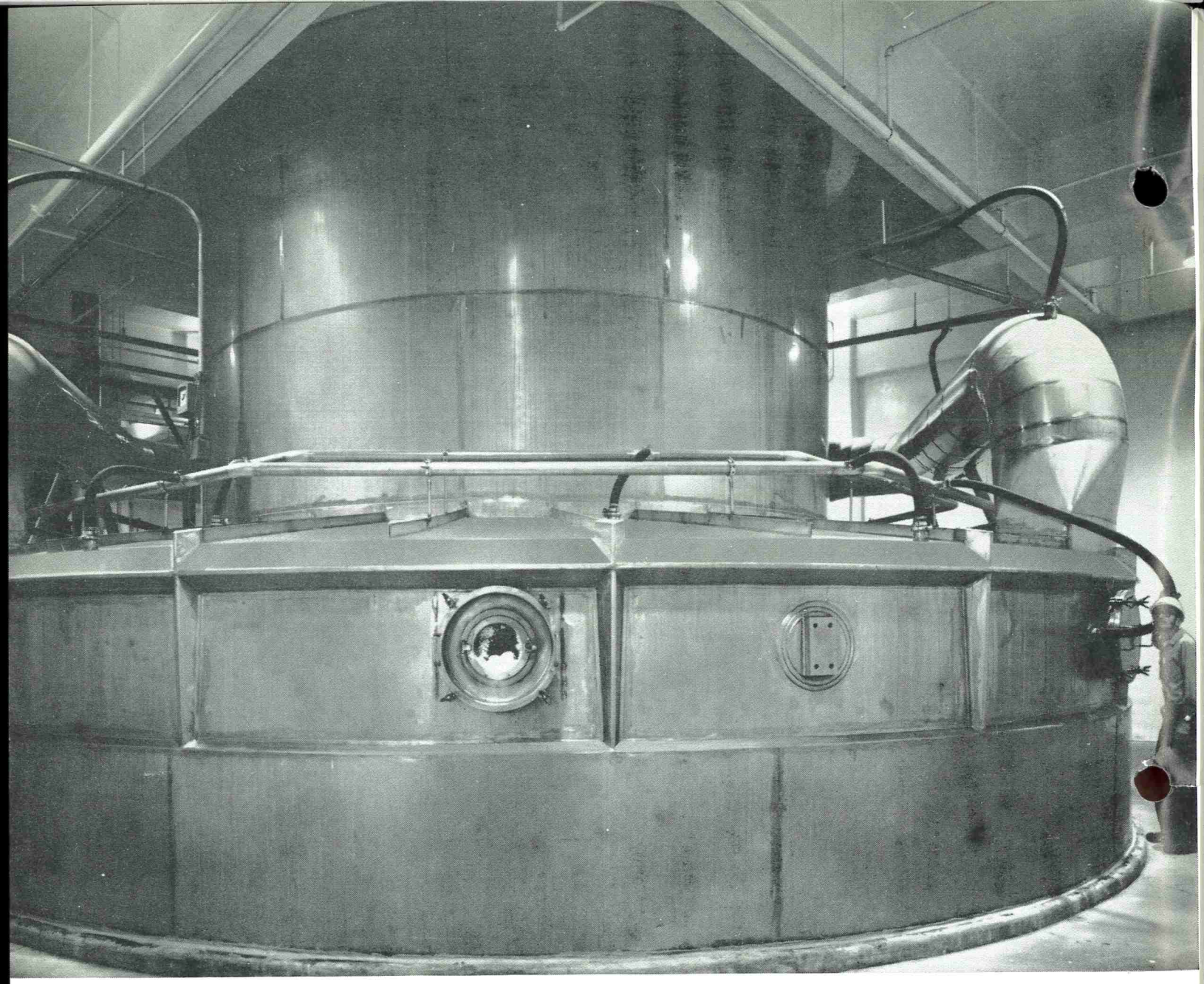
December 1975
Vol. 38, No. 12
Pages 729-816
JMFTA 38(12):729-816 (1975)

Journal of Milk and Food Technology

Official Publication



At Carnation, Jacksonville...

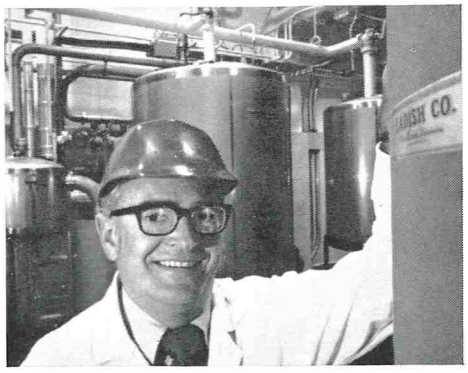
Reduced cleaning time means more production time with Tri-Clover CIP System

This massive 110 ft., 6-story spray dryer, used in the production of Coffee-mate®, would take a week to clean by hand.

At the Carnation Company, Instant Products Div., Jacksonville, Illinois an innovative Tri-Clover clean-in-place system all but eliminates hand cleaning—helping to maximize our customer's investment in productive processing machinery.

Another Tri-Clover CIP system handles the flow of materials through the wet blend process (photo, right). Reliability of these systems has reduced shutdown for cleaning to ⅓ of anticipated time. Savings amount to 5 or 6 hours a week.

At Tri-Clover we *specialize* in process flow control—developing processing and cleaning systems designed to help customers apply innovative machinery—to increase production and reduce unit costs.

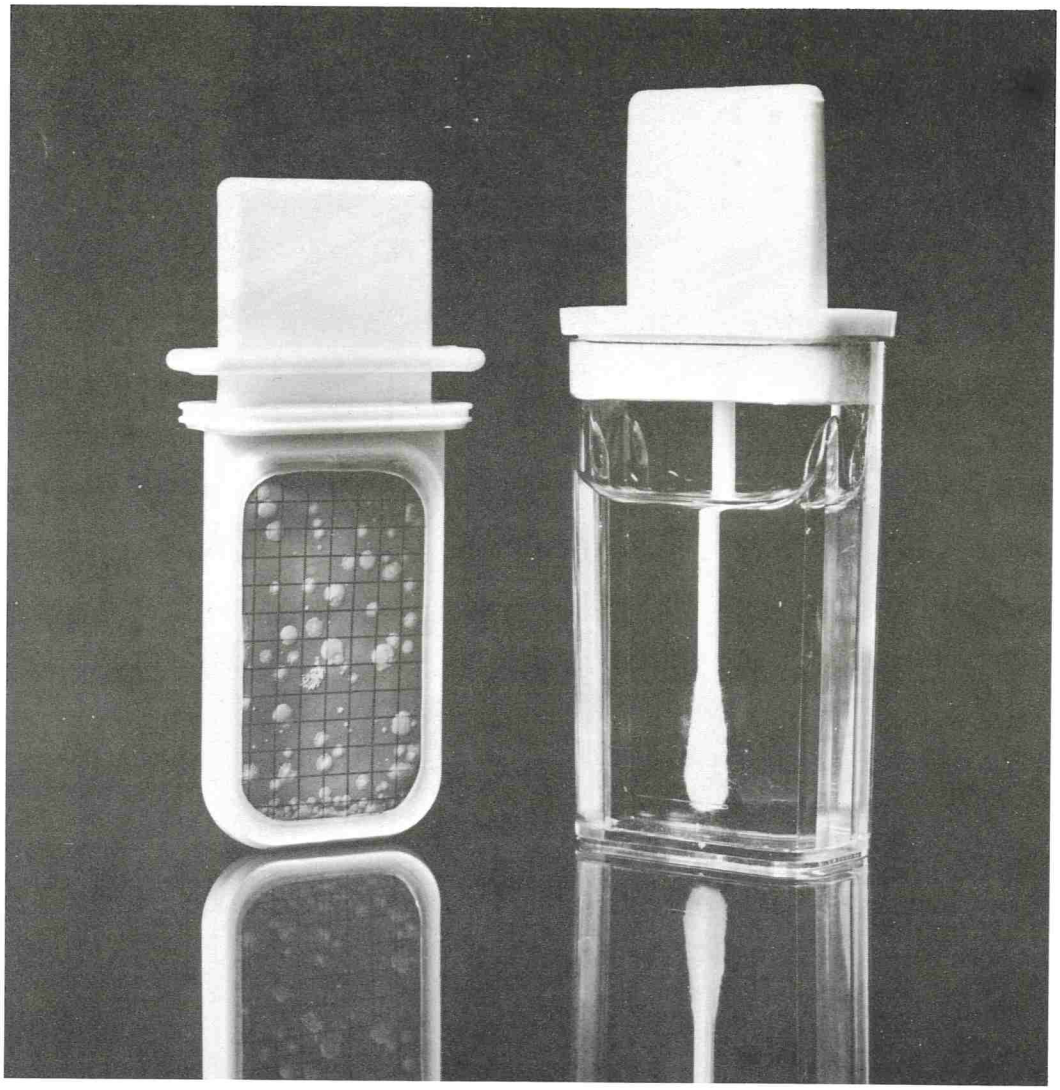


Send for New 12 page Bulletin on Tri-Clover CIP Systems: BULLETIN CIP-75

LADISH CO.

Tri-Clover Division
Kenosha, Wisconsin 53140
and Brantford, Ontario

S-274R



Sanitation

You Can't Control It If You Can't Measure It!

This inexpensive SWAB TEST KIT makes it easy to count bacteria, yeast or mold on sanitized surfaces. You won't need a laboratory or bacteriologist. Just carry several in your pocket.

Use it to find out how well you're controlling your sanitary conditions.

And where your problems are. Use it to gauge progress, evaluate changes and correct inefficient cleaning practices.

You'll find details in our free booklet, "Testing Surface Sanitation With the Swab Test Kit." Send for it. Millipore Corporation Bedford, Mass. 01730*

MILLIPORE®

*Or Call **800-225-1380** Toll Free
In Massachusetts Call (617) 275-9200

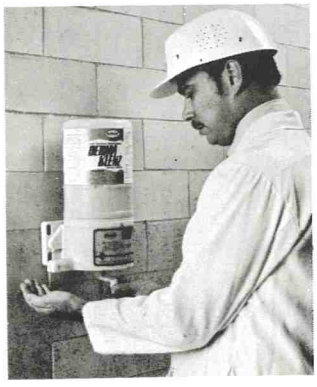
Here's why it won't take you to the cleaners:

First, the KLENZADE® FRASER On-Premise Laundry Program can save you up to 30% over outside contract services. And second, there are no hidden costs. Klenzade Fraser provides total responsibility, from feasibility survey, through architectural planning, supply of equipment and detergents, installation supervision, service and maintenance.

Because this program starts with a feasibility survey, you know the bottom line in advance. It pinpoints every cost, from plant space to detergents to utilities. So you know—up front—what you get, what it costs and what you save.

Klenzade Fraser offers a wide range of specially engineered washers, dryers and utility equipment. For example, the WASHMASTER™ washer-extractor uses computerized push-button programming to custom blend detergents and additives according to your water and soil conditions.

For more information, see your Klenzade Representative, or call Glenn Weavers, Director of Sales.



(Left) New DERMA-KLENZ™ antimicrobial Hand Wash offers fast soil removal, plus bacteria and mold control. Pleasant smelling Derma-Klenz lathers easily, leaves hands and arms clean and soft. Available with dispenser.

(Right) New PORTA-WASHER™ MODEL WM provides convenient high pressure cleaning. Wall-mounted unit installs in remote location to service drop station anywhere in plant. Delivers 3 gpm at 700 psi. Has automatic pressure switch to shut off pump if water supply is cut off.

KLENZADE PRODUCTS DIVISION

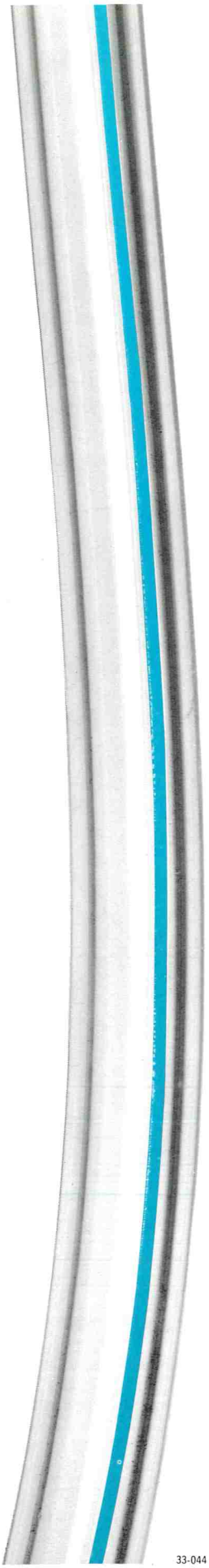
ECONOMICS LABORATORY, INC.
Osborn Building
St. Paul, Minnesota 55102
Telephone: 612/224-4678

KLENZADE®

Transflow M-34R tubing's distinctive Blue Stripe is your best defense against tubings of a lesser stripe.

Here's why.

Transflow® and the Blue Stripe® are registered trademarks of Norton Company, and they're your guarantee that the tubing you buy is the leader in the points that matter to dairymen. Its bore is smooth and dense and won't trap butterfat and milk soil. It's easy to clean, plus its crystal clarity takes the guesswork out of its cleanliness. It'll give you longer life, because it's non-aging. It meets FDA requirements and all criteria in the 3-A Plastics Standard. Perhaps most importantly, Transflow M-34R tubing is tasteless and odorless, guarding your milk's flavor every foot of the way. For the Transflow tubing story in detail, write Norton Company, Plastics and Synthetics Division, Box 350, Akron, Ohio 44309.



NORTON PLASTICS AND SYNTHETICS DIVISION
TUBING AND MOLDED PRODUCTS, AKRON, OHIO 44309 TEL: (216) 633-3224

33-044

The stain in the main goes neatly on the plane...

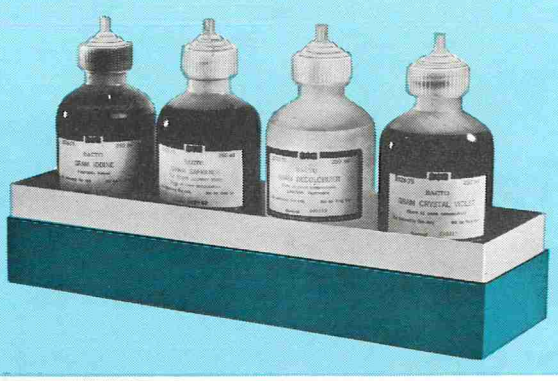
with Bacto-Gram Stain Set.

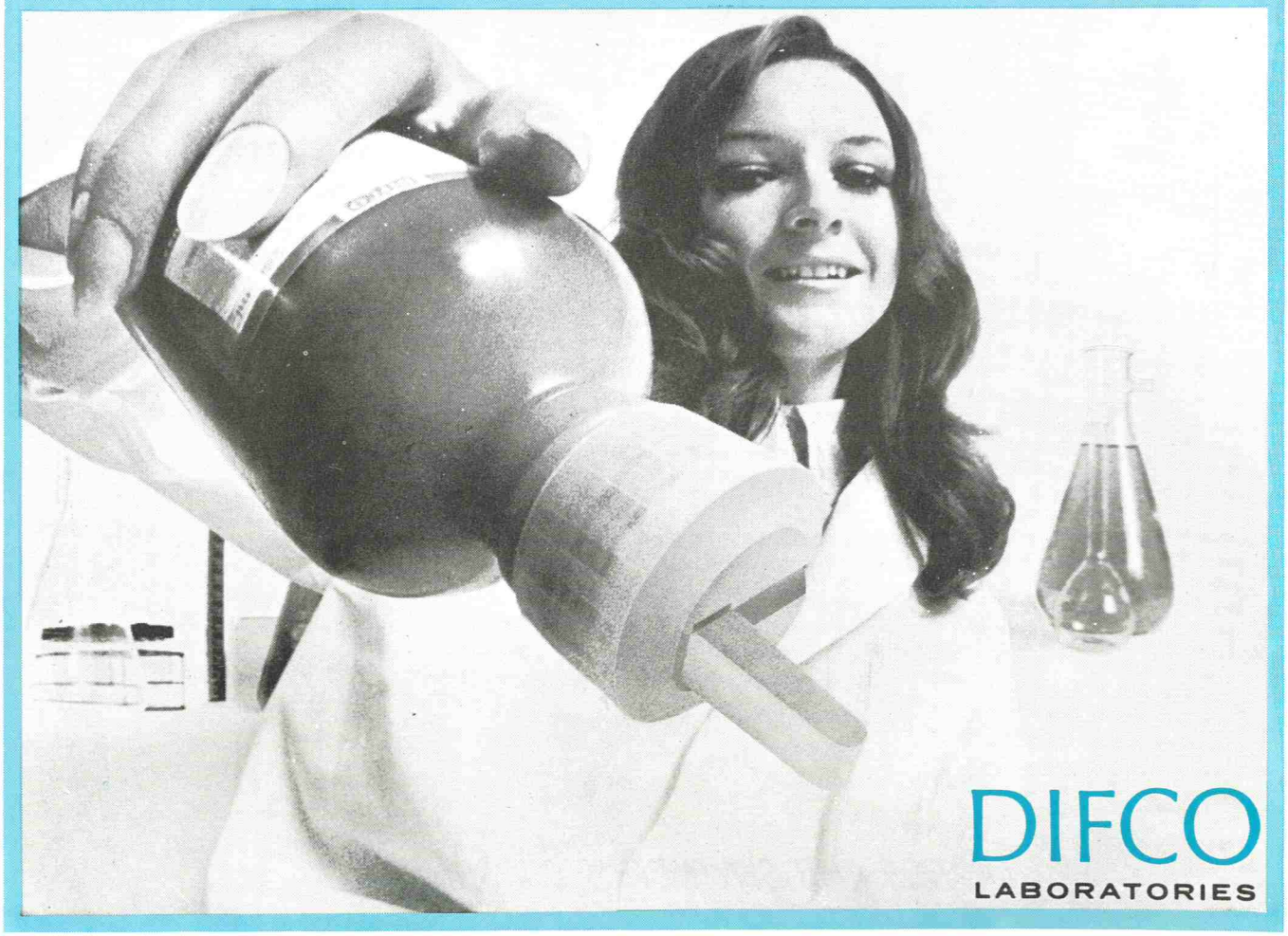
Stains come in 250 ml. plastic bottles, ready to use. The special snorkel-top plastic cap supplied with each bottle puts the stain right where you want it, on the slide, not on hands or lab coat. The base of the set serves as a rack for the stain bottles. When a bottle is empty, simply discard and replace it from the handy economical 250 ml. 6-pack. The replacement eliminates accidental spillage while pouring from one bottle to another.

No time-consuming and messy dilution or preparation necessary. No weighing, filtering, or costly waste of material. Stains will not precipitate. All reagents are carefully standardized; Difco quality assures consistent, reproducible, accurate results every time. End messy stains in your laboratory. Write for literature 3328. P.O. Box 1058A, Detroit, Michigan 48232.

The set includes solutions for the complete procedure:

- Bacto - Gram Crystal Violet
- Bacto - Gram Iodine
- Bacto - Gram Decolorizer
- Bacto - Gram Safranin.





DIFCO
LABORATORIES

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.

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. 38 December 1975 No. 12

Editorial Offices: Dr. Elmer H. Marth, Dept. of Food Science, University of Wisconsin, Madison, Wis. 52706. 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.

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

Subscription Rates: One volume per year individual non-members. Government and Commercial Organizations subscription.

1 yr. Vol. 39, 1976----$32.00
Public and Education Institution Libraries 1 yr.----$32.00
Single Copies----$ 2.50

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

Orders for Reprints: All orders for reprints should be sent to the executive office of the Association, P.O. Box 701, Ames, Ia. 50010.

Membership dues: Membership in the International Association of Milk, Food and Environmental Sanitarians, Inc., is $14.00 per year, and Special Certified Student membership is $5.00 per year, which includes annual subscription to the **Journal of Milk and Food Technology**. All Correspondence regarding membership, remittances for dues, failure to receive copies (notice of failure to receive copies must be reported within 90 days, otherwise single copy rate will apply) of the **Journal**, changes in address and other such matters should be addressed to the Executive Secretary of the Association, Earl O. Wright, P.O. Box 701, Ames, Ia. 50010.

J. Milk Food Technol. Vol. 38, No. 12, Pages 734-737 (December, 1975)

Temperature Control

I. A Procedure for Profiling Temperatures of Dairy Products in Stores

F. W. BODYFELT and W. D. DAVIDSON

Department of Food Science and Technology
Oregon State University, Corvallis, Oregon 97331

(Received for publication January 2, 1975)

ABSTRACT

Temperatures of perishable dairy products in 30 Oregon retail food stores were determined systematically with the aid of a quick response electronic thermometer. Sensitive thermistor surface probes permitted measurement of product temperatures from the exterior surfaces of two adjacent containers to within 0.5 F. A switch box facilitated the simultaneous use of up to six probes for measuring product, air blower, and air "curtain" temperatures at various cabinet shelf locations. Fluid milk temperatures for the outside rows of shelves averaged 4.5 F higher than the inside rows of product. The average temperatures for products in refrigerated display cases were: fluid milk and cream, 43.9 F; cottage cheese, 46.4 F; and hard cheese, 48.8 F. More than 35% of all products checked exceeded the Oregon maximum legal temperature of 45 F and 75% of the products exceeded the generally accepted optimum storage temperature of 40 F. The data indicate a definite need for improvement in temperature control of refrigerated display cases used for perishable dairy products. A form was designed to simplify and efficiently record temperature data for retail food stores.

Keeping quality of perishable dairy products such as fluid milk, cream, and cottage cheese depends on initial quality of the product; on care and techniques employed in processing and distributing; and on storage temperatures. Temperature control is generally considered the key to extended shelf life for dairy products. A common rule-of-thumb followed by the dairy industry is "for each 5 F increase in temperature, the expected shelf life can be reduced by one half." Stated another way, a storage temperature of 35 F should provide 20 days shelf life, 40 F should provide 10 days, but 45 F would provide only 5 days shelf life (*6*). Most psychrotrophic or spoilage type bacteria are capable of comparatively rapid growth at temperatures above 50 F, moderate growth at 46-50 F, slow growth at 41-45 F, and much slower growth at temperatures below 40 F (*7*).

Approximately 85% of all the milk sales in the U.S. occur through retail food stores, hence stores can be considered a most critical link in the distribution chain for milk products. Shea, director of marketing and industry relations for the California Milk Advisory Board has indicated that store temperature control is the number one marketing problem for milk products. Responsible retail food store personnel must be made aware of product storage and handling requirements for perishable dairy products if total quality assurance is to be achieved.

The ability to quickly and accurately detect specific temperature control problems is a very helpful aid in any milk quality assurance program. Bandler (*1*) has demonstrated the application of an electronic temperature sensing device equipped with thermistor type surface, air, and stab probes for evaluating critical temperatures in milk processing and distribution. Recently, Bodyfelt (*3, 4*) has reported on the application and sensitivity of the same electronic thermometer for temperature control evaluation in fluid milk and cottage cheese plants.

This survey was conducted in 30 retail food stores in Oregon to determine temperatures of perishable dairy products. A systematic approach for evaluating the refrigeration performance of store walk-in coolers and dairy display cases was also developed. The data and observations have aided store management in correcting temperature control problems and provided material for conducting educational seminars for store personnel.

MATERIALS AND METHODS

The YSI Model 42SF Tele-thermometer, equipped with flat surface (YSI 408) and air probes (YSI 405) were employed to measure the temperature of dairy products in retail food stores. A YSI Model 4002 switch box (Fig. 1) with 12 jacks for thermistor probes facilitated rapid

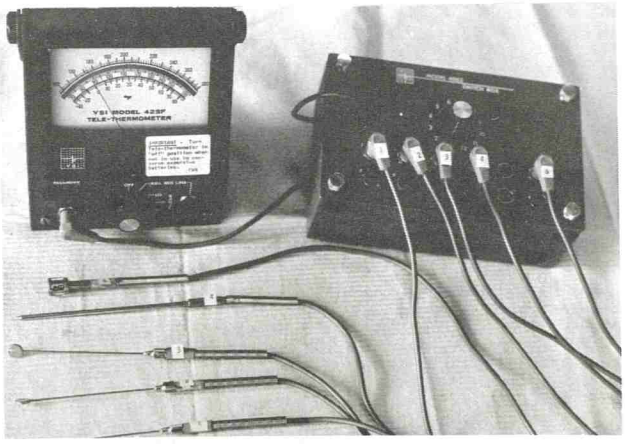


Figure 1. *Electronic thermometer, probes, and switch box used for obtaining temperature data.*

Figure 2. *Placement of surface probes directly between the exterior surfaces of adjacent containers of product.*

determination of product temperatures on the several shelves of the refrigerated display cases and in the walk-in coolers of each store.

The sensitivity and accuracy attained with the Tele-thermometer and surface probe readily permitted temperature measurement from the outer surface of the container. The surface probe was placed directly between the exterior surfaces of two containers of refrigerated product (Fig. 2). The temperature was registered on the direct reading scale of the electronic thermometer within approximately 20 sec. The product temperature was determined by subtracting 0.5 F from the temperature reading of the adjacent container surfaces (*3*). Temperature readings for the entering air from the source (blower) for walk-in coolers and all display cases were measured at a location 1-2 inches from the fan shield or duct, respectively (Fig. 3).

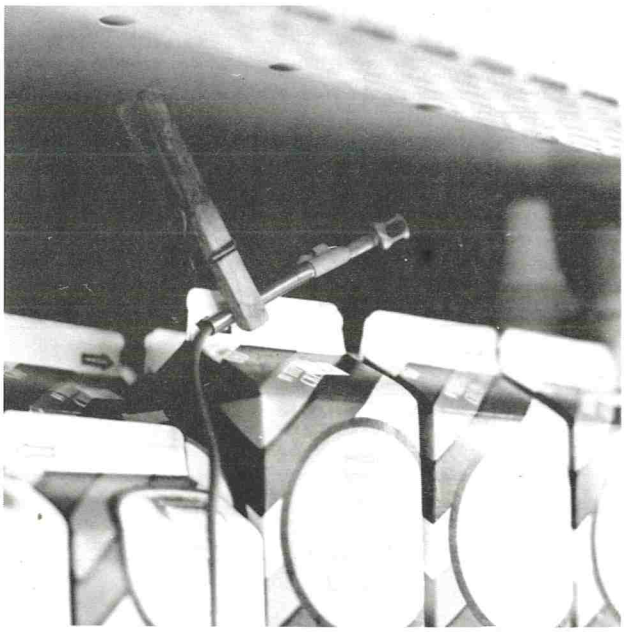


Figure 3. *Technique for positioning probe for measurement of entering air from case blower.*

Permission of the store manager was always secured before proceeding with the survey of dairy product temperatures. The purpose and intent of the activity were carefully explained and discussed with responsible store personnel. The two-man team conducting the survey also indicated that a copy of the recorded data and observations would be provided at the conclusion of the temperature control evaluation of the store.

Data and other pertinent observations were recorded on the form shown in Fig. 4. The temperature control conditions observed throughout the store were critiqued with appropriate store personnel.

RETAIL FOOD STORE

Perishable Dairy Products

TEMPERATURE PROFILE

Store _____ Date _____

Address _____ Time _____

_____ Manager _____

I COLD ROOM (Walk-in Cooler)

Indicated _____ °F Blower air temp._____°F Room (Ave.)_____°F

Product:_____ _____ _____ _____

Temp._____°F _____°F _____°F _____°F

Time in cooler:_____ _____ _____ _____

II DAIRY CASE (°F)	A			B			C		
	Air	Type Product	Prod.Temp. (1) : (2)	Air	Type Product	Prod.Temp. (1) : (2)	Air	Type Product	Prod.Temp. (1) : (2)
Top Shelf			:			:			:
2nd Shelf			:			:			:
3rd Shelf			:			:			:
4th Shelf			:			:			:
5th Shelf			:			:			:
Blower Temp.									
Overload Line			:			:			:
Indicated Temp.									

NOTES: _____

_____

_____

(1) Inside row (2) Outside row

Figure 4. *Form used for recording temperature data for retail food stores.*

RESULTS AND DISCUSSION

Table 1 summarizes the temperature data for 30 retail food stores, for the selected checkpoints for air and products in walk-in coolers and refrigerated display cases. The range in temperature for entering air from the blowers in all walk-in coolers was 26-50 F; four stores had air blowers in excess of 45 F. The highest product temperature recorded in a walk-in cooler was 45 F; the range in temperatures of stored products was 34-35 F. For the 30 coolers, the average temperatures for products and air blowers were 39.1 F and 36.0 F, respectively. From these observations, it is concluded that the air temperature at the blower should not exceed 35 F, if the products are to be maintained below 40 F in the walk-in cooler. However, a temperature of 28-32 F for the air supply would be more satisfactory. By contrast, the Commercial Refrigeration Manufacturers Association guideline (*6*) for air supply temperature appears to be 45 F.

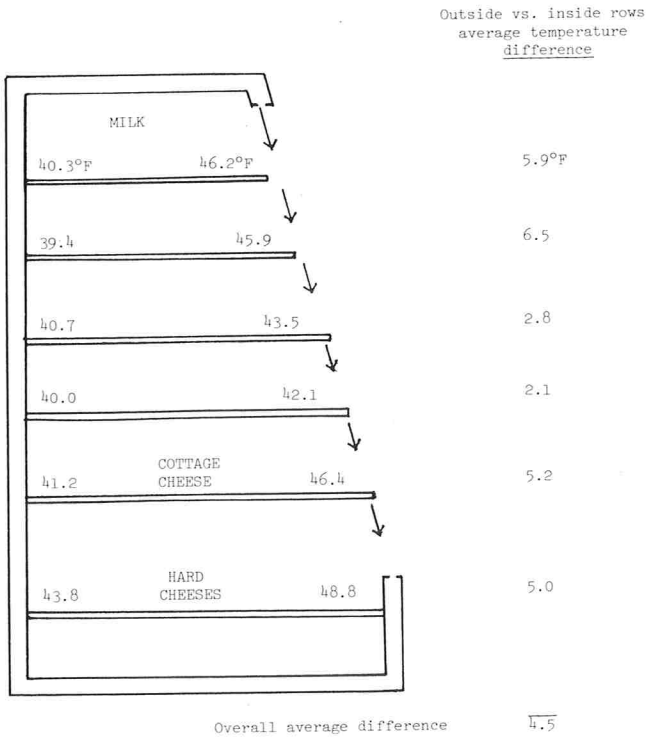


Figure 5. *Average product temperatures related to shelf location and product type for open display refrigerators.*

The average temperatures (Fig. 5) for the outside rows of product in the open display cases were 46.2, 45.9, 43.5, and 42.1 F for the top through the fourth shelves, respectively. Products located in the inside rows averaged 4.5 F lower due to circulation of colder air, greater distances from store ambient temperature, and the effect of heat-generating lights on the outside rows. The average shelf temperature for cottage cheese was 46.4 F and for hard cheeses, 48.8 F.

Table 2 indicates the percentage of recorded temperatures which the authors considered unacceptable for various locations and products, based on the presented criteria. Approximately 27.6% of the product samples in the walk-in coolers exceeded 40 F. No products in the walk-in coolers exceeded the 45 F maximum legal temperature established by the Oregon Department of Agriculture. Over 52% of the blower temperatures exceeded 35 F. The air temperature in 66.7% of the walk-in coolers exceeded 40 F. The frequent number of entries into a walk-in cooler during the day's operation and the extent of air exchange that occurs with each opening of the door account for elevation of the average temperature (42.3 F) of the store room.

A shelf-by-shelf examination (Table 2) of the milk products in the outside (front) row indicated that 79.4, 78.3, 72.0, and 59.3% of the samples exceeded 40 F for the top, second, third, and fourth shelves, respectively. Also, 88% of all cottage cheese samples exceeded the 40 F optimum temperature. When evaluated on legal maximum basis, 48.6, 40.6, 28.1, and 18.5% of the milk and cream samples exceeded 45 F on the top through fourth shelves, respectively. Nearly 69% of all hard cheese and 44% of all cottage cheese samples exceeded the legal maximum temperature of 45 F.

Table 3 groups the observed temperatures for 447 milk product samples in the refrigerated display cases of 30 retail food stores. Only 25.3% of all products were stored at or below 40 F, 39.2% of the products were stored at 41 to 45 F, and 35.5% of the samples were held in excess of 45 F. Also, 15.5% of all products exceeded 50 F. These data suggest that temperature control for this sampling of Oregon food stores was less satisfactory than for a similar study on Pennsylvania food stores by Barnard (*2*).

Temperature comparisons for the two basic types of dairy display cases are shown in Table 4. Six of the 30

TABLE 1. *Summary of temperature data for perishable dairy products for 30 Oregon retail food stores*

	Temperature (F)									
	Walk-in coolers			Refrigerated display cases					Cottage cheese	Hard cheese
	Blower	Room	Product	Blower	Top[a]	2nd[a]	3rd[a]	4th[a]		
Range	26-50	35-54	34-45	24-52	31-61	32-56	35-52	34-51	39-58	38-61
Average	36.0	42.3	39.1	38.1	45.3	44.9	43.4	42.2	46.4	48.8

[a]Shelf

TABLE 2. *The percent of temperature readings considered unacceptable for 30 Oregon food stores*

	Walk-in cooler		
	Blower air[a]	Room air[b]	Product[c]
Above desired maximum	52.1%	66.7%	27.6%
Above legal maximum (45 F)	—	29.8	0

	Refrigerated display case						
	Blower[d]	Top shelf[e]	2nd shelf[e]	3rd shelf[e]	4th shelf[e]	Cottage cheese	Hard cheeses
Above desired maximum	90.0%	79.4%	78.3%	72.0%	59.3%	88.0%	68.8%
Above legal maximum (45 F)	—	48.6	40.6	28.1	18.5	44.0	68.8

Maximum desired temperatures for: [a]Walk-in cooler blower = 35 F; [b]Walk-in cooler room air = 40 F; [c]Milk, cream, and cottage cheese = 40 F; hard cheese = 45 F; [d]Refrigerated case blower = 30 F. [e]Based on product temperatures for outside row of shelves.

TABLE 3. *Temperature data for 447 milk products in refrigerated display cases for 30 Oregon food stores*

Temperature range	Number	Percent
40 F or less	113	25.3
41 to 45 F	175	39.2
Above 45 F[a]	159	35.5
Total	447	100.0

[a]Products in excess of 50 F = 15.5%

TABLE 4. *Temperature comparisons for products in different types of refrigerated display cases*

Shelf	Average shelf temperature (F)	
	Open case[a]	Closed case[b]
Top	46.2	38.1
Second	45.9	38.0
Third	43.6	38.8
Fourth	42.1	39.2
Fifth	—	38.1
Overall Average	44.4	38.4

[a]Data from 24 stores
[b]Data from 6 stores

stores utilized closed display cases, the remaining stores employed open display cases. The results, summarized in Table 4, demonstrate that the closed type cases provided better temperature control. The average product temperature for milk and cream stored in the closed display case was 38.4 F, whereas the average product temperature for the open display case was 44.4 F. For the closed display cases, 75% of the products were stored at or below 40 F; for the open cases, only 23% of the samples were found to be 40 F or lower. At the other extreme, no samples in the closed display cases exceeded 45 F, but 35.6% of the products in the open refrigerated cases exceeded 45 F.

Temperature control problems were noted for three out of every four refrigerated dairy cases in the 30 stores surveyed, if a 40 F maximum is recognized as an objective for an effective quality assurance program for perishable dairy foods. Barnard (2) has stated that a maximum air blower temperature of 30 F, for open type refrigerated dairy cases, is necessary if product temperatures of 40 F or below are to be maintained. Blower air temperatures of 27 out of 30 (90.0%) Oregon food stores exceeded 30 F. Observations in this study indicate that blower air temperatures of 30 F or less are required to maintain products at or below 40 F in open display refrigerators. However, for closed display refrigerators a blower air temperature of 35 F or less is apparently adequate to maintain product temperatures below 40 F.

The electronic thermometer, with a switch box and surface probes, quickly and easily facilitates checking temperatures of perishable dairy products and display refrigerators of retail food stores. A procedure for efficiently obtaining and recording the temperature data was developed.

ACKNOWLEDGMENT

Technical Paper No. 3946. Agricultural Experiment Station, Oregon State University, Corvallis, Oregon 97331.

Mention of trade products by firm name does not imply that they are endorsed or recommended by the Oregon State University over other firms or similar products not mentioned. The authors acknowledge the assistance of Tom Ferguson and Matthew Andrews, graduate assistants in the OSU Department of Food Science and Technology.

REFERENCES

1. Bandler, D. K. 1972. Total temperature control. Amer. Dairy Rev. 34(11):22, 48-51.
2. Barnard, S. E. 1974. Flavor and shelf life of fluid milk. J. Milk Food Technol. 37:346-349.
3. Bodyfelt, F. W. 1974. Temperature control monitoring: A method for fluid milk processing plants. J. Dairy Sci. 57:592-593. (Abstr.)
4. Bodyfelt, F. W. 1974. Temperature control monitoring: A method for cottage cheese plants. J. Dairy Sci. 57:595. (Abstr.)
5. Bradfield, A., and A. H. Duthie. 1966. Influence of the dairy case on quality of milk sold in retail food stores. Bulletin No. 646. Agricultural Experiment Station, University of Vermont, Burlington.
6. Commercial Refrigerator Manufacturers Association. 1967. Voluntary minimum standards for retail food store refrigerators. Health and sanitation bulletin CRS-S1-67. Chicago, Illinois.
7. Hedrick, T. I. 1970. How to keep milk fresh three weeks. Amer. Dairy Rev. 32(7):56-58.
8. Witter, L. D. 1961. Psychrophilic bacteria—a review. J. Dairy Sci. 44:983-1015.

J. Milk Food Technol. Vol. 38, No. 12, Pages 738-744 (December, 1975)

Quality Control Significance of Special Media for Enumeration of Microbial Groups in Cottage-type Cheese

LESTER HANKIN[1], GEORGE R. STEPHENS[2], and WALTER F. DILLMAN[3]

Departments of Biochemistry and Ecology, The Connecticut Agricultural Experiment Station, P.O. Box 1106, New Haven, Connecticut 06504 and Dairy Division, Department of Agriculture, Hartford, Connecticut.

(Received for publication April 15, 1975)

ABSTRACT

Cottage-type cheese samples were examined using specialized media. Nine media were used to provide 21 different types of information and to enumerate bacteria able to excrete lipases, proteases, phosphatases, and acids. Further, these media allow subdivision of the bacteria with specific enzymic attributes into gram negative and positive categories and pseudomonads. A yeast and mold count and a test of those able to grow on violet red bile agar also were made. Tests most useful as predictors of keeping quality were primarily those able to differentiate proteolytic and lipolytic gram negative bacteria and pseudomonads. Microbial tests were also correlated with organoleptic analysis. Manufacturers' code dates (last day of sale) overestimated shelf life about 33% of the time.

Microbial tests on cottage-type cheese usually include a test for the coliform group of bacteria and one for yeasts and molds (*1*). For a coliform count, a solid medium such as violet red bile agar or a liquid medium such as brilliant green lactose bile broth (*1*) is usually used. Acidified potato dextrose agar is commonly used for a yeast and mold count (*1*). All of these tests theoretically help to assess the sanitary conditions under which the product was manufactured and presumably give some evidence of post-pasteurization contamination. None of the tests just mentioned provide data on the degradative abilities of the contaminating microorganisms and these tests do not help assess whether they are mesophiles or psychrotrophs. Although an unusually large count might be related to loss in keeping quality, it is at best only indirect evidence, since clearcut correlations between microbial counts and keeping quality have not been estabished. Further, these media do not measure perhaps the largest population of contaminators in dairy products; i.e., the gram negative bacteria and specifically the psychrotrophic pseudomonads which are considered to be among the most important contaminators in dairy products (*17*).

One measure of the utility of any medium used in quality control testing procedures is the amount of useful information that can be obtained from the use of that medium. Media that provide more than a single set of data would be the most useful. In this paper we report on the suitability of media designed to provide at least two sets, and in some instances three sets, of data. The media described allow enumeration of broad segments of microbial contaminators and furnish information on some important extracellular degradative enzymes produced by these contaminators. The data provided by use of test media in the examination of cottage-type cheese are correlated with keeping quality, i.e., the number of days required for the sample to become unacceptable to the consumer, either because of flavor and aroma deterioration or visible change (e.g. surface growth and discoloration). In some instances several of the test media theoretically allow enumeration of the same groups of microorganisms. Thus, data provided by use of these media were examined to ascertain if comparable counts were obtained.

Additionally, information is provided on the quality of cottage-type cheese, what flavor defects are usually encountered, and how well manufacturers' code dates (last day the product is offered for sale) correlates with the actual time for the sample to reach an unacceptable state.

MATERIALS AND METHODS

Samples

Duplicate samples of 74 cottage-type cheeses were collected during 1973 and 1974 from dairy plants and retail outlets in Connecticut. The samples were refrigerated during transport to the laboratory. One sample was used for microbiological analyses, the other for organoleptic analysis. We collected 55 samples of cottage cheese, 15 of ricotta cheese, nine of baker's cheese and three of mozzarella cheese.

Organoleptic analysis

Samples were refrigerated (2-3 C). Portions removed aseptically were judged at least every other day by a minimum of two persons according to procedures recommended by the American Dairy Science Association and modified for use in the Connecticut Milk Flavor Improvement Program (*4-7*). Samples attaining a score of 36 or less were judged unacceptable.

Microbiological analysis

An 11-g sample in a dilution bottle was shaken with 100 ml of sterile 2% sodium citrate solution. Dilutions for plating were made in sterile distilled water. All inoculations were made by a spread plate technique

[1]*Department of Biochemistry*
[2]*Department of Ecology*
[3]*Dairy Division*

on previously poured and hardened media except that a pour plate technique was used with violet red bile agar. After inoculation plates were incubated at 30 C. Violet red bile agar plates were counted after 24 h. All others, except media designed to detect lipase production, were counted after 48 h. The lipase detection medium was counted after 5 days.

