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# MILK and FOOD TECHNOLOGY

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*Official Publication*

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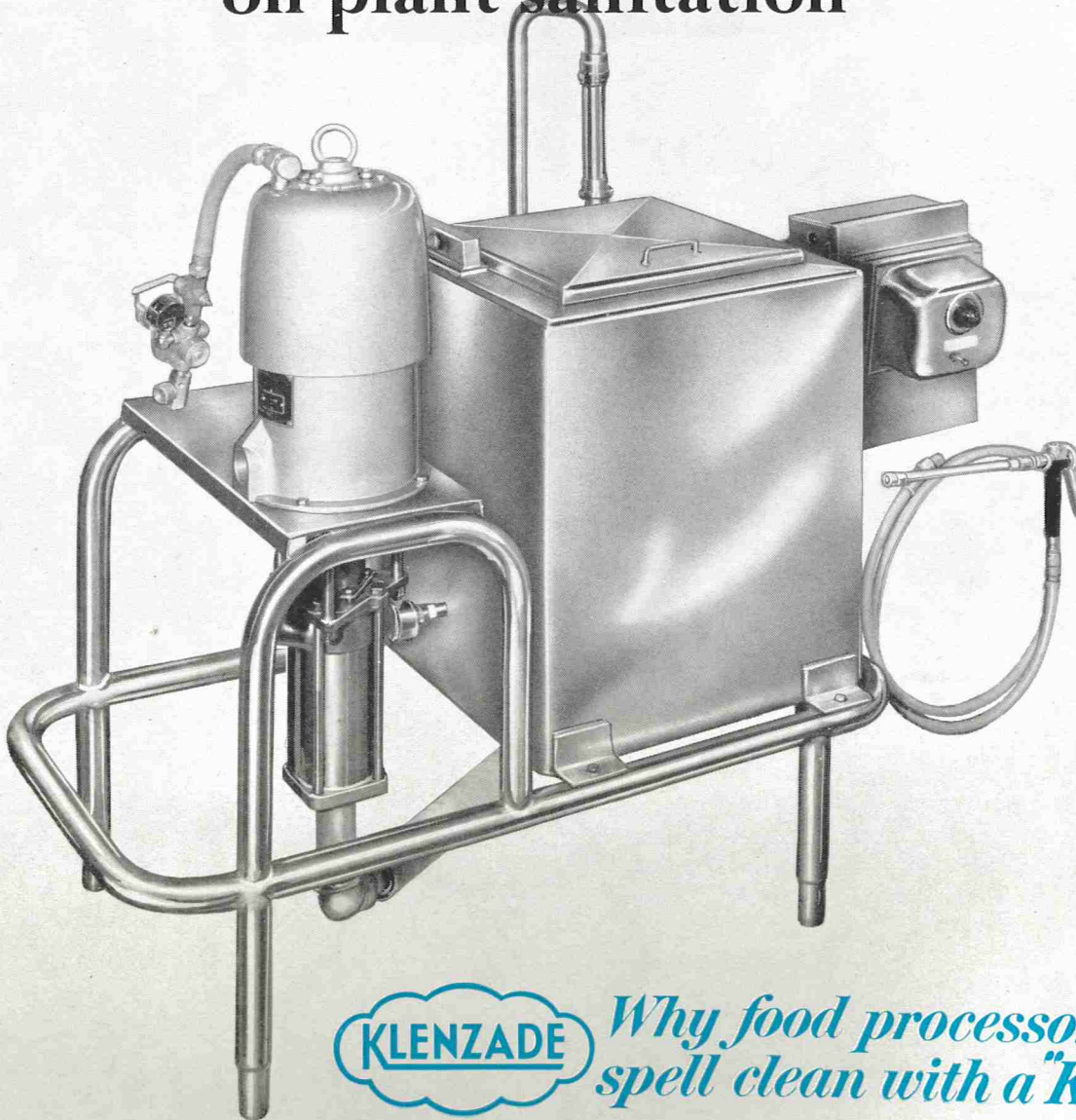
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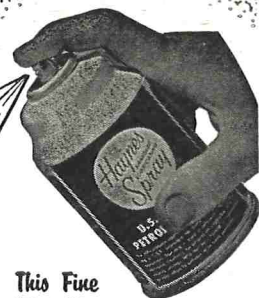
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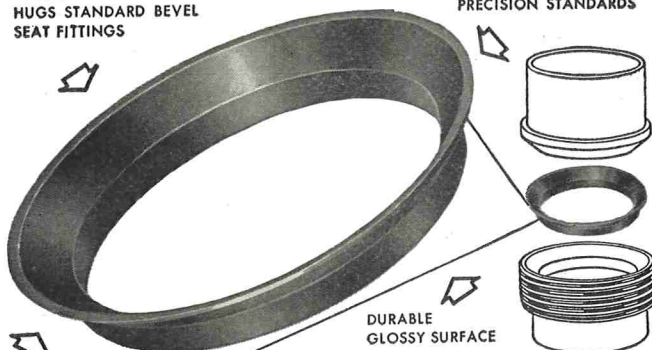


