.

Chemical standards include protein content, moisture, cereal, fat, salt, nitrites. Frequently chemical standards are buyer-seller specifications, or an internal manufacturing specification. However, in addition the Food and Drug Directorate and Meat Inspection Division have minimum and maximum standards to guarantee quality. In Canada the meat regulations specify maximum fat content for three grades of ground beef. For processed meats and prepared meat products, the maximum permitted cereal content of 4% reducing sugars; maximum moisture content of 60%, maximum fat and minimum protein contents are specified. Permitted levels of curing accelerators such as glucono-delta-lactone, calcium-reduced skimmilk powder and sequestrants such as polyphosphates are detailed in the regulations. The Canadian Regulations also standardize chemical composition of vegetable protein-extended and simulated meat products.

In all instances chemical quality standards are relatively static figures, i.e. within experimental error, the results of analyses are the same throughout the entire food chain.

The third type of quality standard is microbiological. The rationale for this type of standard is the significance of microorganisms in foods; they are a source of spoilage and product loss and they are causes of some foodborne epidemics. Microbiological standards may be internal store standards, or buyer-seller specifications; or they may be federal regulations. In this latter case, products which exceed arbitrarily chosen numbers are subject to prosecution under the Food and Drugs Act and Regulations.

The legal justifications for enactment of standards are the responsibilities of public agencies to protect the citizen from fraud, adulteration and health hazard. The Food and Drug Act of Canada reads as follows: "4. No person shall sell an article that (*a*) has in or upon it any poisonous or harmful substances, (*b*) is unfit for human consumption, (*c*) consists in whole or in part of any filthy, putrid, disgusting, rotten, decomposed or diseased animal or vegetable substance, (*d*) is adulterated, or (*e*) was manufactured, prepared, preserved, packaged or stored under unsatisfactory conditions."

In addition, public agencies are obliged to justify consumer confidence. This can be accomplished by exerting indirect pressures on manufacturers and compelling them to adopt methods which conform to the concepts of good manufacturing practices and personal hygiene.

It is widely recognized that microbiological assays have large natural variances, strictly due to the biological distribution of bacteria, and analytical techniques. It is not uncommon to encounter a 10-fold variation for replicates. Thus it is frequently suggested that only a difference in excess of 10-fold is significant (*2,9*). In addition, microbiological populations exhibit a dynamic growth pattern throughout the shelf-life of the meat product. The pattern of growth is that of the traditional population growth consisting of the lag phase, the

logarithmic phase of rapid population increase and the maximum stationary phase. For this reason microbiological standards at the processor are not applicable at the retail or consumer level, and conversely.

AEROBIC PLATE COUNT AS A QUALITY STANDARD

The Aerobic Plate Count has been used as a means of measuring the composite microbiological population before the onset of organoleptic deterioration. However, it needs to be defined carefully to account for the effects of processing, preservation and packaging. It usually is a poor way to predict the probability of a food to contain pathogenic microorganisms.

It is quite true that "unsatisfactory practices may be reflected in high Aerobic Plate Counts." However, the converse "high bacterial counts indicate unsatisfactory practices" is not necessarily true.

With fresh meat, when applied where numbers are not well correlated with quality, the Aerobic Plate Count has little use. High Aerobic Plate Counts for aged beef do not indicate a potential health hazard, or poor sanitation, or handling violations. According to Foster (*6*), Goepfert (*7*) and Hill et al. (*9*), Aerobic Plate Counts of 5×10^6 or 10^7/g are neither quality nor hazard criteria.

The Aerobic Plate Count does not provide differentiation, (i.e. cause and effect relationship), between the organoleptic acceptance and non-acceptance of processed meats. Nor do Aerobic Plate Counts necessarily reflect product deterioration as determined by microbiological quality. In Table 1 the effect of vacuum-packaging on microbial growth and product acceptability is demonstrated. Non-vacuum-packaged ham after 7 days storage was organoleptically unacceptable although the Aerobic Plate Count was 500,000/g. On the other hand, vacuum-packaged sliced ham was acceptable at 21 days of storage, even though the Aerobic Plate Count was 460,000,000/g.

TABLE 1. *Relationship between total count and flavor of sliced cooked ham.*[a]

Days of storage (40 F)	Package	Total count	Flavor score[b]
0	Vacuum	730	9.5
	Non-vacuum	1,400	9.5
7	Vacuum	140,000	8.5
	Non-vacuum	500,000	6.5
14	Vacuum	94,000,000	8.0
	Non-vacuum	27,000,000	6.5
21	Vacuum	460,000,000	7.6
	Non-vacuum	110,000,000	3.8
28	Vacuum	300,000,000	5.9
	Non-vacuum	183,000,000	1.4

[a]From Corlett (*2*).
[b]Average score of a 5 man panel; 10 = excellent, 7 = borderline, 1 = repulsive.

Hill et al. (*10*) found that an Aerobic Plate Count standard of 10^6/g would cause premature removal from the market-place of a very large percentage of luncheon meats which are wholesome and consumer-acceptable.

Members of the Meat Packers Council of Canada found that an organoleptic/chemical/physical/criterion did not correlate well with total Aerobic Plate Count since the product maintains a static Aerobic Plate Count of 10^8/g for a major segment of the durable shelf-life without consumer rejection.

The Council found that the Aerobic Plate Count standard of 10^6/g, when compared to organoleptic/chemical/physical/criterion, could result in a shelf-life reduction of 49-80%. An Aerobic Plate Count standard of 10^7/g could reduce the shelf-life of vacuum-packaged processed meats by 20-66%.

ESCHERICHIA COLI AS A QUALITY STANDARD

E. coli generally is considered to be the classical indicator of fecal contamination. In water, *E. coli* is a direct indicator of the extent of pollution. However, in raw meats, the number of *E. coli* does not necessarily indicate the extent of original contamination since growth may occur at abusive temperatures.

Goepfert (*7*) has stated that meat containing 1,000 (MPN) *E. coli* is no less wholesome than meat with 43 (MPN).

In addition Goepfert and Kim (*8*) concluded in a study: "There is no evidence in scientific literature, nor was any generated in this study, to suggest a correlation between the total Aerobic Plate Count or *E. coli* levels in ground beef and the potential of that material to cause food poisoning."

The Oregon Bacterial Standards Review Committee found that there was no compelling scientific justification to designate *E. coli* as the exclusive indicator of contaminated beef carcasses.

E. coli has several shortcomings as an indicator: (*a*) it is unevenly distributed in meat, (*b*) meat samples high in Aerobic Plate Count tend to suppress the *E. coli* level, and the converse is also true, (*c*) because *E. coli* can grow outside of the host intestine, thrive on a variety of substrates, and survive for long periods of time, the presence of *E. coli* cannot be correlated with extent of initial fecal contamination, and (*d*) presence of *E. coli* cannot be correlated with the presence of pathogens.

PATHOGENS AS QUALITY STANDARDS

Although we generally recognize that pathogens should not be tolerated in foods, even this statement must be considered in the light of practical experience. For fresh meats, a no tolerance level of *Salmonella* is not feasible because sampling techniques automatically involve certain tolerances, determined by sample size and sensitivity of method.

It is not yet commercially possible to market some foods in a condition that approaches the absence of pathogens. For example, most meats contain *Salmonella* at relatively high frequency and no immediate solution is in sight. A sampling plan adjusted to this situation would be more realistic and satisfactory than one based on an immeasurable ideal of complete absence (*12*).

Ingram and Kitchell (*11*) have stated: "Freedom from

pathogens is the aspect which is usually paramount in the international discussion about microbiological standards. There is the implication of the intention, by some public health authority, to use the standards statutorily to exclude foods which fail to comply. The usual phraseology is, 'such and such, a pathogen, or toxin...shall be absent.' But for commercially relevent quantities of material, absolute absence is a condition which can neither be attained by, nor verified by any practical means. It is a figment of the bacteriologist's imagination, in pursuit of which he invents increasingly impracticable methods of examination using larger and larger quantities of food, and makes increasingly difficult demands upon the manufacturer."

BENEFITS OF MICROBIOLOGICAL STANDARDS

Cleaner meat

A statement is frequently made that government standards cause a remarkable improvement in the bacterial quality of meat product. In the Oregon experience proponents of this statement showed that fresh meat samples exceeding 5×10^6 APC declined from 43% to 11% after 19 months of enactment; for processed meats, violations declined from 47% to 34%.

However these claims could not be statistically verified by the data examined by the Meat Bacterial Standards Review Committee of Oregon.

Improved sanitation in retail stores

The Oregon Bacterial Standards Review Committee found no quantitative evidence to show that retail store sanitation improved, although there was a strong feeling that there had been definite improvement.

Longer shelf-life

The effect that bacterial standards have on shelf-life is debatable. If bacterial standards are used as shelf-life criteria instead of organoleptic evaluation for processed meats, the Aerobic Plate Count of 10^7/g could result in a reduction of 20-66% of the shelf-life. For fresh meats the Aerobic Plate Count standards of 10^7/g could remove up to 40% of the ground beef from the market.

Reduced potential health hazard

Although this is a major justification for bacterial standards, "conclusive evidence remains elusive and subject to question" (Oregon Subcommittee).

To our knowledge processed meats and ground beef do not represent a health hazard. In a study by the U.S. Center for Disease Control (*4*), of 1,827 foodborne illness outbreaks, 3.6% (65) were attributed to ground beef, 0.3% (6) to cold cuts, and 1.1% (20) to frankfurters. In most of these cases, specific post-purchase food-handling errors were identified.

The CDC concluded: "these data show that ground beef, cold cuts, and frankfurters are relatively infrequently associated with outbreaks reported to the CDC and that, in outbreaks that were reported for which a place of contamination was identified, the proximate causes with one exception were post-purchase food-handling errors. When these data are considered in the light of the high volume of sales of these products, it is apparent that these meat products are not high-risk foods. Their relative safety is further substantiated by surveys specifically searching for salmonellae."

DISADVANTAGES OF MICROBIOLOGICAL STANDARDS

False sense of security to consumers

If consumers purchase meat from retail stores believing that the program gives them assurance of safety, they may be more inclined to mishandle the product under home conditions. Unsafe practices in the home may be far more disastrous than any practice found in retail stores.

False sense of accomplishment by administrators

If the agencies feel that the program is doing some good, they may be less compelled to follow up with other methods needed to promote meat safety. The industry recognizes that there is no government agency with sufficient staff to police all foods. Thus sampling at retail will be random and only token. It is probable that companies would be found in violation of regulations strictly due to the non-statistical, infrequent sampling scheme, i.e. luck of draw.

Increased cost of meat products

In the long run, the consumer will pay the costs of government and industrial bacterial monitoring. For small processors, the cost factor is severe since they cannot dilute cost through high volume.

It has been established by Corlett, (*3*) that it will cost a plant $35,000-$50,000 for initial laboratory facilities, $15,000 for technician's salary, plus the cost of product removed from human consumption.

The price of the product also will be increased through companies being forced to withdraw their services because they cannot consistently attain the standards. This may result in loss of an economical protein source. The loss of the protein supply increases the demand on other retail cuts — which in turn raises the price; the retailer may strictly refuse to carry such meat products for fear of being subject to legal action if found selling an unacceptable product.

ALTERNATIVES TO MICROBIOLOGICAL QUALITY STANDARDS

As an alternative to microbiological quality standards, industry awareness and education of pertinent workers in hygienic handling of meats will provide optimal quality of meat throughout the entire meat chain. The education of workers may be accomplished through: (*a*) correspondence courses on sanitation such as those offered by the Meat Packers Council of Canada in conjunction with the University of Guelph, or the American Meat Institute, (*b*) statement of good manufacturing practices prepared by trade associations such as that prepared by the Meat Packers Council of Canada for Ground Beef,

and (*c*) a formal manual of company policies and procedures.

In all instances the education program should deal with employee hygiene, work clothing (hairnets, aprons, footwear, gloves), room temperatures, utensils, conditions of raw materials, storage temperatures and times, procedures for thawing or tempering frozen meats, condition of final product, retail display and storage, stock rotation and cleaning procedures (water temperatures, choice of detergents).

Finally, bacterial growth is retarded by low temperatures; thus temperature control is a most significant quality standard for retail meats. Packers operating under Canadian federal inspection are required to promptly chill carcasses to 2-3 C (36-37 F); offal to 1 C (34 F); cutting, boning rooms to operate at 10 C (50 F), and packaging and shipping rooms to operate at 10 C (50 F) or less. Similar temperature control through transportation and retail handling would result in optimal quality of meat products available to the consumer.

Meat storage temperatures and display cases should maintain fresh and processed meats near (2 C (36 F). However, based on work by members of the Meat Packers Council of Canada and Davidson and Bodyfelt (*5*), it may be concluded that the design of some display cases is inadequate to maintain proper refrigeration of product.

These two studies had four similar conclusions. (*a*) The indicating thermometers in display cases tended to reflect the blower temperature rather than product temperature. (*b*) The coldest products were generally located at the bottom of a horizontal display case or the back of a vertical display case. These temperatures averaged 4-5 C warmer than the incoming blower air. (*c*) Products at the load line of a horizontal and a vertical display case averaged 4-6 C warmer than those in the above category (2) i.e. 8-11 C warmer than the blower air. (*d*) Products beyond the load line, depending on volume movement, were from 6-19 C warmer than those in category (*2*).

In conclusion, the failure to hold meat at proper refrigeration temperatures will detrimentally affect the retail quality. Thus adequate temperature control is probably one of the most valuable quality standards for retail meats.

REFERENCES

1. Anon. 1977. Report of the Meat Bacterial Standards Review Committee. January 24, 1977. Submitted to Director L. Kunzman. Department of Agriculture, State of Oregon.
2. Corlett, D. A. 1974. Setting microbiological limits in the food industry. Food Technol. 28(10):34.
3. Corlett, D. A. 1975. Microbiological quality standards workability and limitations. Presented Western Industry Food Conference, March 24, 1975.
4. CDC. 1975. Current trends in microbiological standards for raw ground beef, cold cuts and frankfurters. Morbidity and Mortality. 24(27):1.
5. Davidson, W. D., and F. W. Bodyfelt. 1976. Presentation-6th Food Science Workshop. U.B.C., May 4, 1976.
6. Foster, E. M. 1974. Letter to H. Fugelsten, Hearing Office, North Dakota State Laboratories Department. December 20, 1974.
7. Goepfert, J. M. 1975. Idaho Hearings. June 2, 1975.
8. Goepfert, J. M., and H. U. Kim. 1975. Behavior of selected food-borne pathogens in raw ground beef. J. Milk Food Technol. 38: 449-452.
9. Hill, W. M., J. Reaume, and J. C. Wilcox. 1975. Microbiological standards as a measure of shelf-life. Presented at 35th Annual Meeting, I.F.T., Chicago, Illinois.
10. Hill, W. M., J. Reaume, and J. C. Wilcox. 1976. Total plate count and sensory evaluation as measures of luncheon meat shelf-life. J. Milk Food Technol. 39:759-762.
11. Ingram, M., and A. G. Kitchell. 1970. Chem. Ind. 6:186. per Sulzbacher, W. 1970. Reciprocal Meat Conference. Page 146. Florida.
12. International Committee of Microbiological Standards. 1973. Microorganisms in foods. Their significance and methods of enumerations. University of Toronto Press. Toronto.