Test media

Lipase production by bacterial colonies was detected with the medium described by Sierra (*15*) with sorbitan monolaurate (Tween 20, Fisher Chemical Co., Fairlawn, NJ) as the lipid source. Colonies which produce a lipase are either surrounded by a precipitate of the calcium salt of the liberated fatty acid, or a clear zone when the lipid is completely degraded. The medium described by Martley et al. (*10*) was employed to detect protease production, except that plate count agar (Difco) was used instead of standard methods agar. Colonies which produce a protease are surrounded by a ring of precipitated para-casein. Plate count agar, potato dextrose agar, and violet red bile agar were all commercial preparations (Difco). The potato dextrose agar was acidified to pH 3.5 with tartaric acid.

To indicate acid phosphatase production, the disodium salt of phenolphthalein diphosphate (K and K Laboratories, Plainview, NJ) was added to plate count agar (*2*) to provide a final concentration of 0.1 mg/ml in the medium. The phenolphthalein diphosphate is warmed gently on a hot plate (to avoid hydrolysis) with stirring to effect solution and while still warm, filter sterilized. It was added while warm (rewarmed if necessary) to the previously cooled and tempered (48 C) plate count agar. After incubation of inoculated plates and growth of colonies, the open plate was inverted over a beaker of ammonium hydroxide and colonies which turned pink to red within 60 sec were phosphatase producers. No antibiotics were used in this medium.

Acid production was determined with the medium of Fabian et al. (*3*) to which antibiotics were added. To determine if colonies, on any medium, were oxidase positive, plates were flooded with an oxidase reagent composed of α-napthol and p-aminodimethylanaline oxalate as previously described (*8*), and the characteristic development of a blue color was noted.

Antibiotics used

To exclude gram positive bacteria, chloramphenicol (Chloromycetin, Sigma Chemical Co., St. Louis, MO) and erythromycin (Sigma) were added to media to provide a final concentration of 2.5 and 5.0 μg/ml, respectively (*11*) (Mixture A). Sterilization by autoclaving affects solution. Both antibiotics were prepared in aqueous suspension at the rate of 50 mg/100 ml. The former was added to sterile and tempered (48 C) media at the rate of 0.5 ml/100 ml and the latter at 1 ml/100 ml of media.

A more selective antibiotic mixture was used to allow only pseudomonads to grow (*13, 14*). This mixture (Mixture B) contained penicillin G, novobiocin (Albamycin, Upjohn, Kalamazoo, MI) and cycloheximide (Actidione, Upjohn). To prepare these antibiotics 45 mg penicillin G (75,000 units), 45 mg novobiocin and 75 mg cycloheximide were mixed in a sterile container and 1 ml of ethanol was added. After 30 min, 9 ml sterile distilled water was added. One milliliter of this sterile antibiotic suspension was added to each 100 ml of sterile and tempered media, just before pouring the plates.

Statistical analysis

To facilitate statistical analysis, all microbial counts were transformed as follows. For counts less than 10 the assigned code was 1. For any count X, equal to or greater than 10 the transform was 2n, if $10^n \leq X$ but $<5 \times 10^n$, or $2n + 1$ if $5 \times 10^n \leq X$ but $<10 \times 10^n$. For example a count of 40/g is transformed to 2; a count of 75 is transformed to 3. Such similar transformations on logarithmic intervals have been described previously (*7*). Results from different media theoretically enumerating the same bacterial groups were compared by correlation and linear regression analysis. The relation of microbial counts to keeping quality (i.e., days to go bad from manufacture or collection) was determined by multiple regression analysis (*16*). Prof. G. M. Furnival, Yale University, kindly provided a computer program for rapid screening of independent variables.

RESULTS AND DISCUSSION

Detection of microbial groups and enzymes

What each medium specifically measures is detailed in

TABLE 1. *Test media and groups of organisms enumerated*

Media number	Media description	Antibiotic mixture used in medium	Test applied[1]	Groups detected
1A	Plate count agar with	—[2]	total growth	total gram negative and positive bacteria
1B	phenolphthalein diphosphate	—	reaction of colonies to ammonium hydroxide	bacteria producing phosphatases
2A	Plate count agar	A[3]	total growth	total number of gram negative bacteria
2B		A	oxidase test	oxidase positive gram negative bacteria (presumably pseudomonads)
3A	Sierra's medium with	B	total growth	total number of pseudomonads
3B	Tween 20	B	lipase excretion	pseudomonads excreting lipases
3C		B	oxidase test	oxidase positive pseudomonads excreting lipases
4A	Sierra's medium with	A	total growth	total number of gram negative bacteria
4B	Tween 20	A	lipase excretion	total number of gram negative bacteria excreting lipases
4C		A	oxidase test	oxidase positive lipase excreters (presumably pseudomonads)
5A	Martley's medium	B	total growth	total number of pseudomonads
5B	(Modified)	B	protease excretion	pseudomonads excreting proteases
5C		B	oxidase test	oxidase positive pseudomonads excreting proteases
6A	Martley's medium	A	total growth	total number of gram negative bacteria
6B	(Modified)	A	protease excretion	total number of gram negative bacteria excreting proteases
6C		A	oxidase test	oxidase positive protease excreters (presumably pseudomonads)
7A	Fabian's medium	A	total growth	total number of gram negative bacteria
7B		A	dye reaction (pH change)	total number of gram negative bacteria excreting acids
8A	Violet red bile agar	—	typical colonies	coliform group of bacteria
9A	Acidified potato	—	typical colonies	yeast count
9B	dextrose agar	—	typical colonies	mold count

[1]Tests on each medium are applied sequentially in the order shown.
[2]-Indicates no antibiotics in this medium.
[3]Mixture A contains chloramphenicol and erythromycin.
Mixture B contains penicillin, novobiocin, and cycloheximide.

TABLE 2. *Counts obtained with 21 test media on 74 samples of cottage-type cheese*

Test applied	Media number	Av. transformed count	Numbers per gram (Range)
Total count	1A	8.1	10,000-50,000
Total no. of gram negative bacteria	2A, 4A, 6A, 7A	8.0 ± 0.2	10,000-50,000
Total no. of pseudomonads	2B, 3A, 5A	5.9 ± 0.3	500- 1,000
Proteolytic pseudomonads	5B, 5C, 6C	5.0 ± 0.3	500- 1,000
Lipolytic pseudomonads	3B, 3C, 4C	5.3 ± 0.2	500- 1,000
Proteolytic gram negative bacteria	6B	6.4	1,000- 5,000
Lipolytic gram negative bacteria	4B	6.2	1,000- 5,000
Yeast count	9A	4.6	100- 500
Mold count	9B	3.4	50- 100
Phosphatase producers	1B	6.7	1,000- 5,000
Gram negative acid producers	7B	7.2	5,000-10,000
Coliform bacteria	8A	3.5	50- 100

Table 1. Test media with the same Arabic numeral are the same media and plate. The letter designation following the numeral indicates tests which are applied sequentially to the medium to obtain differential counts of selected microbial groups, either on a biochemical basis (enzyme excretion), gross differentiation (gram negative and gram postive bacteria), or differential count (yeasts and molds). Medium 1 in Table 1 does not contain any antibiotics and is plate count agar to which phenolphthalein diphosphate was added. Thus, this medium provides both a count of gram negative and gram positive bacteria as well as those colonies able to produce a phosphatase. Media 2, 4, and 6 contain antibiotic mixture A which essentially allows most gram negative bacteria to grow. Tests with pure cultures of representative gram negative bacteria that may be present in dairy products have confirmed this. These antibiotics were originally proposed to be used in media to enumerate pseudomonads selectively (*11*), but we have found it to be less selective than suggested. Media 3 and 5 contain antibiotic mixture B which essentially allows only growth of pseudomonads (*13, 14*). Media 8 and 9 are commercial preparations in common use.

Several examples serve to illustrate how the media were used and the information provided by sequential analysis. Counting all colonies on medium 6 (Table 1) provides a total count of gram negative bacteria. Colonies on the same plate that show the typical reaction for proteases are called proteolytic (medium 6B). Colonies that produce a protease were marked on the underside of the plate and the plate was then flooded with the oxidase reagent. Marked colonies which turn blue were called proteolytic pseudomonads (medium 6C). The rationale of designating oxidase positive organisms in dairy products as pseudomonads as well as their potential as psychrotrophs has been discussed previously (*8*). A second example is use of medium 3 which is selective for pseudomonads. Medium 3A (Table 1) provides a total countof pseudomonads. Colonies giving the typical lipase reaction were counted, and thus medium 3B provides a count of lipolytic pseudomonads. These colonies were marked and the plate flooded with the oxidase reagent. Colonies that were oxidase positive provide a confirmed count of lipolytic pseudomonads (medium 3C).

Microbial counts

The actual range of counts provided by the various test media used is shown in Table 2. Since counts were transformed, the average transformed value is shown. Further, since in some instances several test media measured the same group of microorganisms they were grouped together and the standard deviations shown. The range of counts per gram of sample are shown as indicated by the transformed value. The transformed value also shows whether the average count is close to the top or the bottom of the range. For example, for total pseudomonads the transform is 5.9 which indicates a value near the top of the range while a value of 5.0 for proteolytic pseudomonads indicates the lower end of the range. Generally, most counts are fairly low, if one considers 50,000 as not being excessive for cottage-type cheese. It is interesting that the total count of all bacteria is essentially the same as for the total number of gram negative bacteria. Overall we conclude that lipolytic and proteolytic gram negative bacteria account for between 5 and 10% of the total number of gram negative bacteria. Also, most of the pseudomonads detected were able to produce both lipases and proteases. Of considerable interest is the number of phosphatase producers detected. It could be important to determine how these phosphatase producers could affect the phosphatase test which is used to determine proper pasteurization of dairy products.

Correlations among media

If our conclusions that certain test media enumerate the same micro-organisms are correct, then tests numbered 2A, 4A, 6A, and 7A should all give the same measure of the gram negative bacterial flora. Similarly, media 2B, 3A, and 5A should all measure total number of pseudomonads; 3B, 3C, and 4C measure lipolytic pseudomonads and 5B, 5C, and 6C proteolytic pseudomonads. The means of the results obtained from media within these groups agree well (Table 2). As a further test, correlations among results of media within these same groups were calculated (Table 3).

The correlation coefficients, r, in Table 3, are highly significant, indicating high correlation between test media. Medium 8A, violet red bile agar, a "standard"

TABLE 3. *Correlation coefficients*[1] *(r) between media used to enumerate the same groups of bacteria in cottage type cheese (n = 74)*

Total number of gram negative bacteria					Total number of pseudomonads		
	media number[2]					media number[2]	
	4A	6A	7A	8A		3A	5A
2A	.87	.96	.76	.66	2B	.81	.81
4A	—	.85	.74	.69	3A	—	.97
6A	—	—	.76	.62			
7A	—	—	—	.58			

Lipolytic pseudomonads			Proteolytic pseudomonads		
	media number[2]			media number[2]	
	3C	4C		5C	6C
3B	.93	.85	5B	.92	.83
3C	—	.90	5C	—	.81

[1]All correlation coefficients (r) in this table are highly significant.
[2]Refer to Table 1 for media descriptions.

medium used to enumerate the coliform group of bacteria is also highly correlated with our test media, but the r values were lower. It was not unexpected to note that a high correlation exists for this medium since the coliform group undoubtedly comprises a portion of the count obtained on the other media. The highest correlation (r = 0.96) was obtained between media 2A and 6A. Both have the same components except that medium 6A contains added sodium caseinate. Media 3A and 5A, used to enumerate the total number of pseudomonads, contain a different antibiotic mixture than medium 2A. Even so there is a highly significant correlation between these tests. The same situation prevails for lipolytic pseudomonads in that media 3C and 4C contain different antibiotic mixtures and yet the tests are still highly correlated. It is not surprising that media 3B and 3C are significantly correlated since the data for 3C are obtained from 3B, i.e., the same test plate is used. An examination of data for tests for proteolytic pseudomonads shows the same situation to exist. From these analyses, shown in Tables 2 and 3, we conclude that our initial assumptions, that media designed to provide the same information, were correct.

Predicting keeping quality from microbial tests

The ability to predict keeping quality of cottage type cheese from the results of microbial tests would be most desirable. Unfortunately, single microbial tests in common use generally fail to predict adequately, and this failure may be in part due to lack of specificity of the tests. In this study we have enumerated broad groups such as pseudomonads and total numbers of gram negative bacteria. Further, we have utilized tests designed to enumerate, within the broad categories, specific groups of microorganisms that excrete extracellular degradative enzymes.

In all, 21 tests described previously, were performed. Transformed microbial counts from the 21 tests were used, singly or in combination to find which test best estimated the time to go bad from manufacture or from collection. The best results from combinations of one or two variables (i.e., test media) are shown in Table 4.

The two best single estimators of days to go bad from manufacture were measures of proteolytic gram negative

TABLE 4. *Relation of the 2 best single or pairs of 21 microbiological tests to the time required for cottage type cheese samples to become unacceptable (n = 74)*

Variables (groups detected and media no.)[1]	
Time to become unacceptable from manufacture	R^2
Proteolytic gram negative bacteria (6B)	.144
Lipolytic pseudomonads (4C)	.124
Lipolytic pseudomonads (3B) and lipolytic pseudomonads (4C)	.235
Lipolytic pseudomonads (3C) and total gram negative bacteria (4A)	.210
Total on 21 variables	.465
Time to become unacceptable from collection	
Total gram negative bacteria (7A)	.206
Total gram negative and positive bacteria (1A)	.147
Total gram negative and positive bacteria (1A) and total gram negative bacteria (6A)	.262
Total gram negative bacteria (6A) and total gram negative bacteria (7A)	.259
Total on 21 variables	.490

[1]Refer to Table 1 for media descriptions.

bacteria (medium 6B) and lipolytic pseudomonads (medium 4C). The best 2-variable combinations measured lipolytic pseudomonads (media 3B and 4C) whereas, the next best 2-variable combination enumerated lipolytic pseudomonads and total numbers of gram negative bacteria (media 3C and 4A). The combination of all 21 variables accounted for less than half of the observed variability in days to go bad and combinations of two or more variables did not significantly improve the overall R^2 value for the combinations. Nevertheless, it is interesting that an assay of lipolytic pseudomonads (media 3B, 3C, and 4C) appeared most frequently in the best combinations of variables. This suggests that lipolytic pseudomonads have an important effect on keeping quality of cottage-type cheeses held under refrigeration conditions.

On the other hand, variables best related to days to go bad from collection were measures of the total number of gram negative bacteria (media 7A and 6A) and total number of gram negative and positive bacteria (medium 1A).

It is obvious that no quality control or regulatory laboratory routinely would do 21 analyses on a single product. Therefore, based on data obtained from 21 tests on 74 samples as described above, we selected 10 tests (or variables) to use to ascertain the best single and pair of estimators. Selection of variables considered both statistical performance, as determined previously, as well as practical utility. The 10 tests selected were measures of lipolytic gram negative bacteria (medium 4B), lipolytic pseudomonads (medium 4C), total number of gram negative bacteria and positive bacteria (medium 1A), total number of gram negative bacteria (medium 2A), total pseudomonads (medium 2B), proteolytic gram negative bacteria (medium 6B), proteolytic pseudomonads (medium 6C), yeast count (medium 9A), mold count (medium 9B), and a count of the coliform group of bacteria (medium 8A). The latter four were selected because they are "standard" tests for dairy products (*1*); rather than for any potential for estimating keeping quality. For the 10 tests indicated above (media 1, 2, 4, 6,

8, 9) only six test plates are needed since many of the media provide information in two categories as shown in Table 1. Further, media 4B and 4C and 6B and 6C would also provide a total count of gram negative bacteria and medium 1A could also be used to indicate bacteria able to produce a phosphatase. Because the time from manufacture to collection of cheese varied considerably, the data were stratified into two groups, 0 to 3 days and 0 to 7 days from manufacture to collection.

For 28 samples collected 0 to 3 days after manufacture, the test best related to the number of days from manufacture for a sample to become unacceptable was a test for lipolytic gram negative bacteria (medium 4B, Table 5). This test alone accounted for 22% of the observed variability, compared to 41% for all 10 tests. The second best single estimator was the yeast count (medium 9A). Although adding an additional test (the 2-variable combination) accounted for a greater proportion of the observed variability, the increase was not statistically significant. With the exception of the yeast count, no other test generally used in dairy product testing was closely related to the time required from manufacture for the samples to become unacceptable.

TABLE 5. *Relation of 2 best single or pairs of 10 microbiological tests to time required for cottage type cheese samples to become unacceptable*

Variables (group detected and media no.)[1]	R^2
28 samples collected 0 to 3 days after manufacture (dependent variable = days to go bad from manufacture)	
Lipolytic gram negative bacteria (4B)	.216
Yeasts (9A)	.190
Lipolytic gram negative bacteria (4B) and total pseudomonads (2B)	.319
Lipolytic gram negative bacteria (4B) and proteolytic pseudomonads (6C)	.255
Total on 10 variables	.414
51 samples collected 0 to 7 days after manufacture (dependent variable = days to go bad from collection)	
Total gram negative and gram positive bacteria (1A)	.194
Lipolytic gram negative bacteria (4B)	.193
Total gram negative bacteria (2A) and total gram negative and positive bacteria (1A)	.298
Lipolytic gram negative bacteria (4B) and total pseudomonads (2B)	.256
Total on 10 variables	.451

[1]Refer to Table 1 for media description.

For 51 samples collected 0 to 7 days after manufacture, the number of days to go bad from collection was most closely related to tests for the total number of gram negative and gram positive bacteria (medium 1A) and lipolytic gram negative bacteria (medium 4B, Table 5). Although addition of another test (the 2-variable combination) accounted for a larger proportion of the observed variability, the increase was not statistically significant. All of the sets of variables most closely related to days from collection to become unacceptable contained a test that indicated the presence of gram negative bacteria.

Even though a test for lipolytic gram negative bacteria (medium 4B) was a good estimator of time required to become unacceptable, it may not be the test of choice since this test requires 5 days to complete (although 3 days can be used, but with less certainty). Thus, it may not be the most useful test for a manufacturer to use to establish a meaningful code life for his product. However, it does appear that it would be a useful test for monitoring the manufacturing process, since the 5-day period needed to complete the test is well within the usual code period for most samples (Table 6). On the other hand, either the test for lipolytic gram negative or gram negative and positive bacteria may be of more immediate use for the analysis of samples collected at retail outlets, to estimate their remaining shelf life. Nevertheless, this latter test (medium 1A) does not indicate the biochemical abilities of the organisms in the samples as does a test for lipolytic bacteria.

TABLE 6. *Relation of number of days for cottage type cheese to go bad from manufacture or collection and manufacturer's estimate of keeping quality*

Category	Number of samples	Days	
		Average	Range
1. Actual time from manufacture to unacceptability (all samples)	74	17.8	1-41
2. Actual time from collection to unacceptability (all samples)	74	12.4	1-38
3. Manufacturers estimate of keeping quality (code date; last day offered for sale)	56	18.8	7-35
4. Actual time from manufacture to unacceptability (only coded samples)	56	21.3	1-41
5. Actual time from manufacture to unacceptability (only uncoded samples)	18	12.3	3-30

Correlation of microbial and organoleptic tests

An attempt was made to determine if the reason for rejection of samples by the organoleptic test was correlated with the microbiological analyses. Therefore, the data were stratified according to the defect observed when the sample became unacceptable. The dependent

TABLE 7. *Relation of the 2 best single or pairs of 6 microbiological tests to the time required from date of collection for cottage cheese type samples, rejected for a specific flavor, to become unacceptable*

Variables (groups detected and media no.)[1]	R^2
Bitter flavor (n = 18)	
Mold count (9B)	.151
Lipolytic gram negative bacteria (4B)	.013
Mold count (9B) and lipolytic gram negative bacteria (4B)	.212
Lipolytic pseudomonads (4C) and mold count (9B)	.169
Total on 10 variables	.306
Surface growth (n = 15)	
Lipolytic pseudomonads (4C)	.245
Proteolytic pseudomonads (6C)	.180
Lipolytic pseudomonads (4C) and mold count (9B)	.288
Lipolytic gram negative bacteria (4B) and lipolytic pseudomonads (4C)	.275
Total on 10 variables	.399
Old/Lacks freshness (n = 12)	
Yeast count (9A)	.388
Proteolytic pseudomonads (6C)	.188
Lipolytic pseudomonads (4C) and proteolytic pseudomonads (6C)	.532
Yeast count (9A) and proteolytic pseudomonads (6C)	.479
Total on 10 variables	.604

[1]Refer to Table 1 for media description.

variable, days to go bad from collection was regressed on six microbial tests singly and in combinations. Those considered most likely to provide meaningful data were media 4B, 4C, 6B, 6C, 9A, and 9B. Results for three different types of defects are shown in Table 7.

The best single estimator of keeping quality for samples judged as bitter was the mold count (medium 9B, Table 7) which accounted for 15.1% of the observed variability. A poor second best estimator, lipolytic gram negative bacteria (medium 4B) accounted for only 1.3% of the total variability. The best single estimators for samples rejected because of surface growth was a test for lipolytic pseudomonads (medium 4C) and proteolytic pseudomonads (medium 6C) which accounted for 25% and 18% of the observed variability, respectively. The best single estimators for samples judged as old or lacking freshness were the tests for yeasts (medium 9A) and for proteolytic pseudomonads (medium 6C).

Why certain microbial tests were better estimators than others for specific flavor defects is difficult to explain since much depends on the chemical entities elaborated by microorganisms during growth. For surface growth defects, we attempted to differentiate in our testing between that caused by fungi and that by bacteria. Only 3 of 15 samples rejected because of surface growth could be attributed to molds. Even though a mold count accounted for nearly the same amount ($R^2 = .151$) of the total variability as proteolytic pseudomonads (Table 7), it is obvious that for these samples more bacterial than fungal surface growth was encountered and the regression analysis confirms this. The reason why a yeast count is the best single estimator for the old or lacking freshness defect is unclear. It was expected that a total count of pseudomonads would have been the best single estimator, as has previously been shown for this defect in milk (*4, 7*). In this previous study on pasteurized milks, pseudomonads accounted for 17% of the total variability among samples with this defect. However, yeast counts were not made. Although in the present study yeasts accounted for nearly 39% of the total variability, proteolytic pseudomonads accounted for about 19% and lipolytic pseudomonads, 15%. In combination these latter two tests accounted for 53% of the variability (Table 7).

Relation of keeping quality to manufacturers' code

Of the 74 samples examined, 44 were collected from 10 manufacturing plants in Connecticut. The remaining 30 samples, collected in retail outlets, represented nine out-of-state manufacturers. The average time from manufacture to collection was 3.3 days for in-state and 11.3 for out-of-state samples. All but one sample was judged organoleptically acceptable at collection time. The average time to go bad from manufacture for all 74 samples was 17.8 days (line 1, Table 6) and from collection, (line 2) 12.4 days. Time to go bad from manufacture or collection is defined as the time interval in days from date of manufacture, or collection, to the date when the sample was judged to be unacceptable, that is, a flavor score of 36 or less or visible surface growth. The code date (last day to be offered for sale) and the date of manufacture were known for only 56 of the samples. The longevity of these samples, estimated by the manufacturer, was 18.8 days whereas the actual time to go bad from manufacture averaged 21.3 days (lines 3 and 4). The average interval between manufacture and collection was 8.0 days for the 56 coded samples. This probably represents the average age of the product when purchased by the consumer. The average time for 18 uncoded samples to go bad was 12.3 days (line 5). The 9-day discrepancy between coded and uncoded samples (Table 6, lines 4 and 5) is likely due to the difference in manufacturing operations. The 18 uncoded samples were generally from small manufacturers that produce basket- and baker-type cheeses.

Despite the apparent underestimation of longevity by the manufacturer (Table 6, lines 3 and 4), 26.9% of the samples from six Connecticut manufacturers, in fact, were judged unacceptable before the estimated code date expired. For nine out-of-state manufacturers this value was 43.3%. This shows that for Connecticut consumers, purchases of cottage type cheeses, approximately one-third of the time, cannot be expected to remain acceptable to the specified last date of sale. However, the percentages for in- and out-of-state manufacturers are not significantly different.

TABLE 8. *Flavor criticisms of cottage type cheeses in 1973-74 and 1950, and average number of days to go bad from manufacture for 1973-74 samples*[1]

	Present study		
Flavor criticism	% of samples	Av. days to go bad from manufacture	1950 study % of sample
Bitter	24.3	19.4	18.0
Surface growth	20.3	23.6	NR[2]
Old/lacks freshness	16.2	21.8	NR
Rancid	10.8	7.8	NR
Putrid	8.1	20.7	NR
Yeasty	5.4	10.0	25.4
Fruity	5.4	17.8	8.2
No specific comment (or misc. in 1950 study)	4.1	27.7	15.6
Sour (includes acidy in 1950 study)	2.7	19.0	16.4
Oxidized	1.4	5[3]	NR
Unclean	1.4	16[3]	12.3
OK	0	—	1.6
Unnatural	NU[2]	—	2.5

[1]Flavor defect determined after 7-days-storage. Data adapted from Morgan et al. (*12*).
[2]NR indicates this criticism not recorded in 1950 study. NU = this criticism not used in present study.
[3]Single sample.

The reasons for samples to be judged unacceptable is shown in Table 8. Even though three criticisms, bitter flavor, surface growth, and old or lacking freshness (stale) accounted for 60% of rejections, it is important to note that the time to go bad from manufacture for samples with these three criticisms exceeded the average for all samples; 17.8 days (Table 6). Rancid and yeasty flavors accounted for 16% of rejections and the average time to attain these defects was about half that of all 74 samples.

Comparison with earlier studies

A comparison (Table 8) with an earlier study made in 1950 (*12*) showed that after 7-days storage the flavor defects bitter, yeasty, and sour predominated (60% of the samples). This difference in major flavor defects between the 1950 study and the present one may indicate a change, with time, in types of microbial contaminants. In the 1950 study 98% of the samples were unacceptable after 7 days. In the present study 76% remained acceptable 7 days after collection.

It has been pointed out often that psychrotrophic pseudomonads are important to keeping quality of dairy products (*17*). Recently Juffs (*9*) suggested that proteolytic psychrotrophs are important in raw milk in New Zealand. He found that proteolytic psychrotrophs comprised about 7.6% of the total bacterial count of raw milk and 54% of the total psychrotrophic count. With such a large proportion of psychrotrophs and their ability to excrete a wide variety of substances which might cause off-flavors, it is understandable why these organisms are important in keeping quality. Based on previous data that potential psychrotrophs are oxidase positive (*8*) the use of media for enumeration of proteolytic pseudomonads, and other important groups, as reported in this paper, could prove most useful in quality control testing.

ACKNOWLEDGMENTS

We thank Margaret Staba for excellent technical assistance and Matthew Meyer for help in collecting samples.

REFERENCES

1. American Public Health Association. 1972. Standard methods for the examination of dairy products (13th ed.). Amer. Public Health Assoc. New York, N.Y.
2. Barber, M., and S. W. A. Kuper. 1951. Identification of *Staphylococus pyogenes* by the phosphatase reaction. J. Pathol. Bacteriol. 63:65-68.
3. Fabian, F. W., R. C. Fulde, and J. E. Merrick. 1953. A new V-8 medium for determining lactobacilli. Food Res. 18:280-289.
4. Hankin, L., and G. R. Stephens. 1972. What tests usefully predict keeping quality of perishable foods? J. Milk Food Technol. 35: 574-576.
5. Hankin, L., and W. F. Dillman. 1972. Further studies on the flavor quality of retail milk in Connecticut. J. Milk Food Technol. 35: 710-712.
6. Hankin, L., W. F. Dillman, and E. O. Anderson. 1971. An assessment of the flavor quality of whole milk available at commercial outlets. J. Milk Food Technol. 34:244-248.
7. Hankin, L., and E. O. Anderson. 1969. Correlations between flavor score, flavor criticism, standard plate count, and oxidase count on pasteurized milk. J. Milk Food Technol. 32:49-51.
8. Hankin, L., and W. F. Dillman. 1968. A rapid test to find "potentially" psychrophilic organisms in pasteurized dairy products. J. Milk Food Technol. 31:141-145.
9. Juffs, H. S. 1974. Incidence of proteolytic and some other bacterial populations in raw milk produced in south-eastern Queensland. Aust. J. Dairy Technol. 29:99-100.
10. Martley, F. G., S. R. Jayashankar, and R. C. Lawrence. 1970. An improved agar medium for the detection of proteolytic organisms in total bacterial counts. J. Appl. Bacteriol. 33:363-370.
11. Masuronsky, E. B., S. A. Goldblith, and J. Voss. 1963. Differential medium for selection and enumeration of members of the genus *Pseudomonas*. J. Bacteriol. 85:722-723.
12. Morgan, M. E., E. O. Anderson, L. Hankin, L. R. Dowd, and M. J. Foter. 1952. Chemical, bacteriological and organoleptic characteristics of retail cottage cheese. Bull. 284. Storrs Agric. Expt. Station 23 p.
13. Sands, D. C., L. Hankin, and M. Zucker. 1972. A selective medium for pectolytic fluorescent pseudomonads. Phytopathology 62:998-1000.
14. Sands, D. C., and A. D. Rovira. 1970. Isolation of fluorescent pseudomonads with a selective medium. Appl. Microbiol. 20:513-514.
15. Sierra, G. 1957. A simple method for the detection of lipolytic activity of micro-organisms and some observations on the influence of the contact between cells and fatty substrates. Antonie van Leeuwenhoek Ned. Tijdschr. Hyg. 23:15-22.
16. Snedecor, G. W. 1956. Statistical methods (5th ed.). Iowa State College Press. Ames, Iowa 485 p.
17. Witter, L. D. 1961. Pyschrophilic bacteria-a review. J. Dairy Sci. 44:983-1015.

J. Milk Food Technol. Vol. 38, No. 12, Pages 745-746 (December, 1975)

A Research Note

Fungi in Foods

VII. A Comparison of the Surface, Pour Plate, and Most Probable Number Methods for Enumeration of Yeasts and Molds[1]

J. A. KOBURGER and A. R. NORDEN

Food Science Department
University of Florida, Gainesville, Florida 32611

(Received for publication April 15, 1975)

ABSTRACT

It was possible to compare recovery of yeasts and molds from 30 food samples by three methods, employing plate count agar and broth with added antibiotics. Although the pour plate and surface plate methods gave comparable results, the Most Probable Number (MPN) procedure consistently yielded the highest counts. With some of the samples, the MPN method was the only one in which recovery occurred. It thus appears that this procedure is practical for detection of fungi and may be of use in survey work or when analyzing foods containing low numbers of microorganisms.

Recognition of the merits of the MPN technique within the last few years has resulted in a marked increase in its use by food microbiologists. Many current analyses for staphylococci, enterococci, and *Salmonella* start with an enrichment-enumeration step in which features of the MPN are employed. This is particularly true when low levels of contamination are expected and when membrane filtration cannot or should not be used because of certain limitations of the method (*2, 6*).

The surface streak plate method, while overcoming some of the disadvantages of the pour plate, such as exposure of the cells to hot agar and slower growth due to cells being entrapped in the agar, is limited as to sample size. The Most Probable Number (MPN) technique overcomes many of these difficulties, while possibly generating others with respect to space and material usage.

In recognition of the apparent advantages and lack of data regarding the use of the MPN method as applied to yeasts and molds, this study was undertaken to evaluate this enumeration procedure for fungi in comparison to the surface and pour plate methods.

MATERIALS AND METHODS

Fresh and frozen food samples (Table 1) were obtained from retail stores in the Gainesville, Florida area. Fifty-gram samples were blended in 450 ml of phosphate buffered dilution water for 2 min and then diluted as needed (*1*).

Plate Count agar or broth with 100 mg each of chloramphenicol and chlortetracycline HCl per liter (*4*) was used for all analyses. All media were compounded from individual components. The medium for pour plates was prepared and tempered just before use. Plates on which samples were surface inoculated were prepared 24 h before use and left at room temperature to dry. Liquid medium for the MPN procedure was sterilized and cooled; antibiotics were added and the medium was then dispensed into sterile capped tubes in 10-ml aliquots. The tubes were incubated for at least 48 h before use to check for sterility. Tubes and pour plates were inoculated with 1-ml aliquots, whereas 0.5 ml was used on surface inoculated plates. Samples were plated in duplicate and a three-tube dilution series was used for the MPN. Incubation was at 20 C for 5 days.

RESULTS AND DISCUSSION

Initially there was some concern as to whether all yeasts and molds encountered would initiate growth in the MPN tubes because of limited oxygen diffusion. However, both yeasts and molds appeared to initiate growth without difficulty and it was not uncommon for a single mold colony to establish itself on the bottom of a tube. Difficulty was encountered however, in determining the presence of growth in some tubes because of the turbidity caused by the added food sample. The gram stain was the quickest and easiest procedure of those used to attempt to detect growth in turbid media.

It had been anticipated that the surface inoculated plates would yield higher counts (Table 1) than the pour plates due to the cells not being exposed to the warm agar and because of increased breaking up of cell clumps during spreading of the material onto the surface of the agar (*7*). This was not true; both methods gave essentially the same recovery. It can only be hypothesized that fungal cells are not as sensitive to temperature shifts as bacterial cells and that because of the limited growth of fungi that usually takes place in foods, clumping is minimal, or else the clumps are readily dispersed during blending of samples.

Recognizing the bias inherent in the MPN method (*5*), it is possible to explain the generally higher counts

[1]*Florida Agricultural Experiment Stations Journal Series No. 5877.*

TABLE 1. *A comparison of fungal counts conducted by the pour, surface and MPN method*

Samples	Surface plates	Pour plates	MPN
	(Organisms/gram)		
Squid	2,000	1,900	4,600
Black sea perch	3,600	4,300	11,000
Clams	450	420	1,100
Lettuce	140	160	240
Banana	12,000	18,000	24,000
Plums	200	220	240
Buttermilk	10	20	43
Corn flakes	0	0	2,400
Chicken wings	90	100	930
Fresh mullet	1,500	1,400	2,400
Crab cake	62,000	90,000	150,000
Oysters	80	60	460
Yogurt (Boysenberry)	0	0	23
String beans	30,000	29,000	150,000
Pork sausage	9,600	9,900	11,000
Weiners	4,500	4,700	11,000
Tomato	0	10	23
Fish sticks (Frozen)	560	560	1,500
Hamburger	70	70	240
Mixed vegetables (Frozen)	0	0	23
Chicken pot pie (Frozen)	60	30	150
Turkey pot pie (Frozen)	90	100	120
Beef pot pie (Frozen)	90	100	1,100
Creamed chipped beef (Frozen)	30	30	43
Chicken a la King (Frozen)	20	20	23
Gravy with sliced beef (Frozen)	10	20	23
Egg noodles	2,300	2,600	4,600
Elbow macaroni	210	190	240
Dry gelatin	0	0	0
Bran flakes	80	80	460

obtained with this procedure. However, with three of the samples tested, fungi were recovered only by the MPN method. This would indicate that conditions may be somewhat more favorable for growth in broth than on solid media. This factor has been previously noted as one of the attributes of the MPN procedure (*3*). In addition, no attempt was made in this study to take advantage of the capability of this procedure for accommodating large sample volumes, an important feature when working with materials containing low levels of contamination. Membrane filter techniques permit detection of small numbers of microorganisms by concentration, but serious problems have arisen due to poor recovery of certain microorganisms with some membrane filters (*2*).

These data indicate that the MPN procedure for fungi has applicability in a food microbiology laboratory. It has been shown to be at least as efficient in recovery as either the pour or surface plate method and with some samples was able to demonstrate growth that was missed by both the pour and surface inoculated procedures.

REFERENCES

1. American Public Health Association, Inc. 1966. Recommended methods for the microbiological examination of foods. Amer. Public Health Assoc., Inc. New York. p. 205.
2. Dutka, B. J., M. J. Jackson, and J. B. Bell. 1974. Comparison of autoclave and ethylene oxide-sterilized membrane filters used in water quality studies. Appl. Microbiol. 28:474-480.
3. Halvorson, H. O., and N. R. Ziegler. 1933. Application of statistics to problems in bacteriology. 1. A means of determining bacterial populations by the dilution method. J. Bcteriol. 25:101-121.
4. Koburger, J. A. 1970. Fungi in foods. I. Effect of inhibitor and incubation temperature on enumeration. J. Milk Food Technol. 33: 433-434.
5. Oblinger, J. L., and J. A. Koburger. 1975. Understanding and teaching the Most Probable Number technique. J. Milk Food Technol. 38:540-545.
6. Schaeffer, D. J., M. C. Long, and K. G. Janardin. 1974. Statistical analysis of the recovery of coliform organisms on Gelman and Millipore membrane filters. Appl. Microbiol. 28:605-607.
7. Snyder, T. L. 1947. The relative errors of bacteriological plate counting methods. J. Bacteriol. 54:641-654.

J. Milk Food Technol. Vol. 38, No. 12, Pages 747-749 (December, 1975)

The Microbial Flora of Rock Shrimp-*Sicyonia brevirostris*

J. A. KOBURGER, A. R. NORDEN, and G. M. KEMPLER

Food Science Department
University of Florida, Gainesville, Florida 32611

(Received for publication May 19, 1975)

ABSTRACT

The microbial flora of rock shrimp (*Sicyonia brevirostris*) taken off the East Coast of Central Florida was studied. Gram negative bacteria, particularly the *Flavobacterium-Cytophaga* group, represented most organisms isolated from the fresh shrimp. Gram positive organisms were found in greater number after iced storage, with *Planococcus* increasing from 10% of the isolates on fresh shrimp to 40% on the red samples. Examination of the fungal population showed only yeasts to be present, with members of the genus *Rhodotorula* being the only survivors of iced storage.

Rock shrimp is a deep-water, nocturnal species that burrows during daylight hours. The shrimp are found off both coasts of Florida, generally in water of 40-70 m and are harvested in the same manner as *Penaeus* shrimp (*6*). Processing of rock shrimp is more difficult than that of the softer and thinner shelled *Penaeus* shrimp. It has only been recently that equipment suitable for use with this species has been developed. With the advent of this equipment the demand for these shrimp by processors has increased (*8*).

Relatively little is known about rock shrimp; the existing studies are concerned with their biology (*6*) or handling and processing difficulties (*2, 8*). This species is believed to be of increasing economic importance (*8*); thus, an investigation was initiated to determine the aerobic flora normally occurring on these shrimp as well as those organisms found on the shrimp following periods of iced storage.

MATERIALS AND METHODS

Rock shrimp samples were taken off the East Coast of Central Florida aboard the Florida Department of Natural Resources research vessel *Hermando Cortez*. Samples were taken directly from the net and processed within 10 min. Every attempt was made to minimize contact of the samples with any contaminated surface. A description of the samples and sampling stations is given in Table 1.

Samples (500 g) were placed in Whirlpak bags and 50-g portions were weighed out directly into blendor jars. Buffer was added and the sample was blended for 2 min (*1*). Serial dilutions were prepared, and 0.5-ml aliquots were pipetted directly onto the surface of previously prepared plates of media and spread using the hockey stick method. Media used were TCBS agar (Difco) for *Vibrio parahaemolyticus, Plate* Count agar with 0.5% sodium chloride for total counts, and Plate Count agar with 0.5% sodium chloride and antibiotics (*9*) for isolation of fungi. Following distribution of the samples onto the agar surface, plates were allowed to dry for 1 h and then packed into containers for shipment to the laboratory. During approximately the first 16 h of incubation plates were held at ambient temperature (20-25 C). Upon arrival at the laboratory in Gainesville, plates were incubated under controlled conditions. TCBS plates were placed at 35 C for an additional 12 h, whereas both total count and fungal plates were incubated at 20 C for 5 days.

TABLE 1. *Location and description of stations and rock shrimp samples*

Station:	3	5	3	5
Date sampled	Oct. 14-15, 1974		Dec. 6-7, 1974	
Temperature (C)	25.75	25.64	19.74	20.86
Depth (m)	40.70	70.00	42.70	66.10
Salinity	35.82	36.26	36.19	36.28
Location, lat.	28.353	28.351	28.353	28.351
long.	80.130	80.053	80.130	80.053
Distance from shore (miles)	18.8	25.1	18.8	25.1
Fresh samples				
Total count (no./g)	121,000	34,000	78,000	380,000
Fungi (no./g)	900	950	1,000	900
Stored composite samples				
Days stored	9		12	
Total count (no./g)	225,000		3,100,000	
Fungi (no./g)	300		200	

The remaining shrimp from the two stations sampled at each date were composited, placed in ice, and held for sampling at a later date to determine those organisms capable of survival and/or growth during iced storage.

Because of the low numbers recovered, all fungi and typical *V. parahaemolyticus* colonies were picked for further study. Fungi were stored on YM (Difco) agar slants, and the presumptive *V. parahaemolyticus* isolates were maintained on trypticase soy agar with 0.3% added salt. Well separated and representative colonies were picked from the total count plates and stored on trypticase soy agar with 0.5% salt. Usually, a number of colonies equal to the square root of the number of colonies on a countable plate were picked for identification. A total of 118 isolates were picked from the total count plates, 45 from the TCBS plates, and 33 from the fungi plates. For identification, descriptions of the yeasts were taken from Lodder (*10*) and for the bacteria from both the 7th and 8th editions of *Bergey's Manual* (*3, 4*). This was necessary because of the difficulty in identifying some of the isolates when using the 8th edition alone, and accounts for the listing of some genera not included in the new edition. Standard microbiological methods were used throughout (*12*) except that 0.5% salt was added to all media used for identification purposes. Chemical and physical data concerning the stations was obtained with an Interocean-Model 513 recording analyzer.