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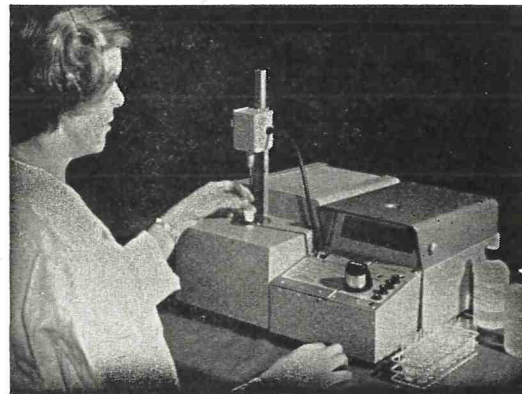
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The Journal of Milk and Food Technology is issued monthly beginning with the January number. Each volume comprises 12 numbers. Published by the International Association of Milk, Food and Environmental Sanitarians, Inc. with executive offices of the Association, Blue Ridge Rd., P. O. Box 437, Shelbyville, Ind.

Entered as second class matters at the Post Office at Shelbyville, Ind., March 1952, under the Act of March 3, 1879.

**EDITORIAL OFFICES:** J. C. Olson Jr., Editor, Dept. Food Science and Industries, University of Minn., St. Paul, Minn. 55101; H. L. Thomasson, Managing Editor, P. O. Box 437, Shelbyville, Indiana 46176.

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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. 30 August, 1967 No. 8

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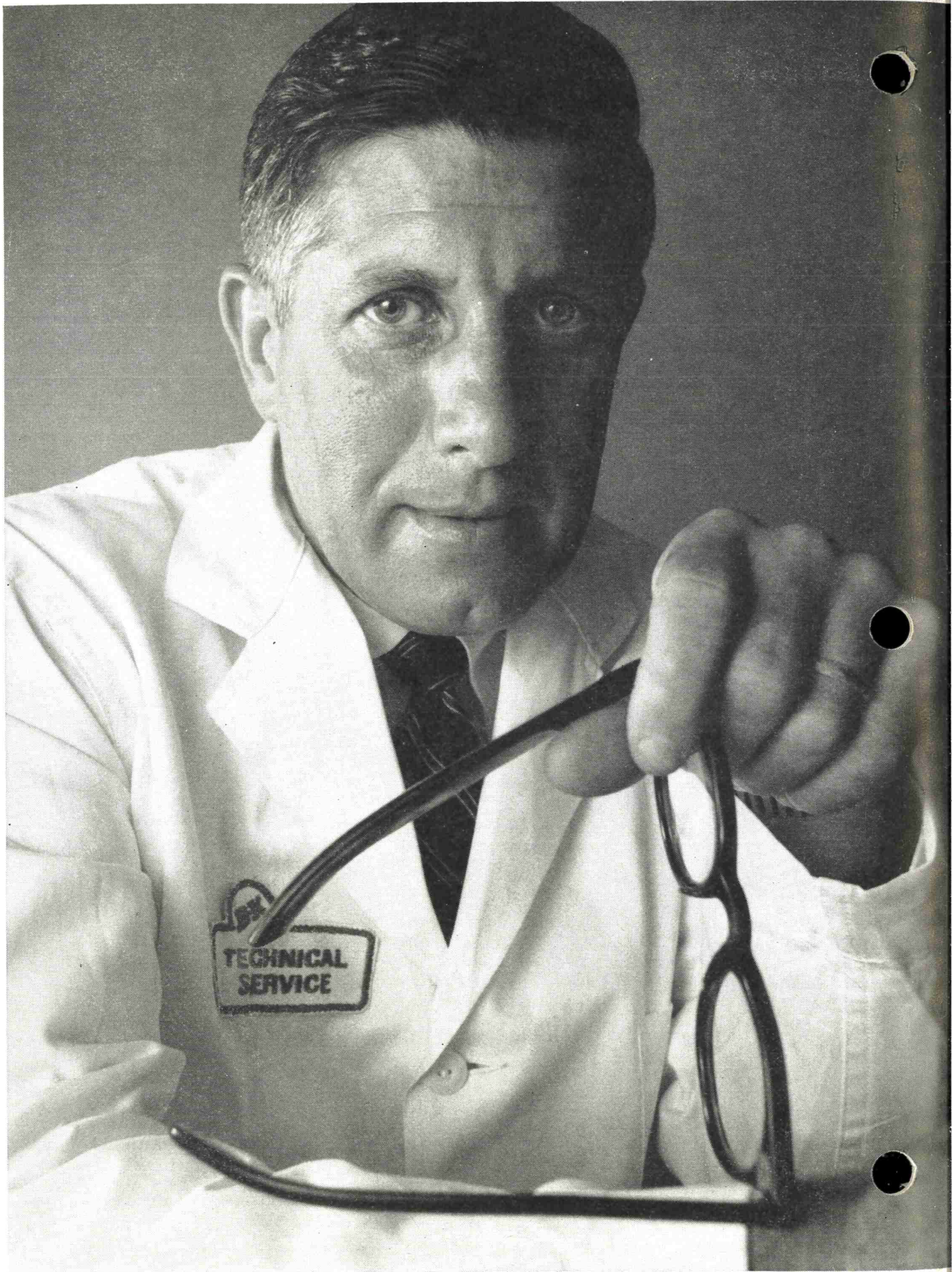
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## PRESIDENTIAL ADDRESS FIFTY-FOURTH ANNUAL MEETING IAMFES

P. R. ELLIKER

*Department of Microbiology  
Oregon State University, Corvallis*

**Note:** For purposes of general orientation of the membership Dr. Elliker has included in his Presidential Address a summary of progress to date toward combining the activities of IAMFES and NAS through the merging of these two organizations into one professional organization of sanitarians. This summary will be especially valuable as reference material for discussions at forthcoming Affiliate Association meetings. In the interest of an informed membership, Affiliate Secretaries are urged to give the widest publicity to President Elliker's Address in announcements of future meetings, newsletters and other appropriate communications.

