

Journal of
Milk and Food
Technology
**Journal of
Food Protection™**

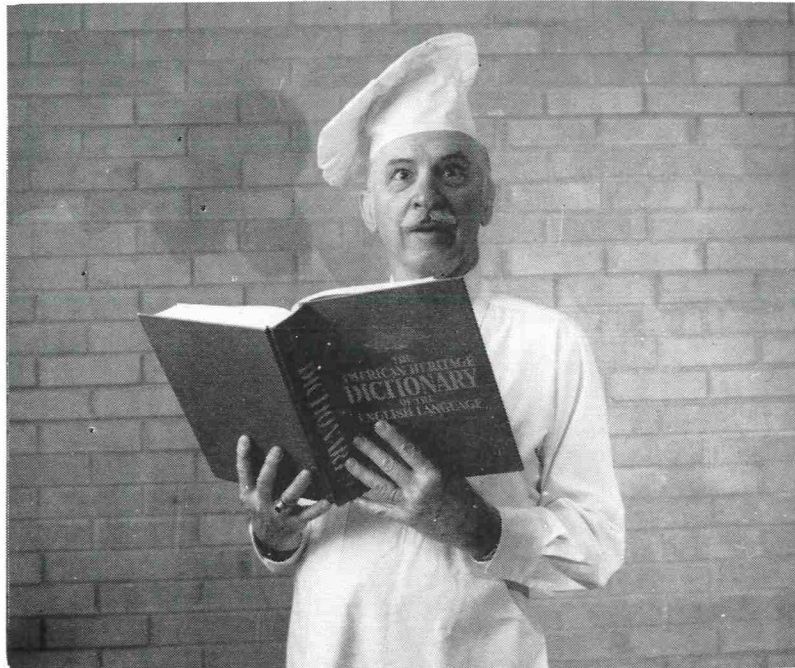
**CALL FOR RESEARCH PAPERS
FOR 1977 IAMFES MEETING**

Contributed research papers will be an important part of the program at the 1977 Annual Meeting of IAMFES scheduled for August 14-18 at the Sioux City Hilton, Sioux City, Iowa. Abstract forms and complete information about presenting papers can be found in this issue.



**Official
Publication**

“CLEANABILITY” isn't in the dictionary but



NSF ASSURES IT IN FOOD SERVICE EQUIPMENT

NSF standards for equipment used in the preparation and storage of foods are *sanitation* standards, and high on the list is cleanability. Rounded corners are specified instead of right angles that trap and hold particles of food. Cracks, crevices and hard-to-clean areas are prohibited. And this is simply the beginning of a long list of expert requirements in the eighteen NSF standards affecting thousands of items of food service equipment made by more than 700 U.S. and foreign manufacturers.

These widely accepted standards have been developed at NSF by joint committees of outstanding public health officials, manufacturers and consumer representatives—backed by the resources of NSF.

State and local authorities have good reason to endorse NSF standards and enforce them. “Cleanability” isn’t in their dictionaries either but it’s high on their list of priorities in helping restaurant operators to protect the public health.

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

NSF

APPLICATION FOR MEMBERSHIP

(Membership open to individuals only)

All memberships on calendar year basis—Membership includes subscription to Journal of Food Protection

Name _____ Date _____
Please Print

Address _____
Zip Code _____
 Renewal
 New
 Re-instatement

Is the above your _____ business _____ home address?

Direct Member Annual Dues \$16.00

Affiliate membership—through an affiliate—\$14.00 plus affiliate dues (contact your local affiliate secretary)

Student Membership \$5.00 with verification of student status. Student subscription effective from receipt of payment to the end of the calendar year.

SUBSCRIPTION ORDER

Name _____ Date _____
Please Print

Address _____
Zip Code _____
 Renewal
 New

Educational Institution & Public Libraries-----\$32.00
Individual Non-Member Subscription-----\$32.00
Government Agencies, Commercial Organizations-----\$32.00

All subscriptions on calendar year basis.

CHANGE OF ADDRESS

Please write to us before you move, 30 days in advance, and mail to Journal of Food Protection, P.O. Box 701, Ames, Iowa 50010.

NAME _____

OLD ADDRESS _____

NEW ADDRESS _____

Effective date _____ Publication _____

PUBLICATIONS ORDER FORM

3-A Sanitary Standards

- () Complete set of softbound -----ea. \$ 8.00
- () Complete set unbound (loose pages) -----ea. \$ 6.50
- () Complete set softbound 3-A & Egg 3-A stds. -----ea. \$10.25
- () Egg 3-A Stds (unbound \$2.25) softbound -----ea. \$ 3.50
- () High Temp-Short Time Standard (Included in complete set) -----ea. \$ 1.50
25 or more \$1.00 ea.
- () 3-A Accepted Practices for Milking Machines (Included in complete set)-----ea. \$.30
100-1,000 \$.25 ea.

Five-Year Service on 3-A Sanitary Standards

All revisions and additions as published

- () 3-A Stds. -----Five years \$ 6.00
- () Egg 3-A Stds. -----Five years \$ 3.00

Methods for Production of High Quality Raw Milk-----ea. \$ 2.00
25-100 copies \$1.75 ea., 100 or more \$1.50 ea.

Procedures to Investigate Foodborne Illness-----ea. \$ 1.50
25-99 copies, \$1.25 ea., 100 or more, \$.90 ea.

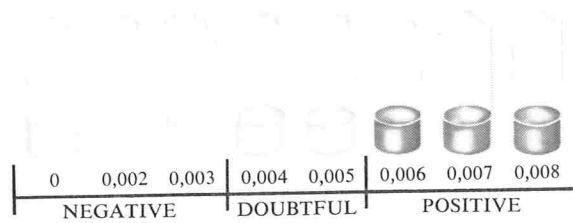
— All prices F.O.B., Ames, Iowa —

Order from: IAMFES, P.O. Box 701, Ames, Iowa 50010

Name: _____ Date _____

Address: _____
Please Print Include Zip Code

Simple!

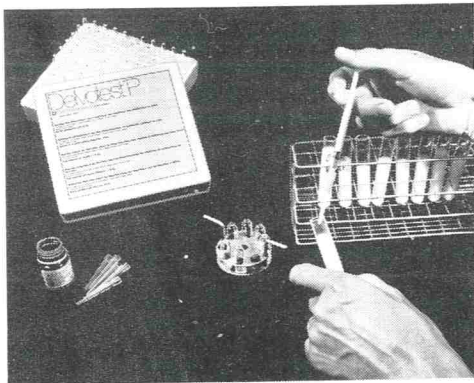


Now available in the U.S.!

A rapid, accurate test for Penicillin residue in milk

Delvotest[®]P

Standard diffusion test for the determination of antibiotic residues in milk



A complete self-contained kit. Contains nutrient-indicator tablets, dosing syringe, disposable syringe tips and ampules containing seeded agar.

It's easy to use. Ampules are broken at the neck, a nutrient-indicator tablet is added, 0.1 ml sample added via syringe, ampule placed in a water bath of 64° (±) 2°C.

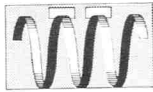
It's rapid. Readings are taken after 2½ hours or after 1½ hour with 1 hour "pre-incubation" period.

It's sensitive. Penicillin concentrations of 0.003 I.U. or less per ml. of milk always yield a negative test result (entirely yellow), while levels of 0.006 I.U. or higher give a positive result (entirely purple). In-between concentrations give yellow-purple and purple colorations.

It's economical. Ideal for screening of loads and practical to perform for one or many determinations.

It's diverse. Also sensitive to most antibiotics used in lactating cattle.

Manufactured by
Gist-Brocades nv
Postbus 1 - Delft
The Netherlands



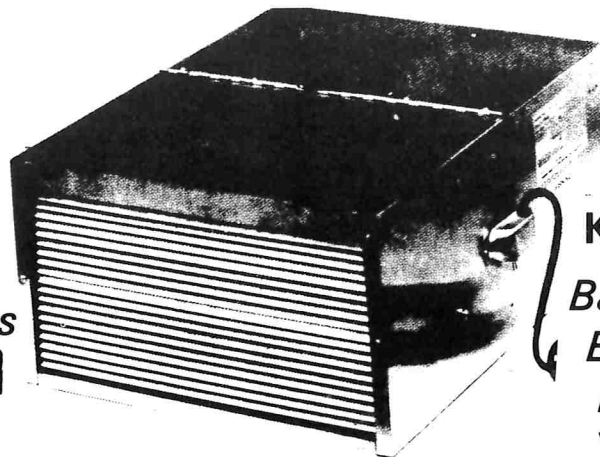
Exclusive distributors for the USA
Enzyme Development Corporation
2 Penn Plaza, New York, N.Y. 10001
Telephone: (212) 736-1580

Sani-Matic Ultraviolet Airborne Bacteria Killing System

APPLICATION

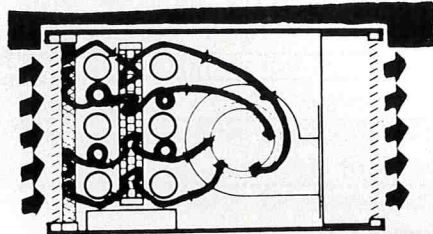
- Test Laboratories*
- Culture - setting rooms*
- Product process areas*
- Preparations - Wrapping*
- Packaging Lines*
- Coolers - Ref. Storage Areas*

This certificate worth \$50 toward



Filter Removes Odors, Smoke, Dust

- KILLS**
- Bacteria*
- E. Coli*
- Mold*
- Yeast*
- Phage*
- Salmonella*
- Virus*



Att: Dir. Marketing
The Schlueter Company, P.O. Box 548, Janesville, Wisconsin 53545

I have immediate interest in the application of your UVBK and wish to learn how to "cash in" on your special offer.

Name _____ Address _____ Zip _____
Company _____ Telephone _____

OFFICERS AND EXECUTIVE BOARD

President, HENRY V. ATHERTON, Dairy Building, University of Vermont, Burlington, Vermont 05401

President-Elect, DAVID FRY, P.O. Box 2113, Orlando, Florida 32802

First Vice-President, HOWARD HUTCHINGS, 624 N. Poplar, Pierre, South Dakota 57501

Second Vice-President, RICHARD P. MARCH, 118 Stocking Hall, Cornell University, Ithaca, New York 14850

Secretary-Treasurer, WILLIAM KEMPA, 1058 Runningbrook Drive, Mississauga, Ontario L4Y 2T2, Canada

Junior Past-President, HAROLD E. THOMPSON, JR. 5123 Holden Street, Fairfax, Virginia 22030

Senior Past-President, P. J. SKULBORSTAD, 2100 South York Road, Oakbrook, Illinois 60521

Editors

DR. ELMER H. MARTH, *Editor*, Dept. of Food Science, University of Wisconsin, Madison, Wisconsin 53706

EARL O. WRIGHT, *Executive Secretary and Managing Editor*, Box 701, Ames, Iowa 50010

BARBARA LEE, *Assistant Executive Secretary and Managing Editor*, Box 701, Ames, Iowa 50010

Editorial Board

J. C. ACTON	Clemson, S.C.
J. A. ALFORD	Beltsville, Md.
F. W. BARBER	Ft. Myers Beach, Fla.
L. R. BEUCHAT	Experiment, Ga.
W. A. BOUGH	Experiment, Ga.
F. L. BRYAN	Atlanta, Ga.
L. B. BULLERMAN	Lincoln, Nebr.
F. F. BUSTA	St. Paul, Minn.
W. S. CLARK, Jr.	Chicago, Ill.
F. M. CLYDESDALE	Amherst, Mass.
N. A. COX	Athens, Ga.
R. W. DICKERSON	Cincinnati, Ohio
W. J. DYER	Halifax, N.S.
J. A. ELLIOTT	Ottawa, Ont.
J. C. FLAKE	Washington, D.C.
D. Y. C. FUNG	University Park, Pa.
S. E. GILLILAND	Raleigh, N.C.
H. S. GRONINGER	Seattle, Wash.
L. G. HARMON	East Lansing, Mich.
W. J. HAUSLER, Jr.	Iowa City, Iowa
N. F. INSALATA	White Plains, N.Y.
D. M. IRVINE	Guelph, Ont.
C. K. JOHNS	Bonita Springs, Fla.
J. A. KOBURGER	Gainesville, Fla.
H. KOREN	Terre Haute, Ind.
D. H. KROPP	Manhattan, Kansas
R. V. LECHOWICH	Blacksburg, Va.
R. T. MARSHALL	Columbia, Mo.
J. R. MATCHES	Seattle, Wash.
D. W. MATHER	Glenview, Ill.
E. M. MIKOLAJCIC	Columbus, Ohio
J. C. OLSON, Jr.	Washington, D.C.
N. F. OLSON	Madison, Wis.
Z. J. ORDAL	Urbana, Ill.
H. PIVNICK	Ottawa, Ont.
D. S. POSTLE	Ithaca, N.Y.
W. D. POWRIE	Vancouver, B.C.
R. B. READ, Jr.	Washington, D.C.
G. W. REINBOLD	Denver, Colo.
G. H. RICHARDSON	Logan, Utah
J. R. ROSENAU	Amherst, Mass.
W. E. SANDINE	Corvallis, Oregon
D. F. SPLITTSTOESSER	Geneva, N.Y.
J. A. TROLLER	Cincinnati, Ohio
B. A. TWIGG	College Park, Md.
C. VANDERZANT	College Station, Texas
J. H. von ELBE	Madison, Wis.
H. W. WALKER	Ames, Iowa
H. B. WARREN	Kansas City, Mo.
K. G. WECKEL	Madison, Wis.
E. A. ZOTTOLA	St. Paul, Minn.

Journal of Milk and Food Technology

INCLUDING MILK AND FOOD SANITATION

Official Publication,

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

Vol. 39	November 1976	No. 11
Research Papers		
A Comparison of Vacuum Packaging Systems and Films on the Physical Characteristics of Beef Cuts S. C. Seideman, Z. L. Carpenter, G. C. Smith, C. Vanderzant and K. E. Hoke		740
Effect of Various Types of Vacuum Packages and Length of Storage on the Microbial Flora of Wholesale and Retail Cuts of Beef S. C. Seideman, C. Vanderzant, M. O. Hanna, Z. L. Carpenter and G. C. Smith		745
Evaluation of Colony Growth of Bacteria and the Surface Environment of Beef R. B. Maxcy		754
Total Plate Count and Sensory Evaluation as Measures of Luncheon Meat Shelf Life W. M. Hill, J. Reaume and J. C. Wilcox		759
Coliform Analyses at 30 C Paul A. Hartman and Philip S. Hartman		763
Identification and Quantification of Fecal Coliforms using Violet Red Bile Agar at Elevated Temperature Harvey Klein and Daniel Y. C. Fung		768
Cooking Inoculated Pork in Microwave and Conventional Ovens H. W. Ockerman, V. R. Cahill, R. F. Plimpton and N. A. Parrett		771
A Research Note. Heat-sensitive Inhibitor(s) Produced in Poor Quality Raw Milk A. H. Duthie, C. G. Woelfel, K. M. Nilson and H. V. Atherton		774
A Research Note. A Method to Measure Pyruvate in Milk J. E. Albrecht and S. R. Tatini		776
General Interest		
Dairy Products as Food Protein Resources J. J. Jonas, T. W. Craig, R. L. Huston, E. H. Marth, E. W. Speckman, T. F. Steiner and S. M. Weisberg		778
3-A Sanitary Standards for Equipment for Packaging Frozen Desserts, Cottage Cheese and Similar Dairy Products Number 23-01		796
3-A Sanitary Standards for Portable Bins for Dry Milk and Dry Milk Products Number 34-00		800
Association Affairs		803
News and Events		806
Index to Advertisers		812

The Journal of Milk and Food Technology is issued monthly beginning with the January number. Each volume comprises 12 numbers. Published by the International Association of Milk, Food and Environmental Sanitarians, Inc. with executive offices of the Association, 413 Kellogg Ave., P.O. Box 701, Ames, Ia. 50010. Printed by Heuss Printing and Signs, Inc., 204 N. Oak, 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.

Page Charge: Effective January 1, 1969 a charge of \$25.00 per printed page will be made for all research papers which are published. See Volume 31, issues 10, 11, or 12 for details.

Orders for Reprints: All orders for reprints should be sent to IAMFES, Inc., P.O. Box 701, Ames, IA 50010.

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 \$2.50 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 \$16.00 per calendar year and include subscription to the JOURNAL OF MILK AND FOOD TECHNOLOGY. 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, Inc., P.O. Box 701, Ames, IA 50010.

A Comparison of Vacuum Packaging Systems and Films on the Physical Characteristics of Beef Cuts

S. C. SEIDEMAN, Z. L. CARPENTER, G. C. SMITH, C. VANDERZANT, and K. E. HOKE¹

*Meats and Meat Chemistry Section, Department of Animal Science
 Texas Agricultural Experiment Station, Texas A&M University
 College Station, Texas 77843*

(Received for publication February 17, 1976)

ABSTRACT

One hundred and fifty beef knuckles, 140 beef ribs, and 60 arm chucks were vacuum packaged by two systems (nozzle and chamber vacuumizing machines) in bags manufactured from films differing in oxygen and moisture vapor transmission rates. Primal cuts in each system were randomly assigned to storage periods of 7, 14, 21, 28, or 35 days. In addition, leaker packages were assigned storage periods of 7, 14, or 21 days. At the termination of each storage period, cuts were evaluated for degree of vacuum, appearance of fat cover, surface discoloration, and total desirability. The combination of a chamber vacuumizing machine and use of a packaging film with a low oxygen transmission rate resulted in superior fat appearance ratings, reduced surface discoloration, and higher total desirability scores. Cuts from leaker packages usually received the lowest ratings for these attributes.

Fresh beef shipment is changing from swinging carcasses to prefabricated primals and subprimal that are vacuum packaged or packaged in some type of modified atmosphere. By 1977, an estimated 70% of the fresh beef supply will be shipped as prefabricated primals and sub-primals and over 65% of the fresh meat will be shipped in vacuum packages (10). Previous studies have indicated that films with low moisture vapor transmission rates can be used successfully for beef quarters and cuts if extended storage periods are not required (7). Other studies (6, 8, 11) have shown the feasibility of vacuum packaging for extended storage periods. Important factors, relative to meat quality after extended storage in vacuum packages, are degree of vacuum achieved and/or maintained as a result of initial degree of vacuum or the permeability of the film. Chamber-type vacuum machines are generally considered capable of achieving a higher degree of vacuum than the nozzle-type vacuum machine (4). Film characteristics such as oxygen transmission rate and resistance to rupture are of paramount importance during shipment and subsequent storage of beef cuts.

This study was initiated to evaluate two vacuum packaging systems (nozzle vs. chamber) and three films with different oxygen permeabilities for use on boneless

(knuckles) and bone-in (ribs and chucks) beef cuts for extended storage periods.

MATERIALS AND METHODS

One hundred and fifty beef knuckles (IMPS 167), 140 beef wholesale ribs (IMPS 109), and 60 arm chucks (0-Bone) were obtained in a commercial packing plant and randomly assigned to three packaging systems. All cuts were very fresh in fat cover, bright in muscle color, free of surface discoloration, and extremely desirable in general appearance. Cuts assigned to treatment A were vacuum packaged in a film with a reported oxygen transmission rate (OTR-cc/100 in²/24 h/23.9 C/50% RH) of 2.28 and a moisture vapor transmission rate (MVTR-g/100 in²/24 h/37.7 C/70% RH) of 0.66 by the use of a nozzle-type, clipseal vacuumizing machine (254 mm of Hg at nozzle). Cuts assigned to treatment B were vacuum packaged in bags with a reported OTR of 0.41 to 0.75 and MVTR of 0.18 to 0.20. Cuts in treatment C were vacuum packaged in films with a reported OTR of 1.09 and MVTR of 0.75. Cuts in treatments B and C were vacuum packaged with the use of a chamber-type vacuumizing machine (heat-seal) with a reported chamber vacuum of 759 mm of Hg. Vacuum packages in treatment A were passed through a shrink tank (hot water for 6 sec at 196 C). Vacuum packages in treatments B and C were passed through a shrink tunnel (hot air for 9 sec at 232 C).

All packages were then boxed and shipped by refrigerated commercial transport to the Texas A&M Meat Laboratory. Upon arrival, 5 days after packaging, the vacuum packages were subjectively scored for degree of vacuum according to a 16-point scale (16 = excellent vacuum; 12 = good vacuum; 9 = marginal; 5 = poor vacuum; 4 = probable leaker; 1 = complete leaker) and randomly assigned to refrigerated storage for 7, 14, 21, 28, or 35 days (Table 1). Vacuum packaged knuckles and ribs that became leakers during transport were assigned to treatment D, termed "leaker" and randomly assigned to storage periods of 7, 14, or 21 days (Table 1). In addition, other extra knuckles were assigned to the "leaker" treatment after intentional puncture of the bags. All cuts were then boxed and stored for the appropriate storage interval at 1 to 3 C.

Upon completion of each storage period, packages were subjectively scored for degree of vacuum. Cuts receiving a vacuum score of 1 (leaker) incurred during storage of treatments A, B, and C were dropped from further study. Table 1 shows the final experimental design and numbers of cuts for the various treatments. Because of the relatively low numbers of each type of cut within each treatment, no attempt was made to determine leaker rates for each system.

The packages assigned to the particular storage period were weighed and opened. Cuts were removed from the package, permitted to drain (to remove purge), and weighed. Bags were washed, dried, and weighed. By difference in weight, percentage purge loss was obtained. Immediately after the 30-min drain period, a three-member experienced panel subjectively evaluated each cut for a number of

¹Transportation and Packaging Research Laboratory, Agricultural Marketing Research Institute, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Md.

TABLE 1. *Experimental design for knuckles, ribs and chucks*

Cut	Storage Interval (Days)	Treatment of cuts ^a			
		A ^b	B ^c	C ^d	D ^e
Knuckles	7	8	8	8	5
	14	8	8	8	5
	21	8	8	8	5
	28	8	8	8	—
	35	8	8	8	—
Ribs	7	6	6	5	9
	14	6	6	4	8
	21	5	7	3	9
	28	8	5	5	—
	35	8	6	4	—
Chucks	7	5	2	6	—
	14	4	2	6	—
	21	6	0	5	—

^aNumbers of samples in each treatment tested after removal of samples in defective bags from treatment A, B and C.

^bTreatment A: film characteristics—OTR = 2.28, MVTR = 0.66, clip-sealed, nozzle-type evacuation.

^cTreatment B: film characteristics—OTR = 0.41 to 0.75, MVTR = 0.18 to 0.20, heat-sealed, chamber-type evacuation.

^dTreatment C: film characteristics—OTR = 1.09, MVTR = 0.75, heat-sealed, chamber-type evacuation.

^eTreatment D: leaker packages derived from treatments A, B and C.

characteristics (1, 8): appearance of fat cover employing a 6-point scale (6 = very fresh; 4 = normal; 3 = some discoloration; 1 = severe or extensive discoloration); surface discoloration according to a 7-point scale (7 = no surface discoloration; 4 = 25 to 50% surface discoloration; 1 = total surface discoloration); total desirability by use of an 8-point scale (8 = extremely desirable; 5 = slightly desirable; 1 = extremely undesirable).

Following primal cut evaluation, one retail steak was removed from each knuckle and rib, placed in a styrofoam tray, overwrapped with polyvinyl chloride film, and placed under simulated retail display conditions (1 to 3 C with 90 ftc of incandescent light). Retail cuts were evaluated by a three-member, experienced panel after 1 and 4 days of retail display. Each retail cut was evaluated for surface discoloration and total desirability.

The microbial analysis of cuts in this study are included in the report of Seideman et al. (9).

Data were analyzed using analysis of variance. When significant ($P < .05$) main effects were observed in the analysis of variance, mean separation analysis was accomplished using the Kramer modification (5) of Duncan's multiple range test (2). Because of high leaker rates of chucks in all treatments, and the ultimate low numbers of samples, statistical assessment of chuck data is questionable.

RESULTS

Vacuum packages in treatment A (vacuum packaged by a nozzle system) were described as being "fair" in

vacuum with numerous residual air spaces and therefore received significantly lower vacuum scores (Table 2) than cuts in treatments B and C (packaged by a chamber system). Cuts in treatments B and C were described as "excellent" in vacuum, having no residual air spaces beneath the film and with the film adhering tightly to the meat surface. Terminal subjective vacuum scores show the importance of a high initial vacuum. Over the entire 35-day study, packages in treatments B and C maintained a significantly higher degree of vacuum than did packages in treatment A.

Knuckles exhibited a higher percentage of purge than ribs and chucks due to the larger lean surface area for knuckles (Table 3). Purge accumulation for knuckles and

TABLE 2. *Subjective vacuum scores for knuckles, ribs and chucks*

Wholesale cut	Subjective vacuum scores ^a					
	Initial ^b			Terminal ^c		
	A	B	C	A	B	C
Knuckles	9.9 ^d	15.9 ^e	15.9 ^e	8.8 ^d	15.3 ^e	15.6 ^e
Ribs	9.9 ^d	15.7 ^e	15.9 ^e	7.2 ^d	15.4 ^e	15.7 ^e
Chucks	6.5 ^d	12.8 ^d	13.2 ^d	4.8 ^d	9.5 ^d	11.6 ^d

^aMeans based on a 16-point scale (16 = excellent vacuum; 12 = good vacuum; 9 = marginal; 5 = poor vacuum; 4 = probable leaker; 1 = complete leaker).

^bEvaluation of package prior to storage.

^cEvaluation of package after designated storage interval.

^{d,e}Means in the same row, under the same subheading, bearing different superscripts differ ($P < .05$).

ribs did not appear to be related to packaging systems or degree of vacuum. Purge significantly increased for knuckles with increasing storage time.

Means for appearance of fat cover (Table 4) for knuckles, ribs, and chucks show a definite advantage for cuts in treatment B (high degree of vacuum, lowest oxygen transmission rate). At short storage periods, cuts in treatment A (low degree of vacuum, highest oxygen transmission rate) and leaker cuts (treatment D) displayed more discoloration of fat cover, presumably due to increased mobilization of purge and/or microbial activity. At longer storage periods, cuts in treatment C (high degree of vacuum, intermediate in oxygen transmission rate) began to develop brown discoloration on chucks and some slight green discoloration on ribs.

Means for surface discoloration of knuckles, ribs and chucks are shown in Table 5. Knuckles in treatment B (high degree of vacuum, lowest oxygen transmission rate) and treatment A (low degree of vacuum, highest oxygen

TABLE 3. *Percent purge loss for knuckles, ribs and chucks stratified according to treatment and storage period*

Storage (days)	Knuckles					Ribs					Chucks				
	Treatment				Order ^a of means	Treatment				Order ^a of means	Treatment			Order ^a of means	
	A	B	C	D		A	B	C	D		A	B	C		
7	0.44 ^e	0.49 ^d	0.69 ^c	0.44 ^b	CBDA	0.63 ^c	1.12 ^b	1.02 ^b	—	BCA	0.20 ^b	0.49 ^b	0.73 ^b	CBA	
14	0.63 ^{de}	1.38 ^{bc}	0.96 ^c	0.62 ^b	BCAD	0.02 ^c	0.12 ^d	1.09 ^d	0.49 ^b	CDBA	0.26 ^b	0.28 ^b	0.29 ^b	CBA	
21	1.47 ^c	0.91 ^{cd}	1.31 ^{bc}	0.99 ^b	ABDC	0.01 ^c	0.01 ^d	0.01 ^d	0.01 ^c	ABCD	0.01 ^b	—	0.45 ^b	CA	
28	1.00 ^{cd}	0.92 ^{cd}	1.66 ^b	—	CAB	1.55 ^b	0.84 ^b	1.02 ^b	—	ACB	—	—	—	—	
35	2.27 ^b	1.97 ^b	1.87 ^b	—	ABC	0.71 ^c	0.48 ^c	0.86 ^c	—	CAB	—	—	—	—	

^aMeans in the same row not underscored by a common line differ ($P < .05$).

^{b,c,d,e}Means in the same column bearing a common superscript do not differ ($P > .05$).

transmission rate) had less surface discoloration ($P < .05$) than cuts in treatment C (high degree of vacuum, intermediate oxygen transmission rate) and leakers (treatment D). Surface discoloration scores for ribs (Table 5) showed that cuts in B and C, both packaged at a high degree of vacuum, had less surface discoloration ($P < .05$) than those cuts in A, packaged with a low degree of vacuum, and cuts from leaker packages (treatment D).

Total desirability of knuckles, ribs, and chucks (Table 6) are based on the over-all appearance of a cut. Because knuckles have a large percentage of the total surface area as lean tissue, the total desirability scores are primarily affected by the amount of surface discoloration of the lean (Table 5). Knuckles in treatment A (low degree of vacuum, highest oxygen transmission rate) and B (high degree of vacuum, lowest oxygen transmission rate)

displayed higher desirability ratings than did cuts in treatment C (high degree of vacuum, intermediate in oxygen transmission rate) except after 7 days of storage. Knuckles from leaker packages (treatment D) had the lowest desirability ratings ($P < .05$).

Total desirability scores for ribs (Table 6) follow the same trends as scores for appearance of fat cover (Table 4) and surface discoloration scores (Table 5). Cuts in B and C, both packaged at a high degree of vacuum, had higher desirability scores ($P < .05$) than those cuts in treatments A and D (leakers).

Surface discoloration ratings (Table 7) for knuckle steaks, after one day of retail display, showed no apparent advantage to any particular system or film. After 4 days of retail display, steaks from cuts in treatment C had more surface discoloration than did

TABLE 4. Means for appearance of fat cover for knuckles, ribs and chucks stratified according to treatment and storage period

Storage (days)	Appearance of fat cover ^a													
	Knuckles					Ribs					Chucks			
	Treatment				Order ^b of means	Treatment				Order ^b of means	Treatment			Order ^b of means
A	B	C	D	A		B	C	D	A		B	C		
7	4.6 ^c	4.8 ^c	4.7 ^c	3.1 ^c	BCAD	2.6 ^c	5.7 ^c	5.4 ^c	3.5 ^c	BC DA	3.4 ^c	4.4 ^c	5.2 ^c	CBA
14	3.6 ^d	4.4 ^d	3.8 ^d	2.9 ^c	B CA D	2.5 ^c	3.0 ^f	2.4 ^d	2.8 ^d	CBDA	2.5 ^c	2.9 ^c	3.3 ^d	CBA
21	3.6 ^d	4.2 ^{de}	3.4 ^d	2.6 ^c	B AC D	2.9 ^c	4.9 ^d	3.7 ^d	2.7 ^d	B CA D	2.6 ^c	—	2.5 ^e	CA
28	3.9 ^{cd}	4.3 ^{de}	3.6 ^d	—	BAC	2.5 ^c	3.7 ^{ef}	2.3 ^e	—	B AC	—	—	—	—
35	3.9 ^d	4.0 ^e	2.7 ^e	—	BA C	1.9 ^c	3.9 ^e	2.9 ^{de}	—	BC A	—	—	—	—

^aMeans based on a 6-point scale (6 = very fresh; 1 = severs or extensive discoloration).

^bMeans in the same row not underscored by a common line differ ($P < .05$).

^{cdef}Means in the same column bearing a common superscript do not differ ($P > .05$).

TABLE 5. Means for surface discoloration for knuckles, ribs and chucks stratified according to treatment and storage period

Storage (days)	Surface discoloration ^a													
	Knuckles					Ribs					Chucks			
	Treatment				Order ^b of means	Treatment				Order ^b of means	Treatment			Order ^b of means
A	B	C	C	A		B	C	D	A		B	C		
7	6.2 ^c	6.2 ^c	6.2 ^c	5.7 ^c	ABCD	2.3 ^c	6.6 ^c	6.5 ^c	3.6 ^d	BC DA	5.5 ^c	5.6 ^c	6.1 ^c	CBA
14	5.7 ^c	6.2 ^c	4.9 ^d	3.1 ^d	BA CD	2.9 ^c	4.9 ^d	5.3 ^f	4.9 ^c	CBD A	4.3 ^{ce}	5.0 ^c	5.1 ^e	CBA
21	5.7 ^c	5.9 ^c	4.2 ^{de}	4.0 ^d	BA CD	3.8 ^c	6.0 ^c	5.9 ^d	4.0 ^d	BC DA	3.1 ^e	—	5.3 ^e	CA
28	5.6 ^c	5.7 ^c	4.5 ^{de}	—	BA C	2.8 ^c	5.4 ^d	6.3 ^c	—	CB A	—	—	—	—
35	5.7 ^c	6.2 ^c	3.8 ^e	—	BA C	3.8 ^c	6.1 ^c	5.6 ^e	—	BC A	—	—	—	—

^aMeans based on a 7-point scale (7 = no surface discoloration; 4 = 25%-50% surface discoloration; 1 = total surface discoloration).

^bMeans in the same row not underscored by a common line differ ($P < .05$).

^{cd}Means in the same column bearing a common superscript do not differ ($P > .05$).

TABLE 6. Means for total desirability for knuckles, ribs and chucks stratified according to treatment and storage period

Storage (days)	Total desirability ^a													
	Knuckles					Ribs					Chucks			
	Treatment				Order ^b of means	Treatment				Order ^b of means	Treatment			Order ^b of means
A	B	C	D	A		B	C	D	A		B	C		
7	6.2 ^c	6.1 ^d	6.2 ^c	5.5 ^c	ACB D	4.0 ^c	6.9 ^c	6.6 ^c	3.8 ^c	BC AD	4.5 ^c	5.3 ^c	6.0 ^c	CBA
14	6.2 ^c	6.9 ^c	5.6 ^c	2.9 ^d	B AC D	3.3 ^{cd}	4.5 ^e	5.3 ^d	4.4 ^c	CBD A	2.6 ^c	4.8 ^c	4.6 ^d	BCA
21	4.9 ^d	5.2 ^f	4.3 ^d	2.9 ^d	BAC D	3.8 ^{cd}	5.7 ^d	5.0 ^d	2.9 ^d	BC AD	3.0 ^c	—	5.1 ^{cd}	CA
28	5.0 ^d	5.6 ^{ef}	4.3 ^d	—	BAC	3.0 ^d	4.9 ^{de}	3.8 ^e	—	BCA	—	—	—	—
35	5.4 ^{cd}	5.6 ^{ef}	3.5 ^e	—	BA C	3.1 ^d	4.4 ^e	3.8 ^e	—	BC A	—	—	—	—

^aMeans based on an 8-point scale (8 = extremely desirable; 1 = extremely undesirable).

^bMeans in the same row not underscored by a common line differ ($P < .05$).

^{cd}Means in the same column bearing a common superscript do not differ ($P > .05$).

steaks from cuts in A and B at the 35-day storage period.

Surface discoloration scores for rib steaks (Table 7) after 1 day of retail display showed that steaks from cuts in leaker packages had more discoloration than did cuts from treatments A and B after 7 and 21 days of storage as wholesale cuts. After 4 days retail display of rib steaks, no definite advantage was noted for any packaging system-film treatment studied.

Mean ratings for total desirability of knuckle and rib steaks are presented in Table 8. Knuckle steaks, after one day of display, showed no differences except after 21 days of storage, cuts in leaker packages were lower in desirability than steaks from other treatments. Similar observations were made after 4 days of display except

that cuts in treatment C had lower desirability ratings after 35 days of storage. Total desirability ratings for rib steaks (Table 8) showed that steaks from leaker packages were lower in desirability at the 7-day storage period than those from intact packages.

DISCUSSION

This study evaluated two vacuum packaging systems (nozzle vs. chamber) and three films used in vacuum packaging. A chamber-type vacuumizing machine was capable of achieving higher vacuum, evidenced by initial subjective vacuum scores, resulting in better film contact to the meat surface. Vacuum packaging with a nozzle-type vacuumizing machine resulted in numerous

TABLE 7. Means for surface discoloration of retail steaks from knuckles and ribs at day 1 and day 4 of retail display stratified according to treatment and storage period

Storage (days)	Surface discoloration ^a of retail knuckles									
	Day 1 of display					Day 4 of display				
	Treatment				Order ^b of means	Treatment				Order ^b of means
A	B	C	C	A		B	C	D		
7	6.6 ^c	6.4 ^d	6.4 ^d	6.2 ^d	ABCD	5.8 ^c	5.5 ^c	5.3 ^c	5.6 ^c	ADBC
14	6.6 ^c	6.9 ^c	6.9 ^c	7.0 ^c	DBCA	5.4 ^c	5.6 ^c	5.4 ^c	5.3 ^c	BACD
21	5.9 ^d	6.1 ^e	6.0 ^e	5.9 ^d	BCAD	5.4 ^c	5.3 ^c	5.4 ^c	3.4 ^d	ACB D
28	6.0 ^d	6.0 ^e	6.0 ^e	—	ABC	5.4 ^c	5.5 ^c	5.4 ^c	—	BAC
35	6.8 ^c	6.6 ^{cd}	6.5 ^d	—	ABC	5.7 ^c	5.7 ^c	4.8 ^c	—	AB C

Storage (days)	Surface discoloration ^a of retail ribs										
	Day 1 of display					Day 4 of display					
	Treatment					Order ^b of means	Treatment				Order ^b of means
AA	B	B	C	CD	D		A	B	C	D	
7	7.0 ^c	7.0 ^c	7.0 ^c	7.0 ^c	6.3 ^d	ABC D	6.7 ^c	6.8 ^c	6.4 ^c	5.6 ^d	BAC D
14	6.8 ^c	7.0 ^c	7.0 ^c	7.0 ^c	7.0 ^c	BCDA	6.3 ^{cd}	6.5 ^c	6.7 ^c	6.4 ^c	CBDA
21	7.0 ^c	7.0 ^c	7.0 ^c	7.0 ^c	6.5 ^d	ABC D	6.4 ^{cd}	6.2 ^c	6.1 ^c	5.7 ^d	ABCD
28	7.0 ^c	6.8 ^c	6.6 ^c	6.6 ^c	—	ABC	6.4 ^{cd}	6.2 ^c	6.1 ^c	—	ABC
35	7.0 ^c	7.0 ^c	7.0 ^c	7.0 ^c	—	ABC	5.8 ^d	6.2 ^c	5.9 ^c	—	BCA

^aMeans based on a 7-point scale (7 = no surface discoloration; 1 = total surface discoloration).

^bMeans in the same row not underscored by a common line differ (P < .05).

^{cde}Means in the same column bearing a common superscript do not differ (P > .05).

TABLE 8. Means for total desirability score of retail steaks from knuckles and ribs at day 1 and day 4 of retail display stratified according to treatment and storage period

Storage (days)	Total desirability of retail knuckles									
	Day 1 of display					Day 4 of display				
	Treatment				Order ^b of means	Treatment				Order ^b of means
A	B	C	D	A		B	C	D		
7	7.1 ^c	7.1 ^c	7.1 ^c	6.3 ^{cd}	ABCD	5.9 ^c	5.5 ^c	5.4 ^c	5.7 ^c	ADBC
14	6.7 ^d	6.8 ^c	7.0 ^c	7.0 ^c	CDBA	5.5 ^c	5.6 ^c	5.3 ^c	5.4 ^c	BADC
21	6.0 ^e	6.1 ^d	6.0 ^d	5.2 ^d	BAC D	5.1 ^c	5.3 ^c	5.2 ^c	2.6 ^d	BCA D
28	6.5 ^{cd}	6.4 ^d	6.8 ^c	—	CAB	5.4 ^c	5.5 ^c	5.4 ^c	—	BAC
35	6.1 ^{de}	6.1 ^d	6.3 ^d	—	CAB	5.4 ^c	5.7 ^c	4.5 ^d	—	BA C

Storage (days)	Total desirability of retail steaks									
	Day 1 of display					Day 4 of display				
	Treatment				Order ^b of means	Treatment				Order ^b of means
A	B	C	D	A		B	C	D		
7	7.3 ^c	7.8 ^c	7.4 ^c	6.3 ^d	BCA D	6.8 ^c	7.1 ^c	6.5 ^c	5.4 ^d	BAC D
14	6.9 ^d	7.2 ^d	7.2 ^c	7.1 ^c	BCDA	6.1 ^c	6.4 ^c	6.7 ^c	6.3 ^c	CBDA
21	7.0 ^d	7.0 ^d	6.7 ^c	6.4 ^d	ABCD	6.3 ^c	6.1 ^c	5.9 ^c	5.6 ^d	ABCD
28	6.9 ^d	6.9 ^d	6.7 ^c	—	ABC	6.3 ^c	6.1 ^c	6.1 ^c	—	ABC
35	7.0 ^d	7.0 ^d	7.0 ^c	—	ABC	5.7 ^c	6.2 ^c	5.9 ^c	—	BCA

^aMeans based on an 8-point scale (8 = extremely desirable; 1 = extremely undesirable).

^bMeans in the same row not underscored by a common line differ (P < .05).

^{cde}Means in the same column bearing a common superscript do not differ (P > .05).

residual air spaces.

Purge can represent 1 to 2% of the weight of vacuum packaged meat (4). Purge accumulation is thought to be dependent on such factors as amount of fat present on the cut, package vacuum (8), rigidity of packaging materials, temperature of product at time of packaging (4), temperature of storage facility, and length of storage interval (8). Within the limits of this study, purge accumulation was not related to degree of vacuum or packaging material, but increased with increasing storage period on cuts having a large lean surface area such as knuckles.

Systems capable of achieving a high vacuum, such as chamber machines, will generally maintain fat cover in a fresh state since a high vacuum results in greater adhesion of film to fat surface. Low vacuum and leakers usually result in fat discoloration either due to the increased mobilization of purge and/or bacterial activity. Discoloration of fat because of increased bacterial activity may result from using films having a high oxygen transmission rate.

Prevention of surface discoloration is very important to the retailer. Discolored areas must be trimmed resulting in a substantial economic loss. High reducing activity of muscle and low film gas permeability retard surface discoloration (6). This study suggests that systems capable of achieving a high degree of vacuum and use of a film with a low oxygen transmission rate will result in less discolored lean. Knuckles have a large lean surface area and are capable of converting more of the residual oxygen trapped in packages after closure to carbon dioxide. This is important with packages that have a low degree of vacuum. Rib cuts have a small area of exposed lean and may be less capable of converting a large quantity of oxygen (in the case of a low vacuum system) to carbon dioxide. Total desirability ratings based on such physical appearance traits as surface discoloration and appearance of fat cover are a reflection of a retailer's overall impression of a primal cut. A system capable of achieving a high vacuum and use of a film with low oxygen transmission will usually result in a more desirable primal cut appearance. Primal cuts with a large lean surface area (knuckles) will be primarily evaluated for surface discoloration. In this instance, oxygen permeability of the film is possibly more important than the degree of vacuum because the respiratory ability of muscle and the microbial population may reduce the proportionate quantity of residual oxygen. Primal cuts with a large surface area of subcutaneous fat will be primarily evaluated on the appearance of fat cover. In this instance, a higher degree of vacuum will result in more desirable appearance ratings because of the close

adherence of film to the surface fat cover. In addition, cuts with a high proportion of the surface as fat tissue are less capable of reducing residual oxygen tensions and may permit growth of more aerobic spoilage bacteria.

Variations in surface discoloration and total desirability ratings of retail steaks from vacuum packaged primal cuts were not influenced by the packaging systems (Tables 7 and 8). However, the importance of identifying leakers and utilizing these cuts before extended storage is evident.

In conclusion, this study indicates the importance of a high degree of vacuum and use of films with low oxygen transmission rates and high resistance to puncture for maintenance of desirable appearance of primal cuts intended for retail operations.

ACKNOWLEDGMENTS

T. A. 12409, Texas Agricultural Experiment Station. The present study was partially supported by the Flexible Packaging Division, Continental Can Co., Inc. and by the Agricultural Marketing Research Institute, Agricultural Research Service, U.S. Department of Agriculture (Contract 12-14-1001-407). The research was a contribution to Western Regional Project, WM-62. Reference to a company or product name is for specific information only and does not imply approval or recommendation of the product by the U.S. Department of Agriculture or the Texas Agricultural Experiment Station to the exclusion of others that may be suitable.

REFERENCES

1. Carpenter, Z.L., S.D. Beebe, G.C. Smith, K.E. Hoke, and C. Vanderzant. 1976. Quality characteristics of vacuum packaged beef as affected by postmortem chill, storage temperature and storage interval. *J. Milk Food Technol.* 39:592-599.
2. Duncan, D.B. 1955. New multiple range for multiple F test. *Biom.* 11:1-42.
3. Jeremiah, L.E., G.C. Smith, and Z.L. Carpenter. 1972. Vacuum packaging of lamb. Effects of storage, storage time and storage temperature. *J. Food Sci.* 37:457-462.
4. Johnson, F.Y. 1974. Chilled vacuum-packed beef. *CSIRO Food Res. Rept.* 34:14-20.
5. Kramer, C.Y. 1956. Extension of multiple range tests to group means with unequal number of replications. *Biometrics* 12:307-310.
6. Pirko, P.C. and J.C. Ayres. 1957. Pigment changes in packaged beef during storage. *Food Technol.* 9:461-468.
7. Rea, R.H., G.C. Smith, and Z.L. Carpenter. 1972. Protective packaging materials for fresh beef shipments. *J. Food Sci.* 37:739-742.
8. Seideman, S.C., Z.L. Carpenter, G.C. Smith, and K.E. Hoke. 1976. The effect of degree of vacuum and length of storage on the physical characteristics of vacuum packaged beef wholesale cuts. *J. Food Sci.* 41:732-737.
9. Seidman, S.C., C. Vanderzant, M.O. Hanna, Z.L. Carpenter, and G.C. Smith. 1976. The effect of various types of vacuum packages and length of storage on the microbial flora of wholesale and retail cuts of beef. *J. Milk Food Technol.* 39:745-753.
10. Shaw, S.T. 1973. Past halfway point, move to boxed beef is accelerating. *Nat'l. Provisioner* 169 (18):12-18.
11. Smith, G.C. S.W. Rape, R.R. Motycka, and Z.L. Carpenter. 1974. Packaging systems for extending the storage life of pork cuts. *J. Food Sci.* 39:1140-1144.

Effect of Various Types of Vacuum Packages and Length of Storage on the Microbial Flora of Wholesale and Retail Cuts of Beef

S. C. SEIDEMAN, C. VANDERZANT, M. O. HANNA, Z. L. CARPENTER, and G. C. SMITH

*Meats and Meat Chemistry Section, Department of Animal Science
 Texas Agricultural Experiment Station, College Station, Texas 77840*

(Received for publication February 17, 1976)

ABSTRACT

Wholesale cuts of beef (knuckles, ribs, chucks) were vacuum packaged in three types of packages with oxygen transmission rates (cc/100 in.²/24 h) of 0.41-0.75, 1.09, and 2.28. One type of package was sealed with a clip; the others were heat sealed. Wholesale cuts were stored for 0-35 days at 1-3 C and retail cuts from ribs and knuckles were observed under retail conditions for 5 days. Differences in psychrotrophic, mesophilic, and lactobacillus counts of knuckles and ribs resulting from differences in type of package usually were not statistically significant. Differences in psychrotrophic counts of retail steaks of knuckles and ribs resulting from differences in type of package used for the primal cuts were not significant. Psychrotrophic and mesophilic counts of cuts in defective packages (leakers) increased faster than those of cuts in intact packages. The initial microbial flora of knuckles, ribs, and chucks consisted primarily of *Moraxella-Acinetobacter* spp. and coryneform bacteria. During the first few weeks of storage at 1-3 C *Lactobacillus* spp. already were dominant on knuckles and *Microbacterium* and *Lactobacillus* spp. predominated on ribs. After 28-35 days of storage *Lactobacillus* spp. were dominant on both knuckles and ribs. The microbial population on chucks after 21 days of storage consisted of *Lactobacillus*, *Microbacterium*, *Enterobacteriaceae*, *Pseudomonas*, *Moraxella-Acinetobacter* spp. and coryneform bacteria. The microflora of cuts from defective packages was comprised of species of *Pseudomonas*, *Microbacterium*, *Lactobacillus*, and *Moraxella-Acinetobacter*.