RESULTS AND DISCUSSION

Fungal counts on the fresh shrimp were low and decreased during iced storage. Yeasts, the only fungi recovered from both fresh and stored samples, are listed

[1]*Florida Agricultural Experiment Stations Journal Series No. 5926.*

in Table 2. Mainly asporogenous yeasts were isolated,

TABLE 2. *Identification of yeasts from fresh and stored samples of rock shrimp (combined data of the two samples for each date)*

	Oct.-74	Dec.-74
Fresh Shrimp:	*Pulluleria pullulans*	*Pulluleria pullulans*
	Torulopsis candida	*Torulopsis candida*
	Rhodotorula minuta	*Rhodotorula minuta*
	Rhodotorula rubra	*Rhodotorula marina*
	Cryptococcus laurentii	*Rhodotorula glutinus*
	Trichosporon cutaneum	*Sporobolomyces alboru-bescens*
	Pichia membranaefa-ciens	
Stored shrimp:	*Rhodotorula rubra*	*Rhodotorula minuta*

with *Pulluleria,* a yeast-like organism, being recovered from all fresh samples. Many of the species and genera found are the same as those reported by Phaff et al. (*11*) as being present on Gulf (*Penaeus*) shrimp. Members of the genus *Rhodotorula* were the only fungi surviving through the period of iced storage.

V. parahaemolyticus was found in low numbers (approx. 200/g) and only during the first sampling period in October. This may be a reflection of the slightly higher water temperature at this time, or else they were missed during the second sampling period because of the low numbers initially present and failure to use an enrichment procedure. As expected, no isolations of this organism were made from the samples following iced storage.

Since this is a different genus of shrimp and newer nonmenclature has been used in describing some of the isolates found in this study, it is difficult to contrast these results with previous studies on the normal flora of shrimp (*7, 13, 14*). However, certain observations can be made. Mostly gram negative bacteria were isolated from the fresh shrimp (Table 3). The *Flavobacterium-Cytophaga* group comprised 36% of the isolates with *Pseudomonas* the next most frequently isolated genus at 16% and *Planococcus,* a motile and pigmented gram positive coccus, representing 10% of the isolates. The remainder of the isolates represented diverse groups of both gram negative and gram positive bacteria. The widely varying flora undoubtedly is attributable not only to the feeding habits of these shrimp but also to the fact that rock shrimp burrow during daylight hours to escape predators. While it was not feasible to wash these shrimp of extraneous material before sampling, a certain degree of washing did occur during the raising of the net. This fact is indicated by the relatively low average count of 153,000/g for the four samples of fresh shrimp.

TABLE 3. *Organisms and frequency of isolation from fresh samples of rock shrimp (combined data for 4 samples)*

Organisms	% of isolates
Achromobacter	2
A. aquamarinus	
A. liquefaciens	
Aeromonas salmonicida	2
Bacillus sphaericus	1
Beijerinckia indica	2
Cytophaga	15
C. aurantiaca	
C. diffluens	
C. hutchinsonii	
Flavobacterium	21
F. balustinum	
F. breve	
F. devorans	
F. helmephilum	
F. indoltheticum	
F. lutescens	
F. rigense	
F. tirrenicum	
Halobacterium salinarium	1
Micrococcus	6
M. colpogenes	
M. cryophilus	
M. ureae	
Planococcus citreus	10
Plesiomonas shigelloides	9
Pseudomonas	16
P. aureofaciens	
P. fluorescens	
P. halestorga	
P. lemoignei	
P. membranoformis	
P. ribicola	
P. stutzeri	
Staphylococcus epidermidis	1
Vibrio	11
V. anguillarum	
V. costicola	
V. parahaemolyticus	
Yeasts	3
Total	100

TABLE 4. *Organisms and frequency of isolation from stored samples of rock shrimp (combined data for the two composite samples)*

Organisms	% of isolates
Alcaligenes metalcaligenes	20
Chromobacterium violaceum	1
Flavobacterium	3
F. devorans	
F. halmephilum	
F. rigense	
F. tirrenicum	
Micrococcus	25
M. cryophilus	
M. varians	
Planococcus citreus	40
Plesiomonas shigelloides	1
Pseudomonas membranoformis	1
Staphylococcus epidermidis	3
Yeasts	6
Total	100

Interestingly gram positive organisms predominated following iced storage (Table 4). Whether this is due to some unusual nutritive or other selective characteristic of these shrimp is not known. However, 68% of the isolates recovered were gram positive cocci, with *Planococcus* increasing from 10% of the isolates on the fresh shrimp to 40% on the stored shrimp. This is in sharp contrast to previous reports on the flora of iced shrimp (*5*). *Alcaligenes metalcaligenes,* the only gram negative bacterium found in large numbers on the stored samples, was not even isolated from fresh shrimp.

The role of the initial flora and the factors influencing subsequent shifts in population need to be investigated further. Little is known of the spoilage potential of several of the gram positive isolates observed on the

stored shrimp, particularly *Planococcus.* This organism is regarded as of marine origin and is obviously capable of increasing in numbers during iced storage. In addition, the significance of such organisms as *Plesiomonas shigelloides, Chromobacterium violaceum,* and *Pseudomonas aeruginosa* that were found either on the fresh or stored rock shrimp needs to be investigated further.

ACKNOWLEDGMENTS

The assistance of the Florida Department of Natural Resources, and in particular Mr. Frank S. Kennedy, in helping us to obtain the samples is greatly appreciated.

REFERENCES

1. American Public Health Association, Inc. 1966. Recommended methods for the microbiological examination of foods. Amer. Public Health Assn., Inc., New York.
2. Bieler, A. C., R. F. Matthews, and J. A. Koburger. 1972. Rock shrimp quality as influenced by handling procedures. Proc. Gulf and Caribbean Fish Inst. 25:56-61.
3. Breed, R. S., E. G. D. Murray, and N. R. Smith. 1957. Bergey's manual of determinative bacteriology. 7th ed. The Williams and Wilkins Co., Baltimore.
4. Buchanan, R. E., and N. E. Gibbons. 1974. Bergey's manual of determinative bacteriology. 8th ed. The Williams and Wilkins Co., Baltimore.
5. Carroll, B. J., G. B. Reese, and B. Q. Ward. 1968. Microbiological study of iced shrimp: Excerpts from the 1965 iced-shrimp symposium. U.S. Dept. of the Interior, Circular 285.
6. Cobb, S. P. The rock shrimp *Sicyonia brevirostris* Stimpson, Update report, Fla. Dept Natural Resources. St. Petersburg, Florida.
7. Cook, D. W. 1970. A study of the bacterial spoilage patterns in iced *Penaeus* shrimp. U.S. Dept. of the Interior Completion Report 2-61-R.
8. Katz, A. 1972. Florida rock shrimp fishery develops previously unmarketable shellfish. Quick Frozen Foods 34:61-72.
9. Koburger, J. A. 1970. Fungi in foods. I. Effect of inhibitor and incubation temperature on enumeration. J. Milk Food Technol. 33:433-434.
10. Lodder, J. 1970. The yeasts: A taxonomic study. North Holland Publishing Co., Amsterdam.
11. Phaff, H. J., E. M. Mrak, and O. B. Williams. 1952. Yeasts isolated from shrimp. Mycologia 44:431-451.
12. Society of American Bacteriologists. 1957. Manual of microbiological methods. McGraw-Hill Book Co., Inc. New York.
13. Vanderzant, C., E. Mroz, and R. Nickelson. 1970. Microbial flora of Gulf of Mexico and pond shrimp. J. Milk Food Technol. 33:346-350.
14. Williams, O. B., L. L. Campbell, and H. B. Reese. 1952. The bacteriology of Gulf Coast shrimp. II. Qualitative observations on the external flora. Texas J. Sci. 4:53-54.

J. Milk Food Technol. Vol. 38, No. 12, Pages 750-758 (December, 1975)

Thermal Inactivation of Conidia from *Aspergillus flavus* and *Aspergillus parasiticus*

II. Effects of pH and buffers, glucose, sucrose, and sodium chloride

M. P. DOYLE and E. H. MARTH

Department of Food Science and the Food Research Institute
University of Wisconsin-Madison, Madison, Wisconsin 53706

(Received for publication June 27, 1975)

ABSTRACT

Conidiospores from one strain of *Aspergillus flavus* and two of *Aspergillus parasiticus* were thermally inactivated in menstrua at pH values of 3.5, 4.5, 5.5, and 6.0. These values were obtained with the following buffering systems: sodium acetate and acetic acid, citric acid and Na_2HPO_4, potassium acid phthalate (KHP)-HCl and KHP-NaOH, and KH_2PO_4 and NaOH. Heating of conidia in a menstruum adjusted to pH 7.0 with KH_2PO_4 and NaOH served as the control. Use of the sodium acetate and acetic acid buffering system resulted in an increase in the rate at which conidia were inactivated when the pH was decreased. Use of the citric acid and Na_2HPO_4 buffering system resulted in increased thermal resistance for the conidia as the pH was decreased; however, the degree of increased thermal resistance was strain dependent. When the KHP-HCl and KHP-NaOH buffers were used, conidia were inactivated more rapidly than in the control at the higher pH values and more slowly than in the control at the lower pH values. An increase in amount of sodium chloride, sucrose, or glucose in the menstruum was accompanied by a decrease in the rate at which conidia were inactivated. Generally, sodium chloride was markedly protective to conidia at a_W values of less than 0.94, whereas the sugars were markedly protective at values below 0.95. Greatest protection at these values was afforded by sucrose.

Resistance of bacterial spores to moist heat is influenced by various factors including the pH and water activity (a_W) of the heating menstruum. Generally, bacterial spores are most resistant to heat at neutral to near-neutral pH values. Buffers such as citrate and phthalate usually reduce the heat resistance of bacterial spores more than does phosphate (*17, 18*).

Furthermore, lowering the a_W value of the heating menstruum increases the heat resistance of bacterial spores and vegetative cells. For example, bacterial spores and vegetative cells became more resistant to thermal inactivation when the amount of sucrose or glucose in the menstruum was increased (*4, 6, 7, 9, 16*). Salts may influence bacterial cells and spores in several ways and hence their effects on heat resistance are not always predictable. When in solution, salts can affect hydration of microbial proteins and stability of microbial enzymes as well as decrease the a_W value (*7*).

The pH and a_W of different foods is variable, depending on their composition. If foods are heated, these factors, in part, will govern the rate at which conidia of toxigenic aspergilli will be inactivated if they are in the product. Inactivation of the conidia will not only help to keep foods from spoiling but also will help to keep products safe, since growth of the toxigenic aspergilli is necessary before aflatoxin is synthesized.

Information is lacking on how pH and a_W influence heat inactivation of conidia produced by toxigenic aspergilli. Consequently, experiments described in this report were done to provide some of that information.

MATERIALS AND METHODS

Two series of experiments were done. The first dealt with how pH and different buffering systems affected thermal inactivation of conidia from *Aspergillus flavus* and *Aspergillus parasiticus.* The second series was concerned with the effects of various amounts of glucose, sucrose, and sodium chloride on thermal inactivation of the conidia.

Selection of cultures

Three cultures were selected for study based on results obtained in previous experiments (*2*). Principal criteria for choosing the cultures were their aflatoxigenicity and degree of thermal resistance. Strains selected included one that is heat sensitive, *A. flavus* NRRL 3353, one with intermediate heat resistance, *A. pararsiticus* NRRL 3315, and one that is markedly heat resistant, *A. parasiticus* NRRL 2999.

Spore suspensions

An inoculum of 0.5 ml of a suspension that contained approximately 10^7 conidia of *A. flavus* or *A. parasiticus* per milliliter was surface plated on modified Moyer's medium (*2*). Cultures were grown for 10 days at 28 C. Conidia were then harvested by adding about 50 ml of sterile distilled water to the surface growth and gently brushing the conidial chains with an inoculating loop. Spores were filtered, observed microscopically for clumping, and washed three times with sterile distilled water to remove any contaminating nutrients. suspensions were adjusted to 10^7 spores/ml by removal or addition of sterile water. Suspensions were then refrigerated and stored at 1-2 C until used. These preparations are designated as 10-day old conidiospores elsewhere in this paper.

Heating apparatus

The apparatus used to determine thermal inactivation rates has previously been described (*2*).

Heating menstrua

Inoculation of the heating menstruum was the same as has been outlined in earlier studies (*2*). All trials utilized a heat treatment at 55 C. To determine how pH and buffers affected inactivation of conidia, several buffering systems were used at different pH values. Table 1 lists the buffering systems used and their pH values. To com-

TABLE 1. *Buffer solutions used and their pH values*

Buffer	pH	Reference
50 ml 0.1 *M* KH_2PO_4 + 29.1 ml 0.1 *M* NaOH + 20.9 ml H_2O	7.0	(*15*)
2.2 ml 0.2 *M* Na acetate • 3 H_2O + 50 ml 0.2 *M* acetic acid + 47.8 ml H_2O	3.5	(*10*)
22.0 ml 0.2 *M* Na acetate • 3H_2O + 50 ml 0.2 *M* acetic acid + 28 ml H_2O	4.5	(*10*)
43.2 ml 0.2 *M* Na acetate • 3 H_2O + 50 ml 0.2 *M* acetic acid + 6.8 ml H_2O	5.5	(*10*)
50 ml 0.1 *M* potassium acid phthalate + 8.2 ml 0.1 *M* HCl + 41.8 ml H_2O	3.5	(*15*)
50 ml 0.1 *M* potassium acid phthalate + 8.7 ml 0.1 *M* HCl + 41.3 ml H_2O	4.5	(*15*)
50 ml 0.1 *M* potassium acid phthalate + 36.6 ml 0.1 *M* NaOH + 13.4 ml H_2O	5.5	(*15*)
20 ml 0.1 *M* citric acid • H_2O + 6.07 ml 0.2 *M* Na_2HPO_4 • $2H_2O$	3.5	(*10*)
20 ml 0.1 *M* citric acid • H_2O + 9.09 ml 0.2 *M* Na_2HPO_4 • $2H_2O$	4.5	(*10*)
20 ml 0.1 *M* citric acid • H_2O + 11.38 ml 0.2 *M* Na_2HPO_4 • 2 H_2O	5.5	(*10*)
20 ml 0.1 *M* citric acid • H_2O + 12.63 ml 0.2 *M* Na_2HPO_4 • 2 H_2O	6.0	(*10*)

pare buffering systems, the pH of each deionized distilled water heating menstruum was adjusted to 3.5, 4.5, 5.5, and, in one instance, 6.0, using each individual buffering system. Only one buffering system was used per heating menstruum.

To determine how sucrose and glucose affected thermal resistance of conidia, concentrations of 10, 30, 45, and 60% (w/w) of each in water were used as heating menstrua. Concentration of sugar, a_w, and molality of each test solution are given in Table 2. The a_w value for

TABLE 2. *Water activity and molality of, and percent (w/w) of sucrose and glucose in solutions used to study thermal inactivation of conidia of aspergilli (12)*

Percent (w/w)	Molality	Water activity
Sucrose		
10	0.32	0.993
30	1.25	0.975
45	2.39	0.949
60	4.39	0.898
Glucose		
10	0.62	0.988
30	2.38	0.956
45	4.55	0.917
60	8.33	0.848

each solution was determined by the method of Norrish (*12*). To determine the effect of sodium chloride on thermal resistance of conidia, concentrations of 0.9, 3.5, 7.0, 10.0, and 16.0% (w/v) were added to the heating menstruum. Concentration of NaCl, a_w, and molality of each test solution are listed in Table 3. The a_w for each solu-

TABLE 3. *Water activity and molality of, and percent (w/v) of NaCl insolutions used to study thermal inactivation of conidia of aspergilli (14)*

Percent NaCl (w/v)	Molality	Water activity
0.9	0.15	0.995
3.5	0.61	0.98
7.0	1.20	0.96
10.0	1.77	0.94
16.0	2.83	0.90

tion was determined as described by Robinson and Stokes (*14*). All test solutions were prepared in 0.1 *M* KH_2PO_4-0.1 *M* NaOH buffer at pH 7.0. All heating menstrua consisted of 999 ml of the respective test solutions.

Sampling and cooling of samples, enumeration of survivors, and analysis of results were identical to those outlined earlier (*2*).

RESULTS AND DISCUSSION

Effects of pH and buffers

Data obtained when the sodium acetate and acetic acid buffer was used are in Fig. 1. Figure 2 summarizes results obtained with the citric acid and Na_2HPO_4 buffer, whereas data in Fig. 3 were observed when the buffer consisted of potassium acid phthalate (KHP)-HCl and KHP-NaOH. Each point in these and all other figures represents the average of duplicate trials. The D-values that were calculated from data in Fig. 1, 2, and 3, are given in Table 4.

When comparing data in Fig. 1, 2, and 3, it is evident that in all instances conidia of *A. flavus* NRRL 3353 were most sensitive to moist heat regardless of buffer system used while conidia of *A. parasiticus* NRRL 3315 were intermediate and those of *A. parasiticus* NRRL 2999 were most resistant to moist heat. However, depending on the buffering system used, conidia from the three strains did vary in degree of thermal sensitivity. For example, Fig. 1 illustrates that conidia of *A. flavus*

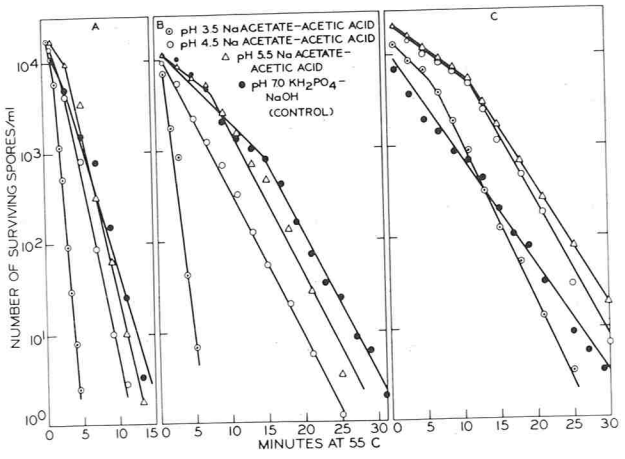


Figure 1. *Heat (moist) inactivation at 55 C of 10-day-old conidiospores of aspergilli. Conidiospores were produced on Moyer's medium and were heated in a distilled water menstruum which was buffered to various pH values using Na acetate and acetic acid. A,* A. flavus *NRRL 3353; B,* A. parasiticus *NRRL 3315; C,* A. parasiticus *NRRL 2999.*

NRRL 3353 and *A. parasiticus* NRRL 2999 consistently increased in thermal sensitivity as the pH was decreased with the Na acetate and acetic acid buffer, whereas conidia of *A. parasiticus* NRRL 3315 were appreciably more sensitive to heat in this buffering system when the pH was below 4.5. An even greater difference in thermal sensitivity is evident among the three strains in which the citric acid and Na_2HPO_4 • $2H_2O$ buffering system was used (Fig. 2). Conidia of *A. parasiticus* NRRL 2999 consistently decreased in thermal sensitivity as the pH was decreased. This same trend was evident for conidia of *A. parasiticus* NRRL 3315; however, the degree of decrease in thermal sensitivity was not as pronounced as with NRRL 2999 when compared to their respective controls. For example, conidia of NRRL 2999 when treated at pH 5.5, were more heat resistant than control conidia, whereas spores of NRRL 3315 were not more heat

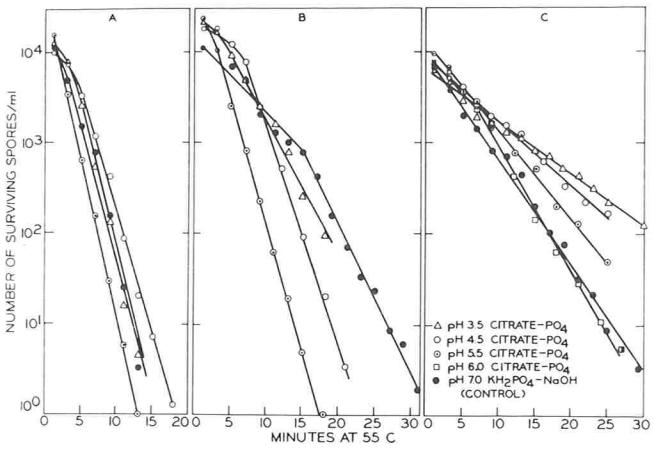


Figure 2. *Heat (moist) inactivation of 55 C of 10-day-old conidiospores of aspergilli. Conidiospores were produced on Moyer's medium and were heated in a distilled water menstruum which was buffered to various pH values using citric acid and Na_2HPO_4. A,* A. flavus *NRRL 3353; B,* A. parasiticus *NRRL 3315; C,* A. parasiticus *NRRL 2999.*

resistant than the control until the pH was reduced to 3.5. Results obtained with conidia of NRRL 3353 were somewhat more erratic than the data from the previous two strains; however, thermal resistance at pH 4.5 was greater than that of the control.

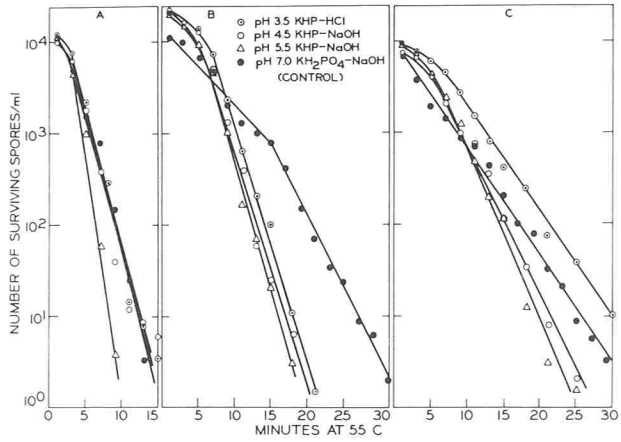


Figure 3. *Heat (moist) inactivation at 55 C of 10-day-old conidiospores of aspergilli. Conidiospores were produced on Moyer's medium and were heated in a distilled water menstruum which was buffered to various pH values using KHP and HCl or KHP and NaOH. A,* A. flavus *NRRL 3353; B,* A. parasiticus *NRRL 3315; C,* A. parasiticus *NRRL 2999.*

Figure 3 gives data obtained with the KHP-HCl and KHP-NaOH buffering systems and the data illustrate a fairly consistant trend, i.e., as the pH was decreased, thermal resistance as increased; however, the degree of thermal resistance was strain dependent. For example, conidia from strain NRRL 2999 increased in thermal resistance as the pH was decreased but thermal resistance was not greater than that of control conidia until a pH value of 3.5 was attained. Conidia from strain NRRL 3353 also increased in thermal resistance as the pH was decreased but in this instance, resistance was greater at pH 4.5 than that of the control. Conidia from strain NRRL 3315 were less responsive under these conditions and increased only slightly in thermal resistance as the pH was decreased. In addition, their thermal resistance never approached the value that prevailed under control conditions.

Sodium acetate and acetic acid buffer. A consistent trend is evident among D-values obtained with the sodium acetate and acetic acid buffering system (Table 4). As the pH of the heating menstruum was lowered, the

TABLE 4. *D-values of 55 C for 10-day old conidiospores of* A. flavus *and* A. parasiticus *produced on Moyer's medium and heated at different pH values achieved by several buffers*

Buffer	pH	D value (min) for strains NRRL 3353	NRRL 3315	NRRL 2999
KH_2PO_4 and NaOH	7.0	3.1	6.4	8.4
Na acetate and acetic acid	3.5	0.9	1.3	5.8
	4.5	2.4	6.1	6.5
	5.5	2.6	6.0	7.8
Citric acid and $Na_2HPO_4 \cdot 2H_2O$	3.5	3.0	6.7	17.7
	4.5	3.7	4.2	14.4
	5.5	2.9	3.7	10.6
	6.0	—[a]	—	7.5
KHP-HCl and KHP-NaOH	3.5	3.3	3.9	8.6
	4.5	3.5	3.9	5.9
	5.5	1.9	3.6	5.2

[a]-indicates not tested.

D-value also decreased. This decrease may be attributed to the increase in concentration of the undissociated acid. Inactivation of microorganisms by organic acids at low pH values without heat has been attributed to the concentration of the undissociated acid (*8*). It is difficult to identify the actual mechanism for inactivation; however, because acetic acid is a relatively small molecule, containing only two carbon atoms, it is possible that the acid is able to penetrate the conidiospore and hasten its inactivation. It is also possible that the hydrogen ion concentration has an effect on stability of the spore's protein. Extremes in pH can cause protein denaturation. As the pH is lowered, the isoelectric point of some of the proteins is reached and those proteins are altered. Hence, occurrence of protein denaturation, which may result from moist heat alone, may be enhanced by the presence of acetic acid because of the lowered pH value. This ultimately could render the conidia more sensitive to heat.

Citric acid and Na_2HPO_4 buffer. The D-values obtained using a citrate-Na_2HPO_4 buffering system are slightly erratic; nevertheless, a trend is evident (Table 4). As the pH was lowered and the amount of citrate increased, the thermal resistance of conidia was increased. This is particularly evident with strain NRRL 2999 which has a $D_{55\ C}$ value of 17.7 min at pH 3.5, while at pH 6.0 the value was 7.5 min.

Since little is known about the physiology of the *A. flavus* conidiospore, an explanation of citrate's protective effect can only be speculative. Citrate is well known for its sequestering ability. It readily binds metals and certain cations. Addition of citrate may sequester

detrimental cations so that their effects are neutralized.

Another possibly important consideration is that citric acid becomes increasingly dissociated as the pH rises and hence the quantity of metal that complexes varies accordingly (*3*). For example, at pH 3.5, citric acid has only one ionized carboxyl ion. This may allow for chelation with cations but does not provide for removal of the cation from the conidiospore. Therefore, the cation and citrate may form a complex which is attached to the conidial wall and possibly to intracellular particles. As the pH is increased, e.g., 4.5, 5.5, and 6.0, the second and third dissociation constants for citric acid for the remaining carboxyl groups are achieved and are surpassed when the pH approaches 6.0. As the second and finally third dissociation constant is achieved, increasing numbers of cations are sequestered and removed from the conidiospore.

It is suggested that a cation-citrate complex is formed which remains attached to the conidiospore at pH values near the acid's first dissociation constant. This cation-citrate grouping may afford a thermally protective complex possibly by stabilizing a heat labile protein. As the pH is raised, less of the complex remains attached to or present in the condiospore so that the conidiospore becomes more sensitive to heat. Deleterious cations are chelated at high pH values; however, the protective cation-citrate complex is not attached to the spore to afford increased thermal protection.

In addition to possibly forming a thermally protective cation-citrate complex, presence of citrate may enhance recovery of debilitated conidia. Moist heat may debilitate the conidiospore to such an extent that it would not be able to germinate unless readily utilizable nutrients were present adjacent to or bound to the spore. Since more of the cation-citrate complex would be present at lower pH values, more citrate for metabolism by the conidiospore would be available under those circumstances. The increased availability of this utilizable substrate may have resulted in increased recovery of heat debilitated conidia.

Hence, the citrate-cation complex may serve a dual purpose in promoting survival of conidiospores by forming a protective complex in and/or around the conidiospore and then providing a readily utilizable substrate for germinating spores.

KHP-HCl and KHP-NaOH buffers. The D-values obtained with the KHP-HCl and KHP-NaOH buffering system are listed in Table 4. It is apparent that thermal inactivation of conidiospores was faster when the pH was at 5.5 rather than at 3.5. KHP is a relatively large molecule. Because of its size, it may not be able to permeate the conidiospore wall. Therefore, KHP would only exert an effect on the spore's external surface.

At pH 5.5, where KHP had its most lethal effect, most of the phthalic acid is in the dissociated ion form. At pH 3.5 and 4.5, the KHP buffering system was not as deleterious and, in some instances, was somewhat more protective. At these pH values, one of the carboxyl groups of phthalate is in its undissociated form. These results are contrary to those obtained using an acetate buffer since greater inactivation of spores occurred when more of the undissociated form of KHP was present. However, since acetate is a small molecule, it may penetrate into the conidiospore while phthalate does not. It is difficult to postulate why the phthalate buffering systems affect the conidiospore the way they do. Further research is needed to provide the answer.

Effects of sodium chloride

Salts can influence the microbial cell and its heat resistance in several ways thus making it almost impossible to predict the results that will be obtained when such substances are present in a heating menstruum. Hence, experiments were done to learn the effects of various amounts of sodium chloride on thermal resistance of conidia from toxigenic aspergilli. Each strain of *Aspergillus* was tested by heating at 55 C in phosphate-buffered deionized distilled water at pH 7.0 which contained 0.0, 0.9, 3.5, 7.0, 10.0 or 16.0% (w/v) sodium chloride. A graphical representation of data for NRRL 3353 is in Fig. 4, for NRRL 3315 in Fig. 5, and for NRRL 2999 in Fig. 6. The D-values calculated from those data are in Table 5.

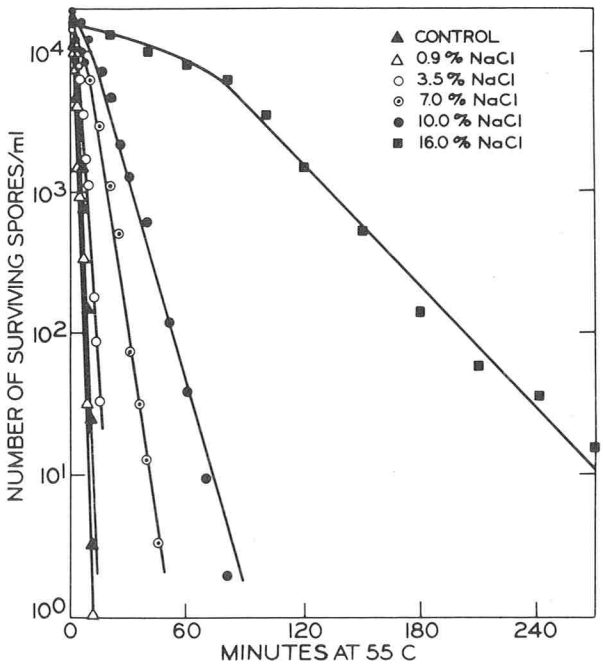


Figure 4. *Heat (moist) inactivation at 55 C of 10-day-old conidiospores of* A. flavus *NRRL 3353. Conidiospores were produced on Moyer's medium and were heated in a distilled water menstruum which was buffered to pH 7.0 using* KH_2PO_4 *and NaOH and to which various amounts of NaCl were added.*

As illustrated by data in Fig. 4, 5, and 6, the degree of conidial thermal sensitivity when in the presence of NaCl is strain dependent. For example, *A. flavus* NRRL 3353 was more sensitive than *A. parasiticus* NRRL 3315 which was more sensitive than *A. parasiticus* NRRL 2999. In addition, a general trend existed for all three strains, i.e.,

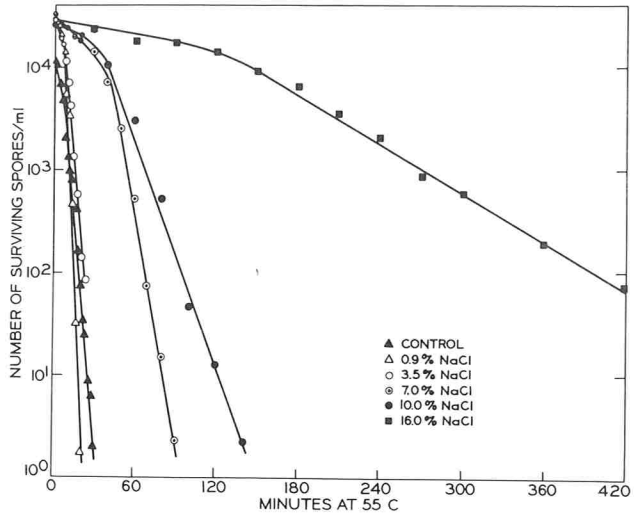


Figure 5. *Heat (moist) inactivation at 55 C of 10-day-old conidiospores of* A. parasiticus *NRRL 3315. Conidiospores were produced on Moyer's medium and were heated in a distilled water menstruum which was buffered to pH 7.0 using KH_2PO_4 and NaOH and to which various amounts of NaCl were added.*

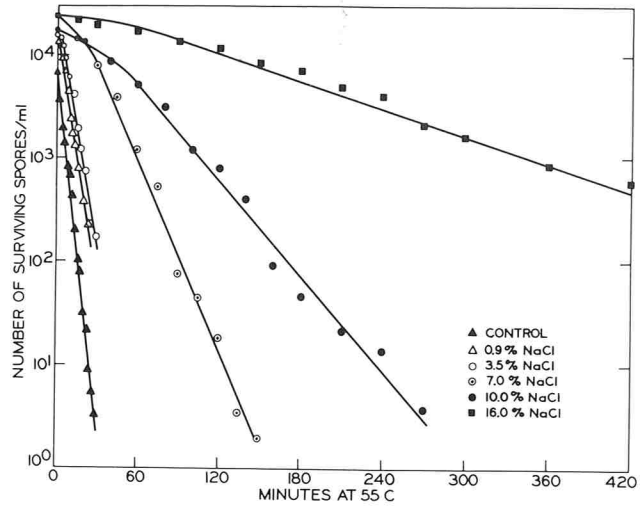


Figure 6. *Heat (moist) inactivation of 55 C of 10-day-old conidiospores of* A. parasiticus *NRRL 2999. Conidospores were produced on Moyer's medium and were heated in a distilled water menstruum which was buffered to pH 7.0 using KH_2PO_4 and NaOH and to which various amounts of NaCl were added.*

TABLE 5. *D-values at 55 C for 10-day old conidiospores of* A. flavus *and* A. parasiticus *produced on Moyer's medium and heated when various amounts of sodium chloride were in the heating menstruum*

Concentration of NaCl (w/v, %)	D value (min) for strain		
	NRRL 3353	NRRL 3315	NRRL 2999
0.0	3.1	6.3	8.4
0.9	2.6	3.6	12.5
3.5	4.3	7.0	12.4
7.0	11.7	13.9	31.6
10.0	18.7	26.4	65.3
16.0	69.8	125.4	230.2

as the amount of salt was increased, the heat resistance of their conidia also increased. This trend was consistent for all amounts of NaCl above 0.9%; however, at this concentration heat inactivation of conidia from two strains of mold was enhanced. As more sodium chloride was added, the difference of osmotic pressure between the interior of the conidiospore and the suspending medium became greater. This change may have provided a more favorable environment for the conidiospore and thereby resulted in decreased leakage of essential spore components during heating (*7*). Ou and Marquis (*13*), for example, noted cell wall contraction in gram positive cocci when they were present in hypertonic sucrose solutions. The bacterial cell wall has also been likened to a heteroporous molecular sieve. When the cell shrinks, it is possible that the pore sizes of the cell wall are reduced, thus minimizing the loss of intracellular components on heating (*5*).

Besides the possibility of reducing the conidiospore's susceptibility to leakage of essential components, the difference in osmotic pressure, because of its dehydrating effects, may enhance protein stability. Presence of sodium chloride, a strongly ionized solute, can cause water to be released from the interior of the conidiospore; the greater the osmotic pressure, the greater the amount of water released from the interior of the conidiospore. Having less water inside the spore means that less water is available to attach to groups within or at the surface of protein molecules with free charges and also, though less strongly, to dipoles such as CO and NH_2 groups (*7*). Therefore, the more water that is removed, the greater the stability of proteins in the conidiospore. This hypothesis agrees with results of an investigation by Gibson (*6*) in which she observed rapid changes in optical density when *Salmonella* or

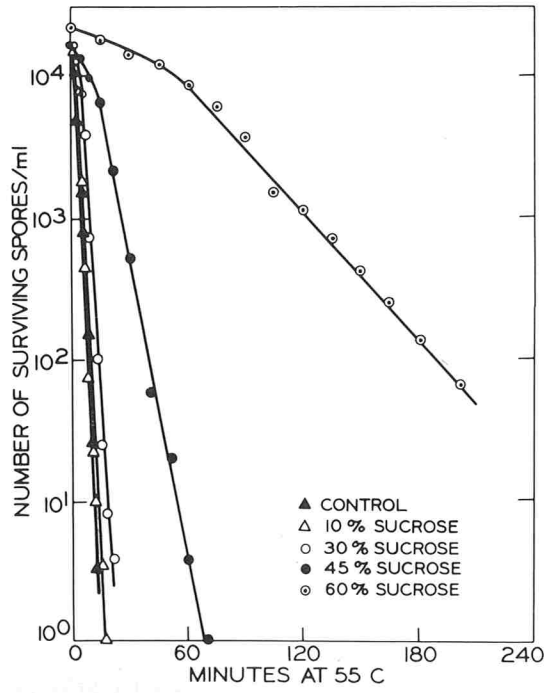


Figure 7. *Heat (moist) inactivation at 55 C of 10-day-old conidiospores of* A. flavus *NRRL 3353. Conidiospores were produced on Moyer's medium and were heated in a distilled water menstruum which was buffered to pH 7.0 using KH_2PO_4 and NaOH and to which various amounts of sucrose were added.*

Saccharomyces cells were present in hypertonic sucrose solutions. She attributed this to an almost instantaneous dehydration of the proteoplasm followed by shrinkage of the whole cell.

Effects of sucrose.

To see how sucrose affects thermal inactivation of *Aspergillus* conidiospores, four concentrations [10, 30, 45, and 60% (w/w)] of the sugar were tested. The various sucrose solutions were prepared in a solvent of phosphate buffered deionized distilled water at pH 7. A graphical representation of data for NRRL 3353 is in Fig. 7, for NRRL 3315 in Fig. 8, and for NRRL 2999 in Fig. 9. The D-values calculated from these data are in Table 6.

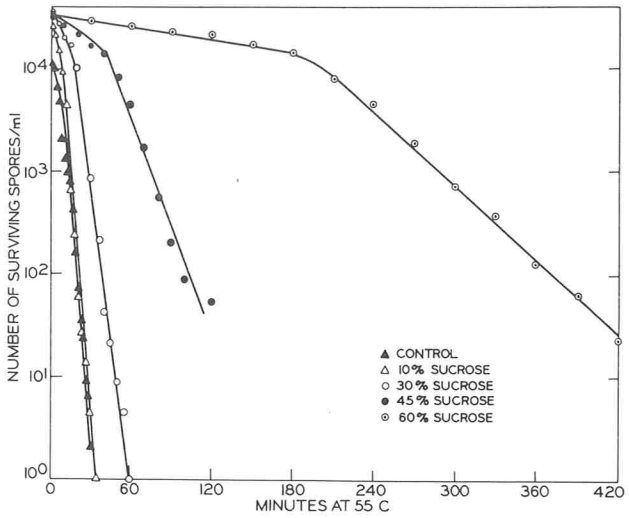


Figure 8. *Heat (moist) inactivation at 55 C of 10-day-old conidiospores of* A. parasiticus *NRRL 3315. Conidiospores were produced on Moyer's medium and were heated in a distilled water menstruum which was buffered to pH 7.0 using* KH_2PO_4 *and NaOH and to which various amounts of sucrose were added.*

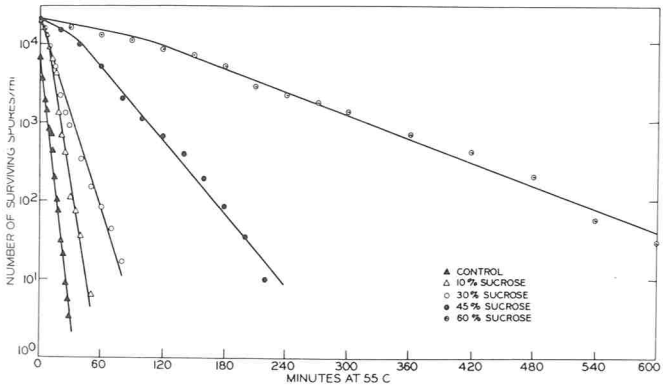


Figure 9. *Heat (moist) inactivation at 55 C of 10-day-old conidiospores of* A. parasiticus *NRRL 2999. Conidiospores were produced on Moyer's medium and were heated in a distilled water menstruum which was buffered to pH 7.0 using* KH_2PO_4 *and NaOH and to which various amounts of sucrose were added.*

When comparing data in Fig. 7, 8, and 9, it is evident that the degree of thermal sensitivity of conidia in the presence of sucrose is strain dependent. Conidia of *A. flavus* NRRL 3353 were least heat resistant while those of *A. parasiticus* NRRL 3315 were intermediate and those of *A. parasiticus* NRRL 2999 were most resistant. In addition, a general trend existed for all three strains and is most pronounced in the most heat resistant strain, NRRL 2999. It is evident that as the amount of sucrose was increased, the heat resistance of the spores also increased. Protection afforded by the presence of sucrose was substantial, particularly when a large amount, such as 60%, was present. For example, with no sucrose in the heating menstruum, the D value for NRRL 2999 at 55 C was 8.4 min; however, when 60% sucrose was present, the D value was 199 min. The mechanism for protection afforded by sucrose may be similar to that for sodium chloride. Increased thermal resistance of conidiospores in the presence of sucrose may be largely a result of dehydration which confers greater stability on protein and possibly reduces loss of spore components.