Editor

Dr. Myhr, distinguished guests and members of the International Association, it is with great pleasure that I greet you this morning with a few words concerning sanitarians and our Association. I would like to emphasize at the outset that I address you with a real sense of pride in the accomplishments and standing of sanitarians, in general, and decided optimism for the future role of the sanitarian and our Association in a rapidly changing environment. For some months I have had a talk roughed out dealing with the fascinating history of sanitation going back to the sanitary regulations of Moses and Hippocrates through the remarkable advances accomplished by sanitarians like yourselves in this century and finally citing problems still remaining and still to develop with a burgeoning population. All we need to indicate as evidence that you will be busy in your respective areas for years to come are items such as the long list of articles, animals, foods and other sources from which salmonellae have been isolated during the past few years and, when all other problems are solved, the tricky sanitation program associated with space exploration.

Another reason for optimism at this time is the improved financial condition of our Association which with good management should continue during future years. Furthermore, considerable progress has been made in efforts to find a common basis of agreement for considering a merger of the two large sanitarian associations in the U. S., the International Association of Milk, Food and Environmental Sanitarians and the National Association of Sanitarians. Before discussing these developments further, I would like to mention one other important item.

It is with regret that we accept the resignation of

Dr. J. C. Olson, Jr. as Editor of the Journal of Milk and Food Technology. Dr. Olson has served this organization in his capacity as Editor with devotion and distinction for many years. On the other hand, we are fortunate that Dr. Elmer H. Marth of the University of Wisconsin has consented to succeed Dr. Olson as Editor effective September 1, 1967. We extend to Joe our sincere appreciation and congratulations and best wishes in his new position as Director of the Division of Microbiology, Bureau of Science, Food and Drug Administration in Washington, D. C. To Elmer, I would like to express, on behalf of the Association, a hearty welcome to the staff and our earnest desire to assist him in every way to continue the excellent editorial standards established for the Journal.

The report of the Executive Director at the business meeting will detail the financial condition of our Association. I will summarize the situation by emphasizing that a long overdue increase in dues has greatly improved our operating budget and will enable services to the organization and membership through the Journal and in other ways that previously were not possible.

### IAMFES—NAS MERGER

#### *Initial considerations.*

In approaching the question of combining activities of IAMFES and NAS, the Executive Board first carefully reviewed past interassociation activity by both organizations. The first question asked by the Board was whether or not such a step would be beneficial to sanitarians and associated professional groups as a whole. The entire Board and most others consulted on the problem have agreed in principle with the idea. Many of our members and, particularly industry representatives have strongly endorsed it.

A second condition preceding such a step is some feeling of confidence and trust that the other Association will cooperate in developing common, useful objectives. There has to be a very practical realization that some give and take by each organization is necessary.

Third, there also has to be some framework in the nature of a tentative written Constitution or By-Laws upon which to build this confidence and exchange

of ideas. During the past year, the Executive Board, through its ad hoc Interassociation Committee, has progressed to this stage. The problem of interassociation cooperation on this objective has been approached in all honesty and sincerity without prejudice and distrust. The result, if culminated, will be a new organization with a new name, new emblem, and new seal, but with present officers of both organizations, a combination of the best features of present constitution and by-laws, as well as present journals and management to launch the program over an interim period.

All that the Executive Board is attempting at the present time is to provide a *tentative set of by-laws* that can be appropriately modified by membership of both organizations. This, in turn, will be presented to both organizations for vote by mail ballot. If approved, the result will be one strong, unified organization with sections for different interest groups. If the vote is negative, both organizations will have to look toward strengthening their individual programs in the best manner possible.

#### *Organizational structure—Tentative By-Laws.*

I would now like to deal more specifically with some questions concerning the proposed merger of IAMFES and NAS that I know are of interest to our membership. Most of this discussion will be based on the Third Draft of the Tentative Proposed By-Laws which represents progress to date in providing a written framework upon which to build a new sanitarian's organization. Copies of this Third Draft have been provided each affiliate representative and additional copies can be made available for those interested in studying them. Let me emphasize again that the Proposed By-Laws represent real compromise to incorporate the best features of both organizations.

*Categories of membership.* There will be two chief categories of membership, active and associate. Active members may vote and hold office in the new organization. Associate members will have all privileges of the organization held by active members, except that they may not vote or hold office. Any person who is employed full-time as a sanitarian or in related educational or industrial activities and has a Bachelor's Degree with a minimum of thirty semester units of academic work in the physical, biological and environmental sciences is eligible to become an active member.

All present active members of IAMFES and NAS on the date of adoption of these by-laws and those who become active members of either association within six months after the adoption of these by-laws shall be considered as active members and will be eligible to vote and hold office. This very generous

provision assures all present and future membership in IAMFES all voting and office privileges in the new organization. Other categories of membership such as fellow, life, retired, honorary, student and sustaining are described.