Mycoplasma Mastitis Alert, *con't from p. 674*

tained. Collect cow samples from all strings associated with positive tank samples. Remove positive cows from the milking string and handle as in 2 above.

4. After four consecutive weekly samplings are negative, test on a monthly basis for several months.

5. Test each clinical mastitis quarter for mycoplasma and bacteria.

6. Test each fresh cow before admission to milking string.

7. Keep mastitis cows separate from fresh cows at all times.

8. Always milk any unknown mycoplasma infected cows last or in a separate milking set-up. Milkers should never milk clean cows after milking infected cows without changing clothes and sanitizing hands.

9. Teat dipping in an approved teat dip should be rigorously followed.

10. Where possible disinfect teat cup clusters in a clean disinfectant solution in herds suspected of having mycoplasma infection.

Reprinted from "Udder Topics," a monthly publication of the National Mastitis Council.

Journal of Food Protection Vol. 42, No. 8, Pages 679-682 (August, 1979)

Consumer Confusion

F. J. FRANCIS

Department of Food Science and Nutrition, University of Massachusetts, Amherst, Massachusetts 01003

(Received for publication December 18, 1978)

ABSTRACT

The degree of consumer understanding, or lack of understanding, may be divided into three groups. The first is the area in which there is a good deal of misinformation being disseminated by groups for economic gain. Examples of this are the diet fads, such as the low carbohydrate diet which has already been resurrected six times under different names. The second area involves knowledge that the public doesn't really want to hear. The best example of this is the guidelines for filth in food published by the FDA. Another related example is the proposed regulations for Fish Protein Concentrate regarding intestinal contents. A third example may be the full ingredient labelling being requested by some groups. A long list of chemical names intimidates some consumers and may actually reduce food credibility. The third area involves technical issues that are so complex that educational efforts have lagged behind the progress of analytical and toxicological advances. The concept of risk/benefit in food components with its attendant difficulties in extrapolating high dosage animal data to low dose data appropriate for humans is the most obvious example.

The degree of consumer understanding, or lack of understanding, may be divided into three groups: (*a*) areas in which there is a good deal of misinformation being disseminated by groups for economic gain or even by well-intentioned but misguided groups who are promoting a way of life, (*b*) areas in which consumers do not really want to hear the truth, and (*c*) areas in which the technical issues are so complex that educational efforts have lagged behind the progress of science.

DELIBERATE DISSEMINATION OF MISINFORMATION

The area of consumer misinformation for economic gain is probably best illustrated by the concern with obesity in the U.S. Possibly as many as 40% of our people are considered to be 15 lb. or more overweight. This translates to over 80,000,000 people, so obviously the market for information, equipment and diets is very large. The total expenditure may be as much as 10 billion dollars. A market of this size obviously will attract entrepreneurs and we see the results almost everywhere. One newspaper seems to have a new diet regime every week. From this source alone, we have the "unlimited food diet", the "Miracle drug which melts your body fat", the "Keep cool diet" which "burns away--fat", a diet which will control the sex of babies, and a diet to make you smarter, all in the course of a few months. Others have diets to make you "immune to fat for the rest of your life", "three foods which can almost miraculously cause you to eat away your sexual problems", and the list goes on. It is not without its humorous side since it has provided grist for the cartoonists. For example, the "blondie" strip has lately printed a series of cartoons on food and diets.

The preoccupation of the American public with obesity, particularly with the degree of success in treatment, is understandable. The degree of long-term success with people with obesity of 40 lb. and over is approximately 1%. With lesser degrees of obesity, say 15 lb. or so, the degree of long term success is still only 5%. Prognosis for the demise of diet regimes would therefore appear to be very poor. This is shown by the degree of reintroduction of the "Low Carbohydrate Diet". It showed up as far back as 1860/70 as the Banting Diet. As late as 1950-66, it was called the Calories Don't Count, the Dupont Diet, the Air Force Diet, the Drinking Man's Diet, the Quick-Weight Loss Diet and the Dr. Atkins Diet Revolution. I'm sure it will be resurrected under many other names.

A number of the above diets are based on sound nutritional concepts, but a number are just foolish. They are catering to the whims of concerned people who would like a magic wand rather than personal discipline. In some instances this may be a combination of an unconscious desire not to want to hear the truth and the persuasive argument of those promoting misinformation. Regardless, this situation is likely to be with us for a long time.

Another area, somewhat related to the above concept, is subliminal advertising. This concept, aptly described by Key (*22,23*), is the deliberate introduction of suggestive items into advertising illustrations to introduce perceptions to the reader. The consumer may be unaware of the reason for his reaction, since the stimulus in the illustration may be below the conscious level of awareness. Most illustrations and responses have a sexual connotation since some would have us believe that we live in a "macho" society. It remains to be seen how effective this form of communication will be in the future.

INFORMATION THE PUBLIC DOESN'T WANT TO HEAR

This area is difficult to define since it is very easy to question one's motives when one is dealing with information to be made available to the public. The area of filth in foods is probably the best example. The FDA for many years had developed tolerance levels for mold, insect eggs, rodent hairs, etc., in food. This is a realistic

approach since it is impossible to remove completely all foreign matter from foods. In recent years some of the activist groups under the influence of the "Freedom of Information" concept, brought sufficient pressure on FDA officials to persuade them to publish their tolerance levels for filth in food. It has been suggested that this action was not designed to further the understanding of science but primarily to embarrass the regulatory officials. Suffice it to say that it certainly did that. But it also encouraged public dismay at the realization that food is not "Simon-pure". Understanding consumers realized this but they would probably prefer not to think about it. Ironically, the pressure to minimize filth in foods may have inadvertantly led to another problem. Pesticide residues from treatments to control insects and rodents may be slightly higher.

A second example in this category is the regulatory decision in the "Fish Protein Concentrate" (FPC) (*29*) program. The program was held up for several years by the position taken by the FDA officials that there should not be any filth in the product. By this they meant the inclusion of intestinal contents from whole fish. The requirement to make FPC from gutted fish increased the price of the final product. Of course, intestinal contents were already allowed in such foods in the human diet as sardines, clams, oysters, etc., but the FDA was not going to allow an increase. The filth requirement resulted in a delay of the program and considerable public education. FPC was predicted to be a panacea for alleviating protein malnutrition in the world. It is practically non-existent today for a variety of reasons, not the least being a shortage of fish.

A third example in this category is the requirement of full ingredient labelling for food. This program may be already starting to backfire because many people read a long list of chemicals and think, "Goodness, should I really eat all those chemicals." The author, on a recent TV panel, experienced a situation in which the moderator read the list of ingredients in a coffee whitener, and asked if we should really eat all those chemicals. I replied with a chemical description of a steak which sounds even more frightening, and asked the moderator if he would eat that. However, this is a self-defeating argument since the public is not ready yet to perceive food as a collection of chemicals. Perhaps it's better that way. Yet public disclosure of ever more minute ingredients, such as in flavorings, will inevitably lead to an unbalanced understanding of "civilized" as opposed to "natural" foods, unless more effort is made to improve consumer understanding of the chemical nature of foods. Unquestionably, foods should contain ingredient-labelling for products containing peanuts, for example, to minimize the problem of those unfortunate individuals with allergies. Yet the information overload, if the ingredient labelling program goes much further, will result in "chemophobia" in much of our population. If we combine this with the double standard employed for "natural" and "processed" foods we have "chemophobic schizophrenia" — a dreaded disease indeed.

A fourth example of the situation where consumers do not really want to know the truth is in the areas of "natural" foods. A mere discussion of the potential toxicological problems in natural foods is unacceptable to many groups. The "natural" movement may be symbolic of a way of life rather than an exercise in the interpretation of modern science, so it is unrealistic to expect an impartial interpretation of modern concepts of food and nutrition. This is not a criticism of food and nutrition concepts as incorporated into a way of life since freedom of expression should be carefully guarded in the U.S. It is unacceptable only when legislation is proposed which infringes on the quantity, quality and cost of food available to the general public. Scientists have a moral responsibility to make food available which is as safe as possible, in reasonable quantities and at a price as low as possible.

COMPLEX TECHNICAL CONCEPTS

Probably the best example of a very complex technical concept in which scientific advances have oustripped the ability to interpret the results is in the estimation of degree of risk in food safety. This is an area of considerable controversy among scientists in many different disciplines today; hence, it is understandable that legislation to cope effectively with this problem is not available. Hopefully it will be in the near future. Consumer understanding will follow when the scientific issues have been resolved to some extent and appropriate legislation is adopted.

The concept of risk and food safety has an interesting beginning. The Federal Food, Drug and Cosmetic Act of 1908 prohibits addition of any poisonous or deleterious substance to food. The Food Additives Amendment of 1938 requires that all additives be proved "safe" before addition to food. The "Delaney Amendment" in 1958 prohibits use of any additive found to produce cancer. The public has interpreted these regulations as indicating that there is no risk in food components. The rigorous enforcement of these regulations by the FDA and constant reassurance of the safety of our food supply has reinforced the concept of absolute safety of the food supply. It comes as something of a shock to learn that absolute safety is just not possible. Events of the past decade have made this conclusion inescapable.

Recent research has generated substantial evidence that carcinogens pervade the whole living environment. Food, as an important part of our environment, has been shown to have its own share of problems. Evidence of carcinogencity in test animals has been shown for cadmium (*24*), calcium (*25*), chromium (*30*), nickel (*31*), egg yolk (*32*), egg white (*31*), lactose (*14*), maltose (*14*), tannic acid (*4*), vitamin D_2 (*10*), and caffeine (*14*). Other trace contaminants in food with evidence of carcinogenicity are benzanthracene (*33*), benzopyrene (*34*), benzacepenanthrylene (*33*), benzofluoranthrene (*34*), diethylstilbesterol (*27*), cyclochloratene (in rice) (*19*), dibenzan-

thracene (*17*), ergot (in rye) (*28*), luteoskyrin (in rice) (*34*), aflatoxins (*34*), pyrrolizidine alkaloids (in cereals) (*34*), safrole (in spices) (*17*), and chloroform (*15*) and carbon tetrachloride (*8*) in water. The above is just a partial list. Under the Biomedical Research and Research Training Amendments of 1978, the National Cancer Institute will be required to issue a comprehensive annual report on this subject. It will include all suspected carcinogens, the nature of exposure, their relative toxicity, any synergistic action, level of exposure in food, approximate number of persons exposed and the identification of any subpopulations which may be at higher risk. When these reports become available any pretense that food is absolutely safe will be impossible. The public is just beginning to appreciate this since a number of influential leaders are taking this stand. For example, Senator Edward Kennedy of Massachusetts has stated:

> "We must begin educating the public to the reality that there is no such thing as absolute safety. Regulation can never completely and totally protect the public. Large segments of the American public already accept this fact. But it is time for persons in position of leadership to strengthen this understanding with more candid discussion on the limits of regulation" (*21*).

A clear concept of the way risk/benefit might be handled was presented by Peter Barton Hutt, former chief counsel with FDA (*14*). First, adequate experimental animal data should be obtained for compounds either already in, or proposed for addition to, the food supply, to extrapolate from high to low doses. Four mathematical models are most widely used, the linear (*13*), multistage (*11*), Mantel-Bryan (*26*) and the Cornfield model (*5*). The linear model is the most conservative and the Cornfield the least. This value obtained from animal data must be interpreted in terms of human response. These methods are commonly used based on mg/kg of body weight, relative body surface areas in cm^2 and the relative lifetime daily intake in mg/kg of body weight of the test animal as compared with humans. The estimates of risk vary widely. For example, with saccharin, using the body surface area scaling factor, the estimated new cancer cases per year were 15 and 3400 for the multistage and linear methods, respectively (*14*). For saccharin, using the linear extrapolation method, the three scaling factors, mg/kg of body weight, body surface area, lifetime intake, respectively yielded 600, 3400 and 15,000 estimated new cancer cases per year (*14*). The differences in the above methods of risk assessment are not likely to be resolved in the near future. Thus the numbers derived from calculation of risk by the above methods may be meaningless in themselves but they provide a comparative estimate of risk. If the methods are standardized, it should be possible to arrive at a figure for a degree of risk for any number of compounds in our food supply, and indeed for any compounds in our environment.

Hutt (*14*) has suggested that, after a degree of risk has been determined for potential toxic chemicals in our food supply, they be classified into high, moderate and low risks. Compounds in the high-risk category should be banned. Compounds in the moderate-risk category should be labelled as such and consumers can make their own choice. Compounds in the low-risk category should be of no concern and be accepted as inevitable daily risks. Clearly it is impossible to either regulate or educate for all of the potential toxic compounds in our food supply. The above concept, albeit a simplified version of an exceedingly complex situation, offers a reasonable approach to a very difficult situation. This is not to suggest that the implementation will be simple or easy (*7*). For example, who will decide the cut-off levels for the definition of high, moderate or low-risks? For the high-risk levels will a decision to ban be implemented with equal dispatch for synthetic compounds such as Red No. 2 or saccharin, as with naturally occurring compounds, such as aflatoxin in peanuts and corn, if indeed they both fall in the high-risk category. These will be difficult decisions.

It is important to separate the concepts of risks determined from epidemiological data and those derived from extrapolation of animal data. Wilson has calculated risks for some occupational hazards based on actual mortality data. These are real risks and are based on actual participation and number of deaths (Table 1). Table 2 presents annual cancer risks as calculated by extrapolation from animal data. It should be possible to develop a means of comparing all risks in human experience and develop the scientific and sociological mechanisms to cope with the situation. When we view the ever-increasing capability of analytical chemists to chase an ever-receding zero, it is high time for more effort to be placed on interpretation of the results.

TABLE 1. *Annual risk of death based on those individual participating or exposed.*[a]

Activity	Individual participant risk per year
Coal mining (black lung)	1/125
Coal mining (accidents)	1/770
Horse racing	1/750
Automobile racing	1/830
Fire fighters	1/1,250
Agriculture	1/1,700
Air line pilot	1/3,300
Football	1/25,000
Drowning	1/53,000
Bicycling	1/100,000
Electrocution	1/200,000
Vaccination	1/330,000
Hurricane	1/2,500,000
Lightning	1/2,500,000

[a]Adapted from Hutt (*14*).

The accumulation of toxicological data on animals for all potentially toxic compounds will clearly strain the scientific resources available. Some priority concepts will have to be established such as the "Decision Tree" approach suggested by the Food Safety Council (*6,12*). Mechanisms to determine the cut-off points for concern will be difficult but attempts have already been

TABLE 2. *Annual cancer risks.*[a]

Product	Individual participant risk per year
Peanut butter (aflatoxin)	
4 tbsp/day	1/25,000
Diet soda (saccharin)	
1 bottle/day	1/100,000
Milk (aflatoxin)	
1 pint/day	1/100,000
Alcohol (ave. of	
smokers and non-smokers)	1/20,000
Charcoal broiled steak	
1/2 lb/week	1/2,500,000
Smoking (cancer only)	1/800
Smoking (all affects)	1/300
Person in room with smoker	1/100,000

[a]Adapted from Hutt (*14*).

published. Examples are the scoring systems (*2,3*) proposed by the FDA for animal drugs and the recent suggestion (*1*) by the Environmental Protection Administration to classify potential carcinogens into four categories. The importance of this type of thinking is such that we should proceed as fast as possible with due regard for good science.