TABLE 6. *D-values at 55 C for 10-day old conidiospores of* A. flavus *and* A. parasiticus *produced on Moyer's medium and heated when various amounts of sucrose were in the heating menstruum*

Concentration (w/w, %)	D value (min) for strains		
	NRRL 3353	NRRL 3315	NRRL 2999
0.0	3.1	6.3	8.4
10.0	3.7	6.4	12.2
30.0	4.6	10.0	25.2
45.0	14.5	28.7	63.4
60.0	65.7	84.2	199.0

Effects of glucose

Glucose solutions were prepared and thermal inactivation rates were determined in the same manner as when sucrose solutions were evaluated. A graphical

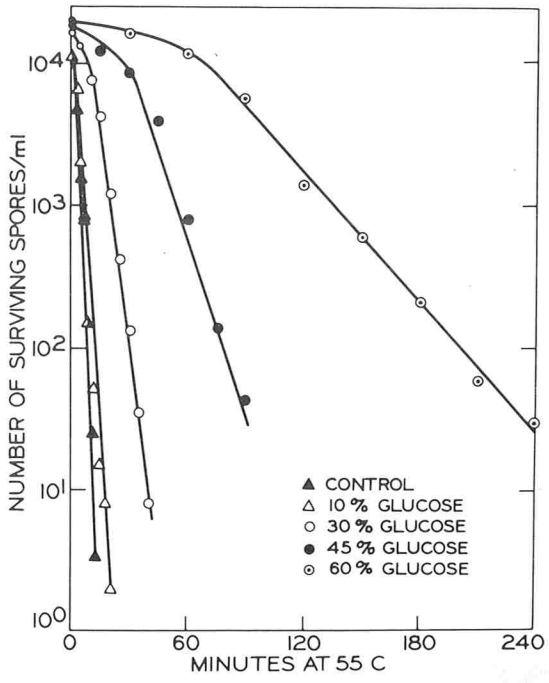


Figure 10. *Heat (moist) inactivation at 55 C of 10-day-old conidiospores of* A. flavus *NRRL 3353. Conidiospores were produced on Moyer's medium and were heated in a distilled water menstruum which was buffered to pH 7.0 using* KH_2PO_4 *and NaOH and to which various amounts of glucose were added.*

representation of data for NRRL 3353 is shown in Fig. 10, for NRRL 3315 in Fig. 11, and for NRRL 2999 in Fig. 12. When data in the three figures are compared, it is

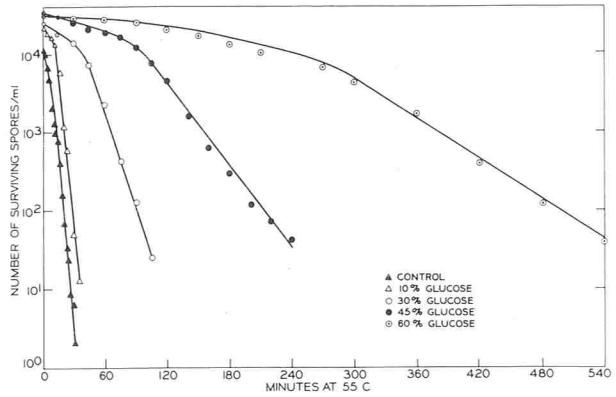


Figure 11. *Heat (moist) inactivation at 55 C of 10-day-old conidiospores of* A. parasiticus *NRRL 3315. Conidiospores were produced on Moyer's medium and were heated in a distilled water menstruum which was buffered to pH 7.0 using* KH_2PO_4 *and NaOH and to which various amounts of glucose were added.*

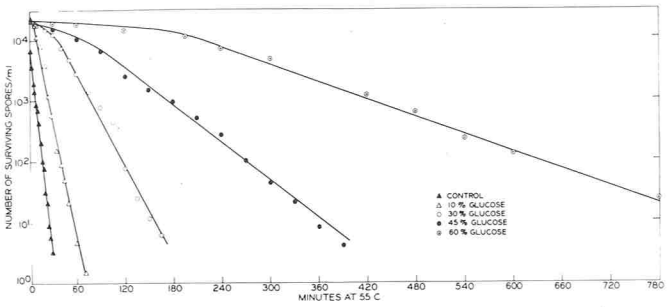


Figure 12. *Heat (moist) inactivation at 55 C of 10-day-old conidiospores of* A. parasiticus *NRRL 2999. Conidiospores were produced on Moyer's medium and were heated in a distilled water menstruum which was buffered to pH 7.0 using* KH_2PO_4 *and NaOH and to which various amounts of glucose were added.*

apparent that strain differences existed, i.e., conidia from strain NRRL 3353 were least heat resistant in the presence of various amounts of glucose, whereas those of strain NRRL 3315 were intermediate in resistance and those of strain NRRL 2999 were most heat resistant. The D-values calculated from these data are in Table 7.

TABLE 7. *D-values of 55 C for 10-day old conidiospores of* A. flavus *and* A. parasiticus *produced on Moyer's medium and heated when various amounts of glucose were in the heating menstruum*

Concentration (w/w, %)	D value (min) for strains		
	NRRL 3353	NRRL 3315	NRRL 2999
0.0	3.1	6.3	8.4
10.0	5.1	7.0	14.5
30.0	9.9	24.2	39.2
45.0	24.4	57.6	95.9
60.0	66.2	117.9	213.9

As with results obtained for sucrose, a general trend exists for all three strains that were heated in glucose solutions. As the amount of glucose was increased, thermal resistance of conidiospores also increased. However, at comparable concentrations (w/w) the thermal resistance of conidiospores treated in glucose was greater than that of conidia heated in solutions of sucrose. This may be explained by the fact that sucrose is a dissaccharide while glucose is a monosaccharide so that when determining weight/weight concentrations, two molecules of glucose are approximately equivalent to one molecule of sucrose. As a consequence, the a_w of glucose at a specific concentration is lower than that of sucrose at the same concentration. This means that an equivalent weight/weight concentration of glucose binds more water than one of sucrose.

Since more water is bound by the glucose it is reasonable to conclude that greater dehydration will occur in the conidiospore and less free water, which is needed for more rapid protein denaturation, will be available. Glucose therefore should be and was more protective to *Aspergillus* conidiospores than an equivalent weight/weight concentration of sucrose when spores were heated under moist conditions.

Comparison of effects of NaCl, sucrose, and glucose on a water activity basis

To compare the effectiveness of the three solutes (sodium chloride, sucrose, and glucose) in affording protection to conidia during heat treatment, D-values of each *Aspergillus* strain were plotted against the a_w of each solute. Figure 13 illustrates this comparison for strain NRRL 3353, Fig. 14 for strain NRRL 3315, and Fig. 15 for strain NRRL 2999. When comparing data in

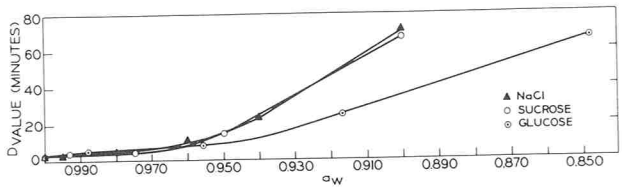


Figure 13. *D-values obtained for* A. flavus *NRRL 3353 at 55 C having NaCl, sucrose, or glucose in the heating menstruum plotted against the water activity* (a_w) *when various amounts of the solutes were in solution.*

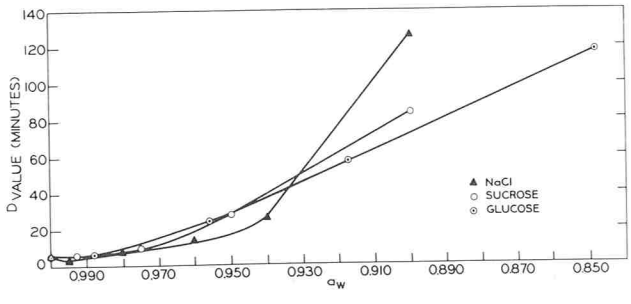


Figure 14. *D-values obtained for* A. parasiticus *NRRL 3315 at 55 C having NaCl, sucrose, or glucose in the heating menstruum plotted against the water activity* (a_w) *when various amounts of the solutes were in solution.*

these figures, it is evident that conidia from strain NRRL 3353 were least resistant to heat when in the presence of each of the three solutes at all a_w values tested. Conidia from strain NRRL 3315 were intermediate in their heat resistance while conidia from strain NRRL 2999 were most heat resistant. The trends were remarkably similar

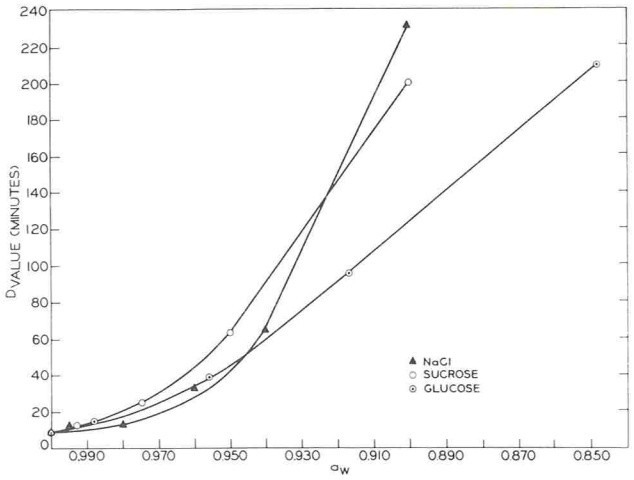


Figure 15. *D-values obtained for* A. parasiticus *NRRL 2999 at 55 C having NaCl, sucrose, or glucose in the heating menstruum plotted against the water activity (a_w) when various amounts of the solutes were in solution.*

for all three strains. At higher a_w values, i.e., from about 0.95 (depending on the strain) and up, sodium chloride was slightly less protective than were either sucrose or glucose; however, as a_w values decreased from about 0.94, the protective effect of sodium chloride was somewhat greater than that of sucrose and much greater than that of glucose. In addition, as $a_w$values decreased from about 0.95, the protective effect of sucrose became substantially greater than that of glucose.

Glucose, sucrose, or sodium chloride solutions at the same a_w value failed to protect the *Aspergillus* conidia to an equivalent degree. This suggests that a_w per se is not the controlling factor in determining thermal resistance. Water activity may have some effect but it is apparent that other factors, such as the chemical nature of the solute, are important in governing heat resistance. Similar results have been observed with bacterial cells. For example, sucrose protected salmonellae from heat destruction far more efficiently than did glycerol at equvalent a_w values down to 0.87 (*4*). The same was true in other studies in which no direct correlation between a_w value and heat resistance was found (*1, 11*), although an increased heat resistance of the microorganism was observed when the a_w of the heating menstruum was lowered.

CONCLUSIONS

From results obtained in this study and in an earlier one (*2*), it can be concluded that thermal sensitivity of conidia is dependent on strain of mold, age of the conidiospore, composition of medium on which the conidiospore was produced, and composition of the material surrounding or adjacent to the conidiospore during heating. The data also indicate that as the amount of glucose, sucrose, and/or sodium chloride in solution is increased, survival of *A. flavus* and *A. parasiticus* conidia can be expected to increase when subpasteurization heat treatments are used. Foods which may support germination of *A. flavus* and *A. parasiticus* conidia as well as production of aflatoxin are of primary concern. Foods such as "no cook" jellies and jams which are high in sucrose but are boiled for only a few minutes might contain surviving conidia. Experiments with such materials are needed to verify that viable conidia are present or absent.

We also noted that there is no consistent correlation between pH and survival of *A. flavus* and *A. parasiticus* conidiospores when heated under moist conditions. Instead thermal sensitivity depends on the various compounds present which contribute to the pH. For example, the presence of acetic acid decreased thermal resistance of conidia, whereas the presence of citric acid increased thermal resistance at the equivalent pH (depending on the strain of mold). Hence it is difficult to generalize about the relationship between the pH values of foods and thermal inactivation of conidia; instead the components which make up a food and determine its pH must be considered.

From our data in this paper and in an earlier report (*2*), it can be concluded that older conidiospores (20 days), conidia grown on a high protein, low carbohydrate substrate, and conidia heated in the presence of Na acetate-acetic acid at pH 3.5 were most susceptible to thermal inactivation. Younger conidiospores (7 to 10 days), conidia grown on a high carbohydrate, low protein substrate, and conidia heated in the presence of glucose, sucrose, or sodium chloride were least susceptible to thermal inactivation.

ACKNOWLEDGMENTS

This work was supported by the College of Agricultural and Life Sciences, University of Wisconsin-Madison; the Food and Drug Administration through Public Health Service grant FD 00143; and by the food industry through contributions to the Food Research Institute. Presented in part at the 62nd Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, Toronto, Ontario, August 11-13, 1975.

REFERENCES

1. Baird-Parker, A. C., M. Boothroyd, and E. Jones. 1970. The effect of water activity on the heat resistance of heat sensitive and heat resistant strains of salmonellae. J. Appl. Bacteriol. 33:515-522.
2. Doyle, M. P., and E. H. Marth. 1975. Thermal inactivation of conidia from *Aspergillus flavus* and *Aspergillus parasiticus.* I. Effects of moist heat, age of conidia, and substrate. J. Milk Food Technol. 38:(In press).
3. Furia, T. E. 1972. Sequesterants in foods. Pages 271-294. In T. E. Furia, ed. CRC handbook of food additives, 2nd ed. Chemical Rubber Co., Cleveland, Ohio.
4. Goepfert, J. M., I. K. Iskander, and C. H. Amundson. 1970. Relation of the heat resistance of salmonellae to the water activity of the environment. Appl. Microbiol. 19:429-433.
5. Gerhardt, P., and J. A. Judge. 1964. Porosity of isolated cell walls of *Saccharomyces cerevisiae* and *Bacillus megaterium.* J. Bacteriol. 87:945-951.
6. Gibson, B. 1973. The effect of high sugar concentrations on the heat resistance of vegetative microorganisms. J. Appl. Bacteriol. 36:365-376.
7. Hansen, N. H., and H. Rieman. 1963. Factors affecting the heat resistance of nonsporing organisms. J. Appl. Bacteriol. 26:314-333.

8. Kostenbauder, H. B. 1968. Physical factors influencing the activity of antimicrobial agents. Pages 44-62. In C. A. Lawrence and S. S. Block, eds. Disinfection, sterilization and preservation. Lea and Febiger, Philadelphia, Pennsylvania.
9. Lee, A. C., and J. M. Goepfert. 1975. Influence of selected solutes on thermally induced death and injury of *Salmonella typhimurium*. J. Milk Food Technol. 38:195-200.
10. Meynell, G. G., and E. Meynell. 1970. Theory and practice in experimental bacteriology. Cambridge University Press. London and New York. p. 66.
11. Moats, W. A., R. Dabbah, and V. M. Edwards. 1971. Survival of *Salmonella anatum* heated in various media. Appl. Microbiol. 21: 476-481.
12. Norrish, R. S. 1966. An equation of the activity coefficients and equilibrium relative humidities of water in confectionery syrups. J. Food Technol. 1:25-39.
13. Ou, L., and R. E. Marquis. 1970. Electromechanical interactions in cell walls of gram positive cocci. J. Bacteriol. 101:92-101.
14. Robinson, R. A., and R. H. Stokes. 1955. Electrolyte solutions. Academic Press, New York and London. p. 461.
15. Sober, H. A. 1970. CRC handbook of biochemistry. Selected data for molecular biology. The Chemical Rubber Co., Cleveland, Ohio. p. J-231.
16. Sugiyama, H. 1951. Studies on factors affecting the heat resistance of spores of *Clostridium botulinum*. J. Bacteriol. 62:81-96.
17. Walker, H. W. 1964. Influence of buffers and pH on the thermal destruction of spores of *Bacillus megaterium* and *Bacillus polymyxa*. J. Food Sci. 29:360-365.
18. Xezones, H., and I. J. Hutchings. 1965. Thermal resistance of *Clostridium botulinum* (62A) spores as affected by fundamental food constituents. Food Technol. 19:1003-1005.

J. Milk Food Technol. Vol. 38, No. 12, Pages 759-761 (December, 1975)

Bacteriological Quality of Selected Delicatessen Foods

NATHOLYN D. HARRIS, STEPHEN R. MARTIN[1], and LORETTA ELLIAS

Departments of Food and Nutrition and Biological Sciences
Florida State University, Tallahassee, Florida 32306

(Received for publication March 20, 1975)

ABSTRACT

Warm and cold delicatessen foods were purchased from local retail stores and were subsequently analyzed for total plate counts, *Staphylococcus aureus* and coagulase positive staphylococci, *Clostridium perfringens,* and coliforms. Foods were analyzed immediately after purchase, after holding 3 h at 37 C, and after holding at 9 C for 4 days. The total viable bacteria, staphylococci, coliform, and *C. perfringens* numbers did not vary much during storage at 37 C for 3 h and 4 days at 9 C. The average total count was 350,000 cells per gram for all foods examined. More than 30% of the staphylococcal colonies tested were coagulase positive. Meat salads contained the highest total counts and warm meats contained the lowest numbers of cells.

During the past few years, there has been an increased consumption of commercially prepared foods. Those foods which receive no treatment such as freezing to prolong their storage life are sometimes referred to as delicatessen foods. Such foods often receive much handling during preparation and may become contaminated from raw materials, food handlers, or from unclean equipment.

No microbiological standards exist for delicatessen foods, but there are guidelines for numbers of bacteria in such products. A total viable count of 100,000/g of food is arbitrarily regarded as the acceptable limit by several researchers (*4, 9, 10*). Low total counts tend to parallel safety from food poisoning pathogens.

The number of research studies designed to assess the safety of delicatessen foods is limited. Christiansen and King (*1*) examined the microbial content of commercially prepared salads and a variety of protein-filled sandwiches in the Raleigh, North Carolina area. They found that 36% of the salad samples and 16% of the sandwiches had total counts greater than 10^6/g. They found coagulase-positive staphylococci in approximately 39% of the chilled salads and 60% of the sandwiches. Samples with high total counts contained coagulase-positive staphylococci.

In a study designed to determine the bacteriological quality of chilled delicatessen foods, Rasmussen and Strong (*7*) found that protein rich salads contained relatively high numbers of total viable bacteria and staphylococci. They found more than 25% of the staphylococcal colonies obtained from high protein salads to be coagulase-positive. Other foods were generally lower in total viable bacteria and pathogens.

The purpose of this study was to assess the bacteriological quality of cold and warm delicatessen foods from two stores in Tallahassee, Florida. A second aspect of the study was to investigate the effect of certain consumer handling practices on the bacteriological quality of the food.

MATERIALS AND METHODS

Samples of the delicatessen-type foods were purchased in the manner of a consumer at random times during the day and on various days. A total of 42 samples were obtained and transported to the laboratory in an insulated carrying case containing bags of frozen refrigerant. Testing was begun immediately upon arrival at the laboratory.

Selection of foods

The types of delicatessen foods chosen were as follows: (*a*) warm main dish meats, (*b*) warm and cold vegetable salads, (*c*) cold sliced meats for sandwiches, and (*d*) cold meat salads.

Holding conditions

The bacteriological quality of the samples was analyzed at three different times. Initially, samples were analyzed immediately after purchase. Second, they were tested after 3 h of holding at 37 C. This holding procedure was used to simulate foods taken to the beach for a picnic or left in a car while doing errands on a hot day. Third, any food not eaten immediately might be refrigerated for several days; thus, samples were analyzed after being refrigerated 4 days at 9 C.

Bacteriological tests

Standard methods agar was used to determine the total plate count. The standard methods plating technique was used and plates were incubated at 35 C for 48 h.

The most probable number (MPN) technique was used to enumerate coliforms. Various dilutions of product were placed in Lauryl Sulfate broth and tubes were checked for gas production. Positive tubes were confirmed by transferring an inoculum to 2% Brilliant Green Bile broth. The MPN was calculated from the AOAC MPN table. E.C. broth was used to test for *Escherichia coli*. The E.C. broth was incubated in a covered water bath at 45.5 C for 24 and 48 h. Tubes with gas were considered positive.

The method of Shahidi and Ferguson (*8*) was used to identify and enumerate *Clostridium perfringens.* Isolates from SFP agar were confirmed by testing for gas and acid production in Lactose motility agar.

Staphylococcus 110 medium with azide was used to test for staphylococci. Gram stains were also observed. Coagulase agar base containing pretested reconstituted coagulase plasma with EDIA was used to test for coagulase production.

[1]*Present address: Land O'Lakes, Inc., 614 McKinley Place, Minneaspolis, Minnesota 55413*

RESULTS AND DISCUSSION

Total plate count

Figure 1 shows the total bacterial counts for the various food groups. The counts ranged from less than 300 to almost one million/g of food. Meat salad had the highest average total plate counts and warm meats had the lowest counts. Holding the foods for 3 h at 37 C or 4 days at 9 C had little effect on total counts. Perhaps larger initial counts would have resulted in growth during the holding periods.

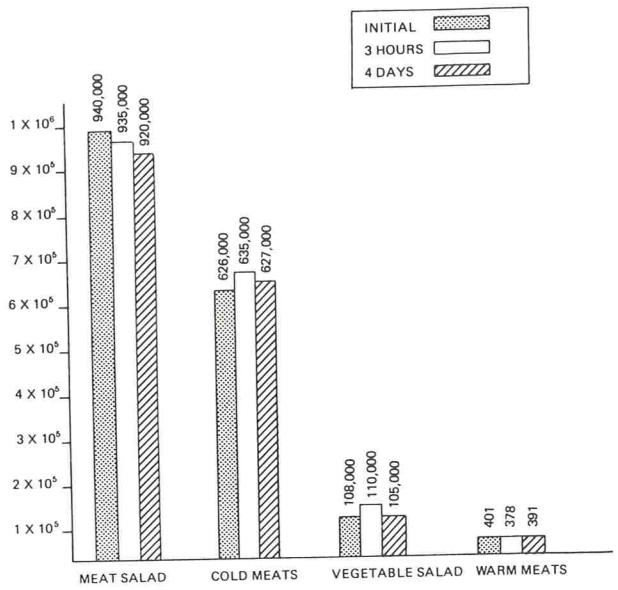


Figure 1. *Comparisonsof total counts of various delicatessen food groups (44 total samples).*

Coliforms

As groups, themeat and potato salads had the highest values for coliforms and cole slaw and barbequed chicken produced lower counts (Table 1). The average number of coliforms was 460 per gram. This is considerably lower than results of other investigators who reported a MPN value of around 2500 per gram (*5, 6, 7, 10*). Fewer than 1% of the coliforms were *E. coli.*

TABLE 1. *Coliforms in selected delicatessen foods*[1] *stored at two temperatures*

Food	No./g			
	Confirmed as *E. coli* (MPN)	Initial count	3 h at 37 C	4 days at 9 C
Corned beef		4.6×10^2	4.6×10^2	4.6×10^2
Potato salad	4×20^0	2.4×10^2	2.4×10^2	2.4×10^2
Potato salad		2.1×10^2	2.1×10^2	2.1×10^2
Potato salad		2.4×10^2	2.4×10^2	2.4×10^2
Baked beans		1.1×10^3	1.1×10^3	1.1×10^3
Cole slaw		9.3×10^1	9.3×10^1	9.3×10^1
B-B-que chicken	3×10^0	7.0×10^0	7.0×10^0	7.0×10^0
Ham salad		1.1×10^3	1.1×10^3	1.1×10^3
Ham salad		4.3×10^2	4.3×10^2	4.3×10^2

[1]Determined by Most Probable Number Technique.

C. perfringens

Meats were tested for *C. perfringens.* Only one corned beef sample was positive and the number of cells in that sample was little affected by holding conditions. The New York City health Department recently reported that 5.7% of meat samples tested contained *C. perfringens.* Other reports (*2, 3*) are in line with the 12.5% incidence of *C. perfringens* in the limited number of samples examined in the present study.

Staphylococci

Ham salad yielded the highest number of staphylococcal colonies (Table 2). Of the 79 colonies tested for coagulase production, 31% were positive (Table 3). These values are slightly higher than those of Rasmussen and Strong (*7*) who found an average of 26% coagulase-positive staphylococci in high protein salads and 21% coagulase positive organisms in all foods tested. As a group, the vegetable salads (cole slaw and potato salad) had the highest percentage of coagulase-positive staphylococci. The holding conditions did not appear to affect the average total staphylococcal counts, however, the percentage of coagulase-positive staphylococci increased from 30.7% to 34.6% during holding for 4 days at 9 C.

TABLE 2. Staphylococcus aureus *in selected delicatessen foods stored at two temperatures*

Food	No./g		
	Initial	3 h at 37 C	4 days at 9 C
Ham salad	3.2×10^4	3.3×10^4	3.2×10^4
Ham salad	3.5×10^2	3.7×10^2	3.5×10^2
Roast beef	<10	<10	<10
Cole slaw	<10	<10	<10
Potato salad	1.5×10^3	1.6×10^3	1.5×10^3
Potato salad	<10	<10	<10
B-B-que chicken	<10	<10	<10
Boiled ham	<10	<10	<10
Corned beef	1.8×10^2	1.8×10^2	1.8×10^2

TABLE 3. *Staphylococci in various delicatessen food groups stored at two temperatures*

Food group	No./g			Percent coagulase positive
	Initial	3 h at 37 C	4 days at 9 C	
Warm meats	<10	<10	<10	0
Cold meats	9.0×10^1	9.0×10^1	9.0×10^1	33
Vegetable salads	5.1×10^2	5.4×10^2	5.0×10^2	44
Meat salads	1.62×10^4	1.67×10^4	1.62×10^4	33

The low number of microorganisms encountered in this study offer evidence that good sanitary practices were followed during preparation and holding of the foods. More investigations must be done in a wide spectrum of food preparation and food service establishments with an emphasis on the many varieties of delicatessen food, other pathogens, and different consumer holding conditions to answer the question as to whether microbiological standards are feasible or needed for delicatessen foods.

REFERENCES

1. Christiansen, L. N., and N. J. King. 1971. The microbial content of some salads and sandwiches at retail outlets. J. Milk Food Technol. 34:289-293.
2. Hall, H., and R. Angelotti. 1965. *Clostridium perfringens* in meat and meat products. Appl. Microbiol. 13:352-357.
3. Hall, H. E., D. F. Brown, and K. H. Lewis. 1967. Examination of market foods for coliform organisms. Appl. Microbiol.
4. Hobbs, B. C., and R. J. Gilbert. 1970. Microbiological standards for food: public health aspects. Chem. 7:215-219.
5. Kereluk, K., and M. F. Gunderson. 1959. Studies on the bacteriological quality of frozen meat pies. Appl. Microbiol. 7:320-323.
6. Raj. H., and J. Liston. 1961. Detection and enumeration of fecal indicator organisms in frozen sea foods. I. *Escherichia coli.* Appl. Microbiol. 9:171-174.
7. Rasmussen, C. A., and D. H. Strong. 1967. Bacteria in chilled delicatessen foods. Public Health Reports 82:353-359.
8. Shahidi, S. A., and A. R. Ferguson. 1971. New quantitative, qualitative and confirmatory media for rapid analysis of food for *Clostridium perfringens.* Appl. Microbiol. 21:500-506.
9. Thatcher, F. S. 1958. Microbiological standards for foods. II. What may microbiological standards mean? Food Technol. 12: 117-122.
10. Zaborowski, H., D. A. Huber, and M. M. Rayman. 1958. Evaluation of microbiological methods used for the examination of precooked frozen foods. Appl Microbiol. 6:97-104.

J. Milk Food Technol. Vol. 38, No. 12, Pages 762-763 (December, 1975)

A Research Note

Some Observations on a Perigo-Type Inhibition of *Clostridium botulinum* in a Simplified Medium

C. N. HUHTANEN

Eastern Regional Research Center[1]
Philadelphia, Pennsylvania 19118

(Received for publication May 29, 1975)

ABSTRACT

A rapid and sensitive assay for Perigo factor was developed using a medium of 0.5% yeast extract and tryptone, 0.2% glucose, 0.12% K_2HPO_4 and 0.1% cysteine • HCl or sodium thioglycollate and vegetative cells of *Clostridium botulinum* type A. Yeast extract or tryptone, together with a reducing agent (cysteine, sodium thioglycollate, or glucose autoclaved with the medium), produced a Perigo inhibitor when autoclaved at 15 psi for 15 min with $NaNO_2$. Tryptone was more active than yeast extract as a source of the Perigo inhibitor; of the reducing agents tested cysteine was more effective in producing Perigo-type inhibition than thioglycollate and either was better than glucose autoclaved with the medium.

A potent inhibitor of vegetative cells of *Clostridium sporogenes* was produced by heating nitrite in a complex medium composed of tryptone, peptone, beef extract (Lab-Lemco), yeast extract, NaCl, K_2HPO_4, glucose, soluble starch, sodium thioglycollate, and brom cresol purple [Perigo et al. (*3*)]. The study reported here was undertaken to develop a more rapid assay for such inhibitors—the original procedure of Perigo et al. requiring 10 days of incubation. The assay medium was then used to identify the component(s) of the medium involved in the formation of the Perigo inhibitor.

MATERIALS AND METHODS

The basic solution used in developing the simpler assay medium contained the chemically defined components of Perigo's medium: 0.5% NaCl; 0.25% K_2HPO_4; 0.2% glucose; 0.1% starch; 0.1% sodium thioglycollate, and 0.008% brom cresol purple. To this basal solution were added 0.5% concentrations of the four crude materials; beef extract, yeast extract, peptone, and tryptone, either individually or in various combinations. A Seitz-filtered, 1.6% solution of $NaNO_2$ was diluted 1:1 serially with sterile water and 0.2 ml of each dilution was added to 5 ml of the medium either before or after autoclaving at 15 psi for 15 min to give final concentrations (ppm) of $NaNO_2$ of 640, 320, 160, 80, etc. to 1.25.

Culture and inoculum

Stock cultures of *Clostridium botulinum* type A (#B1218 from the Northern Regional Research Center, USDA, Peoria, Ill.) were maintained in a cooked meat medium and transferred by needle into a medium containing 0.5% each of tryptone, yeast extract, beef extract, and peptone, 0.2% glucose, 0.1% sodium thioglycollate, and 0.12% K_2HPO_4, at pH 6.6, with an overlay of vaspar. These were incubated 20-24 h at 35 C. The inoculum for the assay was one drop of this culture, previously diluted 1-50 with the assay medium, to 5 ml of medium in 15 × 125 mm culture tubes for a final cell concentration of about 300/ml. Duplicate tubes were incubated at 35 C for 24 h. Further incubation up to one week did not appreciably change the results. Initially, an atmospheric incubator was used while later experiments were carried out in a controlled atmosphere incubator evacuated to 20-27 in. mercury with the vacuum being replaced with nitrogen gas. Growth in the latter system was slightly faster.

Perigo index

Inhibition of growth of the organism was expressed as the lowest concentration of nitrite, in ppm, in the tube which did not allow growth of the organism with turbidity being used as the criterion. The ratio of this minimum inhibitory concentration (mic) of the nitrite added after autoclaving to the mic of nitrite autoclaved with the medium was designated for convenience as the Perigo Index (PI). The PI indicates the magnitude of the heated nitrite effect; for example, a PI of 2 indicates that twice as much unheated nitrite was needed for inhibition than nitrite heated with the medium.

RESULTS AND DISCUSSION

Beef extract, added to the basic, chemically defined solution above, did not support growth and only slight growth was obtained in the medium containing peptone alone. Yeast extract or tryptone alone gave better growth while a combination of yeast extract and tryptone gave nearly as good growth as the complete medium. Further studies indicated that NaCl, starch, and brom cresol purple were not necessary for growth, had no effect on growth inhibition and were therefore eliminated from the medium. The final medium for assay of the "Perigo Inhibitor" consisted of 0.5% yeast extract, 0.5% tryptone, 0.2% glucose, 0.12% K_2HPO_4, with either 0.1% sodium thioglycollate or cysteine • HCl. No pH adjustment was needed with the former while neutralization was needed with cysteine.

Effect of reductants

A comparison of 0.1% cysteine and sodium thioglycollate as reducing agents in the medium for production of the Perigo inhibitor is shown in Table 1. Cysteine was more effective, giving a PI of 64 (first line in Table 1—the complete medium) compared to 16 with

[1]*Agricultural Research Service, U.S. Department of Agriculture.*

TABLE 1. *Effect of separately autoclaved reducing agents or glucose on the formation of Perigo inhibitor*

Components added after autoclaving	Ratio[a] (mic, ppm)
None (complete medium with cysteine autoclaved)	160/2.5 = 64[b]
Cysteine	40/2.5 = 16
Glucose	160/2.5 = 64
Cysteine and glucose	160/80 = 2
None (complete medium with thioglycollate autoclaved)	80/5 = 16
Thioglycollate	40/5 = 8
Glucose	80/5 = 16
Thioglycollate and glucose	80/80 = 1

[a]$NaNO_2$ after autoclaving/$NaNO_2$ before autoclaving.
[b]Perigo Index.
Incubation was in a N_2 atmosphere.

sodium thioglycollate; however, for reasons of economy and availability, the sodium thioglycollate was generally used. Adding separately autoclaved cysteine to the medium autoclaved without cysteine reduced the PI to 16, whereas similar treatment with sodium thioglycollate decreased the PI to 8. This seemed to indicate that a reducing atmosphere was conducive to formation of the Perigo inhibitor. Addition of separately autoclaved glucose to the the otherwise complete medium had no effect on the PI, but if both glucose and the reducing agent were absent during autoclaving, and added later the Perigo inhibitor was not formed. Autoclaving glucose with the medium evidently provided sufficient reductant for the Perigo effect to develop. There was a decrease in mic for unheated nitrite from 160 for the complete medium to 40 when cysteine was added separately. This indicated that autoclaving without cysteine but with glucose in the medium made the unheated nitrite more effective.

Separately added yeast extract and tryptone

The effect of separately autoclaving other components of the medium on the formation of the Perigo inhibitor is shown in Table 2. The PI for the complete medium was 64, the same as in Table 1, although the mic's for the ratio were higher. This may have been due to the difference in incubation conditions or to variation from different batches of sodium thioglycollate which were shown by Huhtanen and Wasserman (*2*) to influence the mic's obtained although the PI's were not appreciably influenced. The absence of yeast extract or tryptone from the medium during heating led to a decrease in the Perigo index from 64 to 16. If in addition to yeast extract or tryptone, cysteine was also added after autoclaving the Perigo index was reduced even further to 2. When $NaNO_2$ was autoclaved in a neutral solution of glucose and K_2HPO_4, with the other ingredients (cysteine, yeast extract, and tryptone) added after autoclaving, the PI of 0.5 indicated that some nitrite was probably destroyed during the heating. The results in Table 2 indicate that Perigo inhibitor is formed when either yeast extract or tryptone, or both, together with a reducing agent and nitrite are autoclaved together. The formation of such a Perigo inhibitor from tryptone and nitrite was also recently reported by Grever (*1*).

TABLE 2. *Effect of separately autoclaved cysteine, yeast extract, or tryptone on the formation of Perigo inhibitor*

Component added after autoclaving	Ratio[a] (mic, ppm)
None (complete medium)	320/5 = 64[b]
Cysteine	320/80 = 4
Yeast extract	320/20 = 16
Yeast extract and cysteine	320/160 = 2
Tryptone	160/10 = 16
Tryptone and cysteine	320/160 = 2
Tryptone and yeast extract	80/40 = 2
Tryptone and yeast extract and cysteine	160/320 = 0.5

[a]$NaNO_2$ after autoclaving/$NaNO_2$ before autoclaving.
[b]Perigo Index.
Incubation was in an atmospheric incubator.

REFERENCES

1. Grever, A. B. G. 1973. Vorming van de Perigo factor in voedingsbodems. Voedingsmiddelentechnologie 4:105.
2. Huhtanen, C. N., and A. E. Wasserman. 1975. Effect of added iron on the formation of clostridial inhibitors. Appl. Microbiol. (In press).
3. Perigo, J. A., E. Whiting, and T. E. Bashford. 1967. Observations on the inhibition of vegetative cells of *Clostridium sporogenes* by nitrite which has been autoclaved in a laboratory medium discussed in the context of sub-lethally processed meats. J. Food Technol. 2:377-397.

J. Milk Food Technol. Vol. 38, No. 12, Pages 764-768 (December, 1975)

Effect of Chemically Adjusted Meat pH and Drying Air Velocity on some Properties of Dry Sausage

W. E. TOWNSEND, C. E. DAVIS, and A. J. MERCURI

Animal Products Utilization and Marketing Research Laboratory
Richard B. Russell Agricultural Research Center
Agricultural Research Service—U.S. Department of Agriculture
P.O. Box 5677, Athens, Georgia 30604

(Received for publication May 27, 1975)

ABSTRACT

Effects of chemically adjusted meat pH (lowered by calcium chloride; raised by sodium hydroxide-sodium citrate), and velocity of air during drying on some properties of a dry type sausage were investigated. During the first 2 days of drying, sausages having initial pH values of 5.5 had a more intense cured meat color than those having initial pH valuesof 5.9 or 6.6. During 21 days of drying, pH 5.5 sausages showed very little change in shape, while sausages with pH values of 5.9 and 6.6 exhibited triangular and twisted-triangular cross-sectional shapes, respectively. Sausages having initial pH values of 5.5 and dried with high air velocity had poor internal cured meat color and texture and developed hollow centers, but not in those having pH values of 5.5, 5.9, or 6.6 and dried with low air velocity. Peelability improved during drying.

Manufacture of dry and semi-dry sausage is an important branch of the meat industry. In 1974, over 220 million lb. of dry and semi-dry sausage products were produced in federally inspected meat plants (*21*).

For centuries, fermentation provided a means of preservation. Keeping qualities of fermented dry and semi-dry sausage are attributable to low pH, low moisture content, and a high salt content. Until recently, all dry and semi-dry sausages were processed by long slow dehydration while flavor developed from bacterial action (*10, 13, 25*).

Because of the economic importance of these types of sausages, considerable effort has been exerted to improve methods of production (*1, 2, 6, 12, 15, 16, 19*). Bacterial starter cultures, generally lactic acid bacteria, e.g. *Pediococcus cerevisiae,* are used in technically progressive plants to speed and direct flavor development (*6*). Nevertheless, use of these cultures in production of certain dry and semi-dry sausages still requires long costly storage with controlled humidity, temperature and air flow, and highly skilled management.

Wierbicki et al. (*23*) reported that addition of soluble edible calcium salts to curing agents lowered pH of comminuted cured meats, thus favoring formation of cured meat color. They reported that pH of comminuted cured meats is lowered by a chemical reaction, whereby calcium ions are exchanged with hydrogen ions in the protein chains to form free acids with the acid residues of the corresponding calcium salts. The reaction decreases hydration of meat proteins, thus increasing free moisture, and promoting dissolution and penetration of the cure into the meat substance, all important for dry curing. A decrease in protein hydration should increase rate of drying sausage, and shorten the processing time. Wierbicki et al. (*23*) did not report any data related to use of soluble edible calcium salts to speed up drying of dry and semi-dry sausage. In contrast, Wierbicki and Tiede (*24*) reported that a brine solution containing sodium hydroxide-sodium citrate raised pH and improved water retention and quality of smoked and canned hams. They reported that sodium citrate dissolves in brine solution to form citrate ions which serve to sequester calcium and magnesium ions. The sodium hydroxide solution increases pH of meat to 6.3-6.8 by completion of cure.

The general importance of air movement patterns and rates during drying of sausages is well recognized (*4, 13*), but little information is available regarding specific effects. Townsend and Davis (*19*) demonstrated that the position of sausage in relation to direction of air flow affected rate of shrinkage as well as other quality characteristics. The velocity of air to which sausages are exposed may also influence the rate of drying as well as other properties of dry sausage. Reduction in processing time that would not adversely affect quality could make the manufacture of dry and semi-dry sausage more profitable.

This study was undertaken to evaluate effects of initial chemical adjustment of pH (by addition of soluble edible calcium salts and sodium hydroxide-citrate solutions) of sausage meat mixtures and velocity of air on rate of dehydration and on some chemical and physical properties of a non-fermented dry sausage.

MATERIALS AND METHODS

Sausage preparation and processing

A non-fermented dry sausage formula was used for our study. Boneless pork shoulders, containing 75 to 80% lean meat, were previously frozen to destroy trichinae to comply with USDA regulations (*20*). The pork was allowed to partially thaw, and was ground once through a 9-mm plate and thoroughly mixed. Cure and seasoning ingredients (Table 1) were then blended into the total meat mixture.

Three 8.1-kg batches of sausages were prepared from this meat mixture. Based on final weight of the individual meat mixture, Batch A

TABLE 1. *Ingredients used in a non-fermented dry sausage*

Ingredient	Quantity
Meat:	
Pork shoulders, 75-80% lean	26.0 kg
Cure:	
Sodium nitrite	4.0 g
Sodium chloride	650.0 g
Sodium ascorbate	14.2 g
Seasonings:	
Sucrose	545.0 g
Heller's Zanzibar Brand Salami	112.0 g
For pH Change[1] (Based on 8.1 kg of meat mix):	
Batch A (pH 5.5, calcium chloride solution)	405 ml
Batch B (pH 5.9, distilled water)	405 ml
Batch C (pH 6.6, sodium hydroxide-trisodium citrite solution)	405 ml

[1]Batch A (82 g calcium chloride in 405 ml distilled water); Batch B (distilled water); Batch C (25 g sodium hydroxide plus 7.5 g trisodium citrate in 1 liter of distilled water).

contained 1% calcium chloride, Batch B contained no calcium chloride or sodium hydroxide-sodium citrate (control), and Batch C contained 0.12% sodium hydroxide and 0.04% sodium citrate. The pH values of the 3 batches were 5.5, 5.9, and 6.6, respectively. Each batch was thoroughly mixed by hand, ground through a 5-mm plate and held at 3.3 C for 48 h. About 400 to 500 g of the ground product from each batch was stuffed into each of 16, 52-mm diameter D.S. casings (Union Carbide[1]). Sausages from each pH level were equally divided into two groups and hung to dry in a walk-in environmental room having excellent temperature and humidity control, but with rather large air velocity gradients. One group was placed in an air-flow of 30 to 40 linear ft/min (LV-Low Velocity) and the second to 110-130 linear ft/min (HV-High Velocity) as determined using an Anemotherm Air Meter (Dynamics Corporation of America, Scranton, Pa.). Sausages were dried for 21 days at 12.9 C (55 F) and 70% RH.

pH determination

pH values were determined on duplicate 10-g samples of the sausage (1:3 ratio, meat to water) blended for 60 sec in a Waring Blendor.