*Publications.* There will be two official publications for at least an interim period to be prescribed by the Executive Board or Governing Council. These will be The Journal of Milk and Food Technology and The Journal of Environmental Health. They will be published six times a year on alternate months. Members would receive both Journals.

*Officers.* Officers of the proposed Association will be President, President-elect, First Vice-President, Second Vice-President, Secretary and Treasurer. Present officers of both organization would alternate in succeeding to the presidency of the new organization and elections for a new second Vice-President would be delayed until the last of the present officers has vacated the office of Second Vice-President. Election of the Second Vice-President will be by mail ballot.

*Sections.* The Association will be divided into the following five sections: *a* Milk and Food, *b* General Environmental Health, *c* Water and Air Pollution, *d* Housing and Urban Development and *e* Health Facilities. Each section will annually elect a Chairman, Vice-Chairman, and Secretary. Division of the Association into Sections is considered an important requirement for IAMFES, because it will permit those interested in milk and food to continue to concentrate on these areas in their annual meetings. Those members of our Association interested in other fields will find a section of their choice and new sections can be added if the need warrants it.

*Management.* There will be an Executive Director to conduct administrative and business affairs of the Association. There will also be an Assistant Executive Director and Managing Editor to supervise printing of publications, handle membership mailings and serve as liaison between the Association and such programs as 3-A Sanitary Standards, Interstate Milk Shippers, National Mastitis Council and The Dairy and Food Exposition.

The Executive Board shall consist of the Immediate Past-President, President, President-Elect, First Vice-President, Second Vice-President, Secretary and one member-at-large appointed by the Board for a single one year term. The Treasurer and Executive Director shall be ex-officio members.

A Governing Council of the Association will consist of the above-named officers, together with Chairmen of Sections and Presidents or other appropriately designated representatives of Affiliate Associations.

*Annual Meeting.* There will be an Annual Educational Conference of the Association to be held at a time and place to be fixed by the Executive Board.

*General Comments.* The above brief account should provide a general picture of progress made toward a Proposed By-Laws for the new Association. It is obvious that the International ad hoc committee, together with NAS representatives have drawn liberally from by-laws of both organizations. Names proposed include International Environmental Health Association, International Association of Sanitarians and International Sanitarians Association. A new Association emblem and a new seal will have to be designed.

The benefits that should follow a merger of these two associations encourage endorsement of this step. The advantages appear to greatly outweigh the disadvantages. There is a place in the proposed new organization for everyone presently employed in the two associations. There will also be a section for

every major interest group in sanitation so that all of our present membership will be served. Furthermore, an analysis of printing costs for both IAMFES and NAS Journals on an alternate monthly basis (6 numbers of each per year) suggests a possible saving of \$10,000 to \$15,000 annually in publication costs alone.

I would like to leave one last suggestion with you for consideration at this meeting and during the coming year. A number of affiliates of IAMFES and NAS may be ahead of the parent societies in their thinking on the merger proposition, and I hope their influence will be felt throughout our organization. I have reference to the interest on the part of IAMFES and NAS affiliates in some states in combining their annual meetings. If additional state affiliates will follow this lead, I am sure they will find a common basis upon which to build one strong, national organization to serve the best interests of the sanitarian and associated professional groups.

---

### AMENDMENT TO 3-A SANITARY STANDARDS FOR MULTIPLE-USE PLASTIC MATERIALS USED AS PRODUCT CONTACT SURFACES FOR DAIRY EQUIPMENT

Serial #2002

*Formulated by*  
*International Association of Milk, Food and Environmental Sanitarians*  
*United States Public Health Service*  
*The Dairy Industry Committee*

The "3-A Sanitary Standards for Multiple-Use Plastic Materials Used as Product Contact Surfaces for Dairy Equipment, Serial #2000," are hereby further amended as indicated in the following:

Section I. Standards for Acceptability, Sub-paragraph (2)

Add the following material to the list of Generic Classes of Plastics:

Acrylonitrile butadiene styrene 0.30 0.45 0.90  
Section I. Standards for Acceptability, Sub-paragraph (2)

In the list of Generic Classes of Plastics substitute the following in place of the present class which is designated, "Fluorocarbons":

Fluorocarbons

\*CTFE, TFE and FEP types 0.05 0.05 0.05  
Vinylidene fluoride types 0.05 0.05 0.15

\*The values shown for CTFE, TFE, and FEP types are not under consideration at this time—no change is indicated here. (These are already included in the Standard). The amendment relates to the arrangement of these values, and to the introduction of the vinyl type with a different value shown for the Solution J. figure.

This amendment shall become effective Oct. 25, 1967.