ACKNOWLEDGMENT

This paper is based on an address given to the Chicago Section of the Institute of Food Technologists, Chicago, Illinois, November 13, 1978.

REFERENCES

1. Anon. 1978. OSHA issues tentative carcinogen list. Chem. Eng. News 56(31):20-22.
2. Anon. 1978. Food Chem. News 20(30):37.
3. Anon. 1978. Food Chem. News 20(32):17.
4. Committee on Food Protection. 1973. Toxicants occurring naturally in foods, 2nd ed. Nat. Acad. Science, Washington, D.C. pp. 329-332.
5. Cornfield, J. 1977. Carcinogenic risk assessment. Science 198: 693-696.
6. Cramer, G. M., R. A. Ford, and R. L. Hall. 1978. Estimation of toxic hazard — A decision tree approach. Food Cosmet. Toxicol. 16:3-8.
7. Culliton, B. J. 1978. Toxic substances legislation: How well are laws being implemented? Science 201:1198-1199.
8. Eschenbrenner, A. B., and C. B. Miller. 1944. Studies on hepatomas: I. Size and spacing of multiple doses in the induction of carbon tetrachloride hepatomes. J. Nat. Cancer Inst. 4:385-388.
9. Fischhoff, B., C. Hehenemser, R. E. Kasperson, and R. W. Kates. 1978. Handling hazards. Environment 20(7):16-20,32-37.
10. Gass, G. H., and W. T. Allaben. 1977. Preliminary report on the carcinogenic dose-response curve to oral vitamin D_2. J. Med. Sci. 5:477-481.
11. Guess, H. A., K. S. Crump, and R. Peto. 1977. Uncertainty estimates for low-dose-rate extrapolation of animal carcinogenicity data. Cancer Res. 37:3475-3480.
12. Hall, R. L. 1978. The decision tree: A systematic approach to determining test priorities. Food Prod. Dev. 12(9):46,48,50.
13. Hoel, D. G., D. W. Gaylor, R. L. Kirshstein, U. Saffiotti, and M. A. Schneiderman. 1975. Estimation of risks of irreversible delayed toxicity. J. Toxicol. Environ. Health 1:133-151.
14. Hutt, P. B. 1978. Food Regulation: Unresolved issues in the conflict between individual freedom and government control of food safety. Food, Drug, Cosmetic Law J. 33:558-589.
15. International Agency for Research on Cancer, Monographs on the evaluation of carcinogenic risks of chemicals to man (IARC). 1973. Survey of compounds which have been tested for carcinogenic activity. IARC 3·91.
16. IARC. 1972. 1:62.
17. IARC. 1973. 3:178.
18. IARC. 1976. 10:51.
19. IARC. 1976. 10:139.
20. IARC. 1976. 10:231.
21. Kennedy, E. M. 1978. Risk/benefit decisions and the public health. 124 Cong. Rec. E 1310, March 15.
22. Key, W. B. 1976. Media sexploitation. Signet Books. New York, N.Y. 234 pp.
23. Key, W. B. 1972. Subliminal seduction. Signet Books, New York, N.Y. 205 pp.
24. Kolonel, L. N. 1976. Association of cadmium with renal cancer. Cancer 37:1982-1987.
25. Krook, L., L. Lutwak, and K. McEntee. 1969. Guest editorial: Dietary calcium, ultimobronchial tumors and osteoporosis in the bull. Amer. J. Clin. Nut. 22:115-116.
26. Mantel, N., N. R. Bohidar, C. C. Brown, J. L. Ciminara, and J. W. Tukey. 1975. An improved Mantel-Bryan procedure for "safety" testing of carcinogens. Cancer Res. 35:865-872.
27. Maugh, T. H., 1978. Chemical carcinogens: The scientific basis for regulation. Science 201:1200-1205.
28. Nelson, A. A., O. G. Fitzhugh, H. J. Morris, and H. O. Calvery. 1942. Neurofibromas of rat ears produced by prolonged feeding of crude ergot. Cancer Res. 2:11-15.
29. Pariser, E. R., M. B. Wallerstein, C. J. Corkery, and N. L. Brown. 1978. Fish protein concentrate: Panacea for protein malnutrition. MIT Press, Cambridge, Mass.
30. Sunderman, F. W., Jr. 1976. A review of the carcinogenicities of nickel, chromium and arsenic compounds in man and animals. Prev. Med. 5:279-94.
31. Szepsenwol, J. 1963. Carcinogenic effect of egg white, egg yolk, and lipids in mice. Proc. Soc. Exp. Biol. Med. 112:1073-1076.
32. Szepsenwol, J. 1964. Carcinogenic effect of ether extract of whole egg, alcohol extract of egg yolk and powdered egg free of the ether extractable part in mice. Proc. Soc. Exp. Biol. Med. 116:1136-1139.
33. Van Duuren, B. L., A. Sivak, B. M. Goldschmidt, C. Katz, and S. Melchione. 1970. Initiating activity of aromatic hydrocarbons in two-stage carcinogenesis. J. Nat. Cancer Inst. 44:1167-1173.
34. Wynder, E. L., and D. Hoffman. 1959. The carcinogenicity of benzafluoranthenes. Cancer 12:1194-1199.

Journal of Food Protection Vol. 42, No. 8, Pages 683-685 (August, 1979)

A Field Topic

Seasonal and Processing Influences on Bacterial Count of Raw and Processed Milk

RUTH FIRSTENBERG-EDEN, BARUCH ROSEN and CHAIM H. MANNHEIM*

Department of Food Engineering and Biotechnology, Technion - Israel Institute of Technology, Haifa, Israel.

(Received for publication November 10, 1978)

ABSTRACT

An increase in total counts of raw milk was detected during summer in comparison to winter. The higher summer temperature did not affect the numbers of coliforms and staphylococci. The microbial contamination of pasteurized milk did not change significantly during the year. All bacterial counts increased during the flow of milk through the various systems of the dairy plant before the pasteurizer. It was shown that pipe lines could cause contamination of milk, especially at the beginning of flow after the line was temporarily shut down. A correlation between numbers of coliforms and staphylococci was found for both raw and pasteurized milk.

Many studies have been devoted to the microbial population of raw milk. The total number of aerobic bacteria has been found to be in the range of 10^3-10^6/ml, the number of psychrotrophic bacteria between 10 and 10^5 and the number of coliform 10-10^5 (*8,15*).

Other researchers (*3,8,11*) have found the number and types of organisms to be influenced by the season of the year. Dempster (*4*) studied the spatial variations in the psychrotrophic microflora of milk plants.

Staphylococcus aureus was usually found in raw milk samples (*2,14*). About 70% of the milk supplied in bulk in Holland has been found to contain 800-1300 coagulase- and DNAase-positive staphylococci/ml (*2*).

Elliott et al. (*5*) surveyed the microbial population of pasteurized milk and showed that the microbial quality of raw milk affected the organoleptic properties of the final product. Hankin et al. (*7*) found that immediately following bottling, pasteurized milk contained 500-1000 bacteria/ml and that the coliform count was usually less than 10/ml. Sheikh and Luedecke (*16*) showed that 4.9% of pasteurized milk samples contained coagulase-positive *S. aureus*.

Cleaning procedures in dairy plants are often inefficient in removing all bacteria from the equipment (*10,13*). Kato et al. (*9*) found many viable cells on the walls of the bulk cooler immediately after automatic cleaning. Marshall and Appel (*12*) surveyed 12 modern fluid milk plants and concluded that relatively large numbers of bacteria are introduced from pipe lines, valves and filling machines into the pasteurized milk.

This paper deals mainly with changes in bacterial counts of milk during processing from raw milk to a finished packaged fluid milk product.

MATERIALS AND METHODS

Sampling

Milk samples were collected aseptically at various points of flow of milk in a commercial dairy plant. A schematic sketch of the plant is shown in Fig. 1. The sampling points were: (*a*) the road tanker immediately after its arrival at the plant, (*b*) the storage tank, before pasteurization, (*c*) the entrance to the clarifying centrifuge, (*d*) the exit of the clarifier, (*e*) after pasteurization at the exit of the flow diversion valve (FDV) before the cooler and (*f*) the final product packaged in plastic bags.

To obtain samples the milk line was momentarily opened at the above points. The first few liters of milk were discarded, then a sample of about 500 ml was poured aseptically into a sterile flask.

Samples were taken from every point 10 times at intervals of 5 to 7 days, on different days of the week, during winter (January - February) and 10 times during summer (August-September). Samples were held in ice water until all microbiological tests were carried out (not later than 2 h after sampling).

In a previous study (*6*), it was found that stagnant zones contribute to bacterial contamination of milk flowing in pipelines.

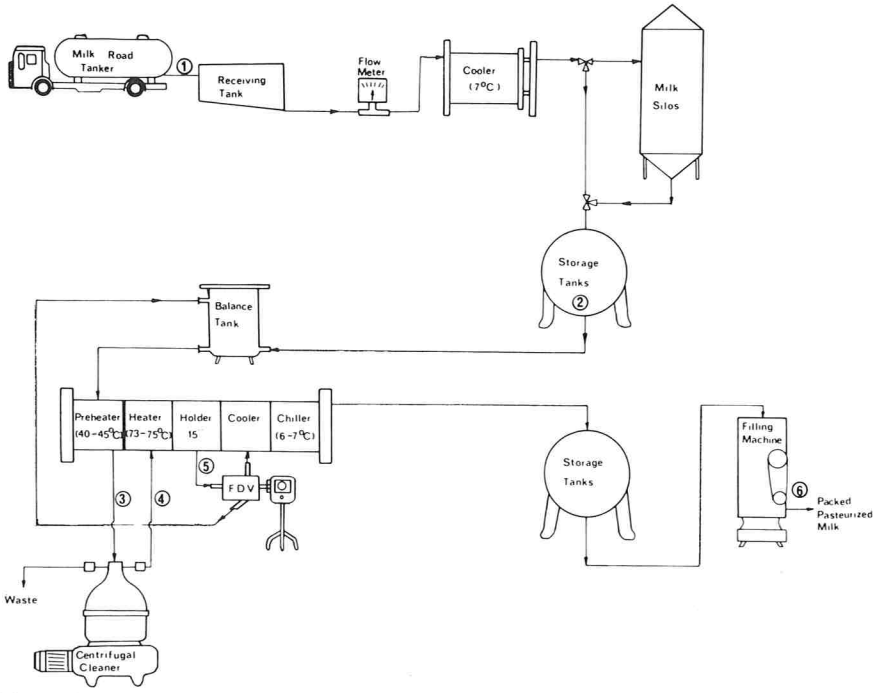


Figure 1. *Schematic description of pasteurized milk line and sampling points in it.*

Therefore milk from pipelines that were shut down for 24 h after automatic cleaning was also examined. Samples from the first milk and from the milk coming through after 10, 20, 30 and 40 min were collected.

Microbiological analyses

The number of aerobic bacteria, coliforms and staphylococci were determined in each milk sample. Standard media and incubation temperatures were used. Aerobic bacteria were counted on Plate Count Agar. The coliforms from raw milk were determined with Violet Red Bile Agar. After pasteurization, the coliforms were counted using the MPN method with enrichment in lactose broth and confirmation in Brilliant-Green Broth. Staphylococci from raw milk were determined directly with Baird-Parker (BP) plates. Staphylococci in samples of pasteurized milk were counted using the MPN method with Tryptic Soy broth + 8% NaCl as an enrichment medium. Bacteria from positive tubes were plated on BP. A few of the colonies from each BP plate were checked for the presence of coagulase enzyme.

RESULTS AND DISCUSSION

The average number of bacteria of the 10 samples taken in winter and the 10 samples taken in summer are given in Table 1. The total aerobic bacteria count was found to be in the same range as that observed by other investigators in different countries (*8, 15*). However, the numbers of coliforms were slightly higher then those reported in the literature. On the other hand, the numbers of staphylococci found in this work were much lower than those found by Bijker et al. (*2*) in raw milk.

Since pasteurization reduced the number of bacteria by some orders of magnitude, statistical analysis of the results was carried out separately for raw milk and for pasteurized milk. The results of the analysis of variance are given in Table 2.

Raw milk

Two-way analysis of variance (*17*) showed that there was a significant difference between total counts in summer and in winter while the numbers of coliforms and staphylococci did not change significantly. In contrast to these results, it was found by Kielwein (*11*) and by Bogdanowicz and Mockiewich (*3*) that the numbers of coliforms change significantly between summer and winter.

For all three groups of bacteria tested there was a significant increase in numbers during the flow of milk in the plant up to the pasteurizer. To investigate which location contributed most to the increase in microorganisms, a Comparison of Contrasts was carried out (*17*). Results, for all three groups of bacteria, showed a significant increase in bacterial counts from the receiving point up to the pasteurizer at all the sampling points, except for the clarifier. This increase might have been caused by growth in stagnant zones or by a contribution of bacteria from the equipment. The clarifier did not reduce significantly the numbers of bacteria. Therefore, this unit which removes extraneous particles from milk did not affect microbial quality of the milk.

Pasteurized milk

Analysis of variance of the number of bacteria immediately after the FDV and the final product during the two seasons was carried out. The results showed that there was no significant difference between the number of bacteria in summer and in winter.

The increase in bacterial counts of the milk between the FDV and the final product was highly significant for coliforms and staphylococci. Anas (*1*) found that some recontamination of pasteurized milk occurred

TABLE 1. *Number of microorganisms in different stages[a] of the milk line.*

Group	Season	Average number of microorganisms per ml					
		Before pasteurizer				After pasteurizer	
		Stage				Stage	
		1	2	3	4	5	6
Total count	Winter	2.28×10^5	1.05×10^6	1.84×10^6	2.58×10^6	5.11×10^3	7.50×10^3
	Summer	2.96×10^5	1.84×10^6	3.74×10^6	4.52×10^6	4.54×10^3	5.98×10^3
Coliforms	Winter	1.10×10^4	3.94×10^4	5.71×10^4	3.91×10^4	0.311	3.94
	Summer	1.42×10^4	3.34×10^4	1.42×10^5	1.36×10^5	0.787	0.64
Staphylococci	Winter	3.11×10^2	7.02×10^2	1.28×10^3	1.61×10^3	0.385	1.07
	Summer	3.56×10^2	1.24×10^3	1.48×10^5	1.86×10^3	0.335	1.27

[a]Stages are shown in Figure 1.

TABLE 2. *Analysis of variance of the results obtained for raw and pasteurized milk.*

Bacterial group	Source of Variation	Raw milk			Pasteurized milk		
		Mean squares	Degrees of freedom	F value	Mean squares	Degrees of freedom	F values
Total count	Location	5.058	3	30.84***	0.205	1	0.45
	Winter/Summer	1.036	1	6.23**	0.056	1	0.12
	Interaction	0.033	3	0.20	0.006	1	0.01
	Error	0.164	72	—	0.459	36	—
Coliform	Location	2.984	3	5.39***	12.002	1	24.90***
	Winter/Summer	1.213	1	2.20	1.568	1	3.25
	Interaction	0.393	3	0.71	0.0005	1	0.01
	Error	0.554	72	—	0.482	36	—
Staphylococci	Location	2.003	3	10.80***	2.608	1	12.43***
	Winter/Summer	0.231	1	1.24	0.0005	1	0.002
	Interaction	0.034	3	0.23	0.046	1	0.22
	Error	0.185	72	—	0.210	36	—

** Significant at 97.5% confidence level.
***Significant at 99.0% confidence level.

in the regeneration and cooling sections of the plate pasteurizer. Other equipment units like storage tanks (*9*), pipe lines and filling equipment (*12*) could also contribute to the increase in bacterial load between the FDV and the final product. The increase of total count between the FDV and the final product was not significant. This could be explained by the fact that most bacteria appearing in the total count of pasteurized milk were not destroyed in the pasteurizer. Apparently conditions in pasteurized milk were not adequate to enable growth of these bacteria. In all instances, the phosphatase test after pasteurization was negative.