Weight loss and sausage composition

Percent weight loss or "shrink" of individual sausages was determined every day during the first 4 days and every 3 to 4 days thereafter. Percentages of moisture, fat, and protein were determined by AOAC methods (*3*) for the initial meat mixture (after addition of ingredients to change pH) and after 7, 14, and 21 days of drying.

Quality characteristics

Several meat scientists familiar with the general quality characteristics of dry sausage subjectively evaluated the external and internal characteristics (color, shape, texture, and peelability) at various times during the drying period.

Statistical analyses

pH and sausage composition data were treated by analysis of variance and the significant differences among means were determined by the Duncan's method (*18*).

RESULTS AND DISCUSSION

pH change

Initial pH values of the three batches of sausage mix-(Batch A—5.5, Batch B—5.9, and Batch C—6.6) did not change during holding at 3.3 C for 48 h. At the level used, calcium chloride did not lower pH of the sausage mixture to the value that would normally be obtained by bacterial fermentation; however, the pH value obtained agrees with that reported by Wierbicki et al. (*23*). The data in Table 2 show the means of the pH values for the different sausage batches and the change in pH during drying. There was, as expected, a highly significant ($P < 0.05$) difference between pH values of each sausage batch. During 21 days of drying, pH of sausages in Batch C decreased 0.3 of a unit while that of Batches A and B did not change. Although not shown in Table 2, air velocity did not significantly affect pH changes during drying.

TABLE 2. *Change in pH values of sausages during drying at 12.9 C and 70% RH*

Sausage batch	Drying time (day) 0	7	14	21	Mean[1]
A (pH 5.5)	5.53	5.64	5.47	5.51	5.54[a]
B (pH 5.9)	5.95	6.01	5.98	5.97	5.98[b]
C (pH 6.6)	6.62	6.51	6.38	6.32	6.46[c]
Mean[1]	6.03[c]	6.05[c]	5.94[b]	5.78[a]	

[1]Means followed by the same letter are not significantly different at $p < 0.05$.

Weight loss and sausage composition during drying

From weights of individual sausages before and during the drying period, percentages of weight loss (shrink) were calculated and averaged. The effect of air velocity during drying on shrinkage is presented in Fig. 1. Drying

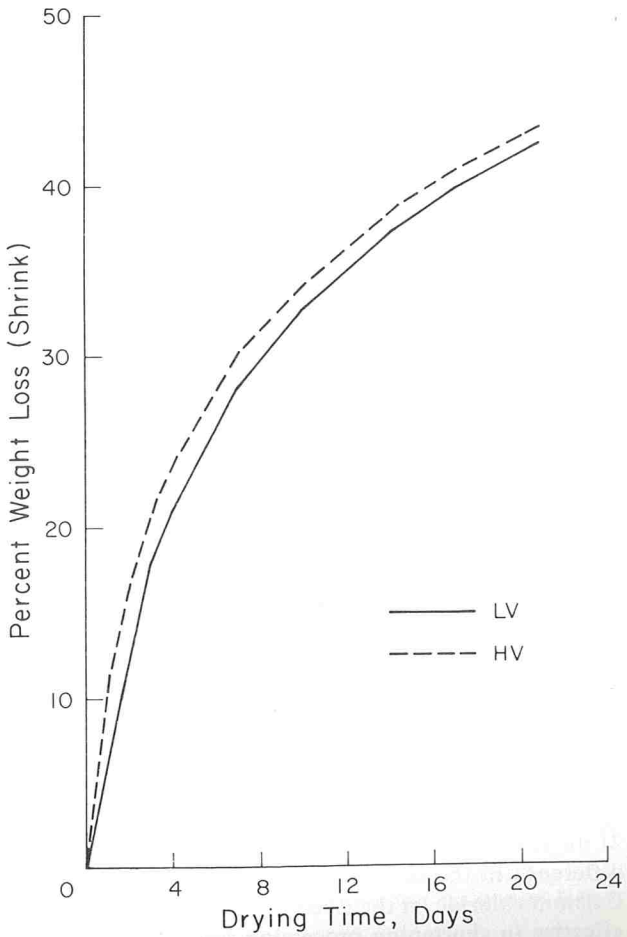


Figure 1. *Percent weight loss (shrink) during drying of sausages exposed to low and high velocities of air.*

[1]*Reference to brand or firm names does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.*

rate in the high velocity area, irrespective of pH, was greater ($P < 0.01$) than in the low velocity area. Commercially, drying sausage to the semi-dry stage (20-25% shrink) normally requires 10 to 25 days and to the dry stage (35-40% shrink) 60 to 90 days (*23*). In these experiments, sausages in the high velocity area could be considered semi-dry after about 3 days, and dry after about 11 days. Sausages in the low air velocity area required about one additional day to reach these stages. In both instances the rate was extremely rapid and relatively constant until about the 4th or 5th day, after which rate gradually decreased.

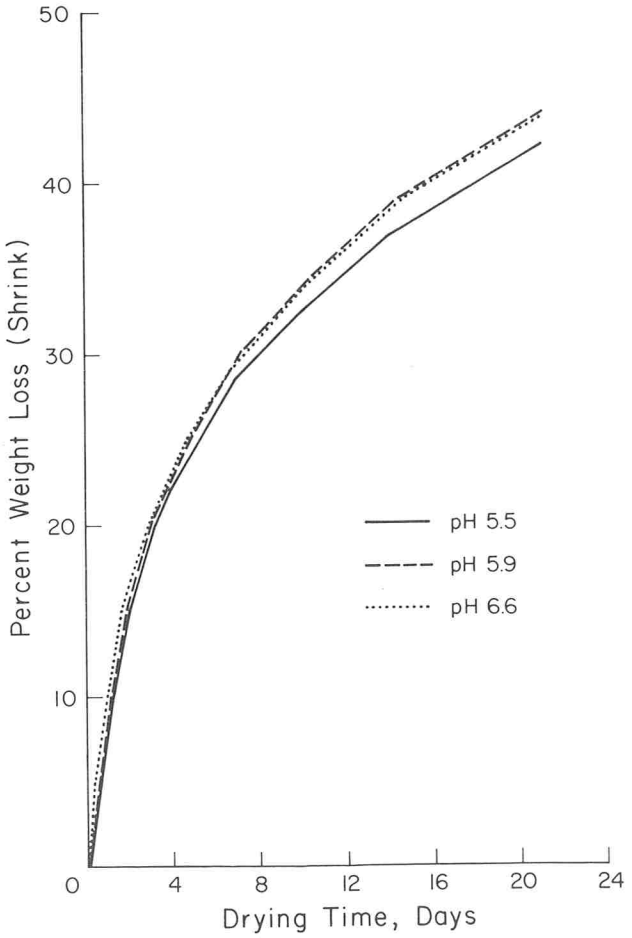


Figure 2. *Effect of initial pH on percent weight loss (shrink) of sausages.*

The effect of initial pH on shrinkage of sausages is shown in Fig. 2. Sausages of all three pH groups shrunk at the same rate until about the 4th or 5th day (22-24% shrink), after which rate of pH 5.5 sausages i.e. those containing calcium chloride, was slower than that of pH 5.9 or 6.6 sausages, resulting in slightly less shrink after 21 days. From a practical standpoint, there would be no difference in the time required to reach the dry stage. Calcium chloride, at the levels used in this study, was not effective in shortening processing time as suggested by Wierbicki (*23*), but tendered to prolong the time required to process sausages to the dry stage. A decrease in protein hydration by the addition of this level of calcium chloride-which according to Wierbicki- should have increased the rate of drying this type of product apparently did not occur. Hamm's report (*9*) that the pH of meat mixtures must be below the isoelectric point (pH 5.0) of tissue proteins before calcium chloride shows any effect in decreasing water holding capacity may partially explain this result.

Sausages in Batch C (pH 6.6) were expected to lose the least moisture because of increased protein hydration due to increased pH (*14, 17, 22*). Schon and Stosiek (*17*) noted that evaporation loss during the storage of dry sausage decreases with increasing pH value.

The rate of drying of pH 6.6 sausages in low velocity air was slower ($P < 0.01$) than pH 5.9 or 5.5. This pH difference was not evident at high velocity.

TABLE 3. *Changes in proximate composition of sausages during drying*[1]

Proximate composition	Drying time (day)			
	0	7	14	21
Moisture, %	60.26^{d}	46.58^{c}	35.75^{b}	30.19^{a}
Fat, %	17.01^{a}	22.75^{b}	27.81^{c}	30.05^{d}
Protein, %	16.19^{a}	20.04^{b}	25.12^{c}	27.53^{d}

[1]Any two means within a line having one of the same letters are not significantly different at $p = 0.05$.

The mean scores for proximate composition of sausages during drying are presented in Table 3. About 50% of the original moisture content was lost during drying. Highly significant ($P < 0.01$) differences were found in moisture, fat, and protein content as drying time increased. As moisture decreased, percentage of fat and protein increased and were nearly doubled at the end of the drying period. No significant ($P < 0.05$) differences were found in proximate composition among the sausage batches and air velocities used in this study.

Quality characteristics

External and internal characteristics (color, shape, texture, and peelability) were observed at various times during the drying period. Sausage surfaces facing the flow of air developed a red cured meat color faster than the opposing surfaces, and sausages developed color faster at HV than LV. This change can be attributed to surface dehydration which increased concentration of cured meat pigments. With fresh meats, Landrock and Wallace (*11*) reported that dehydration increased concentration of meat pigments at the surface which intensified color.

As expected, the cured meat color developed faster in Batch A. Bailey et al. (*5*) and Fox (*8*) reported that nitrosylmyoglobin (cured meat color) formed more rapidly under acidic conditions. After 2 days of drying, the entire surfaces of all sausages were a uniform pink to red cured meat color.

During the first 3 days of drying, all sausages retained their normal cylindrical sausage shape. After 21 days of drying, sausages held in the LV area had firm texture,

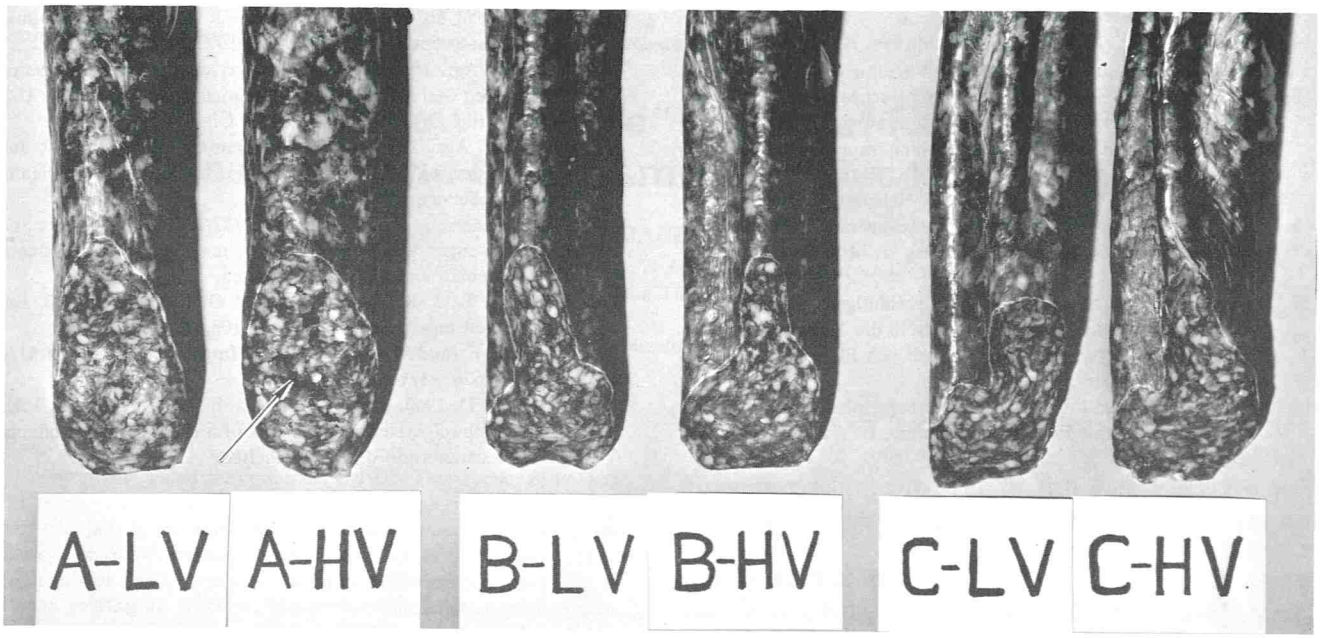


Figure 3. *Effect of chemically adjusted meat pH and drying air velocity on changes in shape of sausage. Arrow points to hollow center of a sausage dried in high velocity air. No hollow centers occurred in those sausages dried in low velocity air. LV and HV refer to low and high velocity air, respectively. A = pH 5.5, B = pH 5.9, C = pH 6.6.*

good cured meat color, and did not exhibit any hollow centers. Sausages with pH values of 5.9 and 6.6 in this area showed triangular and twisted-triangular shapes, respectively, while the shape of the pH 5.5 sausage exhibited very little change (Fig. 3).

In the HV area, the higher pH sausages although having good texture and color also exhibited abnormal shapes (Fig. 3). The pH 5.5 sausages in this area, although retaining a near normal cylindrical shape, exhibited hollow centers (Fig. 3) with the surrounding portion having a granular texture and an unattractive brownish red color. Fox (*7*) attributed this brownish-red color to the oxidation of nitrosylmyoglobin to metmyoglobin.

After 7 days of drying, the ease of casing removal was poor for all sausage groups. After 14 and 21 days of drying, peelability had improved considerably. One explanation might be that up to 7 days the casing shrank at the same rate as the product. Possibly after 7 days the meat shrank more than the casing, and became easy to peel.

The air velocities used in this study were greater than those obtaining in industry where 15-25 air changes per hour is reportedly the common practice (*13*). The results do suggest however, that the drying time for dry sausage (those with pH values of 5.5 and below) can be shortened by increasing air flow above that now employed, without affecting their general quality characteristics. As reported by MacKenzie (*13*), there is no agreement as to the most effective combination of temperature, relative humidity, and air velocity for drying sausage. Further investigations will be required to determine the proper combination of the above three factors.

ACKNOWLEDGMENTS

Mr. Victor Chew is acknowledged for his assistance in interpretation of the data and Mr. Robert Watrous for his technical assistance with the proximate composition analyses.

REFERENCES

1. Acton, J. C., J. G. Williams, and M. G. Johnson. 1972. Effect of fermentation temperature on changes in meat properties and flavor of summer sausage. J. Milk Food Technol. 35:264-268.
2. Acton, J. C., and J. E. Keller. 1974. Effect of fermented meat pH on summer sausage properties. J. Milk Food Technol. 37:570-576.
3. A.O.A.C. 1965. Official methods of analysis. 10th ed. Association of Official Agricultural Chemists, Washington, D.C.
4. ASHRAE Guide and Data Book. 1968. Meat products. p. 289-314. Amer. Soc. of Heating, Refrigeration and Air Conditioning Engineers, Inc., New York, New York.
5. Bailey, M. E., R. W. Frame, and H. D. Nauman. 1964. Studies of the photooxidation of nitrosomyoglobin. J. Agric. Food Chem. 12:89-93.
6. Everson, C. W., W. E. Danner, and P. A. Hammes. 1970. Bacterial starter cultures in sausage products. J. Agric. Food Chem. 18:570-571.
7. Fox, J. B., Jr. 1959. The pigment of cured meats. American Meat Institute Foundation, Bulletin No. 41.
8. Fox, J. B., Jr. 1966. The chemistry of meat pigments. J. Agri. Food Chem. 14:207-210.
9. Hamm, R. 1960. Biochemistry of meat hydration. p. 414-425. In C. O. Chichester, E. M. Mrak, and G. F. Stewart (ed). Advances in food research, Academic Press, New York.
10. Kramlich, W. E., A. M. Pearson, and W. F. Tauber. 1973. Processed meats. AVI Publishing Co., Westport, Conn. 348 p.
11. Landrock, A. H., and G. A. Wallace. 1955. Discoloration of fresh meat and its relationship to film oxygen permeability. J. Food Technol. 9:194-196.
12. Lu, J., and W. E. Townsend. 1973. Feasibility of adding freeze-dried meat in the preparation of fermented dry sausage. J. Food Sci. 38:837-840.
13. MacKenzie, D. S. 1966. Manufacturing processes-fermented (dry) sausage, p. 62-63. Prepared meat product manufacturing, Ameri-

can Meat Institute, Chicago, Ill.
14. Niinivaara, F. P. 1954. Die Bedeutung des Kochsalzes für die Herstellung von Roh und Brühwurst. Fleischwirtschaft 6:357.
15. Niinivaara, F. P. 1968. A study of the influence of fats and their decomposition products on the flavor and aroma of dry sausage, to obtain information for use in developing improved dry sausage. Final Report, Project No. UR-E8-(60)-14, Grant No. *FG*-Fi-138, Institute of Meat Technology, Univ. of Helsinki, Finland.
16. Palumbo, S. A., J. L. Smith, and S. A. Ackerman. 1973. Lebanon bologna. I. Manufacture and processing. J. Milk Food Technol. 36:497-503.
17. Schon, L., and M. Stosiek, 1959. Ein vorläufiger Test zur Frage des Einflusses von Melassebeitfütterung in der Schweinemast auf die Qualität und Verarbeitungsfähigkeit von Fleisch und Speck. Fleischwirtschaft 11:739.
18. Steel, R. G. D., and J. H. Torrie. 1960. Principles and procedures of statistics, McGraw-Hill Book Company, Inc., New York, New York.
19. Townsend, W. E., and C. E. Davis. 1972. Effect of hanging position of some properties of dry sausage. J. Food Sci. 37:633.
20. U.S. Dept. Agr. 1965. Regulations governing the meat inspection of the United States Department of Agriculture. Sec. 318:10. U.S. Govt. Printing Office, Washington, D.C.
21. U.S. Dept. Agr. 1974. Statistical summary. Federal meat and poultry inspection for FY 1974. MPI-1 Animal and Plant Health Inspection Service. Washington, D.C.
22. Wismer-Pedersen, J. 1971. Water. p. 177-207. In J. F. Price and B. S. Schweigert (ed). The science of meat and meat products, W. H. Freeman and Co., San Francisco.
23. Wierbicki, E., J. R. Shackelford, and G. E. Cooper. 1963. Improved cured meat color. U.S. Pat. 3,108,880. Oct. 29.
24. Wierbicki, E., and M. G. Tiede. 1964. Improved meat yields. U.S. Pat. 3,134,678. May 26.
25. Wilson, G. D. 1960. Sausage products. p. 349-372. In J. F. Price and B. S. Schweigert (ed). The science of meat and meat products, W. H. Freeman and Co., San Francisco.

J. Milk Food Technol. Vol. 38, No. 12, Pages 769-773 (December, 1975)

The "New Disease" Status of Human Anisakiasis and North American Cases: A Review[1]

G. J. JACKSON

Division of Microbiology, Food and Drug Administration
Washington, D.C. 20204

(Received for publication June 12, 1975)

ABSTRACT

The first confirmed case of human anisakiasis, i.e., infection with larval anisakine nematodes, occurred in 1955. The practice of holding fish refrigerated for relatively long periods before gibbing and curing, thus enabling larger numbers of anisakine larvae to move from the viscera into edible portions, as well as the increased popularity of raw and semi-raw fish recipes may partially explain the new awareness of human anisakiasis. However, it is probaly not a totally new disease. Presumptive cases are mentioned in the literature as early as 1867. Other cases may have been misdiagnosed because anisakine larvae resemble ascarid larvae in general morphology. To date, Japan ("several hundred cases") and the Netherlands (about 160 cases) have reported the largest number of human infections. Six confirmed cases have been reported from North America. Human infections are acquired from consumption of raw, underheated, insufficiently frozen or lightly marinated fish dishes. The contained anisakine larvae (presumably belonging to genera such as *Anisakis, Phocanema, Porrocaecum,* and *Contracaecum* whose final hosts are mammals or birds) do not mature in humans but can survive long enough to cause pathology. Anisakine larvae remaining free or attached in the human digestive tract may cause irritation, severe inflammation, and ulceration; sometimes the larvae are expelled by coughing or vomiting. Anisakine larvae that penetrate totally into the tissues may stimulate a granuloma formation that surrounds and is thought to kill them. The world incidence of human anisakiasis is just being discovered. Surveys, improved criteria and methods for identifying larval anisakines, and establishment of experimental hosts and culture systems that are practical in the laboratory are needed to determine the extent of infected seafood and to prevent, diagnose correctly, and treat human infections.

Even if one had never heard of anisakiasis previously, a quick perusal of the titles for this symposium might lead one to deduce that the causative agents of the infection are parasitic roundworms, i.e., nematodes, possibly related to common *Ascaris;* that a variety of hosts become infected, presumably by eating raw fish; and that the parasites have also been found in such marine invertebrates as shellfish. However, from the titles alone one can not tell whether the parasite's life requires two hosts (cycling from either a fish or a marine invertebrate to one of their predators) or three hosts (from invertebrate to fish to fish eater). As for the newness of human anisakiasis, or merely its recent discovery, one is apt to have doubts because parasitic animals seldom find another host suddenly and because our latest food habits are seldom without some precedent. A more likely possibility is that human anisakiasis was observed already and forgotten, because 18th and 19th century scientists were wideranging and thorough in their observations, whereas contemporary researchers often have limited knowledge of the old literature. Finally, one is likely to guess that there really ought to be no public health problem if people are advised to cook their seafood.

These conclusions are correct in a general way but do not tell the whole story, nor does this symposium. However, it is intended to familiarize the reader with the more important circumstances of a no longer obscure disease.

THE NEWNESS OF HUMAN ANISAKIASIS

How novel, globally, is human anisakiasis? A Netherlands physician, Dr. M. Straub, is credited with the first confirmed case in 1955. As reported by Van Thiel et al. (*19*), his patient suffered from severe abdominal colic and gastrointestinal distress within 24 h of having eaten lightly salted herring. A laparotomy was done 24 h after the onset of symptoms. Pathological changes were found in the ileum, including a 1.5 cm diameter ulcer from which a 1.3 cm long worm protruded into the intestinal lumen.

Thereafter, similar cases began to be recognized in The Netherlands and the eqidemiological evidence consistently incriminated the eating of uncooked, lightly marinated herring, the so-called Dutch green herring. As late as 1960, the nematodes recovered from human cases were being identified incorrectly. Finally, as Ruitenberg has recorded (*15*), Bruyning compared worms from herring and from patients and found them to be identical. In 1962, Van Thiel classified the incriminated nematodes as larvae of *Anisakis marina* and gave the name anisakiasis to the human disease they cause (*18*). One may dispute Van Thiel's classification at the species level (the correct name is probably *Anisakis simplex*) (*3*)

---

[1]*First of five papers developed from a presentation at the symposium, "Anisakiasis: A New Disease from Raw Fish," held March 14, 1974 under the auspices of the New York Society of Tropical Medicine, at Rockefeller University, New York, N.Y. Other titles in the symposium, all of which will appear in* Journal of Milk and Food Technology, *are as follows: "The Nematodes That Cause Anisakiasis," by B. J. Myers; "The Natural History of Anisakiasis in Animals," by T. C. Cheng; "The Public Health Implications of Larval* Thynnascaris *Nematodes From Shellfish," by D. E. Norris and R. M. Overstreet; and "Experimental Anisakiasis: Cultivation and Temperature Tolerance Determinations," by J. W. Bier.*

or question him for speciating on the basis of only larval forms, but no one now seriously doubts that larval nematodes of the genus *Anisakis* and of some related genera can be transmitted by eating raw or undercooked fish. In the human consumer, these larval nematodes are not able to mature and reproduce, but they may survive long enough to cause pathology.

Considering the fact that most of our pathogenic parasites were known by 1900 and that some of their manifestations had been recognized as distinct disease entities for centuries, it is remarkable that new etiological agents should have been discovered only in the last 20 years. Since neither an electron microscope nor usually even a low power magnifying lens is needed to see anisakine nematodes (Fig. 1), why had anisakiasis not been seen or recognized before?

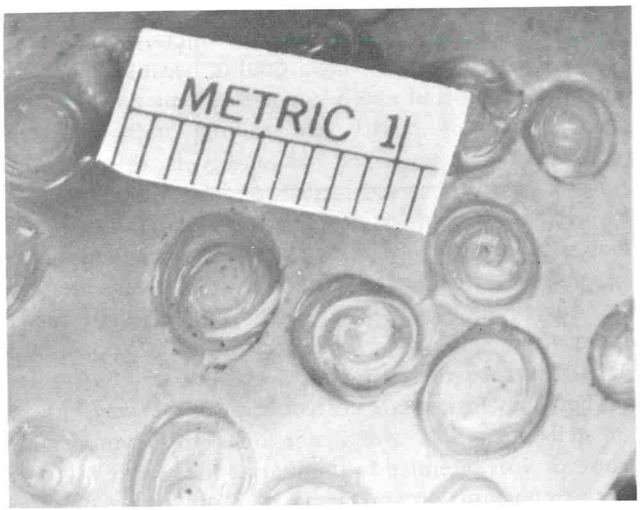


Figure 1. *Anisakis-type of anisakine larvae coiled on the liver of a Pacific Ocean perch* (Sebastes alutus) *caught off Seattle. When the larvae are removed, impressions remain on the surface of the liver. These larvae also occur in the edible musculature of perch and other fish. The Phocanema-type of anisakine larva, typically found in cod fillets, is usually even larger and easier to see.*

One reason is given by Roskam, who postulated a more-than-one hit theory of anisakiasis. According to his explanation as given in Ruitenberg's discussion (*15*), initial infections cause little damage, but repeated infections within about 4 months of each other and located in the same area of the digestive tract tend to sensitize the consumer and thus cause pathology. Before 1955, green herring had not been available commercially in Holland and chances were minimal that significant numbers of people would eat it often enough to become sensitized in the same gut spot. Some experimental evidence has been obtained with rabbits to support the hypothesis of sensitization as an explanation for the increased frequency of human anisakiasis (*6*).

Another reason is given by Van Thiel (*19*) who believes that human anisakiasis began to flourish when refrigeration was brought aboard fishing boats in the middle of the 1950s. Previously, the fish were gibbed and cured on board soon after netting because it was impractical to store fish cold on the boats and transport them whole to shore. Van Thiel argues that this old practice of immediate processing eliminated most of the nematodes because, according to him, most worms move from the mesenteries to the edible portions only after fish die.

Processing and marketing practices and the consequent sensitization of consumers may have increased the number of human cases—especially in The Netherlands—but it can be contended that anisakiasis really is not a new disease. Rather, it has been misdiagnosed or overlooked.

Anisakines are related to and resemble *Ascaris lumbricoides* (a roundworm that develops in humans) as well as other ascarids whose larvae, like those of the anisakines themselves, invade but do not mature in *Homo sapiens.* Since fairly heavy infections with ascarids were common before recent decades and, most likely, not every worm was examined in detail, it might easily have happened that anisakines, when they occurred, were grouped with the more frequent ascardis.

For instance, when Hitchcock (*4*) examined the parasitism of over 100 Eskimos in Alaska, what she describes as ascarid larvae were found in the stools of 10% of the sampled population. The worms were sent for identification to Dickmans at the U.S. Department of Agriculture's Research Center in Beltsville, Maryland. Dickmans thought that there were two possibilities. Either the worms were *Ascaris* larvae, eliminated spontaneously as a consequence of the host's developing immunity or of anthelminthic treatment, or they were anisakine larvae ingested with the flesh of fish and passed from the digestive tract because cooking, freezing, or the unsuitable host environment had impaired their viability. Dickmans did state that two specimens definitely were not *Ascaris lumbricoides* but anisakines. He even suspected that all the worms might be anisakines since there were no adults or eggs, as would be expected in true ascariasis (but not in infections with other ascarids). This illustrates the difficulty of identification.

Perhaps the earliest recorded suspicion that ascarid-like worms from fish could cause human disease (*7, 11*) occurred in 1867. Pfaff, district physician at Jakobshaven on the West Coast of Greenland, sent a nematode to the famous Rudolf Leuckart. The specimen had been vomited by a child from a fishing community, and Pfaff suggested that it might have been consumed with the child's food. Leuckart did not mistake the specimen for *Ascaris lumbricoides.* He designated it a new species, *Ascaris maritima,* but postulated, less correctly, that Greenlanders were alternate hosts for the ascarids that occur in polar bears and seals. Whether he believed the infective stage to be a free egg or a larva encysted in a fish is unclear.

Probably the first scientist to suggest that marine mammals such as seals were the definitive hosts for the larval ascarids in fish (*20*) was Von Linstow in 1876.

Martin (*11*) presented morphological and cultivation evidence in 1921. Reviewing the Greenland case, Martin concluded that the child had acquired the worm by eating a raw fish and that this habit could be a potential source of infections.

Indeed, the Greenland case with its expulsion of the worm by vomiting resembles recent North American cases in which the causative agent has been an anisakine larva of a genus *Phocanema.*

ANISAKIASIS IN THE NETHERLANDS AND JAPAN

The failure to observe anisakiasis in Japan, a culture in which raw fish dishes are traditional, clearly was due to oversight or confusion with ascarids. Not until 1965, as a direct result of the Dutch publications, was it realized that Japan had an anisakiasis problem. In a relatively short time several hundred cases were diagnosed or rediagnosed (*13*).

The Netherlands and Japan have the largest number of recognized anisakiasis cases. Between discovery in 1955 and 1967, there were 149 proven cases in Holland (*15*). In 1968, legislation was introduced that required the freezing of green herring for 24 h. This has reduced the number of Dutch cases dramatically. Japanese recipes require unfrozen raw fish (*16*) and so many types of fish are eaten raw there that, for legislation to be effective, the total national catch would have to be submitted to freezing (*13*). Gutting the fish as soon as they are caught might reduce but would not totally eliminate worms in the edible portions.

RECENT CASES AND RESEARCH IN THE U.S.

In 1972, alerted to reports of human anisakiasis in The Netherlands and Japan, the U.S. Food and Drug Administration decided to study the incidence and pathogenic potential of anisakine nematodes in seafood from territorial fishing waters. Research was begun in-house and sponsored at other institutions. Coincidentally, the first confirmed case of human anisakiasis in North America was described towards the end of the year by Little and MacPhail (*9*). A nematode 31 mm long, identified as an anisakine larva of the genus *Phocanema,* was recovered from an aneurysm of a right common iliac artery that had been removed surgically. The patient, a 76-year-old Massachusetts male, ate fish infrequently and, supposedly, always well cooked. There was no evidence that the larva had caused a cellular reaction. The aneurysm may have been incidental.

Two North American cases of temporary digestive tract infection with an anisakine larva of the genus *Phocanema* were reported from the Atlantic Coast in the 1973 literature (*5, 10*). One patient had eaten an undercooked fish, the other "ceviche," a South American adaptation of a Far Eastern raw fish dish. Later, both persons had felt an irritation in the throat and extricated a live worm. In a third similar case, one from Alaska, the larval nematode was coughed up rather than manually removed (*8*). In a fourth case, from California, a *Phocanema* sp. larva was again expelled by coughing (*2*). What possibly is an earlier North Amerian instance of temporary digestive tract infection and expulsion by coughing was reported (*1*) in 1970. However, the patient, a woman who had recently returned to the U.S. from Tahiti, may have acquired the larval anisakine there or en route rather than on the North American continent. Irritation of the throat and digestive tract, with subsequent manual extrication or expulsion of the worm by coughing or vomiting, is now being associated with infections by *Phocanema* sp. anisakine larvae from North American fish. Possibly the Greenland case of 1867 also involved this type of larva. Deep parenteral penetration by a *Phocanema* sp. larva—the aneurysm case discussed above (*9*)—has been reported just once in North America.

Also, only one case of human infection in North America with an *Anisakis*-type of anisakine larve has been reported (*14*). It occurred in a woman who complained of fever and right lower quadrant pain of 5 days' duration. Roentgen pictures of the abdomen showed the ascending colon to have a thickened wall and the terminal ileum to be inflamed. Surgery was done and the affected parts were removed and examined. The inflammatory process was found to involve the submucosa, muscle, and pericolic fat. There were a necrotizing granulomata and prominent eosinophilic and fibroblastic infiltrates. The ileocecal valve was thickened. The colon and terminal ileum walls were edematous but their mucosa was intact. Finally, an *Anisakis*-type of anisakine larva was identified during histological examination of the surgical specimen. The patient, a Boston woman of Swedish birth, ate fish lightly marinated according to old Scandinavian recipes.

PUBLIC HEALTH IMPLICATIONS

Six confirmed cases, two presumed cases, and a group diagnosis that is difficult to interpret do not in themselves constitute a major public health problem in North America. Even if the number of unrecorded or unrecognized cases is much greater than the reported incidence of human infection, we still must ask whether the estimated total would be big enough to warrant calling anisakiasis to the attention just of professionals, if not of the consumer public.

The eating of raw or undercooked fish is on the increase. Statistics to support this contention are lacking, but circumstantial evidence exists. Recipes for the home preparation of Japanese sushi or sashimi, Latin American ceviche, and Holland green herring have appeared in print. Restaurants that serve these dishes to a general clientele are not difficult to find in most cities. Also influential has been the "natural foods" movement with its emphasis on "not overcooking" to preserve labile nutrients in foods. Gourmets consider that fish, especially salt or brackish water catch, when barely warmed are more nutritious and more delicious than

when they are served hot through and through, and they believe them to be just as safe. Of course, it is the safety that is being questioned here.

If international trade and travel have stimulated the U.S. consumer's taste for "foreign" foods, and if health fashions have prompted the general public's consumption of edibles in a more "natural" state, the traditional eating habits of our ethnic minorities ought also to be of concern. Other U.S. citizens besides those of Dutch, Japanese, or Latin American origin use recipes for raw or undercooked fish. Perhaps the most important area of the U.S. in which fish are eaten raw is Hawaii. People from many parts of Polynesia, Micronesia, Japan, and Southeast Asia have settled there. Visitors to Honolulu report that raw fish dishes from various cultures are practically staples on the menu. Anisakine larvae have been found (*12*) in such reef catch as the aweoweo (*Priacanthus cruentatus*), a fish that is traditionally eaten raw in Hawaii (*17*).

NEEDED RESEARCH

To what extent are the people who eat raw or undercooked fish being exposed to anisakiasis? To answer the question, work must be done.

Surveys are needed to discover the incidence of anisakines in the edible catch of our major fishing waters. This undertaking is no small task if one considers the possible seasonal and geographic variations of parasite incidence that may be encountered even with a single species of fish. An especially difficult part of the undertaking is classifying the types of anisakines recovered, as discussed in this symposium by Myers. Since we currently assume that only those anisakines which mature in mammals or birds (presumably belonging to the genera *Anisakis, Phocanema, Porrocaecum,* and *Contracaecum*) are the potential etiological agents of human anisakiasis, detailed classification is definitely needed. The criteria now in use are morphological. Each nematode must be examined individually with a light microscope and only an experienced observer can decipher the sets of structural details that distinguish the different types. Exploratory work is needed to speed the identification process. Perhaps scanning or transmission electron microscopy will show simple ultrastructural details that separate types. Ultimately, mass identification would be most efficient and it might be possible to use serology or biochemical profiles to discern how many of each type of anisakine exist in a single fish or a netful. For the present, however, we must rely on tedious dissection, recovery, and microscopic procedures.

To work with anisakines in the laboratory, several types of experimental hosts will be required. To assess the pathogenicity of anisakine types and thus test the assumption that only those that mature in mammals are potential etiological agents of human disease, and also to make chemotherapeutic trials, an experimental mammal must be found that reacts to ingested larvae as does the human consumer. So that experimenters need not rely on variable infectious material obtained from commercial fish, the life cycle of each suspect species of anisakine ought to be maintained in the laboratory. This would probably require three additional hosts per species of anisakine but, as shown in the papers of Cheng and of Norris and Overstreet (in this symposium), we do not even know with certainty the complete life cycles of the anisakines in nature. Hosts that are known are often unhandy laboratory animals (e.g., the whale). Success in the cultivation of anisakines, as related by Bier (in this symposium), may help to bypass some of the in vivo experimentation if the in vitro progeny do not lose infectivity. Cultures and laboratory life cycles, of course, may also provide uniform anisakine material to study the inactivation of these parasitic nematodes by means other than cooking. However, until these inactivation studies succeed and have been combined with public education about anisakiasis, there will still be a risk in eating raw fish.

Since the incidence of human anisakiasis is just being discovered, it is unlikely that we can justify massive, immediate expenditures of public health funds on these projected efforts. Yet, predictions that aquafarming and mariculture will provide ever more important sources of animal protein for the human diet make it imperative that we at least begin to know more about those aquatic ascarids, i.e., the anisakine nematodes, about other parasitic animals and all the "neglected microbes" that are potential pathogens in fish and shellfish.

ACKNOWLEDGMENTS

The authors of the papers in this series thank Dr. J. D. Frame, President of the New York Society for Tropical Medicine, for his help in organizing the symposium.

REFERENCES

1. Chitwood, M. 1970. Nematodes of medical significance found in market fish. Am. J. Trop. Med. Hyg. 19:599-602.
2. Chitwood, M. *Phocanema*-type larval nematode coughed up by a boy in California. Am. J. Trop. Med. Hyg. (In press).
3. Davey, J. T. 1971. A revision of the genus *Anisakis* Dujardin. 1845 (Nematoda: Ascaridata). J. Helminthol. 45:51-72.
4. Hitchcock, D. J. 1950. Parasitological study on the Eskimos in the Bethel area of Alaska. J. Parasitol. 36:232-234.
5. Kates, S., K. A. Wright, and R. Wright. 1973. A case of human infection with the cod nematode, *Phocanema* sp. Am. J. Trop. Med. Hyg. 22:606-608.
6. Kuipers, F. C., E. H. Kampelmacher, and F. Steenbergen. 1963. Onderzoekingen over haringwormziekte by Konijnen. Ned. Tijdschr. Geneesk. 22:990-995.
7. Leuckart, R. 1863-76. Die menschlichen Parasiten, Vol. 2. Leipzig. pp. 98, 116-124, 877.
8. Lichtenfels, J. R., and F. P. Brancato. Anisakid larva (*Phocanema* sp.) from the throat of an Alaskan Eskimo. Am. J. Trop. Med. Hyg. (In press).
9. Little, M. D., and J. C. MacPhail. 1972. Large nematode larva from the abdominal cavity of a man in Massachusetts. Am. J. Trop. Med. Hyg. 21:948-950.
10. Little, M. D., and H. Most. 1973. Anisakid larva from the throat of a woman in New York. Am. J. Trop. Med. Hyg. 22:609-612.
11. Martin, O. 1921. Über Ascaridenlarven aus dem Fleische von

Seefischen. Z. Infektionskr. Haustiere 22:13-36.
12. Myers, B. J., and G. J. Jackson. Unpublished 1974 findings.
13. Oshima, T. 1972. *Anisakis* and anisakiasis in Japan and adjacent area(s). pp. 303-393. In K. Morishita, Y. Komiya, and H. Matsubayaski (eds.) Progress of medical parasitology in Japan, Vol. 4. Meguro Parasitological Museum, Tokyo.
14. Richman, R. H., and A. M. Lewicki. 1973. Right ileocolitis secondary to anisakiasis. Am. J. Roentgenol., Radium Therapy Nuclear Med. 119:329-331.
15. Ruitenberg, E. J. 1970. Anisakiasis: pathogenesis, serodiagnosis and prevention. Published doctoral dissertation. Rijkuniversiteit te Utrecht, The Netherlands. 138 pp.
16. Steinberg, R. 1969. The cooking of Japan. Time-Life Books (New York). 208 pp.
17. Titcomb, M. 1972. Native use of fish in Hawaii. The University of Hawaii Press, Honolulu. 175 pp.
18. Van Thiel, P. H. 1962. Anisakiasis. Parasitology 56:16.
19. Van Thiel, P. H., F. C. Kuipers, and R. T. Roskam. 1960. A nematode parasitic to herring, causing acute abdominal syndromes in man. Trop. Geogr. Med. 12:97-115.
20. Von Linstow, O. F. B. 1895. Untersuchungen an Nematoden. Arch. Mikrosk. Anat. Entwicklungsmech. 44:519-524.

J. Milk Food Technol. Vol. 38, No. 12, Pages 774-782 (December, 1975)

The Nematodes That Cause Anisakiasis[1,2]

BETTY JUNE MYERS

Southwest Foundation for Research and Education
P.O. Box 28147, 8848 West Commerce Street
San Antonio, Texas 78284

(Received for publication June 12, 1975)

ABSTRACT

Anisakine nematodes responsible for human anisakiasis have been recognized in fish hosts for over a decade. They are larval stages, relatively simple in form, and lack many morphological features used for identification of the adults (e.g., cephalic structures, spicules, etc.). However, these immature helminths can be sorted into larval types on the basis of the anterior digestive tract formation and the position of the excretory pore.

Problems in identfying larvae, even as to type, are often encountered in tissue sections that may not show those anterior regions containing features of the digestive system which are diagnostic for type sorting. Hence, it is necessary to reconsider the diagnostic value of lateral chords, muscle cells, etc., that are visible in all cross-sections.

Fish which are eaten by the human consumer also contain larval stages of other roundworms (nematodes) than those responsible for human anisakiasis, as well as numerous species of adult nematodes.

The roundworms or nematodes comprise one of the largest and most diverse group of helminths. Some species are parasitic on animals and plants, for all or part of their life cycle; other nematodes are totally free living.

As a group the nematodes show considerable morphological variations; and before discussing the anisakine nematodes, it is necessary to present basic information of the morphology used in classification.