# EVALUATION OF METHODS FOR DETERMINING THE BACTERIAL POPULATION OF FRESH FILLETS

REMEDIOS SILVERRIO AND R. E. LEVIN

Department of Food Science and Technology  
University of Massachusetts  
Amherst, Massachusetts 01002

(Received for publication April 17, 1967)

## SUMMARY

The use of a nutrient broth consisting of 0.5% tryptone, 0.25% yeast extract, and 0.1% dextrose for blending and diluting fish tissue resulted in a 10 fold higher count compared to that obtained when distilled water was used. No specific preference was shown for yeast extract, beef extract or tryptone for yielding maximum total counts between 0.1 - 0.5% concentrations. A fish infusion agar representing a 1:10 dilution of fish tissue in distilled water yielded lower counts than conventional nutrient media. The addition of 0.1 - 0.5% glucose, glycerol, and sodium lactate to a basal medium of 0.1% yeast extract and 0.2% tryptone failed to increase total counts above those obtained with the basal medium alone. The addition of 0.05% - 0.5% sodium citrate to enumerating media markedly suppressed the total count; 0.3% reduced the count by 64% from fresh haddock and by 95% from stale haddock. The addition of 0.1 - 0.5% NaCl to enumerating media did not influence total counts but was essential for pigment formation by *Pseudomonas putrefaciens*. Plate counts determined at 20 and 25 C exceeded those from plates incubated at 3 C from fresh haddock while the reverse was true for stale haddock.

Throughout the literature there is a lack of uniform methodology in determining the total number of aerobic and facultatively anaerobic bacteria on fish. A variety of media, diluents, and incubation temperatures have been used by many workers (4, 6, 7, and 8). This study was designed to determine the influence of various media, diluents, and incubation temperatures on the total bacterial counts from fish.

## MATERIALS AND METHODS

All counts in this study were obtained from unfrozen haddock fillets. Unless otherwise specified Difoco media and ingredients were used throughout. Fish tissue was prepared for total counts by blending 30-g samples with 270 ml of diluent for 2 min. The blending menstruum and that used for serial dilutions was composed of the basal plating medium used in each respective experiment exclusive of the agar unless otherwise stated. Photomicroscopy was performed with a Carl Zeiss standard model GFL light microscope with an apochromatic oil immersion objective (N.A. 1.32) and Nikon camera back. Flagella were detected using the staining method of Leifson (3).

## RESULTS

### *Influence of dilution menstruum on bacterial counts.*

The effect of distilled water versus broth for blending and diluting fish tissue was determined by blend-

ing samples from the same fillet in both distilled water and a broth consisting of 0.5% tryptone, 0.25% yeast extract, and 0.1% dextrose in distilled water at pH 7.0. Serial 1:10 dilutions were then prepared in tubes of the same menstruum used for blending each respective sample and were plated in duplicate using Plant Count agar. Two sample fillets were used. Duplicate samples from each fillet were subjected to the two blending and suspending menstrua. The counts obtained with broth as diluent yielded a 14-fold higher count than distilled water from the first fillet and a 6-fold higher count from the second fillet resulting in an overall 10-fold difference in mean counts between both menstrua (Table 1). These results indicate the necessity of blending and diluting samples in a menstruum of sufficiently high enough solute content to prevent osmotic destruction and damage to the cells in order to obtain maximum counts.

TABLE 1. EFFECT OF DILUENT ON BACTERIAL COUNTS FROM HADDOCK FILLETS<sup>a</sup>

Diluent	Mean counts (X10 <sup>5</sup> ) per g fish			
	Fillet I		Fillet II	
	Trial 1	Trial 2	Trial 1	Trial 2
Distilled water	1.1	1.0	3.9	3.5
Mean	1.1		3.7	
Broth <sup>b</sup>	15.5	18	21.8	22.5
Mean	15.8		22.2	

<sup>a</sup>Incubation was at 20 C for 3 days. Each indicated count is the average of duplicate plates.

<sup>b</sup>Broth used for blending and subsequent dilutions consisted of: 0.5% tryptone, 0.25% yeast extract, and 0.1% dextrose in distilled water at pH 7.0.

### *Studies on media composition.*

The efficiency of a fish infusion agar was evaluated after being prepared by blending 1 part by weight of fresh fillets using equal portions of haddock, flounder and swordfish, and 9 parts distilled water. The mixture was boiled for one-half hour and then strained through 2 layers of cheese cloth and filtered. The

volume was restored with distilled water, 1.5% agar added and the pH adjusted to 7.0. The fish infusion yielded lower counts when compared to media containing tryptone or yeast extract (Table 2) even when 0.1% glucose was added. An additional disadvantage to the use of fish infusion agar was the cloudiness of the resulting medium which made it difficult to discern small colonies. The possibility that a more concentrated fish infusion might have resulted in higher colony counts cannot be discounted, however, its inconvenience in preparation and resulting opacity do not favor its use.

TABLE 2. COMPARISON OF BACTERIAL COUNTS WITH FISH INFUSION AGAR AND TRYPTONE GLUCOSE YEAST EXTRACT AGAR<sup>a</sup>

Agar medium	Mean counts (X10 <sup>4</sup> ) per g fish after incubation time (days) <sup>b</sup>			
	1	2	3	4
0.1% tryptone + 0.1% glucose	15.0	170	197	203
0.5% tryptone + 0.1% glucose + 0.5% yeast extract	21.0	179	208	213
Fish infusion	2.5	102	134	148
Fish infusion + 0.1% glucose	5.5	95	140	164

<sup>a</sup>Incubation temperature was 20 C. Each indicated count is the average of duplicate plates.

<sup>b</sup>Refers to incubation time of plates.