In recent years several reports have been published on the effect of vacuum packaging on the level and type of the microbial flora of meats (1, 3, 10-13, 15, 16, 17). Most of the earlier studies were concerned with processed meats or saran-wrapped ground beef. The principle of the method involves use of a film with a low permeability to oxygen which prevents re-entry of oxygen after evacuation of the air. The residual oxygen is converted to CO₂ possibly by respiration of meat tissue and microbial activity. The gaseous environment in the package, primarily the presence of CO₂, is responsible for suppression of common spoilage bacteria such as *Pseudomonas* and allows development of facultative anaerobes such as *Lactobacillus* species. The rate of development of these species depends upon the temperature at which the meat is stored. In some instances, members of the family *Enterobacteriaceae* constitute a significant part of the microflora of vacuum-packaged poultry (1). In a previous study (17) it

was found that lactobacilli and anaerobic plate counts of wholesale beef cuts (knuckles) stored under high vacuum for 21-35 days at 1-3 C were consistently lower than those of comparable cuts stored under low or intermediate vacuum. This was also true, but much less frequently, for the psychrotrophic and mesophilic counts. The psychrotrophic microflora of cuts stored for 28 days at 1-3 C consisted primarily of *Lactobacillus* spp. The present paper reports the effect of different types of vacuum-packages on the level and type of microbial flora of fresh beef cuts (knuckles, ribs, chucks) over a 35-day storage period at 1-3 C.

EXPERIMENTAL

Samples

Forty-five beef knuckles (IMPS 167), 45 beef ribs (IMPS 109), and 16 beef chucks (the arm portion of IMPS 113) were randomly assigned to three treatments. Samples in treatment A were vacuum packaged with a nozzle-type machine in a barrier bag with a Moisture Vapor

TABLE 1. Numbers of samples^a examined bacteriologically, arranged according to packaging treatment and length of storage

Storage (days)	Treatment of cuts ^b			
	A	B	C	D
Knuckles	0		3 ^c	
	7	2	2	3
	14	3	3	3
	21	3	3	3
	28	3	3	—
	35	3	3	—
Ribs	0		3 ^c	
	7	2	2	3
	14	3	2	6
	21	3	3	6
	28	3	3	—
	35	3	3	—
Chucks	0		4 ^c	
	7	2	1	—
	14	2	2	—
	21	2	—	—

^aNumbers of samples in each treatment tested after removal of samples in defective bags from treatments A, B, C.

^bTreatment A: film characteristics OTR = 2.28, MVTR = 0.66, clip-sealed, Treatment B: film characteristics OTR = 0.41-0.75, MVTR = 0.18-0.20, heat sealed, Treatment C: film characteristics OTR = 1.09, MVTR = 0.75, heat sealed, Treatment D: defective package (leaker).

^cSamples examined before packaging.

Transmission Rate (MVTR = g/100 in.²/24 h/37.7 C/70% RH) of 0.66 and an Oxygen Transmission Rate (OTR = cc/100 in.²/24 h/23.9 C/50% RH) of 2.28. These samples were clip-sealed. Samples in treatments B and C were vacuum packaged in a chamber machine and heat sealed. In treatment B, cuts were packaged in barrier bags with a MVTR of 0.18-0.20 and an OTR of 0.41-0.75 and were heat sealed. Cuts in treatment C were packaged in bags with a MVTR of 0.75 and an OTR of 1.09. All packages (treatments A, B, and C) were passed through a heat tunnel.

Bacterial counts were made on three knuckles, three ribs, and four chucks before application of films and are therefore common to all treatments (Table 1).

Upon arrival at the Meats Laboratory at Texas A&M University, cuts from each treatment were randomly assigned to storage periods of 7, 14, 21, 28, or 35 days at 1-3 C (three knuckles, three ribs and two chucks) per storage interval. In addition, 15 ribs and nine knuckles that had become leakers during transport were designated as treatment D and were assigned to storage intervals of 7, 14, or 21 days. One or two cuts from each treatment (A, B, C) were represented in these samples (treatment D) for each storage period.

At the end of each storage period, leaker packages were separated from intact packages and all packages then were opened and examined.

Retail cuts from ribs and knuckles (about 3 cm thick) were removed from primal cuts, placed in a styrofoam tray and overwrapped with polyvinyl chloride film. The retail cuts were displayed for 5 days under simulated retail conditions (1-3 C with 90 ft-c of incandescent light).

Microbiological

Bacteriological evaluation of the cuts was done by sampling 20 in.² (129 cm²) of the cuts with a sterile cellulose sponge (2 × 2 × 0.5 in.) wetted in sterile 0.1% peptone broth (18). Each knuckle was sampled in the same manner, first 10 in.² (64.5 cm²) of the lean surface and then 10 in.² (64.5 cm²) of the subcutaneous fat surface. Ribs were swabbed on the fat surface only. Chucks were swabbed on the blade surface and on the subcutaneous fat cover. The sponge then was placed in 100 ml of sterile 0.1% peptone broth and squeezed five times. The sample jar then was shaken 25 times and appropriate dilutions were made with sterile 0.1% peptone broth.

Psychrotrophic bacterial counts were made on plate count agar (Difco) with plate incubation at 7 C for 10 days. Mesophilic bacterial counts were obtained with plate count agar with plates incubated at 32 C for 2 days. *Pseudomonas* agar F (Difco) was employed to determine fluorescent pseudomonads. Enumeration of lactobacilli was done by using *Lactobacillus* MRS broth (Difco) with 1.5% agar added. These plates were incubated at 32 C for 5 days. Psychrotrophic counts of steaks after 5 days of retail display were obtained by swabbing 2 in.² (12.9 cm²) with sterile cotton swabs. This technique was used because of the limited surface area available for sampling. Details of the above procedures are given in a previous paper (17).

Distribution of the psychrotrophic microbial flora after the various storage periods was determined by picking about 30-40 colonies from countable plates and placing them on trypticase soy agar (BBL) slants. Incubation of slants was at 25 C for 2-3 days. Diagnostic schemes and procedures to identify the isolates are those published by Vanderzant and Nickelson (20). Except as noted, further identification of isolates of the family *Enterobacteriaceae* was carried out as described by Edwards and Ewing (6). Included were the following tests: nitrate reduction, (Nitrate broth, Difco); indol; MR-VP; Simmon's citrate; motility (Motility medium S, Difco); TSI (BBL); Christensen's urea (Difco); gelatin liquefaction (Nutrient gelatin, Difco); lysine decarboxylase; ornithine decarboxylase; arginine dihydrolase; phenylalanine deaminase; malonate; β -D-galactosidase (ONPG); and selected carbohydrates (1% filter sterilized in phenol red broth base, BBL).

Isolation and identification methods of strains referred to as *Yersinia enterocolitica* are given in a separate paper (8).

Bacteriological count data were analyzed by analysis of variance on log counts [\log_{10} per in.² (6.45 cm²)] of individual samples. Data in Tables 2-10 represent the mean count of the number of samples

indicated in Table 1. Where mean counts were different ($P < .05$), the technique of Duncan (5) was employed for mean separation.

RESULTS

Initially and after 7 days of storage, psychrotrophic counts of beef knuckles (Table 2) in the three types of in-

TABLE 2. Psychrotrophic bacterial counts (log/in.²) of beef knuckles stratified according to treatment and storage interval

Storage (days)	Treatment				Order of means ^e
	A	B	C	D	
0	2.05 ^b	2.05 ^c	2.05 ^c	2.05 ^d	
7	2.21 ^b	2.33 ^c	2.47 ^c	3.76 ^c	DCBA
14	3.19 ^b	2.60 ^c	4.07 ^b	5.52 ^b	DCAB
21	6.09 ^a	5.67 ^b	4.94 ^{ab}	7.00 ^a	DAB C
28	5.83 ^a	6.73 ^{ab}	6.26 ^{ab}		BCA
35	7.48 ^a	7.21 ^a	6.83 ^a		ABC

^{abcd}Means in the same column bearing a common superscript do not differ ($P > .05$).

^eMeans in the same row not underscored by a common line differ ($P < .05$).

tact packages (treatments A, B, C) were low. Largest increases in count in any sampling period occurred between 14 and 21 days for samples in treatment A and B (2.90-3.07 logs) and between 7-14 (1.60 logs) and 21-28 days (1.32 logs) for those in treatment C. Differences in count associated with type of film were not statistically significant. Psychrotrophic counts of leakers (treatment D) increased rapidly and after 21 days reached levels comparable to those attained after 35 days in intact packages.

TABLE 3. Mesophilic bacterial counts (log/in.²) of beef knuckles stratified according to treatment and storage interval

Storage (days)	Treatment				Order of means ^e
	A	B	C	D	
0	2.14 ^c	2.14 ^c	2.14 ^d	2.14 ^c	
7	3.01 ^c	3.29 ^{bc}	2.27 ^d	3.90 ^b	DBAC
14	3.52 ^c	3.08 ^c	4.15 ^c	5.62 ^a	DCAB
21	5.98 ^b	5.65 ^{ab}	5.10 ^{bc}	6.91 ^a	DA BC
28	6.77 ^{ab}	5.93 ^a	6.36 ^{ab}		ACB
35	7.49 ^a	7.08 ^a	6.80 ^a		AB C

^{abcd}Means in the same column bearing a common superscript do not differ ($P > .05$).

^eMeans in the same row not underscored by a common line differ ($P < .05$).

The pattern of changes in mesophilic counts (Table 3) was similar to that of the psychrotrophic counts. Largest increases in count in any one sampling period occurred between 14 and 21 days in samples packaged according to conditions of treatments A and B (2.46-2.57 logs), and between 7-14 days (1.88 logs), and 21-28 days (1.26 logs) for samples in treatment C. In most instances, differences in mesophilic count because of differences in types of packages were not statistically significant. Mesophilic counts of knuckles in defective packages (treatment D) increased much faster than did those of cuts stored in intact packages.

TABLE 4. *Lactobacillus* agar counts (log/in.²) of beef knuckles stratified according to treatment and storage interval

Storage (days)	Treatment				Order of means ^d
	A	B	C	D	
0	1.39 ^b	1.39 ^c	1.39 ^c	1.39 ^c	
7	1.94 ^b	2.38 ^c	2.59 ^c	3.00 ^c	<u>DCBA</u>
14	2.52 ^b	1.40 ^c	3.86 ^{bc}	4.77 ^b	<u>DCA B</u>
21	5.85 ^a	5.39 ^b	3.78 ^{bc}	5.90 ^a	<u>DABC</u>
28	5.29 ^a	6.05 ^{ab}	5.96 ^{ab}		<u>BCA</u>
35	6.95 ^a	6.42 ^a	6.40 ^a		<u>ABC</u>

abcMeans in the same column bearing a common superscript do not differ (P > .05).

^dMeans in the same row not underscored by a common line differ (P < .05).

Lactobacillus counts of beef knuckles (Table 4) were low during the first 2 weeks of storage. Large increases in lactobacilli occurred between 14 and 21 days in the samples in treatments A and B (3.33-3.99 logs) and between 21 and 28 days in those in treatment C (2.18 logs). In most instances, differences in lactobacillus counts because of differences in types of package were not statistically significant. Significant increases in lactobacillus count occurred on samples in defective packages (treatment D) between 7 and 14 and 14 and 21 days.

TABLE 5. Psychrotrophic bacterial counts (log/in.²) of retail knuckle steaks after 5 days of retail display (I-3 C) stratified according to primal cut treatment and storage interval

Storage (days)	Treatment				Order of means ^e
	A	B	C	D	
7	3.14 ^d	3.78 ^c	2.63 ^c	2.88 ^c	<u>BADC</u>
14	5.59 ^c	5.84 ^b	5.55 ^b	5.06 ^b	<u>BACD</u>
21	5.94 ^c	6.53 ^{ab}	7.39 ^a	8.01 ^a	<u>DC BA</u>
28	7.65 ^b	6.98 ^{ab}	7.26 ^a		<u>ACB</u>
35	8.92 ^a	7.30 ^a	7.39 ^a		<u>ACB</u>

abcdMeans in the same column bearing a common superscript do not differ (P > .05).

^eMeans in the same row not underscored by a common line differ (P < .05).

Psychrotrophic counts of retail knuckle steaks (Table 5) usually were higher (14 of 15 samples) than the counts of knuckles from which they were prepared. These differences in count for samples from treatment A ranged from -0.15 to +2.40 logs, for samples in treatment B from 0.09 to 3.24 logs, and for those from treatment C from 0.16-2.45 logs. The extent of these differences could not be associated with one particular type of package. In most instances differences in psychrotrophic counts of retail steaks because of differences in the type of package used for primal cuts were not statistically significant. Psychrotrophic counts of steaks from primal cuts stored in defective packages for 21 days were high. Psychrotrophic, mesophilic, and lactobacillus counts of beef knuckles stored for 35 days were numerically higher in treatment A than those of cuts prepared and stored under conditions of treatments B and C; the same was true for the retail steaks.

TABLE 6. Psychrotrophic bacterial counts (log/in.²) of beef ribs stratified according to treatment and storage interval

Storage (days)	Treatment				Order of means ^d
	A	B	C	D	
0	2.42 ^b	2.42 ^c	2.42 ^b	2.42 ^c	
7	5.08 ^{ab}	4.09 ^b	1.40 ^b	1.97 ^c	<u>AB DC</u>
14	6.49 ^a	5.62 ^a	4.05 ^{ab}	5.00 ^b	<u>ABDC</u>
21	6.33 ^a	6.18 ^a	5.76 ^a	7.23 ^a	<u>DABC</u>
28	7.12 ^a	5.69 ^a	5.94 ^a		<u>ACB</u>
35	6.32 ^a	6.87 ^a	5.93 ^a		<u>BAC</u>

abcMeans in the same column bearing a common superscript do not differ (P > .05).

^dMeans in the same row not underscored by a common line differ (P < .05).

Large increases in psychrotrophic counts (Table 6) of beef ribs (3.20-4.07 logs) occurred during the first 14 days of storage for samples in treatments A and B and after 21 days for cuts stored in treatment C (3.34 logs). With one exception, differences in psychrotrophic counts because of the type of package were not statistically significant. Development of psychrotrophic bacteria on meats in defective bags (treatment D) was rapid and counts after 21 days were similar to those of meats stored for 28-35 days in intact packages.

TABLE 7. Mesophilic bacterial counts (log/in.²) of beef ribs stratified according to treatment and storage interval

Storage (days)	Treatment				Order of means ^d
	A	B	C	D	
0	2.73 ^b	2.73 ^c	2.73 ^b	2.73 ^b	
7	4.55 ^{ab}	4.06 ^b	1.20 ^b	2.99 ^b	<u>ABDC</u>
14	6.32 ^a	6.26 ^a	4.18 ^{ab}	5.23 ^b	<u>ABDC</u>
21	6.25 ^a	5.61 ^{ab}	5.68 ^a	7.21 ^a	<u>DACB</u>
28	6.81 ^a	6.04 ^a	6.17 ^a		<u>ACB</u>
35	6.34 ^a	6.46 ^a	6.18 ^a		<u>BAC</u>

abcMeans in the same column bearing a common superscript do not differ (P > .05).

^dMeans in the same row not underscored by a common line differ (P < .05).

Data on the mesophilic bacterial counts of beef ribs (Table 7) also showed large increases in count (3.53-3.59 logs) after 14 days for ribs stored in treatments A or B and after 21 days (2.95 logs) for samples stored in treatment C. As with the psychrotrophic bacteria, mesophilic counts of beef ribs stored in defective bags increased rapidly. Counts exceeded 10⁷ per in.² after 21

TABLE 8. *Lactobacillus* agar counts (log/in.²) of beef ribs stratified according to treatment and storage interval

Storage (days)	Treatment				Order of means ^d
	A	B	C	D	
0	1.39 ^c	1.39 ^c	1.39 ^c	1.39 ^b	
7	2.38 ^b	1.85 ^c	1.40 ^c	1.50 ^c	<u>ABDC</u>
14	3.96 ^b	3.27 ^{bc}	3.79 ^{bc}	3.09 ^b	<u>ACBD</u>
21	5.23 ^{ab}	5.22 ^{ab}	5.55 ^{ab}	5.70 ^a	<u>DCAB</u>
28	6.14 ^a	5.44 ^{ab}	5.30 ^{ab}		<u>ABC</u>
35	5.84 ^a	6.23 ^a	6.34 ^a		<u>CBA</u>

abcMeans in the same column bearing a common superscript do not differ (P > .05).

^dMeans in the same row not underscored by a common line differ (P < .05).

days of storage. Differences in mesophilic counts as related to type of package were not statistically significant.

Lactobacillus counts of beef ribs were initially low (Table 8), exceeded 10^5 per in.² after 21 days of storage and increased to about 10^6 per in.² after 35 days. Lactobacillus counts on ribs stored in defective bags (treatment D) had increased to $>10^5$ per in.² after 21 days. Type of package had no significant effect on the lactobacilli count.

TABLE 9. Psychrotrophic bacterial counts (log/in.²) of retail rib steaks after 5 days of retail display (1-3 C) stratified according to treatment and storage interval

Storage (days)	Treatment				Order of means ^d
	A	B	C	D	
7	4.05 ^b	2.50 ^c	3.19 ^b	3.65 ^b	<u>AD CB</u>
14	6.25 ^a	5.60 ^b	4.32 ^b	5.55 ^{ab}	<u>ABDC</u>
21	5.98 ^a	5.92 ^b	6.32 ^a	6.94 ^a	<u>DCAB</u>
28	6.69 ^a	6.22 ^b	6.50 ^a		<u>ACB</u>
35	7.63 ^a	7.72 ^a	7.33 ^a		<u>BAC</u>

abcMeans in the same column bearing a common superscript do not differ ($P > .05$).

^dMeans in the same row not underlined by a common line differ ($P < .05$).

Psychrotrophic counts of retail rib steaks (Table 9) were approximately 10^6 per in.² after 14 days in treatment A and after 21 days in treatment B or C. Differences in counts of retail rib steaks as related to differences in type of package in which the primal cuts were stored were not statistically significant. A comparison of the psychrotrophic counts of the retail rib steaks and the wholesale cuts from which they were fabricated showed that counts of the steaks were sometimes (seven of 15 comparisons) lower and sometimes (eight of 15 comparisons) higher than those of the original cuts.

TABLE 10. Psychrotrophic, mesophilic and lactobacillus agar counts (log/in.²) of beef chucks stratified according to treatment and storage interval

Storage (days)	Treatment		
	A	B	C
	<i>Psychrotrophic</i>		
0	2.04	2.04	2.04
7	3.29	3.16	2.23
14	4.23	2.40	5.07
21	6.66	—	5.69
	<i>Mesophilic</i>		
0	2.39	2.39	2.39
7	4.04	2.98	2.93
14	4.14	2.55	5.01
21	6.32	—	5.75
	<i>Lactobacillus</i>		
0	1.46	1.46	1.46
7	3.13	1.70	2.05
14	3.54	1.40	2.98
21	5.29	—	4.58

Psychrotrophic, mesophilic, and lactobacillus counts of beef chucks are presented in Table 10. The data were not analyzed statistically because of the limited number of samples. Considerable increases in counts took place

in samples stored for 21 days in treatments A and C. Counts of chucks under conditions of treatment B were consistently lower than those of comparable samples in treatment A. After 21 days of storage, counts of samples in treatment C were lower than those stored in the packages of treatment A.

Distribution of the psychrotrophic microbial flora of vacuum-packaged beef knuckles, ribs and chucks

Psychrotrophic plate counts and distribution of microbial types of 33 knuckles, 41 ribs, and 15 chucks stored in intact bags and of eight knuckles and 10 ribs from defective bags (leakers) are presented in Tables 11-14.

Knuckles. Psychrotrophic counts of knuckles (Table 11) immediately after packaging were very low and increased to levels ranging from 5.2×10^5 to 5.6×10^6 per in.² after 35 days of storage. The microbial flora of the samples immediately after packaging was dominated by *Moraxella-Acinetobacter*, *Aeromonas*, *Erwinia herbicola*, and *Corynebacterium* species. The significance of this distribution is limited because only two samples were available and the counts were extremely low (20-110/in.²). With few exceptions, *Lactobacillus* spp. dominated the microbial population of beef knuckles stored for 7-35 days, at 1-3 C, followed by *Pseudomonas* (five samples $>25\%$ of isolates) and *Microbacterium* spp. (two samples $>25\%$ of isolates). Among the *Enterobacteriaceae*, *Enterobacter liquefaciens* occurred frequently in vacuum-packaged beef knuckles. Type of packaging film (A, B, or C) had little if any influence on the type of microbial population of beef knuckles stored for 7-35 days at 1-3 C.

Ribs. Psychrotrophic counts of ribs (Table 12) immediately after packaging were very low (80-130/in.²). As storage progressed, counts increased and after 35 days ranged from 4.9×10^5 to 7×10^6 /in.² (6.45 cm²). Maximum counts of 1.4 and 1.8×10^7 /in.² were observed after 28 days of storage in two samples packaged in treatment A. The microbial flora of ribs immediately after packaging was dominated by *Moraxella-Acinetobacter* and *Corynebacterium* species, but the value of this observation is limited because only two samples were available and the counts were very low. In the microbial population of samples stored for 7 days, *Pseudomonas*, *Moraxella-Acinetobacter*, *Enterobacteriaceae*, *Lactobacillus*, or *Corynebacterium* species, either dominated ($>50\%$ of isolates) or formed a significant part ($>25\%$ of isolates) of one or two of six samples. After 14 days of storage, *Lactobacillus* dominated in three and *Microbacterium* spp. in two of six samples. After 21 and 28 days of storage, *Lactobacillus* and/or *Microbacterium* spp. either dominated or formed a significant part of seven of 10 samples (21 days) and seven of nine samples (28 days). *Lactobacillus* spp. completely dominated the microbial flora of samples stored for 35 days. *Enterobacter liquefaciens*, *Enterobacter aerogenes*, *E. herbicola*, *Escherichia coli*, and *Y. enterocolitica* were present among the *Enterobacteriaceae*. *E. liquefaciens*

was more frequently encountered than the other species.

A comparison of the type of microbial flora with respect to difference in type of packaging film showed that after 14, 21, and 28 days *Lactobacillus* spp. were more dominant in the samples packaged under high vacuum (chamber, heat-seal system) than in those packaged under low vacuum (nozzle, clip-seal system). *Microbacterium* and *Lactobacillus* spp. were more dominant in samples packaged in treatment A (low vacuum). After 35 days this difference had disappeared and *Lactobacillus* spp. dominated the microbial flora of samples packaged under either system.

Samples stored under high vacuum (treatment B) for 28 days had a larger percentage of *Enterobacteriaceae* than did comparable samples stored under lower vacuum (treatment A). However, this pattern was not apparent after 7, 14, 21, and 35 days.

Chucks. During storage for 21 days, psychrotrophic counts of chucks (Table 13) increased from 10-130/in.² to 1.1 × 10⁴ – 1.4 × 10⁶/in.² *Moraxella-Acinetobacter* and

Coryneforms (*Microbacterium*, *Corynebacterium*, and *Arthrobacter*) were dominant on samples immediately after packaging. Although the number of chucks examined was limited (12 stored samples), the microbial flora was more varied than with either ribs or knuckles. In order of decreasing importance, *Lactobacillus*, *Microbacterium*, *Enterobacteriaceae* (*E. liquefaciens*), *Pseudomonas*, *Moraxella-Acinetobacter*, *Corynebacterium*, and *Arthrobacter* constituted either a significant or dominant part of the microbial flora. *E. liquefaciens* was a major part of the microbial flora of four samples (27.3-56.2%) stored under high vacuum (treatments B or C).

Leakers

Counts of leakers (Knuckles and ribs) after 21 days ranged from 1.3 × 10⁵ to 6.1 × 10⁷/in.², considerably higher than those of comparable samples stored for the same period in intact bags (Table 14). Distribution of microbial types differed from that observed on samples

TABLE 11. Psychrotrophic plate counts and microbial flora of beef knuckles stored in various film bags for 0-35 days at 1-3 C

Day	Package treatment ^a	PPC/in. ² (6.45 cm ²) ^b	<i>Pseudomonas</i>	<i>Moraxella-Acinetobacter</i>	<i>Flavobacterium</i>	<i>Aeromonas</i>	<i>Enterobacteriaceae</i>				<i>Microbacterium</i>	<i>Corynebacterium</i>	Yeasts	
							<i>Y. enterocolitica</i>	<i>E. liquefaciens</i>	<i>E. herbicola</i>	<i>Lactobacillus</i>				
0		2 × 10				50.0 ^c								
7	A	11 × 10		36.4										
	A	84 × 10 ²		59.5								63.6		
	B	55 × 10	5.4	7.3						14.3	26.2			
	B	16 × 10	100							80.0		7.3		
14	B	41 × 10		4.9	14.6									
	C	78 × 10		14.1	1.3					7.3			2.4	
	A	11 × 10 ³		4.7						1.3				1.3
	C	52 × 10 ²	13.5	5.8										
21	C	31 × 10 ³	4.6											
	A	97 × 10 ³	4.6	1.5						1.9			0.9	
	B	22 × 10 ³	4.6	4.6						1.3				
	B	36 × 10 ⁴	1.4			1.4				4.6				
	B	61 × 10 ³								1.6			0.6	
	C	20 × 10 ³	30.0											
28	C	65 × 10 ⁴	1.5							5.0				
	C	57 × 10 ³	54.4					1.5	1.5	24.6	70.8			
	A	49 × 10 ⁴							1.7	38.6	5.3			
	A	35 × 10 ⁴	5.7					5.7	10.2	85.7	4.1			
	B	42 × 10 ⁴	0.5					0.5		88.6				
	B	41 × 10 ⁴					0.2	0.5	0.5	98.5				
	B	30 × 10 ⁴	1.0	0.3				0.5	2.0	96.8				
	C	84 × 10 ⁴	1.2					0.8	13.3	83.1	1.4			
35	C	13 × 10 ⁴								97.6	1.2			
	A	56 × 10 ⁵	5.4							90.6				
	A	39 × 10 ⁵	10.3							82.1	1.8			
	A	50 × 10 ⁵	6.0							79.5	7.7			
	B	34 × 10 ⁵	11.8							94.0				
	B	24 × 10 ⁵	75.0							25.0				
	B	27 × 10 ⁵	3.0							96.3				
	C	52 × 10 ⁴	78.8				1.9		0.7	7.7	11.5			
	C	93 × 10 ⁴	1.1						1.1	97.8				
	C	20 × 10 ⁵	3.1				2.0		3.1	91.8				

^aA: OTR = 2.28, MVTR = 0.66, clip-sealed
 B: OTR = 0.41 – 0.75, MVTR = 0.18 – 0.20, heat-sealed.
 C: OTR = 1.09, MVTR = 0.75, heat sealed.
^bPPC = Psychrotrophic plate count.
^cPercent.

in intact packages. Ranked in order of decreasing significance, *Pseudomonas*, *Microbacterium*, *Lactobacillus*, and *Moraxella-Acinetobacter* predominated in these samples. *Enterobacteriaceae* (*E. liquefaciens*) did not constitute a significant part of the microbial flora of leakers. Of 263 *Pseudomonas* isolates tested, 65% were non-fluorescent.

DISCUSSION

Initial psychrotrophic, mesophilic, and lactobacillus counts of knuckles, ribs, and chucks were low. Psychrotrophic and mesophilic counts remained low during the first 7-14 days of storage, especially for knuckles and chucks as compared to ribs. Large

populations of psychrotrophic and mesophilic bacteria on knuckles and ribs occurred somewhat earlier on cuts in treatments A and B than on those in treatment C. Lactobacillus counts of knuckles indicated large increases after 14-21 days for cuts in treatment A and B, and between 21-28 days for samples in treatment C. On ribs, large increases in lactobacilli occurred after 21 days. These differences in development of bacterial populations during the early phases of the storage period cannot be explained solely on the basis of differences in the oxygen permeability of packages. The oxygen-permeability (OTR) of the films in order of decreasing permeability were: treatment A (2.28), treatment C (1.09), and Treatment B (0.41-0.75). Thus if

TABLE 12. Psychrotrophic plate count and microbial flora of beef ribs stored in various film bags for 0-35 days at 1-3 C

Day	Package treatment ^a	PPC/in. ² (6.45 cm ²) ^b	Enterobacteriaceae												
			<i>Pseudomonas</i>	<i>Moraxella-Acinetobacter</i>	<i>Aeromonas</i>	<i>Y. enterocolitica</i>	<i>E. liquefaciens</i>	<i>E. aerogenes</i>	<i>E. herbicola</i>	<i>E. coli</i>	<i>Lactobacillus</i>	<i>Microbacterium</i>	<i>Corynebacterium</i>	Coagulase-Staphylococcus	Yeasts
0		8 × 10		50.0 ^c								50.0			
7		13 × 10		61.5								23.1	15.4		
	A	72 × 10 ²		8.3				88.9			2.8				
	A	40 × 10	65.0	7.5						10.0		5.0		12.5	
	A	56 × 10 ³	1.4	30.3					1.8			65.8			
	B	28 × 10 ²		10.7							10.7	7.1	71.4		
	B	18 × 10 ²	16.7	61.1								22.2			
14	B	20 × 10 ²		20.0							60.0	10.0	10.0		
	A	72 × 10 ⁴	4.2								5.6	88.8		1.4	
	A	14 × 10 ⁵									7.0	93.0			
	B	19 × 10 ⁴			1.0		1.6				88.1	9.3			
	B	33 × 10 ³									87.9	12.1			
	C	30 × 10 ⁵	0.2	0.03							96.8	2.9			97.3
21	C	30 × 10 ⁵		0.03						0.3	2.3				
	A	17 × 10 ⁴	4.2	5.3			4.2			58.9	27.4				
	A	18 × 10 ⁵	41.2	1.7				2.3		31.6	22.6	0.6			
	A	22 × 10 ⁴	100												
	B	60 × 10 ⁴	1.2							87.1	0.8	0.8			
	B	57 × 10 ⁴								89.5					
	B	36 × 10 ³	2.8	94.4						2.8					
	B	96 × 10 ⁴	11.5							62.0	16.1	10.4			
	C	17 × 10 ⁵	2.3	14.9						71.3	11.5				
	C	34 × 10 ⁴	23.5	55.9							14.7	5.9			
	C	74 × 10 ³	1.4	1.4						62.1	27.0				
28	A	14 × 10 ⁶	77.5				8.1			14.3	7.1				
	A	18 × 10 ⁶	3.8	14.7			1.1				79.9	1.6			
	A	26 × 10 ⁴	4.6		1.1					41.8	52.5				
	B	13 × 10 ³	7.7			7.7	30.8			30.8					
	B	58 × 10 ⁴	1.7	1.7			6.9			81.0	8.6				
	B	11 × 10 ⁵	4.5			0.8	3.6			83.9	7.1				
	C	25 × 10 ³	80.0							13.3	6.7				
35	C	32 × 10 ⁴	3.4			0.3	0.3			64.5	31.5				
	C	37 × 10 ⁴	1.6		1.9		0.3			94.0	2.2				
	A	36 × 10 ⁵	8.3	5.5			11.1			66.7	8.3				
	A	69 × 10 ⁵	2.9				1.4			78.3	17.4				
	A	18 × 10 ⁵	22.2							55.5	11.1			11.1	
	B	21 × 10 ⁵	10.3		1.4	0.5				67.9	19.9				
	B	42 × 10 ⁵	1.0	15.6			2.2			69.2	12.0				
	B	20 × 10 ⁵	2.0	15.8	1.5					78.6	0.5	1.5			
	C	70 × 10 ⁵					8.6			91.4					
	C	49 × 10 ⁴	7.1		5.1	2.0	14.3	2.0	2.0	65.3	2.0				

^aTreatment A: film characteristics OTR = 2.28, MVTR = 0.66, clip-sealed.

B: film characteristics OTR = 0.41 - 0.75, MVTR = 0.18 - 0.20, heat sealed.

C: film characteristics OTR = 1.09, MVTR = 0.75, heat sealed

^bPPC = Psychrotrophic plate count.

^cPercent.

oxygen-permeability was the only factor governing development of bacteria during the early phase of storage, the rate of increase in counts on cuts would have been greatest in treatment A and least on cuts in treatment B. It is possible that other parameters such as CO₂-permeability may have been involved. In addition, drastic changes probably occur in film characteristics after heating in the shrink tunnel.

Differences in psychrotrophic, mesophilic, and lactobacillus counts of refrigerated vacuum-packaged

cuts because of differences in type of package were not statistically significant. Taylor and Shaw (19) compared bacterial counts of primal beef cuts stored for up to 8 weeks at 1 C in three films ranging in oxygen-permeability from 5-70 cc/m²/24 h/atm. After storage for 4, 6, or 8 weeks, there was no significant difference between the bacterial counts of cuts packaged in the different films. Apparently, all packages had CO₂ levels high enough to inhibit the putrefactive bacteria normally responsible for spoilage of refrigerated meat. Perdue et al. (14)

TABLE 13. Psychrotrophic plate count and microbial flora of beef chucks stored in various film bags for 0-21 days at 1-3 C

Day	Sample treatment ^a	PPC/in. ² (6.45 cm ²) ^b	<i>Pseudomonas</i>	<i>Moraxella-Acnetobacter</i>	<i>Flavobacterium</i>	<i>Aeromonas</i>	<i>E. liquefaciens</i>	<i>Lactobacillus</i>	<i>Microbacterium</i>	<i>Corynebacterium</i>	<i>Arthrobaacter</i>	Yeasts
0	Blade	1 × 10							100 ^c			
	Blade	4 × 10		25.0					25.0		25.0	
7	Arm	13 × 10		92.3						7.7		
	A	14 × 10 ²		32.9				35.7	14.3	17.1		
	A	27 × 10 ²		40.7				22.2		37.0		
	B	48 × 10		8.3			56.2	31.3		4.2		
	B	33 × 10 ²		7.6			12.1	4.6	72.7			
14	C	27 × 10	14.8	22.2			3.7	59.3				
	A	20 × 10 ⁴	6.5	7.5		5.0		20.0	61.0			
	C	26 × 10 ⁴					3.8	80.8	7.7			7.7
21	C	23 × 10 ³	34.8				30.4	13.0	21.7			
	A	47 × 10 ⁴	83.0	8.5					8.5			
	A	14 × 10 ⁵	15.0	71.4				3.6	1.4	8.6		
	C	82 × 10 ⁴	2.4				34.1	56.1	7.4			
	C	11 × 10 ³	54.5		18.2		27.3					

^aTreatment A: film characteristics OTR = 2.28, MVTR = 0.66, clip-sealed.

B: film characteristics OTR = 0.41-0.75, MVTR = 0.18-0.20, heat sealed.

C: film characteristics OTR = 1.09, MVTR = 0.75, heat sealed.

^bPPC = Psychrotrophic plate count.

^cPercent.

TABLE 14. Psychrotrophic plate count and microbial flora of beef knuckles and ribs stored in defective film bags

Day	Sample treatment ^{a,b}	PPC/in. ² (6.45 cm ²) ^c	<i>Pseudomonas</i>	<i>Moraxella-Acnetobacter</i>	<i>Flavobacterium</i>	<i>Aeromonas</i>	<i>E. liquefaciens</i>	<i>Lactobacillus</i>	<i>Microbacterium</i>	<i>Corynebacterium</i>	Yeasts
7	K-B	24 × 10 ²		20.0 ^d				40.0	20.0		20.0
	K-C	71 × 10	2.8	59.2	9.9			5.6	2.8	19.7	
	R-C	18 × 10		33.3				33.3	22.2	11.1	
14	K-A	89 × 10 ²	3.4	4.5		2.2	1.1	43.8	43.8		1.1
	R-A	90 × 10 ⁵	2.9	2.6		0.9			93.3	0.3	
	R-A	16 × 10 ⁴	4.4					95.6			
	K-B	91 × 10 ³	85.7					14.3			
	R-B	33 × 10 ⁴	36.4	6.1				3.0	54.5		
	K-C	24 × 10 ⁴	15.6	4.5		2.9		33.7	42.8		0.4
	R-C	62 × 10 ⁴		19.4				3.2	77.4		
21	K-A	61 × 10 ⁶	100								
	R-A	13 × 10 ⁶	40.2	37.0				19.6	2.4	0.8	
	R-A	54 × 10 ⁵	100								
	K-B	13 × 10 ⁴	20.1								
	R-B	55 × 10 ⁶	52.0				6.3	73.6			
	R-B	76 × 10 ⁵	57.9					4.5	43.5		
	K-C	69 × 10 ⁵	97.1					10.5	31.6		
	R-C	30 × 10 ⁶	47.8	6.4					2.9		
								42.8	3.0		

^aTreatment A: film characteristics OTR = 2.28, MVTR = 0.66, clip sealed, Treatment B: film characteristics OTR = 0.41-0.75, MVTR = 0.18-0.20, heat sealed, Treatment C: film characteristics OTR = 1.09, MVTR = 0.75, heat sealed.

^bK = Knuckle, R = Rib.

^cPPC = Psychrotrophic plate count.

^dPercent.

compared development of total counts and lactic acid bacteria on beef steaks stored for 28 days at 2.2 C in films with oxygen-permeabilities of 0, 31, 37, and 115 cc/m²/24 h/22.8 C/0% RH. Counts were slightly higher as the oxygen-permeability increased. Differences in total counts of samples stored in films with oxygen-permeabilities of 0 vs. 115 cc were about 1 log after 21 days and 1.5 logs after 28 days. Initial counts of the samples were low (10² – 10³ per gram). The effect of this range of differences in oxygen-permeability on microbial population levels and types and hence on meat quality might have been different if initial counts had been higher or if marginal refrigeration had been used during storage.

The rapid increase of psychrotrophic and mesophilic counts of cuts in defective packages can be expected because of the loss of the inhibitory effect of CO₂ and because of the ready availability of oxygen which would support continued growth of gram-negative aerobic bacteria.

Psychrotrophic counts of retail knuckle steaks were usually somewhat higher than counts of wholesale cuts from which they were prepared. No relationship was detectable between counts of retail rib steaks and counts of wholesale cuts from which they were fabricated. Hudson and Roberts (9) reported that bacterial counts of retail cuts of beef (both freshly prepared from vacuum packaged primal cuts and after display for 24 h in refrigerated cabinets) showed a significant correlation with counts of the vacuum packaged primal cuts. In the present study, differences in counts of retail knuckle or rib steaks because of differences in package type employed for the primal cut were not statistically significant.

The most predominant types among the initial microbial flora of knuckles, ribs, and chucks consisted primarily of *Moraxella-Acinetobacter* and coryneform bacteria. In the early phases of storage, *Lactobacillus* dominated on knuckles and *Microbacterium* and *Lactobacillus* spp. dominated on ribs. In the final phase of storage, *Lactobacillus* spp. became dominant on both knuckles and ribs. Although storage of chucks was not extended beyond 21 days, the microbial population at that time was more varied (*Lactobacillus*, *Microbacterium*, *Enterobacteriaceae*, *Pseudomonas*, *Moraxella-Acinetobacter*, *Corynebacterium*, *Arthrobacter*) than that of the knuckles or ribs. Hudson and Roberts (9) reported that *Microbacterium thermosphactum* developed on vacuum-packaged beef in the earlier phases of storage after which *Lactobacillus* spp. became dominant. Gardner et al. (7) showed that lactobacilli and *M. thermosphactum* constituted a significant part of the microflora of pork stored at 2 C. Other researchers (10-12, 15) also have shown that lactic acid bacteria became dominant on vacuum-packaged meats.

Differences in types of the microbial population of knuckles, ribs, and chucks during the early phases (0-21 days) of refrigerated storage may be related to the degree

of evacuation (vacuumization) attained during packaging. Because of their configuration (generally spherical), knuckles are likely to be packaged with fewer non-evacuated cavities inside the package than are cuts which are irregular in configuration. Cavities created by irregularly shaped cuts (ribs and chucks) generally result in a lower degree of vacuum and consequently a greater amount of residual air remaining in the package. Hence, with other factors being the same, increases in CO₂ will occur earlier and the concentration of CO₂ will be higher in packages with the smallest amount of residual air. This in turn would affect the inhibitory activity on the gram-negative aerobic spoilage bacteria (19). Other factors may be associated with differences in microbial populations of knuckles, ribs, and chucks (for example, differences in moisture content). Knuckles have a higher moisture content than either ribs or chucks. In addition, the fabrication process of the cuts may cause some differences in the initial number and type of bacteria present on the cuts. Interactive phenomena among micro-organisms could influence distribution of various types during refrigerated storage. In this study, initial microbial levels and types on knuckles, ribs, and chucks were similar. The microbial flora of cuts from defective bags was varied and consisted primarily of *Pseudomonas*, *Microbacterium*, *Lactobacillus*, and *Moraxella-Acinetobacter* species. These are bacteria commonly found on refrigerated meats stored under aerobic conditions (2, 4).

ACKNOWLEDGMENTS

T.A. 12410, Texas Agricultural Experiment Station. This research was supported by the Flexible Packaging Division, Continental Can Co., Inc., and Agricultural Marketing Research Institute, Agricultural Research Service, U.S. Department of Agriculture (Contract 12-14-1001-407). This project is a contribution to Western Regional Information WM-62. Reference to a company or product name is for specific information only and does not imply approval or recommendation of the product by the U.S. Department of Agriculture or the Texas Agricultural Experiment Station to the exclusion of others that may be suitable.

REFERENCES

1. Arafa, A. S., and T. C. Chen. 1975. Effect of vacuum packaging on microorganisms on cut-up chickens and in chicken products. *J. Food Sci.* 40:50.
2. Ayres, J. C. 1960. The relationship of organisms of the genus *Pseudomonas* to the spoilage of meat, poultry and eggs. *J. Appl. Bacteriol.* 23:471.
3. Baltzer, J. 1969. The relationship between bacterial contamination and growth on meats and the quality of meat products. *Proc. Recip. Meat Conf.* 22:294.
4. Davidson, C. M., M. J. Dowdell, and R. G. Board. 1973. Properties of gram-negative aerobes isolated from meats. *J. Food Sci.* 38:303.
5. Duncan, D. B. 1955. New multiple range and multiple F tests. *Biometrics* 11:1.
6. Edwards, P. R., and W. H. Ewing. 1972. Identification of *Enterobacteriaceae*. 3rd ed. Burgess Publishing Co., Minneapolis.
7. Gardner, G. A., A. W. Carson, and J. Patton. 1967. Bacteriology of prepacked pork with reference to the gas composition within the pack. *J. Appl. Bacteriol.* 30:321.
8. Hanna, M. O., D. L. Zink, Z. L. Carpenter, and C. Vanderzant. 1976. Isolation of *Yersinia enterocolitica* from vacuum-packaged

- beef and lamb. Paper presented at the 36th Annual Meeting IFT, Anaheim, CA.
9. Hudson, W. R., and T. A. Roberts. 1973. A bacteriological survey of enzyme treated vacuum packaged beef. Mem. No. 27. Meat Res. Inst., Langford, Bristol, England.
 10. Ingram, M. 1962. Microbiological principles in prepacking meats. *J. Appl. Bacteriol.* 25:259.
 11. Jaye, M., R. S. Kittaka, and Z. J. Ordal. 1962. The effect of temperature and packaging material on the storage life and bacterial flora of ground beef. *Food Technol.* 16:95.
 12. Johnson, B. Y. 1974. Chilled vacuum-packed beef. *CSIRO Food Res. Quart.* 34:14.
 13. Ordal, Z. J. 1962. Anaerobic packaging of fresh meat. Proc. of Meat Ind. Res. Conf. American Meat Institute Foundation, Chicago. 14:39.
 14. Perdue, R. R., R. O. Kuehne, and W. Brown. 1975. Vacuum packaging to extend shelf life. Paper presented at the 35th Ann. Meetings IFT, Chicago, IL. p. 175.
 15. Pierson, M. D., D. L. Collins-Thompson, and Z. J. Ordal. 1970. Microbiological, sensory and pigment changes of aerobically and anaerobically packaged beef. *Food Technol.* 24:129.
 16. Seideman, S. C., Z. L. Carpenter, G. C. Smith, and K. E. Hoke. 1976. The effect of degree of vacuum and length of storage on the physical characteristics of vacuum packaged beef wholesale cuts. *J. Food Sci.* 41:732.
 17. Seideman, S. C., C. Vanderzant, G. C. Smith, M. O. Hanna, and Z. L. Carpenter. 1976. The effect of degree of vacuum and length of storage on the microflora of vacuum packaged beef wholesale cuts. *J. Food Sci.* 41:738.
 18. Silliker, J. H., and D. A. Gabis. 1975. A cellulose sponge sampling technique for surfaces. *J. Milk Food Technol.* 38:504.
 19. Taylor, A. A., and B. G. Shaw. 1972. Effect of packaging method and material on storage quality of primal beef joints. Mem. No. 14, Meat Res. Inst., Langford, Bristol, England.
 20. Vanderzant, C., and R. Nickelson. 1969. A microbiological examination of muscle tissue of beef, pork and lamb carcasses. *J. Milk Food Technol.* 32:357.

Evaluation of Colony Growth of Bacteria and the Surface Environment of Beef¹

R. B. MAXCY

*Department of Food Science and Technology
University of Nebraska, Lincoln, Nebraska 68583*

(Received for Publication March 22, 1976)

ABSTRACT

Bacterial growth in or on solid and semi-solid foods primarily involves growth of micro-colonies, which are influenced by restrictive factors of little consequence in liquid foods. To evaluate bacterial growth on solid foods, methods were developed to study colony growth with emphasis on the early stages. Pure cultures of *Escherichia coli*, *Lactobacillus bulgaricus*, *Pseudomonas fluorescens*, and *Staphylococcus aureus* had distinctly different microscopic colony appearance. Growth expressed as numbers of cells per colony was exponential and linear when expressed as diameter. Mature colonies had a population density of approximately 10^{10} viable cells. Microcolonies of bacteria were particularly resistant to dispersion and required the presence of physical agents such as sea sand in the dispersion process. Colony growth on small test strips of beef was extremely susceptible to the relative humidity of the environment. Furthermore, availability of nutrients on the surface of beef apparently influenced growth. Pregrowth of *P. fluorescens* followed by destruction with gamma rays enhanced subsequent growth of *L. bulgaricus*. Bacterial growth at the micro-colony stage can present some unique considerations in evaluating the microenvironment of solid foods.

Bacterial contamination of fresh food is assumed to be in discrete units of a single cell or of very small numbers of cells. Food without a continuous liquid phase has restrictive factors for bacterial growth which occurs as colonies. Contaminants of solid food are limited by diffusion of nutrients and auto-inhibitory substances as well as cell motility. Present knowledge of colony growth of contaminants of food is based primarily on analogy to growth on laboratory media developed for enumeration purposes.