METHODS

When nematodes are recovered from fish by dissection or digestion, their viability should first be determined by immersing them in water or Ringer's solution and observing for mobility. For identification, proper fixation of nematodes is essential so that the morphology can be evaluated. It is important to fix specimens soon after removal from the host; they may be cleaned by rinsing in tap water or saline. Fixation and preservation in 10% hot (60 C, not boiling) formalin, or 70% hot (60 C, not boiling) alcohol is recommended. The volume of fixative should be at least 10 times that of the tissue. For best preservation, a vent is made in the body wall with a fine pin (i.e., insect pin) after the nematodes have been in fixative for approximately 1 min. After 24 h transfer them to 5% glycerine alcohol for storage. For pathological specimens, fixation should be in cold, 10% buffered formalin. If material cannot be fixed immediately, it should be refrigerated, since deterioration of internal structures frequently prevents proper identification.

Each vial should be labelled as follows: source (if human, the patient's name or identification number), date of collection, method of preservation, and name of sender. In human cases a covering letter should accompany the specimens giving pertinent information as to possible sources of infection.

Since the size of the worm determines the method used for studying it, one cannot recommend a technique that is applicable for all specimens. Larger specimens are difficult to examine microscopically, and it may be necessary to dissect out the various organs and to prepare en face views (i.e., sever the lips of their bases with a scalpel or razor blade, orient them to give an apical mount in glycerine jelly. Such a preparation will allow the study of the lips, papillae, denticles, etc.

Sometimes larger specimens must also be split and the body cavity filled by pipette with clearing medium (lacto-phenol or phenol, etc.). If the nematodes are small, temporary microscopical mounts may be prepared by clearing the specimens in either lacto-phenol (phenol 10 g, glycerol 10.6 ml, lactic acid 8.2 ml, distilled water 10.0 ml) or phenol (95 parts liquid phenol, 5 parts absolute alcohol). When observations have been completed, the specimen should be washed thoroughly in 70% alcohol before being returned to its vial for storage in 5% glycerine alcohol.

To determine the genus and species of an anisakine nematode, it is necessary to study the principal morphological features: cephalic regions, cuticle, position of the excretory pore, digestive tract, and caudal region. Also to be oberved in females are the shape of the tail and the position of the vulva. In males, the papillae pattern, the spicules, and the size of the gubernaculum should be noted. The study of the caudal region of the male is extremely difficult and requires much manipulation to observe papillae formation, spicules, etc. Dissection of spicules is sometimes necessary.

Larval stages are studied in the same manner

[1]*This paper is the second in a series of five developed from presentations at the symposium, "Anisakiasis: A New Disease from Raw Fish," held under the auspices of the New York Society of Tropical Medicine on 14 March 1974 at Rockefeller University in New York City.*

[2]*The original work included in this paper was supported in part by a contract (No. 223-74-2140) from the Food and Drug Administration, U.S. Department of Health, Education and Welfare.*

although, of course, adult primary and secondary features are absent in most instances; precise classification as to genus and species is impossible.

It is important to emphasize (except for en face preparations, spicules, gubernaculum, and male tail) that a permanent slide should not be made; nematodes are cylindrical and must be "rolled" to detect the various diagnostic features.

At times it is necessary to prepare cross-sections. Standard processing of formalin fixed histological material is adhered to, and a slow infiltration of the embedding medium is recommended. Infiltration is improved if the nematodes are cut into pieces before processing. For those not expert in nematode morphology, it is suggested that the standard fixation and preservation techniques described here be followed and the specimens referred to experts for identification.

ADULT MORPHOLOGY

Nematodes are a variable group and show many morphological variations. They are cylindrical and have an oral opening usually surrounded by lips which have various sensory organs. The mouth is anterior and followed by a cavity, esophagus, intestine, and a rectum which opens exteriorly to the outside through an anus. The surface of nematodes is covered with a cuticle secreted by the hypodermis. Longitudinal muscles are present. Sexes are usually separate; the male system opens directly into the rectum forming a cloaca, while the female reproductive systems open through the vulva. Excretory and nervous systems are present.

The entire body is covered by a transparent, noncellular cuticle secreted by the hypodermis. Externally the markings are varied. Nematodes pass through a series of stages in development separated by molts. In each stage a new cuticle is formed under the old, which eventually is shed, terminating that particular stage. Usually there are five stages, the final being the adult.

Beneath the cuticle lies the syncytium which forms the hypodermis and four longitudinal thickenings known as the dorsal, lateral, and ventral chords. These chords divide the musculature into four uniform quadrants and contain the nuclei of cells that make up the hypodermis.

There are two kinds of muscle: somatic, or unspecialized, and specialized. The somatic musculature lies next to the hypodermis in a single layer. If only a few longitudinal rows of muscle cells (2-5) are present in the interchordal field, the musculature is called meromyarian; if there are many rows of muscle, it is termed polymyarian. Each muscle cell is composed of a sacroplasmic zone containing the nucleus and a network of fibers. The fibrillar zone contains a series of fibrils perpendicular to the cell surface and extending the length of the cell.

There are three types of muscle cells. In platymyarian cells, the fibrils are found only along the margin next to the hypodermis. In coelomyarian muscle, the fibrils are on the side of the cell next to the hypodermis and extend along the sides of the cell. In circomyarian muscle, the fibrils form a layer around the entire cell. There are, in addition, many specialized muscles which have a variety of functions.

The digestive tract is a long, straight tube. The anterior end is surrounded by lips in some, but not all. The first portion of the digestive tract is called the stoma and extends from the oral opening to the beginning of the esophagus. It varies in appearance. Immediately behind the stoma is the esophagus, which is muscular, tri-radiate and, like the stoma and outside surface of the nematode, it is lined with cuticle. There are usually three esophageal glands within the esophageal wall: one dorsal, two subventral. Posterior to the esophagus is the esophageal-intestinal valve that connects with the intestine. The intestine is composed of a single layer of epithelial cells; there is no cuticular lining. Intestinal cells contain numerous and varied inclusion bodies.

The intestine opens into the rectum (cloaca in the male) which, again, is lined with cuticule. The rectum leads into the cuticularly lined anus, which is normally on the ventral side of the body. The portion posterior to the anus is referred to as the tail.

The nematode nervous system is composed of a nerve ring around the esophagus; ganglia and longitudinal nerves are associated with it. In addition there are special nerve branches which connect the nerve ring with papillae, setae, and chemoreceptors. The excretory system is the most varied system.

Sexes are separate in most nematodes. The male reproductive system consists of one or two tubular testes which empty into the vas deferens which, in turn, joins the rectum forming a cloaca. Dorsal to the cloaca is the spicule pouch, which contains the spicule. Spicules differ in structure and are useful to the taxonomist in identification of species. Associated with the spicules is a gubernaculum. The male tail has many sensory papillae whose number and location are also important taxonomic characteristics. In some genera the tail is modified to form caudal alae and bursa.

The female reproductive system consists of one or two tubular ovaries connected with a uterus (or uteri). The uterus terminates in a vagina, which opens to the outside through the ventrally located vulva.

The nematode body cavity, or pseudocoelom, is filled with fluid and thought to be lined with a network of fenestrated membranes that help to support the visceral organs. Associated with the pseudocoelomic membrane are large cells known as coelomocytes.

ANISAKINE MORPHOLOGY

Morphologically the anisakine adults are characterized by a stout, elongated body tapering at both ends. The posterior portion is generally straight in females and curved in the males. Superficially they resemble the familiar pig ascarid, *Ascaris lumbricoides*. Indeed, anisakines are classified among the ascarids (see below).

The cuticle of the anisakine nematodes is characterized by fine striations. In many genera the

cuticle is deeply grooved behind the base of the lips.

The somatic muscles of the anisakines are arranged in radial patterns with the fibrillar portions attached to the hypodermis and the sarcoplasm lying interiorly adjacent to the pseudocoelom. The somatic muscles of the anisakines and other ascarids are termed polymyarian because each quadrant contains numerous (more than five) bundles of fibers. The arrangement of the fibers is coelomyarian—i.e., the fibers are arranged in radial patterns parallel to the cuticle. The fibers are perpendicular to the long axis of the cell.

The oral opening is surrounded by three lips (one dorsal and two subventral). The actual form of the lips varies for the various genera; generally there are two equal papillae on the dorsal lip and one papilla and amphid on each of the subventral lips. Between the lips, interlabia may be present. In many of the anisakine genera, there are rows of denticles termed "dentigerous ridges," on the internal surface of the lips.

The esophagus of the anisakines is divided into two portions, the preventriculus and ventriculus. The ventriculus joins the intestine and preventriculus and may give rise to a posterior projection, termed a ventricular appendix; there may be one or several appendices (Fig. 1).

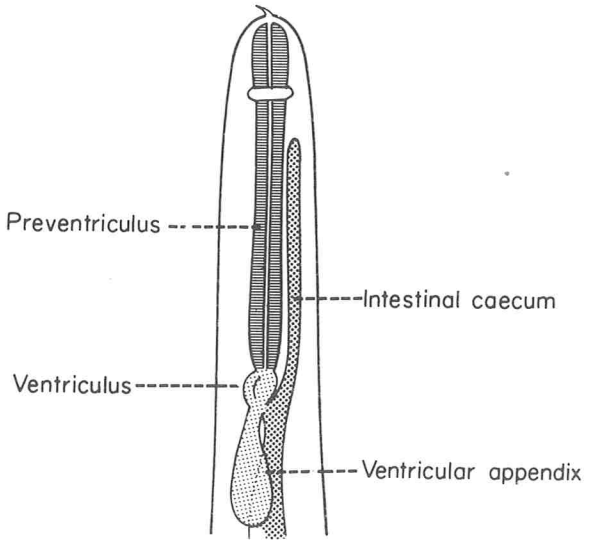


Figure 1. *Digestive tract of the anisakines.*

The intestine is a simple tube lined by a single layer of columnar epithelial cells. In certain genera, it gives rise to an anteriorly directed outpocketing referred to as an intestinal caecum. One genus has multiple caeca. The lumen of the caecum is cytologically similar to the intestine. It is broader at the base and gradually tapers anteriorly into a rounded tip where the lumen ends. A ligament extends anteriorly from the caecum to the body wall and holds the caecum in a fixed position. When this ligament is damaged by fixation, the intestinal caecum becomes distorted. The number of cells in the intestine seen at cross-section varies.

The excretory system and its external opening, the excretory pore, have not been characterized for all anisakine genera. The excretory pore opens either at the base of the subventral lips or at the level of the nerve ring. In some genera, it is asymmetrical and has only one lateral canal. In others the canal has been described as an inverted U shape (*2*). The excretory system has been neglected, and many descriptions fail to detail the morphology of the excretory gland or the position of the excretory pore.

The peripheral nervous system consists of a lattice-work of nerves connecting surface papillae and amphids with each other. The nerve centers are concentrated as a "nerve ring," a series of ganglia that surround the esophagus. A series of anterior and posterior fibers emanate from the nerve ring.

Anisakine nematodes are dioecious and the gonads are a series of tubes. The male gonads are single; the female gonads are paired. The male system consists of a coiled testis, a seminal vesicle, vas deferens, ejaculatory duct that terminates in the cloaca, and of such accessory copulatory organs as spicules and gubernaculum, as well as caudal papillae and in some, postcloacal cuticular ridges. There are usually two spicules that vary in shape. A gubernaculum is present in only a few genera.

The tip of the tail of certain species bear numerous spines.

The female genital organs are the ovaries, oviducts, seminal receptacles, uterus, vagina, and vulva. The vulva is on the ventral side, and generally appears in the anterior half of the body. The eggs vary in size and in shell structure. They are generally passed to the outside in an unsegmented condition.

CLASSIFICATION

The anisakine nematodes are members of the order Ascarida and suborder Ascaridina (*3*). The Ascaridina consist of three families: (*a*) Ascaridae, of which the largest intestinal nematode of primates, *Ascaris lumbricoides* Linnaeus, 1758, is a member; (*b*) Toxocaridae, containing the well-known dog ascarid, *Toxocara canis* Werner, 1782, responsible for human visceral larval migrans, and; (*c*) Anisakidae, of which the anisakine nematodes are members. The family Anisakidae contains 24 genera (Table 1) which are found as adults in fish, amphibia, reptiles, birds, and mammals. Life cycles are usually indirect, and may involve free living stages, a series of transport or paratenic host, as well as obligatory intermediate host(s). Larval stages have been reported from coelenterates, arthropods, annelids, fish, amphibians, birds, and mammals. These larval stages have been recognized since the late 17th century, but they have only been associated with human disease in the last decade. Only some types of anisakine larvae from fish have been implicated in human anisakiasis.

TABLE 1. *Classification of Anisakidae*

Family Anisakidae
Subfamily Anisakinae
Genera: *Anisakis* Dujardin, 1845
Acanthocheilus Molin, 1858
? *Belanisakis* Maplestone, 1932
? *Cloeoascaris* Baylis, 1923
Contracaecuum, Railliet et Henry, 1912
Dujardinascaris Baylis, 1947
? *Heligmus* Dujardin, 1845
Heterotyphlum Spaul, 1927
Ichtyanisakis Gendre, 1928
? *Metanisakis* Mozgovoy, 1950
Multicaecum Baylis, 1923
Paradujardinia Travassos, 1933
? *Paranisakiopsis* Yamaguti, 1941
Paranisakis Baylis, 1923
Phocascaris Host, 1932
Phocanema Myers, 1959
? *Polycaecum* Maplestone, 1930
Porrocaecum Railliet et Henry, 1912
? *Pseudanisakis* (Layman and Borokova, 1926) Mozgovoy, 1950
Pseudoterranova Mozgovoy, 1950
Raphidascaris Railliet et Henry, 1915
Raphidascaroides Yamaguti, 1941
Terranova Leiper and Atkinson, 1914
Thynnascaris Dollfus, 1933

? - Of questionable generic status.

CHARACTERIZATIONS OF LARVAL ANISAKINE NEMATODES

If the larvae found in fish hosts show the characteristic features of the digestive tract typical for anisakines (ventriculus and intestine), the larvae may be further sorted into types on the basis of characteristics shown in Fig. 2, 3, and 4. Other features, such as overall shape of the digestive tract and ratios of length to widths of the various tract portions, may further serve to subdivide these groups; but these additional criteria have yet to be confirmed by comparison with known specimens obtained from experimental host.

The position of the excretory pore, if known, already serves as a valuable diagnostic tool in helping to sort the anisakine nematodes as to type. However, it is sometimes difficult to locate. Unfortunately, many of the diagnostic features of adults are lacking and larvae cannot be classified as to genus and species, only as to a general "type."

Rather than add new terminology for the assessment of these types, the generic name will be used loosely (sensu lato).

Anisakis (sensu lato) larvae

Diagnosis: ventriculus present; anteriorly projecting intestinal caecum absent.

Anisakis (sensu lato), in which the excretory pore opens at the base of the lips (Fig. 2), has attracted the greatest attention in its association with human anisakiasis. *Anisakis, Acanthochielus,* and *Paranisakiopsis* are the three genera members possessing this larval type with the excretory pore opening at the base of lips. Only the genus *Anisakis* reaches its adult stage in marine mammals.

Japanese workers (*13*) have recognized three *Anisakis* larval subtypes, the most common one being Type I. It is characterized as having a prominent ventral boring tooth, short tail, mucron, esophagus with a long ventriculus that ends obliquely at its junction with the intestine. The *Anisakis* Type II larva is rare and characterized as having a long, tapered tail, short ventriculus, which has a horizontal junction with the intestine, boring tooth same as Type I, and no tail mucron. *Anisakis* Type III has been reported only once, and described as a stout larva with ventriculus as in Type II and a short tail with a tiny mucron.

These differences in the ventriculus also appear in adult forms of *Anisakis, Acanthochielus,* and *Paranisakiopsis* (*15*). Differences in the tail structure might also serve as morphological characteristics to separate the larval types; however, different methods of fixation and preservation may alter these morphological characteristics.

It appears that *Anisakis* (sensu lato) larvae, whose excretory pore characteristically opens at the level of the

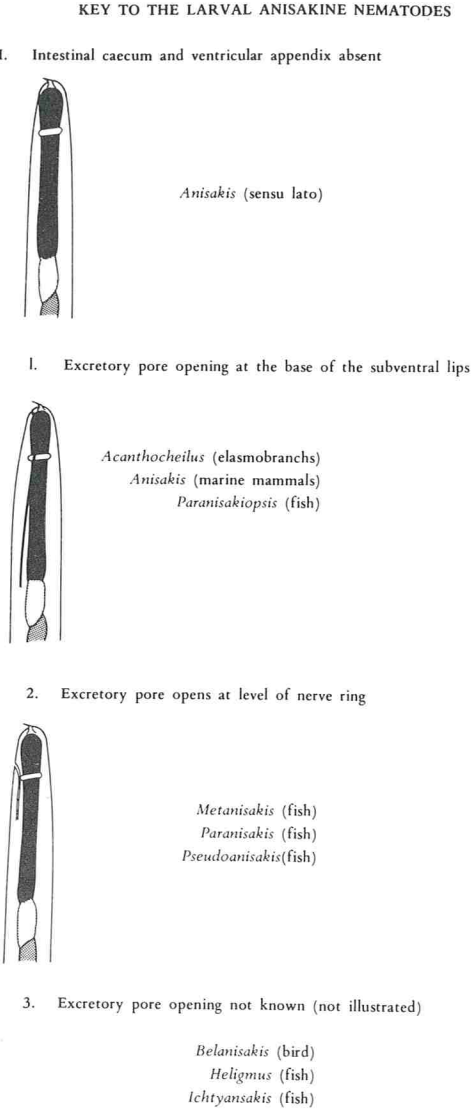


Figure 2. *Key to the larval anisakine nematodes (Part I).*

nerve ring, belong to genera that mature in fish and elasmobranch hosts.

It is quite possible that a more critical analysis of the ventriculus shape could serve to further distinguish the genera at a larval stage.

For the detailed diagnoses of genera with *Anisakis* (sensu lato) larvae, see Appendix I.

Phocanema (sensu lato) larvae

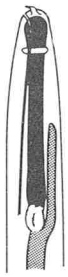
Diagnosis: ventriculus present; anteriorly projecting intestinal caecum present (Fig. 3).

Key to the Larval Anisakine Nematodes (continued)

II. Intestinal caecum present, ventricular appendix absent

Phocanema (sensu lato)

1. Excretory pore opening at the base of the subventral lips



Phocanema (marine mammals)
Terranova (elasmobranchs)

2. Excretory pore opening at the level of the nerve ring

Paradujardinia (sirenia)
Porrocaecum (birds, fish)
Pseudoterranova (marine mammals)

3. Excretory pore opening not known (not illustrated)

Cloeoascaris (semiaquatic mammals)

Figure 3. *Key to the larval anisakine nematodes (Continued, Part II).*

The *Phocanema* (sensu lato) larval type, known as the "codworm," attracted much attention in the late 1940's and in the 1950's since the presence of large numbers in the flesh of cod and other food-fish lowered their marketability (*11*). Recently, several human infections with *Phocanema* (sensu lato) have been documented in North America (*4, 6-8*). In all of these cases, identification of *Phocanema* as the causative agent has been based on the recovery of the entire nematode.

These larvae, in which the excretory pore opens at the base of the lips, represent two genera: *Phocanema,* which occurs as adults in seals; and *Terranova,* reported as adults in fish and elasmobranchs. However, differentiation of these genera by their larval stages is difficult. Our criteria for separation are not critical enough to allow us to predict the ones responsible for human infections, namely those destined to reach maturity in a marine mammal. The necessity for making this diagnosis is indicated by the human cases.

The Japanese recognize two types of this larva. Oshima (*13*), in his review, only presents a brief description of what he terms the *Terranova* type A larva from the squid. He considers it a possible causative agent of human anisakiasis, but does not discuss differences or similarities between it and type B from fish.

The genus *Phocanema* was established by Myers (*10*) with *P. decipiens* as type species on the basis of adult characteristics: cephalic structures and male tail. There has been much confusion in the literature, and there have been many misquotes. *Terranova,* Leiper and Atkinson, 1914, is a valid genus based on the description of the type species *T. anarctica* from an elasmobranch *Mustelus anarcticus.* The generic rank has been recognized by Russian (*9*) and other workers, and *T. anarctica* must remain the type species. Yamaguti (*15*) incorrectly cited *T. decipiens* (Krabbe, 1878) as the type species. In the literature, the genera *Porrocaecum* and *Terranova* have been used incorrectly for *Phocanema decipiens.*

Paradujardinia, Pseudoterranova, and *Porrocaecum* are genera with *Phocanema* (sensu lato) larvae having an excretory pore at the level of the nerve ring. No. human cases have been reported and the adults of these genera do not develop in mammals.

The genus *Pseudoterranova* closely resembles *Terranova* and was originally placed in that genus but removed by Mozgovoy (*9*) on the basis of the presence of a gubernaculum and position of the excretory pore. He considered it to occupy an intermediate position between *Porrocaecum* and *Terranova.* Cephalic structures, characteristics of the male tail, separate the adult stages of these three genera.

For diagnoses of genera with *Phocanema* (sensu lato) larvae, see Appendix II.

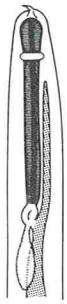
Contracaecum (sensu lato) larvae

Diagnosis: ventriculus and ventricular appendix present; anteriorly projecting intestinal caecum present (Fig. 4).

Larval stages of the *Contracaecum* (sensu lato), in which the excretory gland opens at the base of the lips, are probably *Phocascaris* (reported as adults from seals) and/or *Contracaecum* (reported as adults in marine mammals and birds). These two genera are separated in

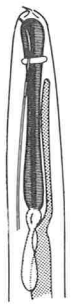
Key to the Larval Anisakine Nematodes (continued)

III. Intestinal caecum and ventricular appendix present



Contracaecum (sensu lato)

1. Excretory pore opening at the base of the subventral lips



Contracaecum (marine mammals and birds)
Phocascaris (marine mammals)

2. Excretory pore opening at the level of the nerve ring

Thynnascaris (fish)
Heterotyphlum (fish)

Figure 4. *Key to the larval anisakine nematodes (Continued, Part III).*

the adult stages on the basis of cephalic structures. However, as larval stages in the fish host, these two genera may be difficult to separate. Both could be potential human pathogens. One actual case has been reported (*14*).

Thynnascaris and *Heterotyphlum,* characterized by the opening of the excretory pore at the level of the nerve ring, occur as adults in fish and are probably not able to survive in mammals. Larval stages of *Thynnascaris* have been reported from shrimp, squid, and fish (*2*).

For diagnoses of genera with *Contracaecum* (sensu lato) larvae, see Appendix III.

Raphidascaris (sensu lato) larvae

Diagnosis: ventriculus and ventricular appendix present; anteriorly projected intestinal caecum absent (Fig. 5).

The *Raphidascaris* type of larva has not been incriminated in human anisakiasis. It occures in genera such as *Raphidascaris* and *Raphidascaroides,* which reach adulthood in fish.

For diagnoses of general which have *Raphidascaris* (sensu lato) larvae, see Appendix IV.

Multicaecum (sensu lato) larvae

Diagnosis: ventriculus and multiple ventricular appendices present; anterior projecting intestinal caecum present.

The larval stages of *Multicaecum* (sensu lato) are unknown and probably will not be implicated in human anisakiasis. However, for completeness of the review of the anisakine nematodes, diagnoses of genera that might have *Multicaecum* (sensu lato) larvae are given in Appendix V.

DISCUSSION

As can be seen from the foregoing descriptions, the definite assignment of larval stages to genus, let alone species, is not possible. This situation may be solved by a more precise characterization of the larval stages that are frequently encountered. However, this does not aid in separating rare forms. Other methods may contribute to their identification, but exploration of biochemical profiles, serology, and ultrastructure criteria is still in infancy.

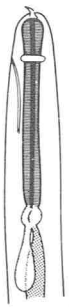
At present we must be satisfied with assigning

Key to the Larval Anisakine Nematodes (continued)

IV. Intestinal caecum absent, ventricular appendix present

Raphidascaris (sensu lato)

1. Excretory pore opening at the level of the nerve ring



Raphidascaris (fish)
Raphidascaroides (fish)

Figure 5. *Key to the larval anisakine nematodes (Continued, Part IV).*

collected material to broad groupings based on morphological characteristics known to be present in the early forms which hatch from the egg. This, too, may present problems as the intestinal caecum is not formed in this early larval stage.

It is hereby recommended that any forms which can survive at 35 C and above in a physiological solution be considered as potential human pathogens.

As larval stages the anisakines have been described in the literature since 1767. There are over 100 descriptions of anisakine larval stages, and even today their classification is too often based on arbitrary measurements. As an example, the length of the intestinal caecum of a larval *Thynnascaris* is approximately 2.5 times the length of the ventriculus in living specimens and in most fixed specimens, but in some fixed specimens this length is reduced to less than the length of the ventriculus (*1*). This drastic change in caecal length is presumably due to the breakingof the ligament which connects the caecum to the body wall in normal specimens, but which has been severed during fixation. The ligament is readily observable in many normal specimens but has not been observed in specimens with a short caecum.

Three types of anisakine larvae have been implicated in human disease: *Anisakis* s.l., *Contracaecum* s.l., *Phocanema* s.l. (= *"Terranova"*) (*13*), and possibly *Porrocadecum* s.L. (*4*).

In tissue sections from pathological specimens, one may not have all of the specimen available for identification. The Japanese (*13*) have elucidated the following identification criteria based on tissue sections; they are even more tenuous than criteria mentioned previously for whole specimens.

The criteria are((*a*) the number of muscle cells in a quadrant of the body; (*b*) the number of intestinal cells; (*c*) maximum body width; and (*d*) shape of the lateral chords.

Oshima (*13*) has reviewed these for *Anisakis, Terranova* (= *Phocanema*), and *Contracaecum* larvae. He found that there was an overlap as to the number of muscle cells in quadrant of the body, and also in body width. However, the number of intestinal cells (less than 100 in *Anisakis* and more than 100 in *Terranova* (= *Phocanema*)), and lateral chord structure (y-shaped in *Anisakis* and butterfly shaped in *Terranova* (= *Phocanema*)) could serve as diagnostic characteristics separating these two types. The lateral chords are of hypodermal origin and contain a great part of the nerve cells of the body. In the simplest nematodes, there are four chords: one dorsal, two lateral, and one ventral. Modifications of this arrangement are characteristic for various groups of nematodes (*5*). Although more thorough study is needed of many specimens to determine the limits of variations which may exist, the morphology of the lateral chords, which extend the entire length of the nematode and would be observed in all cross-sections, could be a valuable characteristic for diagnosis of these nematodes when observed in pathological material.

CONCLUSIONS

Recognized since 1767, the nematodes that cause human anisakiasis are inadequately characterized; and our knowledge of their relationships to host is poorly understood.

We do not yet know the distribution and incidence of these larvae in food fish of North America. There is a complete void of information on the percentage of fish parasitized and the location of the larva in the fish host. Not all larvae in muscle are *Anisakis* or *Phocanema,* nor are all those in viscera *Anisakis* or *Contracaecum.* Mixed infections do occur.

At present each larva must be examined morphologically to determine which type is present. Likewise, marine mammals may be infected with mixed adult populations of the genera *Anisakis, Contracaecum, Phocanema,* and *Phocascaris.* Unfortunately, there are no shortcuts to identification of specimens.

Many genera and species listed as members of Anisakidae need a critical review to determine their validity. Some are monotypic and have only been reported once. However, until the species of the various genera are studied critically, they will remain in the literature. To date, cephalic structures and the digestive tract formation constitute the best morphological criteria for separation of the genera.

REFERENCES

1. Bier, J. Personal communication.
2. Chitwood, B. G., and M. G. Chitwood. 1950. An introduction to nematology, Sec. 1., Anatomy. Washington, B. G. Chitwood, Pub.
3. Chitwood, M. B. 1969. The systematics and biology of some parasitic nematodes. Chem. Zool. 3:223-244.
4. Chitwood, M. B. 1970. Nematodes of medical significance found in market fish. Am. J. Trop. Med. Hyg. 19:599-602.
5. Chitwood, M. B., and R. Lichtenfels. 1972. Identification of parasitic metazoa in tissue sections. Exp. Parasitol. 32:407-519.
6. Kates, S., K. A. Wright, and R. Wright. 1973. A case of human infection with the cod nematode *Phocanema* sp. Am. J. Trop. Med. Hyg. 22:606-608.
7. Little, M. D., and J. MacPhail. 1972. Large nematode larva from the abdominal cavity of a man in Massachusetts. Am. J. Trop. Med. Hyg. 21:948-950.
8. Little, M. D., and H. Most. 1973. Anisakid larvae from the throat of a woman in New York. Am. J. Trop. Med. Hyg. 22:609-612.
9. Mozgovoi, A. A., 1953. Ascaridata of animals and man, and the diseases caused by them. Vol. 2:616 pp (Osnovy Nematologie).
10. Myers, B. J. 1959. *Phocanema,* a new genus for the anisakid nematode of seals. Can. J. Zool. 37:459-465.
11. Myers, B. J., 1970. Nematodes transmitted to man by fish and aquatic animals. J. Wildlife Dis. 6:266-271.
12. Norris, D. E., and R. M. Overstreet. 1976. The public health implications of larval *Thynnascaris* nematodes from shellfish. J. Milk Food Technol. 39: (In press).
13. Oshima, T. 1972. *Anisakis* and anisakiasis in Japan and adjacent area. p. 305-393. *In:* K. Morishita, and Y. Komiya, (eds.) Progress of med. parasitology in Japan. Vol. 4. Meguro Parasitology Museum, Tokyo.
14. Schaum, E., and W. Müller, 1967. Die Heterocheilidiasis eine Infektion des Menschen mit Larven von Fish Ascariden. Dtsch. Med. Wochenschr. 92:2230-2233.
15. Yamaguti, S., 1961. Systema helminthum. Vol. III, Pts. 1 and 2. Interscience, New York.

APPENDIX I

Anisakis Dujardin, 1845

Synonyms: *Stomachus* Goeze (in Zeder, 1800)
Filocapsularia Deslongchamps, 1824
Peritrachelius Diesing, 1851
Conocephalus Diesing, 1860

Generic diagnosis: Three lips each bearing a bilobed anterior projection which carries a single dentigerous ridge; interlabia absent; excretory pore opening base subventral lip; esophagus with anterior muscular portion and posterior ventriculus, the latter being oblong and sometimes sigmoid, or else as broad as long; no esophageal appendix or intestinal caecum; vulva in middle or first third of body; spicules of male unequal; preanal papillae numerous; postanal papillae including a group of three or four pair set close to tip of tail on ventral side.

Type species: *A. simplex* (Rudolphi, 1809) Baylis, 1920.
Parasites of marine mammals.

Metanisakis Mozgovoy, 1950

Generic diagnosis: Lips simple with two transverse ridges; interlabia absent; excretory pore opening at level of nerve ring; anterior esophagus muscular followed by glandular ventriculus; vulva in anterior fourth of body; spicules equal or subequal, long, slender; gubernaculum absent.

Type species: *M. rajae* (Yamaguti, 1941) Mozgovoy, 1950
Parasites of elasmobranchs.

Paranisakis Baylis, 1923

Generic diagnosis: Interlabia small, dentigerous ridges absent; excretory pore opens anterior to the nerve ring; vetriculus oval; ventricular appendix and intestinal caecum absent; vulva in middle of body; male tail conical, transversely striated, ventral surface preanal region longitudinally ribbed; spicules subequal, alate; gubernaculum massive.

Type species: *P. squatinae,* Baylis, 1923
Parasites of elasmobranch.

Icthyanisakis Gendre, 1928

Generic diagnosis: Lateral alae from base of lips to near tail; lips well defined, separated from body by festoons; each with four longitudinal ridges, dentigerous ridges absent; interlabia well-developed; anterior esophagus muscular followed by small glandular ventriculus; vulva in anterior quarter of the body; spicules short, equal, alate; gubernaculum absent.

Type species: *I. monodi* Gendre, 1928
Parasites of fish.

Belanisakis Maplestone, 1932

Generic diagnosis: Cervical alae present; interlabia and dentigerous ridges present; esophagus with posterior muscular ventriculus; intestinal caecum and ventricular appendix absent; vulva anterior to middle of body; spicules stout, equal; gubernaculum short.

Type species: *B. ibidis* Maplestone, 1932
Parasites of birds.

Heligmus Dujardin, 1845

Generic diagnosis: Head rounded, three lips; esophagus musculuar with bulbular ventriculus; vulva anterior one-third of body; single alate spicule.

Type species: *H. longicirrus* Dujardin, 1845
Parasites of fish.

Acanthocheilus Molin, 1858

Generic diagnosis: Lips with four forward projecting denticles arranged in two separate paris; excretory pore probably opens close to base of subventral lips; esophagus claviform followed by glandular ventriculus. Vulva in anterior of the body. Spicules short and slender.

Type species: *Acanthocheilus quadridentatus* Molin, 1850
Parasites of elasmobranches.

Pseudoanisakis (Layman and Borovkova, 1926) Layman and Borovkova, 1950

Synonym: *Anacanthocheilus* Wülker, 1929

Generic diagnosis: Lips with a single transverse dentigerous ridge; interlabia absent; esophagus with a glandular ventriculus; vulva close to anterior extremity. Spicules equal, long slender.

Type species: *P. rotundata* (Rudolphi, 1819) Mozgovoy, 1950
Parasite of marine fish.

Paranisakiopsis Yamaguti, 1941

Generic diagnosis: small nematodes; interlabia prominent, about two-thirds as long as the lips-dentigerous ridges absent; esophagus muscular with a glandular ventriculus; ventricular appendix and intestinal caecum absent; excretory duct opens at behind baseof subventral lips; vulva anterior to middle of the body; spicules long, equal, or subequal; gubernaculum absent.

Type species: *P. corlorhynchi* Yamaguti, 1941
Parasites of marine fish.

APPENDIX II

Dujardinascaris Baylis, 1947

Snonyms: *Dujardinia* Gedoelst, 1916 nec Quatrefages, 1844, nec Gray, 1858

Generic diagnosis: Lips with broad membranous margins, with large toothlike structures capable of being interlocked; interlabia and interlabial grooves present; excretory pore opens at level of nerve ring; spherical-shaped ventriculus; ventricular appendix absent; intestinal caecum present; vulva in anterior half of body; spicules equal, short; gubernaculum usually present.

Type species: *D. helicina* (Molin, 1860) Baylis, 1947
Parasites of reptiles and fish.

Phocanema Myers, 1959

Generic diagnosis: Three prominent, fleshy, well-developed bilobed lips; dentigerous ridges present; interlabia absent; excretory pore opening at the base of the subventral lips; esophagus with anterior muscular portion and posterior ventriculus; vulva opens in anterior third of body; anteriorly projecting intestinal caecum present; spicules subequal; gubernaculum absent; three postanal dentigerous ridges present.

Type species: *Phocanema decipiens* (Krabbe, 1878) Myers, 1959
Parasites of marine mammals.

Terranova Leiper and Atkinson, 1914

Generic diagnosis: Lips "poorly" demarked from neck region; bearing anterior bidentate projection with dentigerous ridges, interlabia absent; esophagus with anterior muscular portion and posterior ventriculus; intestinal caecum present; vulva in anterior half of the body; spicules equal or subequal; gubernaculum present or absent.

Type species: *T. anarctica* Leiper and Atkinson, 1914
Parasites of elasmobranchs, crocodiles.

Porrocaecum Railliet et Henry, 1912

Generic diagnosis: Three lips; dentigerous ridges and interlabia present; excretory pore opens at level of nerve ring; esophagus with anterior muscular portion and posterior ventriculus of oblong shape, the latter short in genotype but in other species bent at an angle so as to open into the intestine laterally; intestinal caecum present; vulva near middle of body; spicules equal, gubernaculum usually absent.

Type species: *P. crassum* (Deslongchamps, 1824) Railliet et Henry, 1912
Parasites of birds and fish.

Paradujardinia Travassos, 1933

Generic diagnosis:Dorsal lip octagonal in outline, lip pulp gives rise to small conical, anterior processes which project forward and inward; well-developed interlabia, dentigerous ridges absent; excretory pore

opens at level of nerve ring; ventriculus spherical; intestinal caecum narrow; vulva preequational; spicules short; gubernaculum absent.

Type species: *P. halicoris* (Owen, 1833) Travassos, 1933.
Parasites of sirenia.

Pseudoterranova Mozgovoy, 1950

Generic diagnosis: Lips similar in shape, internally projecting bilobed part with long teeth in the dentigerous ridges; interlabia absent; excretory pore "apparently" at level of nerve ring; ventriculus present; intestinal caecum only slightly longer than ventriculus; vulva a short distance posterior to esophagus; spicules small, unequal; gubernaculum present.

Type species: *P. kogiae* (Johnston and Mawson, 1939) Mozgovoy, 1950
Parasites of whales.

Cloeoascaris Baylis, 1923

Generic diagnosis: Each lip provided with a pair of large conical teeth on its inner surface; interlabia absent, collar-like fold of cutucle surrounds the neck, between this and the bases of the lips is an area covered with small spines; esophagus with a small rounded ventriculus, intestinal caecum present; vulva in anterior half of the body; spicules short, slender, and equal.

Type species: *C. spinicollis* Baylis, 1923
Parasites of semiaquatic land mammals.

APPENDIX III

Contracaecum Railliet et Henry, 1912
Synonyms: *Kathleena* Leiper and Atkinson, 1914
Cerascaris Cobb, 1928
Iheringascaris Pereira, 1935
Amphicaecum Walton, 1929

Generic diagnosis: Lips with antero-lateral projections; dentigerous ridges absent; interlabia present usually well-developed; excretory pore opening base of subventral lips; esophagus with anterior muscular portion and reduced posterior ventriculus with a posterior appendix; anterior projecting intestinal caecum present; vulva in anterior region of the body; male without definite caudal alae; numerous preanal papillae, spicules long alate, equal or subequal gubernaculum absent.

Type species: *C. spiculigerum* (Rudolphi, 1809) Railliet et Henry, 1912
Parasites of marine mammals and birds.

Heterotyphlum Spaul, 1927

Generic diagnosis: Lips asymmetrical each with two unequal papillae; dentigerous ridges and interlabia absent; excretory pore opens at level of nerve ring; ventriculus reduced; intestinal caecum present; vulva in anterior half of body; male tail is conical and short with papilliform tip; spicules long, slender, and unequal.

Type species: *H. himantolophi* Spaul, 1927
Parasites of fish and birds.

Thynnascaris Dollfus, 1933

Generic diagnosis: Lips with oblique cuticular ridges; interlabia short and compressed, interlabial grooves arise from interlabia and extend around base of the lips; esophageal appendix and intestinal caecum present; vulva opening in middle third of body; spicules long, equal, or unequal; gubernaculum absent.

Type species: *T. legendrei* Dollfus, 1933.
Parasites of fish.

Phocascaris Höst, 1932

Generic diagnosis: Lips with three deep incisions directed toward oral aperture; interlabia absent; dentigerous ridges present; excretory pore opens at base of ventral lips; ventriculus short, ventricular appendix and intestinal caecum present; vulva in anterior region of the body; spicules long, slender, subequal; gubernaculum absent.

Type species: *P. phocae* Höst, 1932

APPENDIX IV

Raphidascaris Railliet et Henry, 1915
Synonyms: *Hysterothylacium* Ward amd Magath, 1916
Ichthyascaris Wu, 1949

Generic diagnosis: Well-developed cuticular expansions on subventral lips; interlabia and dentigerous ridges absent; excretory pore opens at the level of the nerve ring; esophagus with a small ventriculus, ventricular appendix, intestinal caeca absent; vulva in anterior half of the body; spicules equal, alate; gubernaculum absent.

Type species: *R. acus* (Bloch, 1779) Raillet et Henry, 1915
Parasite of fish.

Raphidascaroides Yamaguti, 1941
Synonyms: *Ryjikovascaris* Mozgovoy, 1950

Generic diagnosis: Lips with lateral cuticular expansions, interlabia present, dentigerous ridges occasionally absent; excretory pore opens level of nerve ring; ventriculus spherical, ventricular appendix; intestinal caecum absent; vulva anterior to mid-body; spicules equal alate, gubernaculum absent.

Type species: *R. nipponensis* Yamaguti, 1941
Parasite of marine fish.

APPENDIX V

Multicaecum Baylis, 1923

Generic diagnosis: Lips rounded, labial processes weakly developed; interlabia small with well-marked interlabia grooves at the base; excretory pore opens at level of nerve ring; esophagus with a small ventriculus from which arise two anterior and two posterior appendices; intestinal caecum present; vulva in anterior one-third of body; spicules equal or subequal; gubernaculum present.

Type species: *M. agili* (Wedl, 1862) Baylis, 1923
Parasites of crocodiles.

Polycaecum Maplestone, 1930

Generic diagnosis: Lips not well-defined; interlabia absent; annular swelling behind lips; short ventriculus from which arise two anterior and three posterior appendices; intestinal caecum present; vulva near middle of body.

Type species: *P. gangeticum* Maplestone, 1930
Parasites of crocodiles.

J. Milk Food Technol. Vol. 38, No. 12, Pages 783-785 (December, 1975)

Presidential Address

PARNELL J. SKULBORSTAD

Babson Bros. Company
2100 South York Road, Oak Brook, Illinois 60521

(Received for publication August 12, 1975)

In 1975 the International Association of Milk, Food and Environmental Sanitarians (IAMFES) continued to move forward despite a general recession in the economic community. Without in any way diminishing the importance of radical changes made in 1974, it must be noted that results from those changes have come to fruition in 1975. In most important respects, this association has outperformed other organizations with similar interests; it has also performed well in comparison with previous years.