In order to determine the optimum concentration of yeast and beef extracts to be incorporated in enumerating media a basal medium was prepared consisting of: 0.5% tryptone, 0.1% glucose and 1.5% agar in distilled water at pH 7.0. The addition of 0.1 - 0.5% yeast and beef extracts to the basal medium failed to yield significantly higher counts compared to those obtained with the unsupplemented basal medium (Table 3).

To obtain additional information on the organic nitrogen requirements for maximum colony development a basal medium consisting of 0.1% yeast extract, 0.1% glucose and 1.5% agar in distilled water was supplemented with 0.1 - 0.5% tryptone and casein hydrolysate. The results failed to show a consistent and significantly higher number of colonies when these supplements were added to the basal medium (Table 4).

To evaluate the effect of various carbon and energy sources on total counts a basal medium consisting of 0.1% yeast extract, 0.2% tryptone, and 1.5% agar distilled water at pH 7.0 was supplemented with 0.1 - 0.5% glucose, glycerol, sodium lactate and sodium citrate. None of the four carbon compounds used yielded consistently higher counts than the basal medium alone (Table 5). Of notable significance

TABLE 3. EFFECT OF MEDIUM WITH VARIOUS LEVELS OF YEAST AND BEEF EXTRACTS ON THE BACTERIAL COUNTS FROM HADDOCK FILLETS

Medium	Mean counts (X10 <sup>4</sup> ) per g fish	
	Fillet I	Fillet II
Basal medium (BM) <sup>b</sup>	180	40.5
BM + 0.5% yeast extract	200	57.5
BM + 0.3% yeast extract	130	47.5
BM + 0.2% yeast extract	150	49.0
BM + 0.1% yeast extract	200	48.0
BM + 0.5% beef extract	210	29.5
BM + 0.3% beef extract	200	42.0
BM + 0.2% beef extract	120	45.5
BM + 0.1% beef extract	140	36.0

<sup>a</sup>Incubation was at 20 C, for 4 days. Each indicated count is the average of one or more dilutions plated in duplicate.

<sup>b</sup>Basal medium (BM) consisted of 0.5% tryptone, 0.1% glucose and 1.5% agar.

TABLE 4. EFFECT ON TOTAL COUNT OF VARIOUS CONCENTRATIONS OF TRYPTONE AND CASEIN HYDROLYSATE ADDED TO A BASAL MEDIUM OF 0.1% YEAST EXTRACT, 0.1% GLUCOSE AND 1.5% AGAR AT PH 7.0<sup>a</sup>

Nitrogen supplement	Mean count (X10 <sup>3</sup> ) per g fish	
	Fillet I	Fillet II
Basal medium (BM)	129	535
BM + 0.5% tryptone	148	500
BM + 0.3% tryptone	120	520
BM + 0.2% tryptone	113	595
BM + 0.1% tryptone	106	525
BM + 0.5% casein hydrolyzate	133	620
BM + 0.3% casein hydrolyzate	137	430
BM + 0.2% casein hydrolyzate	148	590
BM + 0.1% casein hydrolyzate	143	485

<sup>a</sup>Plates were incubated at 20 C for 4 days.

was the dramatic reduction in counts due to the presence of sodium citrate; 0.3% caused a 64% reduction in count with fresh fish (Trial I, Table 5) and a 95% reduction in count with stale fish (Trial II, Table 6).

The rate and number of colonies developing during continued incubation of the plates containing 0.2% carbon compounds (Table 5) and the composite GGCL supplement are plotted in Figure 1. The rate of colony formation is notably suppressed with sodium citrate in the medium in counts from both fresh and stale haddock.