Contribution of pipelines to the microbial contamination

To evaluate the possible contamination of milk coming from pipelines, a line that was left idle for 24 h after automatic cleaning and sanitizing with chlorine was opened and the milk collected after different periods of time. This was done for different pipe lines. A typical result of such an experiment is shown in Fig. 2. It is clear that at the beginning of flow all the bacterial counts of the milk were higher by 1-2 orders of magnitude than after 30-40 min. This behavior was probably due to removal of bacteria from stagnant zones, as shown previously (*6*).

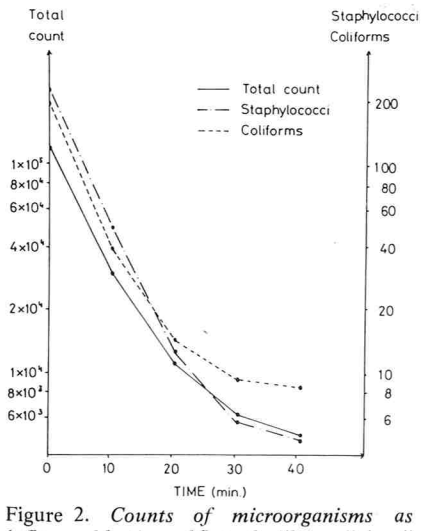


Figure 2. *Counts of microorganisms as influenced by time of flow of milk in a "clean" line.*

Relation between number of coliforms and staphylococci in the dairy plant

The correlation between numbers of coliforms and staphylococci inside the dairy plant was tested (120 pairs of data were compared). The results are given in Fig. 3. The regression coefficient (r) was 0.911, showing a significant correlation between these two groups in the plant. Since the origin of these two groups of organisms in milk is considered by some to be different, these results suggest that the sanitary conditions within the plant apparently control the numbers of these two groups.

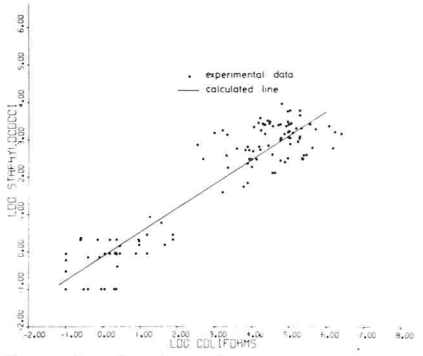


Figure 3. *Correlation between staphylococci and coliforms in milk.*

CONCLUSIONS

The higher ambient temperatures encountered during summer affected only the total count of raw milk but not the coliform and staphylococcus counts. This was probably due to the efficient cooling systems. Inside the dairy plant there was a significant increase of all types of bacteria examined, as the milk flowed through the equipment. Post-pasteurization contamination was observed.

REFERENCES

1. Anas, K. E. 1977. Bacterial content of milk after passage through different sections of a plate pasteurizer. Nordisk Mejeriindustry 4:324-326.
2. Bijker, P. G. H., H. Mol, and H. M. Vincentie. 1976. An investigation of the occurrence of staphylococci in raw milk. Tijdschrift voor Diergeneeskunede 101: 365-371.
3. Bogdanowicz, A., and A. Nockiewicz. 1973. Studies on the microflora of raw milk and sour cream. Roczniki Panstwowego Zakladu Higieny 24:731-733.
4. Dempster, J. F. 1968. Distribution of psychrophilic microorganisms in different dairy environments. J. Appl. Bacteriol. 31: 290-301.
5. Elliott, J. A., D. B. Emmons, and A. R. Yates. 1974. The influence of the bacterial quality of milk on the properties of dairy products. A review. Can. Inst. Food Sci. Technol. J. 00:32-39.
6. Firstenberg-Eden, R., C. H. Mannheim, and B. Rosen. 1978. Removal of material from stagnant zones in dairy piping. Food Sci. Technol. 11:(In press)
7. Hankin, L., W. F. Dillman, and G. R. Stephens. 1977. Keeping quality of pasteurized milk for retail sale related to code date, storage temperature, and microbial counts. J. Food Prot. 40:848-853.
8. Kalina, G. P., N. S. Koroleva, and V. F. Semenikhina. 1974. Enterococci in raw and pasteurized milk. Food Sci. Technol. Abs. 6:3 p. 307.
9. Kato, Y., K. Shimazaki, and K. Sukegawa. 1976. Examinations of bacteria in raw milk stored in a bulk cooler. Research Bulletin of Obihiw University 10:165-171.
10. Kaufmann, O. W., T. I. Hendrick, I. J. Pflug, L. G. Pheil, and R. A. Keppeler. 1960. Relative cleanability of various stainless steel finishes after soiling with inoculated milk solids. J. Dairy Sci. 43: 28-41.
11. Kielwein, G. 1977. The comparison of the aerobic gram-negative flora in delivered milk and its relation to dairy products. Alimenta 16:41-48.
12. Marshall, R. T., and R. Appel. 1973. Sanitary conditions in twelve fluid processing plants as determined by use of the rinse filter method. J. Milk Food Technol. 36:237-241.
13. Maxcy, R. B. 1969. Residual microorganisms in "clean-in-place" systems for handling milk. J. Milk Food Technol. 32:140-143.
14. Olson, J. C., Jr., E. P. Casman, E. F. Baer, and J. E. Stone. 1970. Enterotoxigenicity of *Staphylococcus aureus* cultures isolated from acute cases of bovine mastitis. Appl. Microbiol. 20:605-607.
15. Randolph, H. E., B. K. Chakroborty, O. Hampton, and D. L. Bagart. 1973. Microbial counts of individual producer and commingled grade A raw milk. J. Milk Food Technol. 36:146-151.
16. Sheikh, M. I., and L. O. Luedecke. 1974. *Staphylococcus aureus* in commercial processed fluid dairy and non-dairy products. J. Milk Food Technol. 37:329-332.
17. Winer, B. J. 1962. Statistical principles in experimental design. McGraw-Hill Book Company, Inc. New York.

Journal of Food Protection Vol. 42, No. 8, Pages 686-689 (August, 1979)

A Field Topic

Beyond the One-shot Clean-up

CHARLES F. VOGT

Clean City Commission, 20th Floor, City Hall, Kansas City, Missouri 64106

(Received for publication September 11, 1978)

ABSTRACT

The Clean City Commission of Kansas City, Missouri, in conjunction with Keep America Beautiful, Inc., New York, has devised a four-front approach for sustaining litter reduction through changing behaviors and attitudes. These measures consist of devising ordinances that assign responsibilities for pick-up and containment of trash, introducing new technology and waste handling methods to improve solid waste clean-up and disposal, increasing educational awareness of the environment, and teaching persons new habits which can be learned and sustained and providing a fail-safe measure by which proper enforcement of litter ordinances can be maintained. To do this, the Commission is getting the facts about littering, involving the people, focusing on results of successful anti-litter projects, planning systematically through goals and objectives, and providing positive reinforcement (recognition) for jobs well done.

Since my background includes mostly work in the field of journalism, I would like to share with you some headlines and stories that relate to this discussion. You may have heard some of these, for they are quite recent.

News item: Things are getting tight at Loose Park...park usage has increased more than 50% this summer according to park administrators. Debris and litter are scattered throughout the parks...and a duck pond is no longer fit for ducks but instead is filled with cans and bottles.

News item: Woman found bitten by rats... a 67-year-old lady was listed in poor condition...having been found in her apartment with several rat bites on her body... her apartment was full of trash and bits of food.

News item: Litter source...man's best friend? In a random sampling of Kansas City, Missouri residents, dogs were listed as the #1 source of the litter problem. It was noted that although Kansas City has one of the best refuse collection divisions, it relies on plastic trash bags for collections.

News item: City spends millions to clean streets and parks. More than 4 million dollars will be spent this year on picking up litter and trash around Kansas City.

and the list goes on. Just what kind of society are we living in today that allows a lady to be bitten by rats or the taxpayer to be bitten by the litterbug? Well, let's take a look.

TODAY'S SOCIETY

If one talks to a political scientist, the most common explanation given is that today's society has become stratified. Those with the ability to move, leave the city and move to the suburbs. The result is the loss of the tax base they once provided. The cities become the graveyards of the people who cannot pay the price to leave. Thus with limited resources, the cities are forced to provide for people who cannot....or will not ...help themselves.

Social scientists from Stanford Research Institute explain the situation a little differently. They quote statistics that state that the increase in our society's mobility and affluence had lead to an inappropriate allocation of resources to remedy problems caused by this phenomenon. I will let the statistics speak for themselves.

Between 1960 and 1975, our population has increased about 16%, automobiles, 44%, inner-city passenger traffic, 78%, drivers under 25, 93%, recreational area visits, 350% and national park and seashore visits, 2,000%. In stating these figures, there is a direct relationship between the mobility and affluence increases and the substantial increases in consumption of food, beverages and other products while en route. Both as pedestrians and motorists.

This lifestyle development has been accompanied by similar magnitudes of growth in specialized outlets to serve these needs. Evidence in this respect shows that between 1960 and 1975 food oulets grew 470% and convenience stores more than 900%.

Compare these figures with allocations for city services, adjusted for inflation. In 1960, street cleaning and garbage collection was $7.00 per capita; in 1975, $8.30. Street cleaning on highways, $34.00 per capita in 1960 compared with $35.00 per capita in 1975. Local parks and recreation areas, $8.00 per capita in 1960; in 1975, $13.50.

What should be added in comparing these data is that parks and highways have increased in overall land area and use since 1960 and the per capita base has changed these as well, to reflect population increases.

What can be inferred from these data is that first, life-style trends will continue to rise in respect to mobility and affluence. As this happens, services to meet the demand for these developments will increase. Through that demand, given the increase in disposable containers for quick service food, we have raised the littering potential of the population.

Second, due to the rapid growth of the population affluence and mobility, litter-related maintenance activities of public agencies have not been able to keep pace with the litter generation rates and accumulations in recent years, except in communities where special priority has been assigned to this and other cleanliness functions.

Thus through an ongoing population, stratification and move towards affluence and mobility, cities and other public agencies are losing

the battle against litter. When faced with these statistics and interpretations, many a public works official, health department official, or city sanitarian has said to himself "Is there an answer?" I would like to suggest one.

AN ORGANIZATION — AN ANSWER

How many remember the television commercial with the Indian shedding a tear with the slogan "It's a crying shame?" If you do, you may have noticed that the message was brought to you by the National Advertising Council and a group called Keep America Beautiful, Inc.

Keep America Beautiful (KAB) is just that....a not-for-profit, international public service organization working with citizen groups, governmental agencies, academic institutions and private industry to stimulate individual involvement in improving the environment.

In its 25th year, KAB has solicited and trained more than 130 communities in ways they can approach and deal with the litter problem. These methods have been drawn together in what KAB calls the clean community system, a system that, if followed and adapted to local surroundings, can bring results. But what of results? Many anti-litter programs are one-shot clean-ups, short-term remedies that provide immediate results, but then, after the anti-litter troops have left and the media cameras have focused elsewhere, return to their former littered states.

If you have a child and have ever taken him or her out on one of these clean-ups, or have been in one yourself, you known how discouraging it can be to return to the location one week later, if you are willing to, and see the area all littered again.

The clean community system, once adopted, is a community-owned and -operated attempt to stage a continuous attack on the litter problem on four fronts. These emphasize a behaviorally based approach to changing people's littering behavior and attitudes. It costs very little to become certified and operate the program. Once you are, the challenge to meet the littering problems can be shared by all.

THE CLEAN COMMUNITY SYSTEM

The first step in complying with the clean community system is setting up the commission itself. In Kansas City, a special task force who attended a KAB workshop made recommendations to the mayor as to who they thought should be included in the commission. In addition, the 12-member City Council was given an opportunity to pick one each of the commission which totals 30 members. Diversity is the key, and Kansas City has just that.

The first front of the clean community system is the revision and review of ordinances and laws that relate to containment of litter itself. Whose responsibility is it to clean sidewalks, alleyways or vacant lots? What is a good definition of litter? What kind of notices should be delivered before a fine is submitted and issued?

Not all these questions can be answered in one presentation. But let me say that through the clean community system, Kansas City has been able to draw from the experience of more than 20 cities that have submitted to us copies of ordinances that they have used to successfully contain litter. With area resources like these, cities can draw from a variety of good resources to draw up better laws. One cannot talk of revisions in litter ordinances without mentioning two things which lend themselves to controversy. The first issue is one of the courts. Why is it that so many cases are referred to the municipal courts in this country with ample evidence of violations and notices to affirm these violations, and when submitted to the courts are either continued or dismissed?

Case in point. Recently one of the city's attorney's assigned to the Property Conservation Division of Public Works failed to convict a property owner in violation of numerous litter and trash violations. Get this--because the property owner had incorporated and could not be proven the actual owner of the properties. The charges against the "corporation" representative were dismissed.

The state law on the matter states that officers or registered agents of the corporation cannot be held liable for acts of the corporation. Clearly this law will encourage the absentee slum landlord to seek incorporation. Such laws and practices must be changed or else the job of the sanitarian, and the lawyer (who also handles complaint cases and discrimination cases) will be all the more difficult.

The second item in discussing revisions in the laws, not just for cities, but for states as well, is what is called the bottle legislation. As those concerned with food handling and packaging understand, the battle is going on throughout the country regarding bottle legislation. Environmentalists claim bottle and can deposits are the only answer. The bottle and can industry claims that its alternative is best — that of taxation of businesses that contribute to the litter situation, such as fast-food restaurants, quick-service grocery chains and the more remotely related litter producers, the newspaper and napkin industries.

My only comment on the controversy is that of a question. Who are we trying to get to stop littering? The grocery store owner or the pedestrian and motorist? Somehow taxation of industry does not change attitudes; it only taxes those who are already taxed heavily and forces them to pay for the pick-up of litter that government must provide. Will not the over-taxed fast-food restaurant owner say that it is not his responsibility to clean up any longer since the city or the county or the state is getting paid to do it? If the taxation bills succeed in Colorado, California, Washington and elsewhere, perhaps we could see a greater decrease in litter, as the bottle bills have demonstrated. Perhaps not.

In any event, Keep America Beautiful may stand one way on this issue, but members of the clean community

system are not compelled to join with it. Each city, county and state taking on the model program may decide its own course of action.

The second front the commission under the clean community system is attacking is that of updating sanitation equipment and improving operations. Even the best equipment, when not used well, can prove more of a handicap in litter control than a benefit. This second frontal attack is as much an operational one as an additional technological one.

Case in point. As I mentioned earlier, a citizens' attitude survey of Kansas City residents showed that our most noteworthy source of litter in the city was our favorite friend, the dog.