In reviewing the year now coming to an end, one word dominates our current situation, and that word is "service." Service to our affiliates and their membership, service to the public, service to the food industry, service to the scientific community, and last but not least, service to our own cause. While no single criterion exists for measuring the service we perform, it has to be recognized that we are not communicating as we should with those persons who hunger for leadership from this organization. They need to know what this organization is doing, what its goals are, where it's going, how it's going to get there, and what it has accomplished on the way. They are looking for answers and recommendations that they can apply in their everyday activities and responsibilities. It seems to me that the IAMFES is charged with and responsible for a certain amount of leadership in the food industry. We have to help the affiliates fulfill their objectives. By improper communication and *doing* nothing really constructive we are merely saying to them that we are not qualified or not concerned enough to provide information where it can be applied. I say we ought to take charge and get something done and justify the existence of the IAMFES. The accuracy of this generalization is critically important to the immediate future and a longer look over the next 5 years should provide some interesting challenges for those who make an effort to get involved and want to get involved.

I would propose that we set up a committee or hire a consulting and research firm to undertake a study to determine the future direction of the IAMFES. A study of our history to see if any useful lessons might emerge with respect to our educational efforts, membership programs, committee assignments, journal policies, meeting arrangements, etc. With findings of a study such as this in mind we might see what is in store for the balance of this decade and hopefully improve our performance.

PERFORMANCE IN 1975

I am pleased to be able to report that our overall financial condition in 1975 will show a substantial improvement over 1974. The improved financial performance is, of course, a most welcome development, for only a financially healthy organization can continue to provide necessary service to its members and the public. I am well aware that this organization is not established as a commercial venture, but unquestionably financial problems do have an adverse effect on our performance.

Most of the credit for our recent financial improvement is due to the very capable management by our Executive Secretary, Earl Wright. Without the burden of serving as both President and Executive Secretary, he has performed magnificently. Obviously, his dedication and total commitment during a very trying transitional period has been beyond the requirements of his position.

During the year we have continued the efforts to work closer with the affiliate organizations, but much is left to be desired in this area. We have added a new affiliate, approved by the Executive Board on May 15, 1975, in St. Louis, Missouri. The Alberta Association of Milk, Food, and Environmental Sanitarians joined IAMFES with 12 members. Lawrence McKnight of the University of Alberta, Department of Food Science, is the president of the new Canadian association. I would like to extend a most hearty welcome and hope the relationship is beneficial to all concerned.

Our membership continues to grow largely due to the efforts of a fine membership committee, chaired by Harold Heiskell. Time does not permit a detailed report, however, we have 513 new members with a net increase in membership of 182 for the fiscal year. Increasing membership should be the concern of all members since it increases our capacity for effective performance. As we improve our performance, undoubtedly we will attract additional affiliate interest.

Committee activity in 1975 has been inconsistent mostly due to travel restrictions and limited funds. This area has to be a major concern to all members. The Executive Board has studied the problem and through proper consultation with committee chairmen has recommended changing the structure and profile of several committees to improve representation at meetings during the year, obviously increasing

performance. We have 28 committees functioning within our organization. Unquestionably our success in the future is dependent on hard-working committees, and I want to thank all committee chairmen and members for their efforts the past year. An excellent example of committee performance is the 1973-1975 Farm Methods Report.

Preliminary discussions on educational programs sponsored by the IAMFES were conducted. The need has been established for a vehicle to convey information and programs developed to members that cannot attend meetings.

The association continued its cooperation with the National Environmental Health Association. A joint meeting of officers was held in Washington, D.C. on June 12-13, 1975. The purpose of the meeting was to explore common grounds of both organizations and suggest positive steps necessary to examine potential problems and ultimate desirability of unification. A proposed time table for this purpose was approved by the joint executive boards. The proposal is a sincere attempt to serve the best interests of the public and members of both organizations.

It provides an opportunity to: (*a*) follow through with the earlier efforts of our colleagues; (*b*) respond to the 1974 report of the IAMFES Affiliate Council which stated the "balloting concerning the merger is premature at this time" and requested that the Executive Board of IAMFES meet with the executive board of NEHA to consider the possibility of a "joint conference;" (*c*) respond to the request of local IAMFES affiliates that further investigation be conducted before final unification is proposed; (*d*) assure that each organization maintains its primary objectives and the interests of its members and the public; (*e*) provide the time necessary to compile and disseminate the facts about each respective association in an organized manner to assure ultimate satisfaction on the part of all members; and (*f*) study mechanics of a joint annual conference and compatibility of memberships and determine a format for a program that will satisfy the needs of both. The proposed time table is certainly not a cure-all for the problems to be encountered in the next 4 years, but it is a starting point and work program to get things going.

THE JOURNAL

The *Journal of Milk and Food Technology* continues to be our numbr one ambassador. With distribution in over 70 countries and a circulation of over 4,000, it is recognized as one of the finest publications in the food industry and academic community. We have changed the make-up of the Journal Management Committee slightly, hopefully strengthening it and making it more effective. We have endeavored to get some younger members involved, supported by some very extensive experience, knowing that we will get more input and more results. As most of you know, printing of the *Journal* was moved to Ames, Iowa, on January 1, 1975, enabling us to make several changes and make the *Journal* more attractive. Considerable progress has been made toward having an equal amount of space devoted to research, technical, and non-technical papers. The table of contents has reflected this progress since our February, 1975 issue. The ultimate goal of a balanced publication needs the support and participation of all members before it can be realized. We continue to get outstanding research papers for publication and about 70% of the papers in Volume 37 reported research findings. It also was among the largest to be published, exceeded only by Volume 35 in 1972.

A QUICK LOOK AT 1976

Unquestionably travel restrictions due to rising costs and limited funds will curtail committee activity. The situation at the moment is clearly a matter of concern at the governmental and institutional levels. Industry is also watching its travel costs more carefully.

I am not sure what the proper remedy is, but we have to face the problem immediately. Possible geographical realignment of all committees, including co-chairmen on each, to get better representation at meetings throughout the year should be considered. This would also aid in perpetuating committee activities and programs as changes in chairmanships are assigned.

We have to continue our membership thrust. We cannot afford a "no growth" year. While we have had favorable results the past 2 years, we cannot rest on our laurels. It is necessary that our membership efforts are supported by another program for the affiliates. We need a closer working relationship with similar organizations on the national level. We must continue cooperating with the affiliates and also improve our communications with them. Indeed, the association may have some problems, but they are not insurmountable.

A LONGER LOOK AT THE NEXT FIVE YEARS

While it is difficult not to be concerned as 1976 begins, a longer look over the next 5 years provides several encouraging signs amid some persistent problems. First, the demographic factors are favorable. There will be more people, thus a rising need for the services we perform. Second, more young people are looking favorably on food and environmental careers. Our student program is weak and totally inadequate, but this can be resolved. Our relationship with educational institutions that offer specific degree programs in environmental and public health has to be reviewed and improved. They should be invited to participate at our annual meetings with appropriate displays offering information on specific courses and programs that are available. We cannot afford to ignore the young people. We have to do whatever is necessary to get them involved in the IAMFES. Inclusion of research papers in the annual meeting program has proved to be an excellent vehicle to serve this purpose and should be expanded.

A third favorable factor is our financial situation. We have shown that we can control costs and increase performance. While some may question this statement, we are moving to a budget program for the future. A budget committee will be assigned for 1976, consisting of several Past Presidents and the Executive Secretary. Their responsibility will be to establish an operating budget with several priorities. The travel problem will not disappear. Faced with this problem we have no alternative but to explore the feasibility of establishing a fund for travel purposes. Administration of the fund should be the responsibility of the First and Second Vice Presidents. Obviously, time does not permit an extended discussion of this immediate problem, but it has to be handled. We will have to concentrate on this important subject in the coming months.

A fourth favorable factor is that we know where we are going. There is an awareness of our problem areas and needs, and they must be surmounted. We should have a comprehensive educational package that includes training guides and lesson plans on sanitary standards, equipment standards, and biological aspects of sanitation. The ground work should be done in 1976 with a 4-year program to follow. Clearly the IAMFES must make every reasonable effort to assume leadership in the industry.

CONCLUSIONS

I appreciate the opportunity this organization has provided me to get involved in the problems and opportunities of the food and environmental industry. I believe deeply in the cause and ability of the IAMFES to get the job done. This is a unique organization that is active and strong because of the diligence and dedication of its members. It is a professional organization that can take pride in what it has accomplished. In summary, I am pleased that 1975 has turned out to be a good year, but I am concerned that we did not accomplish more. I am optimistic—very optimistic—that the IAMFES will realize its many opportunities and overcome with time the problems it faces.

J. Milk Food Technol. Vol. 38, No. 12, Pages 786-795 (December, 1975)

The Sixty-Second Annual Meeting of IAMFES

Royal York Hotel, Toronto, Ontario, Canada, August 10-13, 1975

The Ontario Milk and Food Sanitarians Association and the Department of Food Science of the University of Guelph, Guelph, Ontario cooperated to make arrangements for a successful 62nd Annual Meeting of IAMFES. From registration through the Awards Banquet, the meeting was well organized and was held in facilities that were more than adequate to meet the needs of registrants. Approximately 400 IAMFES members and guests attended the meeting. Additional registrants attended the 1975 Regional Meeting of the National Mastitis Council which was held on August 14th at the Royal York Hotel. The general format of the 62nd Annual Meeting followed that of the 61st Annual Meeting. The schedule appeared to meet the needs of committees and the Affiliate Council and provided ample time for exchange of information at technical sessions. As usual, the Executive Board of IAMFES met before, during, and after the regular sessions of the Annual Meeting.

Figure 1. *Action at the registration desk.*

MEETINGS OF THE EXECUTIVE BOARD

The Executive Board heard numerous reports during its sessions. Included were the following:

(*a*) *Financial report.* Executive Secretary E. O. Wright reported that the total income for July 1, 1974 to June 30, 1975 was approximately $93,000 and expenses for the same period were approximately $86,000. The actual net income was $7,015; the *Journal of Milk and Food Technology* generated $5,629 of the total net income. Income increases over the previous year were noted in affiliate dues, annual meeting income, interest income, advertising, subscriptions, sale of reprints, and page charges. The net income of 1974-1975 more than covered the net loss ($4,657) that was encountered during 1973-1974.

(*b*) *Membership.* H. Y. Heiskell, Chairman of the Membership Committee, reported that 513 new members were gained for IAMFES during the past year and that this resulted in a net gain of 194 members. Five states—Kentucky, Missouri, Florida, Oregon, and Wisconsin were winners in the membership contest.

(*c*) *Electionof officers.* Dr. A. N. Myhr noted that 533 ballots were cast to elect a second Vice-President and Secretary-Treasurer. Mr. H. E. Hutchings of South Dakota was elected as the Second Vice-President and Professor R. P. March was reelected as Secretary-Treasurer.

Figure 2. *The Executive Board and Editors of IAMFES (top row, left to right): Dr. E. H. Marth, Editor,* Journal of Milk and Food Technology; *W. F. Wilson, Senior Past-President during 1974-1975 and now retired from the Board; D. D. Fry, First Vice-President; Dr. H. V. Atherton, President-Elect; H. Hutchings, Second Vice-President; (front row, left to right): E. O. Wright, Executive-Secretary, Managing Editor, and Senior Past-President; P. J. Skulborstad, Junior Past-President (President, 1974-1975); H. E. Thompson, President; and Prof. R. P. March, Secretary-Treasurer.*

(*d*) *Awards.* According to W. F. Wilson, Chairman of the Committee on Awards and Recognition, numerous outstanding candidates were nominated for all awards given by the Association. The committee selected the following as recipients of awards: Honorary Life, A. E. Parker; Citation, Dr. A. R. Brazis; Sanitarians, S. C. Rich; Educator-Industry, Dr. K. G. Weckel; Shogren Award, Illinois Affiliate.

(*e*) *Farm Methods Committee.* Chairman M. W. Jefferson stated that a complete committee report was prepared this year. The report will appear in a future issue of the *Journal of Milk and Food Technology.*

(*f*) *International Dairy Federation.* Harold Wainess is the unofficial IAMFES representative to IDF. IDF is concerned, on a worldwide basis, with many of problems

that are dealt with by IAMFES. Future meetings of IDF include Austria in 1975 and Canada in 1976.

(*g*) *Editor, Journal of Milk and Food Technology.* According to Dr. E. H. Marth, Editor, Volume 37 (1974) was among the largest ever published. In 1974, 102 papers were published; of these 70% reported research findings, 20% dealt with technical topics of general interest, and 10% considered nontechnical matters. Volume 37 was the second in which the number of papers dealing with non-dairy foods (48%) exceeded that of those concerned with dairy foods (41%). Volume 38 (1975) will probably be the largest that ever was published both in terms of total pages and number of research papers. Review papers continue to be an important part of the material published in the Journal. Changing of printers (from Franklin Press in Franklin, Indiana to Heuss Printing and Signs, Inc. in Ames, Iowa) was accomplished with no difficulty. Since the change was made, the Journal is in the hands of readers during the month that a given issue is dated.

(*h*) *Journal Management Committee.* Dr. R. B. Read, Jr., Chairman of the committee, presented the following recommendations: (1) appoint an assistant editor to work on the nontechnical content of the Journal, (2) establish sustaining members for IAMFES, (3) increase the size of page numbers and of letters in Journal indexing on the front cover, also reduce white space on pages where this is possible; (4) change the name of the Journal to *Journal of Food Protection,* (5) increase subscription rate to at least double that of the member rate, and (6) include schedule for meeting of this committee in the annual meeting Program.

Figure 3. *The Journal Management Committee discusses policies for the* Journal of Milk and Food Technology. *Left to right: Paul J. Pace, Dr. E. H. Marth, Dr. A. N. Myhr (back to camera), Dr. Brian McCarthy, and O. M. Osten (back to camera).*

(*i*) *Committee on Communicable Diseases Affecting Man.* According to Dr. F. Bryan, Chairman, the 3rd edition of *Procedure for the Investigation of Foodborne Diseases* should be published in 1976, perhaps by March. The third edition will be completely revised and updated. Approximately 90,000 copies of the first and second editions have been sold. The committee is considering a future companion manual on waterborne diseases.

(*j*) *Sanitarians Joint Council.* R. Belknap, the IAMFES representative to the Council, distributed copies of the completed proposed Model Act, Registration of Sanitarians. The Council for State Sanitarians Registration Agencies will endore the Model Act. The Council is now planning to develop a code of ethics for sanitarians. In 1976 the Council will meet in Nashville, Tennessee in conjunction with the annual Meeting of the National Environmental Health Association.

(*k*) *Representative to National Mastitis Council.* According to A. E. Parker, the National Mastitis Council will again meet in conjunction with the 1976 Annual Meeting of IAMFES in Arlington Heights, Illinois. The National Mastitis Council increased dues for individuals from $5 to $10 but failed to agree to change its name to the International Mastitis Council.

(*l*) *Committee on Food Equipment Sanitary Standards.* Karl Jones, Chairman, reported on activities of this committee. A prepared report is available for all persons at the meeting. The committee lost several members and is seeking to replace them.

(*m*) *Committee on Sanitary Procedures.* D. B. Whitehead reported that 12 standards for equipment were considered during the past year. Four of the standards dealt with egg processing equipment.

Action taken by the Executive Board include the following: (*a*) agreed that only one honorary life membership be given each year and that a Certificate of Recognition be awarded annually at the discretion of the board to all worthy candidates; (*b*) unless funds for two awards become available, beginning in 1976 the Educator-Industry award will go to a candidate from industry and that in succeeding years it be alternated between the two groups; (*c*) a certificate of appreciation will be prepared for H. Y. Heiskell to recognize his outstanding work as Chairman of the Membership Committee; (*d*) a budget committee will be formed, (*e*) a representative of IAMFES will be appointed to attend a National Conference on Sanitation Aspects of Food Facilities Planning, (*f*) any advertisements which reflect a policy contrary to that of IAMFES will not be accepted for the Journal of Milk and Food Technology; (*g*) the prize in the 1976 membership contest will be $100 for each affiliate with a net gain of 25 or more members, each winning state must send a representative to the Annual Meeting and to the Affiliate Council meeting, (*h*) the president of IAMFES will take steps so that the IAMFES representative to NSF need not vote on NSF standards since IAMFES does not want to be in a position of approving a standard which may not be completely acceptable, (*i*) subscription (non-member) rate for the Journal shall be increased to $32 per year; (*j*) Drs. E. H. Marth and K. G. Weckel are to prepare a statement on the proposed change in the name of the Journal; (*k*) sustaining membership was established and P. J. Skulborstad will work out details of a program for instituting sustaining memberships; and (*l*) funds received by IAMFES from the 1975 Annual Meeting will go into a separate travel fund.

The fall meeting of the Executive Board will be held November 19-21 at the Arlington Park Hilton, Arlington Heights, Illinois.

AFFILIATE COUNCIL

The 1975 Affiliate Council meeting was the best attended, liveliest, and most productive of any Affiliate Council meeting in the recent past. Attendance ranged between 30 and 40 during most of the session.

The Affiliate Council heard Mr. Robert O'Malley of Mutual of Omaha Insurance Company discuss a life insurance plan available to members of IAMFES. The Council requested that each Affiliate Secretary be provided with a packet of information on the insurance plan so this information can be provided to members at the annual meetings of the Affiliates.

H. Y. Heiskell, Chairman of the Membership Committee, reported on progress by his committee. He urged greater efforts by the Affiliates in securing new members. The Affiliate Council was enthusiastic about future membership contests. By unanimous vote the Council recommended that in the next contest: (*a*) a set number (25) net increase in members be used as the basis for winning, and (*b*) a set amount of money be given as the price rather than an air ticket.

Earl O. Wright discussed the need to broaden the base of membership in IAMFES. Efforts to gain members should be extended to students, food scientists and technologists, and to persons in allied fields. The Council voted to continue the policy of initiating new memberships either in January or July, depending on when the Affiliate has its annual meeting.

After a discussion of awards by W. F. Wilson, the Council voted unanimously to recommend that only a single honorary life membership be given annually and that an achievement award be created and given to deserving members.

The Journal of Milk and Food Technology was discussed by its Editor, Dr. E. H. Marth. He emphasized the need for more local or affiliate news and for news about and accomplishments of sanitarians in the field. Dr. R. B. Read, Jr., Chairman of the Journal Management Committee, stressed that non-research general interest papers are needed for the Journal.

Figure 4. *Two presidents meet, (left) Dr. F. O. Blackwell, president of the National Environmental Health Association, and P. J. Skulborstad, president of IAMFES.*

P. J. Skulborstad, IAMFES president, discussed plans for future cooperation with the National Environmental Health Association (see Presidential Address). The Council passed a motion which commended the long-range step-by-step plan outlined by Skulborstad. The Council also favored the expression "unification" over the term "merger."

Officers of the Affiliate Council for 1974-1975 were Erwin Gadd (Missouri), Chairman, and Howard Hutchings (South Dakota), Secretary. Officers for 1975-1976 are: Erwin Gadd, Chairman, and John W. Zook (Kansas), Secretary.

TECHNICAL SESSIONS

Research papers again made up a major part of the technical program at the Annual Meeting. This year 18 research papers were given on such topics as: relationship of results obtained with the Milk Quality Gauge to those of the Wisconsin Mastitis Test, survival

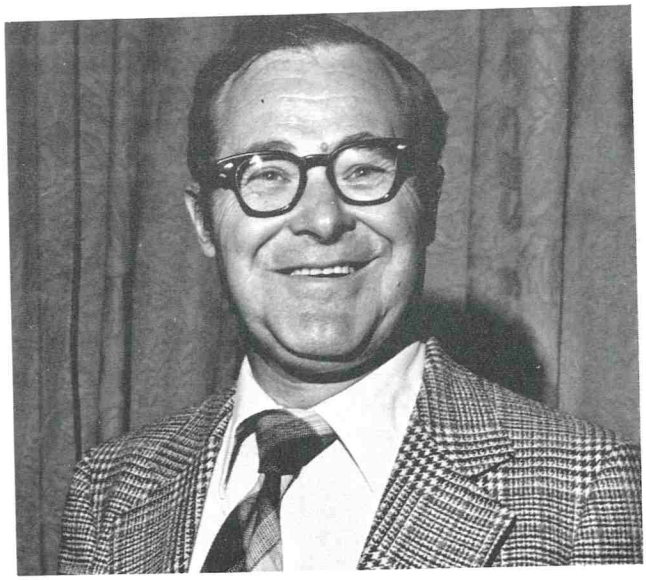


Figure 5. *The keynote speaker, Dr. A. B. Morrison, discussed food safety in the seventies.*

of foot-and-mouth disease virus in casein, heat inactivation of conidia from toxigenic aspergilli, levels of heavy metals in mushrooms, aflatoxin produced on smoked mullet, microbiology of the wild rice fermentation, sensitivity of guinea pigs to diets containing methyl mercury and selenium, persistence of enteric organisms in soil and on vegetables, normal flora of rock shrimp, efficacy of chemical cleaners and sanitizers, behavior of *Enterobacter* in skimmilk during the lactic fermentation, conditions for the Standard Plate Count of milk, rinse-filter method to determine sanitary condition of farm milk pipelines, microbiology of retail ground beef, food poisoning in Canada, feeding apple pomace to dairy cattle, removal of bisulfite from shrimp, and recovery from milk of compounds responsible for light-induced flavor.

Figure 6. *Speakers Dr. R. L. Bradley, Jr. (left) and Dr. R. R. Zall (center) with session chairman, H. E. Thompson (right).*

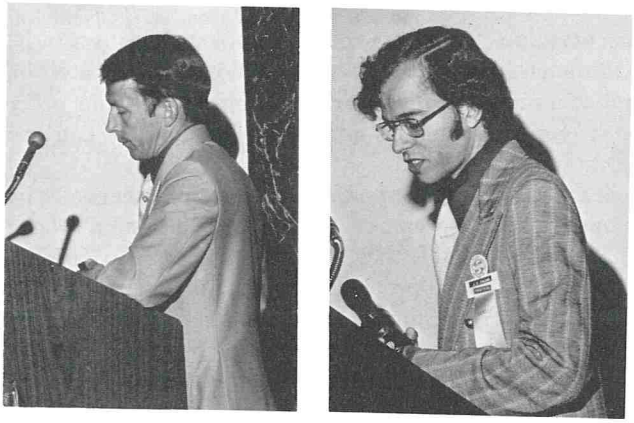


Figure 7. *Papers were given at the Annual Meeting by Dr. R. T. Marshall (left) and J. F. Frank (right).*

Figure 8. *Some of the papers at the Annual Meeting were given by (left to right): David E. Hartley, Dr. E. Todd, Ms. Margaret Cheney, Dr. John Koburger, and William F. Bower.*

Invited papers discussed such subjects as: methods to assess post-pasteurization contamination, the federal food service program, in defense of packaging, microbial standards for cheese, national (Canada) sanitation training program, fate of animal viruses in liquid farm wastes, bacterial quality of hamburger, trends in vending, Northeast Dairy Practices Council, microbial

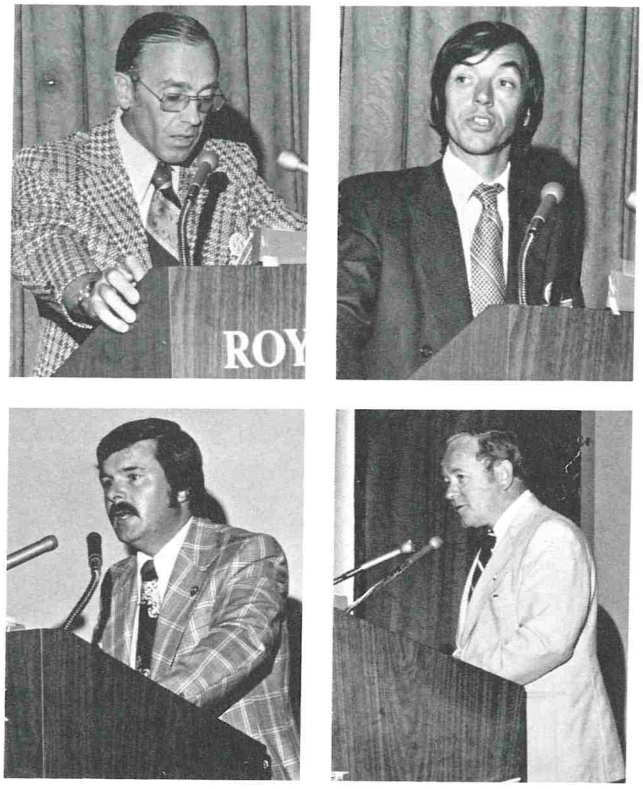


Figure 9. *Speakers at the Annual Meeting included Dr. D. H. Kleyn (top left), Dr. D. L. Collins-Thompson (top right), R. J. Davies (bottom left), and E. M. Biggs (bottom right).*

standards for meat, bulk milk transport washing, food production integrated into natural geochemical cycles, organization of environmental health programs, detergents and sanitizers as contaminants of milk, food protection in the seventies, water and waste management, federal regulations for Grade A milk and milk products, and nutrition in Canada.

Abstracts of all the research papers and most of the invited papers appeared on pages 634-639 of Volume 38 (October, 1975) of the *Journal of Milk and Food Technology*. Most papers given at the annual meeting will or have appeared in the *Journal of Milk and Food Technology*.

BUSINESS MEETING

President P. J. Skulborstad called the business meeting to order at 10:20 a.m. on August 13, 1975 with about 125 persons in attendance. Much of the business meeting was conducted with background music of bagpipes and drums as a result of a parade that passed by the Royal York Hotel.

Reports at the business meeting were given by E. O. Wright (Executive Secretary and Managing Editor), Dr. E. H. Marth (Editor, *Journal of Milk and Food Technology*), Dr. R. B. Read, Jr. (Journal Management Committee), Karl Jones (Food Equipment Sanitary Standards), Dr. F. L. Bryan (Communicable Diseases Affecting Man), Dale Termunde (Farm Methods), D. B. Whitehead (Sanitary Procedures), H. Y. Heiskell

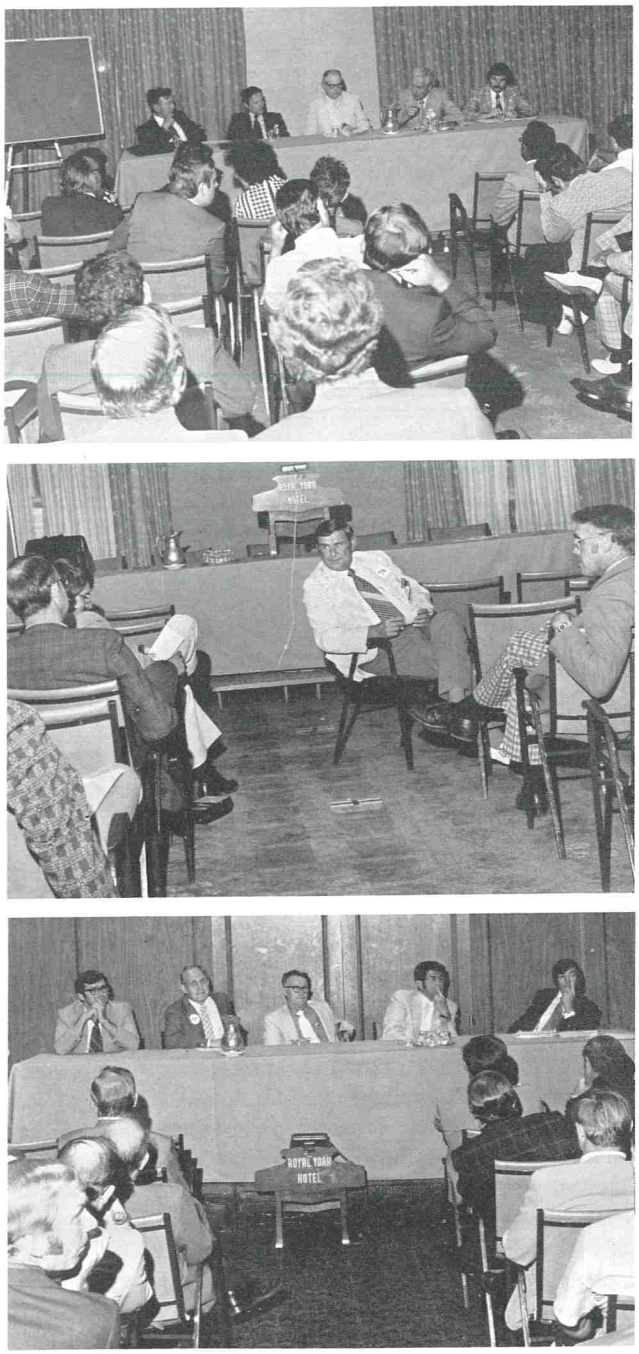


Figure 10. *The evening discussion groups continue to be a popular part of the Annual Meeting. Top, food sanitation discussion session; at the speakers' table are (left to right): Dr. H. Pivnick, Prof. C. L. Duitschaever, W. F. Bower, D. E. Hartley, and R. J. Davies. Center, W. F. Wilson leads the discussion on environmental sanitation. Bottom, milk sanitation session; at the speakers' table (left to right): Dr. R. L. Richter, Prof. R. P. March, H. E. Thompson, Dr. R. R. Zall, and Dr. D. L. Collins-Thompson.*

(Membership), R. Belknap (Sanitarians Joint Council), Harold Wainess (International Dairy Federation), and Erwin Gadd (Affiliate Council). Highlights of reports by these persons appear elsewhere in this discussion of the Annual Meeting and hence are not repeated here.

Dr. A. R. Brazis (Applied Laboratory Methods) reported that the subcommittee on laboratory methods

Figure 11. *Some of the reports at the business meeting were given by (back row, left to right): E. O. Wright, E. Gadd, O. M. Osten, K. Jones, D. B. Whitehead, Dr. R. B. Read, Jr., Dr. E. H. Marth; (front row, left to right): D. Termunde, H. Y. Heiskell, Prof. R. P. March, Dr. A. R. Brazis, and R. Belknap.*

to examine foods has been reactivated with C. N. Huhtanen as Chairman. Gene Ronald is chairman of the subcommittee on methods to examine water and other environmental samples and this subcommittee can use additional members.

The Committee on Food Protection, headed by Charles Felix, prepared resolutions on solid waste management guidelines for containers, a national conference on food protection, and training programs for food service personnel. The complete text of the resolutions appears at the end of this report.

Felix also reported on the Keep America Beautiful National Advisory Council. He recommended that the president of IAMFES be the representative to the 1976 meeting of this Council.

Harold Wainess, reporting for the Committee on Baking Industry Equipment, indicated that Vincent Foley will not continue on this committee and that new committee members are needed. Wainess stated that 56 manufacturers are now making equipment that complies with BISSC standards but that other manufacturers produce equipment that does not meet the standards. Copies of BISSC standards are available from Ray Walter, Executive Secretary, BISSC, 521 Fifth Avenue, New York, N.Y. 10017.

According to Dr. K. G. Weckel, who reported for the 3-A Symbol Council, a 3-A Sanitary Standards number will be required on all approved equipment unless the equipment is too small to space for the number is not available. As of July 31, 1975 there were 159 3-A authorizations in effect. The Council is making provisions to accept applications from foreign companies.

R. P. March moved that Article 4, Section I of the Constitution be modified to permit the Secretary-Treasurer to advance through the Executive Board and thus hold each office. The motion was seconded and carried with one dissenting vote. March then moved that the words "Secretary-Treasurer" in Article 7, Section I be replaced with "Executive-Secretary." The motion was seconded and carried unanimously. Finally, March

moved that Article 2, Section B of the by-laws be changed to transfer certain responsibilities from the Secretary-Treasurer to the Executive-Secretary. This motion was seconded and carried unanimously. Details of these constitutional changes appear in the January and May issues of Volume 38 (1975) of the *Journal of Milk and Food Technology.*

O. M. Osten, Chairman of the Resolutions Committee, presented the following resolutions that were adopted by the membership.

RESOLUTION NO. 1

CONCERNING: The U.S. Environmental Protection Agency "Solid Waste Management Guidelines for Beverage Containers"

WHEREAS: Single service containers were introduced and required as a public health measure and,

WHEREAS: Single-use containers have improved sanitation in the handling of many foods and beverages and,

WHEREAS: The introduction of single service containers for food and beverages has reduced the infestation by insects and rodents of the food service environment in public buildings, and

WHEREAS: Food and beverage plants have improved their sanitation through the use of single-use food and beverage containers and,

WHEREAS: The elimination of multi-use containers has reduced the contamination of the food and beverage plant effluent by toxic chemicals, through elimination of the cleaning and sanitizing process and,

WHEREAS: The danger from glass breakage to both employee and consumer has been eliminated by the use of single-use beverage containers and,

WHEREAS: The U.S. Environmental Protection Agency "Solid Waste Management Guidelines for Beverage Containers" proposes to eliminate the use of single service containers and cup vending machines in Federal installations,

NOW THEREFORE BE IT RESOLVED: That the International Association of Milk, Food, and Environmental Sanitarians views these Guidelines as a regressive step in food, beverage, and environmental sanitation and,

BE IT FURTHER RESOLVED: That a copy of this Resolution be forwarded to Mr. Russell Train, Administrator of the U.S. Environmental Protection Agency, and to the Hearing Officer following publication of the proposed EPA Guidelines in the Federal Register.

RESOLUTION NO. 2

CONCERNING: The proposed 1976 National Conference on Food Protection

WHEREAS: The 1971 National Conference on Food Protection inaugurated a new concept in food protection in laying the groundwork for cooperation between governmental agencies, industry, consumer groups, and educational institutions and,

WHEREAS: Five years have elapsed since the first national conference was held and,

WHEREAS: Many of the recommendations of the conference have not as yet been implemented and,

WHEREAS: The conference was limited primarily to microbiological hazards, additional areas of food protection now need to be considered,

NOW THEREFORE BE IT RESOLVED: That the International Association of Milk, Food, and Environmental Sanitarians, a sponsor of the first national conference, recommends that another national conference be held in 1976 and,

BE IT FURTHER RESOLVED: That a copy of this Resolution be sent to the Executive Secretary of the American Public Health Association and to the Chairman of the Section on Environment of APHA.

RESOLUTION NO. 3

CONCERNING: Training programs for food service personnel

WHEREAS: The food service worker plays an important role in the protection of the consumer and,

WHEREAS: Management has the major role in training and supervising food service workers in safe food handling practices and,

WHEREAS: Management can only fulfill its responsibilities in promoting and overseeing food handling practices if it has the necessary understanding of correct procedures and the reasons for them and,

WHEREAS: Many cities and states are instituting manager, operator certification and self inspection programs and,

WHEREAS: The International Association of Milk, Food, and Environmental Sanitarians has a primary interest in the highest quality of food protection and,

WHEREAS: The consumer will ultimately benefit from improved food service handling practices,

NOW THEREFORE BE IT RESOLVED: that the International Association of Milk, Food, and Environmental Sanitarians support the concept that a national uniform training program be developed through the cooperative efforts of the food service industry, and regulatory agencies for implementation by the food service industry to improve and unify food handling practices and further protect the consumer, and to provide reciprocity among all programs and,

BE IT FURTHER RESOLVED: That a copy of this Resolution be sent to Dr. Alexander Schmidt, Commissioner of the U.S. Food and Drug Administration.

RESOLUTION NO. 4

CONCERNING: Farm Methods Committee Report for 1973-75 as accepted by the International Association of Milk, Food, and Environmental Sanitarians, 1975

WHEREAS: The 1973-75 Farm Methods Committee report as accepted by the International Association of Milk, Food, and Environmental Sanitarians 1975 makes a number of recommendations concerning:

1. Antibiotics, pesticides and other adulterants
2. Cleaning and sanitizing of farm milk equipment
3. Plastic use on farms in equipment
4. Testing for cleanliness of milk production
5. CIP cleaning of milker units in parlors and milking barns
6. Cleaning and sanitizing of farm milk pick-up tanks, milk transports, silo storage and cooling tanks, and storage tanks
7. Sampling of milk in transport tanks, storage tanks, and farm milk pick-up tankers
8. Water treatment and water supply protection
9. Animal waste management
10. Uniform and informative teat dip labeling

and,

WHEREAS: These recommendations could be used as guidance and direction in revising or updating the 1965 Pasteurized Milk Ordinance and Code, and,

WHEREAS: The Secretary of U.S. Department of Health, Education, and Welfare and the Commissioner of Food and Drug Administration have requested recommendation on the update or revision of the 1965 Pasteurized Milk Ordinance for the United States,

THEREFORE BE IT RESOLVED: That the International Association of Milk, Food, and Environmental Sanitarians request the Commissioner of Food and Drugs consider the 1973-75 Farm Methods Committee report as a recommendation and guide in updating or revising the 1965 Pasteurized Milk Ordinance, and that a copy of the Report be sent to the Commissioner of Food and Drug Administration.

RESOLUTION NO. 5

Harold Heiskell has served the International Association of Milk, Food, and Environmental Sanitarians in a distinguished manner for four years as National Membership Chairman and,

WHEREAS: During those years, the work of Harold Heiskell and his committee has substantially increased the membership of the Association and,

WHEREAS: This Membership committee Chairman was a crowning example of 38 years of dedicated service to our Association,

NOW THEREFORE BE IT RESOLVED: that the International Association of Milk, Food, and Environmental Sanitarians bestow upon Harold Heiskell its deep appreciation and highest honors for his dedicated services.

RESOLUTION NO. 6

WHEREAS: The Ontario Milk and Food Sanitarians Association and the University of Guelph Department of food Science have again displayed the Canadian hospitality at the second annual meeting to be held in Toronto as hosts of the 1975 annual meeting of the International Association of Milk, Food, and Environmental Sanitarians and,

WHEREAS: All needed facilities for the successful meeting were aniticpated and supplied by the Ontario Association and the University of Guelph Department of Food Science and,

WHEREAS: Excellent coordination among the Industry, Educational, and Regulatory members of the Ontario Association was accomplished within the highest standard of the International Association of Milk, Food, and Environmental Sanitarians and,

WHEREAS: The 1975 meeting was an outstanding success in attendance, accomplishment, and fellowship of members,

THEREFORE BE IT RESOLVED: That the International Association of Milk, Food, and Environmental Sanitarians adopt the resolution of gratitude to the Ontario Association and further that an appropriate copy of the resolution be sent to the Ontario Association as well as being published in the *Journal of Milk and Food Technology.*

RESOLUTION NO. 7

WHEREAS: The Royal York Hotel of Toronto, Ontario, was the site of the 1975 International Association of Milk, Food, and Environmental Sanitarians annual meeting, and

WHEREAS: The facilities for the membership's use and personal comfort were outstanding, and

WHEREAS: The personnel of the Royal York Hotel did extend themselves individually to provide for all our needs,

THEREFORE, BE IT RESOLVED: That an appropriate expression of gratitude be sent to the Toronto Royal York Hotel.

Figure 12. *P. J. Skulborstad (right) installs Howard Hutchings (center) as second vice-president and Professor R. P. March (left) as secretary treasurer.*

Figure 13. *Members of the Illinois Affiliate are promoting the 63 Annual Meeting of IAMFES which is scheduled for August 8-11, 1976 at the Arlington Heights Hilton Hotel, Arlington Heights, Illinois. Left to right: John Dean, Dale Termunde, Charles Price, and Robert Kroslak.*

Figure 14. *H. E. Thompson (left), new president of IAMFES, receives the gavel from Charles Felix, editor of* Environmental News Digest. *P. J. Skulborstad is in the foreground.*

Figure 15. *P. J. Skulborstad (left) receives the Past-President's Award from H. E. Thompson.*

Figure 16. *Dr. C. K. Johns served as president of IAMFES in 1934-1935. He received a "birthday" cake to commemorate the 40th anniversary of his presidency.*

Figure 17. *Dr. E. H. Marth (standing) addresses a luncheon meeting of the Milking Machine Manufacturers' Council.*

Figure 18. *Some views at the annual awards banquet.*

Figure 19. *Ladies attending the meeting enjoyed visiting in the Ladies Hospitality Room.*

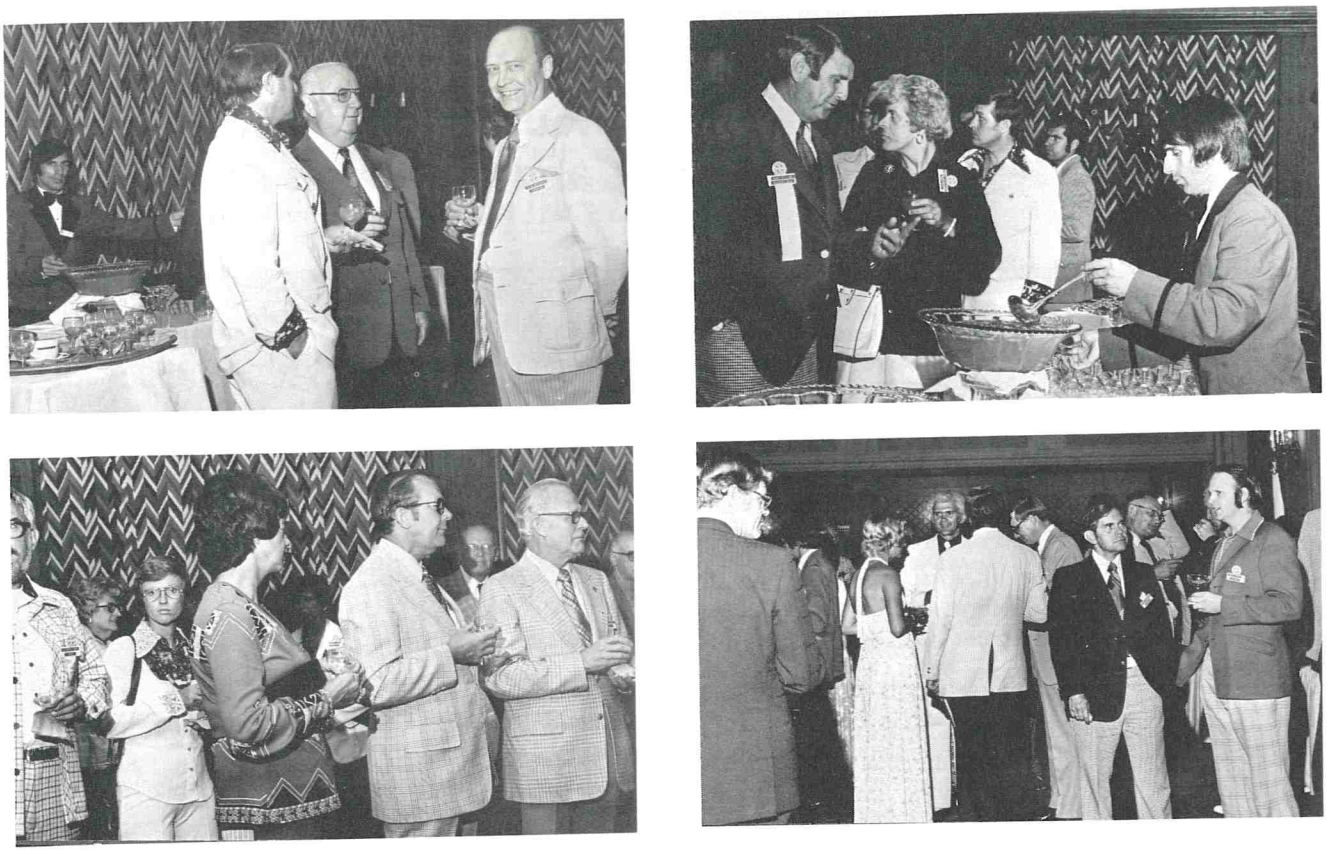


Figure 20. *Sociability at the receptions that were attended by many of the registrants at the 62nd Annual Meeting of IAMFES.*

J. Milk Food Technol. Vol. 38, No. 12, Pages 796-799 (December, 1975)

IAMFES Recognizes Meritorious Achievements by Brazis, Parker, Rich, Weckel, and the Illinois Affiliate

Each year IAMFES honors several members and one affiliate organization for meritorious service. Honors given to individual members are in the form of four awards designated as the Citation Award, Honorary Life Membership, Sanitarians Award, and Educator-Industry Award. The Shogren Award is given annually to the affiliate organization with the most outstanding program during the past year. Recipients of awards are selected by the Committee on Awards and Recogniztion.