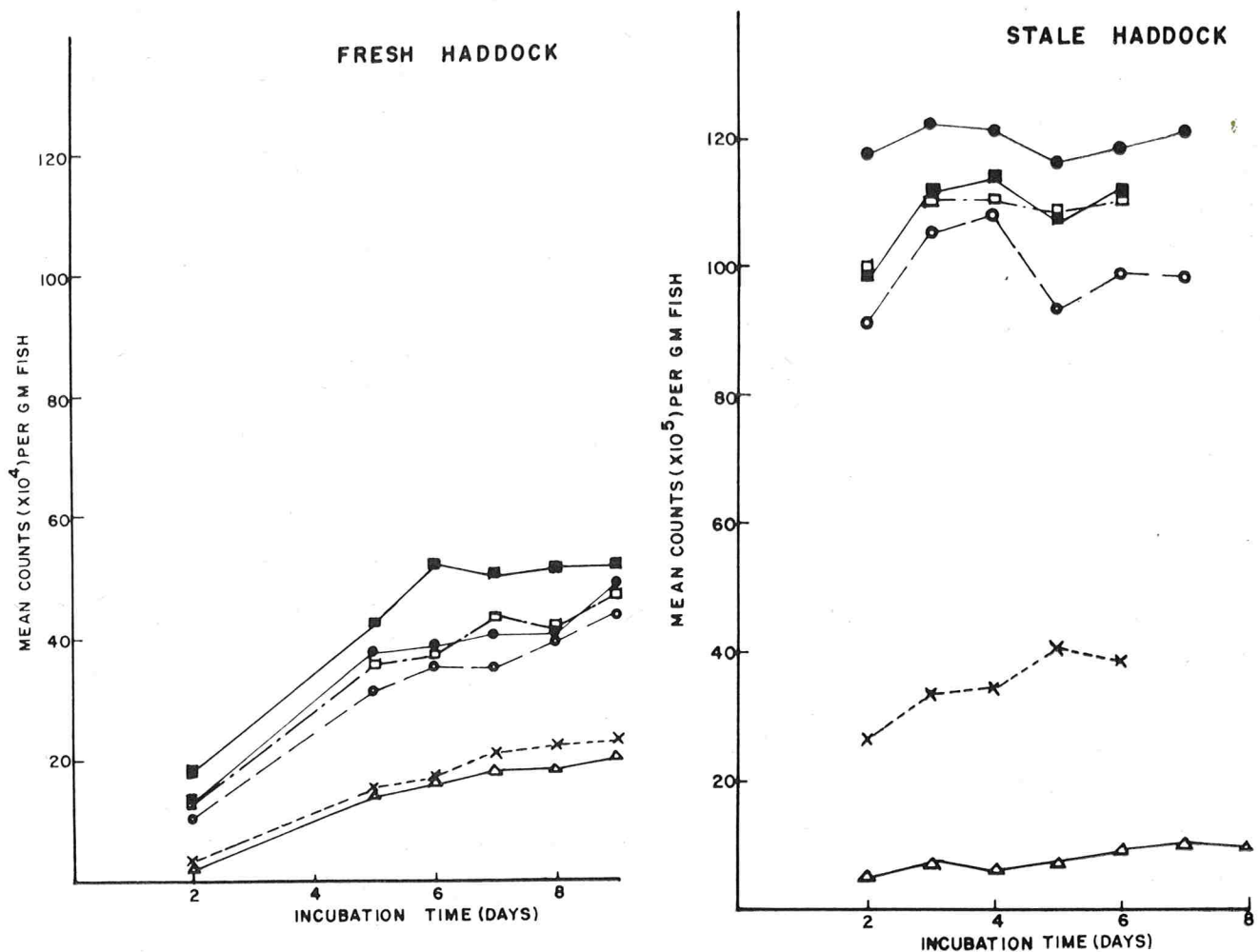


Figure 1. Effect of various carbon sources on bacterial counts from fresh and stale haddock. ● basal medium (BM) (see Table 5); ■ BM + 0.2% sodium lactate; □ BM + 0.2%

glycerol; O BM + 0.2% glucose; × BM + 0.1% sodium lactate, 0.1% sodium citrate, 0.1% glycerol, and 0.1% glucose; Δ 0.2% sodium citrate.

The addition of 0.1 - 3.5% NaCl to a basal medium of plate count agar failed to have any consistent influence on the total number of developing colonies (Table 6). The use of sea water in place of distilled also failed to yield a significantly higher count. The presence of 0.1 - 3.5% NaCl did give rise, however, to the development of reddish brown pigmented colonies which were not as intensely pigmented in the absence of salt. Such colonies often constituted over 50% of the population. Several of these pigmented colonies were streaked onto plate count agar with and without salt. Resulting colonies were notably pigmented in the presence of salt while those on salt-free agar either were slightly pigmented or lacked pigment entirely. The organism was facultatively psychrophilic, gram negative, proteolytic in gelatin deeps, produced  $H_2S$  and was polarly flagellated (Figure 2), indicating it to be *Pseudomonas putrefaciens* which Castell et al. (2) reported isolating from fresh and frozen cod fillets. The readily observed presence of

this intense fish spoilage organism afforded by the addition of salt in the enumerating medium and the observation by Castell et al. (2) that with some strains little or no growth occurs in culture media in the absence of salt indicate the desirability of incorporating at least 0.1% NaCl.

#### Effect of incubation temperature on total counts from haddock fillets.

Fish of high quality designated as fresh, and fish stored at 3 C for 7 days after procurement designated as stale were used. Counts at 3 C exceeded those at 20 and 25 C only from stale fish (Table 7, Figure 3), confirming the observation by Castell et al. (1). This higher count at 3 C from stale fish constituted a 24% increase over the count at 20 C and a 30% increase over the count at 25 C. The count at 3 C exceeded the count at 30 C from fresh fish by 20% and by 49% from stale fish. With fresh fish the count at 3 C exceeded the count at 35 C by 85%; with stale fish the count at 3 C exceeded the count at 35 C by 96%.

TABLE 6. EFFECT OF VARIOUS SALT LEVELS ON PLATE COUNTS FROM HADDOCK FILLETS<sup>a</sup>

Medium	Mean counts (10 <sup>5</sup> ) per g fish <sup>b</sup>					
	I Fresh	II Fresh	III Fresh	IV Fresh	V Fresh	VI Stale
Basal medium <sup>c</sup> (BM)	5.6	2.2	5.9	7.6	1.06	113
BM + 3.5% NaCl	6.3	2.2	5.4	3.9		
BM + 2.0% NaCl	7.4	1.6	6.8	5.1		
BM + 1.0% NaCl	6.9	1.9	5.3	9.4	1.01	110
BM + 0.5% NaCl	7.0	2.2	6.4	10.1		
BM + 0.25% NaCl	10.4	2.0	7.5	9.6	1.16	110
BM + 0.1% NaCl	2.7	6.7	8.9			
BM + sea water	6.8					

<sup>a</sup>Six fillets (I-VI) from several sources were used. The incubation time at 20 C was 5 days for plates in Trial I, 6 days for plates in Trials II, III, IV, and V, and 4 days in Trial VI. <sup>b</sup>Each indicated count is the average of duplicate plates in Trials I and II, and 4 plates in Trials III, IV, V, and VI.