On laboratory media, colony size and characteristics are dependent on numerous environmental conditions (6). Studies of colony growth have dealt primarily with macroscopic observations (10, 11, 14). Some early interest in microscopic size colonies was shown by Frost (4) in his work to develop a plate count based on microscopic observations. Hoffman and Frank (7) studied colony development of *Escherichia coli* with particular emphasis on the genealogy. The previous observations, however, did not consider the dynamics of

initial population increases of 10 to 100 times. This magnitude of increase in population is of great interest for studying bacterial contaminants of food.

Determination of the factors affecting the early stages of colony growth on food apparently is an important, unstudied area in food microbiology. Comparison of colony growth on laboratory media and on fresh beef seemed a logical approach to build on present knowledge and to involve a challenging food product.

METHODS

Cultures and dilutions

Pure cultures of *E. coli*, *Staphylococcus aureus*, and *Pseudomonas fluorescens* were grown in nutrient broth (Difco) at 32 C for 16-18 h. *Lactobacillus bulgaricus* was grown in microinoculum broth (Difco) for approximately 24 h at 32 C. Dilutions of the pure cultures were made in phosphate buffer (2) so that a capillary tube delivering approximately .005 ml provided an average inoculum of 1.2 colony-forming units (CFU). Thus, 30% of the trial inocula were without a CFU, and by calculation using the Poisson distribution 36, 22, 9, 3, and 1%, respectively, contained 1, 2, 3, 4, 5 cells per inoculum. Those trials without a CFU were discarded. General procedures for determining numbers were those shown in *Recommended Methods for the Microbiological Examination of Foods* (2).

Humidity control

Desiccator jars were used as compartments to generate the desired relative humidity (RH). Distilled water covering the bottom of a desiccator provided the maximum RH. Lower RH, to be specified in the results, was obtained by substituting saturated salt solutions as recommended by Winston and Bates (15).

Meat samples

Beef with an average of less than 100 microorganisms per gram was obtained by previously described aseptic methods (8) from 2 to 3 days post-slaughter quarters of beef. The meat was subsequently frozen and held frozen until used. Portions of approximately 10 mg were shaved from the frozen blocks to serve as microbial carriers. When there was an inherent contaminant unlike the inoculum, it was apparent on plating and the results of the experiment were not used. Use of the small meat samples facilitated studying behavior of individual CFU and represented a common problem of dispersing contaminants through sawing of meat.

Recovery of cells

To determine numbers of cells in a colony, the supporting medium was included in transfer to a tube with 10 ml of phosphate buffer (2). Dispersion of young colonies required more physical action than that

¹Published as Paper No. 3861, Journal Series, Nebraska Agricultural Experiment Station.

attained by the commonly used blender method recommended in *Standard Methods* (2). Details on the requirements for dispersion are given with data in the Results. Plating was on plate count agar (Difco; PCA) with incubation at 32 C.

RESULTS

Early stages of colony growth

CFU growth on the surface of PCA periodically was observed microscopically, beginning when there were approximately 50 cells, until the colonies were mature. Early growth of *E. coli* appeared as palisades as described by Hoffman and Frank (7). *P. fluorescens* exhibited similar palisades except that the terminal rods appeared to be separating, which was suggestive of branching. *L. bulgaricus* developed as independent chains reaching in all directions. *S. aureus* colonies were circular. There was no apparent space between the cells of the young colonies. These results are contrary to the report of Frost (5), who observed micro-colonies as loosely arranged clusters. However, his procedures involved drying and staining. Such treatments would be expected to change the physicochemical relations between the cells in a colony.

The compact nature of the young colonies apparently resisted breaking with a blender for enumeration by the plate count. When there were 50 to 100 cells as estimated by microscopic observation, the most commonly used method of dispersion by a mechanical blender (2) indicated less than 10% of the cells were giving rise to colonies. Various systems of disrupting the colonies were tried. Inclusion of anionic and nonionic surfactants in the process of dispersion showed no effect. Physical disruption of a colony and the supporting medium by grinding with a mortar and pestle or by agitation with sea sand on a vortex mixer gave results in agreement with the microscopic observations of the young colonies.

Colony dispersion for enumeration of bacteria

To determine whether resistance by colonies to dispersion was a common phenomenon of bacterial colonies associated with beef, 20 different isolates were taken from commercial ground beef. These isolates were grown from CFU on PCA and colonies were evaluated at various ages up to 48 h. With a total of 145 observations, inclusion of sea sand and agitation on a vortex mixer gave higher counts than agitation without sea sand (significant by the "t" test at 99.9% level). The difference in dispersion was general for all the isolates though observations were not adequate to prove comparative relative resistance to dispersion of the various isolates.

To determine the repeatability of the methodology for determining numbers in each colony, observations were made with 24-h colonies of *E. coli*. The mean count of nine colonies was 110×10^6 with a standard deviation of 4.8×10^6 , which was a coefficient of variation of 4.4%.

For all subsequent observations on colony growth, approximately 1 g of sterile sea sand was included in the test tube with 10 ml of phosphate buffer for a 1-min treatment on a vortex mixer.

Colony dispersion for enumeration of bacteria in ground beef

Samples of fresh and frozen ground beef were used for comparison of the standard method using a blender (2) for dispersion of bacteria and inclusion of a step with sea sand on a vortex mixer. The latter method with four samples and duplicate plating resulted in an average count of 18% more than with the standard method of dispersion with a blender. The "t" test of difference indicated significance at the 99% level.

Colony growth to macroscopic size

When individual CFU grew to near their maximum size, the numbers of cells increased geometrically in the early stages, but slowed markedly after the colony was approximately one-third maximum size. Diameter as an expression of colony growth was linear to near the maximum size (Fig. 1, 2, 3). The data exemplify typical

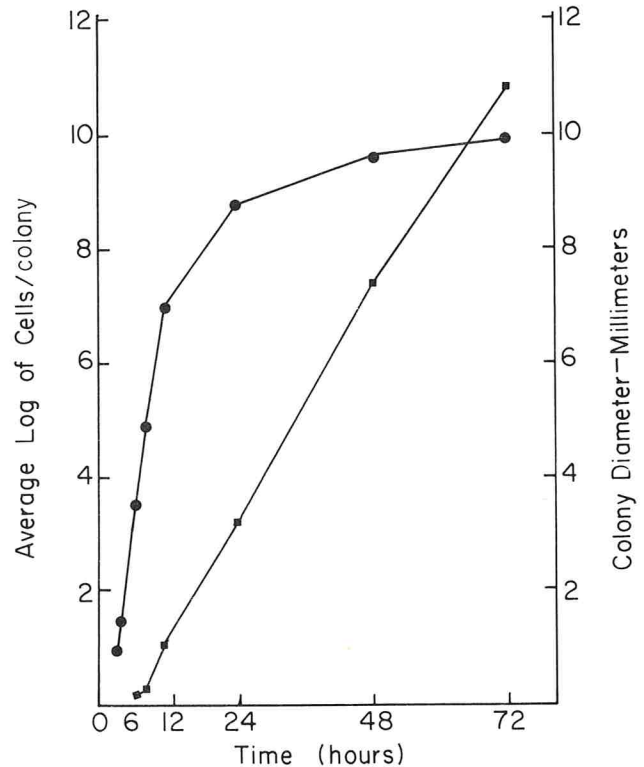


Figure 1. Growth pattern of colonies of *E. coli* at 37 C on plate count agar. Circles indicate numbers of cells and squares indicate the colony diameter.

growth patterns. Rate of growth expressed either as increase in population or as increase in colony diameter was temperature dependent.

Comparative surface and subsurface growth of *E. coli* with plate count agar was determined after 48 h at 32 C. The mean count for subsurface growth was 15.1×10^7 with a standard deviation of 3.7×10^7 . The mean count for surface growth was 4.9×10^9 with a standard deviation of 1.4×10^9 . Subsurface growth produced approximately 3% as many cells as surface growth.

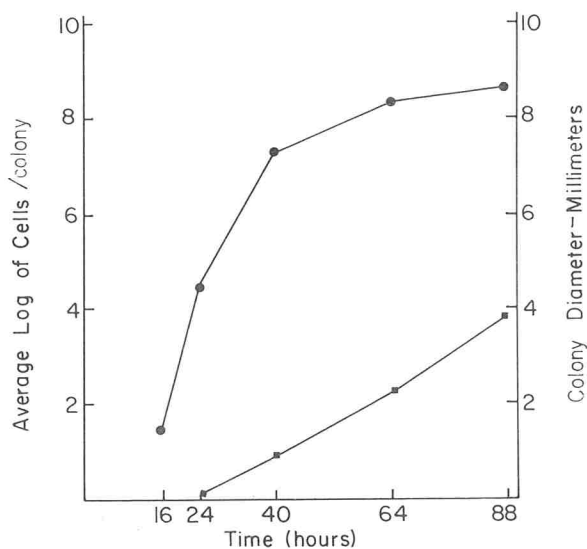


Figure 2. The growth pattern of colonies of *P. fluorescens* at 20 C on plate count agar. Circles indicate numbers of cells and squares indicate the colony diameter.

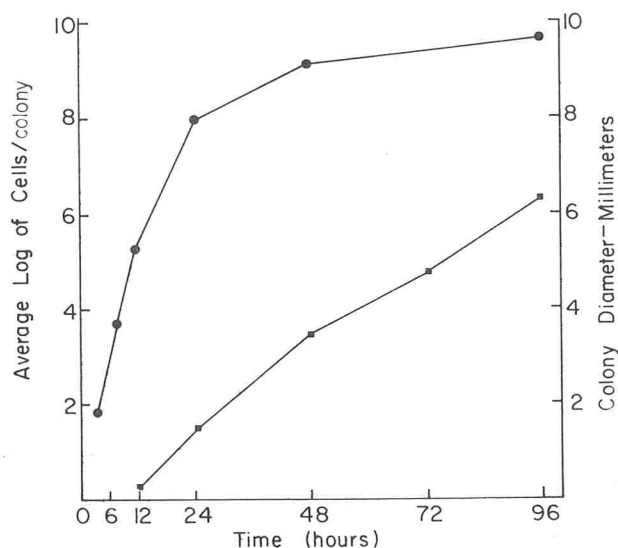


Figure 3. The growth pattern of colonies of *S. aureus* at 32 C on plate count agar. Circles indicate numbers of cells and squares indicate the colony diameter.

Assuming comparable longevity for cells in each environment this relation is expected with a 1-mm diameter spherical subsurface colony and a 4-mm diameter hemispherical surface colony.

Colony growth on beef

When approximately 10-mg samples of beef were incubated on PCA, there was no growth attributable to 18 of 22 samples. With three test samples there was a single colony, and one sample showed mixed contamination. This frequency of occurrence of contaminants was expected in light of previous results on total counts using these techniques (9). It was therefore necessary to make frequent checks to see that the pure culture inoculum was the CFU being studied. Experiments indicating

organisms other than the pure culture under observation were discarded.

To determine the fate of isolated contaminants on small pieces of meat, 10-mg pieces of meat were placed in sterile 15 × 60 mm petri plates and inoculated with a CFU of *E. coli*, *S. aureus*, or *P. fluorescens*. Test samples were incubated in 25 and 60% RH for various times up to 48 h at 32 C and for long times at lower temperatures to 10 C. There was no growth on any bacteria under these conditions. Apparently the meat dried and prevented growth of the inoculum.

To minimize drying as a factor in restricting growth, trials were made with approximately 10-mg pieces of inoculated meat in open inverted sterile 15 × 60 mm petri dishes over distilled water. At 32 C six of 23 samples showed growth of *E. coli*, but 940 was the highest plate count for any test piece irrespective of the time of incubation. These numbers are indeed low when compared to the population of more than 100×10^7 cells per colony when grown on PCA. *S. aureus* grew at 32 C in 24 h to produce an average log of cells per test piece of 8.14, which was not significantly different from the growth rate on PCA as shown in Fig. 3. There was no growth of *P. fluorescens* at 32 C irrespective of the time of incubation. With incubation for up to 7 days at 10 C, there was some growth of *P. fluorescens*, but the results were erratic and the population density of the test pieces was never more than 1% of the count per colony on plate count agar.

Further tests to study the effect of drying on colony growth on meat were made by inoculating CFU of *E. coli* onto 10-mg samples of beef in sterile petri dishes. The test strips were then covered with aluminum foil squares 25 × 25 mm and sealed around the edges with petroleum jelly. After 24 h at 32 C, the count per CFU was 2.4×10^8 , which was 260,000 times the growth on uncovered test strips and 55% of the count obtained by growing colonies on plate count agar for 24 h at 32 C.

To simulate a larger piece of meat yet retaining the ability to manipulate a small piece and CFU as had been done with PCA, a 6-mm thick piece of meat obtained aseptically was used as a carrier for 10-mg test pieces. A parallel experiment involved placing the test pieces of meat on solidified PCA. The inoculum was *E. coli* and incubation was at 32 C in a desiccator jar with distilled water in the bottom. The results of four replications are presented in Fig. 4. There was considerable lag and variability during the early hours of incubation. PCA as the carrier for test strips gave greater growth response than with beef as the carrier.

Metabiotic effect of *P. fluorescens* growth on subsequent colony growth of *L. bulgaricus* on beef

Fresh beef excised with aseptic techniques was inoculated by swabbing with a 24-h old culture of *P. fluorescens*. The meat was incubated for 72 h at 2 C after which it was frozen and irradiated with 500 Krad using a cobalt-60 source to kill the *P. fluorescens* (9). Test samples of approximately 10 mg of the irradiated beef

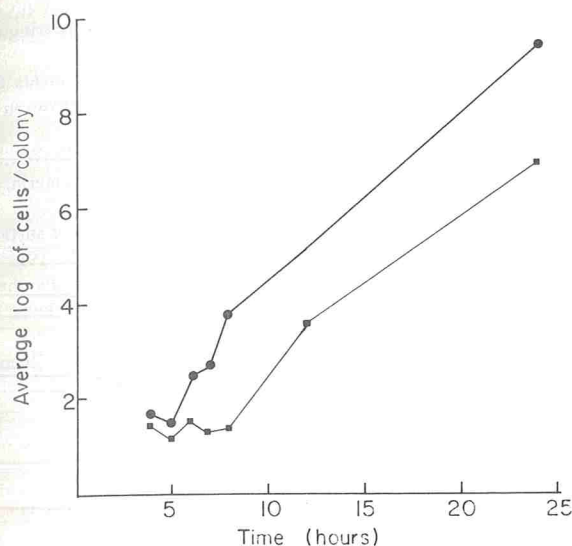


Figure 4. Growth of *E. coli* on beef incubated at 32 C. Circles indicate growth on test strips with plate count agar as the carrier. Squares indicate growth on test strips with beef as the carrier.

were placed on solidified 1.5% agar and inoculated with a CFU of *L. bulgaricus*. Control consisted of parallel samples excluding the treatment with *P. fluorescens* and incubation. After 40 h at 20 C, the numbers of *L. bulgaricus* were determined by plating on PCA. With nine tests on samples where there had been previous growth of *P. fluorescens* the average count was 8,200. The average of six tests on the control was 68. The "t" test indicated significance at the 99.99% level.

DISCUSSION

Growth of colonies of bacteria on plate count agar provides guides for methods and some parameters of expectation for observations of growth associated with solid foods, e.g., beef. However, growth of colonies on beef is much more difficult to observe than growth on plate count agar, because it is not possible to obtain a microscopic view of very early colony growth.

It is generally assumed that age of colonies has no influence on the ease of disruption for counting purposes. This concept likely had its origin in the early work of Frost (5), who observed relatively young colonies arising from milk. His report depicted the cells in an apparent loose cluster but his results were based on observations of dried, stained colonies. Furthermore, his data were based on observations of colonies with well over 100 individual cells, which represented colonies with larger numbers than found to be particularly difficult to disrupt in the work reported in the present paper. Our work with still smaller colonies indicated very early colony growth involves some physicochemical phenomena that influence the dispersion of cells. These phenomena may influence some commonly accepted counting procedures as well as the spread of contamination.

It was surprising to find such extreme limitations in

the growth of contamination on a small piece of meat. There is a general consensus that such small pieces of meat, as generated from sawing, when left unattended in a meat processing plant would give rise to profuse microbial contamination. Yet, in this work, growth of *E. coli* and *P. fluorescens* was limited even under the most favorable conditions of near 100% RH (an atmosphere over distilled water). *S. aureus* grew well only when the meat was in an environment of near 100% RH. Both surrounding atmosphere and diffusion control directly affected the availability of water and indirectly the availability of nutrients to bacteria for surface growth. Bacteria associated with dairy foods and the micro-environment of surface contamination have been shown to be highly sensitive to evaporation and available moisture (3, 8). Loss of moisture alone, however, does not account for the difference in growth on meat and on plate count agar. Availability of nutrients appears to be an influential factor though meat is considered to be a good growth medium. Colony growth has been shown to be highly susceptible to available nutrients (10, 13).

Growth conditions for bacteria, in many ways, are markedly different for colony growth than for growth in liquid media. Mobility of cells, accumulation of auto-inhibitory substances, and availability of nutrients are influential factors. Colonies on solid media, therefore, are highly susceptible to micro-environmental changes, which influence bacterial growth on meat.

With solid food systems, bacterial growth is rightfully assumed to be in colony form, but the colonies are probably very small in comparison to a mature colony on plate count agar. If a single colony on solid food grew to maturity and attained numbers equal to those occurring in a colony on plate count agar, there would be enough bacteria to produce organoleptic spoilage in 200 g of food. The extrapolation is based on the concept that 10^{10} cells occur in a colony and growth of approximately 50×10^6 bacterial cells per gram in a food produce a detectable off-flavor (1, 9, 12). Colony growth in or on food must, therefore, be limited to the very early stages of the growth patterns as presented in Fig. 1, 2, and 3.

Growth of numbers of bacteria in a colony is geometric in much the same pattern as in the well known pattern of bacterial growth in liquid media. Growth expressed as colony diameter, however, is a linear relation with time and represents a steady state of growth of bacterial colonies (10). Thus growth proceeds at the periphery of the colony outward while within the colony the population remains stable. This characteristic has potential practical significance in terms of ease of disruption of colonies of certain species. Colony disruption may influence bacterial distribution during meat processing and may influence enumeration procedures.

ACKNOWLEDGMENT

Appreciation is due Vickie Martinkus and Elva Steinbruegge for technical assistance.

REFERENCES

1. Adameic, M., D. S. Clark, and M. Yaguchi. 1970. Effect of psychrotolerant bacteria on the amino acid content of chicken skin. *J. Food Sci.* 35:272-275.
2. APHA. 1966. Recommended methods for the microbiological examination of foods. 2nd ed. American Public Health Association, Inc., New York, N.Y.
3. Barnhart, H. M., Jr., R. B. Maxcy, and C. E. Georgi. 1970. Effect of humidity on the fate of the microflora of milk on stainless steel surfaces. *Food Technol.* 24(12):67-71.
4. Frost, W. D. 1916. A rapid method of counting living bacteria in milk and other richly seeded materials. *J. Amer. Med. Ass.* 66:889-890.
5. Frost, W. D. 1919. A new method for the study of bacteria. *J. Amer. Med. Ass.* 72:343-344.
6. Hoffman, H. 1964. Morphogenesis of bacterial aggregations. *Ann. Rev. Microbiol.* 18:111-130.
7. Hoffman, H., and M. E. Frank. 1961. Form and internal structure of cellular aggregations in early *Escherichia coli* microcultures. *J. Gen. Microbiol.* 25:353-364.
8. Maxcy, R. B. 1973. Nature and cause of yellow film occurring on dairy equipment. *J. Dairy Sci.* 56:164-167.
9. Maxcy, R. B., and N. P. Tiwari. 1973. Irradiation of meats for public health protection. pp. 491-503. In *Radiation preservation of food*. International Atomic Energy Agency, Vienna.
10. Palumbo, S. A., M. G. Johnson, V. T. Rieck, and L. D. Witter. 1971. Growth measurements on surface colonies of bacteria. *J. Gen. Microbiol.* 66:137-143.
11. Pirt, S. J. 1967. A kinetic study of the mode of growth of surface colonies of bacteria and fungi. *J. Gen. Microbiol.* 47:181-191.
12. Punch, J. D., J. C. Olson, Jr., and E. L. Thomas. 1965. Psychrophilic bacteria. III. Population levels associated with flavor or physical change in milk. *J. Dairy Sci.* 48:1179-1183.
13. Rieck, V. T., S. A. Palumbo, and L. D. Witter. 1973. Glucose availability and the growth rate of colonies of *Pseudomonas fluorescens*. *J. Gen. Microbiol.* 74:1-8.
14. Stokes, J. L., and H. G. Bayne. 1957. Growth rates of *Salmonella* colonies. *J. Bacteriol.* 74:200-206.
15. Winston, P. W., and D. H. Bates. 1960. Saturated solutions for the control of humidity in biological research. *Ecology* 41:232-237.

Total Plate Count and Sensory Evaluation as Measures of Luncheon Meat Shelf Life

W. M. HILL, J. REAUME, AND J. C. WILCOX

*Armour Food Company, Research Division
Scottsdale, Arizona 85260*

(Received for publication January 19, 1976)

ABSTRACT

The relationship between sensory attributes and total numbers of bacteria was investigated in a study involving 600 samples of spiced luncheon meat, cooked salami, bologna, and chopped ham. The vacuum packaged products were purchased from 60 markets throughout the United States. Samples were subjected to microbiological and sensory evaluation within a few days after purchase and once a week during a 3-week refrigerated storage period. Based upon an off-flavor intensity scale of 1 = none to 7 = extremely strong, the samples having total plate counts (TPC) of $< 10^4$, 10^4 , 10^5 , 10^6 , 10^7 , and 10^8 per gram had mean taste panel scores (TPS) of 2.00, 2.25, 1.92, 2.06, 1.93, and 3.17, respectively over the 3-week storage period. A microbiological standard of 10^6 TPC would terminate shelf life of these products at 24-28 days after manufacture. Establishment of a total bacterial population of 10^8 per gram with a corresponding decrease in sensory quality did not occur until 46-60 days after manufacture.

Shelf life may be defined as the period a product can be stored under specified conditions without significant deterioration in quality. The consumer employs various sensory attributes to judge the quality of foods. To the consumer, then, the shelf life of a perishable food such as ready-to-eat luncheon meats has ended when the appearance, odor, or taste becomes unacceptable.

Total plate count microbiological standards have been placed upon meat products by several state regulatory agencies (6). It is argued that these standards improve the quality of meats available to the consumer at the retail level (3). Thus, to some regulatory agencies, the shelf life of perishable meats has ended when the product contains a total number of bacteria in excess of that allowed by the microbiological standard.

To be of value, a microbiological standard must provide some benefit to the consumer. Therefore, the relationship between the consumer's sensory measurement of quality and shelf life and a regulatory agency's microbiological measurement of quality and shelf life is worthy of investigation.

There have been several studies conducted on the growth of microorganisms in vacuum packaged sliced luncheon meats during refrigerated storage. Brooks and Henrikson (2) examined 350 retail samples representing seven varieties of luncheon meat. The number of aerobic bacteria per gram ranged from a low of about 300 to a

high of over 300,000,000 with mean values ranging from 700,000 to over 18,000,000. At the time of plating, 343 of the samples were judged organoleptically acceptable. The authors concluded that total plate counts did not provide a clear-cut differentiation between organoleptically acceptable and unacceptable samples. Allen and Foster (1) found that signs of spoilage in sliced luncheon meats, if they appeared at all, were not detectable until after the bacterial population exceeded 10,000,000 per gram. Similar results were reported by Kempton and Bobier (7) who observed that bologna and cooked ham were stable in odor and appearance throughout a 15-week storage study even though a maximum of 100,000,000 bacteria per gram was attained in 3 to 4 weeks.

The studies which provided valuable information in the growth patterns and types of organisms occurring in luncheon meats under controlled conditions (1, 7) may not accurately reflect the condition of these products as they are available to the consumer from supermarket shelves due to variable transportation and retail stocking practices. Other studies (2), while measuring product quality as perceived by the consumer, often lack information on the age of the product at the time of analyses.

The objective of our study was to further evaluate the relationship between total plate counts, organoleptic attributes, and age of luncheon meats obtained at retail and held under refrigeration for up to 3 weeks.

MATERIALS AND METHODS

Collection of samples

The Armour products were removed from the display cases of as many pre-selected stores as necessary to obtain a sufficient number of samples representing three product code dates per city surveyed. The products were purchased by personnel of an independent research service organization, packed in insulated shipping containers, and sent air freight to the Armour Food Research Laboratory. Product temperatures in the shipping container of less than 10 C were considered acceptable.

Storage of samples

The samples were held in a 3-4 C cooler until one week before analysis when selected packages were placed in a lighted display case, simulating retail conditions. Case temperature was adjusted to 4-7 C.

Sampling

Two packages of product from each city/code date group were analyzed within 2 days after receipt and again each week thereafter for 3 weeks. The packages were removed from the display case and sampled for microbiological analyses. The remainder of the product was then taken to the Home Economics Section for immediate sensory evaluation.

Microbiological analyses

Packages were opened aseptically and a wedge cut through the slices of product to obtain a 33-g sample. The sample was blended with 300 ± 2 ml of water in a Waring Blendor jar for 2 min. Plates were poured with Difco Plate Count Agar and incubated at 30 C for 48 h.

Sensory evaluation

Ten taste panel judges for each product (12 for bologna) were selected from laboratory personnel. The judges were trained to detect flavor changes in their assigned products. Since these studies were conducted over a 4-year period, most panelists evaluated all four of the products. The product slices were cut in half, rolled and secured with a plastic toothpick. Two sets of samples were served to the judges at each test session. Each set included three code dated samples purchased in one city. Samples were placed on aluminum foil trays, under code, in predesigned randomized order. Judges scored the samples on a 7-point off-flavor intensity scale with 1 equal to no off-flavor, 4 equal to moderate off-flavor, and 7 equal to extremely strong off-flavor.

To approximate consumer acceptance of the samples, the off-flavor intensity scale was arbitrarily divided into two ranges. A score < 3.5 was considered acceptable while a score ≥ 3.5 was deemed unacceptable. The criteria of product failure have been discussed by Gacula (4) and Gacula and Kubala (5).

RESULTS AND DISCUSSION

The results of the microbiological and sensory evaluations of bologna, spiced luncheon meat, cooked salami and chopped ham are shown in Tables 1-5.

As would be expected, the total plate counts (TPC) and off-flavor taste panel scores (TPS) increased with storage time (Table 5). Initially, 69% of the samples analyzed contained less than 10^6 TPC and the means TPS for the initial samples was 1.72. After 3 weeks of storage, 71% of the samples exceeded 10^6 TPC and the mean TPS for the 3-week samples was 2.47. Also, the number of samples having TPS ≥ 3.5 increased from 3.3% initially to 16.7% after 3 weeks of storage. While storage time is related to both total counts and sensory characteristics of vacuum packaged luncheon meats, we did not observe a cause and effect relationship between total counts and off-flavor development. Indeed, there often was a decrease in the off-flavor scores at the four sampling periods with increasing bacterial numbers up to 10^8 per gram. This trend is shown in Fig. 1 which is a three-way plot of storage time, taste panel scores, and total numbers of bacteria. Initially, there was little difference in taste panel scores of samples containing up to 10^8 TPC. However, after 3 weeks of storage, samples con-

TABLE 1. Taste panel scores (TPS) and total plate counts (TPC) for bologna stored at 4-7 C.

Range TPC/g	Storage time (Weeks)														
	Initial			One			Two			Three			Total		
	No. of samples	Mean TPS	% TPS ≥ 3.5	No. of samples	Mean TPS	% TPS ≥ 3.5	No. of samples	Mean TPS	% TPS ≥ 3.5	No. of samples	Mean TPS	% TPS ≥ 3.5	No. of samples	Mean TPS	% TPS ≥ 3.5
LT 10^4	28	1.73	10.7	8	2.54	37.5	7	2.38	28.6	4	2.34	50.0	47	2.04	21.3
10^4 - 10^5	5	1.73	0	3	1.56	0	2	1.67	0	4	2.67	50.0	14	1.95	14.3
10^5 - 10^6	5	1.13	0	5	1.60	0	6	1.56	0	4	2.00	0	20	1.55	0
10^6 - 10^7	13	1.56	7.7	11	2.24	9.1	7	2.05	0	10	1.70	0	41	1.87	4.9
10^7 - 10^8	9	1.56	0	24	1.74	0	25	2.07	4.0	27	1.78	0	85	1.83	1.2
10^8 - 10^9	0	—	—	9	2.70	33.3	13	3.15	38.5	11	3.49	54.5	33	3.14	45.5
TOTAL	60	1.61	6.7	60	2.06	11.7	60	2.24	13.3	60	2.19	16.7	240	2.03	12.1

TABLE 2. Taste panel scores (TPS) and total plate counts (TPC) for spiced luncheon meat stored at 4-7 C.

Range TPC/g	Storage time (Weeks)														
	Initial			One			Two			Three			Total		
	No. of samples	Mean TPS	% TPS ≥ 3.5	No. of samples	Mean TPS	% TPS ≥ 3.5	No. of samples	Mean TPS	% TPS ≥ 3.5	No. of samples	Mean TPS	% TPS ≥ 3.5	No. of samples	Mean TPS	% TPS ≥ 3.5
LT 10^4	10	2.38	0	0	—	—	0	—	—	0	—	—	10	2.38	0
10^4 - 10^5	2	2.10	0	8	2.65	25.0	4	3.00	50.0	5	2.84	40.0	19	2.72	31.6
10^5 - 10^6	11	1.85	0	1	1.80	0	4	2.50	0	3	2.33	0	19	2.06	0
10^6 - 10^7	7	2.11	14.3	14	2.24	21.4	9	2.51	33.3	13	2.26	0	43	2.28	16.3
10^7 - 10^8	2	2.30	0	8	2.10	12.5	11	2.02	9.1	9	2.49	11.1	30	2.20	10.0
10^8 - 10^9	0	—	—	1	3.80	100.0	4	3.20	50.0	2	3.30	50.0	7	3.31	57.1
TOTAL	32	2.12	3.1	32	2.34	21.9	32	2.39	25.0	32	2.49	12.5	128	2.34	15.6

TABLE 3. Taste panel scores (TPS) and total counts (TPC) for cooked salami stored at 4-7 C.

Range TPC/g	Storage time (Weeks)														
	Initial			One			Two			Three			Total		
	No. of samples	Mean TPS	% TPS ≥ 3.5	No. of samples	Mean TPS	% TPS ≥ 3.5	No. of samples	Mean TPS	% TPS ≥ 3.5	No. of samples	Mean TPS	% TPS ≥ 3.5	No. of samples	Mean TPS	% TPS ≥ 3.5
Lt 10^4	14	1.67	0	0	—	—	0	—	—	0	—	—	14	1.67	0
10^4 - 10^5	9	1.18	0	4	1.65	0	5	2.84	20.0	4	2.65	25.0	22	1.91	4.5
10^5 - 10^6	2	1.20	0	14	1.51	0	7	2.71	0	8	2.78	12.5	31	2.09	3.2
10^6 - 10^7	5	1.40	0	5	1.56	0	7	2.51	14.3	11	2.31	0	28	2.06	7.1
10^7 - 10^8	4	1.30	0	5	1.60	0	6	2.47	16.7	0	—	—	15	1.87	6.7
10^8 - 10^9	0	—	—	6	2.17	0	9	3.11	33.3	11	3.40	45.5	26	3.02	30.8
TOTAL	34	1.43	0	34	1.66	0	34	2.75	17.7	34	2.81	20.6	136	2.16	9.6

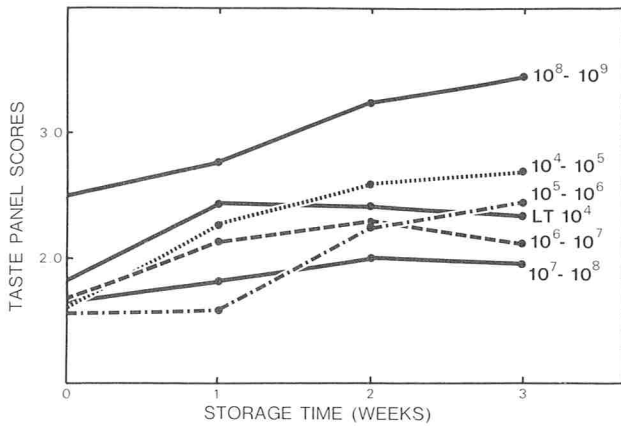


Figure 1. Mean taste panel scores for bologna, spiced luncheon meat, cooked salami, and chopped ham.

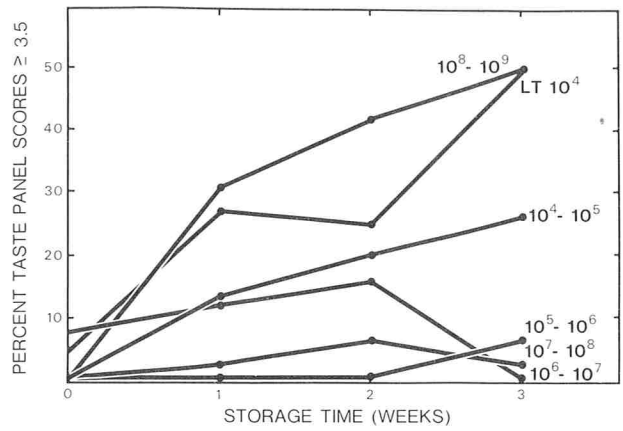


Figure 2. Percent taste panel scores ≥ 3.5 for bologna, spiced luncheon meat, cooked salami, and chopped ham.

taining less than 10^6 TPC had higher off-flavor scores than those samples having total counts of 10^6 - 10^8 per gram. Off-flavor development was consistently greater in those samples having 10^8 - 10^9 TPC.

The percent of samples having off-flavor scores ≥ 3.5 is plotted with storage time and total counts in Fig. 2. At each of the weekly testing periods there was less "consumer rejection" of the luncheon meat samples with 10^5 - 10^6 , 10^6 - 10^7 and 10^7 - 10^8 TPC than those samples containing less than 10^4 , 10^4 - 10^5 and 10^8 - 10^9 TPC.

The apparently greater "consumer acceptance" of luncheon meats containing progressively higher numbers of total bacteria (up to 10^8 TPC) may be the result of lactic acid fermentation processes. Lactic acid producing bacteria, which are the dominant microflora of vacuum packaged luncheon meats (1, 7), may act as oxygen scavengers and thus inhibit oxidative reactions which form off-flavors. Or, the lactic acid may simply mask the off-flavors yielding a more acceptable product up to the point where the acid itself becomes objectionable. Lactic

cultures are commonly employed to create specialized products by the dairy and dry sausage industries. Reddy and Chen (6) have shown that the sensory shelf life of raw ground beef is improved by the addition of lactic cultures. There seems little reason to believe that the growth of lactic bacteria in luncheon meats would not create flavor changes desired, and perhaps even expected, by the consumer.

The relationship between shelf life as defined by microbiological standards and shelf life as perceived by the consumer can be estimated from the data presented in Fig. 3. Linear regression analyses of total plate counts and product age from the date of pack yield best fit growth rates of the bacteria in the four luncheon meat products. While chopped ham has a relatively stable microbial population, the off-flavor scores for this product still increased at a rate (Table 4) comparable to that of the other products. The regression lines shown for bologna, cooked salami, and spiced luncheon meat are similar. Assuming a microbiological standard of 10^6

TABLE 4. Taste panel scores (TPS) and total plate counts (TPC) for chopped ham stored at 4-7 C

Range TPC/g	Storage time (Weeks)												Total		
	Initial			One			Two			Three			No. of samples	Mean TPS	% TPS ≥ 3.5
	No. of samples	Mean TPS	% TPS ≥ 3.5	No. of samples	Mean TPS	% TPS ≥ 3.5	No. of samples	Mean TPS	% TPS ≥ 3.5	No. of samples	Mean TPS	% TPS ≥ 3.5	No. of samples	Mean TPS	% TPS ≥ 3.5
LT 10^4	9	1.73	0	3	2.13	0	1	2.60	0	0	—	—	13	1.89	0
10^4 - 10^5	7	1.91	0	7	2.54	14.3	9	2.49	11.1	10	2.66	10.0	33	2.43	9.1
10^5 - 10^6	2	1.60	0	3	1.67	0	1	2.60	0	1	1.80	0	7	2.10	0
10^6 - 10^7	0	—	—	3	2.33	0	2	1.20	0	2	2.10	0	7	1.94	0
10^7 - 10^8	4	1.89	0	2	2.30	0	6	2.00	0	5	1.96	0	17	1.99	0
10^8 - 10^9	2	2.50	0	6	3.27	50.0	5	3.72	60.0	6	3.50	50.0	19	2.56	47.4
TOTAL	24	1.88	0	24	2.52	16.7	24	2.53	16.7	24	2.64	16.7	96	2.39	12.5

TABLE 5. Taste panel scores (TPS) and total plate counts (TPC) for bologna, spiced luncheon meat, cooked salami, and chopped ham stored at 4.7 C

Range TPC/g	Storage time (Weeks)												Total		
	Initial			One			Two			Three			No. of samples	Mean TPS	% TPS ≥ 3.5
	No. of samples	Mean TPS	% TPS ≥ 3.5	No. of samples	Mean TPS	% TPS ≥ 3.5	No. of samples	Mean TPS	% TPS ≥ 3.5	No. of samples	Mean TPS	% TPS ≥ 3.5	No. of samples	Mean TPS	% TPS ≥ 3.5
LT 10^4	61	1.82	4.9	11	2.43	27.3	8	2.41	25.0	4	2.34	50.0	84	2.00	11.9
10^4 - 10^5	23	1.62	0	22	2.28	13.6	20	2.60	20.0	23	2.70	26.1	88	2.25	13.6
10^5 - 10^6	20	1.58	0	23	1.59	0	18	2.27	0	16	2.44	6.3	77	1.92	1.3
10^6 - 10^7	25	1.68	8.0	33	2.14	12.1	25	2.28	16.0	36	2.11	0	119	2.06	8.4
10^7 - 10^8	19	1.65	0	39	1.82	2.6	48	2.10	6.3	41	1.96	2.4	147	1.93	3.4
10^8 - 10^9	2	2.50	0	22	2.76	31.8	31	3.24	41.9	30	3.45	50.0	85	3.17	41.2
TOTAL	150	1.72	3.3	150	2.11	12.0	150	2.47	17.3	150	2.47	16.7	600	2.19	12.3

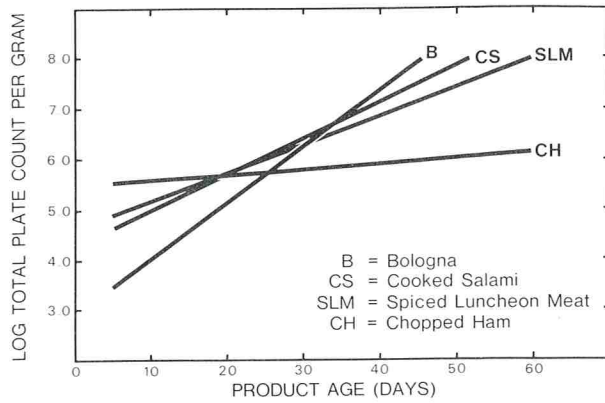


Figure 3. Linear regression analysis of total plate counts and product age.

TPC, the regulatory shelf life of bologna, cooked salami, and spiced luncheon meat would be 24-28 days beyond the date of pack. The consumer shelf life for these products, based upon sensory data, would be 46-60 days beyond the date of pack. Obviously, microbiological standards could cause the premature removal from the marketplace of a very large percentage of luncheon meat

products which are no doubt wholesome and the consumer would find totally acceptable.

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. Brooks, R. F., and R. L. Henrickson. 1956. Microflora of pre-packaged luncheon meats. *Univ. of Missouri Agr. Exp. Sta. Res. Bull.* 611. Columbia, Missouri.
3. Carl, K. E. 1975. Oregon's experience with microbiological standards for meat. *J. Milk Food Technol.* 38:483-486.
4. Gacula, M. C., Jr. 1975. The design of experiments for shelf life study. *J. Food Sci.* 40:399-403.
5. Gacula, M. C., Jr., and J. J. Kubala. 1975. Statistical models for shelf life failures. *J. Food Sci.* 40:404-409.
6. Johnston, R.W. 1975. Microbiological criteria, and update, proceedings of the Meat Industry Research Conference. pp. 115-118.
7. Kempton, A. G., and S. R. Bobier. 1970. Bacterial growth in refrigerated vacuum packed luncheon meats. *Can. J. Microbiol.* 16: 287-297.
8. Reddy, S. G., and M. L. Chen. 1975. Influence of lactic cultures on the biochemical, bacterial and organoleptic changes in beef. *J. Food Sci.* 40:314-318.

Coliform Analyses at 30 C¹

PAUL A. HARTMAN and PHILIP S. HARTMAN²

Department of Bacteriology
 Iowa State University, Ames, Iowa 50011

(Received for publication November 18, 1974)

ABSTRACT

The literature directly pertinent to coliform analyses at 30 C was reviewed. Original data were presented on effects of time of incubation of violet red bile (VRB) agar plates at 30 C and the effects of autoclave-sterilization and storage of VRB agar. When boiled VRB agar was used, an incubation period of at least 24 h was required for dairy products; an incubation period of 48 h was required for water, frozen vegetables, and other materials that contained coliforms that were slow in growth initiation. Additional observations should be made after incubation of plates for another 24 h to discover if substantial increases in colony count occurred. If autoclave-sterilized VRB agar was used, the recommended incubation periods should be extended for an additional 12-24 h. The pH of prepared VRB agar should be 7.2. A pH of 6.9 or below was indicative of extensive flaws in the method of preparation and/or storage, although some deterioration occurred in the absence of marked pH changes.

In 1953, 32 and 35 C were designated (1) as optional incubation temperatures for the Standard Plate Count. A temperature of 35 C was retained, however, for incubation of plates and tubes for coliform counts. This situation stimulated Lawton (15) to examine 32 vs 35 C for incubation of violet red bile (VRB) agar plates. A statistical analysis of data obtained on 416 samples revealed that there was "no difference between the count obtained on a sample after incubation (for 24 h) at 32 C or at 35 C." Because fewer than 15% of the samples examined (15) contained appropriate numbers of colonies for valid statistical analyses, the results could be questioned. Nevertheless, the study showed that any differences in coliform count due to incubation temperature were minimal. Therefore, 32 C was adopted for coliform analyses where it has remained through the most recent edition of *Standard Methods* (2).

Consideration now is being given (26) to lower the recommended incubation temperature for the Standard Plate Count (SPC) to 30 C, and the rationale behind this decision has been presented (26). Thus, a situation analogous to that which arose in the past is faced: Can reliable coliform analyses be conducted at 30 C? A survey was made of literature pertinent to 30-C

incubation for coliform analyses of foods, especially dairy products. In making this survey, we attempted to answer three specific questions: (a) Will 30-C incubation result in identical, higher, or lower coliform counts than incubation at other temperatures. (b) Is the coliform flora recovered at 30 C the same as the flora obtained when incubation temperatures of 32 C or higher are used? (c) With incubation at 30 C, will incubation times have to be increased? Thomas (30) published a review over 20 years ago, summarizing studies conducted in Europe, where 30 C has been used (13, 18, 33, 34) with apparent success.

Low-temperature incubation of tubes and plates for coliform analyses was first suggested in the 1930s. Prompted by reports that some coliforms produce gas at 30 C, but not at 37 C (12), Murray (20, 21; *J. Appl. Bacteriol.* 19:v, 1956) examined 30 vs 37 C for coliform analyses of raw and pasteurized milk. Substantially more gas-positive tubes were obtained in MacConkey's broth incubated at 30 than at 37 C. This phenomenon of increased coliform recovery at 30 C (see also 5, 17, 23) was not limited to gas production, as was demonstrated by several authors (11, 28, 32). Violet red bile (VRB) agar plates incubated at 30 C for 24 h yielded significantly higher counts than plates incubated at 37 C (11, 32). The results of these studies, and of additional studies reported in the next paragraph, indicated that lowering the incubation temperature to 30 C should result in similar or higher (but not lower) coliform counts than are obtained at 32, 35, or 37 C. Variabilities of replicate colony counts of milk on countable plates of VRB agar did not exceed those of total colony counts on plate count agar (31). Coliform counts made on VRB agar incubated at 30 C for 20-24 h correlated well with counts in MacConkey's broth incubated for 72 ± 3 h at 30 C (6, 22).

Murray (20, 21) examined the reason for increased recovery of coliforms in MacConkey's broth when 30 C was used as an incubation temperature. Certain isolates were unable to produce gas at 37 C in the selective medium, but did produce gas at 30 C. These isolates were coliforms (see also 36), as were isolates from VRB-agar plates incubated at 30 C (11, 24, 32). When cultures of various identified and named coliforms were

¹Journal Paper No. J-8053 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa; Project No. 2018. Supported by Public Health Service grant FD-00592 from the Food and Drug Administration.

²Present address: Division of Biological Sciences, 105 Tucker Hall, University of Missouri, Columbia, MO 65201.

mixed, inoculated into milk, and incubated at 17, 22, 30, and 37 C (16), "*aerogenes-cloacae*" types usually multiplied more rapidly than did *Escherichia coli* at 17 and 22 C, but *E. coli* outgrew the "*aerogenes-cloacae*" types at 30 and 37 C. A temperature of 30 C was satisfactory for growth of both the mesophilic and psychrotrophic coliform types. Thus, 30 C detected more psychrotrophic coliforms (17, 25, 27, 39) than incubation at higher temperatures, although a relatively small percentage of mesophilic types did not produce positive results at 30 C. Taylor et al. (29) recommended that, "Where a full examination is to be made for all types of coli-aerogenes bacteria," sets of tubes should be incubated at both 30 and 37 C, but a standard temperature of 30 C should be used for all tests unless otherwise stated. MacKenzie (17) observed that a 30-C coliform test of raw milk samples was superior to a 30-C SPC, when compared with the same counts made at 37 C, in detecting general conditions of production on the farm.

Conflicting data are available regarding the time of incubation required at 30 C to achieve near-maximal coliform counts. Definitive studies on the growth rates of coliform bacteria at different temperatures were made as early as 1908 by Barber (3). Generation times of a single strain of *E. coli* in broth were about 36, 30, 25, 22, and 19 min respectively, at temperatures of 27, 30, 32, 35, and 37 C. A number of other investigators (25, 27, 30, 39) obtained similar, but sometimes slower, growth rates when various coliforms were cultured in dairy products. Boniece and Mallmann (4) compared 32, 35, 37, and 39 C for primary isolation of coliforms from water by using lactose broth; 32 and 35 C were superior to the higher temperatures. At both 32 and 35 C, 145 water samples yielded positive tests after 48 h of incubation; after only 24 h of incubation, however, there were 116 positive samples at 32 C and 134 positive samples at 37 C. In Europe, an incubation period of 72 h at 30 C is recommended for MacConkey's broth tubes (13, 18, 33). Moussa et al. (19) obtained many more coliform/*Enterobacteriaceae*-positive results from dehydrated or frozen foods on five different MPN media after an incubation period of 48 h than after 24 h at 30 C. Thus, 24 h is too short an incubation period at 30 C for maximum recovery of coliforms from many types of samples by the MPN method.