What was the city to do? Members of the Kansas City, Missouri Commission, a 30-member layperson group appointed by the mayor, looked into the problem. One operational problem noticed from the start was that although there was a high correlation between trash collection days and the number of dogs loose in the streets (even though Kansas City has a leash law), no radio contact had been made between the Animal Control Division and the Refuse Collection Division. Radio control would most likely lead to the more effective identification of loose dogs, but previous attempts to get the two divisions in two different departments together had failed. Why? Because one could not depend on the other and thus had lost the desire to continue cooperation further.

As soon as the commission learned of the impasse, it set up a meeting between the two groups and soon the two were discussing their mutual problems. It was agreed in the first meeting that a new attempt would be made to work together once the Animal Control Division received new trucks and manpower. When it did, the cooperative venture paid off, and now both are in constant contact, willing to help out each other. Call it a watch-dog program, call it a miracle or call it whatever. The fact remains that the commission came in and presented a neutral side to the argument and some alternatives were finally accepted.

Motivation is the key in refuse collection as it is in other fields. Without proper motivation, the job gets old fast and the desire is lost. One of the important functions of the clean city commission is providing positive reinforcement for jobs well done. This can be in the form of performance awards or public recognition with a news story. Any pat on the back goes a long way.

Before leaving this subject of motivation and positive reinforcement, let me add that training can also be a good motivational tool. Encourage sanitarians to go to school or to specialized training classes. With the turnover in sanitarians, training becomes a matter of style rather than procedure. A person taught under another sanitarian's guidance is often prone to inherit both the good and the bad. Try blending the two, procedure and experience, into one training session. It makes a lot of sense. If I ever get to it, I have promised our environmental inspection unit an introductory slide presentation to indoctrinate its new personnel.

The third frontal attack in the clean community system is continuous public relations and education. Just what makes up an educational public relations campaign?

With litter containment we are talking about a vast audience — adults and children of all ages. We need materials that appeal to all of them. Brochures will suffice for the adult, as will training workshops that discuss the seven sources of litter: uncovered trucks, residential trash uncovered and put out improperly, the same for commercial trash, loading and unloading docks, construction and demolition sites and the motorist and pedestrian. Such workshops tell about the littering norms or habits we have regardless of our good intentions. Even I find myself wanting to litter sometimes when I can't find a receptacle.

After each presentation, we ask the group, whether it be hotel maintenance workers or community group members, to comment on what they would like to do to end the sources of litter in their environment. You would be surprised to see how most people react to the workshop. Many have never thought of the sources of the litter problem, just blamed others. The workshop enables them to define their own needs. It is one step beyond the public information media campaign.

The media can help. Don't ever doubt their power to get out the word. Be sure they know what you are doing to publicize an event well in advance. The media can be your best friends...or your worst enemies. Keep them informed and they will work with you, not against you.

When mentioning continuous education, we cannot ignore our most challenging audience, the children, who will be the adults of tomorrow. Will they continue to litter, or will the *Give a Hoot, Don't Pollute* slogans and many more stay with them as adults? This is the fundamental question every city within the clean community system has to ask itself — can we reach the children? Their future is ours. The future that we study and plan for begins today. Such is the charge of the Kansas City Clean City Commission in regard to continuous education.

The fourth component is the ability to have effective code and ordinance enforcement. Although the Clean Community System considers this a last resort, it is very important. Like the case of the city vs. the slumlord corporation, residents of a city wanting a clean city can not be denied due process of law without some form of deterrent for the constant offender. Courts should not be used just as a means to punish the public with huge fines for infractions of the law. They should also be able to rule on requests of 'injunctive relief', such as the judge ordering a violator to clean up his property within a certain period.

Even with fines and court orders, the sanitarian still finds his job more frustrating when the case is prolonged for another 30 to 60 days before the violation is abated.

I have two suggestions that we are looking into here in Kansas City which have been demonstrated successfully in other cities. Why not a litter ticket, much like a parking ticket, that fines the offender on the spot? This could be used by policemen who see a violator while en route. Do parking violators get a chance to move their car with a meter maid standing there ready to write a ticket? I am afraid not; they get the ticket.

My other suggestion is to set up a special Environmental Court and Litter Control Unit. The City of Indianapolis has recently created the first Environmental Court which will hear all cases stemming from violations of city codes pertaining to the environment, including those on weed, litter and junk control, air pollution, water pollution, health and safety, fire prevention and unsafe buildings. Such a court would free the prosecutor, as in our earlier example, to do strictly environmental work rather than share duties in several unrelated areas. Both ideas can work in Kansas City, and they can work in your city as well.

These ideas came from the tremendous freedom-of-information network exchange that cities within the Clean Community System have access to as much as they need. People learn from people. Cities, counties, and even states can learn from others.

The four components of the Clean Community System to change littering behavior have now been described. The city cannot do all these things alone, nor can any governmental unit given the fiscal restraints many are faced with today all around the country.

IN CONCLUSION

In conclusion, any successful program, whether it be within the guidelines of the Clean Community System or any other system, needs to concentrate on results once the people are involved. The key accountability aspect, and accountability is a key word these day, is the method by which the clean community system evaluates itself. Using a scientific litter measurement technique approved by the American Public Works Association, called the Photometric Index, pictures are taken of randomly selected areas of the city to determine a baseline of measurement before the community effort begins. Once the programs on all four fronts begin, similar random areas are photographed to determine what change, if any, has taken place in the quantifiable amounts of litter. If there is a reduction, then the commission's or committee's efforts are paying off. Such methods of accountability clearly demonstrate to the funding source for the program that the money is being used effectively.

As you might wonder, cities participating in this system since 1974 have seen up to 70% reductions in litter since they took their baseline measurements. That is a substantial decrease in litter which makes this whole program worthwhile. Within the entire Clean Community System, results can be compared with other cities and more successful cities with successful programs can be used for assistance. Since the program began in Kansas City in April of 1977, we have seen a 12% reduction in litter since our baseline measurement was taken. Such a reduction is considerable since the program itself cost the city just under $22,000 and actually generated more than $50,000 in in-kind donations from the private sector.

Only through positive attempts to contain litter and encourage litter containment in everyday life can we hope to remedy the situation in our resource-scarce cities and counties. There is no written rule that says government must pick up after all mankind. Only through sharing the responsibility can we help keep our land clean and beautiful.

ACKNOWLEDGMENT

Presented at the 65th Annual Meeting of the International Association of Milk, Food and Environmental Sanitarians, Kansas City, Missouri, August 13-16, 1978.

Energy--A Vital Resource for the U.S. Food System

- Production agriculture uses 3% of the energy consumed by the U.S. Our total food system uses 16.5% of the nation's energy.
- Fertilizer and pesticide manufacture represent over 1/3 of the energy used in production agriculture.
- About 89% of the energy invested in production agriculture is associated with crops; the remaining 11% is used for livestock production.
- Milk cooling and water heating account for at least 50% of the farmstead energy requirements on most dairy farms. New equipment and techniques developed by agricultural engineers can reduce the energy needed for heating water for milking operations by about 65%. Also, heat extracted from cooling milk can be recovered and used to heat water. These two factors combined with energy produced from biomass or solar panels can eliminate the need for purchased energy to heat water.
- American farmers could annually produce about 4 quads of energy from crop residue and more than 2 quads from manure while producing food for the nation and for export. (A quad equals 10^{15} Btu or about 172 million barrels of oil. Total U.S. energy usage is about 77 quads per year.)
- Diesel tractors average about 38% higher horsepower-hr per gallon than comparable gasoline models (as of January, 1977). Diesel engines, however, have a higher unit cost because it costs more to manufacture them.
- In 1976, 93.2% of the 153,373 farm tractors sold by Farm and Industrial Equipment Institute member companies were diesel-powered. This compares to 86.6% in 1973 and 62.7% in 1969.

con't p. 692

3-A Accepted Practices for a Method of Producing Steam of Culinary Quality

Number 609-00

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

It is the purpose of the IAMFES, USPHS and DIC in connection with the development of the 3-A Sanitary Standards program to allow and encourage full freedom for inventive genius or new developments. Methods of producing steam of culinary quality heretofore or hereafter developed which so differ in material, fabrication and installation or otherwise as not to conform with the following practices, but which, in the opinion of the operator, manufacturer or fabricator are equivalent or better, may be submitted for the joint consideration of IAMFES, USPHS, and DIC, at any time.

A.

SCOPE

A.1

These 3-A Accepted Practices cover a method of producing steam of culinary quality. These practices are not intended for heating of rinse water or the sterilizing of equipment.

B.

DEFINITIONS

B.1

Culinary Steam: Shall mean steam that will be used with product.

B.2

Product: Shall mean milk, milk products, cheese, ice cream, ices and formulated dairy products.

B.3

Safe Water: Shall mean water from a supply properly located, protected and operated and shall be of a safe sanitary quality. The water shall meet the standards prescribed in the Public Health Service Drinking Water Standards which are found in the Code of Federal Regulations, Title 42, Chapter 1, Part 72, Subpart J.[1]

B.4

Product Contact Surfaces: Shall mean surfaces in contact with culinary steam from point B on Figure One to the point of attachment to the equipment in which it will be used.

C.

MATERIALS

C.1

Product contact surfaces shall be of stainless steel of the AISI 300 series[2] or corresponding ACI[3] types (See Appendix, Section G), or equally corrosion-resistant metal that is non-toxic and non-absorbent, except that:

C.1.1

Rubber and rubber-like materials may be used for multi-use gaskets.

C.1.2

Rubber and rubber-like materials when used for the above specified applications shall comply with the applicable provisions of the 3-A standard for rubber and rubber-like materials, Number 18-00.

C.1.3

Plastic materials may be used for multi-use gaskets.

C.1.4

Plastic materials when used for the above specified applications shall comply with the applicable provisions of the 3-A standard for plastic materials, Number 20-11.

C.1.5

Single-service sanitary-type gaskets may be used in connections which must be disassembled for cleaning.

D.

FABRICATION

D.1

Product contact surfaces not designed to be mechanically cleaned shall be readily accessible for cleaning and inspection either when in an assembled position or when removed. Removable parts shall be readily demountable.

D.2

The piping assembly for direct steam injection is shown in Figure One. The piping assembly for direct steam injection shall include (1) a filtering device capable of removing particles 5 microns in size and larger, (2) an entrainment separator capable of

---

[1] *Code of Federal Regulations, published annually, are for sale by the Superintendent of Documents, U.S. Goverment Printing Office, Washington, DC 20402.*

[2] *The data for this series are contained in the following reference: AISI Steel Products Manual, Stainless & Heat Resisting Steels, December 1974, Table 2-1, pp. 18-19. Available from: American Iron & Steel Institute, 1000 16th St., NW, Washington, DC 20036.*

[3] *Alloy Casting Institute Division, Steel Founders' Society of America, 20611 Center Ridge Road, Rocky River, OH 44116.*

removing particles 10 microns in size and larger and (3) a means of sampling the steam or condensate downstream of point A on Figure One. Stainless steel pipe and fittings or sanitary tubing and fittings shall be used between points A and B, and sanitary tubing and fittings only shall be used between point B and the processing equipment.

D.2.1

The piping assembly may include the other units shown on the drawing with the following exceptions:

D.2.2

The location of the filtering device and the entrainment separator may be interchanged.

D.2.3

Additional valves, orifices, strainers, gauges and piping may be used for control and convenience in operation.

D.2.4

The location of the steam throttling valve is not restricted to the position indicated on the drawing.

D.2.5

The steam pressure (reducing) valve may be omitted if not required.

D.2.6

Coil springs having product contact surfaces shall have at least 3/32 inch openings between coils including the ends when the spring is in a free position. Coil springs shall be readily accessible for cleaning and inspection.

D.3

The piping assembly for air space heating or defoaming is shown in Figure Two.

E.

BOILER FEED WATER

E.1

Source: Safe water or water supplies acceptable to the regulatory agency having jurisdiction shall be used.

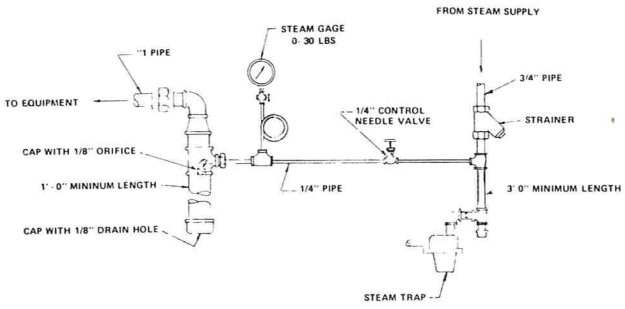


FIGURE 2-PIPING ASSEMBLY FOR AIR SPACE HEATING OR DEFOAMING

E.2

Treatment: Most boiler feed waters must be treated to prevent corrosion and scale in boilers and/or to facilitate sludge removal for proper boiler care and operation. Boiler feed water treatment and control shall be under the supervision of trained personnel or a firm specializing in industrial water conditioning. Such personnel shall be informed that the steam is to be used for culinary purposes. Pre-treatment of feed waters for boilers or steam generating systems to reduce water hardness before entering the boiler or steam generator by ion exchange or other acceptable procedures is preferable to addition of conditioning compounds to boiler waters. The list of boiler water additives that may be safely used in the preparation of steam that will be in contact with product will be found in the Code of Federal Regulations, Title 21, Chapter 1, Part 173, Subpart D, Section 173.310[1]. Boiler compounds containing cyclohexylamine, diethylaminoethanol, hydrazine, morpholine, octadecylamine and trisodium nitrilotriactetate shall not be permitted for use in steam in contact with milk and milk products.

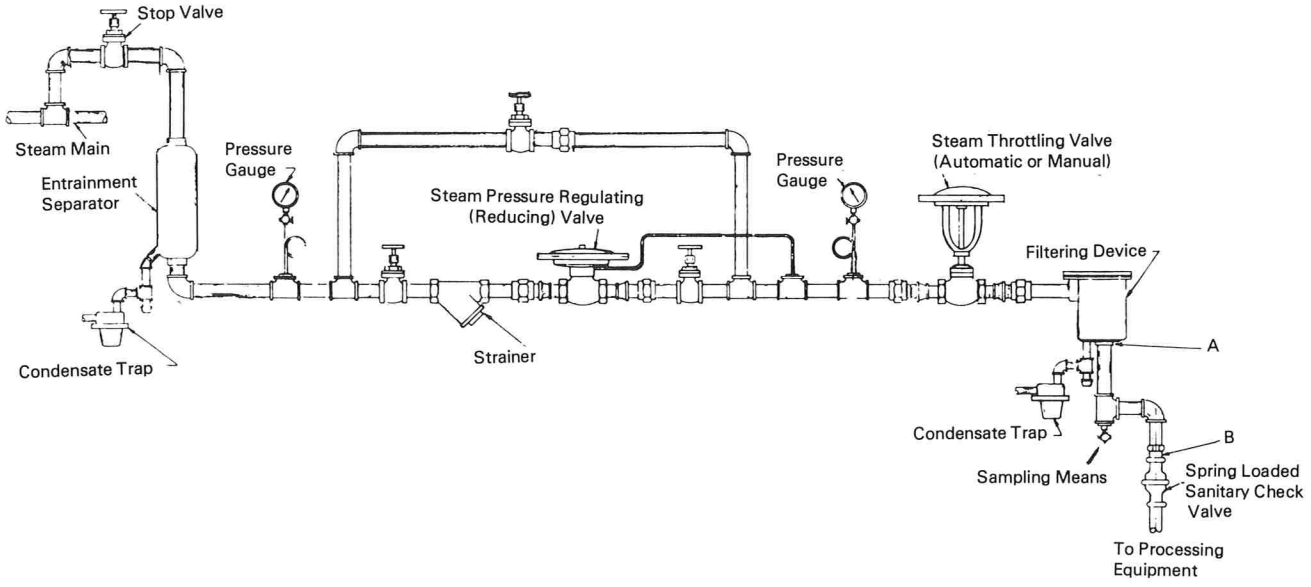


FIGURE 1- PIPING ASSEMBLY FOR DIRECT STEAM INJECTION

Greater amounts of boiler water treatment compounds shall not be used than the minimum necessary for controlling boiler scale or other boiler water treatment purposes and no greater amount of steam shall be used than necessary.