The citation Award is given annually to a member of IAMFES who has contributed substantially to the growth, advancement, and status of the Association. A member at or near retirement who has contributed substantially to the programs of IAMFES may be selected as an Honorary Life Member.

The Sanitarians Award, which consists of a plaque and $1,000, is given annually to a member of IAMFES who has done outstanding work in public health during the preceding 7 years. Funds for this award are provided jointly by the Pennwalt Corporation, Economics Laboratory, and the Diversey Corporation.

The Educator-Industry Award was instituted in 1973 to recognize major contributions to food safety and hygiene by an IAMFES member in industrial or academic work. This award consists of a plaque and $1,000, and is funded by the Milking Machine Manufacturers Council of the Farm and Industrial Equipment Institute.

CITATION AWARD TO DR. A. RICHARD BRAZIS

Dr. A. R. Brazis was born in Bridgeport, Connecticut and lived there until he graduated from Norwich University in Northfield, Vermont in 1949. He received the M.S. and the Ph.D. degrees from the University of Missouri in 1951 and 1954, respectively. He was an Assistant Instructor in the Dairy Husbandry Department at the University of Missouri between 1952 and 1954. During World War II, Brazis served as a non-commissioned officer in a tank company in the First Cavalry Division of the U.S. Army. Following return to civilian status in 1946, Dick returned to Norwich University to receive his B.S. degree. During his last two years at the University of Missouri, Brazis was commissioned into the inactive reserve of the Commissioned Corps, United States Public Health Service. Following receipt of his doctorate from the University of Missouri in 1954, he was placed on active duty with the U.S. Public Health Service Commission Corps. Dick served as a microbiologist with the Water Supply and Pollution Control Program at the Robert A.

Figure 1. *W. F. Wilson (left) presents the Citation Award to Dr. A. R. Brazis.*

Taft Sanitary Engineering Center in Cincinnati, Ohio from 1954 until 1959 when he transferred to the Milk Sanitation Research Unit in the Milk and Food Research Section at the same Center. From 1959 to 1965, Dr. Brazis conducted research and in addition assisted in the PHS program for approval of State and territorial laboratories as well as standardization and certification of State and territorial laboratory survey programs. From 1966 to date, Brazis has been actively engaged in coordination FDA-District-State Milk and Food Laboratory Approval Programs. He currently is Chief, Laboratory Development Section, Division of Microbiology (FDA).

Dr. Brazis is a registered microbiologist of the American Board of Microbiology. He received the commendation medal from the Public Health Service for exemplary performance of duty in 1963. He is Chairman of the Applied Laboratory Methods Committee of the International Association of Milk, Food, and Environmental Sanitarians; has served as Chairman of the Subcommittee on Screening Tests of the National Mastitis Council; has served as a member of the Standard Methods Subcommittee on Coliform Bacteria, American Public Health Association; and is a member of the National Mastitis Council Research Committee. In addition to membership in several other honorary and professional societies, he has also participated in several committees that are working on standardization of

procedues for examination of milk and food.

During Brazis' tenure as Chairman of the Applied Laboratory Committee, the committee has been particularly active and has studied analytical problems as they relate to the microbiological analyses of milk and milk products. These studies have resulted in several papers that were published in the *Journal of Milk and Food Technology.*

HONORARY LIFE MEMBER—ARTHUR E. PARKER

Arthur E. Parker, recipient of the 1975 Honorary Life Member Award, was chief of the Milk Sanitation Section in the Multnomah County (city of Portland) Health Department until his recent retirement. Art is a Registered Sanitarian in the State of Oregon. Before Parker became involved with regulatory contol of the milk supply, he held various positions in different segments of the dairy industry. Art also served in the U.S. Navy where he gained experience in vessel boiler operations.

Parker is a long-time member of IAMFES and has served with distinction on the Dairy Farm Methods Committee. He co-authored "Methods for Production of High-Quality Raw Milk" which was published by IAMFES in 1972. This book was an outgrowth of annual reports that were prepared for 15 years by the Dairy Farm Methods Committee. Art also has served for several years as the representative of IAMFES to the National Mastitis Council. Although officially retired, Art continues to provide IAMFES with his insights and expertise in the field of milk sanitation.

Parker also has been active in the Oregon Association of Milk, Food, and Environmental Sanitarians. He was a charter member of the Oregon Affiliate and also served on its board of directors, and as its president, vice-president, and representative to the IAMFES Affiliate Council.

Art's meritorious work also has been recognized by the folks in Oregon. He received commendation awards from the mayor of Portland and from Multnomah County.

SANITARIANS AWARD TO SAMUEL C. RICH

Samuel C. Rich is Program Manager and Sanitarian Milk Specialist Supervisor in the Environmental Health Division of the Mecklenburg County Health Department, North Carolina. He received the Sanitarians Award, in part, for his efforts to insure safe food in food service establishments and a safe milk supply in Mecklenburg County.

Rich has devoted his entire professional career of over 40 years to milk and food hygiene. During that time he held the following positions: 1934-1937, sanitarian, Beauford County Health Department, Washington, N.C.; 1937-1941, district sanitarian, North Carolina State Board of Health, Raleigh; 1941, field representative for Coble Dairy Products, Lexington, N.C.; 1941-1945, U.S. Army Sanitary Corps Medical Department, 1946-present, with the Mecklenburg County Health Department.

Figure 2. *W. F. Wilson (left) presents the Sanitarians Award to Samuel Rich.*

As Program Manager for the Consumer Services Section of the Mecklenburg County Health Department, Rich was responsible for organizing the Section and continues to be responsible for making it work. He provides professional and technical supervision for the following programs: milk quality control and sanitation; food, lodging, and the institutional sanitation; rodent control; solid waste and vector control; recreational sanitation; public water supply; on-premises waste disposal and water supply; soil science consultation; and pesticide and hazardous chemical control. Rich prepared local codes for all of the programs just listed and was instrumental in getting the codes adopted in the county.

Sam has been particularly active in milk sanitation. He implemented several programs that were coordinated with and became lead programs in state-wide interagency cooperative efforts. These programs include: (*a*) elimination of pesticides from the milk supply, (*b*) developing a training program for newly employed milk sanitarians and newly appointed milk sanitation supervisors, (*c*) evaluating newly developed equipment and procedures for milk sanitation and on the basis of such results provide guidance to agencies in other counties and to the State, and (*d*) prevention and control of mastitis (and abnormal milk).

In the program to control abnormal milk, Rich enlisted the aid of North Carolina State University to do somatic cell counts on bulk tank milk and he established the first regression equation for converting results of the Wisconsin Mastitis Test to valid estimates of numbers of somatic cells. Sam also promoted educational efforts by fieldmen, sanitarians, and university personnel so that concerned persons were informed about the importance of somatic cells in milk and how the presence of excessive numbers could be controlled.

Mr. Rich has served as the executive committee of the Interstate Milk Shipments Conference and program

planning committees for the local dairy technology society and for the annual fieldmen and sanitarians' conference at North Carolina State University.

Because of the success of his programs and his willingness to help others, Samuel C. Rich has earned the unofficial status as "counselor and advisor" to sanitarians and other health officials throughout North Carolina.

EDUCATOR-INDUSTRY AWARD TO DR. K. G. WECKEL

Dr. Kenneth G. Weckel was born in Stark County, Ohio and "grew-up" in a small milk business in Canton, Ohio. He attended the University of Wisconsin where he majored in Dairy Industry and received the B.S., M.S., and Ph.D. degrees in 1931, 1932, and 1935, respectively. Some of Weckel's training also was in Biochemistry and Bacteriology. Upon receiving the Ph.D. degree, Weckel joined the faculty of the Department of Dairy Industry at the University of Wisconsin as an Assistant Professor. He became Associate Professor in 1941 and Professor in 1945. At present Dr. Weckel is Professor of Food Science and Associate Director of the University-Industry Research Program at the University of Wisconsin-Madison.

Figure 3. *Dr. K. G. Weckel (left) receives the Educator-Industry Award from W. F. Wilson.*

Weckel has served on many committees of IAMFES and currently is a member of the Journal Management Committee. Ken also was president of IAMFES in 1951 and of the Wisconsin Affiliate in 1945 and 1947. Professor Weckel is Chairman of the 3-A Symbol Administration Council and has been the IAMFES representative to that group since its inception. He is a Registered Sanitarian in the State of Wisconsin.

At the University of Wisconsin Weckel has taught various courses in the Department of Food Science. Among these are courses in food technology, food sanitation, and food packaging. For the past 13 years, Dr. Weckel has supervised the Candy Technology Short Course given annually at the University of Wisconsin.

During his professional career of over 40 years, Ken has done research on food protection, food technology, and nutritional fortification of milk and dairy products. Recently he received the Governor's Award (State of Wisconsin) for developing a sterile processed food product that can be administered to patients via a gastric tube.

Weckel also has served in an advisory capacity to various organizations related to the dairy, fruit, vegetable, confectionery, and refrigerated warehouse industries. During the past 3 years, Dr. Weckel has worked with governmental agencies and laboratories in Central and South America. His work with these groups has involved surveying existing facilities for processing of food.

Additional details about Dr. Weckel's professional achievements can be found on page 585 of Volume 37 (1974) of the *Journal of Milk and Food Technology.* They appeared at that time because Professor Weckel received the Honorary Life Member Award from IAMFES in 1974.

SHOGREN AWARD TO ILLINOIS AFFILIATE

The Shogren Award was developed by the Affiliate Council of IAMFES to annually recognize the affiliate organization with the most outstanding program. A questionnaire is sent annually to the secretary of each affiliate organization. Completion of the questionnaire serves to enter the organization in the competition and provides the information used by the Committee on Recognition and Awards to select the winner. This year the award went to the Associated Illinois Milk, Food, and Environmental Sanitarians. Mr. Robert A. Coe, Secretary, accepted the award.

Figure 4. *W. F. Wilson (left) presents the Shogren Award to R. W. Coe as the representative of the Illinois Affiliate.*

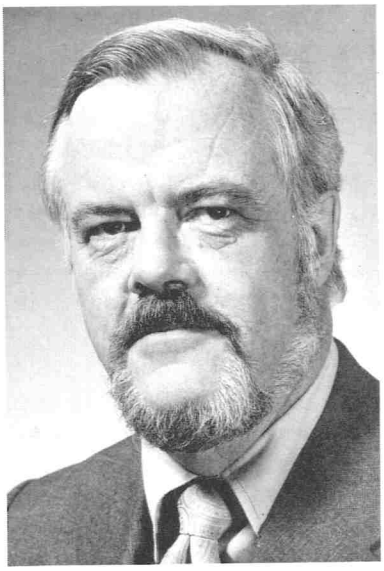
Major factors considered by the committee in selecting the affiliate organization to receive this award include: number of members, activities of the affiliate during the year, percentage of members that also belong to IAMFES, number of papers authored by members that were published in the *Journal of Milk and Food Technology,* number of members that attend the annual meeting of IAMFES, and preparation and circulation of a newsletter.

Association Affairs

NOMINATION FOR OFFICE OF IAMFES INC. 1976-1977

(Notice to membership—ballots will be mailed to paid members as of December 31, 1975)

For Secretary-Treasurer



K. G. Savage

K. G. Savage is director, Dairy Division, Canada Department of Agriculture. In this position his work encompases control of corporation weight, labelling and grade identification of domestically produced, exported and imported manufactured dairy products. He is also responsible for the inspection and registration of dairy plants, liason with provincial governments, federal departments and producer and processor organizations.

Mr. Savage has a B.S. from University of Manitoba. He earned his M.S. at University of Manitoba in 1953, and has a diploma in public administration at the University of British Columbia in 1963.

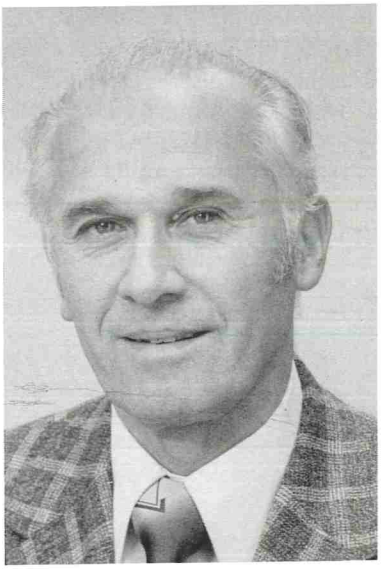
Between university sessions Mr. Savage has been employed in a variety of dairy plants in Canada in both and production capacities.

He joined the British Columbia Department of Agriculture in 1951 as a dairy inspector located in Vancouver, British Columbia, Canada. He was appointed Dairy Commissioner, of that department in 1961. Mr. Savage became closely involved in integration of dairy legislation and administration of regulatory service and extension activities related to dairying. He also developed correspondence short courses for dairy plant personnel; central bacteriological and chemical laboratory; Infra-Red Milk Analysis program for milk recording and producer payment.

Mr. Savage is past Chairman Canadian National Committee of the International Dairy Federation and present Executive Committee member of the International Dairy Federation. Mr. Savage is also Chairman Provincial-Federal Dairy Committee, and Chairman of the National Milk Quality Steering Committee of Canada.

He holds membership in International Association of Milk, Food and Environmental Sanitarians; Agricultural Institute of Canada; Canadian Institute of Food Technology; Institute of Public Administration of Canada.

Mr. Savage was born in Winnipeg, Manitoba, Canada on April 1, 1926. He is married and has two daughters age 24 and 14 years.



William Kempa

William Kempa was born and raised on a dairy farm in Chatfield, Manitoba. Following graduation from Teulon High School in 1935, he worked for three years in a local creamery during the summer months and in a local general store during the winter months. In 1938 he

enrolled at the University of Manitoba and was granted a B.S.A. Degree in Dairy Science in 1942.

Later that year he also completed a course in Public Health Inspection and received a Certificate in Sanitary Inspection (Canada) from the Canadian Public Health Association.

After joining The Royal Canadian Army Medical Corps in October 1942 he qualified and worked as hospital laboratory technician. Two years later he transferred to the Infantry Corps for overseas duties. In Great Britain he was assigned to the Ambulance Corps until the end of hostilities.

On return to Canada in May 1946 he accepted the position of Senior Dairy & Milk Inspector with the City of of Regina Health Department, Regina, Saskatchewan, a position he held until 1967. On September 1, 1967 he accepted a new challenge as director and in April 1971 as the first Chairman of the newly established Public Health Inspection Department at the Ryerson Polytechnical Institute, in Toronto, Ontario. Since September 1975, his efforts have been directed to teaching duties in areas of milk and food sanitation, institutional sanitation, occupational health, accident prevention, alcoholism, drug abuse and excessive smoking.

His education was resumed in 1951 at the University of Minnesota School of Public Health from which institution he obtained a Master of Public Health Degree.

In 1959 Bill was the first Canadian to receive the Sanitarian of the Year Award from the International Association of Milk and Food Sanitarians, Inc. at the 46th Annual Meeting held in Glenwood Springs, Colorado.

He has been a regular attender at the Annual Meetings of the I.A.M.F.E.S., having attended all but seven meetings since 1953, and missed only one since 1963. In 1975 he was General Chairman of the Local Arrangements Committees, for the 62nd Annual Meeting of the I.A.M.F.E.S. held in Toronto, and is currently President of the Ontario Milk and Food Sanitarians Association.

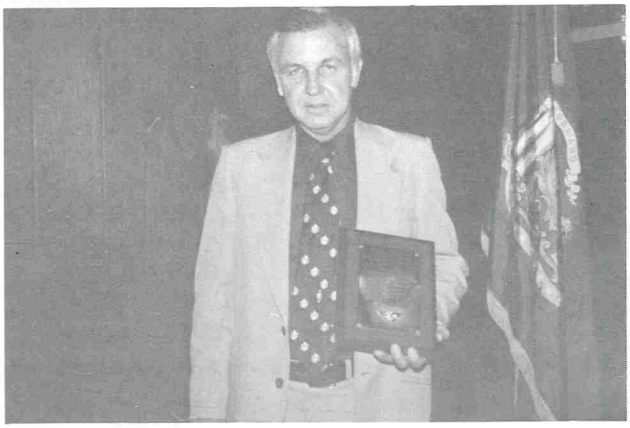
Keenly interested in the promotion of environmental health, he is a member of the Canadian Institute of Public Health Inspectors, and the Canadian Public Health Association. He was President of the Saskatchewan Branch, C.P.H.A. in 1963.

Bill lives with his wife Naida, R.N. in Mississauga, Ontario. They have four children, Linda, age 22, who is away for post graduate studies in Scotland, Bryan 20, Rodney 17, and Delwin 14. His hobbies are camping, gardening, bowling, photography and coin and stamp collecting.

Wisconsin Educational Conference

The 31st Annual Educational Conference of the Wisconsin Association of Milk and Food Sanitarians was held on September 25-26, 1975, at the Holiday Inn at Tomah, Wisconsin. This conference was held in conjunction with the 12th annual meeting of the Wisconsin Association of Dairy Plant Fieldmen and it was the first year that the Institute of Food Technology met with us. Approximately 200 were in attendance during the two day meeting.

First Row L. to R. Robert Cassens, President Elect IFT; Robert Lindsay, President IFT; John Shoup, Sec. Treas. IFT; Elmer Marth, President WAMFS. Second Row: Leonard Rudie, 1st Vice Pres. WAMFS; Lyle Cuff, President WADPF; Earl Brancel, Sec. Treas. WADPF. Third Row: Clifford Mack, Pres. Elect WAMFS; Don Raffel, Sec. Treas WAMFS; Ward Peterson, Past Pres. WAMFS.



The Wisconsin Association of Milk and Food Sanitarians presented their annual Sanitarian of the Year Award to Mr. Paul Pace, Microbiologist with the City of Milwaukee. Mr. Pace has been with the city since 1952. Among his many accomplishments has been the study of occurrence of *clostridium botulinum* in whitefish from Lake Michigan that were to be used for the production of smoked fish. He has contributed to and coordinated a program for evaluating the Microbiological quality of certain delicatessen salads. He is involved in many professional organizations.

News and Events

Fall Meeting—Montreal Contest Winners

Ohio State University narrowly emerged as winner in the 54th Intercollegiate Dairy Products Evaluation Contest in Montreal, Canada, on Oct. 21. Strong challenges from Oregon State University, the Universities of Minnesota and Missouri and South Dakota State University provided spirited competition. Student judges from each of the five universitites finished first in the individual products, winning wrist watches for their efforts.

David Dzurec of Ohio State University was the top All-Products judge followed by Peggy Matthews of the University of Missouri and Rick Keane of the University of Minnesota.

The Master of Ceremonies at the Awards Breakfast, Arthur W. Nesbitt, introduced all coaches and teams, announced all winners and at the conclusion of the program presented the Dairy Remembrance Award of $100 to David Dzurec of Ohio State.

John R. Jackson, president of the National Dairy Council of Canada, delivered an interesting address. Jackson, who graduated from Iowa State University in the late '40s and judged on the Iowa State team, traced the provincial differences in Canada and noted that opportunities abound for careers in the food field. He outlined the preparations for the Summer Olympic Games in Montreal in 1976 and cordially invited attendance.

Harold E. Meister, contest superintendent, presented the Coach-of-the Year award to Professor Walter Slatter of Ohio State.

By winning the Butter Cup for the third successive year South Dakota State University permanently retired the cup and a new one will be placed in competition in 1976.

National Conference on Interstate Milk Shipments will Re-Convene Conference

Chairman H. H. Vaux and the Executive Board have voted to re-convene the non-terminally adjourned Conference at the Chase-Park Plaza Hotel, St. Louis, Missouri, extending January 18-22, 1976.

The tentative program calls for full Council deliberations to begin at 2:00 p.m. on Sunday afternoon, January 18. Council meetings will continue all day on both Monday and Tuesday, and on Wednesday morning. Wednesday afternoon will be used to have the Council reports printed and ready for the business session on Thursday morning. A brief general session will be held on Tuesday morning, beginning at 9:00 a.m.

Since this will be a re-convened Conference the official delegates will be the same as last spring—unless the Executive Secretary hears to the contrary.

The main order of business for the Council sessions will be the discussion of the proposed changes in the Pasteurized Milk Ordinance. Anyone wishing to register comments regarding any of the proposed changes before the meeting in St. Louis must send his/her comments to John Speer, Program Chairman, before December 1, 1975. He will then assign the comments to the proper Council for consideration. Mr. Speer's address is: c/o Milk Industry Foundation, 910-17th St., N.W., Washington, D.C. 20006.

Erratum

3-A Sanitary Standards for Farm Milk Cooling and Holding Tanks

Number 13-06

Published in Journal of Milk and Food Technology
Vol. 38, No. 10, October 1975

D.
FABRICATION

D.5

Gaskets shall be removed. Any gasket groove or gasket retaining groove shall not exceed ¼ inch in depth or be less than ¼ inch wide except those for standard O-Rings smaller than ¼ inch.

Journal of Milk and Food Technology
Index to Volume 38

AUTHOR INDEX

CONTENTS, VOLUME 38

January (No. 1)

February (No. 2)

March (No. 3)

April (No. 4)

May (No. 5)

June (No. 6)

July (No. 7)

August (No. 8)

September (No. 9)

October (No. 10)

November (No. 11)

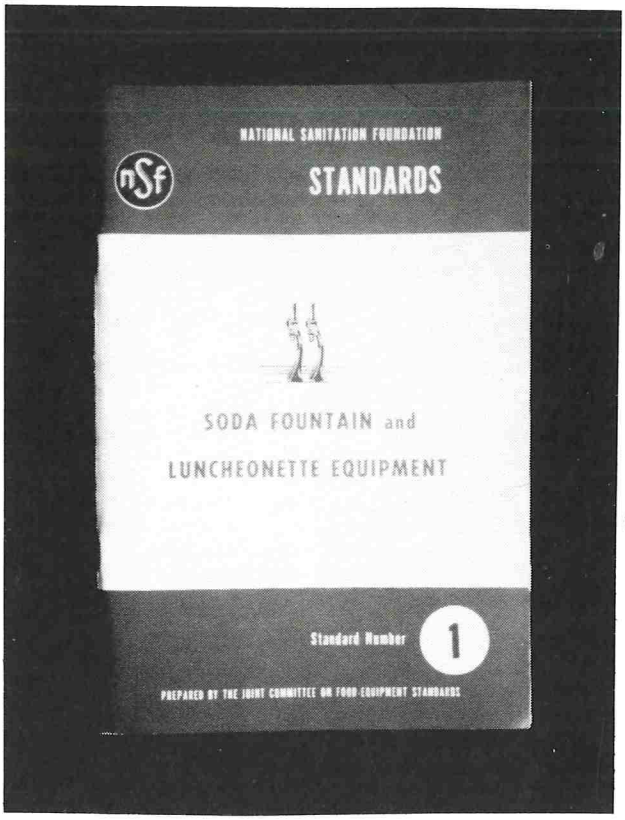
December (No. 12)

SUBJECT INDEX

A Landmark in food service sanitation— NSF STANDARD 1

The advent of this standard for soda fountain and luncheonette equipment in 1952 gave health professionals a powerful new weapon for cleanliness in public eating places. It was now possible to identify an item of food service equipment in terms of adherence to third party standards of sanitation. Health officers and their staffs could accept a unit of NSF-marked soda fountain and luncheonette equipment with the assurance that:

- Its performance fulfilled the requirements of a sanitation standard that had been written with the active participation of outstanding public health officials
- It had been examined and tested in an impartial testing laboratory for adherence to the standard
- Its production was subject to unannounced factory visits by NSF field personnel and the random selection of any completed unit for testing by NSF against the standard.



That's how it was in 1952 with Standard 1 and that's how it is today with 18 NSF standards for food service equipment. This is why such a high percentage of city, county, district and state health jurisdictions strongly support NSF standards.

NSF

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

Government officials and professional workers concerned with public health and environmental quality are invited to write for the NSF Publications List. It is free. It lists all NSF standards and criteria as well as listings, reports and NSF literature.

THE ONLY *Approved*
SANITARY METHOD OF APPLYING
A U. S. P. LUBRICANT
TO DAIRY & FOOD
PROCESSING EQUIPMENT

Haynes-Spray

U.S.P. LIQUID PETROLATUM SPRAY

U.S.P. UNITED STATES PHARMACEUTICAL STANDARDS

CONTAINS NO ANIMAL OR VEGETABLE FATS. ABSOLUTELY NEUTRAL. WILL NOT TURN RANCID—CONTAMINATE OR TAINT WHEN IN CONTACT WITH FOOD PRODUCTS.

SANITARY—PURE
ODORLESS—TASTELESS
NON-TOXIC

This Fine Mist-like **HAYNES-SPRAY** *should be used to lubricate:*

SANITARY VALVES
HOMOGENIZER PISTONS — RINGS
SANITARY SEALS & PARTS
CAPPER SLIDES & PARTS
POSITIVE PUMP PARIS
GLASS & PAPER FILLING
MACHINE PARTS
and for ALL OTHER SANITARY
MACHINE PARTS which are
cleaned daily.

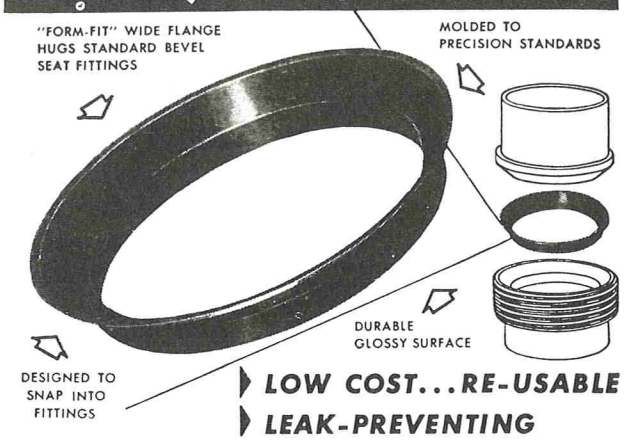
The Modern HAYNES-SPRAY Method of Lubrication Conforms with the Milk Ordinance and Code Recommended by the U. S. Public Health Service

The Haynes-Spray eliminates the danger of contamination which is possible by old fashioned lubricating methods. Spreading lubricants by the use of the finger method may entirely destroy previous bactericidal treatment of equipment.

PACKED 6-12 oz. CANS PER CARTON SHIPPING WEIGHT—7 LBS.

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

HAYNES SNAP-TITE GASKETS



LOW COST...RE-USABLE
LEAK-PREVENTING
NEOPRENE GASKET for Sanitary Fittings

Check these SNAP-TITE *Advantages*

- Tight joints, no leaks, no shrinkage
- Sanitary, unaffected by heat or fats
- Non-porous, no seams or crevices
- Odorless, polished surfaces, easily cleaned
- Withstand sterilization
- Time-saving, easy to assemble
- Self-centering
- No sticking to fittings
- Eliminate line blocks
- Help overcome line vibrations
- Long life, use over and over

Available for 1", 1½", 2", 2½" and 3" fittings.
Packed 100 to the box. Order through your dairy supply house.

THE HAYNES MANUFACTURING CO.
4180 Lorain Avenue • Cleveland 13, Ohio

HAYNES
SELF-CENTERING
SNAP-TITE® *Gaskets*

SIZES 1" - 1½"
2" - 2½" - 3"

*MADE FROM
TEFLON®

"The Sophisticated Gasket"

THE IDEAL UNION SEAL FOR BOTH VACUUM AND PRESSURE LINES

Gasket Color . . . slightly off-white

SNAP-TITE self-centering gaskets of TEFLON are designed for all standard bevel seat sanitary fittings. They SNAP into place 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

APPLICATION FOR MEMBERSHIP

(Membership open to individuals only)

All memberships on calendar year basis—Membership includes subscription to Journal of Milk & Food Technology

Name _____ Date _____
Please Print

Address _____ ☐ Renewal
☐ New
_____ Zip Code _____ ☐ Re-instatement

Is the above your ____ business ____ home address?

Direct Member Annual Dues $14.00
Affiliate membership—through an affiliate—$12.00 plus affiliate dues (contact your local affiliate secretary)
Student Membership $5.00 with verification of student status

SUBSCRIPTION ORDER

Name _____ Date _____
Please Print

Address _____ ☐ Renewal

_____ Zip Code _____ ☐ 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 Milk and Food Technology, P.O. Box 701, Ames, Iowa 50010.

Name _____

OLD ADDRESS _____

_____

NEW ADDRESS _____

_____

Effective date _____ Publication _____

PUBLICATIONS ORDER FORM

3-A Sanitary Standards

( ) Complete set bound (durable cover) ---------- ea. $ 8.00
( ) Complete set unbound (loose pages) ---------- ea. $ 6.50
( ) Complete set bound 3-A & Egg 3-A stds. ---------- ea. $10.25
( ) Egg 3-A Stds (unbound $2.25) bound ---------- 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 25c each

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.

Procedure for Investigation of Foodborne Disease Outbreaks ---------- ea. $ 1.00
25-100 copies, 75c ea., 100 or more, 65c ea.

—All prices F.O.B., Ames, Iowa—

Name: _____ Date _____

Address: _____
Include Zip Code

_____
Please Print

NATIONAL MASTITIS COUNCIL ANNUAL MEETING

February 16-18, 1976

EXECUTIVE INN — LOUISVILLE, KENTUCKY

Everyone interested in prevention and control of bovine mastitis is cordially invited to attend the 15th Annual Meeting of the National Mastitis Council.

Vice President and Program Chairman Dr. R. D. Mochrie has planned an outstanding program for this meeting. Subject matter of interest to all segments will be presented, as indicated in this program preview:

Dr. F. H. S. Newbould, University of Guelph, Canada, will up-date international concepts of dealing with mastitis problems with his report on the International Dairy Federation Seminar on Mastitis Control held in Reading, England in April 1975.

Mastitis-induced changes in milk composition will be discussed by Dr. L. H. Schultz, University of Wisconsin. The effects of mastitis on processing properties in milk will be presented by Dr. R. L. Richter, University of Florida. Mr. D. C. Jordan, Colorado State University, will discuss incentive payments for high quality milk.

Teat dip formulations will be reviewed by Mr. C. R. McDuff, Economics Laboratories. Iodine in milk resulting from feed or from udder applications will be discussed by Dr. R. W. Hemken, University of Kentucky. An evaluation of Coulter Counter-Chemical method, WMT and DMSCC will be presented by Dr. D. R. Thompson, University of Minnesota. The regulatory aspects of teat dips and udder washes will be discussed by Dr. Howard Meyers, Bureau of Veterinary Medicine, Food and Drug Administration.

Milking parlor performance will be discussed by Dr. W. G. Bickert, Michigan State University. Milking machine problems will be reviewed by Mr. K. C. Kirby, Hi-Life Rubber.

Antibiotic Problems will be dealt with in a panel discussion at the evening session. Dr. J. J. Jarrett, veterinarian, Rome, Georgia will serve as moderator. Panelists include: Dr. H. M. Trabosh, U.S. Department of Agriculture, Mr. J. R. Quayle, Wake County (NC) Health Department, Mr. J. B. Smathers, Maryland and Virginia Milk Producers Association, and a number of dairymen who will present farm practice views.

A panel discussion: Implementation of a Mastitis Management Program will conclude the annual program. Dr. J. H. Nicolai, University of Kentucky will moderate. Panel participants will be: Dr. J. R. Kunckel, veterinarian, St. Michael, Minnesota, Mr. J. W. Barnes, Michigan MPA, Mr. J. K. Webb, AMPI, and Dr. A. N. Bringe, Extension Service, University of Wisconsin.

Make your plans to attend this excellent meeting. It will start at 8:45 a.m. on February 17 and will adjourn at noon on February 18. Request advance registration form from the National Mastitis Council, 910-17th Street, NW, Washington, DC 20006.

Send request for room reservation directly to the Executive Inn, Watterson Expressway at Fairgrounds, Louisville, KY 40213.

Burdet Heinemann, *President*
National Mastitis Council

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.

Notice of Vacancy

Chairman, Department of Food Science and Nutrition, University of Missouri-Columbia. *Qualifications:* Ph.D. in Food Science or related field; excellence in scholarship, research, communications, organization and administration; knowledge of Land Grant College system; understanding of food industry. Should qualify individual for rank of Professor. *Apply:* Dr. R. E. Baldwin, Chairman, Search Committee, University of Missouri-Columbia, Food Science and Nutrition, 219 Gwynn Hall, Columbia, Missouri 65201. Affirmative Action/Equal Opportunity Employer.

Index to Advertisers

HELP CONSERVE ENERGY

BUY - SPECIFY & USE

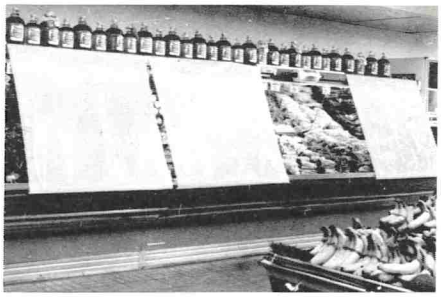
ELIASON ECONO-COVERS®

Patented — no spring — night covers — self storing for all types of
REFRIGERATED OPEN FRONT — OPEN TOP & FREEZER CASES

the ELIASON authored, published and copyrighted MANUFACTURER'S warranty/guarantee of Sanitary, Safety and Performance SPECIFICATIONS plan is a DIRECT COMMITMENT to USERS and PUBLIC HEALTH that it's FOOD, HEALTH and SAFETY related equipment and products will fulfill the LAW, valid REGULATIONS and GUIDELINES "without reference to none law" sales seals, labels, stars and certifications by others.

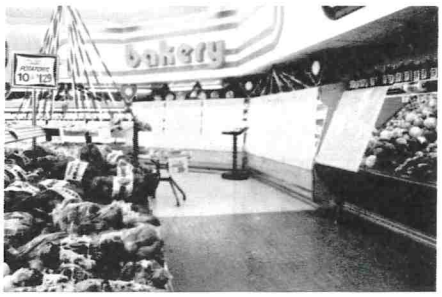
Carl Eliason
President

FREEZERS AND MULTI SHELF



OPEN PRODUCE

OPEN TOP MEAT



OPEN BAKERY-PRODUCE

NORMALIZE ENVIRONMENT · PROMOTES GOOD HOUSEKEEPING
·ECONOMICAL · PAY FOR THEMSELVES

ECONO-COVERS $AVE DOLLAR$

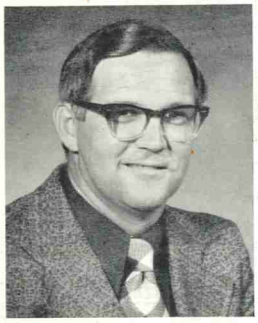
· SAVE TIME AT CLOSING & OPENING
· SANITARY FOOD PROTECTION · SAVE FOOD IN POWER
SHUT OFFS / BREAKDOWNS / BROWN OUTS

· A GENUINELY PRACTICAL REFRIGERATOR CASE ACCESSORY·
· ROLLS UP · ROLLS DOWN · LIKE WINDOW SHADE · PERFORATED·
· Reduce Fresh Meat re-wraps · Produce fresher, tastier and crisper longer ·
·Saves Labor · Helps Humidity · Cleaner Frozen Foods · Protects quality of
Dairy & Milk Products · Longer shelf life for Deli, Bakery & Food Specialties ·

WRITE OR CALL FACTORY FOR SPECS & PRICES

ELIASON CORPORATION
P.O. BOX 2128 KALAMAZOO, MICH. 49003
· Easy Swing DOORS · ECONO-COVERS · COMMERCIAL REFRIGERATORS
PHONE: 616/327-7003

Dairy authorities speak out on better cow milking



Dr. Robert D. Appleman
Professor of Animal Science
University of Minnesota

Automatic take-off milking units: They can save and protect.

There are two primary reasons why an investment in more mechanized milking is being considered by many dairymen. One is to reduce labor. The other is to improve udder health and maintain production of high quality milk.

LABOR TAKES A BIG BITE

Labor accounts for 15 to 30 percent of all costs in a dairy operation. About 55 percent of this labor is expended in the milking operation. In general, the total labor cost to produce 100 pounds of milk in a herd averaging 12,000 pounds per cow annually when labor* is valued at $3.00 per hour approaches $2.50 per cwt. in 30-cow herds; $2.10 in 50-cow herds; $1.68 in 100-cow herds; $1.13 in 250-cow herds; and $.91 in 500-cow herds**.

With an investment to modernize milking parlors, including unit take-off, it is not unusual to substantially lower the labor costs of producing milk.

Many of the milking chores are repetitious and result in drudgery. According to our studies, 5 to 10 percent of the milker's chore time is spent removing the milking unit. On top of that, the typical milker spends from 12 to 30 percent of his time machine-stripping cows.

THE OPERATOR IS A BUSY MAN

Proper stimulation of cows in a milking parlor is important to good milk letdown. Recent New Zealand work shows there is a loss of up to 1,000 pounds of milk per cow yearly when cows are not properly stimulated. In many barns the milker cannot effectively handle as many milking units as today's economy demands. Frequently, washing and stimulation time is limited to less than 15 seconds per cow because the milker is too "busy" with machine stripping or handling other units. The result is slow milking combined with considerable overmilking. Automatic unit take-off should improve this situation. Addition of automated prep stalls will help even more, provided they function properly.

SOME RESEARCH RESULTS

Research studies comparing automatic take-off and conventional milking units involving 550 cows in a Louisiana herd resulted in these conclusions:

1. Automatic unit take-off significantly reduced the number of quarters infected with mastitis.
2. Automatic unit take-off reduced udder irritation as evidenced by lower CMT scores.
3. The men operating the automatic take-off units reduced their walking distance in the parlor by more than 25%.

Dr. Nelson Philpot, leader of this study, says one should not expect miracles. Automatic take-off units do not make a poor operator better. They do, however, allow a conscientious operator to do an even better job on more cows.

According to dairymen using this equipment, proper maintenance and proper operation of equipment is even more important with non-automated systems. The ability, cooperative attitude and location of your local serviceman should become a primary factor in deciding whether to install this more sophisticated and expensive equipment.

MASTITIS PREVENTION NOT SIMPLE

Dairymen should not necessarily expect a reduction in the number of cows requiring treatment for clinical mastitis (gargot). In a marginal system, more cases may result because the significance of a single variable is not the same in every milking system or in every situation.

Frank Smith, California milking system specialist, illustrates this point well. He indicates that too many researchers and educators have attempted to over-simplify the cause of mastitis. In turn, they have over-simplified its prevention. *The concept of a direct, independent relationship shown in Figure 1 is incorrect.* Figure 2 arranges these same variables in a manner which is sequential, additive, and interdependent.

Figure 1

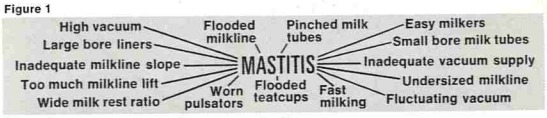
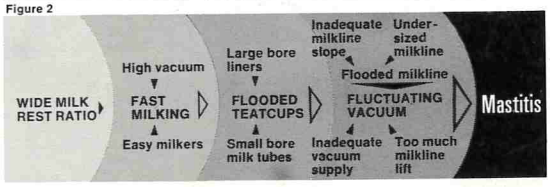


Figure 2



As mentioned earlier, installing automatic unit take-off may allow one to milk cows faster and reduce overmilking. However, if such a change resulted in flooded milk lines and fluctuating vacuum, the incidence of mastitis might increase rather than decrease. Providing all other deficiencies in the system were corrected, automatic take-off would prove highly beneficial.

AUTOMATIC TAKE-OFFS A COMMON SIGHT?

Where cost of this mechanization is not excessive and such installations prove to be reasonably trouble-free over time, I'm sure that automatic take-off units will become an increasingly more common sight on dairy farms.

*For our purposes, the labor figures include all dairy chore labor, feeding labor, and the raising of offspring. Field labor isn't included.

**In 250-cow and 500-cow herds, we assume the existence of a parlor and a free-stall barn with mechanized feeding and waste handling.