The only definitive study with VRB agar was conducted by Thomas and co-worker (32); these investigators counted VRB agar plates of 73 raw milk samples after both 20-24 and 48 h of incubation. An average increase of only 4.6% in counts was obtained by extending the incubation period, which "indicates that the incidence of slow acid forming bacteria is generally low." In England, incubation of VRB agar plates for a period exceeding 24 h is discouraged (18) because it would "allow colonies other than coli-aerogenes to develop." We could not locate data in the literature that would substantiate this claim, and present in the

following sections results of preliminary experiments that suggest that incubation of VRB agar plates at 30 C for 24 h or less is inadequate to obtain near-maximal counts.

MATERIALS AND METHODS

To examine the effects of incubation time on coliform counts at 30 C, samples of cottage cheese, raw milk, surface water, and frozen vegetables were plated on VRB agar. The VRB agar was sterilized in an autoclave at 121 C for 5 min, and the medium was used within 2 weeks by remelting it at 121 C for 5 min. Standard plating procedures (2) were used, except that 1.0- and 10.0-ml pipettes were used to transfer 0.1- and 1.0-ml aliquots into replicate plates. Seven plates of appropriate dilutions were used for each sample. Colony counts were made after incubation for 23-26 h and again after incubation for 30-36, 44-50, and about 72 h at 30 C. The percentage increases in counts were calculated.

To determine whether the increased numbers of coliform-like colonies that were obtained between about 24 and 48 h of incubation were true coliforms, colonies from the cottage cheese, milk, and water samples were verified. Colonies that were not counted after 23-26 h of incubation but were positive by 44-50 h of incubation were selected at random and streaked onto eosin methylene blue (EMB) agar. Except for isolates from water, a colony from each EMB-agar plate was checked for gram reaction and gas production in lactose broth.

Plating efficiencies obtained on freshly-prepared, boiled VRB agar also were compared with batches of medium that had been sterilized for 5, 10, 15, or 30 min; sterilized for 15 min and refrigerated; or sterilized for 15 min and held at room temperature in the light for 1-2 months. Three coliform-positive samples of each of three types of food were plated in quadruplicate. Colonies were counted after 24 ± 2 h and again after 48 ± 4 h. Counts obtained on the boiled medium were set at 100%, and mean counts obtained on the other treatments were adjusted to relative percentage values. Because of the results obtained in other studies (9), the pH values of the media also were determined at the time of use.

RESULTS

Examples of the effect of incubation time on coliform counts at 30 C are presented in Table 1 to illustrate the

TABLE 1. Examples of increased coliform counts that were obtained when VRB agar plates were incubated at 30 C for more than 24 h^a

Type of material	Sample number	Percentage increase in counts			Total
		30-36 h	44-50 h	72 h	
Cottage cheese	1	5.9	0.4	b	6.3
	2	4.3	0.1	b	4.4
	3	8.1	3.2	b	11.3
Raw milk	1	4.6	3.9	0.9	8.4
	2	11.9	10.1	2.1	24.1
	3	18.6	11.3	1.8	32.7
Water	1	16.9	12.5	24.2	53.6
	2	19.2	21.6	14.3	55.1
	3	19.8	13.2	40.0	73.0
Vegetables	1	c	46.3	4.9	51.2
	2	c	70.0	1.7	71.7
	3	c	79.5	1.2	80.7

^aThe results are expressed as percentage increase in counts experienced at three incubation times, compared with the preceding incubation time; the 30-36 h counts were based on counts obtained at 23-26 h. Three examples are given for each type of material that was examined.

^bThe plates were overgrown with lactic acid bacteria after incubation for 72 h.

^cPlates were not counted at 23-26 h because colonies were too small; the percentage increases in counts at 44-50 h were based on counts made at 30-36 h.

variety of data that were obtained. As was expected, there was considerable variation between samples of the same type in the times of appearance of positive colonies.

Furthermore, substantial differences were observed in the times of appearance of positive colonies, depending on the type of material that was examined. When 22 cottage cheese samples were examined (data for three samples are shown in the table), the mean increase in counts was 6.2% after 30-36 h and an additional mean increase of 3.8% was obtained after incubation for 44-50 h. The total mean increase was 10.0%. VRB-agar plates of cottage cheese samples were overgrown with lactic acid bacteria after incubation for about 72 h. Coliform counts of 11 raw milk samples increased an average of 11.8% between 23-26 and 30-36 h, an additional 10.2% after 44-50 h and another 1.5% by 72 h of incubation; the total mean increase was 23.5%. Some colonies from water samples developed very slowly on VRB agar. Only three samples were examined in detail; mean increases of 18.6, 15.8, and 26.2% were obtained after the specified incubation periods. The total increase in coliform counts after incubation for 72 h was 60.6% over counts obtained at 23-26 h. The most interesting observation on the water samples was that an almost continuous increase in countable colonies was obtained during continued incubation of VRB agar plates. When 11 samples of frozen vegetables were examined, however, most of the increases in counts were obtained between 30-36 and 44-50 h of incubation. Many colonies were too small to count after incubation for 23-26 h, but a 69.9% increase in counts was obtained by 44-50 h, compared to counts made at 30-36 h; an additional 2.6% mean increase in counts was obtained after incubation for 72 h.

Of 50 positive colonies (2) from cottage cheese samples that were examined, 100% were coliforms, and at least 92% of 50 colonies from milk and 96% of 50 colonies from water were coliforms. Thus, most of the colonies that arose after extended incubation at 30 C were coliforms, as defined in Materials and Methods.

Data obtained on three samples each of cottage

cheese, raw milk, and frozen vegetables and plated with VRB agar exposed to various treatments are shown in Table 2. Autoclave-sterilization of VRB agar often resulted in decreased yields and retardation of colony formation of coliforms from dairy products. Increased counts frequently were obtained by delaying final enumeration of plates until incubation had proceeded for 48 h, especially if the VRB agar had been sterilized in the autoclave. Only with samples of frozen vegetables did autoclave-sterilization of VRB agar often result in increased, rather than decreased, counts (see also references 7 and 8). These data, and other experiments not reported here, indicate that autoclave-sterilization of VRB agar results in decreased recoveries of coliforms from some foods. Oversterilization, or extended storage of sterilized VRB agar (Table 2), can result in significant decreases in recovery.

DISCUSSION

As stated in the literature review, 30-C incubation of coliform tests should result in similar or higher counts than are obtained at temperatures of 32 C or higher. Furthermore, coliform flora that are isolated from specific environmental samples are fairly similar at incubation temperatures within the range of 30-37 C. Increased counts, if obtained at 30 C, are primarily a result of increased recovery of psychotrophic coliforms. Only a few mesophilic strains that produce gas in broth or acid in agar at 37 C fail to do so at 30 C.

During the studies reported herein, we did not notice that incubation of VRB-agar plates for a limited period exceeding 24 h resulted in growth of large numbers of bacteria other than coliforms, as has been claimed (18). Colonies could be counted on all plates of the various types of samples examined after 48 h of incubation at 30 C. With the exception of cottage cheese samples, incubation for up to 72 h at 30 C did not result in overgrowth by noncoliforms.

Our data also differ somewhat from those reported by Thomas et al. (32). They obtained an average increase of only 4.6% when plates of raw milk samples were incubated for 48 h, rather than 20-24 h, at 30 C. We obtained increases in counts of 10% and 22% when VRB agar plates of cottage cheese and raw milk samples, respectively, were incubated for an additional 24 h.

Our discovery that coliforms in water develop slowly on VRB agar is in agreement with the statement of Thomas et al. (35) that, when VRB agar was inoculated with soil suspensions, coli-aerogenes colonies developed more slowly than when milk and milk products were examined. Water and soil types might be expected to have similar growth rates. They recommended (35) incubation for 48 h at 30 or 37 C for enumeration of soil types on VRB agar. We obtained substantial increases in positive colonies when incubation at 30 C was extended to 72 h (Table 1).

We recommend incubation of plates of freshly boiled VRB agar at 30 C for the following periods: (a) Dairy

TABLE 2. pH values and percentage recoveries^a of coliforms after incubation for 24 and 48 h at 30 C on VRB agar that was prepared in different ways

Treatment	pH ^b	Cottage cheese		Raw milk		Frozen vegetables	
		24 h	48 h	24 h	48 h	24 h	48 h
Freshly boiled	7.2	100	100	100	100	100	100
Autoclaved 5 min	7.2	85	86	98	102	112	131
Autoclaved 10 min	7.0	81	83	85	92	92	100
Autoclaved 15 min	7.1	89	93	81	96	116	131
Autoclaved 30 min	6.9	93	93	79	92	98	108
Stored in cold	6.8	71	75	56	66	70	80
Stored room temp	6.8	85	88	67	80	69	84

^aThree samples of each type of food were plated by using four plates per dilution per treatment. Counts obtained on freshly boiled VRB agar were set at 100%, counts obtained on VRB agar exposed to other treatments were adjusted on a percentage basis, and then the mean percentages for the three samples were calculated.

^bpH values were recorded on each medium immediately before use.

products require an incubation period of at least 24 h, with an additional observation at 48 h to discover if a substantial increase in colony count occurred. The 20-h minimum incubation period in some recommendations (13, 18) is insufficient, unless the VRB agar is being used for confirmation. (b) Samples of water, soil, and frozen vegetables require an incubation period of at least 48 h, with an additional observation at 72 h to discover if a substantial increase in colony count occurred. When autoclave-sterilized VRB agar is used at 30 C, the minimum recommended incubation periods should be extended by 12-24 h. Our suggestions for extension of incubation times for coliform tests at 30 C parallel suggestions made to extend the incubation times when 30-C methylene blue reduction (37) and resazurin (38) tests are made on dairy products.

Jensen and Hausler (14) recently reported that discrepancies in counts were obtained when comparisons were made between boiled and autoclaved VRB agars; plates were incubated at 32 C for 18-24 h. They recommended use of only boiled agar. As shown in the present study, extension of the incubation period might have reduced the discrepancies observed by Jensen and Hausler (14); however, their conclusion is still valid. The use of violet red bile 2 (VRB-2) agar, which can be sterilized in the autoclave and is satisfactory for the recovery of stressed coliforms (10), might be a logical alternative. VRB-2 agar also effected large increases in coliform counts of surface water samples (10), probably because large proportions of the coliforms in these environments are slow in colony development (Table 1).

Periodic pH checks should be made of VRB agar before use. pH values of 6.9 or below are definitely indicative of deterioration of the medium, although some deterioration can occur in the absence of marked pH changes (Table 2). Oversterilization and/or extended storage of prepared medium, even in the refrigerator, should be avoided. VRB agar in dehydrated form is stable for years, however, if kept dry (9).

The results reported herein are preliminary, and the recommendations are tentative. Confirmation by other investigators is needed.

ACKNOWLEDGMENT

We thank Dr. C. K. Johns for pointing out some deficiencies in the original manuscript and for many helpful suggestions.

REFERENCES

1. American Public Health Association. 1953. Standard methods for the examination of dairy products, 10th ed. American Public Health Assoc., New York.
2. American Public Health Association. 1972. Standard methods for the examination of dairy products, 13th ed. American Public Health Assoc., Washington, D.C.
3. Barber, M. A. 1908. The rate of multiplication of *Bacillus coli* at different temperatures. *J. Infect. Dis.* 5:379-400.
4. Boniece, J. R., and W. L. Mallmann. 1950. The optimum incubation temperature for the primary isolation of coliform organisms. *J. Am. Water Works Assoc.* 42:155-160.
5. Crossley, E. C. 1946. The coliform flora of milk and dairy products. *J. Dairy Res.* 14:233-282.
6. Druce, R. G., N. B. Bebbington, K. Elson, J. M. Harcombe, and S. B. Thomas. 1957. The determination of the coli-aerogenes content of milk and dairy equipment by plating on violet red bile agar incubated at 30°. *J. Appl. Bacteriol.* 20:1-10.
7. Hartman, P. A. 1958. The selectivity of autoclave-sterilized violet red bile agar. *Food Res.* 23:532-535.
8. Hartman, P. A. 1960. Further studies on the selectivity of violet red bile agar. *J. Milk Food Technol.* 23:45-48.
9. Hartman, P. A., P. S. Hartman, and K. T. Frey. 1975. Longevity of dehydrated violet red bile agar. *Appl. Microbiol.* 29:295-296.
10. Hartman, P. A., P. S. Hartman, and W. W. Lanz. 1975. Violet red bile 2 agar for stressed coliforms. *Appl. Microbiol.* 29:537-539, 865.
11. Higginbottom, C. 1962. Bacteriological studies on raw milk before and after the adoption of bulk milk cooling on the farm. *Proc. 16th Intl. Dairy Congr. A (Sect. I-2):*426-432.
12. Hiscox, E. R. 1934. A coliform organism isolated from milk. The effect of temperature on the fermentation of lactose. *J. Dairy Res.* 5:233-235.
13. International Dairy Federation. 1966. Standard routine method for the count of coliform bacteria in raw milk. *Intl. Std. FIL-IDF39*, Brussels.
14. Jensen, J. P., and W. J. Hausler, Jr. 1975. Productivity of boiled and autoclaved violet red bile agar. *J. Milk Food Technol.* 38:279-280.
15. Lawton, W. C. 1955. A comparison of 32°C. and 35°C. as incubation temperatures for the coliform count of milk and cream. *J. Milk Food Technol.* 18:288-289.
16. Malcolm, J. F. 1939. The enrichment of *aerogenes-cloacae* types in milk held at low temperature: With observations on the relative rates of growth of *aerogenes-cloacae* and *B. coli* types in milk at different temperatures. *J. Dairy Res.* 10:410-425.
17. McKenzie, D. A. 1952. Observations on milk testing standards in Scotland. *Dairy Indust.* 17:1098-1100.
18. Ministry of Agriculture, Fisheries and Food. 1968. Bacteriological techniques for dairy purposes. Her Majesty's Stationery Office, London.
19. Moussa, R. S., N. Keller, G. Curiat, and J. C. deMan. 1973. Comparison of five media for the isolation of coliform organisms from dehydrated and deep frozen foods. *J. Appl. Bacteriol.* 36:619-624.
20. Murray, J. G. 1953. A comparison of 30° and 37° as incubation temperatures in the presumptive coli-aerogenes test for raw and pasteurized milk. *J. Appl. Bacteriol.* 16:24-26.
21. Murray, J. G. 1959. Incubation temperature in the presumptive test for coli-aerogenes group in milk. *Proc. 15th Intl. Dairy Congr.* 3(Sect. 5):1345-1348.
22. Orr, M.J., R.M. McLarty, M.E. McCance and S. Baines. 1964. Alternate day collection of bulk milk. *Dairy Indust.* 29:169-173.
23. Oxley, C. D. 1938. Observations on the occurrence of milk samples yielding a positive reaction for acid and gas in bile-salt lactose broth when incubated at 30°C. *Proc. Soc. Appl. Bacteriol.*, pp. 15-16.
24. Panes, J. J., and S. B. Thomas. 1959. The multiplication of coli-aerogenes bacteria in milk stored at 3-5°. *J. Appl. Bacteriol.* 22:272-277.
25. Panes, J.J., and S.B. Thomas. 1968. Psychrotrophic coli-aerogenes bacteria in refrigerated milk: A review. *J. Appl. Bacteriol.* 31:420-425.
26. Roughley, F. R., C. K. Johns, and K. L. Smith. 1974. The influence of time and temperature of incubation on the plate count of milk. *J. Milk Food Technol.* 37:209-212.
27. Skelton, W. R., and L. G. Harmon. 1964. Growth rate of coliform organisms in cottage cheese and reconstituted nonfat dry milk. *J. Milk Food Technol.* 27:197-201.
28. Smillie, D. M., M. J. Orr, and R. M. McLarty. 1958. Bulk milk collection in Wigtownshire. A survey of the bacteriological results during the first year of operation. Part II. *Dairy Indust.* 23:329-333.
29. Taylor, C. B., L. F. L. Clegg, W. J. Dowson, E. R. Hiscox, and T.

- Richards. 1949. The classification of the coli-aerogenes bacteria. Report of the Coliform Sub-Committee. Proc. Soc. Appl. Bacteriol. 2:3-16.
30. Thomas, S. B. 1955. Coli-aerogenes bacteria in raw milk. J. Appl. Bacteriol. 18:331-357.
31. Thomas, S. B., D. L. Bowen, and N. B. Bebbington. 1958. Further investigations on the coli-aerogenes colony count at 30° C. Dairy Indust. 23:30-33.
32. Thomas, S. B., D. L. Bowen, N. B. Bebbington, E. Mackenzie, and M. Beavan. 1957. Examination of milk by the coli-aerogenes test on violet-red bile agar at 30°. Dairy Indust. 22:831-833.
33. Thomas, S. B., and R. G. Druce. 1972. The incidence and significance of coli-aerogenes bacteria in refrigerated bulk collected milk. Dairy Indust. 37:593-598.
34. Thomas, S. B., R. G. Druce, A. Davis, and J. S. Bear. 1966. Bacteriological aspects of bulk milk collection. J. Soc. Dairy Technol. 19:161-169.
35. Thomas, S. B., R. G. Druce, and K. Elson. 1960. An ecological study of the coli-aerogenes bacteria of surface soil. J. Appl. Bacteriol. 23:169-184.
36. Thomas, S. B., P. M. Hobson, and K. Elson. 1959. Coli-aerogenes bacteria from farm dairy equipment and raw milk. J. Appl. Bacteriol. 21:58-64.
37. Thomas, S. B., and B. F. Thomas. 1973. The development of dye reduction tests for the bacteriological grading of raw milk-Part I. Methylene blue reduction test (2). Dairy Indust. 38:425-427, 429.
38. Thomas, S. B., and B. F. Thomas. 1974. The development of dye reduction tests for the bacteriological grading of raw milk-Part 2. Resazurin test (3). Dairy Indust. 39:113-116.
39. Witter, L. D. 1961. Psychrophilic bacteria—a review. J. Dairy Sci. 64:983-1015.

Identification and Quantification of Fecal Coliforms using Violet Red Bile Agar at Elevated Temperature

HARVEY KLEIN and DANIEL Y. C. FUNG

Department of Microbiology
The Pennsylvania State University, University Park,
Pennsylvania 16802

(Received for publication March 17, 1976)

ABSTRACT

A procedure was developed for identification and quantification of fecal coliforms by colony size obtained in Violet Red Bile agar incubated at 44.5 ± 0.5 C for 24 h in an air incubator. Five strains of *Escherichia coli*, 5 strains of *Enterobacter aerogenes*, 37 coliform isolates from sewage, and 76 coliform isolates from water were tested by this procedure. The practical usefulness of this procedure was studied by comparative quantification of 11 water samples using this procedure, the Millipore method, and MPN method. Statistical analysis showed that this procedure is as reliable as the other procedures for enumeration of fecal coliforms. The advantages of this method over the commonly used methods of enumeration of fecal coliforms are ease of operation, simplicity, and speed.

The presence of fecal coliforms in water and food has been shown to indicate the presence of fecal contamination (3). At present techniques accepted by the American Public Health Association for detection of fecal coliforms in water are the E.C. medium method and the membrane fecal coliform test, and various biochemical tests such as the IMViC tests (2). Huffman recently showed that the membrane fecal coliform test is not reliable for enumeration of *Escherichia coli* (4), bacteria which make up as much as 90% of the fecal coliform group (3). The E.C. medium technique requires 48 h to complete. To obtain quantitative results using the E.C. medium method, the multiple tube, most probably number (MPN) technique must be used. This procedure has the disadvantage of not always giving results as accurate as the pour plate method at high organism densities (1). Also the procedure is relatively cumbersome to use. Another drawback to both the membrane filtration and the E.C. medium methods is the demand of very precise temperature of incubation (44.5 ± 0.2 C), which requires expensive water baths with precision controls. In the literature several different temperatures have been cited (2, 5) for growth and enumeration of fecal coliforms, all in the 43.0 to 45.5 C range.

Violet Red Bile (VRB) agar has been used to enumerate coliforms from dairy products at 32 C with no attempt to separate fecal from nonfecal coliforms (1). *Standard Methods for the Examination of Dairy Products* states that on VRB agar both fecal and nonfecal coliforms yield colonies that are 0.5 mm or

larger when incubated at 32 C; however, M. Klein (President, Garden State Lab., Irvington, New Jersey, personal communication) showed that only fecal coliforms will yield colonies that are 0.5 mm or larger usually with a halo of 1-2 mm in diameter when incubated at 44.5 C after 24 h of incubation. This paper describes a more detailed investigation on the validity of using colony size differences to separate fecal from non-fecal coliforms incubated at 44.5 C after 24 h using VRB agar (ETVRB test-Elevated Temperature Violet Red Bile Agar test).

MATERIALS AND METHODS

Test organisms and samples

Five strains of *Escherichia coli* (ATCC 12701, 12795, 12805, 12807, and 12810) were obtained from the culture collection of the Department of Microbiology, The Pennsylvania State University. Five strains of *Enterobacter aerogenes* (#153, 196, 207, 239, and 247) were kindly supplied by Dr. S. D. Kominos, Mercy Hospital, Pittsburgh, PA. All organisms were grown overnight in nutrient broth (Difco) at 37 C. All organisms when streaked on Eosine Methylene Blue agar (EMB, Difco) proved to be pure and gave typical reactions.

To obtain a mixed population of fecal and non-fecal coliforms, the overnight cultures of five strains of *E. coli* and the five strains of *E. aerogenes* were mixed and diluted to 10^{-6} . One ml of this diluted mixture was delivered into a standard petri dish after which 12-14 ml of sterile tempered (45 C) VRB agar (Difco) was poured into the plate and then an overlay of 2 to 4 ml was poured. In each set of experiments four plates were prepared; two were incubated at 37 C and the other two at 44.5 C in an air incubator for 24 h. Temperature fluctuation in the air incubator was about ± 0.5 C of the set temperature. Dark red colonies over 0.5 mm in diameter on plates held at 37 C were interpreted as positive for coliform organisms. For plates held at 44.5, dark red colonies larger than 0.5 mm with a zone of 1 mm around them were first identified as fecal or non-fecal coliforms by colony size (Fig. 1) then streaked on EMB agar and incubated for 24 h at 37 C to confirm as either fecal coliform (*E. coli*) or non-fecal coliform (*E. aerogenes*). Colonies from the 37-C plates were also streaked to obtain the total number of *E. coli* and *E. aerogenes*. It is not possible to differentiate the two groups by size at 37-C incubation.

Coliform isolates were also obtained from different locations of the local sewage treatment plant by use of VRB agar and incubation temperatures of 37 and 44.5 C. Those colonies suspected as coliforms and/or fecal coliforms were confirmed by re-streaking on EMB agar and incubated at 37 C. A total of 37 such isolates were evaluated. In a similar study of fecal coliforms in water in samples from swimming pools and wells in the New Jersey area, 76 colonies were isolated and tested on EMB agar for confirmation.

To evaluate the ETVRB test for counting fecal coliforms, 11 water

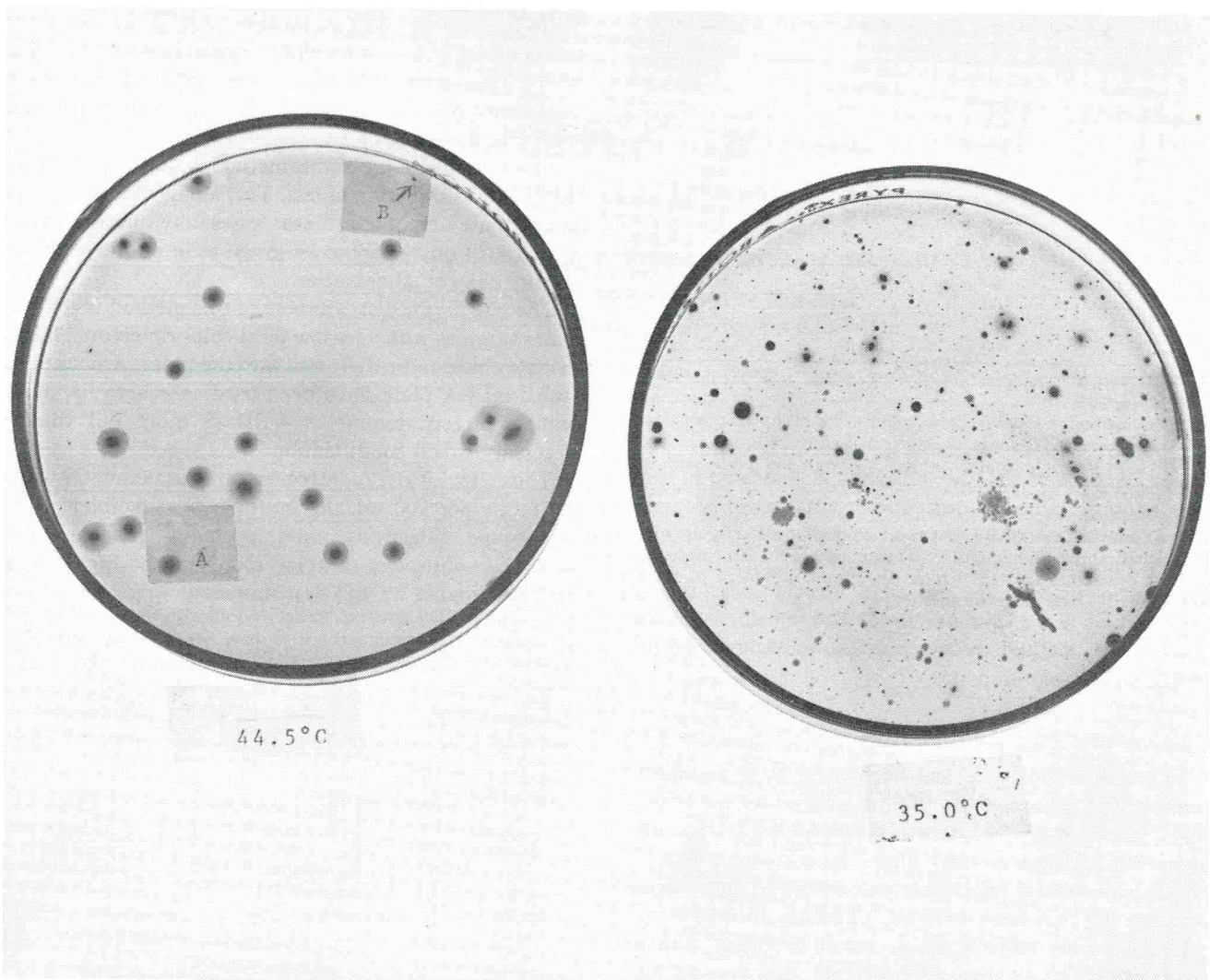


Figure 1. *Fecal coliform and non-fecal coliform on VRB plates. Fecal coliforms formed larger colonies with a halo (A) while non-fecal coliforms formed smaller colonies (B) and no halo on the VRB plate incubated at 44.5 C. On the VRB plate incubated at 35 C, fecal and non-fecal coliform cannot be differentiated according to size.*

samples were tested using this procedure and the standard Millipore method (2). The 5-tube, three dilution series (10 ml, 1 ml, and 0.1 ml) MPN method using the E.C. medium at 44.5 C was also done on five of the above samples. Confirmation tests were done by streaking liquid from turbid tubes on EMB agar.

RESULTS

Table 1 shows the percentage accuracy of the ETVRB test in identification of fecal coliform. Of the 322 colonies designated as fecal coliforms on the ETVRB test 318 were confirmed to be fecal coliforms on EMB agar, giving an overall percentage accuracy of 98.8%. Of the 144 colonies designated as non-fecal coliforms on ETVRB test, 142 were confirmed as such in EMB, giving an overall percentage accuracy of 98.6%. These data showed that the ETVRB procedure is highly reliable as a one-step identification of fecal-coliforms from the primary isolation plate.

The acceptability of this method in enumerating fecal coliforms in water samples is presented in Table 2. Statistical analysis showed that the correlation

TABLE 1. *Identification and confirmation of coliforms using elevated incubation of Violet Red Bile agar*

Colonies on VRB agar for 24 h at 44.5 C	Identified ^a	Confirmed	% Accuracy
<i>Laboratory cultures</i>			
Fecal coliforms	216	215	99.5
Non-fecal coliforms	137	136	99.2
<i>Sewage water</i>			
Fecal coliforms	30	30	100.0
Non-fecal coliforms	7	6	85.7
<i>Water samples</i>			
Fecal coliforms	76	73	96.1

^aIdentification: Dark red colonies >0.5 mm diameter with halo = fecal coliforms

Dark red colonies <0.5 mm diameter = non-fecal coliforms

Confirmation: Metallic green sheen on EMB = fecal coliforms

No metallic green sheen on EMB = non-fecal coliforms

coefficient of this method and the Millipore method is 0.87, which is significant at the 1% level (6). Analysis of variance of all three methods on the five samples showed that the variation is not significant at 1% level indicating

TABLE 2. Quantification of fecal coliforms in water samples by the ETVRB Millipore, and MPN methods^c

Water sample (No.)	ETVRB ^a	Millipore method	MPN method
1	160	150	220
2	500	180	140
3	230	117	348
4	180	140	348
5	300	240	348
6	9100	9500	ND ^b
7	7100	6400	ND
8	60	30	ND
9	140	90	ND
10	420	150	ND
11	960	670	ND

^aETVRB = elevated temperature Violet Red Vile agar, incubated at 44.5 C for 24 h.

Millipore method = Fecal coliform count using Millipore equipment for membrane filtration; incubated at 44.5 C for 24 h.

MPN = Most Probable Number using E. C. broth and 5 tube system; incubated at 44.5 C for 24 h.

^bND = Not determined

^cCorrelation coefficient of the first two methods for all 11 samples is 0.87. Significant at 1% level. Analysis of variance of the 3 methods for the first 5 samples showed that no significant difference of these methods at 1% level of significance (6).

that all three methods provided the same bacterial count within acceptable limits (6).

DISCUSSION

Violet Red Bile agar has been used as a standard medium for detection of coliform organisms for many years in the dairy industry (1). Initial research work demonstrated that if VRB plates were held at 44.5 C non-fecal coliform organisms did not grow into large colonies and that fecal coliform organisms formed large colonies usually more than 1.0 mm in diameter with a large, usually more than 3.0-mm wide halo around the colony. The zone is indicative of lactose fermentation, a requirement of fecal coliform organisms. A small number of organisms in the 0.5 to 1.0-mm range gave a green metallic sheen on EMB but all had a large 2.0-mm zone around them.

One of the advantages of this method is that no special apparatus other than an air incubator set at 44.5 C is needed. This eliminates the need for filters, vacuum pumps, numerous test tubes, and the precision water

baths required in other fecal coliform tests. Although the temperature of the air incubator varied between 44.0 and 45.0 C, no noticeable effect was observed; non-fecal organisms never formed colonies larger than 0.5 mm with a large halo.

Another advantage of this method is that only 24 h are needed for completion of test. This along with the simple pour plate technique are especially useful when numerous samples are to be tested as in public health or quality control laboratories.

The one major disadvantage of this method is that for water samples with very low fecal coliform counts, larger samples have to be delivered into the plate. Amounts up to 5.0 ml per plate have been used satisfactorily, along with a larger amount of VRB (5 more ml) that is necessary to aid solidification.

Thus, for water samples with less than 20 fecal coliforms per 100 ml, the multiple tube method or the membrane filtration would probably provide more accurate results. For samples with expected higher fecal coliform counts we feel that this method is superior.

ACKNOWLEDGMENT

We are greatly indebted to Mathew Klein, Garden State Laboratories, Irvington, New Jersey, for the preliminary research as well as subsequent suggestions and discussions concerning this method.

REFERENCES

1. American Public Health Association. 1972. Standard methods for the examination of dairy products, 13th ed. American Public Health Association Inc., Washington, D.C.
2. American Public Health Association. 1971. Standard methods for the examination of water and wastewater, 13th ed. American Public Health Association Inc., Washington, D.C.
3. Geldreich, E. E., and R. H. Bordner. 1971. Fecal contamination of fruits and vegetables during cultivation and processing for market. A review. J. Milk Food Technol. 34:184-195.
4. Hufman, J. B. 1974. Evaluating the membrane fecal coliform test by using *Escherichia coli* as the indicator organism. Appl. Microbiol. 27:771-776.
5. National Research Council, Sub-committee on Food Microbiology. 1971. Reference methods for the microbial examination of foods. National Academy of Sciences.
6. Snedecor, G. W., and W. G. Cochran. 1967. Statistical methods, 6th ed. The Iowa State University Press, Ames, Iowa.

Cooking Inoculated Pork in Microwave and Conventional Ovens¹

H. W. OCKERMAN, V. R. CAHILL, R. F. PLIMPTON and N. A. PARRETT

The Ohio State University, Columbus, Ohio 43210 and Ohio
 Agricultural Research and Development Center, Wooster, Ohio 44691

(Received for publication April 1, 1976)

ABSTRACT

High quality (grayish-pink, firm, and dry) and pale, soft, and exudative (P.S.E.), aseptic pork muscle tissue was individually inoculated with *Bacillus subtilis*, *Leuconostoc mesenteroides*, and *Pseudomonas putrefaciens*. These organisms were allowed to grow (48 h) in the pork tissue. The tissue was cooked by microwave and by conventional oven heating until internal temperatures of 60, 68, 77, 85 C were reached. Pork quality did not significantly affect bacterial destruction in this experiment. *B. subtilis* proved to be the most heat tolerant, *L. mesenteroides* intermediate, and *P. putrefaciens* the least in both cooking methods. Cooking temperature significantly reduced bacterial numbers and oven cooking was more effective at reducing percent survival than microwave for all three species.

In today's society consumers and the food industry are interested in methods that will reduce the time necessary to prepare food. Microwave cooking is one approach to this accelerated preparation. When microwave cooking is used to replace conventional cooking and the product is observed and tasted, the flavor, color, tenderness, and final product acceptability are altered. One of the main reasons for cooking is to destroy microorganisms that may be present in the product. It is generally agreed that destruction of microorganisms treated with microwave energy is primarily due to the time-thermal effects (6, 10, 11, 14). If this is true, cooking meat to the same internal temperature with microwave would destroy fewer microorganisms than conventional cooking due to the reduced time effect. To help evaluate this problem some research workers (18, 19) have suggested multi-stage cooking of meat products. However, other research workers (9) have indicated microbial destruction by factors other than thermal energy, when the product is cooked with microwaves. The question of microwave microbiological destruction compared with conventional cooking has not been explored to any great depth using food products which cook at different rates. The first objective of this research was to determine whether any microbiological difference existed between microwave and conventional cooking when a meat product was used as a substrate for selected microorganisms.

Pork quality and particularly the condition known as

pale, soft, and exudative (PSE) tissue has received a great deal of biochemical attention but little research has been directed toward the relationship of muscle quality and microbiological protection (or lack of) during cooking.

The second objective of this research was to investigate the microbiological behavior of selected strains of microorganisms with both methods of cooking using different qualities of tissue as the substrate.

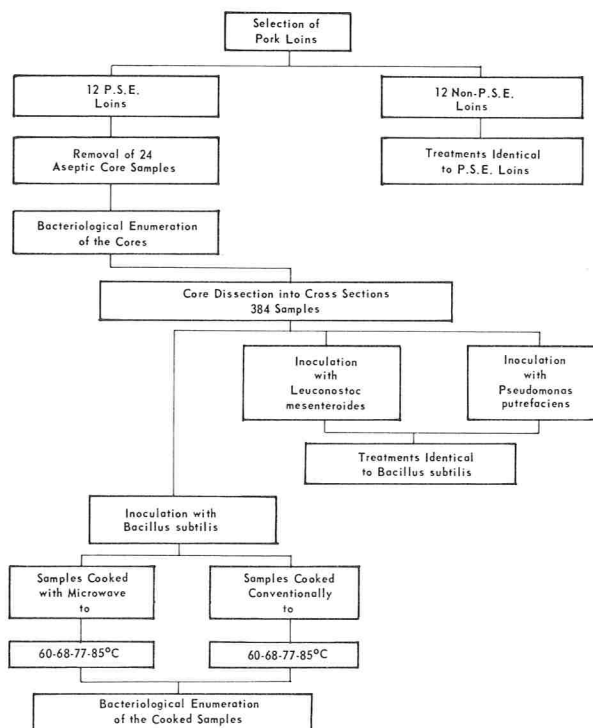


Figure 1. Schematic diagram of experimental design.

MATERIALS AND METHODS

Figure 1 displays a schematic diagram of the experimental design. Twelve commercial loins which were firm, had a grayish-pink color, were free from bruises and defects, and had an unbroken pleura membrane were selected as non-P.S.E. (Pale, Soft, Exudative) loins. Twelve loins which were pale in color, soft, and exudative but which were otherwise free of defects were selected as P.S.E. loins. *Bacillus subtilis*, *Leuconostoc mesenteroides*, and *Pseudomonas putrefaciens* were selected as test organisms because they have been isolated from meat products and soil and represent a range in growth temperature as

¹Approved for publication as Journal Article No. 44-76 of the Ohio Agricultural Research and Development Center, Wooster, Ohio 44691.

well as physical and biochemical characteristics.

The loins were "aseptically" sampled by a coring technique described by Hone (13). Air movement was reduced to a minimum, sampling equipment was sterilized, and the work table surface was sanitized. An external slice was removed with sterile equipment and the coring device was inserted into the loin. The 50 ± 10 -g tissue sample was then stored in a sterile container until dissected. The samples were aseptically cut into 10 portions and the end sections were used to determine unintentional microbial contamination. Each remaining cross section was placed in a sterile petri dish, weighed, and inoculated with 1/10 ml of the inoculum of the appropriate organism (*B. subtilis* or *L. mesenteroides* or *P. putrefaciens*). Approximately 3/4 of the inoculum was injected into the sample and 1/4 placed on the surface. The samples were then stored at 3-5 C to allow microbial growth for 48 ± 2 h.

Samples were cooked in the petri dish containers to internal temperatures of 60, 68, 77, and 85 C. The conventional oven was maintained at 121 ± 12 C and the internal temperature of microwave cooked samples rose at the rate of 4.5 C per sec.

Microbiological analyses were determined on inoculum, on samples before cooking, and on samples after completion of cooking. The pour plate technique with Tryptone Glucose Extract Agar (Difco) was used and *B. subtilis* samples were incubated at 37 ± 1 C for 48 h and *L. mesenteroides* and *P. putrefaciens* were incubated at 22 ± 2 C for 48 h (5, 7). These recommended incubation temperatures are within the optimum range listed for these microorganisms.

The microbiological counts per gram were transformed to logarithms (a zero count was considered as one) and calculations and analysis (12) were conducted on the transformed data.

RESULTS AND DISCUSSION

Using this core technique 35% of the samples were sterile, 64% contained less than 5 organisms/g and the maximum number found in any of the 48 cores was 106 organisms/g. This contamination level appears higher than the isolator techniques (4, 16, 17) but lower than the sterile surgical technique (3). The level of microbiological contamination found for the core samples supports the research reports (1, 15, 16, 20) that most muscle tissue is

sterile or relatively free of microorganisms until contaminated postmortem. There was no initial significant difference in bacterial numbers between the PSE and non-PSE samples.

The tissue samples were inoculated with an average of 4.9×10^7 *B. subtilis*, 4.8×10^7 *L. mesenteroides* and 7.0×10^7 *P. putrefaciens* organisms/ml. These grew in tissue to an average of 1.7×10^8 , 5.8×10^7 , 8.3×10^7 organisms/g, respectively. There was no significant difference in the increase in number of microorganisms between PSE and non-PSE loins.

The microbiological destruction due to cooking is graphed in Fig. 2. There was no significant difference in the destruction rate of microorganisms in PSE and non-PSE loins. This may have been due to the very large number of microorganisms destroyed by cooking making the influence of PSE very negligible. As would be expected, there is a significant ($P < .01$) difference in microbiological numbers when compared at the three internal cooking temperatures. This significance was evident when all three species were evaluated independently.

When conventional cooking was compared with microwave cooking, there was less destruction of microorganisms with the microwave method for all three species at each final internal temperature and it is suggested that this is due to the drastically reduced time factor. The longest time required for a microwave sample to reach 85 C was 24 sec in contrast to the shortest conventional samples that required 720 sec to reach 60 C. These results are in agreement with reported (2) ham cooking data indicating that after cooking and storage microwave treated samples had a much higher microbiological count than conventionally cooked hams. It should be pointed out that the type of sample and consequently the heating rate can have a tremendous influence on the conclusions obtained. The results of this investigation do not agree with research when microorganisms were heated in a liquid suspension (10) and suggested that the two cooking techniques are equally effective. However, due to the high specific heat of water and the smaller samples used, the time factor was quite similar in the research samples which were heated in a small quantity of liquid (10).

When the three test species were compared using microwave cooking, particularly at the higher final temperatures, *B. subtilis* was the most resistant to thermal death, *L. mesenteroides* intermediate in resistance, and *P. putrefaciens* least resistant. Due to the lack of difference at the two lower microwave cooking temperatures, counts for *B. subtilis* and *L. mesenteroides* were not significantly different using this method of cooking.

The same order of resistance with all microorganisms tested was maintained when conventional cooking was analyzed. This was also true when a combined analysis was used. The higher survival rate of *B. subtilis* over the other two species is supported by the research of

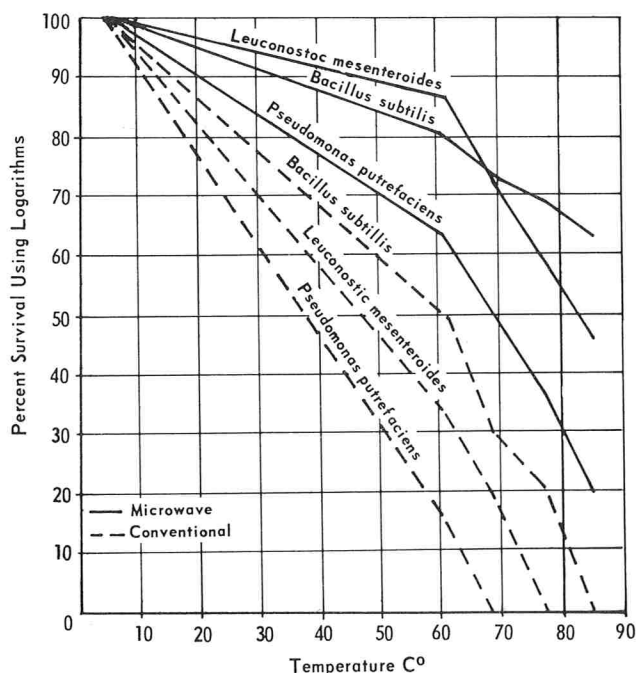


Figure 2. Survival rate for *Leuconostoc mesenteroides*, *Bacillus subtilis*, and *Pseudomonas putrefaciens* during cooking by microwave and conventional methods.

Goldblith and Wang (11). The order of survival also agrees with the maximum growth temperatures of: *B. subtilis*, 50 C; *L. mesenteroides*, 42 C; and *P. putrefaciens*, 37 C as reported by Breed et al. (5). In a more recent edition (8) Buchanan et al. reported the maximum growth temperature of *L. mesenteroides* to be only 37 C but indicated that slimy cultures could withstand much higher temperatures.

This research suggests that microwave cooking to an internal temperature end point is not as effective as conventional cooking when comparing microbiological destruction within meat tissue. It should be pointed out, however, that in this study abnormally high numbers (average 1.0×10^9) of microorganisms were distributed throughout the sample before cooking and when a less drastic load is used the thermal destruction due to microwave cooking may be adequate.

REFERENCES

1. Ayres, J. C. 1956. The source and significance of microorganisms on meats. Proc. Reciprocal Meat Conference 9:164-173.
2. Bengtsson, N. W., and W. Green. 1970. Radio-frequency pasteurization of cured hams. J. Food Sci. 35:681-687.
3. Borton, R. J. 1969. The effects of four species of bacteria on some properties of porcine muscle protein. Ph.D. Thesis, Michigan State University, East Lansing, Michigan.
4. Bothast, R. J., H. W. Ockerman, and V. R. Cahill. 1968. Improved procedures for meat sanitation. J. Milk and Food Technol. 31:340-343.
5. Breed, R. S., E. G. D. Murray, and N. R. Smith. 1957. Bergey's manual of determinative bacteriology. 7th ed. Williams and Wilkins Co., Baltimore, Maryland.
6. Brown, G. H., and W. C. Morrison. 1954. An exploration of the effects of strong radio-frequency fields on microorganisms in aqueous solution. Food Technol. 8:361-366.
7. Bryan, A. H., C. A. Bryan, and C. G. Bryan. 1968. Bacteriology-principles and practice. Barnes and Noble. N.Y.
8. Buchanan, R. E., and N. W. Gibbons. 1974. Bergey's manual of Determinative bacteriology. 8th ed. Williams and Wilkins Co., Baltimore, Maryland.
9. Carroll, D. E., and A. Lopey. 1969. Lethality of radio-frequency energy upon microorganisms in liquid, buffered, and alcoholic food systems. J. Food Sci. 34:320-324.
10. Delany, E. K., H. J. Van Zante, and P. A. Hartman. 1968. Microbial survival in electronically heated foods. Microwave Energy App. Newsl. 1(3):11-14.
11. Goldblith, S. A., and D. I. C. Wang. 1967. Effect of microwaves on *Esherichia coli* and *Bacillus subtilis*. App. Microbiol. 15:1371-1375.
12. Harvey, W. R. 1968. Least-squares analysis of data with unequal subclass numbers. Agri. Res. Ser. 20-8. U.S.D.A.
13. Hone, J. D., H. W. Ockerman, and V. R. Cahill. 1975. A rapid method for the aseptic collection of tissue. J. Milk Food Technol. 38:664-666.
14. Lechowich, R. V., L. R. Beuchat, K. I. Fox, and F. H. Webster. 1969. Procedures for evaluating the effects of 2,450 megahertz microwaves upon *Streptococcus faecalis* and *Saccharomyces cerevisiae*. Appl. Microbiol. 17:106-110.
15. Meyer, R. C., E. H. Bohl, and E. M. Kohler. 1964. Procurement and maintenance of germ-free swine for microbial investigations. Appl. Microbiol. 12:295-300.
16. Ockerman, H. W., V. R. Cahill, K. E. Davis, and C. E. Davis. 1964. Tissue storage without contaminants. J. Animal Sci. 23:142-146.
17. Ockerman, H. W., and V. R. Cahill. 1967. How to get bacteria-free meat samples. National Provisioner. 156(10):35-38.
18. Wantanabe, W., and N. W. Tape. 1969. Microwave processing of wieners 1. Composition and method of preparation. Can. Inst. Food Technol. J. 2:64-68.
19. Wantanabe, W., and N. W. Tape. 1969. Microwave processing of wieners 2. Effect of microorganisms. Can. Inst. Food Technol. J. 2: 104-107.
20. Zender, R. C., C. Lataste-Dorolle, R. A. Collet, P. Rowinski, and R. F. Mouton. 1958. Aseptic autolysis of muscle: biochemical and microscopic modification occurring in rabbit and lamb during aseptic and anaerobic storage. Food Res. 23:305-326.