Tannin is also frequently added to boiler water to facilitate sludge removal during boiler blow-down. This product, although included in the list of approved boiler additives, has been reported to give rise to odor problems, and for this reason should be used with caution.

E.3

Containers of boiler water additives for use in the processing plant must be clearly labeled as to the identity of the material that is added to boiler feed water.

F.

BOILER OPERATION

A supply of clean, dry and saturated steam is necessary for proper equipment operation; therefore, boilers and steam generation equipment shall be operated in such a manner as to prevent foaming, priming, carry-over and excessive entrainment of boiler water into the steam. Carry-over of boiler water additives can result in the production of product off-flavors. Manufacturers' instructions regarding recommended water level and blow-down should be consulted and rigorously followed. The blow-down of the boiler should be carefully watched, so that over-concentration of the boiler water solids and foaming are avoided. It is recommended that periodic analyses be made of condensate samples. Such samples shall be taken from the condensate outlet of the final steam separating equipment or the line between the final steam separating equipment and the point of the introduction into the processing equipment.

APPENDIX

G.

STAINLESS STEEL MATERIALS

Stainless steel conforming to the applicable composition ranges established by AISI[2] for wrought products, or by ACI[3] for cast products, should be considered in compliance with the requirements of Section C.1 herein. Where welding is involved the carbon content of the stainless steel should not exceed 0.08%. The first reference cited in C.1 sets forth the chemical ranges and limits of acceptable stainless steels of the 300 series. Cast grades of stainless steel corresponding to types 302, 303, 304, and 316 are designated CF-20, CF-16F, CF-8, and CF-8M, respectively. These cast grades are covered by ASTM[4] specifications A296-68 and A351-70.

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

Energy - A Vital Resource, *con't from p. 689*

- Nearly 75% of the energy used in the non-production sectors of the food system is in the form of natural gas and oil, yet electricity is the primary energy source for food marketing.
- Transportation of agricultural products accounts for more than 2% of total U.S. energy consumption.
- About 65% of energy usage in retail food stores is directly associated with refrigeration. Space heating with oil or natural gas for an average supermarket uses 37,660 BTU/100 ft^2 of selling space per day; electricity usage in an average supermarket averages 280,400 Btu/100 ft^2 of selling space.
- Food service establishments account for about 2.8% of all energy used in the nation. Some half million food service establishments including restaurants, schools, hospitals, clubs and other institutions prepare and serve food to U.S. consumers away from home.
- The annual cost of energy used in the home for food preservation and preparation amounts to $6.5 billion. Studies indicate that households with nonemployed homemakers expend 6-10% more total energy than households where adult members work full-time (this is only partially attributed to home food preservation and preparation).
- Cooking and refrigeration combined account for about 8% of energy used by households. Space heating and cooling account for 40%; water heating for 6%; and automobile transportation for 42%.

Information compiled by the American Society of Agricultural Engineers, excerpted from "A Public Policy Issues Study Report: Energy - A Vital Resource for the U.S. Food System, Facts and Ideas in Agriculture."

Journal of Food Protection Vol. 42, No. 8, Pages 693-698 (August, 1979)

Holders of 3-A Symbol Council Authorizations on August 20, 1979

Questions or statements concerning any of the holders of authorizations listed below, or the equipment fabricated, should be addressed to Earl O. Wright, Sec'y.-Treas., P.O. Box 701, Ames, Iowa 50010.

01-06 Storage Tanks for Milk and Milk Products

28 Cherry-Burrell Corporation (10/ 3/56)
(unit AMCA Int'l)
575 E. Mill St.
Little Falls, New York 13365

102 Chester-Jensen Company, Inc. (6/ 6/58)
5th & Tilgham Streets
Chester, Pennsylvania 19013

2 CREPACO, Inc. (5/ 1/56)
100 C.P. Avenue
Lake Mills, Wisconsin 53551

117 DCI, Inc. (10/28/59)
St. Cloud Industrial Park
St. Cloud, Minnesota 56301

76 Damrow Company (10/31/57)
196 Western Avenue
Fond du Lac, Wisconsin 54935

115 DeLaval Company, Ltd. (9/28/59)
113 Park Street South
Peterborough, Ontario, Canada
(not available in USA)

109 Girton Manufacturing Company (9/30/58)
State Street
Millville, Pennsylvania 17846

114 C. E. Howard Corporation (9/21/59)
P.O. Box 2507
City of Industry, California 91746

127 Paul Mueller Company (6/29/60)
P.O. Box 828
Springfield, Missouri 65801

31 Walker Stainless Equipment Co. (10/ 4/56)
Elroy, Wisconsin 53929

02-08 Pumps for Milk and Milk Products

214R Ben H. Anderson Manufacturers (5/20/70)
Morrisonville, Wisconsin 53571

212R Babson Bros. Co. (2/20/70)
2100 S. York Rd.
Oak Brook, Illinois 60621

29R Cherry-Burrell Corporation (10/ 3/56)
(unit AMCA Int'l)
2400 Sixth St., Southwest
Cedar Rapids, Iowa 52406

63R CREPACO, Inc. (4/29/57)
100 CP Avenue
Lake Mills, Wisconsin 53551

205R Dairy Equipment Company (5/22/69)
1919 South Stoughton Road
Madison, Wisconsin 53716

65R G & H Products, Inc. (5/22/57)
5718 52nd Street
Kenosha, Wisconsin 53140

145R ITT Jabsco, Incorporated (11/20/63)
1485 Dale Way
Costa Mesa, California 92626

314 Len E. Ivarson, Inc. (12/22/78)
3100 W. Green Tree Road
Milwaukee, Wisconsin 53223

26R Ladish Co., Tri-Clover Division (9/29/56)
9201 Wilmot Road
Kenosha, Wisconsin 53140

303 E. R. Mitchell Co., Inc. (3/ 8/78)
(Mfg. by Howard Pneumatic Eng. Co. Ltd.)
576 Haddon Ave.
Collingswood, NJ 08108

236 Megator Corporation (5/ 2/72)
(Mfg. by Howard Pneumatic Eng. Co. Ltd.)
125 Gamma Drive
Pittsburgh, Pennsylvania 15238

319 Mono Group, Inc. (3/21/79)
(Mfg. by SSP Pumps Ltd.)
847 Industrial Drive
Bensonville, IL 60106

241 Puriti S. A. (9/12/72)
Alfredo Noble #39, Industrial Pte. de Vigas
Tlalnepantla, Mexico
(not available in USA)

148 Robbins & Myers, Inc. (4/22/64)
Moyno Pump Division
1345 Lagonda Avenue
Springfield, Ohio 45501

306 Stamp Corp. (5/ 2/78)
1309 Culmen St.
Madison, WI 53713

72R L. C. Thomsen & Sons, Inc. (8/15/57)
1303 43rd Street
Kenosha, Wisconsin 53140

219 Tri-Canada Ltd. (2/15/71)
P.O. Box 4589
Buffalo, NY 14240

52R Viking Pump Div. (12/31/56)
Houdaille Industries, Inc.
406 State Street
Cedar Falls, Iowa 50613

5R Waukesha Foundry Company (7/ 6/56)
1300 Lincoln Ave.
Waukesha, Wisconsin 53186

04-03 Homogenizers and High Pressure Pumps of the Plunger Type

247 Bran and Lubbe, Inc. (4/14/73)
1241 Rand Rd.
Des Plaines, IL 60016

87 Cherry-Burrell Company (12/20/57)
(unit AMCA Int'l)
2400 Sixth Street, Southwest
Cedar Rapids, Iowa 52404

37 CREPACO, Inc. (10/19/56)
100 CP Avenue
Lake Mills, Wisconsin 53538

75 Gaulin, Inc. (9/26/57)
44 Garden Street
Everett, Massachusetts 02149

237 Graco Inc. (6/ 3/72)
P.O. Box 1441
Minneapolis, Minnesota 55440

309 General Dairy Equipment (7/19/78)
(Mfg. by Rannie A/S, Denmark)
434 Stinson Boulevard
Minneapolis, Minnesota 55413

256 Hercules, Inc. (1/23/74)
2285 University Avenue
St. Paul, Minnesota 55114

282 Knudsen Corporation (11/ 8/76)
(Mfg. by Gaulin Inc.)
715 N. Divisadero Street
Visalia, California 93277

05-13 Stainless Steel Automotive Milk Transportation Tanks for Bulk Delivery and/or Farm Pick-up Service

131R Almont Welding Works, Inc. (9/ 3/60)
4091 Van Dyke Road
Almont, Michigan 48003

70R Brenner Tank, Inc. (8/ 5/57)
450 Arlington
Fond du Lac, Wisconsin 54935

40 Butler Manufacturing Co. (10/20/56)
900 Sixth Ave., Southeast
Minneapolis, Minnesota 55114

66 Dairy Equipment Company (5/29/57)
1919 South Stoughton Road
Madison, Wisconsin 53716

45 The Heil Company (10/26/56)
3000 W. Montana Street
Milwaukee, Wisconsin 53235

297 Indiana Tank Co., Inc. (8/29/77)
P.O. Box 366
Simmitt, Indiana 46070

305 Light Industrial Design Co. (3/23/78)
3726 Halverstick Rd.
Sumas, WA 98295

201 Paul Krohnert Mfg., Ltd. (4/ 1/68)
811 Steeles Avenue
Milton, Ontario, Canada L9T 2Y3
(not available in USA)

85 Polar Tank Trailer, Inc. (12/20/57)
Holdingford, Minnesota 56340

121 Technova Inc. Gosselin Division (12/ 9/59)
1450 Hebert c.p. 758
Drummondville, Quebec, Canada
(not available in USA)

189 A. & L. Tougas, Ltee (10/ 3/66)
1 Tougas St.
Iberville, Quebec, Canada
(not available in USA)

47 Trailmobile, Div. of Pullman, Inc. (11/ 2/56)
701 East 16th Avenue
North Kansas City, Missouri 64116

25 Walker Stainless Equipment Co. (9/28/56)
New Lisbon, Wisconsin 53950

08-17 Fittings Used on Milk and Milk Products Equipment and Used on Sanitary Lines Conducting Milk and Milk Products

291 Accurate Metering Systems, Inc. (6/22/77)
1731 Carmen Drive
Elk Grove Village, IL 60007

79R Alloy Products Corporation (11/23/57)
1045 Perkins Avenue
Waukesha, Wisconsin 53186

245 Babson Brothers Company (2/12/73)
2100 South York Road
Oak Brook, Illinois 60521

284 Bristol Engineering Company (11/18/76)
210 Beaver Street
Yorkville, Illinois 60560

301 Brown Equip. Co., Inc. (12/ 6/77)
9955-9 1/4 Ave.
Hanford, California 93230

82R Cherry-Burrell Company (12/11/57)
(unit AMCA Int'l)
2400 Sixth Street, Southwest
Cedar Rapids, Iowa 52406

260 CREPACO, Inc. (5/22/74)
100 CP Avenue
Lake Mills, Wisconsin 53551

322 The DeLaval Company Ltd. (7/16/79)
113 Park St. So.
Peterborough, Ontario
Canada K9J 3R8

304 EGMO Ltd-Israel (3/16/78)
(Martin Silver P.E.)
406 Kinderamack Rd.
River Edge, NJ 07661

271 The Foxboro Company (3/ 8/76)
Neponset Street
Foxboro, Massachusetts 02035

67R G & H Products, Inc. (6/10/57)
5718 52nd Street
Kenosha, Wisconsin 53140

203R ITT-Grinnell Company, Inc. (11/ 7/68)
DIA-FLO Div
33 Centerville Rd.
Lancaster, Pennsylvania 17603

34R Ladish Co., Tri-Clover Division (10/15/56)
9201 Wilmot Road
Kenosha, Wisconsin 53140

287 Koltek OY (1/14/77)
Kotinummentieiz
SF-00700 Helsinki 70
Finland
(not available in USA)

239 LUMACO (6/30/72)
Box 688,
Teaneck, New Jersey 07666

200R Paul Mueller Co. (3/ 5/68)
P.O. Box 828
Springfield, Missouri 65801

295 Precision Stainless Products (8/11/77)
(Mfg. by Toyo Stainless Co. Ltd.)
5636 Shull St.
Bell Gardens, CA 90201

242 Puriti, S.A. (9/12/72)
Alfredo Nobel #39 Industrial Pte de Vigas
Tlalnepantla, Mexico
(not available in USA)

149R Q Controls (5/18/64)
Occidental, California 95465

73R L. C. Thomsen & Sons, Inc. (8/31/57)
1303 43rd Street
Kenosha, Wisconsin 53140

300 Superior Stainless, Inc. (11/22/77)
211 Sugar Creek Rd.
P.O. Box 622
Delvan, Wisconsin 53115

191R Tri-Canada, Ltd. (11/23/66)
P.O. Box 4589
Buffalo, NY 14240

250 Universal Milking Machine (6/11/73)
Div. of Universal Cooperatives
407 First Ave, So.
Albert Lea, Minnesota 56007

278 Valex Products (8/30/76)
9421 Winnetka
Chatsworth, California 91311

86R Waukesha Specialty Company, Inc. (12/20/57)
Darien, Wisconsin 53114

Inlet and Outlet Leak Protector Plug Valves for Batch Pasteurizers

09-07 Instrument Fittings and Connections Used on Milk and Milk Products Equipment