A Research Note

Heat-sensitive Inhibitor(s) Produced in Poor Quality Raw Milk¹

A. H. DUTHIE, C. G. WOELFEL, K. M. NILSON, and H. V. ATHERTON

*Department of Animal Sciences, Agricultural Experiment Station
 University of Vermont, Burlington, Vermont 05401*

(Received for publication March 19, 1976)

ABSTRACT

As bacterial numbers in raw milk increased to millions and pH decreased to 6.3 and lower, inhibitory substances were produced that gave positive tests by the *Bacillus subtilis* disc assay and *Sarcina lutea* cylinder plate method. These substances were inactivated by heat treatment. The inhibitor was not lactic acid. Results of our study emphasized that all laboratories that test raw milks for antibiotics must be certain to heat samples before reporting a positive test result for inhibitor. If a heat-sensitive inhibitor is found in raw milk, one should evaluate the quality control program.

After a state regulatory agency and a Food and Drug Administration laboratory found penicillin in an interstate shipment of nonfat dry milk in 1974, fluid milk and dairy product plants in Vermont increased their surveillance of raw milk supplies for the presence of antibiotics. Dairymen, too, became more cautious of their milk supply before it left the farm. In fact, many dairymen who questioned human error in the treatment of their cows took a sample from their bulk tank and had it tested for antibiotic. Only if the test was negative would they allow the tank load to be collected.

On several occasions dairymen in Vermont have been fined because their milk samples tested positive for antibiotic, but they or the fieldman could not explain the reason for a positive test. These dairymen in question were not chronic offenders.

One such incident occurred during the summer of 1975. A dairyman received a positive report for antibiotic from a sample of milk taken from his bulk tank. But he said that he had not treated any cows within a month and no fresh cows had been added to the milking herd within 3 weeks of the positive bulk tank sample. An unheated sample from the same load that tested positive by the processing plant was verified as positive for inhibitor but negative for penicillin in our laboratory.

While reviewing this particular inhibitor problem, the sample was tasted and scored "poor" with defects of

malty, high acid, and unclean. The sample had a pH of 6.1. Further discussion with the dairyman uncovered that refrigeration for his bulk tank was turned off for 10 to 12 h before the samples were taken. Milk of such poor quality should never have been picked up by the bulk tank driver. But the point that caught our attention was that poor flavor and a positive test for inhibitor were found on the same sample. Several studies were conducted to determine if this observation was a coincidence.

MATERIALS AND METHODS

Raw milk from a bulk storage tank and cottage cheese starter culture were supplied by our University Dairy Plant. Reagent-grade lactic acid (85%) was used to lower the pH of milk samples.

Antibiotics were determined by the disc assay (1) and cylinder (2) methods. Bacterial numbers of the samples were estimated by the direct microscopic method (1).

RESULTS AND DISCUSSION

Raw milk was divided into two samples. Sample one was refrigerated and sample two was held at room temperature until its pH decreased to 6.1. The refrigerated sample gave a negative test for both inhibitor and penicillin by the *Bacillus subtilis* disc assay method. Sample two showed a positive test for inhibitor, but negative test for penicillin. After sample two was heated to 82 C for 3 min, it was negative for inhibitor. The experiment was repeated twice with identical results. These data confirmed our earlier observation of the farmer's bulk tank sample. When normal microorganisms in raw milk increased in sufficient numbers to decrease the pH of milk to 6.1, heat-sensitive inhibitor(s) were produced.

We wondered if lactic acid was responsible for the inhibitory effect. Various amounts of lactic acid were added to raw milk samples to decrease the pH by 0.1-unit increments from 6.5 to 6.1. The samples were divided into two sets: one set was heated to 82 C for 3 min; the other was left at room temperature. Neither set of

¹Vermont Agricultural Experiment Station Journal Article No. 354.

Sixty-Fourth Annual Meeting of IAMFES

Sioux City Hilton Hotel, Sioux City, Iowa
August 14-18, 1977

Instructions to Prepare Abstracts of Contributed Papers

Procedure

1. Use the printed Abstract form that appears on the other side of this page. Complete the form using a typewriter equipped with a reasonably dark ribbon.
2. Type in the title, capitalize the first letter of the first word and of any proper nouns.
3. List authors and institution(s). Capitalize first letters and initials. Indicate with an asterisk the author who will present the paper. Give complete mailing address of the author who will present the paper.
4. Type the Abstract *double-spaced*, in the space provided on the Abstract form.
5. Mail *two* copies of the Abstract before February 15, 1977 to:

Mr. E. O. Wright
Executive Secretary, IAMFES
P.O. Box 701
Ames, Iowa 50010

6. Enclose *two* self-addressed standard post cards. One will be used to acknowledge receipt of the Abstract and the other to notify the speaker about the scheduling of the paper. Two cards must be included with *each* Abstract that is submitted.

Content of the Abstract

The Abstract should describe briefly: (a) the problem that was studied, (b) methods used in the study, (c) essential results obtained, and (d) conclusions. Statements such as "results will be discussed" should not appear in an Abstract.

Oral Presentations

Papers will be scheduled so a speaker has a maximum of 15 minutes, including discussion. Hence the actual presentation should be no more than 11-13 minutes so that time for discussion will be available. Projectors for 2 × 2 inch slides will be available. If the speaker needs other projection equipment, Mr. E. O. Wright (address given earlier) should be contacted as soon as possible.

Subject Matter for Papers

Papers should report results of applied research in such areas as: food, dairy, and environmental sanitation and hygiene; foodborne disease hazards; food and dairy microbiology; food and dairy engineering; food and dairy chemistry; food additives; food and dairy technology; food service and food administration; food and dairy fermentations; quality control; mastitis; environmental health; waste disposal, pollution, and water quality.

Additional Abstract Forms

Extra copies of the Abstract forms may be obtained from Mr. E. O. Wright (address given earlier).

Membership in IAMFES

Membership in IAMFES is NOT necessary for presenting a paper at the annual meeting.

(OVER)

Annual Meeting
INTERNATIONAL ASSOCIATION OF MILK,
FOOD, AND ENVIRONMENTAL SANITARIANS, INC.

ABSTRACT FORM

Title _____

Authors _____

Institution and Address _____

Please type abstract, double-spaced, in the space provided above.

Sixty-Fourth Annual Meeting of IAMFES

Sioux City Hilton Hotel, Sioux City, Iowa
August 14-18, 1977

Instructions to Prepare Abstracts of Contributed Papers

Procedure

1. Use the printed Abstract form that appears on the other side of this page. Complete the form using a typewriter equipped with a reasonably dark ribbon.
2. Type in the title, capitalize the first letter of the first word and of any proper nouns.
3. List authors and institution(s). Capitalize first letters and initials. Indicate with an asterisk the author who will present the paper. Give complete mailing address of the author who will present the paper.
4. Type the Abstract *double-spaced*, in the space provided on the Abstract form.
5. Mail *two* copies of the Abstract before February 15, 1977 to:
Mr. E. O. Wright
Executive Secretary, IAMFES
P.O. Box 701
Ames, Iowa 50010
6. Enclose *two* self-addressed standard post cards. One will be used to acknowledge receipt of the Abstract and the other to notify the speaker about the scheduling of the paper. Two cards must be included with *each* Abstract that is submitted.

Content of the Abstract

The Abstract should describe briefly: (a) the problem that was studied, (b) methods used in the study, (c) essential results obtained, and (d) conclusions. Statements such as "results will be discussed" should not appear in an Abstract.

Oral Presentations

Papers will be scheduled so a speaker has a maximum of 15 minutes, including discussion. Hence the actual presentation should be no more than 11-13 minutes so that time for discussion will be available. Projectors for 2 x 2 inch slides will be available. If the speaker needs other projection equipment, Mr. E. O. Wright (address given earlier) should be contacted as soon as possible.

Subject Matter for Papers

Papers should report results of applied research in such areas as: food, dairy, and environmental sanitation and hygiene; foodborne disease hazards; food and dairy microbiology; food and dairy engineering; food and dairy chemistry; food additives; food and dairy technology; food service and food administration; food and dairy fermentations; quality control; mastitis; environmental health; waste disposal, pollution, and water quality.

Additional Abstract Forms

Extra copies of the Abstract forms may be obtained from Mr. E. O. Wright (address given earlier).

Membership in IAMFES

Membership in IAMFES is NOT necessary for presenting a paper at the annual meeting.

(OVER)

Annual Meeting
INTERNATIONAL ASSOCIATION OF MILK,
FOOD, AND ENVIRONMENTAL SANITARIANS, INC.

ABSTRACT FORM

Title _____

Authors _____

Institution and Address _____

Please type abstract, double-spaced, in the space provided above.

samples inhibited the growth of *Sarcina lutea* or *B. subtilis* at any of the pH values. Lactic acid was not responsible for inhibition at pH values between 6.5 and 6.1.

It is common knowledge that lactic acid bacteria produce antibiotics (3). Our final experiment was designed to determine if a starter culture that lowered the pH of milk to 6.3 and 6.1 would produce inhibitory substances. Starter culture (1.5%) was added to raw milk. As the pH decreased, samples were taken at selected values and frozen. On the day of analyses, samples were thawed, pH values recorded, bacterial numbers estimated, and samples assayed by the *S. lutea* cylinder and *B. subtilis* disc methods.

TABLE 1. Heat-sensitive inhibitor(s) from starter culture in raw milk

Samples	pH	Number of bacteria estimated	Assay ^{a,b}	
			Inhibitor	Penicillin
Control raw milk	6.5	none in 20 fields	negative	negative
Raw milk + starter culture	6.3	15 million	positive	negative
Raw milk + starter culture	6.1	33 million	positive	negative

^aFive replicate tests by the *Bacillus subtilis* disc assay method.

^bNine replicate tests by the *Sarcina lutea* cylinder plate method.

Data in Table 1 show that as bacterial numbers from a starter culture added to raw milk increased to millions and pH decreased to 6.3 and 6.1, compounds were synthesized that inhibited growth of *B. subtilis* and *S. lutea*. The inhibitor(s) was not penicillin, but it was heat sensitive. Hurst (3) reported that the best known inhibitor produced by *Streptococcus lactis* was nisin. Nisin or another family of naturally occurring antibiotics could be responsible for inhibition observed in these experiments.

Most dairy industry laboratories heat raw milk samples before testing for antibiotics. This procedure saves time because it distinguishes between antibiotics used in mastitis treatment preparations and those produced by microorganisms in milk. However, some dairy laboratories test unheated milk because it saves on energy to heat and cool the samples as well as on labor. Laboratory directors indicate that samples are saved and if a positive test for antibiotic is found, those specific samples are heated and retested.

Results of our study reemphasize the importance of heat treatment for samples that test positive for inhibitor. A positive test could result from naturally occurring antibiotics produced by microorganisms in the milk supply. If the dairy industry laboratory gets positive results when testing raw milk for antibiotics but does not heat the sample, it would be wrong to reject a dairyman's supply of milk unless the sample is heated and retested. This retesting could indicate that the only problem is that the milk is of poor quality.

ACKNOWLEDGMENT

We thank Lionel S. Destremps for excellent technical assistance. This study was supported by the Walker Research Fund and Vermont Agricultural Experiment Station project Hatch 272.

REFERENCES

1. American Public Health Assoc. 1972. Standard methods for the examination of dairy products, 13th ed. Am. Publ. Health Assoc., Washington, D.C.
2. National Center for Antibiotic and Insulin Analysis. 1974. Antibiotic residues in milk, dairy products, and animal tissues; Methods, reports, and protocols. Food and Drug Administration, Washington, D.C.
3. Hurst, A. 1972. Interaction of food starter cultures and food-borne pathogens: The antagonism between *Streptococcus lactis* and spore forming microbes. J. Milk Food Technol. 35:418-423.

A Research Note

A Method to Measure Pyruvate in Milk

J. E. ALBRECHT and S. R. TATINI

*Department of Food Science and Nutrition
 University of Minnesota, St. Paul, Minnesota 55108*

(Received for publication March 18, 1976)

ABSTRACT

A simple procedure to measure pyruvate in fluid milk products was developed. A supernatant fluid from test milk was prepared by clotting with rennet followed by centrifugation. The supernatant fluid was then boiled, centrifuged again, and finally membrane-filtered. This filtrate was then used in the spectrophotometric assay of pyruvate via the NADH-NAD⁺ mediated conversion of pyruvate to lactate by lactate dehydrogenase (LDH). This procedure recovered nearly 100% of pyruvate added to various milks, and detected a difference of 1 ppm of pyruvate ($p < 0.01$).

Recently, accumulation of pyruvate in milk has been proposed as an index of bacterial activity in milk during storage (2, 3). Pyruvate was measured in these studies using an automated procedure with the Technicon Autoanalyzer^R. As the cost of this instrument may be beyond the reach of a smaller dairy processor, a less expensive procedure for pyruvate analysis would make this test more readily utilizable at the dairy plant level. This report describes such a procedure for the measurement of pyruvate in fluid milk product.

MATERIALS AND METHODS

Apparatus

A Beckman ACTATM III UV-Visible Spectrophotometer equipped with an automatic sampling system was used for the assay. A Sorvall RC-2 Automatic Refrigerated Centrifuge was used in sample preparation. Samples were filtered through membrane filters of 0.8 μ m (Cat. #HAWP02500) pores, using a Multi-Sample Manifold (Cat #3025) both from Millipore Corporation, Bedford, Mass., 01730. An Oxford^R Macro-Set Transfer Pipetting System (Oxford Laboratories, 1149 Chess Drive, Fosta City, California, 94404) was used in measuring samples.

Reagents

The following reagents were obtained from Sigma Chemical Company (P.O. Box 14508, St. Louis, Mo. 63178): (a) nicotinamide adenine dinucleotide, reduced form (NADH), Cat #N8129 (b) pyruvic acid, sodium salt, Cat. #2256; (c) lactate dehydrogenase (E.C. 1.1.1.27), ammonium sulfate suspension, Cat. #L2500; and (d) Tris (Hydroxymethyl) Aminomethane, TRIZMA^R, Cat. #T1502. Commercial rennet was obtained from Hansen (Chr. Hansen's Laboratories, 9015 W. Maple, Milwaukee, Wis., 53214).

Preparation of solutions

The stock solution of lactate dehydrogenase was diluted to a final concentration of approximately 6000 to 12,000 units per ml with 0.05 M Tris buffer (pH 7.0), and stored at 4 C. The NADH solution used in the assay was prepared fresh by dissolving the reagent in the ratio of 0.45 mg of NADH to 1.0 ml of Tris buffer (pH 7.0). Both of these solutions

were kept in an ice-water bath during the assay.

A standard 1000 ppm pyruvate solution was prepared by dissolving 550 mg of sodium pyruvate in a total volume of 500 ml of distilled water in a volumetric flask and stored at 4 C. This solution was diluted with .05 M Tris Buffer (pH 7.0) in the following manner to yield the desired ppm values:

Preparation of pyruvate solution		
ml of stock (1000 ppm pyruvate)	ml of Tris Buffer	ppm of pyruvate
5	995	5
10	990	10
20	980	20
30	970	30

The commercial rennet solution was diluted (three parts of undiluted rennet to five parts of buffer) with 0.05 M Tris buffer (pH 7.0) and stored at 4 C.

Preparation of samples for assay

To 5 ml of milk placed in a 15 × 150 mm centrifuge tube, 0.05 ml of the diluted rennet solution was added. The tubes were placed in a 45-C water bath until a solid clot formed (usually within 5 min). Following centrifugation at 10,000 rpm for 10 min, the supernatant fluid was steamed in another centrifuge tube for 5 min. After recentrifugation as above, each sample was filtered through a membrane filter (0.80 μ m). This filtrate was used for the spectrophotometric assay (1) by adding 2 ml of the filtrate to 1 ml of the NADH solution in a cuvette equipped with a micro-stirrer. Following a 2-min mixing period, the initial absorbance at 340 nm was recorded. To each cuvette, 0.05 ml of the lactate dehydrogenase solution was added and the absorbance was again recorded after a 2-min reaction time. The concentration of pyruvate was determined as follows.

$$\mu\text{m pyruvate/ml of sample} = \frac{\text{Change in absorbance}}{E_{340\text{nm}}^{\text{mM}} \text{ NADH} \times \text{Dilution factor}}$$

$$E_{340\text{nm}}^{\text{mM}} = 6.2$$

$$\text{Dilution factor} = 0.67$$

$$1 \mu\text{m pyruvate/ml} = 110 \text{ ppm}$$

Pyruvate was added to pasteurized whole, skim, and chocolate milks as well as raw whole milk and Tris buffer to yield a 1, 2, or 3 ppm concentration of added pyruvate. After thorough mixing, a 15-ml aliquot was withdrawn from each preparation and analyzed for pyruvate according to the above procedure.

RESULTS AND DISCUSSION

Table 1 summarizes the data on recovery of pyruvate added to raw milk and pasteurized whole, skim and chocolate milks. Even though the recoveries shown

TABLE 1. Recovery of added pyruvate from tris buffer and various dairy products

Added ppm	n	\bar{X} ppm	S.D.	Recovery ^a		Coefficient of variation S.D. ($\frac{\quad}{\bar{X}}$)
				ppm	%	
<i>Raw milk</i>						
0	12	3.04	.09			3%
1	12	3.95	.06	0.91	91%	2%
2	12	4.89	.09	1.85	93%	2%
<i>Pasteurized whole milk</i>						
0	20	3.16	.12			4%
1	20	4.32	.24	1.16	116%	6%
2	20	5.34	.17	2.18	109%	3%
<i>Pasteurized skim milk</i>						
0	16	3.43	.08			2%
1	16	4.46	.12	1.03	103%	3%
2	16	5.53	.15	2.10	105%	3%
3	16	6.60	.13	3.17	106%	2%
<i>Pasteurized chocolate milk</i>						
0	16	2.20	.19			9%
1	16	2.99	.14	0.79	79%	5%
2	16	4.10	.16	1.90	95%	4%
<i>Tris buffer</i>						
0	11	0.05	.11			24%
1	4	1.35	.06	0.90	90%	4%
2	4	2.64	.05	2.19	110%	2%

^aInitial pyruvate + Added pyruvate - Initial pyruvate

ranged between 90 and 116% in milks, a similar variation in percent recovery was also observed with the Tris buffer. Therefore, it would appear that essentially all the pyruvate added to the milks was recovered in the filtrate.

Data from replicate analyses of pyruvate from each milk were subjected to analysis of variance to determine

if a difference of 1 ppm of pyruvate could be detected by this procedure. The method was able to detect a difference (significant at 1% level) of 1 ppm of pyruvate even in the presence of 2 to 3 ppm of pyruvate already present in the milks.

Twenty-four samples of milk were analyzed for pyruvate in each trial. This number was determined by the number of slots in the centrifuge head. Generally, the time required for preparation of filtrate and subsequent assay for each trial was 2 h. Though this procedure does not match the number of samples analyzed/h by the Autoanalyzer^R, approximately 100 samples can be analyzed per day by the method described here.

Since 1 ppm of pyruvate is calculated from a change in absorbance of 0.04, any u.v. spectrophotometer with this sensitivity could be used. In addition, a table-top centrifuge could probably be substituted for the refrigerated centrifuge used here. Therefore, with a minimum expenditure of about \$1,000 one can establish this procedure at the dairy plant level.

ACKNOWLEDGMENT

This work was supported by the Dairy Quality Control Institute, Inc., St. Paul, Minnesota. Scientific Journal Series Paper No. 9447, Minnesota Agricultural Experiment Station, St. Paul, Minnesota 55108.

REFERENCES

1. Sigma Chemical Company. 1971. The ultraviolet determination of pyruvic acid. Technical Bulletin #726. 13 p.
2. Tolle, A. 1973. Pyruvat als zentrale Schlüsselsubstanz des bakteriellen Katabolismus in Milch. Arch. Lebensmittelhyg. 24:149-154.
3. Tolle, A., W. Heeschen, H. Wernery, J. Reichmuth, and G. Suhren. 1972. Die Pyruvatbestimmung-ein neuer Weg zur Messung der bakteriologischen Wert von Milch. Milchwissenschaft 27:343-352.

Dairy Products as Food Protein Resources

J. J. JONAS, T. W. CRAIG¹, R. L. HUSTON², E. H. MARTH³, E. W. SPECKMANN²,
 T. F. STEINER⁴, and S. M. WEISBERG⁵

*Kraftco Corporation, Research and Development
 Glenview, Illinois 60025*

(Received for publication June 16, 1976)

ABSTRACT

An overall, continuous downward trend in milk production and utilization can be observed in the U.S. during the last 30 years. The per capita dairy product consumption decreased by about 10% in every decade. Since dairy products supply an important portion of the nation's protein, and 75% of our calcium needs, it seems to be important to understand the causes of this decline. To overcome the decline, the rationale of a coordinated technological, nutritional, and marketing research approach is discussed in this paper. The future of the dairy problem is analyzed from three interrelated viewpoints: (a) product research to meet consumer needs, (b) nutrition research to promote consumer health, and (c) marketing research to explore consumer motivation.

The interrelationship of the world's and our nation's protein supplies presents a problem which has grown in significance during the last decade. The emergence of the acute energy crisis has made the situation even more complex. Since 1972, world food shortages have affected our internal protein food commodity price structure, even though of all countries we possess the greatest capacity for food protein production.

The heart of the problem is to maintain reasonable domestic prices for protein in the face of constraining forces of energy, prices, and environmental effects, as well as the sharply increasing protein demand in the world. The challenge to our nation is to optimally use our land, the climatic advantages, and the technical know-how to maximize our agricultural productivity at the lowest possible cost. We must also maintain the competitiveness of our export potential without endangering the domestic standard of living, both with respect to the quality of proteins and their price structure.

Since dairy products supply an important portion of the nation's protein needs, it was thought to be important to find out where the dairy producing system stands in this stressed economic situation, and what tasks are ahead for the dairy industry to remain strong and productive.

This paper considers the present contributions by and

¹Foremost Foods Company, Dublin, California 94566

²National Dairy Council, Rosemont, Illinois 60018

³Department of Food Science, University of Wisconsin, Madison, Wisconsin 53706

⁴Kraftco Corporation, Corporate Marketing Research, Glenview, Illinois 60025

⁵League for International Food Education, Washington, D.C. 20016

the future of the U. S. dairy industry as a supplier of proteins for the nation. The favorable biological protein output-to-feed energy ratio of milk production by ruminants compared to those of other animal proteins should give the dairy industry an additional impetus to enhance its output efficiency. In the domestic market, this should lead to price stability. In foreign trade, U. S. dairy proteins should provide a means for improving our balance of payments. Since dairy proteins, when used in small quantities, can improve the quality of vegetable proteins, they can be of great significance in alleviating the nutritional protein shortages which face some areas of the world. Technological, economic, and marketing research are needed to find the optimal solutions, in global terms, for problems related to dairy protein utilization. Furthermore, whey utilization is discussed with regard to environmental considerations and improved economics of cheese and casein production.

Research and development programs to strengthen the dairy protein field are discussed from technological, nutritional, and marketing points of view. The objectives of these multi-disciplinary approaches are to define product research orientations for meeting consumer needs, to establish nutrition research directions for promoting consumer health, and to project marketing research to explore consumer motivation.

DAIRY PROTEINS AND DAIRY PRODUCTS IN THE U. S. FOOD MARKET AND IN THE AMERICAN DIET

Major Dairy Products

The total trade volume for food expenditures in the U.S. was 132.2 billion dollars in 1973 (99, 100). Dairy products accounted for 19 billion dollars, i.e., about 14.4% of the total expenditures (Fig. 1). This business volume was derived from 118 billion pounds of milk as raw material. Figure 2 shows the breakdown of utilization of the yearly milk production in terms of fluid milk, butter, cheese, and other uses in the period 1965-1974 (105). These data indicate that total dairy product consumption is decreasing with respect to fluid milk and butter, but increasing significantly with respect to cheese.

Figure 3 illustrates the trend of the last 15 years in daily per capita consumption of dairy protein (37) when compared to total protein consumption. These data

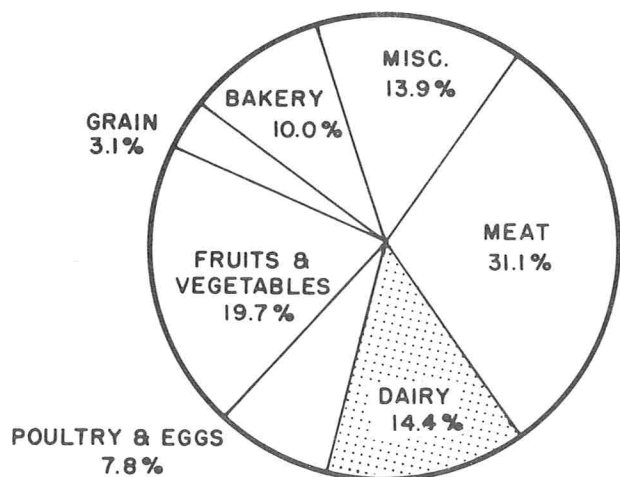


Figure 1. Distribution of U.S. food expenditures in 1973. The total trade volume of food in the U.S. was 132.2 billion dollars in 1973. The dairy products' share was 19 billion dollars, i.e., about 14.4% of the total expenditures. Source: U. S. Dept. Agriculture. Agr. Economic Report No. 138, page 82. (99,100).

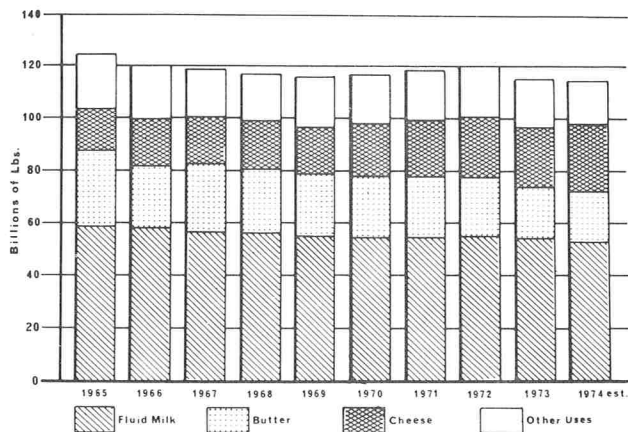


Figure 2. Milk production and utilization, United States 1965-1974. The dairy products were derived from 118 billion pounds of milk. The use of this raw material is shown as fluid milk, butter, cheese, and miscellaneous other dairy products. Source: U.S. Dept. of Agriculture. Agr. Economic Rept. 278, page 8-15 (105).

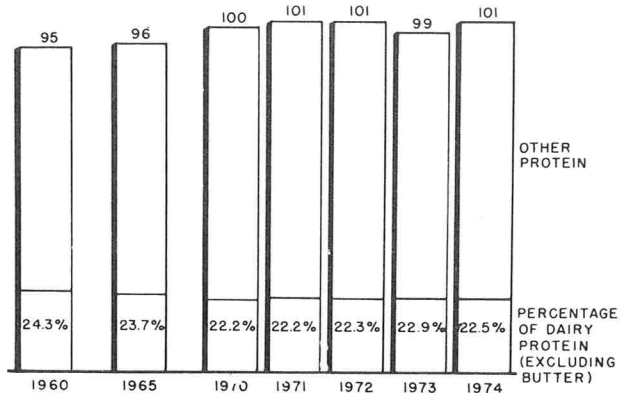


Figure 3. Total food and dairy protein in grams available in the U.S. per capita, per day, between 1960-1974. The total per capita daily protein disappearance increased from 95 grams in 1960 to 101 grams in 1974; the dairy protein's share decreased from 24.3% to 22.5% respectively. Source: U.S. Dept. of Agriculture, 1975. National Food Situation (NFS-154), page 26-32 (37).

indicate that, whereas the per capita daily total protein intake increased from 95 g in 1960 to 101 g in 1974, the dairy protein's share dropped from 24.3% to 22.5%. Since dairy proteins are uniquely rich sources of other important nutrients, continuation of this trend is considered unfavorable from the public health point of view.

It is important to note that, among the major dairy products, cheese shows strong competitive strength. Figure 4 illustrates this point. In the period 1965-1974,

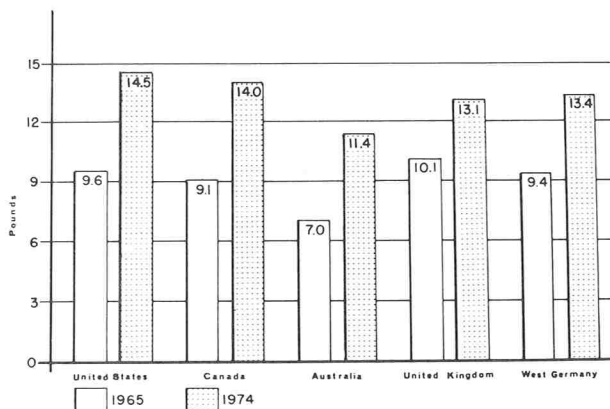


Figure 4. Per capita cheese consumption in several countries, 1965-1974. Cheese consumption increased sharply, not only in U.S., but also in other countries. Source: U.S. Dept. of Agriculture, 1974a. Dairy Situation DS-352, page 16 (101).

the per capita cheese consumption (101) increased sharply.

The nutritional significance of milk and milk products (37) for the nation is demonstrated in terms of critical nutrients and their percentage share in the American diet (Table 1). Besides proteins, dairy products supply

Table 1. Share of milk nutrients in the American diet

In 1975, dairy products (excluding butter) supplied the following percentages of nutrients for the U.S. population:

Protein	22.0%	Riboflavin	39.8%
Fat	13.2%	Vitamin B ₁₂	19.8%
Carbohydrates	6.7%	Vitamin A value	12.7%
Calcium	74.5%	Vitamin B ₆	10.2%
Phosphorus	34.7%	Thiamine	9.0%
Magnesium	21.1%		

Source: Friend and Marston (37).

key nutrients which are important for health; included are calcium, phosphorus, and certain vitamins. For example, 75% of the calcium, and 35% of the phosphorus required by the U. S. consumer are obtained from dairy products. In addition, most of the vitamin D in the American diet comes from milk which is fortified with this nutrient.

These data emphasize the singularity of milk as a supplier of key nutrients for our diet. The public health aspect of the relative richness of milk in calcium and phosphorus in particularly important because there is no other comparable calcium food source available in our dietary. Dairy products supply only 11% of the total daily caloric requirements, but considerably more of key nutrients. This indicates that dairy products have an

excellent nutrient density.

Nutrients in milk

Milk proteins. The proteins of cow's milk comprise about 3.5% of the milk. The major protein component is casein, which, because of its nutritional value, is routinely used as a reference protein. The nutritional value of casein is based on its amino acid balance and phosphorus content. The relative surplus of lysine makes milk proteins valuable for supplementing plant proteins.

To prevent milk from spoiling, i.e., to conserve milk nutrients, most liquid milk is pasteurized, sterilized, treated at ultra high temperatures (UHT) or boiled before consumption. While all forms of heat treatment produce some denaturation of the proteins, i.e., rupture of crosslinkages holding together the polypeptide chains, denaturation of milk proteins does not alter their nutritive values.

Milkfat. Like all fats, milkfat is of high caloric value. It exists in a finely dispersed state in milk and is readily assimilated. The fatty acids in oils and fats which are many in number and structure, can be classified as saturated, monounsaturated, and polyunsaturated. Present nutritional and medical knowledge inclines to the view that about 1/3 of a person's total intake of fatty acids (about 2% of the total caloric intake) should be in the polyunsaturated form (49). Cow's milkfat contains 36% of its fatty acids in the monounsaturated form but only 3-4% as polyunsaturated, and it has been suggested that some vegetable oil rich in polyunsaturated fatty acids, such as soybean oil, should be included in the diet (113).

The role of unsaturated fatty acids in milkfat and their effect on blood cholesterol level has been much discussed in relation to cardiovascular disease, but there is insufficient evidence to condemn consumption of moderate amounts of milkfat⁶ (3, 5, 53, 59, 128).

Lactose. Like milkfat, lactose is a contributor to the energy value of milk. Recently, several studies have appeared on lactose intolerance resulting from lactase deficiency in certain ethnic groups. The following excerpt from the United Nation's Protein Advisory Group (PAG) statement No. 17 addresses this issue (92):

"During the last few years, reports have appeared in the world medical literature on the occurrence of low intestinal lactase (exact term: β -galactosidase) activity on large groups of apparently healthy, non-white populations in different parts of the world. Some of the reports and many articles in the lay press have concluded that milk consumption by these people may lead to untoward reactions in the form of gastrointestinal disturbances ('milk intolerance') and may interfere with proper utilization of milk nutrients.

It would be highly inappropriate, on the basis of present evidence, to discourage programmes to improve milk supplies and increase milk consumption

among children because of the fear of milk intolerance."

Vitamins in milk. Milk contains small amounts of the fat-soluble vitamins E and K. However, it is a major source of vitamins A and D, as a result of fortification. Since milk occupies a unique position in the diet of the young, it is an excellent food for preventing rickets. The optimal combination of calcium and phosphorus and the presence of fats makes milk an obvious choice as a carrier for supplemental vitamins A and D. Milk provides significant amounts of vitamins B₁, B₂, and B₁₂. Although it is low in niacin, milk provides sufficient tryptophan for formation of niacin equivalents in man.

Nutrition surveys (87, 109) indicate that many individuals are consuming less than recommended amounts of several major nutrients, i.e., vitamins A and C, calcium, iron, and riboflavin. It is apparent that dairy products can fulfill a very important role in supplying some of these same nutrients to the human diet. Thus, it must be concluded that milk makes an important contribution to the human diet, in particular, as regards feeding of children.

Dairy products and calcium requirements

The contribution of dairy products to the dietary calcium intake in the U. S. is especially significant in view of declining per capita consumption of these foods. Calcium is one of the major mineral nutrients consumed in less than recommended amounts. It has been determined that 30% of the American public consumes 20-40% less than the currently recommended 800 mg of calcium per day (1). This may, in part, account for a trend toward decreased bone density with advanced age which has been noted by researchers in recent years. A high phosphorus intake because of increased red meat and soft drink consumption may also be involved. A decrease in bone density may in turn be linked to higher incidences of osteoporosis and periodontal disease.

It has been estimated that about 6 million spontaneous fractures because of osteoporosis occur annually in the U. S. among people 45 or more years old. Almost 5 million of these fractures occur in women. The unfortunate thing about bone disorders is that most of the calcium has been withdrawn from the skeleton before deficiency symptoms are detected, which means that it is too late for corrective measures to have any significant influence. An insufficient intake of calcium-containing food in the teenage years, especially among young women, sets the stage for greater bone problems with increasing age. Child bearing plus the stress of lactation coupled with poor dietary habits leads to poor skeletal health. A 10-year study (1) on the clinical manifestation of inadequate bone health emphasizes the importance of maintaining proper calcium intake throughout life.

Several factors have been suggested as affecting calcium absorption and utilization. Among these are nutrients common to milk such as lactose (64, 69, 70), lysine (114), and phosphopeptides of casein (79). Hence, information now available points toward the efficacy of

⁶Also see "Nutrition research to promote consumer health" later in this paper.

milk in the management and prevention of bone demineralization and certain nutritional disease states as well as being a critical food for the general nutritional health of normal individuals.

Cost of proteins in various foods

The retail cost of dairy proteins is relatively low: 20 g of dairy protein (about the daily per capita consumption) cost 24 cents (107). This cost is about twice as much as that of proteins in dry beans and half as much as the same amount of protein in sirloin beefsteak. These data are based on retail prices in January, 1976 (Table 2).

TABLE 2. Cost of 20 grams of protein at January, 1976, prices

Food	Market unit	Price per market unit ¹	Part of market unit to give 20 grams of protein ²	Cost of 20 grams of protein
Dry beans	lb	\$0.56	.24	\$0.14
Peanut butter	12 oz	.71	.23	.16
Bread, white, enriched	lb	.36	.51	.18 ³
Beef liver	lb	.82	.24	.20
Hamburger	lb	.89	.24	.22
Eggs, large	doz	.89	.25	.23
Chicken, whole, ready-to-cook	lb	.64	.37	.24
Milk, whole fluid	half gal	.82	.29	.24 ⁴
Turkey, ready-to-cook	lb	.75	.35	.26
Tuna, canned	6.5 oz	.62	.44	.28
Bean soup, canned	11.5 oz	.30	.96	.28
Chicken breasts	lb	1.13	.25	.29
American process cheese8 oz	.86	.38	.33
Pork, picnic	lb	1.04	.32	.34
Chuck roast of beef, bone in	lb	1.03	.35	.36
Ham, whole	lb	1.48	.29	.43
Round beefsteak	lb	1.98	.22	.43
Ocean perch, fillet, frozen	lb	1.23	.36	.45
Frankfurters	lb	1.24	.36	.45
Liverwurst8 oz	.78	.60	.47
Salami8 oz	.93	.50	.47
Rump roast of beef, boned	lb	1.91	.26	.49
Sardines, canned	4 oz	.52	.94	.49
Pork loin roast	lb	1.49	.33	.50
Haddock, fillet, frozen	lb	1.53	.35	.54
Ham, canned	lb	2.32	.24	.56
Sirloin beef steak	lb	2.06	.28	.58
Bologna8 oz	.83	.73	.60
Rib roast of beef	lb	1.94	.33	.64
Pork chops, center cut	lb	1.89	.35	.66
Veal cutlets	lb	3.18	.21	.68
Pork sausage	lb	1.56	.52	.81
Porterhouse beef steak	lb	2.53	.34	.85
Lamb chops, loin	lb	2.85	.31	.88
Bacon, sliced	lb	1.77	.52	.93

¹Average retail prices in U.S. cities, Bureau of Labor Statistics, U.S. Department of Labor.

²One-third of the daily amount recommended for a 20-year old man. Assumes that all meat, including cooked fat, is eaten.

³Bread and other grain products, such as pasta and rice, are frequently used with a small amount of meat, poultry, fish, or cheese as main dishes in economy meals. In this way, the high quality protein in meat and cheese enhances the lower quality of protein in cereal products.

⁴Although milk is not used to replace meat in meals, it is an economical source of good quality protein. Protein from non-fat dry milk costs about half as much as from whole fluid milk.

Source: Agricultural Research Service, U.S. Department of Agriculture, 1976. (107).

Dairy By-products

Consumption of casein and whey products (9, 103, 106, 123), as well as the contribution of protein by these

materials, is shown in Table 3. Casein, caseinates, and whey solids consumed as components of human food contributed approximately 173,628,000 lb. of high quality protein to the American diet in 1974.

TABLE 3. U.S. consumption of food grade casein and whey products (pounds $\times 10^3$)

Year	Casein		Whey	
	Total	Protein contribution	Total	Protein contribution
1969	116,107	107,980	—	—
1970	135,288	126,423	292,952 ^a	35,154
1971	105,939	98,523	319,383 ^a	38,326
1972	105,401	98,023	376,652 ^a	45,198
1973	112,828	104,930	501,231 ^b	60,148
1974	113,328	105,395	568,611 ^b	68,233

^aFAO figures

^bUSDA figures

Casein and whey products have a wide range of functionality which is useful in food technology. The caseins and caseinates offer high concentrations of proteins with exceptional water binding capacity, fat emulsifying properties, whipping ability, ability to develop viscosity, and solubility in neutral or alkaline solutions. Perhaps most important, all this is provided by a product that is very bland. The milk protein coprecipitate products exhibit much the same properties as casein, but have somewhat limited solubility. Whey protein differs considerably in its functionality from casein and coprecipitated milk protein. Whey and, more particularly, whey protein concentrates, offer a high quality protein that absorbs little water and thus develops minimal viscosity in solution; is soluble in acid solutions; and has fat emulsifying capacity, whipping ability, and bland flavor.

A recent marketing research report by Cornell University lists major uses of casein in various food products (44). Coprecipitates have found their greatest use and acceptance in the baking industry where a high-quality, functional, carbohydrate-free milk protein is required (46).

Whey products, at present, are widely used in food products in the U.S. Increasing availability of whey, coupled with development of professional sales/marketing forces by U.S. companies, has been instrumental in gaining acceptance of whey by food processors. Several general references on whey utilization in food products are available (7, 72, 73, 75, 76, 118, 124). Use of whey in dairy products (16, 36, 95), bakery products (6, 23, 41, 42), candy products (93, 115), snacks (57), meat products (86), and beverages (51, 52) have all been described in the recent literature. In addition, whey serves as a major component of milk replacer blends that are enjoying increasing popularity in the baking industry (62).

Nutritional qualities of isolated milk proteins

The nutritional qualities of isolated milk proteins have been studied (19, 34, 98, 111). One important consideration in expanded protein utilization is the ability of casein, coprecipitate, and, more particularly,

whey protein concentrates to complement and supplement vegetable proteins. For example, Table 4 shows the effect on protein efficiency ratio (PER) when

TABLE 4. *Effect of whey protein supplementation on soy protein value*

Source of protein	PER ^a
Whey protein	3.4
Isolated soy protein	1.4
Whey protein: Soy isolate (1:9)	2.0
Whey protein: Soy isolate (1:3)	2.7
Whey protein: Soy isolate (1:1)	2.9
Casein	2.5

^aPER: Protein efficiency ratio; g of gain/g of protein consumed.

increasing amounts of whey protein are added to a commercial isolated soy protein (68, 68a). Similar effects are seen with corn, wheat, rice, potatoes, and other vegetable proteins (33, 35, 126). Figure 5 shows the increase in balanced protein (meets FAO profile) in

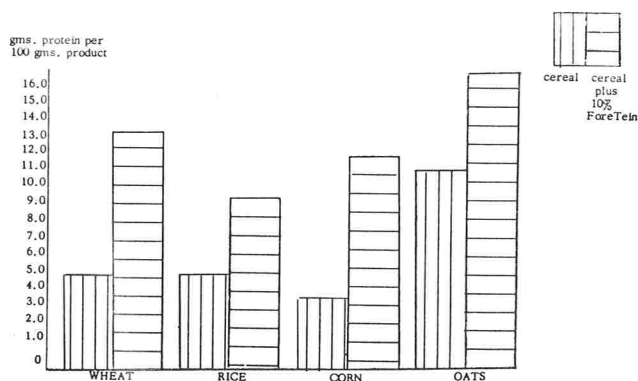


Figure 5. *Whey protein addition improves the amino acid balance of cereals. The quantity of protein, meeting the Food and Agricultural Organization's recommended amino acid profile, is increased by the addition of 10% whey protein concentrate (ForeTein*) to wheat, rice, corn, and oats. These data demonstrate that a relatively small quantity of whey protein is capable of improving the amino acid composition of the respective protein mixture for human needs.*

* ForeTein is a specially processed edible whey product in which the protein has been concentrated through partial lactose removal and demineralization; ForeTein contains 35% protein. Courtesy: Foremost Foods Co., (32).

cereal products when supplemented with 10% whey protein concentrate (22). These data demonstrate that a relatively small quantity of whey protein is able to improve the amino acid balance of the final protein mixture for human needs.

Structure of Dairy Farming

The structure of dairy farming, both in terms of productivity of livestock and size of individual operations, changed considerably during the 1945-1973 period. Yields increased from 4,800 to 10,000 lb. of milk per cow per year. This increased productivity was balanced by the sharp decline in the number of cows — from 25 million to 11 million.

Similarly, the number of dairy enterprises, declined, but the size of the herd increased on surviving farms. This trend is expected to continue resulting in fewer and

larger farms with more productive cows (105).

Structure of Dairy Processing Industry

During the last two decades, there has been a rapid decline in the number of processing plants, but this was balanced by a sharp increase of output per modern plant. These larger dairy product plants produced significant proportions of the total output. For instance, in 1972, 11 cheese plants, or 2% of the total number, produced about 21% of total cheese.

The dairy industry has adequate production capacity to meet domestic needs. Compared to the actual production of dairy products in 1973, the potential capacity to produce cheese was about 119%; for butter 130%; and for dry milk 166%. The modernized cheese plants dry their whey by means of large capacity spray driers which can also be used to produce non-fat milk. The dairy processing industry employs about 200,000 people in the United States (105).

Export-import Status of Dairy Products in the US.

U. S. milk production declined 3.5% in 1973, resulting in the lowest annual output since 1952, as well as a pronounced shift in the pattern of imports and exports of dairy products (102). The value of exports fell by two-thirds while that of imports nearly doubled, leading to a \$270 million deficit in trade in dairy products—the largest such deficit on record.

Milk available for manufacturing declined. The quantity used for cheese production expanded because of greater demand and higher prices. This increase caused a marked decrease in milk available for production of butter and non-fat dry milk. For the first time, the United States in 1973 became a net importer of both butter and non-fat dry milk, which had previously been substantial export items. Dairy exports, with the exception of non-fat dry milk, were small in 1974, further decreasing by 6% in 1975.

The value of dairy product imports reached a record \$318 million in 1973, up 90% over the previous year. This substantial increase in imports reflects falling U. S. milk production, decreased output of butter and non-fat dry milk, and greater consumer demand for cheese. Increased 1973 import authorizations were allowed to alleviate the tight supplies of dairy products. Dairy imports in 1975 totaled 1.7 billion lb. of milk equivalent, well below the 2.9 billion lb. imported during 1974. This decline was entirely the result of lower imports of cheeses. An overview of the dairy imports (81, 108) between 1940-1974 is shown in Table 5, column 4 (81).

Dairy Products in World Trade

During the 1970's, demand for milk and milk products will increase faster than production in the world as a whole and especially in developing countries. In contrast to this, consumption of milk and butter in the milk producing developed countries seems to be decreasing. Cheese consumption, on the other hand, is generally on the increase. As a result, there will be a tendency for dairy prices to rise in the developing countries, but some

TABLE 5. Milk supply and distribution. U.S. specified years, 1940-1974

Year	Milk equivalent ¹														
	Supply				Distribution										
	Production ² (mil. lb.)	Beginning commercial stock (mil. lb.)	Imports (mil. lb.)	Total supply (mil. lb.)	Ending commercial stocks (mil. lb.)	Commercial exports and shipments (mil. lb.)	Fed To calves (mil. lb.)	Department of Agriculture					Total military consump- tion ³ (mil. lb.)	Total civilian consump- tion (mil. lb.)	Per capita civilian consump- tion (lb.)
Beginning stocks (mil. lb.)								Ending stocks (mil. lb.)	Exports and shipments (mil. lb.)	Net purchases for export (mil. lb.)	Beginning stocks (mil. lb.)	Ending stocks (mil. lb.)			
1940	111,512	2,723	290	114,525	2,681	745	2,994	—	—	—	—	—	—	108,105	818
1950	117,302	2,990	459	120,751	2,117	1,204	3,286	2,370	1,637	1,003	270	1,766	111,108	740	
1960	123,109	3,730	604	127,443	4,192	977	2,548	430	1,208	52	830	2,532	116,364	653	
1961	125,707	4,192	760	130,659	4,992	891	2,432	1,208	4,911	41	2,744	2,472	116,128	641	
1962	126,251	4,992	795	132,038	4,338	775	2,330	4,911	7,818	943	3,850	2,969	117,776	641	
1963	125,202	4,338	915	130,455	4,132	897	2,245	7,818	5,556	4,596	2,334	2,964	117,883	632	
1964	126,967	4,132	830	131,929	4,317	720	2,152	5,556	973	6,734	2,151	2,007	119,582	632	
1965	124,180	4,317	923	129,420	3,918	760	2,061	973	538	1,598	1,163	2,819	118,699	620	
1966	119,912	3,918	2,791	126,621	4,813	1,144	1,980	538	46	64	428	2,376	116,736	604	
1967	118,732	4,813	2,908	126,453	4,258	763	1,891	46	3,994	61	4,009	2,117	113,415	581	
1968	117,225	4,258	1,780	123,263	3,983	881	1,821	3,994	2,724	890	380	3,295	113,663	577	
1969	116,108	3,983	1,621	121,712	3,897	942	1,745	2,724	1,447	477	800	2,696	113,232	569	
1970	116,962	3,798	1,874	122,634	3,705	949	1,696	1,447	2,098	41	692	2,419	113,173	561	
1971	118,532	3,705	1,346	123,583	3,565	1,063	1,544	2,098	1,539	1,963	1,404	2,031	113,876	558	
1972	119,904	3,565	1,694	125,163	3,493	1,263	1,641	1,539	2,005	884	1,350	1,671	115,745	561	
1973	115,385	3,493	3,860	122,738	4,732	1,213	1,595	2,005	476	79	1,450	1,257	115,391	554	
1974	115,416	4,732	2,932	123,080	5,580	1,133	1,595	476	306	25	144	1,137	113,780	543	

¹Milk equivalent of milk and cream and manufactured dairy products, including butter, computed on basis of fat content. Milk equivalent means expressing any given dairy product in terms of the amount of milk of standard butterfat content that would be required to produce the product.