321 Anderson Instrument Co., Inc. (6/14/79)
R.F.D. #1, Fulton, New York 12072

315 Burns Engineering, Inc. (2/ 5/79)
10201 Bren Road, East
Minnetonka, MN 55343

206 The Foxboro Company (8/11/69)
Neponset Avenue
Foxboro, Massachusetts 02035

285 Tank Mate Company (12/ 7/76)
1815 Eleanor
St. Paul, Minnesota 55116

32 Taylor Instrument Process Control (10/ 4/56)
Div. Sybron Corporation
95 Ames Street
Rochester, New York 14601

246 United Electric Controls (3/24/73)
85 School Street
Watertown, Massachusetts 02172

10-00 Milk and Milk Products Filters Using Disposable Filter Media, As Amended

35 Ladish Co., Tri-Clover Division (10/15/56)
9201 Wilmot Road
Kenosha, Wisconsin 53140

296 L. C. Thomsen & Sons, Inc. (8/15/77)
1303 43rd St.
Kenosha, Wisconsin 53140

11-03 Plate-type Heat Exchangers for Milk and Milk Products

316 Agric Machinery Corp. (2/ 7/79)
P.O. Box 6
Madison, NJ 07940

20 A.P.V. Company, Inc. (9/ 4/56)
395 Fillmore Avenue
Tonawanda, New York 14150

30 Cherry-Burrell Corporation (10/ 1/56)
(unit AMCA Int'l)
2400 Sixth Street, Southwest
Cedar Rapids, Iowa 52404

14 Chester-Jensen Co., Inc. (8/15/56)
5th & Tilgham Streets
Chester, Pennsylvania 19013

38 CREPACO, Inc. (10/19/56)
100 CP Avenue
Lake Mills, Wisconsin 53551

120 DeLaval Company, Ltd. (12/ 3/59)
113 Park Street
South Peterborough, Ontario, Canada
(not available in USA)

279 The Schluter Co. (8/29/76)
(Mfg. by Samuel Parker Ltd.)
112 E. Centerway
Janesville, WI 53545

17 The DeLaval Separator Company (8/30/56)
Alfa-Laval Dairy Div.
P.O. Box 430
Somerville, NJ 08876

15 Kusel Dairy Equipment Company (8/15/56)
820 West Street
Watertown, Wisconsin 53094

12-04 Internal Return Tubular Heat Exchangers, for Milk and Milk Products

248 Allegheny Bradford Corporation (4/16/73)
P.O. Box 264
Bradford, Pennsylvania 16701

243 Babson Brothers Company (10/31/72)
2100 S. York Road
Oak Brook, Illinois 60521

103 Chester-Jensen Company, Inc. (6/ 6/58)
5th & Tilgham Street
Chester, Pennsylvania 19013

307 G&H Products, Inc. (5/ 2/78)
5718-52nd St.
Kenosha, WI 53141

217 Girton Manufacturing Co. (1/23/71)
Millville, Pennsylvania 17846

252 Ernest Laffranchi (12/27/73)
P.O. Box 455
Ferndale, California 95536

238 Paul Mueller Company (6/28/72)
P.O. Box 828
Springfield, Missouri 65801

96 C. E. Rogers Company (3/31/64)
P.O. Box 188
Mora, Minnesota 55051

13-06 Farm Milk Cooling and Holding Tanks

240 Babson Brothers Company (9/ 5/72)
(Mfg. by CREPACO, Inc.)
2100 S. York Road
Oak Brook, Illinois 60521

11R CREPACO, Inc. (7/25/56)
100 CP Ave.
Lake Mills, Wisconsin 53551

119R DCI, Inc. (10/28/59)
St. Cloud Industrial Park
St. Cloud, Minnesota 56301

4R Dairy Equipment Company (6/15/56)
1919 South Stoughton Road
Madison, Wisconsin 53716

92R DeLaval Company, Ltd. (12/27/57)
113 Park Street
South Peterborough, Ontario, Canada

49R The DeLaval Separator Company (12/ 5/56)
Alfa-Laval Dairy Div.
P.O. Box 430
Somerville, NJ 08876

10R Girton Manufacturing Company (7/25/56)
Millville, Pennsylvania 17846

95R Globe Fabricators, Inc. (3/14/58)
3350 North Gilman Rd.
El Monte, California 91732
(not available in USA)

179R Heavy Duty Products (Preston), Ltd. (3/ 8/66)
1261 Industrial Road
Preston, Ontario, Canada
(not available in USA)

12R Paul Mueller Company (7/31/56)
P.O. Box 828
Springfield, Missouri 65801

249 Sunset Equipment Co. (4/16/73)
293 Como Ave.
St. Paul, Minnesota 55103

16R Zero Manufacturing Company (8/27/56)
Washington, Missouri 63090

16-04 Evaporators and Vacuum Pans for Milk and Milk Products

164R Anderson IBEC (4/25/65)
19609 Progress Drive
Strongsville, Ohio 44136

254 Anhydro, Inc. (1/ 7/74)
165 John Dietsch Square
Attleboro Falls, Massachusetts 02763

132R A.P.V. Company, Inc. (10/26/60)
137 Arthur Street
Buffalo, New York 14207

263 C. E. Howard Corporation (12/21/74)
240 N. Orange Avenue
City of Industry, California 91746

107R C. E. Rogers Company (8/ 1/58)
P.O. Box 118
Mora, Minnesota 55051

277 ConTherm Corp. (8/19/76)
P.O. Box 352
Newbury Port, MA 01950

294 DeLaval Separator Co. (7/19/77)
1600 County Rd. F.
Hudson, WI 54016

186R Marriott Walker Corporation (9/ 6/66)
925 East Maple Road
Birmingham, Michigan 48010

273 Niro Atomizer Inc. (5/20/76)
9165 Rumsey Road
Columbia, Maryland 21044

299 Stork-Bowen Engr. Co. (11/16/77)
(Mfg. by Stork-Friesland B.V.)
P.O. Box 898
Somerville, New Jersey 08876

311 Wiegand Evaporators, Inc. (8/28/78)
5565 Sterrett Place
Columbia, Maryland 21044

17-06 Fillers and Sealers of Single Service Containers For Milk and Milk Products

192 Cherry-Burrell Corporation (1/ 3/67)
(unit AMCA Int'l)
2400 Sixth St., Southwest
Cedar Rapids, IA 52404

137 Ex-Cell-O Corporation (10/17/62)
2855 Coolidge,
Troy, Michigan 48084

220 Hercules, Inc., Package Equipment Div. (4/24/71)
2285 University Ave.
St. Paul, Minnesota 55114

281 Purity Packaging Corporation (11/ 8/76)
4190 Fisher Road
Columbus, Ohio 43228

211 Steel & Cohen (Twin-Pak, Inc.) (2/ 4/70)
745 Fifth Avenue
New York, New York 10022

19-03 Batch and Continuous Freezers, For Ice Cream, Ices and Similarly Frozen Dairy Foods, As Amended

286 O.G. Hoyer, Inc. (12/ 8/76)
201 Broad St.
Lake Geneva, WI 53147
(Mfg. by O.G. Hoyer A/S of Denmark)

146 Cherry-Burrell Company (12/10/63)
(unit AMCA Int'l)
2400 Sixth Street, Southwest
Cedar Rapids, Iowa 52404

141 CREPACO, Inc. (4/15/63)
100 CP Avenue
Lake Mills, Wisconsin 53551

22-04 Silo-Type Storage Tanks for Milk and Milk Products

168 Cherry-Burrell Corporation (6/16/65)
(unit AMCA Int'l)
575 E. Mill St.
Little Falls, New York 13365

154 CREPACO, Inc. (2/10/65)
100 CP Avenue
Lake Mills, Wisconsin 53551

160 DCI, Inc. (4/ 5/65)
St. Cloud Industrial Park
St. Cloud, Minnesota 56301

181 Damrow Company, Division of DEC (5/18/66)
International, Inc., 196 Western Ave.
Fond du Lac, Wisconsin 54935

262 DeLaval Company Limited (11/11/74)
113 Park Street
South, Peterborough, Ontario, Canada

156 C. E. Howard Corporation (3/ 9/65)
240 N. Orange Ave., Box 2507
City of Industry, CA 91746

155 Paul Mueller Co. (2/10/65)
P.O. Box 828
Springfield, Missouri 65801

312 Sanitary Processing Equip. Corp. (9/15/78)
Butternut Drive
East Syracuse, New York

165 Walker Stainless Equipment Co. (4/26/65)
Elroy, Wisconsin 53929

23-01 Equipment for Packaging Frozen Desserts, Cottage Cheese and Milk Products Similar to Cottage Cheese in Single Service Containers

174 Anderson Bros. Mfg. Co. (9/28/65)
1303 Samuelson Road
Rockford, Illinois 61109

209 Doboy Packaging Machinery (7/23/69)
Domain Industries, Inc., 869 S. Knowles Ave.
New Richmond, Wisconsin 54017

302 Eskimo Pie Corp. (1/27/78)
530 E. Main St.
Richmond, Virginia 23219

258 Hercules, Inc. (2/ 8/74)
2285 University Ave.
St. Paul, Minnesotta 55114

24-00 Non-Coil Type Batch Pasteurizers

161 Cherry-Burrell Corporation (4/ 5/65)
(unit AMCA Int'l)
575 E. Mill St.
Little Falls, New York 13365

158 CREPACO, Inc. (3/24/65)
100 CP Avenue
Lake Mills, Wisconsin 53551

187 DCI, Inc. (9/26/66)
St. Cloud Industrial Park
St. Cloud, Minnesota 56301

177 Girton Manufacturing Co. (2/18/66)
Millville, Pennsylvania 17846

166 Paul Mueller Co. (4/26/65)
P.O. Box 828
Springfield, Missouri 65601

25-00 Non-Coil Type Batch Processors for Milk and Milk Products

275 Bepex Corporation (7/12/76)
150 Todd Road
Santa Rosa, California 95402

162 Cherry-Burrell Corporation (4/ 5/65)
(unit AMCA Int'l)
575 E. Mill St.
Litlle Falls, New York 13365

159 CREPACO, Inc. (3/24/65)
100 CP Avenue
Lake Mills, Wisconsin 53551

188 DCI, Inc. (9/26/66)
St. Cloud Industrial Park
St. Cloud, Minnesota 56301

167 Paul Mueller Co. (4/26/65)
Box 828
Springfield, Missouri 65801

202 Walker Stainless Equipment Co. (9/24/68)
New Lisbon, Wisconsin 53950

26-01 Sifters for Dry Milk and Dry Milk Products

229 Russell Finex Inc. (3/15/72)
156 W. Sandford Boulevard
Mt. Vernon, New York 10550

173 B. F. Gump Division (9/20/65)
Blaw-Knox Food & Chem. Equip. Inc.
750 E. Ferry St., P.O. Box 1041
Buffalo, NY 14211

185 Rotex, Inc. (8/10/66)
(Mfg. by Orville Simpson Co.)
1230 Knowlton St.
Cincinnati, Ohio 45223

176 Koppers Company, Inc. (1/ 4/66)
Metal Products Division
Sprout-Waldron Operation
Munsy, Pennsylvania 17756

172 SWECO, Inc. (9/ 1/65)
6033 E. Bandini Blvd.
Los Angeles, California 90051

27-01 Equipment for Packaging Dry Milk and Dry Milk Products

313 WPM Systems, Inc. (10/10/78)
Div. of St. Regis Paper Company
3990 Acoma St.
Denver, Colorado 80216

28-00 Flow Meters for Milk and Liquid Milk Products

272 Accurate Metering Systems, Inc. (4/ 2/76)
1731 Carmen Drive
Elk Grove Village, Illinois 60007

253 Badger Meter, Inc. (1/ 2/74)
4545 W. Brown Deer Road
Milwaukee, Wisconsin 53223

223 C-E IN-VAL-CO, Division of Combustion Engineering, Inc. (11/15/71)
P.O. Box 556, 3102 Charles Page Blvd.
Tulsa, Oklahoma 74101

265 Electronic Flo-Meters, Inc. (3/10/75)
1621 Jupiter Rd.
Garland, TX 75042

226 Fischer & Porter Company (12/ 9/71)
County Line Road
Warminster, Pennsylvania 18974

224 The Foxboro Company (11/16/71)
Neponset Avenue
Foxboro, Massachusetts 02035

320 Max Machinery, Inc. (3/28/79)
1420 Healdsburg Ave.
Healdsburg, CA 95448

270 Taylor Instrument Company Division (2/ 9/76)
Sybron Corporation, 95 Ames Street
Rochester, New York 14601

29-00 Air Eliminators for Milk and Fluid Milk Products

30-00 Farm Milk Storage Tanks

257 Babson Bros. Co. (2/ 7/74)
(Mfg. by CREPACO, Inc.)
2100 S. York Road
Oak Brook, Illinois 60521

31-00 Scraped Surface Heat Exchangers

274 Contherm Corporation (6/25/76)
P.O. Box 352
Newburyport, Massachusetts 01950

322 Cherry Burrell (7/26/79)
2400 6th St. SW
Cedar Rapids, IA 52406

290 CREPACO, Inc. (6/15/77)
100 So. CP Ave.
Lake Mills, WI 53551

32-00 Uninsulated Tanks for Milk and Milk Products

264 Cherry-Burrell Company, (1/27/75)
(unit AMCA Int'l)
575 E. Mill St.
Little Falls, NY 13365

268 DCI, Inc. (11/21/75)
P.O. Box 1227
St. Cloud, Minnesota 56301

33-00 Polished Metal Tubing for Dairy Products

310 Allegheny Bradford Corporation (7/19/78)
P.O. Box 264
Bradford, PA 16701

289 Ladish Co., Tri-Clover Division (1/21/77)
9201 Wilmot Road
Kenosha, Wisconsin 53140

308 Rath Mfg. Co. Inc. (6/15/77)
2505 Foster Ave.
Janesville, WI 53545

35-00 Continuous Blenders

292 Waukesha Foundry Div. ABEX Corp. (8/24/77)
1300 Lincoln Ave.
Waukesha, WI 53186

36-00 Colloid Mills

293 Waukesha Foundry Div., ABES Corp. (8/24/77)
1300 Lincoln Ave.
Waukesha, WI 53186

37-00 Pressure and Level Sensing Devices

318 Anderson Instrument Co., Inc. (4/ 9/79)
R.D.#1 Fulton, N.Y. 12072

317 C-E Invalco Division of Combustion (2/26/79)
Engineering, Inc.
P.O. Box 556
Tulsa, OK 74101

Coming Events

Aug. 20-22--FREEZING OF BAKED AND UNBAKED PRODUCTS. American Institute of Baking, 1213 Bakers Way, Manhattan, KS 66502. Contact: Darrell Brensing, AIB, 913-537-4750.

Aug. 29-31--FOURTH INTERNATIONAL IUPAC SYMPOSIUM ON MYCOTOXINS AND PHYCOTOXINS. Co-sponsored by World Health Organization and Swiss Society for Analytical and Applied Chemistry. Lausanne, Switzerland. For participation and poster presentation, contact: Prof. P. Krogh, Dept. of Veterinary Microbiology, School of Veterinary Medicine, Purdue University, West Lafayette, IN 47907 or Prof. D. Reymond, IUPAC, Case postale 88, 1814 La Tour de Peilz, Switzerland.

Sept. 6--WASHINGTON MILK SANITARIANS - OREGON MILK SANITARIANS, Joint Meeting. Vancouver, WA. Contact: Lloyd Leudecke, Dept. of Food Science and Technology, Washington State University, Pullman, WA 99163.

Sept. 9-11--FOOD WAREHOUSE SANITATION WORKSHOP. Sponsored by Food Sanitation Institute. Sheraton Post Inn, Cherry Hill, NJ. Contact: H. C. Rowe, Food Sanitation Institute, EMA, 1701 Drew St., Clearwater, FL 33515.

Sept. 10-13--2nd INTERNATIONAL CONFERENCE ON FOOD SERVICE SYSTEMS DESIGN. Harrogate, England. Contact: G. Glew, Catering Research Unit, Procter Dept. of Food Science, The University of Leeds, LS2 9JT, England.