²Production on farms plus allowance for milk produced by cows not on farms through 1955; beginning 1956, farm production only.

³Includes any quantities used by military in civilian feeding programs abroad.

Source: U.S.D.A. (81)

downward pressure on prices is expected in milk producing countries.

This projection certainly is not too favorable for the traditional dairy business. It indicates a world trade impasse in terms of dairy products if the present-day product and market structure is maintained. However, it leaves an opportunity for new international trade between dairy and non-dairy areas through a search for joint ventures in countries where the dairy product industry is on a sound economic basis. Development of a new dairy-based food product mix is required which might satisfy local consumer needs and nutritional requirements more effectively than the existing conventional milk, cream, butter type products.

The United Nation's statistical survey reveals (31) that favorable opportunities for dairy product development and trade exist in a few developed, non-dairying countries. These have adequate economic strength for import and demand is upward. Japan is at the top in this category. In Japan, the 1961-63 average per capita annual dairy consumption in terms of milk equivalent was 26 kg whereas by 1975 it is expected to rise to the level of 90 kg. Similarly, the Mediterranean oil producing countries, Spain, Israel, and Lebanon, have increasing demand, projected from 117 kg to 163 kg per capita per year. Very substantial dairy demand is projected for the USSR from the level of 198 kg to 258 kg. These tendencies point to the need for increased efforts toward dairy production in non-dairying countries and careful reconsideration of production targets and price policies in surplus countries.

Significant participation by the U. S. dairy industry in an extended world trade of dairy proteins would require reduction of our dairy raw material production costs to a level approximately equivalent to that of New Zealand. Development of low-cost dairy analogues, based on vegetable proteins, might represent an alternate solution to stimulate U. S. participation in the world-wide dairy product trade.

POTENTIAL FOR EXPANDED DEVELOPMENT

Retail Dairy Products

Expanded U. S. production of most traditional dairy foods is probably not economically feasible under present and foreseeable conditions. The capability for expansion certainly exists. As noted earlier, dairy product processing capacity is already 15% to 50% above our present needs. Past experience indicates that, in spite of capital, manpower, and environmental constraints, an increase in the government support price of milk would bring forth higher output from dairy farmers. But the demand for milk and milk products has been declining steadily since 1960 (Table 5). Per capita consumption by the U. S. civilian population of all dairy products dropped from 653 lb. per year in 1960 to 543 lb. in 1974 (81). Although the U. S. population was growing, total milk production decreased from about 123 billion lb. in 1960 to about 115 billion lb. in 1974 (81). A further

decline in per capita consumption to 1985 has been predicted by the Stanford Research Institute (13). Even if total demand for dairy products were to increase, it could well be supplied by imports, mainly from New Zealand where milk can be produced more cheaply than in the U. S. (105).

As fabricated dairy substitutes gain in variety and quality, their consumer acceptance will increase, and their adverse effects on U. S. sales of genuine dairy products will intensify. It is estimated that, by 1985, milk farmers' sales will be lower by \$1.2 billion than they would be without the growth of fabricated substitute products. About 1.5 million fewer cows will be required in 1985 as diet and health considerations, and price competition from substitute products intensify the downward tendency of per capita dairy product consumption (13).

To compensate for this situation, dairy companies are expected to aggressively promote sales of fluid low-fat and skim milk and low-fat forms of cream cheese, other cheeses, and yogurt. Producers will concentrate much attention on improving the taste of these products. Overall, low-fat milk-based dairy products are expected to show an annual growth rate of 4.3%. It is expected that dried whey products will be developed for home use and subsequently introduced in retail marketing by 1985.

If consumers continue to prefer low-fat products, efforts to alter the nutrient composition of milk should become increasingly important. New feed-management methods resulting in protein-rich milk with polyunsaturated milkfat are being studied (10, 30, 45, 61, 71). Development through breeding of cows able to produce milk with more protein and less fat than at present could be another significant step in this direction.

Industrially used Dairy Products

The principal determinants of expanded utilization and production of dairy protein concentrates, caseins, and whey are economic and regulatory rather than technological. Milk marketing economics control the relative product flows. For example, it has not been economically attractive in recent years for manufacturers in the U. S. to produce casein or caseinate from the domestic milk supply. As a consequence, the U. S. is totally dependent on imported material to meet food processors' demand for this functional protein. The relatively new coprecipitated milk protein products likewise are not produced domestically, but are available only as imported products principally from Australia and New Zealand.

Market-determined prices backed by U. S. Government support prices for manufactured dairy products (non-fat dry milk, butter and cheese) are instrumental in determining production for domestic cheese and whey. Whey production is directly determined by interaction of the forces of supply and demand in the U.S. cheese market. Cheese consumption has been increasing on a per capita basis in the U. S. (80). Table 6 gives production figures for 1966-1974 for hard

cheese, cottage cheese, and accompanying whey production. On a solids basis, the 28,848 million lb. of fluid whey produced in 1974 in the U. S. converts to 1,731,000,000 lb. of dry whey equal to 208,000,000 lb. of high quality protein. Comparing these values to consumption figures shown in Table 3 of 568,611,000 lb. of dry whey gives an overall food whey utilization of 33%. Adding whey consumed as animal feed (535,643,000 lb.) gives an overall utilization figure of 64%. It is estimated that approximately 70-74% of the fluid sweet whey in the U. S. is presently recovered and utilized while only 20% of the fluid acid whey is recovered as a usable product. Thus, the presently annual unused whey production in the U. S. is approximately 623,160,000 lb. of dry whey solids equivalent to 75,000,000 lb. of protein.

TABLE 6. *Cheese and whey production, U.S.^a (lb. × 10⁶)*

	Cheese	Sweet whey ^b	Cottage cheese	Acid whey ^c
1966	1,918	15,344	861	4,305
1967	1,973	15,784	868	4,340
1968	2,002	16,016	901	4,505
1969	2,134	17,072	955	4,775
1970	2,281	18,248	1,039	5,195
1971	2,404	19,232	1,089	5,445
1972	2,664	21,312	1,115	5,575
1973	2,853	22,824	1,099	5,495
1974	2,981	23,848	1,000	5,000

^aSource: Milk Facts 1974 (80).

^bBased on 8 lb. of whey produced per pound of cheese.

^cBased on 5 lb. of whey produced per pound of cottage cheese.

CONSTRAINTS LIMITING PRODUCTION AND USE; HOW THESE MAY BE OVERCOME

The question of availability of adequate dairy protein resources to meet domestic needs has been raised often in recent years. This issue has become interrelated with energy problems, environmental quality considerations, socio-economic constraints, legal and regulatory barriers, and consumer attitudes. Each of these factors is examined and their possible resolution discussed.

Biological and Fossil Energy Requirements

While it is recognized that dairy foods are desirable and reasonably priced, there exists a general misconception concerning the energy cost of animal production enterprises and the efficiency with which farm animals can produce food protein. The data in Table 7 support the contention that protein production in the form of milk is one of the most efficient of all animal production enterprises (94). Note that, when no concentrates are fed, the dairy cow still manages to produce protein in an efficient manner, while not competing with man for highly productive land and grain. However, it appears that yields from selected high-producing animals might warrant use of grain in some types of feeding programs. As one increases grain concentrate usage, the efficiency of food protein production (grams per mega-calorie of digestible energy) also increases. This is an important consideration when one realizes that, among all domestic livestock

enterprises, ruminants are least competitive with humans for foodstuffs (84, 85).

Management of dairy cattle for animal protein production requires trucks, tractors, manure movers, and other equipment. The fossil fuel input for the management operation (88) requires about 1.2×10^6 Kcal per hectare for production of 59 kg of milk protein. The milk protein production per man-hour of labor is about 2.4 kg.

Oftentimes we hear the criticism that cows are wasteful and not ecologically consistent with today's world food situation. It is true that row crops can produce a greater number of calories per hectare and are, therefore, more efficient energy converters. Blaxter (17) compared the yield of key nutrients from an acre (about two-fifths of a hectare) of land used in the wheat production and milk production and found protein production approximately equal, but milk provides more B vitamins (except for nicotinic acid), calcium, and phosphorus. Cows can use several fibrous plant portions (often produced on land unsuitable for row crops) which cannot be utilized by man; included are pasture grasses, corn stalks, hay, silage, etc. (11, 84). Several inedible by-products from the grain milling industry can also be utilized by ruminants (48, 50), as can cellulose, or saw-dust from the lumber industry (112). Hence, in this day of "recycling," we have at our disposal a very useful recycling tool in the form of the dairy cow which can convert inedible food stuffs (roughage and non-protein nitrogen) into high quality protein foods useful to man. When new ways are sought for production of protein (e.g., cultivation of suitable yeasts using petroleum or methane as substrates), it is important to bear in mind that the cow is already able to produce high-value proteins using only simple nitrogen compounds and carbohydrates.

TABLE 7. *Efficiency with which farm animals produce food protein*

Food product	Level and/or rate of output	Protein ¹ production (g/Meal of digest. energy)
Eggs	200 eggs/yr	10.1
Broiler	1.6 kg/12 wks; 1.4 kg feed/.5 kg gain	11.9
	1.6 kg/10 wks; 1.1 kg feed/.5 kg gain	13.7
Pork	1.6 kg/8 wks; 1.0 kg feed/.5 kg gain	15.9
	91 kg; 2.7 kg feed/.5 kg gain	5.0
	91 kg/6 mos; 1.8 kg feed/.5 kg gain	6.4
	91 kg; 1.1 kg feed/.5 gain	8.7
Milk	Biolog. limit (?); 0.9 feed/.5 gain: No losses	12.1
	3,600 kg/yr; (No concentrates)	10.5
	5,400 kg/yr; (25% concentrates)	12.8
	9,000 kg/yr; (50% concentrates)	16.3
	13,600 kg/yr; (65% concentrates)	20.5
Beef	500 kg/15 mos; 3.6 kg feed/.5 kg gain	2.3
	500 kg/12 mos; 2.3 kg feed/.5 kg gain	3.2
	Highly intensive system; No losses	4.1

Source: Ried and Anrique, 1974. (94).

¹These data represent the overall efficiency with which dietary energy is converted to food, as they include the energy cost of reproduction, rearing of breeding stock, and mortality as well as that of production.

Thus, there can be a fruitful relationship between milk production and cereal production for human use as has been instinctively recognized by man for many millennia. What is needed is a more complete understanding and full development of the potential inherent in this relationship. There need be no conflict of interest between direct human use of food crops and production of dairy products.

Environmental Impacts by Dairy Cattle and Dairy Plants

A plausible argument can be made for increased direct food use of oilseed, legume, and cereal crops in preference to conversion of such crops to food by means of animals. It can be agreed in general terms that direct use of oilseeds, legumes, and cereals as foods is more efficient than is their indirect conversion to animal products (47). However, an examination of land usage shows that, of the total world land area, 13.4 billion hectares, only 1.4 billion hectares are under permanent crop production. Of the remainder, 2.9 billion hectares are grasslands (permanent meadow and pasture), 4.0 billion are forested; and 5.1 billion are unclassified. Only 11% are in permanent crop production, while 22% are in permanent meadow and pasture. Thus, land available for ruminants is double that suitable for crops. The forested land can also provide grazing for ruminants. Moreover, land under permanent crops can provide forage and crop refuse in large amounts for ruminant grazing.

Grasslands provide a potential cover for the soil, preventing soil erosion. They also restore fertility to the soil when rotated after cereal crops have been grown. Thus, to provide an intelligent ecological balance for the best use of land, animal agriculture is indispensable. In the United States in 1968, 66% of the feed needs of dairy cattle came from grassland crops. In Europe, Australia, and New Zealand, probably still larger percentages of feed come from forage.

If large herds are concentrated into a small milk producing area, environmental problems can arise. However, proper waste recycling methods are already being developed to minimize the potential pollution of ground water. Use of manure from animals, such as the dairy cow, as a feed ingredient also offers a potential for reducing environmental pollution. This use is currently being explored.

Dairy wastes, mainly whey, if released into streams, are a significant environmental threat. Today, the dairy industry is equipped with proper technology by which whey is recovered in the plant, concentrated, and utilized.

Socioeconomic Impacts

There are problems in milk production and marketing (105). Milk is a highly perishable commodity. It must be handled under sanitary conditions and marketed widely, either for retail consumption or for manufactured products. Milk production is characterized by a strong

seasonal pattern, but consumption is relatively constant throughout the year. This seasonal imbalance between supply and demand for milk has created problems in milk pricing and marketing with inherent instability in milk prices. Dairy producers requested government intervention to stabilize milk marketing conditions. The Agriculture Marketing Agreement Act of 1937 and the Agricultural Act of 1949 resolved this problem by introducing a price support program. According to this program, the Commodity Credit Corporation offers to buy car lots of butter, cheese, and non-fat dry milk at announced prices. Thus, when necessary, the Commodity Credit Corporation removes the excess milk from the market in the form of storable products at equitable prices. In this fashion, a temporary over-supply of milk is avoided, prices are stabilized, and waste, because of perishability, is prevented.

Constraints in Milk Protein Concentrates, Isolates, and in Whey Production and Utilization

An excellent summary of the economic factors involved in whey utilization can be found in work reported by Groves (40). The amount of casein, coprecipitate, cheese (whey), and non-fat dry milk produced in the U. S. is determined principally by economics, in that manufacturing milk will flow to the outlet of highest return to the producer. Government support pricing policy can play a major role in shifting this flow as desired. In the absence of deliberate government manipulation in favor of one product over another, the market will determine actual production figures.

Utilization of these protein sources is determined by several factors, including economic, [market price (a function of production/distribution costs) versus competitive protein sources] and technological/functional properties of value to U. S. food producers, including nutrition. Specifically, the increasing cost of energy will favor less energy-intensive processes (20). For example, costs involved in spray drying may encourage greater utilization of fluid whey concentrates. Already, less energy-intensive membrane techniques such as reverse osmosis have begun to play a role in whey utilization.

The concern with environmental quality will be a factor working in favor in increased whey utilization and recovery. The organic nutrients of whey, if they go unused, create an enormous burden on sewage systems and waterways. The biological oxygen demand (BOD) of whey ranges from 32,000 to 60,000 ppm. Specific values for cottage cheese whey are between 30,000 and 45,000 ppm, depending primarily on the specific cheese-making process used. Every 1,000 gal per day of raw whey discharged as waste can impose a sewage load equal to the amount from 1,800 people. Every 1,000 gal of raw whey discharged directly into a stream requires for its oxidation the dissolved oxygen in over 4,500,000 gal of unpolluted water (129). The newer membrane processes now being applied to whey can result in BOD reduction of raw whey up to 97% or from an initial value of about

35,000 mg/l to less than 1,000 mg/l (77).

The Environmental Protection Agency (EPA) guidelines for dairy processing plant effluent water quality will be a strong force for consolidation of cheese operations into larger plants better able to meet those guidelines. This consolidation will in turn increase the attractiveness of whey recovery and utilization.

Whey utilization is restricted by certain government regulations. Presently, whey and whey products are excluded from a variety of consumer foods. For example, USDA regulations presently prohibit whey from being used in all but a small portion of the comminuted meats being produced in the U. S. Likewise, FDA regulations currently limit whey utilization in frozen desserts to 25% replacement of the milk solids. Another example is the FDA prohibition of whey or modified whey in macaroni products. In view of the highly complementary nature of whey and wheat protein, this restriction makes little sense. While some progress has recently been made in terms of less restrictive standards, significant market outlets remain closed to whey and present regulations serve as a disincentive toward further whey utilization.

Consumer and trade acceptance of whey as a food ingredient continues to grow in the U. S. One factor limiting even greater trade acceptance, however, is the large amount of lactose usually found in whey compared to the amount found in non-fat dry milk (73 versus 55%). While lactose as a purified food and pharmaceutical ingredient has enjoyed strong sales in recent years, its presence in whey can contribute to both functional and digestive problems in certain consumer applications (54). Membrane separation techniques have been used to produce lactose-reduced whey protein concentrates. However, additional approaches to whey products with reduced amounts of lactose should be encouraged. Two such approaches presently being investigated by both industrial and government researchers include lactose hydrolysis (119, 120, 121) and whey fermentation to produce single cell protein (12, 15, 110). Table 8 summarizes the current positive and negative factors influencing further whey utilization in the U. S.

TABLE 8. *Factors influencing increased whey utilization*

<i>Encouraging</i>	<i>Discouraging</i>
Environmental quality	Energy requirements
Nutrition awareness	Lactose intolerance
Process engineering	Government regulations
Consumer acceptance	

Constraints in Marketing of Milk Products to Consumers and their Acceptance

Although consumption of some dairy products has increased, total U. S. civilian consumption of all dairy products combined in 1973 was down approximately to the level of 1960 in spite of a population increase of 25 million people. It is projected that total consumption will continue to drop.

The reasons for these trends appear to be: (a) the impact of low calorie and low cholesterol diets which single out milkfat as an undesirable dietary component;

(b) competition from other nutritious beverages such as tomato juice or citrus juice; (c) increased competition from soft drinks which are extensively advertised; (d) development of substitute products such as coffee whiteners, margarine, and others; (e) need for flavor improvement; (f) relatively low level of spending for advertising, research, and development; and (g) the out-dated basis upon which the price paid to the farmer for milk is determined.

It seems worthwhile to consider why some dairy products have gained and others have lost favor. Cottage cheese and low-fat fluid milk clearly fit into the modern pattern of reducing diets or diets recommended for health purposes. Recently, yogurt has been greatly improved in quality and flavor. Cheeses have grown in favor no doubt because of improved quality and especially because of major improvements in packaging. Another major reason for the increased sale of cheese products is effective advertising and promotion. Frozen desserts have maintained their position because they combine great variety and acceptable flavor and texture.

Dried skim milk has achieved large usage and is now a major dairy product. It has been incorporated into many institutional products, and has also become an important part of reducing and health diets. In terms of domestic usage, the dairy industry consumes approximately 25% of the total annual production of non-fat milk solids in the manufacture of ice cream, cottage cheese, cultured buttermilk, and chocolate drinks. Retail purchases for the home take another 25% in the form of cream sauces, soups, desserts, and beverages. The baking industry uses 14% of the annual supply to improve flavor, texture, color, and nutrition of bread. Meat products, confections, commercial soups, soft drinks, and institutions take most of the balance.

Spurred by recent steep increases in the price of non-fat milk solids, one may expect analogues consisting, for example, of whey-soy flour combinations to begin displacing non-fat milk solids in some appropriate applications as in processed meats or confections.

The dairy industry has not been as aggressive in advertising, sales promotion, and research as other branches of the food industry. Figures published in 1970 (4) indicate that the soft drink and beverage industry spent 70 cents per capita on promotion, coffee 27 cents, all dairy products 24 cents, margarine alone 15 cents. Store level advertising expenditures for dairy products averaged 1.5% of dairy product sales, compared to an average of 3.1% for all product sales. The dairy industry will have to look to itself, and not to retailers, for effective dairy sales and promotion programs (24).

It has been pointed out that only about 0.1% of annual dairy sales income is invested in research and development as compared with 3% for the average industrial expenditure (63). It is surprising to note (65) that, even quite recently, the flavor of milk has appeared as a troublesome consumer issue. The soft drink industry seems to be more conscious of the sales significance of

good and uniform flavor.

Traditionally, farmers have been paid for their milk according to its milkfat content. In the past, milkfat has been prized as food by humans, and skim milk could not be sold much above the cost of production. Recently, there has been a trend toward improving the beverage quality of milk by increasing the non-fat solids and emphasizing the nutritional benefits of increased protein. It is now proposed that milk pricing be based on both milkfat and non-fat solids, especially protein. The equitable execution of such a plan in various milk marketing areas is a complex matter. However, the trend in this direction is unmistakable.

Impact of Marketing Regulations

The dairy industry in the United States has been overregulated. This has resulted in serious problems because regulatory constraints have prevented the industry from tailoring many of its products to meet new consumer demands and from prompt adoption of technological improvements (66). For example, some regulations were designed to protect the industry from the competition of non-dairy products (26). Butter is defined by an Act of Congress, and to add any new ingredient to modify butter would require a new Act of Congress. Imitation cream can legally have the shelf life increased by simple addition of an acceptable preservative, but the law forbids addition of a preservative to regular cream. A multiplicity of sanitary regulations which are a carry-over from the early days when milk was produced and marketed on a very localized basis still remain in force.

There is a need for greater sensitivity to marketing considerations in the making of regulatory policy (39). Greater emphasis should be placed on whether present regulations (other than those concerned with health) hinder or help the sales of dairy products. Regulatory standards should allow the use of approved optional ingredients that can make products better fit consumer needs and should permit labelling which presents the product in its best light while remaining fully informative to the consumer.

Great advances have been made in applying food technology to the development of new families of food products. Examples are the array of snack foods and textured vegetable protein foods. The former sharp dividing lines between segments of the food industry are fading because of technological advances and changing food habits. To keep pace with this changing scene, dairy industry regulations will need constant study and modification. How fast these modifications come will depend on: (a) the expressed needs and desires of consumers, and on economic pressures; (b) the advent of imitation products and dairy product analogues; and (c) the determination of food processors to sense and meet consumer needs and to engage in vigorous promotion and marketing of new food blends.

PRODUCT, NUTRITION AND MARKET RESEARCH DIRECTIONS

Research and development activities are reviewed from technological, nutritional, and marketing points of view. The objectives of these multidisciplinary reviews are to define product research orientations for meeting consumer needs, to establish nutrition research directions for promoting consumer health, and to project marketing research to explore consumer motivation and market structure changes. In each of these respective disciplines, the major lines of current and future research are discussed in appropriate sub-units. In the course of these discussions, the three disciplinary viewpoints are interrelated.

Current and Future Product Research to Meet Consumer Needs

Current trends

(a) In the United States, the trend toward pasteurized, low-fat, high solids, fluid milk continues (117). The potential here will not soon be exhausted because of continuing public concern with dieting. The non-fat milk solids added to these products do much to improve the flavor.

(b) Cultured milk products, such as yogurt, have had considerable acceptance in many countries. No doubt, one important reason for this has been the extension of safer shelf life. This results from the suppression of undesirable microorganisms by lactic acid producing cultures. These cultures have attractive organoleptic properties and health benefits. It seems reasonable that consumption of these yogurt foods will continue to grow and that there will be additional product types. Buttermilk and yogurt products are well adapted for inclusion of a variety of fruit and berry flavors. This opens the prospect of a larger place for these items in the beverage and dessert areas of the food industry.

(c) The conventional cheeses are acceptable largely because of highly prized organoleptic properties. However, they are high in milkfat and in calories. It may be expected that soft skim milk cheeses, low in milkfat, will receive increasing developmental attention. Such cheese fits well into modern dietary patterns and meets the newer nutritional requirements.

Quark is the name for a fresh, uncured bulk cheese, widely produced and eaten in Germany. This product resembles cottage cheese in composition, but its production is much simpler. It is coagulated like cottage cheese and is simply centrifuged to remove the whey. It is then cooled and packaged in bulk. It now serves as an ingredient of protein foods, may be blended with various butter or vegetable fat emulsions, and mixed with spices or herbs.

It should be remembered that nearly all cheeses achieve their prized organoleptic properties as a result of microbiological processes. Surely research which seeks new cheese varieties via microbiology is warranted.

(d) Aseptically processed and packaged dairy-type

products have had a rather slow growth. However, products with improved flavor and better texture are now appearing and achieving increasing acceptance. The inherent advantages of aseptic processing are preservation of good flavor and texture, and long shelf life. It can be expected that special aseptic dairy products will be developed. These can stand the necessary price markup that may result from increased processing and packaging costs.

(e) New types of foods based on dairy products have been the subject of recent research. One such food consists of a meat analogue made by deep fat frying of milk curd precipitated by means of calcium chloride. The texture is similar to that of meat (127). Another related product is a textured food made from skim milk, resembling cooked ground meat or hamburger (89).

Crisp snack products have been developed incorporating sizable amounts of dairy products (74). Such snacks would have better nutritive value than the conventional snack. Puffed snacks can also be made from milk curd.

A cheese, "tvarah," developed in Czechoslovakia, requires no whey separation and, therefore, permits total utilization of the milk. This is a fresh cheese, high in milk fat, made by fortifying condensed milk with cream (67). It is interesting to note that a cheese manufactured without whey was already under consideration earlier by Dahlberg (25). Cream, with 40-45% milkfat fortified with skim milk powder after suitable heat treatment and homogenization, was inoculated with starters. The resulting gelled Neufchatel type cheese is resistant to syneresis.

An interesting spread (8) for home and bakery use has been developed in Japan consisting of butter fortified with raisins which have been presoaked in lactic acid. In Brazil, a chocolate ice cream on a stick, enriched with additional protein has been evaluated successfully in school lunches by a food company. The Australian and New Zealand whole milk "biscuits" consist essentially of highly compressed whole milk powder modified with various fruit flavors and food colors. They may be regarded, in a sense, as confections with optimum nutrition.

(f) Milk protein concentrates and isolates can now be produced in a variety of ways. Such products should be suitable as components of the new textured protein products derived from oilseeds.

(g) Dairy by-product research is of great significance to increase the processing economy of cheese manufacture. Research directed at improved whey processing (less energy-intensive separation processes) has been carried out for some time and has even reached the stage of Environmental Protection Agency (EPA) demonstration grants. Adoption of membrane processes by manufacturers should proceed at an accelerating pace as a result of increased energy costs for conventional processing operations and improved functionality of products with resulting greater consumer acceptance and market demand.

Research and development in the future can probably generate the largest return on investment if aimed at increasing whey utilization. This can involve a variety of approaches from basic to more applied studies.

Identification of future research directions

The following are some directions for product development based on a review of the problems and opportunities now confronting the dairy industry.

(a) Develop new methods for processing fresh milk which increase useful storage life, reduce bulk, and make milk a product suitable for worldwide trade.

(b) Conduct basic research on milk proteins to find methods by which the water binding capacity of the colloidal protein particle can be preserved during various processing conditions.

(c) Conduct basic research on interaction between milk and vegetable proteins results of which could be applied to preparation of high quality dairy analogues.

(d) Develop methods for automation and acceleration of cheese manufacture.

(e) Develop dairy analogues based on alternate protein sources (e.g., oilseeds or cereal proteins). Find economically, nutritionally, and organoleptically acceptable applications for the dairy analogues.

(f) Upgrade acceptable protein resources for dairy product analogue manufacture both in flavor and functionality.

(g) Chemical modification of whey protein and its effect on whey protein properties; whey protein fractionation and characterization; fundamental investigation of whey sensory properties and the contribution of whey components to the overall sensory perception of whey.

(h) Adjust product types to current consumer demand and develop a "fun drink" with dairy protein content.

Nutrition Research to Promote Consumer Health

The significance of milk and dairy products for the American diet was reviewed by Briggs (18) and Phillips and Briggs (87). The work postulates that a decline in milk consumption is conducive to poor nutritional status. Nutritional research strengthened with product development, and an innovative promotional-educational program are required to reverse the downward trend in dairy product consumption. It is important to recognize that, although milk is one of nature's most perfect foods, it is at best, perfectly designed only for the suckling young within the particular species (14). It is known, however, that certain health problems are associated with milk consumption and nutritionally oriented research activity should be focused in these critical areas.

Nutrition research topics (96) needing greater investigation by the dairy industry include: (a) What is the role of milk-fat in the diet? Are the saturated fatty acids and cholesterol as consumed in recommended daily amounts of dairy foods related to coronary heart disease? Is dietary cholesterol needed early in life for optimal development? (b) What is the tolerance of Caucasian and

non-Caucasian people to lactose consumed in recommended daily amounts of dairy foods and is there an effect on nutrient utilization? (c) What is the role of calcium, phosphorus, vitamin D, and perhaps other milk nutrients in the prevention, mitigation and cure of bone disorders such as osteoporosis and periodontitis? (d) Are there interactions between the components of milk which affect its nutritional value? Is the whole better than the sum of its parts? (e) Compared to traditional dairy products, what is the nutritional value, the potential use value, and role of products which resemble or imitate these foods? (f) What nutritional and health benefits are derived from cultured dairy products? How do cultured dairy foods relate to maintaining overall gut health, especially in regard to the interplay of micro-organisms in dairy cultures and intestinal microflora? (g) Iron deficiencies in the human diet have come to be recognized worldwide. If technological problems associated with addition of iron salts to milk can be solved, fresh pasteurized milk might become a logical vehicle for providing iron, especially for infants, children, and the elderly.

These are some of the general nutritional and health concerns about dairy foods. Such concerns can influence national nutrition policy and will affect governmental feeding programs which now include dairy foods.

Two important nutrition research problems, related specifically to the protein component of dairy products, deserving long-range attention are: (a) the effect of dietary protein on blood cholesterol levels and coronary heart disease development, and (b) a nutritional evaluation of genuine and analogue dairy products with respect to a study of nutrient interactions between dairy proteins and other milk nutrients as well as interactions between proteins from dairy and vegetable sources.

Dietary protein and cholesterol

Early work demonstrating a role for diet in the development of atherosclerosis concluded that lesions appearing in the aorta of animals bore a resemblance to those in humans and were in part due to the effects of animal proteins on arterial walls. Others, however, felt the aortic lesions were due to dietary lipids and, of course, this thinking has been proclaimed by many researchers today as the prominent force in development of coronary heart disease (97, 122). Subsequent studies, however, have continued to demonstrate a relationship between dietary protein, plasma cholesterol levels in rabbits, and the onset of coronary heart disease (43, 78). A recent comprehensive review (21) of this subject indicates that dietary protein and carbohydrates can affect serum lipid levels in humans as well as other animal species and the subsequent development of atherosclerosis. Whether or not there exists a true cause and effect relationship, however, is not known and there exists a lack of agreement as to the effect of non-lipid dietary components on serum cholesterol levels and coronary heart disease. The data would indicate, however, that it is desirable to support further studies on

the effects of non-lipid dietary components, such as protein, on coronary heart disease.

It is now well-recognized that statistical relationships exist between several factors and the incidence of coronary heart disease. It is also true that unknown etiological factors still exist and that coronary heart disease is probably influenced by undetected factors (60). One of these unknowns may have to do with development of serum antibodies in response to certain foods, hence opening the possibility for an immunological basis of atherogenesis (27, 28, 90). For example, a recent report (29) suggests that a high percentage of patients suffering from myocardial infarction possessed antibodies to dried milk and possibly egg. Mortality was markedly increased in patients suffering from myocardial infarction and who possessed significant amounts of antibodies in blood samples taken soon after infarction. The presence of these antibodies seemed to be a good predictor of death and were consequently thought to have a possible relationship with myocardial infarction through an immunological mechanism. The results, therefore, seemed to support an immunological hypothesis of coronary heart disease and atheroma. It would be prudent, therefore, to support basic research in this area to confirm or deny these observations.

Nutrient interactions

It is noted that the public is interested in both natural foods and engineered imitations. Milk and other foods are, in fact, natural foods in that little is done during processing to adulterate them. Research in the area of nutrient interactions may demonstrate heretofore unrealized benefits of consuming such natural foods in human nutrition, while at the same time, data can show the problems and limitations of attempting to duplicate the complex array of nutrients found in conventional foods like milk. For example, a need exists for further elucidation of the functions and metabolism of various lipids because they play an important role in protein metabolism, but mechanisms remain unknown.

The interactions of milk's lipid profile with milk protein may be unique and inimitable. If ratios or the physiological forms of nutrients are altered, do nutrient relationships change and, if so, what are the optimal distributions of nutrients in milk? Such information is needed before imitation products can claim "nutritional equivalency" to foods they are supposed to supplement or replace.

Another area of nutrient interactions relates to the future supply of nutrients for mankind. Some nutritionists advocate fortifying foods with limiting essential nutrients so as to improve efficiency of utilization and quality. For example, proteins from a variety of sources can be combined to arrive at a mixture of amino acids optimal for human growth and development. While it is true that high quality protein sources can be used to upgrade the quality of other food proteins, it may also be true that we will have to extend the excellent nutrient profile of conventional foods (i.e.,

milk, meat, eggs) in the future with plant food sources. Before this can be done, however, we must know more about how nutrients from a variety of sources will interact when combined and/or subjected to a variety of processing measures (i.e., heating, flaking, rolling, etc.).

Utilizing correct research inputs now to better understand interactions between nutrients will allow us to efficiently maximize the use of foods already supplying high quality proteins as well as significant amounts of other essential nutrients.

Current and Future Marketing Research: Exploring Consumer Motivation

Marketing research has been an important tool of the dairy industry, specifically by: (a) identifying some of the causes of declining dairy consumption; (b) defining consumer needs; and (c) forecasting future demand. Marketing research should continue to make a contribution to the industry.

Question areas in which published marketing research is inadequate and which represent vital consumer issues for the future include: (a) What do consumers understand about dietary fat (cholesterol, polyunsaturates, etc.)? (b) What fat-related imagery surrounds various protein sources? (c) What specific modifications in diet are made, if any, by people concerned about dietary fat? (d) What are the satisfactions and dissatisfactions with low-fat products and imitation dairy products? (e) How great a premium, if any, are consumers willing to pay for low-fat products? (f) What segments of the population represent the greatest markets for low-fat dairy products? (g) What conditions or factors will promote or retard continued growth of low-fat or imitation dairy products? What are the probabilities that these conditions or factors will be present? (h) What are consumer reactions to positionings/names of "imitation" or modified dairy products? (i) What do consumers understand about vegetable proteins (soy protein and others)? (j) What are the characteristics and attitudes of consumers who purchase extended or analogue meat products? (k) What price spread between dairy based and non-dairy based milk and derivative products is necessary to create demand? (l) What are consumer reactions to various extended or analogue dairy products?

These questions will need to be addressed by the industry in more depth than is evident in published matter. The findings of such research should define the gap between public understanding of the nutritional worth of dairy products and their actual value. It is conceivable that the gap is a wide one and that some form of public education will be needed to eliminate the misunderstanding.

ALTERNATE PROTEIN SOURCES FOR DAIRY PRODUCT ANALOGUES

An Overview of Dairy Analogues

The food protein raw material supply and demand balance in the past year clearly indicated that neither the

U.S. nor the rest of the world can any longer depend entirely on traditional protein sources for human nutrition. The short supply of animal proteins has pointed toward more rational use of unconventional proteins as supplements or partial replacements of animal proteins in foods (58).

A wide range of protein-containing raw materials, of both animal and vegetable origin, are available for dairy analogue manufacture. Materials derived from animal sources include skim milk powder, edible casein and caseinate salts, coprecipitated milk proteins (casein plus serum proteins), whey proteins, and fish protein concentrate. Raw materials of vegetable origin include soy and other oilseed flours, concentrates, and isolates. Leaf protein concentrate and proteins obtained from single-cell fermentation products are possible replacement candidates, but their use in dairy analogues has been barely studied (125).

The FAO/WHO/UNICEF Protein Advisory Group (PAG) adopted the following statement on this subject (91):

"The PAG believes that certain protein concentrates, isolates and extracts, nutritionally suitable oils, and acceptable carbohydrates are useful in proper combination as major ingredients of milk substitutes where the resulting products are not nutritionally inferior to milk or corresponding milk products. Whereas PAG favors efforts to stimulate milk production in protein-deficient areas, in those situations where animal milk is not available or is too costly, the production and use of any clearly labeled nutritious milk substitutes or toned milk product as a protein source should be encouraged. Research and development aimed at improving the quality and lowering the cost of such products should be intensified."

Continued research activity is required by product developers before consumer satisfaction can be anticipated at a level believed to be adequate for commercial success. Moreover, governmental laws or regulations may require that the vegetable analogues of the dairy products be placed on the market with full disclosure of the nature of their origin, composition, and nutritional equivalency. This principle should be observed also when the vegetable protein sources are used only as extenders at a 10-30% addition level. Although their market positioning and their roles in the dietary patterns of various subcultures might be different, the analogues should possess adequate nutritive values commensurate with their dietary role, have satisfactory functionality when used in manufactured food formulations, and be aesthetically acceptable in the form offered for human consumption.

Imitation, Hybrid, and Analogue Dairy Products

Products derived from milk have been rigorously standardized by Federal and State legislations throughout the United States. However, certain "dairy-like" (analogues, imitations, filled, etc.) products are also permitted for sale provided appropriate

regulations and classifications are recognized. At present, the regulations concerning, for instance filled milk products are in a state of change, and for dairy analogues, are undefined. Consequently, in the following discussion, the terms used in product categorization will recognize both the prevailing legalities and the possible new options. *Imitation* dairy products contain no milk as such. Examples are coffee whiteners and whipped toppings. They are usually based on sodium caseinate which, while derived from milk, is considered to be a chemical substance. Isolated soy protein as the sodium salt has also been used in imitation dairy products. Generally a *filled* milk product is made by adding to skim milk a bland vegetable oil or fat, and a few tenths of one percent of monoglycerides to aid in emulsification. The blend is then homogenized. The filled milk usually contains about 3.5% oil or fat. This product may be pasteurized or sterilized. Mellorines are filled milk products resembling ice milk or ice cream. They range in fat content from about 4 to 12%.

Coffee whiteners, especially in the form of spray dried powders, have achieved a substantial market in the United States. They belong to the category of convenience foods because they are easy to use, do not require refrigeration, and have a long shelf life. Coconut fat is generally the major fat component in whitener. Because of their stability, whipped toppings also achieved a substantial market. They can be obtained as dry, stable powders of long shelf life to which only water need be added for reconstitution. They are extensively applied in institutions such as bakeries, hospitals, schools, and restaurants.

Advances in food science have made possible the production of dairy product analogues of constantly improving organoleptic properties. The excellence of today's margarine is an outstanding example. Similar improvements are evident in imitation sour cream, coffee whiteners, and whipped toppings.

Over the years, local and federal regulations have exerted a strong restraining influence on development of "hybrid" types of dairy products or dairy product analogues. With respect to protecting the nutritive contribution of milk to the diet, some labelling regulations may have been and may still be warranted. Coffee whiteners and whipped toppings in their present nutritionally unbalanced form were forced on the industry by the Filled Milk Act, without which most of those products now using sodium caseinate would have used skim milk solids with vegetable fats instead, and they would have been nutritionally better balanced.

Similarly, one can conceive of hybrid protein foods based on dairy products processed together with oilseed and cereal products, reasonable in price and of excellent nutritional value. A whole array of cheese spreads and even cheese analogues with the flavor and texture of traditional cheese can be visualized. The PL 480 donated food programs, which provided corn-soy-milk or whey-soy-drinks, offer an example of hybrid foods of

excellent nutrition suitable for many food needs of various ages. The milk product content, though modest, enhances the nutritive value of the blend in terms of milk protein and milk minerals.

However, one must keep in mind that the flavors and textures of dairy products are delicate (116) and are extremely difficult to duplicate. There are also serious questions of flavor stability in hybrid products. No doubt, these considerations have hindered development of such products as much as restrictive regulations because the American public is very quality conscious. Nevertheless, the rapidly increasing price of dairy products and expected shortages will surely provide a strong incentive for development of hybrid products and analogues. These shortages will also lead to pressure for modifying restrictive regulations.

An excellent summary of the major differences between milk and milk products and their imitations and the most important pros and cons concerning the distribution of imitation products has been provided by the American Academy of Pediatrics Committee on Nutrition in a report entitled "Filled Milks, Imitation Milk and Coffee Whiteners" (2). The conclusions and recommendations of this neutral body of scientists read as follows:

"Imitation milk products or non-dairy 'white beverages' are being developed and evaluated in many countries where milk and other high-quality protein sources are scarce. These efforts to provide nutritional supplements and to extend the available food supplies are to be commended, and research in these areas should not be stifled. If imitation milk products are to replace milk in the diet, they must contain adequate quantities of the essential nutrients to approximate the qualities inherent in milk.

The products available to the American population have not been consistent in offering the same nutritional benefits as fluid milk. Additional information about the qualitative and quantitative composition of these products is needed before physicians in developed countries can unqualifiedly accept them.

The use of imitation milks should not necessarily be viewed as a potential nutritional hazard for the population, unless they are substituted as formula for infants. Imitation milks can be used as a more nutritious beverage than many beverages now consumed by children. However, certain nutritional risks are likely if imitation milks are used as a replacement for milk in the diets of children in the absence of other suitable sources of essential protein, minerals, and vitamins. The same may be true if these products are used in the belief that they are supplying the same nutrients as skim milk or well formulated filled milks.

Informative labelling and suitable standards are needed to minimize any nutritional hazards that might result from the indiscriminate use of poorly formulated products.

The same publication also contains observations as

regards consumer awareness of milk substitutes in a certain area in the U.S.A.: 61% of those using substitutes believed that milk substitutes were nutritionally equivalent to milk, while only 10% of the users thought there was a difference. The remaining 29% of these consumers were unaware of the nutritional value of the milk substitutes.

The first imitation milks on the market varied widely in ingredient composition and also had lower protein and calcium contents than does cow's milk. In newer formulations, attempts have been made to correct these deficiencies. Chemical and biological assay methods for the study of imitation dairy products are discussed by Mitchell and Hu (82).

Market Penetration of Analogue Dairy Products

Imitation and synthetic dairy products have a worldwide penetration potential (125). The price differential between dairy analogues (substitutes) and genuine dairy products is believed to be an important factor in their future acceptance, assuming equal functional and organoleptic properties. For this reason, a quantitative ingredient composition and cost comparison of a number of dairy products and their respective analogues was studied (83). The data indicate that, in all items, the analogue products are expected to be significantly below the price of the genuine product.

Market penetration predictions have been published by two independent investigators. One by USDA's Gallimore (38) and the other by Stanford Research Institute (13). The USDA study (38) estimated 2-3% penetration for most imitation products. Penetration level as high as 10% is assumed for cream type products. The Stanford Research Institute study (13) projected the potential sales losses of natural dairy products caused by the assumed appearance of selected imitations, for the period 1975-1985. This study concluded that dairy companies may be forced more and more to enter the substitute products market as a defensive action.

ACKNOWLEDGMENT

This paper is an abbreviated version of a report on dairy proteins prepared by the authors as part of a larger National Science Foundation study on the present status, future potentials, and research needs of U.S. protein resources.

The authors are indebted to the National Science Foundation for permission to publish their findings. The National Science Foundation's full report will be published late in 1976. A summary volume, entitled *Protein Resources and Technology: Status and Research Needs*, is presently available from the U.S. Government Printing Office. (Stock number D38-000-00251-1, price \$1.75).

REFERENCES

- Albanese, A. A., A. H. Edelson, and E. J. Lorenza, Jr. 1975. Problems of bone health in the elderly. *New York State J. Med.* 75: 326-336.
- American Academy of Pediatrics. 1972. Filled milks, imitation milks and coffee whiteners. Committee on Nutrition. 49:770-775.
- Anonymous. 1968. Milkfat and heart disease. *Milk Ind.* 63(6):29.
- Anonymous. 1970. The media, money and the manufacturer — 1969. Supermarketing. July. Page 19.
- Anonymous. 1971. American Heart Association revises chart on cholesterol. *J. Dairy Sci.* 54(7):8-10.
- Anonymous, 1972. Whey in the bakery world. *Baking Ind.* 138(1707):30-32.
- Anonymous, 1972. Emphasis on whey utilization is growing as more profitable outlets are being sought. *Dairy Record* 73(2): 28-30.
- Anonymous. 1972. Novel dairy products in Japan. *Modern Dairy* 51(10):6.
- Anonymous, 1974. Whey — an important potential protein source. *Monthly Bull. of Agr. Economics & Statistics* 23(4):12-19.
- Anonymous. 1974. Now-polyunsaturated meat and dairy products. *Food Processing* 35(10):17-24.
- Anonymous. 1975. Ruminants as food producer, now and in the future. Council for Agricultural Science and Technology. Special Publication No. 4, March 1975.
- Anonymous. 1975. Fermentation process turns whey into valuable protein. *Chem. Eng.* 82(6):36-37.
- Anonymous. Undated. Dietary foods. Long Range Planning Service. Stanford Research Institute.
- Ashton, W.M. 1972. The components of milk, their nutritive value and effect of processing — Part 2. *Dairy Ind.* 37:602-611.
- Bechtle, R.M. 1974. Conversion of whey solids to an edible yeast cell mass. U. S. Patent 3,818,109.
- Bills, D.D. 1974. The right of whey. *Dairy Ice Cream Field* 157(10):148-151.
- Blaxter, K.L. 1968. The animal harvest. *Science* J.4:53-59.
- Briggs, G.M. 1975. Milk and its role in the American diet. In symposium: Milk and dairy products for the American diet. *J. Dairy Sci.* 57:729. (Presented in Pullman, Washington June 27, 1973).
- Bunce, G.E., and K.W. King, 1969. Amino acid retention and balance in the young rat fed varying levels of lactalbumin. *J. Nutr.* 98:159-167.
- Canada Department of Agriculture, 1974. Proceedings of the whey utilization symposium.
- Carrol, K.K., and R.M.G. Hamilton. 1975. Effects of dietary protein and carbohydrate on plasma cholesterol level in relation to atherosclerosis. *J. Food Sci.* 40:18-23.
- Craig, T.W. 1973. Whey/protein vegetable combinations — achieving nutritional benefits. *Professional Nutritionist* 5(3):2-3.
- Craig, T.W., and J.C. Colmey, 1971. Milk and milk products for use in bakery products. *Bakers Dig.* 45(1):36.
- Cropp, R.A., and T.F. Grat. 1975. How effective is promotion and advertising? *Am. Dairy Rev.* 37(12):34B-34D.
- Dahlberg, A.C. 1927. A new method of manufacturing cream cheese of the Neufchatel type. *J. Dairy Sci.* 10:106-116.
- Davids, R. 1968. *Readers' Digest* 93:93-96.
- Davies, D.F. 1969. An immunological view of atherogenesis. *J. Atheroscler. Res.* 10:253-259.
- Davies, D.F. 1971. Milk protein and other food antigens in atheroma and coronary heart disease. *Am. Heart J.* 81:289-290.
- Davies, D.F., B.W. Rees, A.P. Johnson, P.C. Elwood, and M. Abernathy. 1974. Food antibodies and myocardial infarction. *Lancet* 1:1012-1014.
- Erickson, S. 1975. Producing polyunsaturated milk fat. *Food Prod. Dev.* 9(4):122-123.
- Food and Agricultural Org. of U.N. 1968. Agricultural commodity projections for 1975 and 1985. Milk and milk products. *Monthly Bull. of Agr. Economics and Statistics.* 17(6):6-13.
- Foremost Foods Company, 1970. ForeTein, a high protein demineralized whey protein (brochure), San Francisco.
- Forsum, E. 1975. Use of a whey protein concentrate as a supplement to maize, rice and potatoes: A chemical and biological evaluation using growing rats. *J. Nutr.* 105:147-153.
- Forsum, E., and L. Hambræus, 1972. Biological evaluation of whey protein fraction. *Nutr. Metabol.* 14:48.
- Forsum, E., L. Hambræus, and I. Siddiqi, 1973. Fortification of wheat by whey protein concentrate, dried skim milk, fish protein concentrate and lysine monohydrochloride. *Nutr. Rep. Int.* 8(1): 39-48.
- Frazeur, D.R. 1967. The use of wheys in frozen desserts. *Ice Cream Field Trade J.* 149(8):22+.