Sept. 10-14--FOOD PROCESSORS ADVANCED MICROBIOLOGY SHORT COURSE. University of California, Davis. Fee $200. Contact: John C. Bruhn, Dept. of Food Science and Technology, University of California, Davis, CA 95616, 916-752-2192.

Sept. 11-12--AMERICAN CULTURED DAIRY PRODUCTS INSTITUTE, Annual Meeting and Conference. Southeast Holiday Inn, Madison, WI. Contact: C. Bronson Lane, ACDPI, P.O. Box 7813, Orlando, FL 32854.

Sept. 11-14--MAINTENANCE MANAGEMENT. American Institute of Baking, 1213 Bakers Way, Manhattan, KS 66502. Contact: Darrell Brensing, AIB, 913-537-4750.

Sept. 13-14--INTERNATIONAL SYMPOSIUM ON ANIMAL AND HUMAN INFLUENZA. Ecole Nationale Veterinaire D'Alfort, 7, avenue du General de Gaulle, 97 704 Maisons-Alfort cedex, France. Contact: Ch. Pilet, Secretariat of the Dept. of Microbiology, Ecole Nationale Veterinaire d'Alfort.

Sept. 13-14--MINNESOTA SANITARIANS CONFERENCE. Earle Brown Continuing Education Center, St. Paul Campus, University of Minnesota. Mastitis control, "STOP" program, quality control and energy conservation on the farm will be discussed. Awards banquet, 7 p.m., Sept. 13, Eagles Club No. 33, St. Paul. Contact: Edward A. Kaeder, Publicity Chairman, Minnesota Sanitarians Assoc., Mid-America Dairymen, Inc., 2424 Territorial Road, St. Paul, MN 55114, 612-646-2854.

Sept. 17-19--BAKING PRODUCTION TECHNOLOGY. Sponsored by American Institute of Baking. Dunfey Dallas Hotel, Dallas, TX. Fee: $300 per person. Contact: Registrar, AIB, 1213 Bakers Way, Manhattan, KS 66502, 913-537-4750.

Sept. 18-20--PROCESSING AND QUALITY ASSURANCE UPDATE FOR THE CITRUS INDUSTRY. University of Florida, Gainesville. Sponsored by Cooperative Extension Service, Institute of Food and Agricultural Sciences, and IFT, Florida Section. Contact: R. F. Matthews, Food Technologist, Florida Cooperative Extension Service, 325 Food Science Bldg., University of Florida, Gainesville, FL 32611.

Sept. 18-20--WESTPACK '79. Convention Center, Anaheim, CA. Contact: Clapp & Poliak, Inc., 245 Park Ave., New York, NY 10017.

Sept. 19-20--NEW YORK STATE ASSOCIATION OF MILK & FOOD SANITARIANS, Annual Meeting. Holiday Inn Arena, 2-8 Hawley Street, Binghamton, NY 13901. Sponsored by NYSAMFS, Cornell University Food Science Dept., New York State Dept. of Health, New York State Dept. of Agriculture and Markets. Contact: R. P. March, 124 Stocking Hall, Ithaca, NY 14853, 256-4550.

Sept. 19-20--WISCONSIN ASSOCIATION OF MILK AND FOOD SANITARIANS, Annual Meeting. Madison, WI. Sponsored by WAMFS, Wisconsin Dairy Plant Fieldmen's Association, Wisconsin Dairy Tech Society, Wisconsin Environmental Health Association, and Wisconsin Institute of Food Technologists. Contact: Neil Vassau, 4702 University Ave., Madison, WI 53705.

con't p. 630

News and Events

IAMFES Election Results Announced

Results of the 1979 IAMFES, Inc. election were announced recently by Harold J. Barnum, Chairman of the Nominating Committee.

Dr. Robert T. Marshall, Professor of Food Science and Nutrition at the University of Missouri, Columbia, was elected to the post of Second Vice President. Dr. A. Richard Brazis, Corporate Microbiologist, Research and Development Laboratory, Fairmont Foods Company, Omaha, was elected Secretary-Treasurer.

Both men will assume their new duties for the Association at the Annual Meeting this month in Orlando, FL.

A total of 699 ballots were cast for second vice president and 690 for secretary-treasurer.

YOU'LL FIND ADDITIONAL News and Events items on pages 650, 663, 665, 674, 678, 689, 692, and 700.

Mastitis Book Available

Current Concepts of Bovine Mastitis, published by the National Mastitis Council, Inc., is available through *Hoard's Dairyman*, Fort Atkinson, WI, 53538.

The book, now in its second edition, discusses the microorganisms that cause bovine mastitis, the nature and development of the disease, detection of the disease and abnormal milk. Also discussed are factors influencing susceptibility to the disease, the environment and its contribution to mastitis, procedures for preventing the spread of infection, treatment, and procedures for control of specific udder infections.

MMFEHA Holds First Annual Meeting

Meeting for the first time as a combined association of the state IAMFES and NEHA affiliates, the Missouri Milk, Food and Environmental Health Association held its annual meeting March 26-29 at the Lodge of the Four Seasons in Lake Ozark, MO.

The membership elected as its leaders for the following year these persons: Jim Gifford, president; Ray Lange, first vice-president; John Stark, second vice-president; Bill Kenley, treasurer; Erwin Gadd, secretary; and Eugene Viets and Ron Blumer, auditors.

J. C. Hounschell Director, Joplin City Health Dept., was named "Sanitarian of the Year" at the awards banquet.

In addressing a food session during the three-and-a-half day meeting, John Farquhar emphasized that "good sanitation is good business." Farquhar is Vice President, Educational and Technical Affairs, Food Marketing Institute, Washington, DC. "Show the manager his role in good food sanitation practices," he stressed. "Continually approach sanitation from the economic angle. Emphasize its cost effectiveness," he explained.

Executive Board members of the Missouri Milk, Food and Environmental Health Assoc., are, left to right, Erwin Gadd, Ray Lange, Jim Gifford, Eugene Viets, Ron Blumer, and Bill Kenley.

Dr. Frank Bryan, Chief of Foodborne Disease Training for the Center for Disease Control, Atlanta, GA, noted that primary factors in foodborne disease are inadequate cooling of food, inadequate hot storage, and preparation of food long before serving. These are primarily time and temperature problems, he explained. "We must emphasize during consultations and inspections the identification of factors that contribute to outbreaks of foodborne disease in preference to looking for minor violations of sanitation codes," he noted. "We do not see dirty walls, floors, and dishes as the main problem, although they often receive high priority" in inspections, he explained.

"Quality Control in Franchised Chains," was discussed by Jim Baumgartner, co-owner, McDonald's, Jefferson City, MO. He noted that the most difficult sanitation problem is trying to teach employees not to touch food after scratching their hair or faces. Sanitation standards are like any other area of company operations, he noted, adding, "the top of the line for one company might be the bottom of the line for another."

Other presentations during the meeting included the STOP program, electronic milk cell counting, pasteurization and UHT, NIFI applied sanitation certification, arthropod disease vectors and their control, and innovations in private sewage treatment.

CLEHA Elects Leaders

Jack Hatlen, Professor of Environmental Health, University of Washington (Seattle), has been elected chairman of the U.S. Conference of Local Environmental Health Administrators.

Other officers elected at the Conference, June 24-26, at Charleston, South Carolina are: Dr. Bailus Walker, Jr., Chairman-Elect, Administrator, Environmental Health Administration, Washington, D.C.; Harry Marsh, Vice Chairman, Director, Environmental Health, Lexington, Kentucky; Richard Roberts, Secretary, Director, Environmental Health, San Bernardino, California.

The Conference of Local Environmental Health Administrators (CLEHA) is a national professional organization dedicated to improved management of environmental health services through exchange, development and dissemination of information about environmental health administration.

Index to Advertisers

THE ONLY *Approved* SANITARY METHOD OF APPLYING A U. S. P. LUBRICANT TO DAIRY & FOOD PROCESSING EQUIPMENT

Haynes Spray

U. S. P. LIQUID PETROLATUM SPRAY

U.S.P. UNITED STATES PHARMACEUTICAL STANDARDS

CONTAINS NO ANIMAL OR VEGETABLE FATS. ABSOLUTELY NEUTRAL. WILL NOT TURN RANCID—CONTAMINATE OR TAINT WHEN IN CONTACT WITH FOOD PRODUCTS.

SANITARY—PURE

ODORLESS—TASTELESS

NON-TOXIC

This Fine Mist-like **HAYNES-SPRAY** *should be used to lubricate:*

SANITARY VALVES
HOMOGENIZER PISTONS — RINGS
SANITARY SEALS & PARTS
CAPPER SLIDES & PARTS
POSITIVE PUMP PARTS
GLASS & PAPER FILLING MACHINE PARTS
and for ALL OTHER SANITARY MACHINE PARTS which are cleaned daily.

The Modern HAYNES-SPRAY *Method of Lubrication Conforms with the Milk Ordinance and Code Recommended by the U. S. Public Health Service*

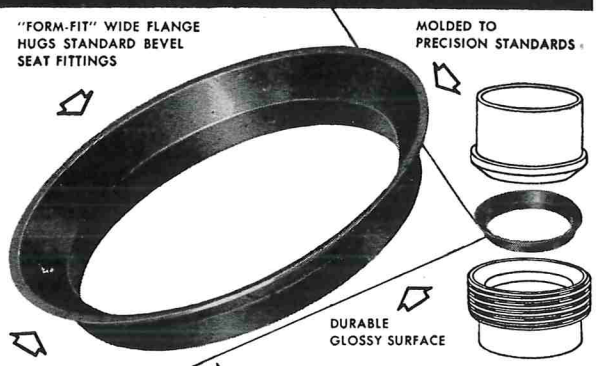
The Haynes-Spray eliminates the danger of contamination which is possible by old fashioned lubricating methods. Spreading lubricants by the use of the finger method may entirely destroy previous bactericidal treatment of equipment.

PACKED 6-12 oz. CANS PER CARTON
SHIPPING WEIGHT—7 LBS.

THE HAYNES MANUFACTURING CO.
4180 Lorain Ave. • Cleveland, Ohio 44113

HAYNES-SPRAY INGREDIENTS ARE APPROVED ADDITIVES AND CAN BE SAFELY USED AS A LUBRICANT FOR FOOD PROCESSING EQUIPMENT WHEN USED IN COMPLIANCE WITH EXISTING FOOD ADDITIVES REGULATIONS.

HAYNES SNAP-TITE GASKETS



LOW COST...RE-USABLE

LEAK-PREVENTING

NEOPRENE GASKET for Sanitary Fittings

Check these SNAP-TITE *Advantages*

- Tight joints, no leaks, no shrinkage
- Sanitary, unaffected by heat or fats
- Non-porous, no seams or crevices
- Odorless, polished surfaces, easily cleaned
- Withstand sterilization
- Time-saving, easy to assemble
- Self-centering
- No sticking to fittings
- Eliminate line blocks
- Help overcome line vibrations
- Long life, use over and over

Available for 1", 1½", 2", 2½" and 3" fittings.
Packed 100 to the box. Order through your dairy supply house.

THE HAYNES MANUFACTURING CO.
4180 Lorain Avenue • Cleveland 13, Ohio

HAYNES SELF-CENTERING SNAP-TITE® Gaskets

SIZES 1" - 1½" 2" - 2½" - 3" - 4"

Gasket Color . . . slightly off-white

*MADE FROM **TEFLON®**

"The Sophisticated Gasket"

THE IDEAL UNION SEAL FOR BOTH VACUUM AND PRESSURE LINES

SNAP-TITE self-centering gaskets of TEFLON are designed for all standard bevel seat sanitary fittings. They SNAP into place providing self-alignment and ease of assembly and disassembly.
HAYNES SNAP-TITES of TEFLON are unaffected by cleaning solutions, 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

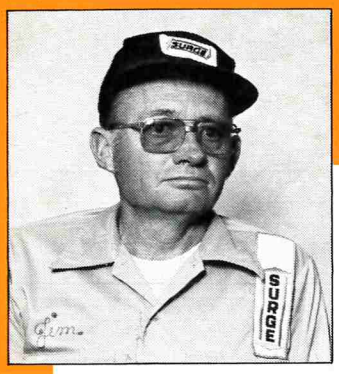
For Use in Dairies — Ice Cream Plants — Breweries — Beverage Plants — Bakeries — Canneries — Packing Plants

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

SOMEONE YOU SHOULD KNOW IN THE DAIRY INDUSTRY



Jim Rahr, Dairy Sanitation Routeman

Jim Rahr works for Botens Dairy Supply, in Cuba, N.Y. and has been a routeman for nine years. Before that he was a dairyman with one of the largest herds in Allegany Co., N.Y. A graduate of New York State Agriculture Technical Institute, and the Surge Training Center in Illinois, Jim offers his views on the value of a dairy route sanitation program.

"For a dairyman trying to make a living from his commitment to the dairy industry, a routeman is his link to the dairy equipment dealership. The routeman is the dealership in the field, sharing the latest information on good milking practices, improved equipment and better sanitation.

Qualities Of a Routeman

"A good routeman needs three basic qualities to help him succeed: Honesty, knowledge of his customer's business, and respect for that customer. A man is only as good as his promise, and if my promise is no good, that's the way I'm perceived. This means when I say I'll deliver a part or merchandise, I make sure I deliver it on time as promised.

"Dairymen expect me to know about their business. Sometimes I feel like a walking encyclopedia, but to help a man do a better job, you have to know the things that can help him. My years as a dairyman helped greatly, and I still keep up on the latest dairy information.

"Respect for the dairyman may be the most important part of a routeman's job. You have to remember the dairyman is a businessman, and his beliefs are part of his livelihood. You might see a way to do something differently to help him out, but you tell him from a position of respect, not superiority.

How We Help

"Since we're bringing the dealership to the dairyman, we can help in a number of ways right there. Our services include:

- Testing the dairyman's water and prescribing the best detergent to meet his needs.
- Making sure service is available to him when his equipment needs attention.
- Leaving enough supplies so the dairyman won't run out and have his operation suffer.
- Delivering supplies in bulk, at the lowest price we can offer.
- Informing him on the latest information which can help improve his operation.
- Checking important details such as vacuum pump oil and vacuum controls to make sure they're working properly.

"In addition, there are some intangible values which only a routeman who's involved with his customer can offer. Like suggesting help from an outside source such as a vet or extension specialist. Understanding the dairyman's thinking helps me serve him better. Once he understands I'm only in business if he is, he knows I want to help him succeed.

"You can't be pushy, but you want the dairyman to understand the importance of things like changing inflations often enough to protect his herd, and using the right products for proper sanitation. I try to think to myself, if I were this dairyman, what would I want to know to help improve my business? This helps me explain things without seeming pushy or like a hardline salesman.

"Another important service which helps both routeman and dairyman is the records kept on supplies used. When I was on the farm, I was a stickler for record keeping and I still believe in it. My records assure the dairyman enough supplies without overstocking. I also know how often inflations need to be replaced.

"We want to help the dairyman help his cows' performance. In my dairy, I had a sign in my parlor which read, 'Every Cow Is A Lady, Treat Her As Such.' I modified that as a motto for our dealership to say, 'We Are The Milking Cow's Friends.' I truly believe a route program is worthwhile for the dairyman and his herd or I wouldn't be in it, and you can take my word on that!"

Babson Bros. Co., 2100 South York Road, Oak Brook, Illinois 60521