37. Friend, B., and R. Marston. 1975. Nutrition review. National Food Situation. NFS-154 Nov. 1975. Page 26-32. Economic Res. Serv. U.S.D.A.
38. Gallimore, W.W. 1972. Synthetics and substitutes for agricultural products. Projections for 1980. U.S.D.A. Economic Res. Service, Marketing Res. Dept. No. 947. Page 57.
39. Glaser, E. 1972. Sterilized dairy analogue products. *J. Milk Food Technol.* 35:464-466.
40. Groves, F. 1972. An economic analysis of whey utilization. University of Wisconsin, Madison, Staff Paper Series #48.
41. Guy, E. J., H.E. Vettel, and M. J. Pallansch, 1974. Effect of cheese whey protein concentrates on the baking quality and rheological characteristics of sponge doughs made from hard red spring wheat flour. *Cereal Sci. Today* 19:551-556.
42. Habighurst, A.B. 1972. Replaces non-fat dry milk in wide range of foods. *Food Processing* 33(7):34.
43. Hamilton, R.M.G. 1972. Effects of diet on plasma cholesterol of rabbits and other young animals. Ph.D. Thesis. Univ. of Westn. Ont., London, Ont.
44. Hammonds, T.M., and D.L. Call, 1970. Utilization of protein ingredients in the U.S. food industry. A.E. Res. 320 & A.E. Res. 321, Cornell University, Ithaca, New York.
45. Harrap, B.S. 1973. Recent developments in the production of dairy products with increased levels of polyunsaturation. *Australian J. Dairy Technol.* 28:101-104.
46. Henderson, J.O., R.A. Buchanan, 1973. Manufacture of CF 1: A sterilized dairy-based carbohydrate-free infant food. *Australian J. of Dairy Technol.* 28:7-11.
47. Hodgson, R.E. 1971. Place of animals in world agriculture. *J. Dairy Sci.* 54:442-447.
48. Hodgson, R.E. 1973. That fluid called milk. *J. Dairy Sci.* 56: 500-502.
49. Holland, R.F. 1969. Flavor, nutritional values of filled and imitation milks. *Am. Dairy Rev.* 31(5):60-61,69.
50. Holmes, W. 1970. Animals for food. *Proc. Nutr. Soc.* 29:237-243.
51. Holsinger, V.H. 1973. Fortifying soft drinks with cheese whey protein. *Food Technol.* 27(2):59-60, 64-65.
52. Holsinger, V.H., L.P. Posati, and E.D. DeVilbiss, 1974. Whey beverages: a review. *J. Dairy Sci.* 57:849-859.
53. Hursh, L.M. 1975. Coronary heart disease: risk factors and the diet debate. National Dairy Council, Rosemont, Ill.
54. Hurt, H.D. 1972. Lactose intolerance. *Cultured Dairy Prod. J.* 7(4):7-10.
55. Ignatowski, A. 1908. Influence de la nourriture animal sur l'organisme des lapins. *Arch. of Med. Exp. Anat. Patholog.* 20:1.
56. Ignatowski, A. 1908. Über die Wirkung des tierischen Eiweisses auf die Aorta und die parenchymatösen Organe der Kaninchen. *Virchow's Arch. für Pathol. Anat. Physiologie und Klin. Med.* 198:248.
57. Jaynes, H.O., and C.T. Chiang, 1974. High protein snack utilizes whey protein concentrate. *Food Prod. Dev.* 8(9):32, 34.
58. Jonas, J. J. 1975. Impact of vegetable proteins on dairy products. *J. Milk Food Technol.* 38:39-43.
59. Kaunitz, H. 1975. Dietary lipids and arteriosclerosis. *J. Am. Oil Chem. Soc.* 52:293-297.
60. Keys, A., C. Aravanis, H. Blackburn, F.S. Van Buchem, R. Buzina, B.S. Djordjevic, R. Fidanza, M.F. Karvonen, A. Menotti, V. Pudda, and H.L. Taylor. 1972. Probability of middle aged men developing coronary heart disease in five years. *Circulation* 45: 815-828.
61. Kieseker, F.G. 1975. Polyunsaturated milk fat products. *Australian J. Dairy Technol.* 30(1):7-10.
62. Kinsella, J.E. 1971. Chemistry of dairy powders with reference to baking. Pages 148-213 In C.O. Chichester, E.M. Mrak, and G.F. Stewart (eds.). *Advances in food research*, Vol. 19. Academic Press, New York and London.
63. Kinsella, J. E. 1972. Dairy foods: trends, research and development. *J. Milk Food Technol.* 35:142-147.
64. Kline, O.L., J.A. Keenan, C.A. Elvehjem, and E.B. Hart. 1932. Lactose in nutrition. *J. Biol. Chem.* 98:121-131.
65. Lake, G. 1971. Twenty year review of the dairy industry in the United States. *J. Dairy Sci.* 54:1708-1713.
66. Lane, C.B. 1970. A critical look at our conservative dairy industry. *J. Milk Food Technol.* 33:249-254.
67. Lang, A., and F. Lang. 1972. Recent developments in dairy research in Czechoslovakia. *Milk Ind.* 71(6):28-30.
68. Lefaire, J., L. Tremblay, and J. P. Julien, 1972. Rôle de complémentarité des protéins solubles du lait pour les protéins de la fève soja. *Can. Inst. Food Sci. Technol. J.* 5:87-92.
- 68a. Lefaire, J., L. Tremblay, and J. P. Julien, 1972. Rôle de complémentarité des protéines solubles du lait pour les protéins de la fève soja, erratum. *Can Inst. Food Sci. Technol. J.* 5:221. (French)
69. Lengemann, F.W. 1959. The site of action of lactose in the enhancement of calcium utilization. *J. Nutr.* 69:23-27.
70. Lengemann, F.W., and C.L. Comar. 1961. Distribution of adsorbed strontium-85 and calcium-45 as influenced by lactose. *Am. Physiol.* 200:1051-1054.
71. Libman, J. 1975. An udder discovery: Cows fed new feed give low-fat milk. *Wall Street J.*, May 15. Pages 1,18.
72. Mann, E.J. 1969. Whey processing and utilization. *Dairy Ind.* 9: 507-508.
73. Mann, E.J. 1973. Some aspects of whey utilization. *Dairy Ind.* 38: 77-78.
74. Mann, E. J. 1973. New dairy products. *Dairy Ind.* 38:179-182.
75. Mann, E. J. 1974. Whey utilization, Part 1. *Dairy Ind.* 39:303-304.
76. Mann, E. J. 1974. Whey utilization; Part 2. *Dairy Ind.* 39:343-344.
77. Marshall, P.G., W.L. Dunkley, and E. Lowe, 1968. Fractionation and concentration of whey by reverse osmosis. *Food Technol.* 22(8):37-44.
78. Meecker, D., and H.D. Kestan. 1941. Effect of high protein diets on experimental atherosclerosis of rabbits. *Arch. Pathol.* 31:147.
79. Mellander, O. 1962. Phosphopeptides; chemical properties and their possible role in the intestinal adsorption of metals. Pages 265-276. In R.H. Wasserman (ed.) *Transfer of calcium and strontium across biological membranes*. Proc. Conference, Ithaca, N.Y. Academic Press, New York 1962.
80. Milk Industry Foundation. 1974. *Milk facts*, Washington, D.C.
81. Milk Industry Foundation. 1975. *Milk facts*. Washington, D.C. Page 22.
82. Mitchell, M.E., and M. Hu. 1975. Protein value of two imitation milks. *J. Am. Diet. Assoc.* 67:564-569.
83. Moede, H.H. 1970. Marketing margins for selected dairy and non-dairy products. *Dairy Ice Cream Field.* 153(1):80-86.
84. National Dairy Council. 1970. Role of dairy products in world food problems. Publication 41:1 (Rosemont, Ill.)
85. National Dairy Council. 1976. Role of the dairy cow in providing food. National Dairy Council Publication 47:1 (Rosemont, Ill.)
86. Neer, K.L., R.F. Plumpton, Jr. and H.W. Ockerman. 1974. Bologna product characteristics as influenced by various sources and levels of cottage cheese whey. *J. Food Sci.* 39:993-996.
87. Phillips, M.C., and G.M. Briggs. 1975. Milk and its role in the American diet. *J. Dairy Sci.* 58:1751-1763.
88. Pimentel, D. 1975. Protein production, energy/land limitations and population pressure. Dept. of Entomology and section of Ecology and Systematics, Cornell Univ. Ithaca, N.Y.
89. Pinkstone, P. J., and T. J. Claydon. 1971. Burger-type products from texturized skim milk curd. *Food Prod. Dev.* 5(7):87-90.
90. Poston, R.N., and D.F. Davies. 1974. Immunity and inflammation in the pathogenesis of atherosclerosis. *Atherosclerosis* 19:353-367.
91. Protein Advisory Group, U.N. 1970. PAG Statement on milk substitutes, No. 6. PAG Compendium E5. Halsted Worldmark, New York.
92. Protein Advisory Group, U.N. 1972. PAG Statement No. 17. Low lactase activity and milk intake. PAG Compendium E 349-351. Halsted Worldmark, New York.
93. Rash, K.E. 1969. Milk substitutes made from whey for candy provide savings in ingredient, processing cost. *Candy Ind.* 133(11):32, 34.

94. Ried, J.T., and R. Anrique. 1974. Biological efficiency of animal production, energy utilization and fat deposition. Symposium. National Research Council, Washington, D.C. Unpublished.
95. Singleton, A.D. 1973. Whey usage in dairy products. *Dairy Ice Cream Field* 156(2):30-32.
96. Speckmann, E.W. 1975. The National Dairy Council's nutrition program and its relationship to a National Nutrition Policy. *J. Milk Food Technol.* 39:166-175.
97. Stucky, N.W. 1912. Über die Veränderung der Kaninchenaorta bei der Fütterung mit verschiedenen Fettsorten. *Zentralbl. allgem. Pathol. und pathol. Anat.* 23:910.
98. Tomarelli, R.M., and F.W. Bernhart. 1962. Biological assay of milk and whey protein compositions for infant feeding. *J. Nutr.* 78:44-50.
99. U.S. Department of Agriculture. 1973. Distribution of U.S. food expenditures in 1973. *Agr. Economics Dept. No. 138.* Page 82.
100. U.S. Department of Agriculture. 1973. Food consumption, prices, expenditures. Supplement for 1973 to *Agricultural Economic Report No. 138.* Economic Research Service. Page 37-38.
101. U.S. Department of Agriculture. 1974. Dairy situation DS-352. Sept. 1974. Page 16.
102. U.S. Department of Agriculture. 1974. Foreign agr. circular (dairy) FD 3-74. August 1974. Pages 1-2.
103. U.S. Department of Agriculture. 1974. Agricultural statistics. Page 391.
104. U.S. Department of Agriculture. 1975. The cost of meats and meat alternates. *Agr. Res. Service.* Febr. 1975.
105. U.S. Department of Agriculture. 1975. The impact of dairy imports on the U.S. dairy industry. *Economic Research Service, Agr. Economic Rept. No. 278.* Pages 8-15.
106. U.S. Department of Agriculture, 1975. Dairy situation. *Econ. Res. Serv.* March. Page 27.
107. U.S. Department of Agriculture, 1976. Cost of 20 grams of protein at January, 1975, prices. *Agr. Res. Service.*
108. U.S. Department of Agriculture, 1976. Dairy situation DS-359. Page 28. March, 1976.
109. U.S. Department of Health, Education and Welfare. 1972. Ten-State nutrition survey, 1968-1970. Publication No. HSM-72 8131.
110. Vananuvat, P., and J.E. Kinsella, 1975. Production of yeast protein from crude lactose. *J. Food Sci.* 40:336-341.
111. Vaughan, D.A. 1970. Nutritional aspects of whey as food. In *Whey Utilization Conference Proceedings.* U.S. Department of Agriculture, Page 78-92.
112. Virtanen, A.I. 1967. Milk production on a protein-free and protein-poor food. *Netherlands Milk Dairy J.* 21:223-244.
113. Waite, R. 1973. Manufactured milk. *Nutr. Food Sci.* 30:6-8.
114. Wasserman, R.H., C.L. Colmar, J.C. Schooley, and F.W. Lengemann. 1959. Interrelated effects of L-lysine and other dietary factors on the adsorption of calcium-45 in the rat and chick. *J. Nutr.* 62:367-376.
115. Webb, B.H. 1966. Whey — a low-cost dairy product for use in candy. *J. Dairy Sci.* 49:1310-1313.
116. Webb, B.H. 1969. New dairy product development. *J. Dairy Sci.* 52:1693-1698.
117. Webb, B.H. 1970. New dairy foods, opportunities and obstacles. *Food Eng.* 42(8):104-113.
118. Webb, B.H. 1972. Recycling whey for profitable uses. *Amer. Dairy Rev.* 34(6):32A, 32D.
119. Weetal, H.H. 1973. Immobilized enzymes: Some applications to foods and beverages, Part I. *Food Prod. Dev.* 7(3):46, 49+.
120. Weetal, H.H. 1973. Immobilized enzymes: Some applications to foods and beverages, Part II. *Food Prod. Dev.* 7(4):94, 96+.
121. Weetal, H.H., N.B. Havewala, W.H. Pitcher, Jr. C.C. Detar, W.P. Vann, and S. Yaverbaum, 1974. The preparation of immobilized lactase and its use in the enzymatic hydrolysis of acid whey. *Biotechnol. Bioeng.* 16(3):295-313.
122. Wesselkin, M. W. 1913. Über die Ablagerung von fettartigen Stoffen den Organen. *Virchow's Arch. für Pathol. Anat. Physiol. und Klin. Med.* 212:225.
123. Whey products Institute. 1975. U.S. Department of Agriculture/Production of certain whey products, Jan.-Dec. 1974. Chicago.
124. Wingerd, W.H. 1971. Lactalbumin as a food ingredient. *J. Dairy Sci.* 54:1234-1236.
125. Winkelman, F. 1974. Imitation milk and imitation milk products. *Food and Agr. Org. of United Nations, Rome.*
126. Womack, M., and D. Vaughan, 1972. Whey and whey products as cereal supplements. *J. Dairy Sci.* 55:1081-1084.
127. Wong, N.P., and O.W. Parks. 1970. New high-protein food from milk. *J. Dairy Sci.* 53:978-979.
128. Yudkin, J. 1970. Milk and heart disease: The evidence is not convincing. *Milk Ind.* 66(6):18-20.
129. Zall, R.R., R.L. Goldsmith, D. J. Goldstein, and B.S. Horton, 1971. Membrane processing of 300,000 pounds per day cottage cheese whey for pollution abatement, Phase II. Presented at *Water Quality Eng. for Ind. Symp. A I Ch E 64th Annual Meeting.*

3-A Sanitary Standards for Equipment for Packaging Frozen Desserts, Cottage Cheese and Similar Dairy Products

Number 23-01

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. Frozen desserts, cottage cheese and similar dairy products packaging equipment specifications heretofore or hereafter developed which so differ in design, material, fabrication, or otherwise as not to conform with the following standards, but which, in the fabricator's opinion are equivalent or better, may be submitted for the joint consideration of the IAMFES, USPHS, and DIC, at any time.

A. SCOPE

A.1

These standards cover the sanitary aspects of equipment for performing the functions of holding, mechanically opening, forming, dispensing, filling, closing, sealing, or capping containers, or wrapping the product, and all parts which are essential to these functions. These standards do not pertain to the container, nor to other integral equipment embodied on certain machines which perform such functions as container fabricating or container overwrapping.

A.2

In order to conform with these 3-A Sanitary Standards, equipment for packaging frozen desserts, cottage cheese and similar dairy products shall comply with the following design, material and fabrication criteria.

B. DEFINITIONS

B.1

Product: Shall mean frozen desserts, cottage cheese and products such as sour cream, yogurt, whipped butter, and whipped cream cheese, including added ingredients.

B.2

Container: Shall mean a packaging enclosure, including its body, cap, cover or closure, or a wrapper, capa-

ble of holding the product.

B.3

Holding, Opening, Forming and Dispensing Equipment: Shall mean the equipment for holding, mechanically forming, opening and dispensing the containers.

B.4

Filling Equipment: Shall mean the equipment for mechanically filling the container with the product.

B.5

Capping, Closing, Sealing and Wrapping Equipment: Shall mean the equipment for mechanically capping, closing and sealing the container, or wrapping the product.

B.6

Surfaces

B.6.1

Product Contact Surfaces: Shall mean all surfaces which are exposed to the product, surfaces from which liquids may drain, drop, or be drawn into the product or into the container, and surfaces that touch the product contact surfaces of the container.

B.6.2

Non-Product Contact Surfaces: Shall mean all other exposed surfaces.

B.7

Mechanical Cleaning or Mechanically Cleaning: Shall denote cleaning solely by circulation and/or flowing chemical detergent solutions and water rinses onto and over the surfaces to be cleaned, by mechanical means.

B.8

Engineering Plating: Shall mean plated to specific dimensions or processed to specified dimensions after plating.¹

C.

MATERIALS

C.1

Product contact surfaces shall be of stainless steel of the AISI 300 series² or corresponding ACI³ types (See

¹QQ-C-320a-Federal Specification for Chromium Plating (Electrodeposited), July 26, 1954. Available from: General Services Administration, Seventh and D Streets NW, Room 1643, Washington, D.C.
 QQ-N-290-Federal Specification for Nickel Plating (Electrodeposited), April 5, 1954, and Amendment 1, December 13, 1961. Available from: General Services Administration, Seventh and D Streets NW, Room 1643, Washington, D.C.

²The 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 Street NW, Washington, D.C. 20036.

³Alloy Casting Institute Division, Steel Founders' Society of America, 20611 Center Ridge Road, Rocky River, OH 44116.

Appendix, Section E.), or metal which under conditions of intended use is at least as corrosion-resistant as stainless steel of the foregoing types and is non-toxic and non-absorbent, except that:

C.1.1

Optional metal alloy may be used but only in applications requiring disassembly and manual cleaning. (See Appendix, Section F. for the composition of an acceptable optional metal alloy).

C.1.2.

Those surfaces of holding, forming, opening, dispensing, closing, capping, sealing or wrapping equipment which touch the product contact surfaces of the container or from which liquids may drain, drop or be drawn into the container may be made of a non-toxic, non-absorbent metal that is corrosion resistant under conditions of intended use or may be made of metal made corrosion-resistant and wear-resistant by a covering of an engineering plating of chromium or nickel or an equally corrosion and wear-resistant non-toxic metal.

C.1.3

Rubber or rubber-like materials or plastic materials may be used for filling nozzles, plungers, bonded or removable gaskets, diaphragms, sealing rings, drip shields, protective caps for sanitary connections, container opening, dispensing, capping and closing parts, filling valve members, seals and parts used in similar applications. Plastic materials may be used for short flexible transparent connectors.

C.1.4

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.1.5

Plastic materials when used for the above specified applications shall comply with the applicable provisions of the 3-A standard for plastic materials, Number 20-00, as amended.

C.1.6

The final bond and residual adhesive, if used, of bonded rubber and rubber-like materials and bonded plastic materials shall be non-toxic.

C.1.7

Silver soldered or brazed areas and silver solder or braze material shall be non-toxic and corrosion resistant.

C.1.8

Single service gaskets of a sanitary type may be used.

C.2

Non-product contact surfaces shall be of corrosion-resistant material or material that is rendered corrosion-resistant. If coated, the coating used shall adhere. Non-product contact surfaces shall be relatively non-absorbent, durable and cleanable. Parts removable for cleaning having both product contact and non-product contact surfaces shall not be painted.

D.

FABRICATION

D.1

Product contact surfaces shall be at least as smooth as a No. 4 finish on stainless steel sheets free of imperfections such as pits, folds and crevices. (See Appendix, Section G.).

D.2

Permanent joints in metallic product contact surfaces shall be continuously welded. If it is impractical to weld, they may be silver soldered or brazed. An exception is made to the foregoing for product connections which may have rolled-on sanitary pipeline ferrules or flanges. Welded or silver soldered or brazed areas of product contact surfaces shall be at least as smooth as No. 4 finish on stainless steel sheets free of imperfections such as pits, folds and crevices.

D.3

The minimum thickness of engineering plating shall be 0.0002-inch for all product contact surfaces, except that when the parts listed in C.1.2 are to be plated are other than stainless steel the minimum thickness of the engineering plating shall be 0.002-inch.

D.4

Product contact surfaces shall be easily accessible, visible for inspection, and readily cleanable, either when in an assembled position or when removed. Removable parts shall be readily demountable. Fillers designed to be mechanically cleaned shall be accessible for manual cleaning and inspection.

D.5

Product contact surfaces shall be self-draining or self-purging except for normal clingage. The bottom of the hopper shall have a minimum pitch of 1/8 inch per foot toward the plane of the outlet(s).

D.6

The product hopper shall be equipped with a cover having a drop-flange which overlaps the rim of the hopper by at least 3/8 inch. The edges of openings in the hopper cover shall extend upward at least 3/8 inch or be fitted with a permanently attached sanitary pipeline connection conforming to D.13. Openings in the hopper cover, except those fitted with a permanently installed sanitary pipeline connection, shall be provided with covers having a downward flange of not less than 1/4 inch so designed as to prevent liquid from entering the hopper. Covers shall be self-draining.

D.7

The filling equipment shall be so designed that adjustments necessary during the operation may be made without raising or removing the hopper cover(s).

D.8

Gaskets:

D.8.1

Gaskets having a product contact surface(s) shall be removable or be bonded.

D.8.2

Bonded rubber and rubber-like gaskets and bonded plastic gaskets shall be bonded in such a manner that the bond is continuous and mechanically sound, and so that when exposed to the conditions encountered in the environment of intended use and in cleaning and bactericidal treatment, the rubber and rubber-like material or the plastic material does not separate from the base material.

D.9

Gasket grooves or gasket retaining grooves shall not exceed 1/4 inch in depth or be less than 1/4 inch wide except those for standard O-Rings smaller than 1/4 inch.

D.10

Radii

Internal angles of 135° or less on product contact surfaces shall have radii of not less than 1/4 inch, except that:

D.10.1

Where smaller radii are required for essential functional reasons, such as those in filler nozzles, the radii shall be not less than 1/32 inch.

D.10.2

The radii in gasket grooves or gasket retaining grooves, except for those for standard 1/4 inch and smaller O-Rings, shall be not less than 1/8 inch.

D.10.3

The radii in grooves for standard 1/4 inch O-Rings shall be not less than 3/32 inch and for standard 1/8 inch O-Rings shall be not less than 1/32 inch.

D.11

Covers, diverting aprons, shields or guards shall be provided and shall be so designed and located to prevent liquid or other contaminants from draining or dropping into the container or product, or onto product contact surfaces.

D.12

There shall be no threads on product contact surfaces.

D.13

Sanitary fittings and connections shall conform with the applicable provisions of the 3-A standard for sanitary fittings, Number 08-17, except that sanitary fittings made of optional metal alloy shall not be used if the filler is designed for mechanical cleaning.

D.14

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.15

Equipment for producing air under pressure and/or air piping which is supplied as an integral part of the filling equipment shall comply with the applicable provisions of the 3-A accepted practices for supplying air under pressure, Number 604-03.

D.16

Supports:

The means of supporting the filling equipment shall be legs or casters, or the equipment shall be mounted on a slab or island and shall comply with the applicable provisions of the following:

D.16.1

Legs or casters shall provide a clearance between the lowest fixed point on the filling equipment and the floor of at least 4 inches when the base outlines an area in which no point is more than 12 1/2 inches from the nearest edge of the base, or a clearance of at least 6 inches when any point is more than 12 1/2 inches from the nearest edge.

D.16.2

Legs, if provided, shall be smooth with rounded ends and have no exposed threads. Legs made of hollow stock shall be sealed.

D.16.3

Casters, if provided, shall be easily cleanable, durable and of a size that will permit easy movement of the equipment.

D.16.4

If the equipment is to be mounted on a slab or island, the base shall be designed for sealing to the slab or island. (See Appendix, Section H.).

D.17

A guard(s) required by a safety standard that will not permit accessibility for cleaning and inspection shall be designed so it (they) can be removed without tools.

D.18

Non-product contact surfaces shall be smooth, free of pockets and crevices and be readily cleanable and those to be coated shall be effectively prepared for coating.

APPENDIX

E.

STAINLESS STEEL MATERIALS

Stainless steel conforming to the applicable composition ranges established by AISI² for wrought products, or by ACI³ 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 303, 304, and 316 are designated CF-16F, CF-8, and CF-8M, respectively. These cast grades are covered by ASTM⁴ specifications A296-68 and A351-70.

F.

OPTIONAL METAL ALLOY

An optional metal alloy having the following minimum and maximum composition is deemed to be in com-

⁴Available from American Society for Testing and Materials, 1916 Race Street, Philadelphia, PA 19103.

pliance with C.1.1 herein.

Zinc	- 8% maximum
Nickel	-19 1/2% minimum
Tin	-3 1/2% minimum
Lead	- 5% maximum
Iron	- 1 1/2% maximum
Copper	- the balance

An alloy of the composition given above is properly designated "nickel silver," or according to ASTM⁴ Specification B149-70, may be entitled "leaded nickel bronze."

G.

PRODUCT CONTACT SURFACE FINISH

Surface finish equivalent to 150 grit or better as obtained with silicon carbide, is considered in compliance with the requirements of Section D.1 herein.

H.

SLABS OR ISLANDS

When the equipment is designed to be installed on a slab or island, the dimensions of the slab or island should be such that the base of the equipment will extend beyond the slab or island at least 1 inch in all horizontal directions. The slab or island should be of

sufficient height so that the bottom of all product connections are not less than 4 inches above the floor. The surface of the slab or island should be coated with a thick layer of waterproof mastic material, which will harden without cracking. The junction of the equipment base and the slab or island should be sealed.

I.

INFORMATION PLATE

Manufacturers should provide an information plate in juxtaposition to the name plate giving the following information or the information should appear on the name plate:

- (1) If the filling equipment is or is not designed for mechanical cleaning.
- (2) A statement that to prevent corrosion the recommendations of the filler manufacturer should be followed with respect to time, temperature and the concentration of specific cleaning solutions and chemical bactericides.

These standards are effective January 22, 1977 at which time the "3-A Sanitary Standards for Equipment for Packaging Frozen Desserts, Cottage Cheese and Similar Milk Products, Serial #2300" are rescinded and become null and void.

U.S. POSTAL SERVICE STATEMENT OF OWNERSHIP, MANAGEMENT AND CIRCULATION <small>(Required by 39 U.S.C. 3685)</small>		
1. TITLE OF PUBLICATION Journal of Milk & Food Technology		2. DATE OF FILING Oct. 1, 1976
3. FREQUENCY OF ISSUE Monthly	A. NO. OF ISSUES PUBLISHED ANNUALLY 12	B. ANNUAL SUBSCRIPTION PRICE \$32.00 per yr \$25.00 6mo sub \$14.00 membership
4. LOCATION OF KNOWN OFFICE OF PUBLICATION (Street, City, County, State and ZIP Code) (Not printers) 413 Kellogg, Ames, Iowa 50010 Story County		
5. LOCATION OF THE HEADQUARTERS OR GENERAL BUSINESS OFFICES OF THE PUBLISHERS (Not printers) 413 Kellogg, Ames, Iowa 50010 Story County		
6. NAMES AND COMPLETE ADDRESSES OF PUBLISHER, EDITOR, AND MANAGING EDITOR		
PUBLISHER (Name and Address) International Association of Milk, Food and Environmental Sanitarians, Inc. 413 Kellogg, Ames, Iowa 50010 (PO Box 701)		
EDITOR (Name and Address) Dr. F. H. Marth, Babcock Hall, U. of Wisconsin, Madison, WI 52706		
MANAGING EDITOR (Name and Address) Earl O. Wright, 413 Kellogg, Ames, Iowa 50010 (PO Box 701)		
7. OWNER (If owned by a corporation, its name and address must be stated and also immediately thereunder the names and addresses of stockholders owning or holding 1 percent or more of total amount of stock. If not owned by a corporation, the names and addresses of the individual owners must be given. If owned by a partnership or other unincorporated firm, its name and address, as well as that of each individual must be given.)		
NAME ADDRESS International Association of Milk, Food and Environmental Sanitarians, Inc., 413 Kellogg - PO Box 701 Ames, Iowa 50010		
8. KNOWN BONDHOLDERS, MORTGAGEES, AND OTHER SECURITY HOLDERS OWNING OR HOLDING 1 PERCENT OR MORE OF TOTAL AMOUNT OF BONDS, MORTGAGES OR OTHER SECURITIES (If there are none, so state)		
NAME ADDRESS NONE		
9. FOR COMPLETION BY NONPROFIT ORGANIZATIONS AUTHORIZED TO MAIL AT SPECIAL RATES (Section 132.122, FPM) The purpose, function, and nonprofit status of this organization and the exempt status for Federal income tax purposes (Check one)		
<input checked="" type="checkbox"/> HAVE NOT CHANGED DURING PRECEDING 12 MONTHS <input type="checkbox"/> HAVE CHANGED DURING PRECEDING 12 MONTHS (If changed, publisher must submit explanation of change with this statement.)		
10. EXTENT AND NATURE OF CIRCULATION	AVERAGE NO. COPIES EACH ISSUE DURING PRECEDING 12 MONTHS	ACTUAL NO. COPIES OF SINGLE ISSUE PUBLISHED NEAREST TO FILING DATE
A. TOTAL NO. COPIES PRINTED (Net Press Run)	4258	3800
B. PAID CIRCULATION 1. SALES THROUGH DEALERS AND CARRIERS, STREET VENDORS AND COUNTER SALES	NONE	NONE
2. MAIL SUBSCRIPTIONS	3933	3467
C. TOTAL PAID CIRCULATION (Sum of B1 and B2)	3933	3467
D. FREE DISTRIBUTION BY MAIL, CARRIER OR OTHER MEANS SAMPLES, COMPLIMENTARY, AND OTHER FREE COPIES	52	52
E. TOTAL DISTRIBUTION (Sum of C and D)	3985	3519
F. COPIES NOT DISTRIBUTED 1. OFFICE USE, LEFT OVER, UNACCOUNTED, SPOILED AFTER PRINTING	273	281
2. RETURNS FROM NEWS AGENTS	NONE	NONE
G. TOTAL (Sum of E, F1 and 2—should equal net press run shown in A)	4258	3800
11. I certify that the statements made by me above are correct and complete.		SIGNATURE AND TITLE OF EDITOR, PUBLISHER, BUSINESS MANAGER OR OWNER Earl O. Wright, Mgr. Editor
12. FOR COMPLETION BY PUBLISHERS MAILING AT THE REGULAR RATES (Section 111, Postal Service Manual) 39 U.S.C. 3626 provides in pertinent part: "No person who would have been entitled to mail matter under former section 4359 of this title shall mail such matter at the rates provided under this subsection unless he files annually with the Postal Service a written request for permission to mail matter at such rates." In accordance with the provisions of this statute, I hereby request permission to mail the publication named in Item 1 at the phased postage rates presently authorized by 39 U.S.C. 3626.		
SIGNATURE AND TITLE OF EDITOR, PUBLISHER, BUSINESS MANAGER, OR OWNER Earl O. Wright, Mgr. Editor		

3-A Sanitary Standards for Portable Bins for Dry Milk and Dry Milk Products

Number 34-00

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. Portable dry milk bin specifications heretofore or hereafter developed which so differ in design, material and construction, or otherwise, as not to conform to the following standards but which, in the fabricator's opinion, are equivalent or better, may be submitted for the joint consideration of the IAMFES, USPHS, and DIC at any time.

A.

SCOPE

A.1

These standards cover the sanitary aspects of portable bins for storage, transportation and handling of dry milk and dry milk products in bulk. They do not pertain to (1) stationary bins, (2) bins (tanks) installed on automotive highway equipment, and (3) piping and appurtenances that are not attached to the bin.

A.2

In order to conform with these 3-A Sanitary Standards, dry milk bins shall comply with the following design, material and fabrication criteria.

B.

DEFINITIONS

B.1

Product: Shall mean the dry milk or dry milk product which is stored and/or transported in this equipment.

B.2

Dry Milk Bins: (Referred to hereinafter as "bins"). Shall mean portable bins in which products are stored and/or transported.

B.3

Product Contact Surfaces: Shall mean all surfaces that are exposed to the product or from which liquids and/or solids may drain, drop or be drawn into the product.

B.4

Non-Product Contact Surfaces: Shall mean all other exposed surfaces.

B.5

Mechanical Cleaning or Mechanically Cleaning: Shall denote cleaning, solely by circulation and/or flowing

chemical detergent solutions and water rinses onto and over the surfaces to be cleaned, by mechanical means.

C.

MATERIALS

C.1

Product contact surfaces shall be of stainless steel of the AISI 300 series¹ or corresponding ACI² types (see Appendix, Section E.), aluminum alloys conforming to the Aluminum Association³ designations 5052 and 6061, or metal which under conditions of intended use is at least as corrosion-resistant as stainless steel of the foregoing types and is non-toxic and non-absorbent, except that:

C.1.1

Rubber and rubber-like materials may be used for gaskets, inspection port covers and parts used in similar applications. These materials shall comply with the applicable provisions of the 3-A standard for rubber and rubber-like materials, Number 18-00.

C.1.2

Plastic materials may be used in sight and/or light openings and for gaskets, inspection port covers and parts used in similar applications. These materials shall comply with the applicable provisions of the 3-A standard for plastic materials, Number 20-00, as amended.

C.1.3

Rubber and rubber-like materials and plastic materials used for bonded gaskets having product contact surfaces, shall be of such composition as to retain their surface and conformation characteristics when exposed to the conditions encountered in the environment of intended use and in cleaning and bactericidal treatment.

C.1.4

The final bond and residual adhesive, if used, of bonded rubber and rubber-like material and bonded plastic

¹The 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 Street NW, Washington, D.C. 20036.*

²Alloy Casting Institute Division, *Steel Founders' Society of America, 20611 Center Ridge Road, Rocky River, OH 44116.*

³Aluminum Association, 420 Lexington Avenue, New York, NY 10017.

material shall be non-toxic.

C.2

Non-product contact surfaces shall be of corrosion-resistant material or material that is rendered corrosion-resistant. If coated, the coating used shall adhere. Non-Product contact surfaces shall be relatively non-absorbent, durable and cleanable. Parts removable for cleaning having both product contact and non-product contact surfaces shall not be painted if in cleaning they will be immersed in water and/or a cleaning or sanitizing solution.

D.

FABRICATION

D.1

Product contact surfaces shall be at least as smooth as a No. 4 finish on stainless steel sheets free of imperfections such as pits, folds and crevices (see Appendix, Section F.). Aluminum sheets with a mill finish free of imperfections such as slivers and scratches and also stainless steel sheets with a No. 2B finish free of imperfections such as pits, folds and crevices are considered to meet this finish criterion.

D.2

Permanent joints in product contact surfaces shall be continuously welded. Product contact surfaces of welded areas shall be at least as smooth as a No. 4 finish on stainless steel sheets free of imperfections such as pits, folds and crevices.

D.3

Bins that are to be mechanically cleaned shall be designed so that all product contact surfaces and all parts not removed during cleaning can be mechanically cleaned and can be visually inspected.

D.4

Product contact surfaces not designed to be mechanically cleaned shall be easily accessible for cleaning and inspection either when in an assembled position or when removed.

D.5

Parts that must be removed for cleaning shall be readily removable and easily dismantled.

D.6

Product contact surfaces shall be self-draining or self-purging, except for normal clingage when the bin is in the inverted or dump position.

D.7

Sanitary fittings shall conform to the applicable provisions of the 3-A standard for sanitary fittings, Number 08-17.

D.8

Thermometer connections, when provided, shall conform to the applicable provisions of the 3-A standard for instrument fittings, Number 09-07.

D.9

Gaskets having a product contact surface shall be removable or bonded.

D.10

Bonded rubber and rubber-like gaskets and bonded

plastic gaskets having a product contact surface shall be bonded in such a manner that the bond is continuous and mechanically sound and so that when exposed to the conditions encountered in the environment of intended use and in cleaning and bactericidal treatment the rubber or rubber-like material or the plastic material does not separate from the product contact surface.

D.11

Gasket retaining grooves in product contact surfaces shall be no deeper than their width.

D.12

The radius of the internal angle at the junction of the top, sides and bottom shall be not less than 1/2 inch. All other internal angles of 135° or less on product contact surfaces shall have radii of not less than 1/4 inch, except that:

D.12.1

The radii in gasket-retaining grooves, except those for standard 1/4-inch and smaller O-Rings, shall be not less than 1/8 inch.

D.12.2

The radii in grooves for standard 1/4-inch O-Rings shall be not less than 3/32 inch and for standard 1/8-inch O-Rings shall be not less than 1/32 inch.

D.13

There shall be no exposed threads on product contact surfaces.

D.14

An opening of sufficient size so that all product contact surfaces may be cleaned and/or inspected shall be provided. The dimension of the opening shall be not less than 15 inches by 20 inches oval, 18 inches in diameter or if generally rectangular, 14 inches by 20 inches.

D.15

Covers and/or Doors

Covers and/or doors for openings in bins shall comply with the applicable provisions of the following:

D.15.1

Covers and doors shall be sufficiently rigid to prevent buckling. Handles for covers and doors, if provided, shall be durable, of sanitary design, welded in place or formed into the cover material and conveniently located. Covers and doors shall be of the lift-off type or shall be of the outside swing type.

D.15.2

Threads or ball joints shall not be employed within the bin to attach an outside swing type cover and its appendages. The cover and its appendages, if any, shall be readily removable.

D.15.3

Hinged covers and doors for top openings shall be designed so that they can be maintained in an open position.

D.15.4

Hinged covers shall be designed so that when opening the cover, any liquid or dry material on the exterior will not enter the bin.

D.15.5

Covers and doors for top openings shall be self-draining in the closed position, shall be close fitting and shall have downward flanges of not less than 3/8 inch on all edges or shall be gasketed.

D.15.6

Hinges shall be demountable and readily cleanable. They shall not be of a continuous (piano) type.

D.16

Sight and light openings, when provided, shall be of such design and construction that the inner surfaces drain inwardly; and if the bin is designed for mechanical cleaning, the inner surface of the plastic shall be relatively flush with the inner surface of the bin. The exterior flare shall be pitched so that liquids cannot accumulate. The plastic shall be readily removable. The inside diameter of the opening shall be at least 3 3/4 inches.

D.17

Bins shall be supported on legs and/or casters that will provide a clearance between the lowest part of the bin and the floor of at least 4 1/2 inches. An exception is made to this 4 1/2 inch clearance requirement (1) for members provided on bins for rigidity and/or door protection that extend downward below the bottom of the bin and (2) for casters. Bins which have a member for rigidity and/or door protection which does not provide a 4 1/2 inch clearance between the lowest part of the member and floor shall be open on three sides and the clearance between the underside of the member and the floor shall be at least 1 1/2 inches. Legs, if provided, shall be smooth with rounded ends and have no exposed threads. Legs made of hollow stock shall be sealed. Casters shall be easily cleanable, durable and of a size that will permit easy movement of the bin.

D.18

Non-Product contact surfaces shall be free of pockets and crevices and be readily cleanable and those to be coated shall be effectively prepared for coating.

APPENDIX

E.

STAINLESS STEEL MATERIALS

Stainless steel conforming to the applicable composition ranges established by AISI for wrought products, or by ACI for cast products, should be considered in compliance with the requirements of Section C.1 here-

in. Where welding is involved, the carbon content of the stainless steel should not exceed 0.08 percent. The first reference cited in C.1 sets forth the chemical ranges and limits of acceptable stainless steel of the 300 series. Cast grades of stainless steel corresponding to types 303, 304 and 316 are designated CF-16F, CF-8, and CF-8M, respectively. These cast grades are covered by ASTM⁴ specification A296-68 and A351-70.

F.

PRODUCT CONTACT SURFACE FINISH

Surface finish equivalent to 150 grit or better as obtained with silicon carbide is considered in compliance with the requirements of Section D.1 herein.

G.

SUGGESTED CLEANING PROCEDURES

G.1

Dry Cleaning

G.1.1

Removable parts, if any, should be dismantled. The product contact surfaces and external surface(s) of the bin and those of any parts that are removed should be thoroughly vacuum or dry brush cleaned.

G.1.2

Vacuum cleaning is preferred to brush cleaning or cleaning with air under pressure as it decreases the dust drift problem to other areas of the plant.

G.1.3

Brushes or vacuum cleaner fittings used for cleaning product contact surfaces shall not be used for cleaning external surfaces or for other uses which might result in contamination. Such brushes and special fittings should be stored in an enclosed cabinet when not in use. (For protection and housekeeping consideration, such cabinets preferably should be of non-wood construction and should have open mesh metal shelving.)

G.2

Wet Cleaning: Removable parts, if any, should be dismantled. The surfaces of the bin should be rinsed with clear water and then thoroughly hand brushed with a general purpose dairy cleaner. Following brushing, the surfaces of the bin should be rinsed with clear water to remove soil and cleaning solution. It is recommended that the water used for rinsing following brushing be heated to at least 170°F (76.7°C) in order to sanitize the bin and to aid the subsequent drying. All parts should be allowed to air dry completely before using the bin.

These standards are effective January 22, 1977.

⁴Available from American Society for Testing and Materials, 1916 Race Street, Philadelphia, PA 19103.

Association Affairs

AFFILIATES OF

International Assn. of Milk, Food and Environmental Sanitarians

ALBERTA ASSOCIATION OF MILK, FOOD AND ENVIRONMENTAL SANITARIANS

Pres., N. Tiwari-----Edmonton
 Past Pres., L. M. McKnight-----Edmonton
 Pres.-Elect, F. Brinkland-----Edmonton
 Sec'y., E. J. Bittner, Dairy Division, Alberta Agriculture, Regional Office, Box 330, Vermilion, Alberta TOB 4M0 Canada
 Tres., J. E. Hoskins, Wetoka Health Unit, Wetaskiwin, Alberta, Canada
 Directors:
 R. Elliott-----Stettler
 L. Montgomery-----Calgary

ARIZONA ASSOCIATION OF MILK, FOOD AND ENVIRONMENTAL SANITARIANS

Pres., George H. Parker-----Phoenix
 Sec'y., Jerry Williams, 7536 West Acome Dr., Peoria, Ariz. 85346

CALIFORNIA ASSOCIATION OF DAIRY AND MILK SANITARIANS

Pres., Harold Y. Heiskell-----Sacramento
 First Vice-Pres., Richard L. Ayers-----Tulare
 Second Vice-Pres., Wayne Baragry-----Riverside
 Past Pres., Hugh H. Bement-----La Mirada
 Sec'y.-Treas., Manuel N. Abeyta, 314 Tocoloma Avenue, San Francisco, CA 94134

CONNECTICUT ASSOCIATION OF DAIRY AND FOOD SANITARIANS

Pres., Carl W. Jekanowski-----Wethersfield
 Vice-Pres., Kenneth J. Flanagan-----
 Sec'y., Julia A. Marshall-----Middletown
 Tres., Walter F. Dillman, Room 281, Dept. Of Agric., State Office Building--Hartford, CT 06115
 Asst. Tres., Henry Wilson-----Collinsville
 Board of Governors:

G. VanWormer-----Simsbury
 P. R. Vozzola-----West Granby
 B. Cosenza-----Storrs
 W. Bryant-----Newington
 W. W. Ullmann-----Hartford
 E. L. Johnson-----Hartford
 H. Hall-----Stratford
 J. Redys-----Hartford
 J. Marshall-----Middletown
 K. J. Flanagan-----
 J. G. Hanna-----
 D. Sheilds-----
 R. M. Parry-----Vernon

FLORIDA ASSOCIATION OF MILK AND FOOD SANITARIANS

Pres., Jay B. Boosinger-----Tallahassee
 Pres.-Elect, Tom Hart-----Fort Myers
 Sec'y.-Treas., John Miller, Upper Fla. Milk Producers Assoc., P.O. Box 6962, Jacksonville, FLA 32205

Board of Directors:

Dr. Ron Richter-----Gainesville
 Lupe Wiltsey-----Miami
 W. A. Brown-----Tampa
 Charles Vogelgesang-----Miami
 S. O. Noles-----Jacksonville
 Dr. L. A. Scribner-----Orlando
 Joseph L. Hayes-----Tampa

IDAHO ENVIRONMENTAL HEALTH ASSOCIATION

Pres. Stephan E. Bastian-----Preston
 Vice-Pres., Harold R. Hyer-----Boise
 Sec'y.-Treas., Jack Palmer, Bingham Co. Health Unit, Box 829, Blackfoot, ID 83221

ASSOCIATED ILLINOIS MILK, FOOD, AND ENVIRONMENTAL SANITARIANS

Pres., Charles Price-----Chicago
 Pres.-Elect, Lewis Schultz-----Springfield
 First Vice-Pres., John Oberweis-----Aurora
 Second Vice-Pres., John Dolan-----Chicago
 Sec'y.-Treas., Robert Coe, 2121 West Taylor St., Chicago, IL 60612

Sergeant-at-Arms, Dale Termunde-----Oak Brook
 Auditor, Joe Heneghan-----Chicago
 Auditor, Ray Moldenhauer-----Springfield
 Junior Past Pres., George Muck-----Rockford
 Senior Past Pres., Harold McAvoy-----Springfield

INDIANA ASSOCIATION OF SANITARIANS, INC.

Pres., Thomas H. Dorsey-----Indianapolis
 Pres.-Elect, Rick Lopez-----Muncie
 First Vice-Pres., David McSwane-----Bloomington
 Second Vice-Pres., Harry Werkowski-----Hammond
 Sec'y.-Treas., Earnest Parker, Indiana State Board of Health, 1330 W. Michigan St., Indianapolis, IN 46207
 Senior Past Pres., Paul Welch-----Terre Haute
 Junior Past Pres., Thomas Atkinson-----Richmond

IOWA ASSOCIATION OF MILK, FOOD AND ENVIRONMENT SANITARIANS, INC.

Pres., Chris Singelstad-----Cedar Rapids
 Pres.-Elect, Erwin Johnson-----Cedar Rapids
 First Vice-Pres., Carl Webster-----Cedar Falls
 Second Vice-Pres.,
 Don Larson-----Cedar Rapids
 Sec'y.-Treas., H. E. Hansen, State Health Department, Robert Lucas Building, Des Moines, Iowa 50319
 Faculty Advisor, Dr. William LaGrange-----Ames
 Advisor, Earl O. Wright-----Ames
 Immediate Past Pres., John Halbach-----Cedar Falls

KANSAS ASSOCIATION OF SANITARIANS

Pres., Don Bechtel-----Manhattan
 First Vice-Pres., James Pyles-----Topeka
 Sec. Vice-Pres., Tom Rodgers-----Dearing
 Sec'y.-Treas., John W. Zook, 1231 Wood Ave., Wichita 67212

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

Pres., James Spillman-----Louisville
 Past Pres., Bruce Lane-----Louisville
 Pres. Elect, Harry Marsh-----Lexington
 Vice Pres., Barry Kinslow-----Louisville
 Sec'y.-Treas., David Atkinson, 1016 Pinebloom Dr., Lexington, KY 40504

Directors:

Max Weaver-----Region:
 Bland Dorris-----Midwestern
 David Adams-----Midwestern
 Gene Morrell-----N. Central
 Terrance Haley-----N. Central
 Marvin Fornash-----N. Central
 James T. Harrill-----N. Central
 Bill Anderson-----S. Central
 Berford Turner-----Eastern
 J. B. Bowman-----Eastern

MICHIGAN ENVIRONMENTAL HEALTH ASSOCIATION

Pres., Richard Hunter-----Big Rapids
 Pres.-Elect, James Akers-----Kalamazoo
 Past Pres., Phillip Kirkwood-----Lansing
 Secretary, James Szejda, Ottawa County Health Dept., 414 Washington St., Grand Haven, MI 49417
 Tres., Michael Vanden Heuvel-----Muskegon
 Board of directors:
 Charles R. Newell-----Durand
 James P. Robertson-----Grand Blanc
 Oscar B. Boyea-----Pontiac
 K. Durwood Zank-----Charlotte
 Michael D. Farnsworth-----Monroe
 Thomas Nogel-----Ionia

MINNESOTA SANITARIANS ASSOCIATION

Pres., Arnold O. Ellingson-----Fergus Falls
 Vice-Pres., Peter Patrick-----St. Paul
 Sec'y.-Treas., Roy E. Ginn, Dairy Quality Control Institute, Inc., 2353 North Rice Street, St. Paul, MN 55113

Directors:

Mr. Douglas E. Belanger-----Minneapolis
 Mr. Arnold O. Ellingson-----Fergus Falls
 Mr. James H. Francis-----St. Paul
 Mr. Harold A. Johnson-----Minneapolis
 Mr. Walter H. Jopke-----Minneapolis
 Mr. Ing H. Lein-----Minneapolis
 Mr. Hugh Munns-----St. Paul
 Mr. James A. Rolloff-----New Ulm
 Mr. Charles B. Schneider-----Minneapolis

MISSISSIPPI ASSOCIATION OF SANITARIANS

Pres., Jimmy Wooten
 Pres.-Elect, Charlie Crews
 First Vice-Pres., Charles Howard
 Sec. Vice-Pres., Clinton Van Devender
 Past Pres., A. R. Russell
 Senior Past Pres., Paul M. Rankin
 Sec'y.-Treas., Homer Smith, P.O. Box 368, Jackson, MS 39205

MISSOURI ASSOCIATION OF MILK AND FOOD SANITARIANS

Pres., James Jameson-----Marshfield
 First Vice-Pres., Chester Edwards-----St. Joseph
 Second Vice-Pres., Joe Edmondson-----Columbia
 Past Pres., Michael Sanford-----Columbia
 Sec'y.-Treas., Erwin P. Gadd, Bureau of Community Sanitation, Missouri Div. of Health, Box 570, Jefferson City, MO 65101

NATIONAL ASSOCIATION OF DAIRY FIELDMEN

Pres., Sidney H. Beale, 1931 N. Every Road, Mason, MI 48854
 First Vice-Pres., William E. Stallard, 5379 Temple Court, Madison, WI 53705
 Second Vice-Pres., Clifford Mack, 371 Fifth Street, Prairie Du Sac, WI 53578
 Sec'y.-Treas., Arthur W. Johnson, Route #1, Box 683, Lake Geneva, WI 53147

NEW YORK ASSOCIATION OF MILK AND FOOD SANITARIANS

Pres., William Y. Perez-----Albany
 Pres.-elect, Albert J. Lahr-----Rochester
 Past-pres., Maurice Guerrette-----Albany
 Exec. Sec'y., R. P. March, 118 Stocking Hall, Cornell University, Ithaca, NY 14853

Directors:

Charlotte W. Hinz-----Buffalo
 Howard I. Cobb-----Syracuse
 William K. Jordan-----Ithaca
 Gaylord B. Smith-----Fultonville

ONTARIO MILK & FOOD SANITARIANS ASSOCIATION

Pres., John Wilson-----Simcoe
 Vice-Pres., Cyril L. Duitschaever-----Guelph
 Past Pres., William Kempa-----Toronto
 Sec'y., Gail Evans-----Islington
 Tres., M. A. (Vic) Amer, Gaylea Foods, 21 Speedvale Avenue West, Guelph, Ontario, Canada

Directors:

John Atkinson-----Ann Mayer
 Phil Glass-----Roger Wray
 Bill Breid
 Auditor:
 J. Raithby

Association Affairs

ONTARIO MILK AND FOOD SANITARIANS EASTERN BRANCH

Pres., Bob CarknerWinchester
First Vice-Pres., Jack Nicol.....Perth
Sec. Vice-Pres., Rheel Meilleur.....Ingleside
Treas., Ken Burnett.....Kemptville
Sec'y., Marvin Beach, Box 114, Kemptville, Ontario
Directors:
 Grant Cameron.....Alexandria
 Eves Leroux.....Balderson

OREGON ASSOCIATION OF MILK AND FOOD SANITARIANS

Pres., Terry SuttonPortland
Vice-Pres., Jim EyreEugene
Sec. Treas., Floyd Bodyfelt, Wiegand Hall 240,
 Dept. of Food Science & Technology, Oregon
 State University, Corvallis, Oregon 97331
Directors:
 Matthew V. AndrewsCorvallis
 Jim BlackSalem
 Walter IermanMcMinnville
 Donald RaistakkaPortland

PENNSYLVANIA DAIRY SANITARIANS ASSOCIATION

Pres., Don M. Breiner
Pres.-Elect., Ray Ackerman
First Vice-Pres., John Blyholder
Second Vice-Pres., John Boore
Sec'y.-Treas., Albert Gottfried, 4319 Pottsville Pike,
 Reading, PA 19605
Association Advisers: Stephen Spencer, Sidney
 Barnard, George H. Watrous, George W. Fouse
Executive Committee: Association Officers and ap-
 pointed representatives of regional associations

RHODE ISLAND DAIRY AND FOOD SANITARIANS

Pres., Richard Chambers.....Providence
Sec'y., Maury Dunbar.....Foster, R.I. 02825
Treas., Vincent Mattered, R. I. Dept. of Health, 2843
 South County Trail, East Greenwich, R.I. 02818

SOUTH DAKOTA ENVIRONMENTAL HEALTH ASSOCIATION

Pres., Edward MichalewiczBrookings
Pres.-Elect., Arnie BrownBrookings
Sec'y.-Treas., Thomas Goninon, 902 South Jay,
 Aberdeen, South Dakota 57401
Director: Lawrence ThompsonReliance
Past Pres., Robert WermersRapid City
Directors:
 Casper TwissPine Ridge

VIRGINIA ASSOCIATION OF SANITARIANS AND DAIRY FIELDMEN

Pres., L. T. LesterMeadowview
First Vice-Pres., D. E. Henderson.....Marion
Second Vice-Pres., L. C. Morgan.....Bedford
Int'l. Representative, A. N. Smith.....Flint Hill
Sec'y.-Treas., W. H. Gill, 6702 Van Buren Avenue,
 Richmond, VA 23226

WASHINGTON ASSOCIATION OF MILK SANITARIANS

Pres., Clayton Gustafson.....Vancouver
Pres.-Elect., William Brewer.....Seattle
Past Pres., Fred Froese.....Moses Lake
Sec'y.-Treas., Lloyd Lueddecke, NW 312 True St.,
 Pullman, WA 99163

WISCONSIN ASSOCIATION OF MILK AND FOOD SANITARIANS

Pres., Clifford Mack.....Prairie du Sac
Pres.-Elect., Leonard Rudie.....Appleton
1st Vice-Pres., Harlin Fiene.....Sauk City
2nd Vice-Pres., Norman Kirschbaum.....Madison
Past Pres., Elmer Marth.....Madison
Sec'y.-Treas., Don Raffel, 4702 University Avenue,
 Madison, WI 53705

WISCONSIN STUDENT AFFILIATE

Pres., Mark KubaEau Claire
Vice-Pres., Terry BrandenburgEau Claire
Treas., Marge Uebele.....Eau Claire
Sec'y., Linda Rott, 457 Summit Ave., Eau Claire,
 WI 54701



Members of the Iowa Association of Milk, Food and Environmental Sanitarians met in September to solidify plans for the 1977 IAMFES Annual Meeting. Seated are (left to right): David Kuick, Duane Hagedon (General Chairperson), Jim Burkett (Co-chairperson), Arthur Steinberg, Carl Webster, Dale Cooper and D. H. Wilke. Standing are (left to right): Ralph Smith, David H. Peper, Alan R. Larson, Richard Lane, Don L. Martensen, Bill Read and H. E. Hansen.

New York Association Holds 53rd Annual Conference

Two "firsts" for the New York State Association of Milk and Food Sanitarians took place at the Association's 53rd Annual Conference in Rochester. Mrs. Charlotte W. Hinz, Laboratory Director of Upstate Milk Cooperatives, became the first woman ever elected to the Association's Executive Board. Mrs. Hinz has been chairperson of the NYSAMFS Laboratory Practices Committee since 1972. In another "first," Mr. William Y. Perez was elected President-elect of the Association, but automatically assumed the office of President, which had been vacant due to the resignation of the 1976 President-elect. Mr. Perez is with the New York State Department of Health.

Nearly 400 persons attended the three-day conference, which was held jointly with the Food Science Department of Cornell University. Some program topics included sessions on sanitation of milk and food products, energy conservation, sampling in over-the-road tankers and a mini-course on low-acid food processing.

The following awards were presented at the awards banquet: The *Emmet R. Gauhn Award* for out-

standing service and leadership in behalf of the Association was given to Ray H. Bliss; Mildred M. Frank was the recipient of the *Paul B. Brooks Award* for outstanding contribution to the Association's affiliates; Albert B. Karasz received the *Howard Marlatt Award* for outstanding service in the field of laboratory technology; the *Dr. Theodore H. Reich Award* for outstanding service in the field of milk sanitation and quality control was awarded to Robert J. Guinther.

E. Harvey Davis, one of the Association's oldest active members, received a special award in recognition of his 40 years of service to the Association. Honorary Life Memberships were awarded to Dr. Norman Bartz, Charles H. Cleaveland, Ralph Bernstein, Marion C. Saltsman, Richard R. Franklin, Ray H. Bliss and Kenneth R. Leach.

Officers for 1976-1977 are William Y. Perez, President; Albert J. Lahr, President-elect; and Maurice Guerrette, Past-president. Mrs. Charlotte W. Hinz and Howard I. Cobb joined William K. Jordan, R. P. March and Gaylord B. Smith on the Executive Board.

Washington Affiliate Committee Reports

The following are reports of the Pipeline, Bulk Handling and Farm Building Committees of the Washington Association of Milk Sanitarians. Other affiliate members may find their conclusions to be of interest.

Pipeline Committee

- In one area of the State, P.V.C. plastic pipe solution line was installed on a *trial* basis. This type of pipe has not been accepted by (3A) Standards, U.S.P.H.S.—FDA or the State Department of Agriculture. Further such installations are not allowed and the existing ones will be debited on milk rating surveys until replaced. (3A) Standards require that all solution lines be of stainless steel or other equally corrosion resistant metal or resistant glass (Pyrex). Any joints must be smooth and flush. Research has indicated that acid cleaners can leach the P.V.C. from the pipes and contaminate the milk.
- The following pipeline size, slope and number of milking units were recommended as the *minimum* that would be accepted:

1-1½"	single slope	2 units
1-1½"	double slope	4 units
2"	single slope	4 units
2"	double slope	8 units
2-2½"	single slope	5 units
2-2½"	double slope	10 units
3"	single slope	9 units
3"	double slope	18 units

Bulk Handling Committee

- Committee recommended the installation of recording thermometers and interval timers on all new tanks to give 3 minutes of agitation every 30 minutes. The suggestion was made that tanker drivers be responsible for changing charts on the recording thermometers. No decision was reached on this recommendation.
- The Executive Committee agreed with Bulk Handling

Committee that all sight glasses be of standard size (1") and be made of Pyrex or acceptable plastic material. These sight glasses should be designed so that no milk is left in the lines after gauging.

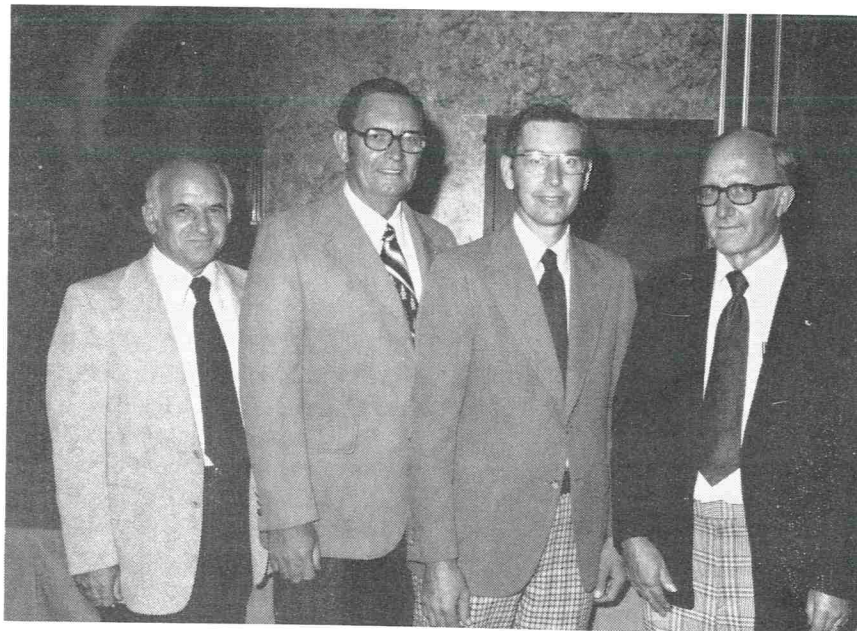
Farm Building Committee

- New PMO Code will include changes that will undoubtedly

permit storing and cleaning units in open type parlors and carrouseles.

The current PMO Code is not clear about storing of pesticides in the same general area as the milking units. The Survey Officer has been debiting farms that are currently following this practice.

Minnesota Affiliate Holds Annual Meeting



MSA officers for 1976-1977 are pictured (left to right): Peter Patrick, Vice-president; Arnold Ellingson, President; Roy E. Ginn, Secretary-Treasurer; and Edward Kaeder, Past-president.



Awards Chairperson James Francis (left) presents Honorary Life Membership to George Steele.



Dennis Bergquist (left) receives the 1976 Achievement Award from Edward Kaeder.

The University of Minnesota at St. Paul was the scene of the annual meeting of the Minnesota Sanitarians Association. More than 125 industry, education and regulatory representatives attended the two-day conference and received up-to-date information on food, dairy and environmental issues.

Joe Larson of Sparta Brush spoke at the Awards Banquet, at which recognition was given to outstanding affiliate members. The Honorary Life Membership was presented to

George Steele, recently-retired Director of Food Inspection and Research and Development of the Minnesota Department of Agriculture. Dennis Bergquist, Director of the Dairy Herd Management course at Hutchinson Vocational Technical School, was the recipient of the 1976 Achievement Award. Bergquist, an outstanding young dairyman, was given a plaque which read "in recognition of exceptional interest and concern for the sanitarian profession."

News and Events

Calendar of Events

January 12-13, 1977. DAIRY PROCESSORS CONFERENCE. Quality Inn Motel, Madison, Wisconsin. Sponsored by the Food Science Department, University of Wisconsin-Madison.

February 9-10, 1977. DAIRY INDUSTRY CONFERENCE. Center for Tomorrow, Ohio State University, Columbus, Ohio. For information contact: John Lindamood, Dept. of Food Science and Nutrition, 2121 Fyffe Road, Ohio State University, Columbus, OH 43210.

February 13-16, 1977. INTERNATIONAL EXPOSITION FOR FOOD PROCESSORS. Superdome, New Orleans, Louisiana. Sponsored by the Food Processing Machiner and Supplies Associa-

tion, 7758 Wisconsin Avenue, Washington, DC 20014.

March 21-25, 1977. MID-WEST WORKSHOP IN MILK AND FOOD SANITATION. Center for Tomorrow, Ohio State University, Columbus. For information contact: John Lindamood, Dept. of Food Science and Nutrition, 2121 Fyffe Road, Ohio State University, Columbus, OH 43210.

May 10-12, 1977. SECOND INTERNATIONAL POWDER & BULK SOLIDS HANDLING & PROCESSING SHOW. O'Hare International Trade & Exposition Center and the Regency O'Hare, Rosemont, IL. For information contact: Aaron Kozlov, Industrial & Scientific Conference Management, Inc., 222 West Adams St., Chicago, IL 60606. (312) 263-4866.

Toxic Substances Seminar

One of the most knowledgeable groups ever assembled of top officials from Government and the private sector will participate in the Toxic Substances Laws and Regulations Seminar, December 9-10, 1976, in Washington, D.C.

The program reads like a "who's who" in the field. The impressive list of notables includes: Congressman John Murphy who chairs the House subcommittee responsible for the current Toxic Substances legislation; Marshall Lee Miller, former Deputy Administrator, OSHA; Richard Denney, Associate General Counsel, EPA; David Hickman, Covington & Burling; J. Clarence Davies, Conservation Foundation; Dr. Irving Selikoff of Mount Sinai School of Medicine; Drs. Marvin Schneiderman and Umberto Saffiotti of the National Cancer Institute; Sheldon Samuels, Director of Health, AFL-CIO; William Butler, Environmental Defense Fund; Michael Brownlee, Senate Commerce Committee staff; Glenn Schweitzer, Director, Office of Toxic Substances, EPA; John G. Behun, Mobil Chemical; V. K. Roe, Dow Chemical; and other distinguished leaders.

This comprehensive group will analyze the current laws and regulations, explore the scientific basis and needs for regulations, review the union and industry reaction to the problem, identify the potential penalties both civil and criminal for failure to comply, and most importantly, learn all about the pending toxic substances legislation. The comprehensive sessions on the new legislation will focus on legislative background, how EPA and industry will implement its mandates plus a discussion on the future prospectus for achieving its goals.

The meeting should be of major interest to anyone (lawyers and non-lawyers) concerned with the many substances known considered toxic.

For a complete brochure on the seminar, contact Nancy McNerney, Government Institutes, 4733 Bethesda Avenue, NW, Washington, D.C. 20014. (301) 657-2922.

News and Events

Interstate Milk Shippers Procedures Revised

The monograph, "Procedures Governing the Cooperative Federal-State Program for the Certification of Interstate Milk Shippers" has been revised and is now available. The booklet contains the basic procedures adopted by the first conference of interstate milk shippers in 1950, and the revisions made at subsequent conferences through 1975. Single copies are available from the Dairy and Lipid Products Branch HFF-415, Food and Drug Administration, Public Health Service, U.S. Department of Health, Education and Welfare, Washington, D.C. 20204.

Illinois and Washington, D.C. Sign Reciprocal Agreement on Sanitation Certification

The first reciprocal agreement in the nation between health departments to recognize each other's foodservice sanitation certification has been signed by officials of Illinois and the District of Columbia.

Under terms of the agreement, a foodservice manager or supervisor certified for knowledge of sanitation in Illinois or the District of Columbia will now be permitted to work in either jurisdiction without further examinations. Both jurisdictions have enacted mandatory programs for the certification of supervisory foodservice personnel.

Dr. Bailus Walker, Jr., Director, Department of Environmental Services, District of Columbia stated that 6,150 District of Columbia citizens have completed training and been certified in Washington, D.C.

Dr. Joyce C. Lashof, Director of the Illinois Department of Public Health, said, "The state health department has so far issued approximately 2,500 certificates to Illinois citizens who have successfully completed the foodservice sanitation training."

In addition to Washington, D.C. and Illinois, the NIFI course, *Applied Foodservice Sanitation*, is recognized as fulfilling the requirements for sanitation education in 12 states with voluntary certification programs.

Mandatory sanitation certification is already in effect in the District of Columbia, and will be required in Illinois effective July 1, 1978. Training courses are currently being offered throughout the state at community colleges, four campuses of Chicago City College, and through the cooperation of the Chicago & Illinois Restaurant Association, the Illinois Department of Public Health, and the National Institute for the Foodservice Industry.

NIFI is the not-for-profit educational foundation established by the industry to advance professionalism in the foodservice industry.

Laughlin and Baird Re-elected BISSC Leaders

The 59th Meeting of the Baking Industry Sanitation Standards Committee was held at The Hyatt Regency, Washington, D.C., September 9-11, 1976. Representatives from all segments of the industry, as well as government health officials were in attendance.

Mr. Paul E. Laughlin, Director of Environmental Health and Service, NABISCO, Inc., East Hanover, New Jersey, was unanimously re-elected BISSC Chairman. Mr. J. Allen Baird, Executive Vice President, Mrs. Baird's Bakeries, Inc., Abilene, Texas, was unanimously re-elected BISSC Vice Chairman. Raymond J. Walter, Attorney-at-Law, continues as the Secretary-Treasurer and Counsel of BISSC with Executive Offices at 521 Fifth Avenue, New York, New York 10017.

The Baking Industry Sanitation Standards Committee was organized in 1949, to promote sanitation in the baking industry through the development, approval and publication of Sanitation Standards for Bakery

Equipment and Machinery. This was a joint effort on the part of six (6) national baking industry organizations: The American Bakers Association, the American Institute of Baking, the American Society of Bakery Engineers, the Associated Retail Bakers of America, the Bakery Equipment Manufacturers Association and the Biscuit & Cracker Manufacturers Association.

The members of these organizations, together with sanitation officials as consultants from the American Public Health Association, International Association of Milk and Food Sanitarians, U.S. Food and Drug Administration, U.S. Public Health Service, U.S. Department of Agriculture and the National Environmental Health Association, have, to date, developed and promulgated 37 Sanitation Standards for Bakery Equipment.

Through the cooperative efforts of the baker, engineer, equipment manufacturer, and their respective Associations, together with government and industry health and sanitation officials, great strides have been made in bakery equipment sanitation.

The next regular meeting of BISSC is scheduled to be held at the Hyatt Regency Chicago Hotel, Chicago, Illinois, March 3rd-5th, 1977.

Food Protection Conference Proceedings Available

Proceedings of the Conference entitled "Food Protection Paradox—

Should We Legislate or Educate?," held at the University of Minnesota May 4, 1976 are now available for purchase. Conference topics and speakers included: Risks/Benefits in Food Protection—Dr. Theodore P. Labuza, Professor, Department of Food Science and Nutrition, University of Minnesota; America's Food Supply & The Safest in the World—Dr. Howard R. Roberts, Director, Bureau of Foods, FDA, Washington,

News and Events

D.C.; The Impact of Food Regulations—Dr. Raymond Moshy, Vice President, Hunt-Wesson Foods, Inc.; Dr. Richard Feltner, Assistant Secretary of Agriculture for Marketing and Consumer Service, United States Department of Agriculture; The Nutritional Impact of Our Changing Food Supply—Dr. Mark Hegsted, Professor, Department of Nutrition, School of Public Health, Harvard University; Are Food Additives Over-regulated?—Dr. Bernard L. Oser, Bernard L. Oser Associates, Inc., Forest Hills, N.Y.; Educational Advertising and Other Forms of Communication Directly With the Consumer—Dr. Howard

Bauman, Vice President, Science and Technology, The Pillsbury Company; Legislation, Nutrification or Education—Dr. Paul Lachance, Professor, Department of Food Science and Nutrition, Rutgers—The State University, New Jersey; and Regulations—A Boon for Consumers—Jane Wyatt, Director of Consumer Affairs, State of Oregon.

Copies may be ordered directly from Dr. E. A. Zottola, 136 Meat Science Lab, University of Minnesota, St. Paul, Minnesota 55108. Cost is \$5.50 per copy. Checks should be made payable to the University of Minnesota and must accompany the order.

NIFI Advisory Committee on FDA Project Names Boyd Marsh Chairman



Boyd Marsh, Commissioner, Environmental Health Services, Cleveland Health Department (extreme right, wearing white shirt), was appointed Chairman of the Project Coordinating Committee of the National Institute for the Foodservice Industry. The Committee is assisting NIFI in developing a plan for implementing a uniform national foodservice sanitation training and certification program under a contract with the U.S. Food and Drug Administration. Committee members include representatives from regulatory agencies, industry,

education, and trade associations.

Attending the meeting were (clockwise from Marsh): James Meany, Chief Sanitary Officer, Chicago Department of Health; Robert Riley, General Manager, California Restaurant Association; Leroy Stratton, Director of Consumer Health Protection, Illinois State Department of Public Health; Andrew Poledor, Director of Public Health and Safety, National Restaurant Association; Martin Harder, Vice President, Marriott Corporation; Henry Montague, President, Michigan Restaurant Association;

Stanley Kubu, Vice President, Burger King Corporation; Charles Sandler, NIFI Director of Educational Information; Dr. Chester G. Hall, NIFI Executive Vice President; Charles Dee Clingman, NIFI Director of Sanitation Certification; Susan Rosenberg, Mr. Clingman's secretary; Carol Carlson, Executive Assistant to the President, ARA Services Inc.; Harold Kelly, NIFI Director of Operations; Art Graham, Director, and James McEntaffer, Manager, Quality Control and Sanitation, Pizza Hut Inc.; Charles Gossett, National Director of Environmental Health, L-K Enterprises, and David Hartley, Director of Public Health, National Automatic Merchandising Association. Committee members not present were Bailus Walker, Director of Department of Environmental Services, Environmental Health Administration, Washington, D.C., and Michael Leisure, Assistant Professor of Environmental Health, Illinois State University.

The next meeting of the Project Coordinating Committee has been scheduled for Chicago in mid-December.

Affiliate Meetings

CONNECTICUT—January 1977.
Hartford Area.

ILLINOIS—December 1976. Elgin.

KENTUCKY — February 22-23,
1977. Stouffer's Inn, Louisville.

MISSOURI—April 1977. Ramada
Inn, Columbia.

ONTARIO—March 1977. Holiday
Inn, Highway #427, Etobicoke.

EASTERN ONTARIO—November
1976. Kemptville.

PENNSYLVANIA — June 13-15,
1977. State College.

VIRGINIA—March 8-9, 1977. Don-
aldson Brown Center, VPI & SU,
Blacksburg.

Procedures to Investigate Foodborne Illness

Recommended By

**INTERNATIONAL ASSOCIATION OF MILK, FOOD AND
ENVIRONMENTAL SANITARIANS, INC.**

COPIES OBTAINABLE FROM

International Association of Milk, Food and Environmental Sanitarians, Inc.
P.O. Box 701, Ames, Iowa 50010

Price: Single copies \$1.50 each

10-99 copies \$1.25 each

100 or more \$.90 each

METHODS FOR PRODUCTION OF HIGH QUALITY RAW MILK

**(A Summary of Annual Reports Prepared From 1955 to 1970 by
the IAMFES Dairy Farm Methods Committee)**

COMPILED AND EDITED BY

J. C. FLAKE, A. E. PARKER, J. B. SMATHERS, A. K. SAUNDERS AND E. H. MARTH

COMPILED AND EDITED BY

**INTERNATIONAL ASSOCIATION OF MILK, FOOD, AND
ENVIRONMENTAL SANITARIANS, INC.**

COPIES OBTAINABLE FROM

International Association of Milk, Food, and Environmental Sanitarians, Inc.
P.O. Box 701, Ames, Iowa 50010

*Prices: Single Copies \$2.00 each—25-100 Copies \$1.75 each,
100 or More Copies \$1.50 each*

**PROGRAM
DAIRY PROCESSORS CONFERENCE**

Department of Food Science
University of Wisconsin-Madison
Madison, Wisconsin 53706
Wednesday, January 12, 1977
Milk Products versus Alternates
Professor M. P. Dean, Chairman

- 1:30 p.m. Welcome Dean Robert Bray
University of Wisconsin-Madison
- 1:45 Developments in Dairy Ingredient and Dairy Product
Alternates—Fats Donald E. Miller
Glidden-Durkee Division, SCM Corporation
- 2:20 Developments in Dairy Ingredient and Dairy Product
Alternates—Proteins Albert R. Kemp
Crest Foods Company
- 2:55 **COFFEE BREAK**
- 3:15 Use of Alternates in Simulated Dairy Products
..... George A. Muck
Dean Foods Company, Inc.
- 3:50 Evaluation of Functional Properties of Milk
Components as Food Ingredients W. James Harper
Ohio State University
- 5:00 **SOCIAL HOUR**
- 6:30 Wisconsin Dairy Tech Society Dinner
Who Will Feed the World? David P. Dickson
University of Wisconsin-Madison

Thursday, January 13, 1977

Whey Utilization

N. F. Olson, Chairman

- 9:00 a.m. Utilization of Liquid and Dry Whey and Whey
Permeates in Swine Feeding Robert H. Grummer
University of Wisconsin-Madison
- 9:35 Utilization of Whey Constituents in Reminant
Nutrition Hugh E. Henderson
Calor Agriculture Limited
- 10:10 **COFFEE BREAK**
- 10:30 Benefits of Applying Whey to Crop Land
..... Arthur E. Peterson
University of Wisconsin-Madison
- 11:05 Consumer Acceptance of Whey and Whey Products in
Foods Warren S. Clark, Jr.
Whey Products Institute

Thursday, January 13, 1977

Fluid Milk and Ice Cream

W. C. Winder, Chairman

- 9:00 a.m. Frozen Whipped Cream Robert L. Bradley, Jr.
University of Wisconsin-Madison
- 9:35 New Provisions of the Revised Grade A Pasteurized
Milk Ordinance Charles D. Price
Public Health Service, HEW
- 10:10 **COFFEE BREAK**
- 10:30 Ultra High Temperature Short Time Pasteurization
Re-evaluated E. L. Thomas
University of Minnesota
- 11:15 Status of Colors in Dairy Products ... Joachim von Elbe
University of Wisconsin-Madison

Thursday, January 13, 1977

Update on Dairy Plant Operations

Clyde H. Amundson, Chairman

- 1:30 p.m. Energy Conservation in Dairy Processing
..... B. J. Gaffney
Land O'Lakes, Inc.
- 2:10 Energy Outlook for Wisconsin Food Processors
in the Future C. J. Cicchetti
Emergency Energy Assistance, Wisconsin
- 2:50 Dairy Waste Management to Meet E.P.A.
Timetable James Harper
Ohio State University
- 3:30 Conference Wrap-up Kenneth G. Weckel
University of Wisconsin-Madison
- 3:45 **ADJOURN**



Avoid manual waste and overuse of detergents with KLENZADE® GENERAL CLEANER DISPENSING SYSTEM. Unit uses four venturi feeders to automatically blend liquid detergents, wetting agents and water conditioners. Lets you custom blend cleaning solutions for specific cleaning jobs.

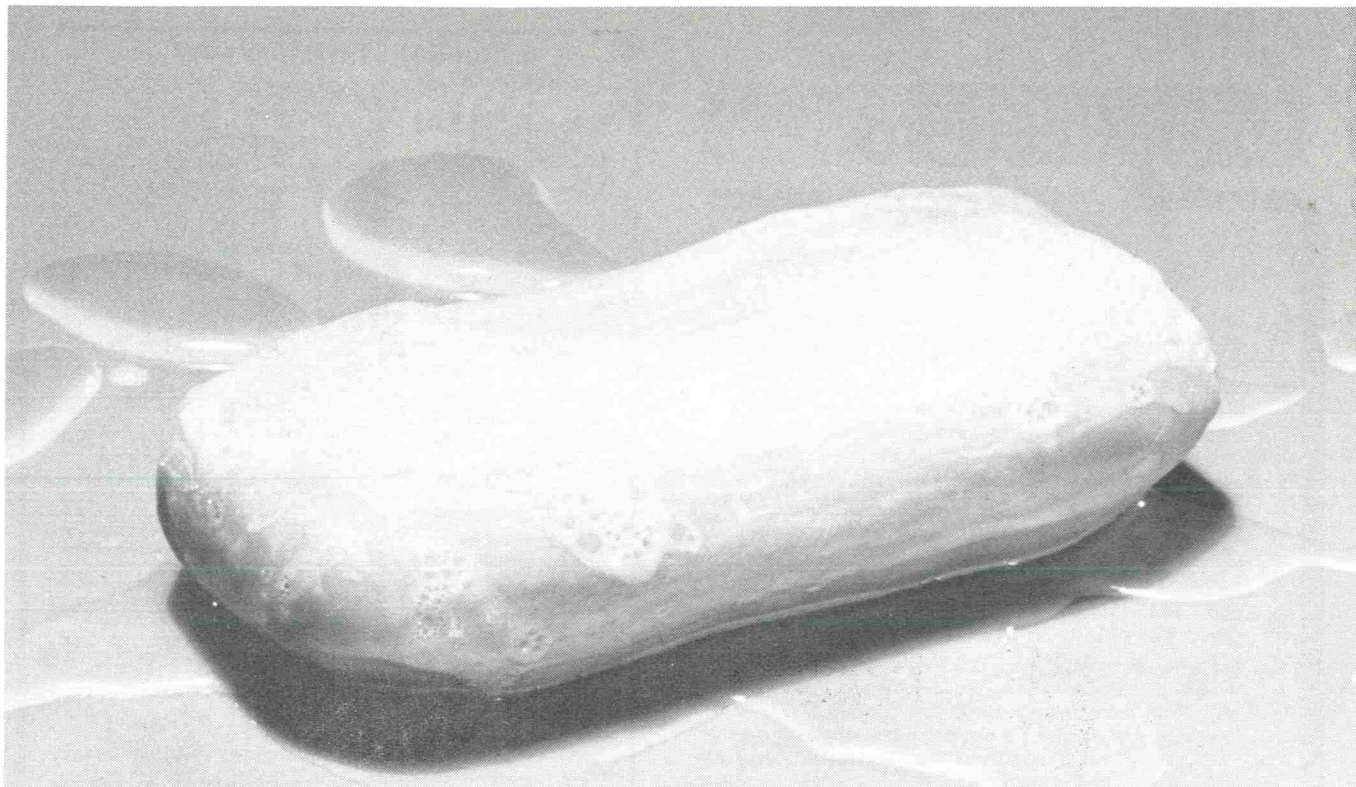


Klenzade DERMA-KLENZ™ Hand Wash removes soils, provides broad spectrum bacterial inhibition. Antimicrobial formula aids in total plant sanitation programs. Derma-Klenz lathers easily and has special emollients to help prevent skin irritation. Available in dispenser.



Uniforms, beef shrouds, cheese cloths, all can be handled with the KLENZADE-ECON SYSTEMS On-Premise Laundry Program. Here's total responsibility—from feasibility survey through planning, equipment and product supply, installation, service and maintenance. Using this program can save up to 30% over outside services.



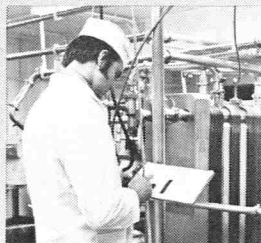


There's a lot of difference between soap and sanitation.

The Klenzade Difference.



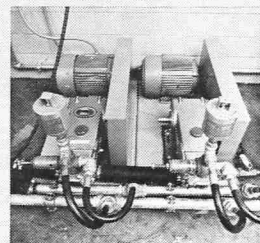
Klenzade sanitation starts with a plant survey...



... determines recommended cleaning and sanitizing products...



... establishes Standardized Cleaning Procedures for consistent results...



... with efficient, economical application equipment.

Soap is simply a compound of alkali and fats. It's a simple product for use on simple soils. Food soils, however, can present very complex cleaning problems. Included are soils such as those caused by proteins, fats, minerals, acids, sugars, bacteria, even heat-hardened soils.

Because of this wide range of soils, KLENZADE® has developed an equally wide range of detergents and sanitizers. These products are formulated to take full advantage of the chemistry of cleaning. Klenzade believes that fast, thorough and economical cleaning should rely on the powers of

chemistry rather than on elbow grease. Food soils are not just washed away. First they have to be penetrated and then dissolved, peptized or emulsified.

In our experience, there's no miracle detergent that is all things to all processors. Your plant is different. The soil conditions are different. The water is different. Even the necessary cleaning

procedures are different. That's why plant sanitation involves more than just selling soap. Klenzade brings all of these clean-up variables together in a program tailored to your particular sanitation needs. This program can help you protect food flavors and assure product quality.

Your Klenzade Representative has full details. He'll explain how our Technical Services Department can help with your special problems and why Standardized Cleaning Procedures are so important for consistent cleaning results. See him soon, or call Glenn Weavers, Director of Sales.



KLENZADE PRODUCTS DIVISION
Dept. 210
ECONOMICS LABORATORY, INC.
Osborn Building, St. Paul, Minnesota 55102
Telephone: 612/224-4678

Applications Engineer

GROW WITH THE PIONEERING LEADER IN LIQUID/SOLIDS SEPARATIONS TECHNOLOGY

With DORR-OLIVER, you'll be in an environment of engineering excellence, one you'll find truly responsive to the creative and technical ideas you can bring to this challenging position.

The successful candidate will have a Masters in Chemical Engineering, plus a solid background in food and dairy processes. Any membrane experience will be a plus.

Principal responsibilities will be handling development and management of prototype processes and application of our IOPOR Ultra-Filtration Systems.

We offer an excellent starting salary and benefits including relocation expenses. Position in our attractive, campus-style Southern Connecticut Headquarters provides a choice of suburban or metropolitan living, convenient to New York, New Jersey, or Connecticut.

For prompt, confidential consideration, send resume including salary history to: Sue Norton, Employment Representative



77 Havemeyer Lane, Stamford, Connecticut 06904
An equal opportunity employer M/F

CLASSIFIED AD

For Sale

Single Service milk sample tubes. For further information and a catalogue please write, Dairy Technology, Inc., P.O. Box 101, Eugene, Oregon 97401.

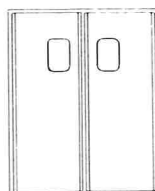
Index to Advertisers

Babson Bros.	Back Cover
Dorr-Oliver	812
Eliason Corporation	812
Enzyme Development Corporation	738
Haynes Manufacturing	Inside Back Cover
Klenzade Products	810, 811
National Sanitation Foundation	Inside Front Cover
Schlueter Company	738

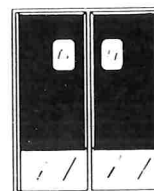
* "for SAFETY in doorways"
BUY OR SPECIFY Patented NO SPRING

ELIASON Easy Swing® DOORS

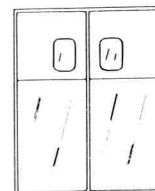
Self-Closing NO SPRING Double Action FOR Service and Convenience Doorways in FOOD SERVICE, SUPERMARKETS and other STORES, COOLERS, INSTITUTIONS, WAREHOUSES & PLANTS



LWP 3
LIGHTWEIGHT ALUMINUM



SCP 8
MEDIUM WEIGHT SOLID CORE



SCC 1
GASKETED COOLER

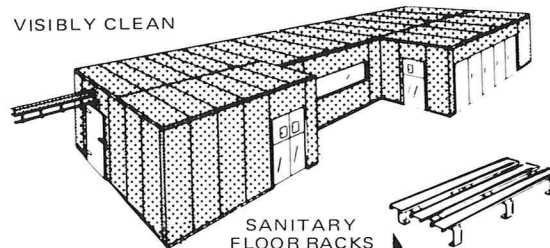
The above illustrations represent just a few standard doors shipped complete ready to install. Catalog lists hundreds of options, accessories and models.

* For Best in Food Protection Safety

BUY OR SPECIFY ELIASON WALK-IN COOLERS-FREEZERS

STEEL, RUSTPROOFED, FIREPROOFED, SANITARY.

VISIBLY CLEAN



SANITARY FLOOR RACKS and other accessories

Sectional. Many Stock Heights, Unlimited sizes and Arrangements of Individual or Composite Rooms. Galvanized, Enamel on Galvanized and Nickel Stainless Steel. NO FLAMABLY HAZARDOUS, TOXIC PLASTICS ALLOWED.

* HELP CONSERVE ENERGY

with Patented

ELIASON® ECONO-COVERS



Protects foods. Reduces rewraps. For ALL Open Refrigerator and Freezer Cases. USERS and CONSUMERS benefit with NIGHT CURTAINS.

*

FREE to ALL users of Food EQUIPMENT and PUBLIC HEALTH SANITARIANS. ELIASON authored, published, copyrighted "MANUFACTURERS warranty/guarantee of Sanitary, Safety, Performance SPECIFICATIONS PLAN" that obsoletes illegal sales seals, low criteria of so called voluntary Industrial Standards Enforcers.

A "Manufacturers direct commitment to user and valid Regulatory responsibility", not a rip-off to protective societies!

WRITE FOR USEFUL GUIDELINES.

ELIASON CORPORATION
P.O. BOX 2128 KALAMAZOO, MICH. 49003
Easy Swing DOORS · ECONO-COVERS · COMMERCIAL REFRIGERATORS
PHONE: 616/327-7003

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

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.



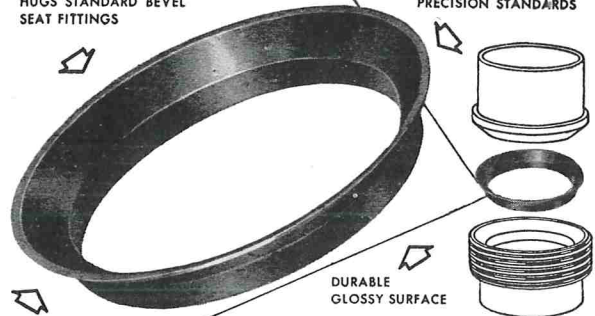
This Fine
Mist-like
HAYNES-SPRAY
should be used to lubricate:

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.

HAYNES SNAP-TITE GASKETS

"FORM-FIT" WIDE FLANGE
HUGS STANDARD BEVEL
SEAT FITTINGS

MOLDED TO
PRECISION STANDARDS



DESIGNED TO
SNAP INTO
FITTINGS

DURABLE
GLOSSY SURFACE

▶ **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



Gasket Color . . .
slightly off-white

HAYNES
SELF-CENTERING
SNAP-TITE
Gaskets

* MADE FROM
TEFLON[®]

SIZES 1" - 1½"
2" - 2½" - 3" - 4"

"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

Dairy authorities speak out on better cow milking



Stephen B. Spencer
Extension Dairy Specialist,
Pennsylvania State University

What's your score on vacuum?

An inadequate vacuum pump can affect the operation of the milking units drastically. Excessive "drop-off," slower milking, incomplete milking and an increase in the incidence of mastitis are likely results.

As a dairyman, your net dollar income depends on the vacuum pump and it probably is used more hours each year than most farm tractors.

Few people would hitch six plows behind a four plow tractor and head for the field to do a day's work. Yet many of these same people milk cows with a short vacuum supply and never question whether the pump is adequate.

Vacuum pumps used to be rated according to the number of units that could be operated. Today we measure the pump capacity in terms of Cubic Feet of air per Minute (CFM). Just as "horsepower" is more reliable than "plow rating" as an indication of tractor size, "CFM" is more reliable than "unit rating" when sizing a vacuum pump.

CFM output depends upon vacuum level. As vacuum level increases, the CFM output decreases. It's important to operate the system at the vacuum level specified by the manufacturer or the output of the pump will be altered.

The important consideration of any vacuum pump is the CFM output at the milking vacuum level. The pump must have adequate CFM output to meet the vacuum requirements of the system and provide sufficient reserve to maintain a constant vacuum level.

Vacuum Requirement

The milking unit is the most important of the machine components which admit air into the system. The air consumption of milking units varies depending upon shell and inflation size, pulsation rate and length and size of pulsated air tubes. Typically, the air requirement of a milking unit while it is not milking is three to four CFM. The pulsator consumes 50 to 70% of this volume. Considerably larger air pumping capacity (a reserve) must be provided to make a milking system operational. Other components which consume air are such things as vacuum operated door openers, milk metering devices, and the vacuum regulator. The requirements of each component must be added together to determine the system requirements.

Vacuum Reserve

The vacuum reserve is the air pumping capacity which remains after the vacuum requirement of all components has been satisfied. That's the problem. We've thought in terms of the vacuum reserve as the amount of CFM capacity that's *left over*. We really should be thinking in terms of a *base reserve* for the operator(s) before we begin to compute the system requirements.

The reserve is all-important in order to maintain vacuum stability. The reserve is necessary in order to make allowances for operator usage and possible leaks in the system or other contingencies.

The most important reason for an adequate vacuum reserve is to provide for the amount of air that the operator

will use. The operator is the largest user of the vacuum reserve. Some operators are very wasteful of the available reserve. This occurs as units are being attached and removed. Improper unit adjustment is also a significant factor. When teat cups start to leak and "squeal" during milking, the vacuum reserve is depleted rapidly.

Some operators may deplete vacuum reserve as much as 30 or more CFM for short periods of time. The careful operator will use but half that amount during the milking process. The real test of any milking system is when a milking unit falls off. It takes huge reserves of air just to keep the remaining units on the cows. Reserve tanks aid a little during these occurrences but basically the vacuum pump must be relied upon to maintain vacuum level. It all adds up to the fact that an adequate pump is a *must* for every dairyman.

Research in Ireland, Wisconsin, Pennsylvania and California indicates that inadequate vacuum reserve is associated with higher leucocyte counts. In plain language it means that mastitis can result if your vacuum pump isn't large enough.

What's your Vacuum Score?

Don't make a mistake and just assume that your pump is putting out enough air. Have it checked with an air flow meter once a year. Many dealers are equipped to do this for you.

And how will you know for sure that they're not just trying to sell you a pump? Frankly, I've found most dealers to be very reliable in this respect.

There have been many different recommendations about pump sizes. It's hard to give one that's exactly right for each system. Here's a guide for you to check your vacuum needs. It's based upon the New Zealand Standard. The American Standard would give values equal to one-half of the New Zealand Standard.

For bucket users:

Allow 4 CFM per unit + 20 CFM base reserve.

For pipeline users:

Allow 5 CFM per unit + 40 CFM base reserve for the first operator and 20 CFM for each additional operator.

The resulting CFM values would give you the minimum size vacuum pump capacity. If your system has more than this, fine. If you have less vacuum capacity than this you should carefully investigate your vacuum needs.

This method of determining vacuum capacity is different from what you may have seen before. A 50 percent reserve is commonly used. While a 50 percent reserve may be satisfactory on a system of six or more units, our field studies indicate that using a 50 percent reserve is not adequate for the smaller system.

Remember this: There is no substitute for an adequate vacuum system. Make sure you know your score on your vacuum needs.

"You're a step ahead with Surge"



Babson Bros. Co., 2100 S. York Rd., Oak Brook, Illinois 60521

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