<sup>c</sup>Basal medium used was plate count agar.

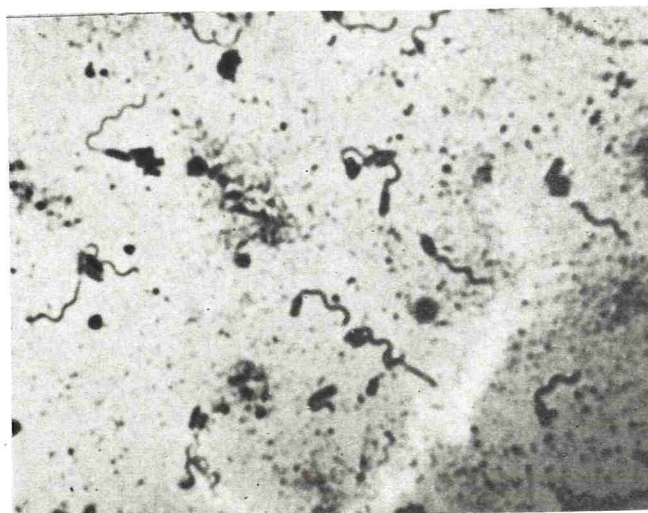


Figure 2. Flagella stain of *Pseudomonas putrefaciens* isolated from haddock fillet on salt agar. X 4,440.

These results confirm the observation by Shewan (5) that most organisms on fish are psychrophilic. Castell et al. (1) concluded that the lack of relationship between counts at 37 C and those at or near 0 C showed that the former has no value in estimating the keeping time of fillets stored at or near 0 C. The counts at 20 and 25 C appear to be a satisfactory compromise among all the incubation temperatures used for maximum enumeration at one temperature within the shortest incubation time. Castell et al. (1) in correlating counts at 25 C with those at 2 C

concluded that the former gave a rough estimate of the keeping time of fillets stored at 0 C but individual results at 25 C especially when the counts are excessive cannot be relied upon to correlate with counts at 2 C.

There is no doubt that the potential refrigerated storage life of a fillet is reflected most closely by enumeration of the psychrophilic flora (1). The higher counts obtained from stale fish on incubation of the plates at 3 C compared to the lower counts at 20 C can only be explained by the selective growth of obligate psychrophiles during refrigerated storage. If fillets are excessively mishandled or contaminated during processing high mesophilic counts resulting will not be indicated by incubation of the plates at 3 C. A more meaningful determination of the bacterial quality of fillets will therefore be afforded by counts at both 3 and 20 C and by using the resulting ratio as an additional index of quality.

TABLE 5. EVALUATION OF MEDIA WITH VARIOUS CARBON SOURCES<sup>a</sup>

Carbon source	Mean counts (X10 <sup>5</sup> ) per g fish			
	Trial I	Trial II	Trial III	Trial IV
Basal Medium (BM) <sup>b</sup>	4.4	119	3.9	22.2
BM + 0.5% glucose	—	—	3.8	—
BM + 0.4% glucose	—	—	2.6	—
BM + 0.3% glucose	3.8	100	2.6	—
BM + 0.2% glucose	4.0	100	2.9	20.9
BM + 0.1% glucose	4.2	104	3.4	—
BM + 0.5% glycerol	—	—	3.4	—
BM + 0.4% glycerol	—	—	3.7	—
BM + 0.3% glycerol	4.7	130	3.8	—
BM + 0.2% glycerol	4.2	110	3.3	23.9
BM + 0.1% glycerol	4.6	107	4.1	—
BM + 0.5% sodium lactate	—	—	3.9	—
BM + 0.4% sodium lactate	—	—	3.7	—
BM + 0.3% sodium lactate	5.4	108	3.7	—
BM + 0.2% sodium lactate	5.2	112	3.9	22.8
BM + 0.1% sodium lactate	3.8	114	3.2	—
BM + 0.5% sodium citrate	—	9.5	—	—
BM + 0.4% sodium citrate	—	8.0	—	—
BM + 0.3% sodium citrate	1.6	5.8	—	—
BM + 0.2% sodium citrate	1.9	7.3	—	—
BM + 0.1% sodium citrate	2.8	14.2	—	—
BM + 0.05% sodium citrate	—	49.2	—	—
BM + GGCL <sup>c</sup> supplement	2.3	39.0	—	—

<sup>a</sup>Each indicated count is the average of duplicate plates in Trials I and II, Triplicate in III and 4 plates in IV. Incubation was at 20 C for 8 days for Trial I and 6 days for Trials II, III, and IV. Fish samples were procured fresh in Trials I, III, and IV and held until stale for Trial II.

<sup>b</sup>Basal Medium (BM) consisted of 0.1% yeast extract, 0.2% tryptone, 1.5% agar, and distilled water at pH 7.0.

<sup>c</sup>GGCL supplement consisted of 0.1% glucose, 0.1% glycerol, 0.1% sodium lactate, and 0.1% sodium citrate.

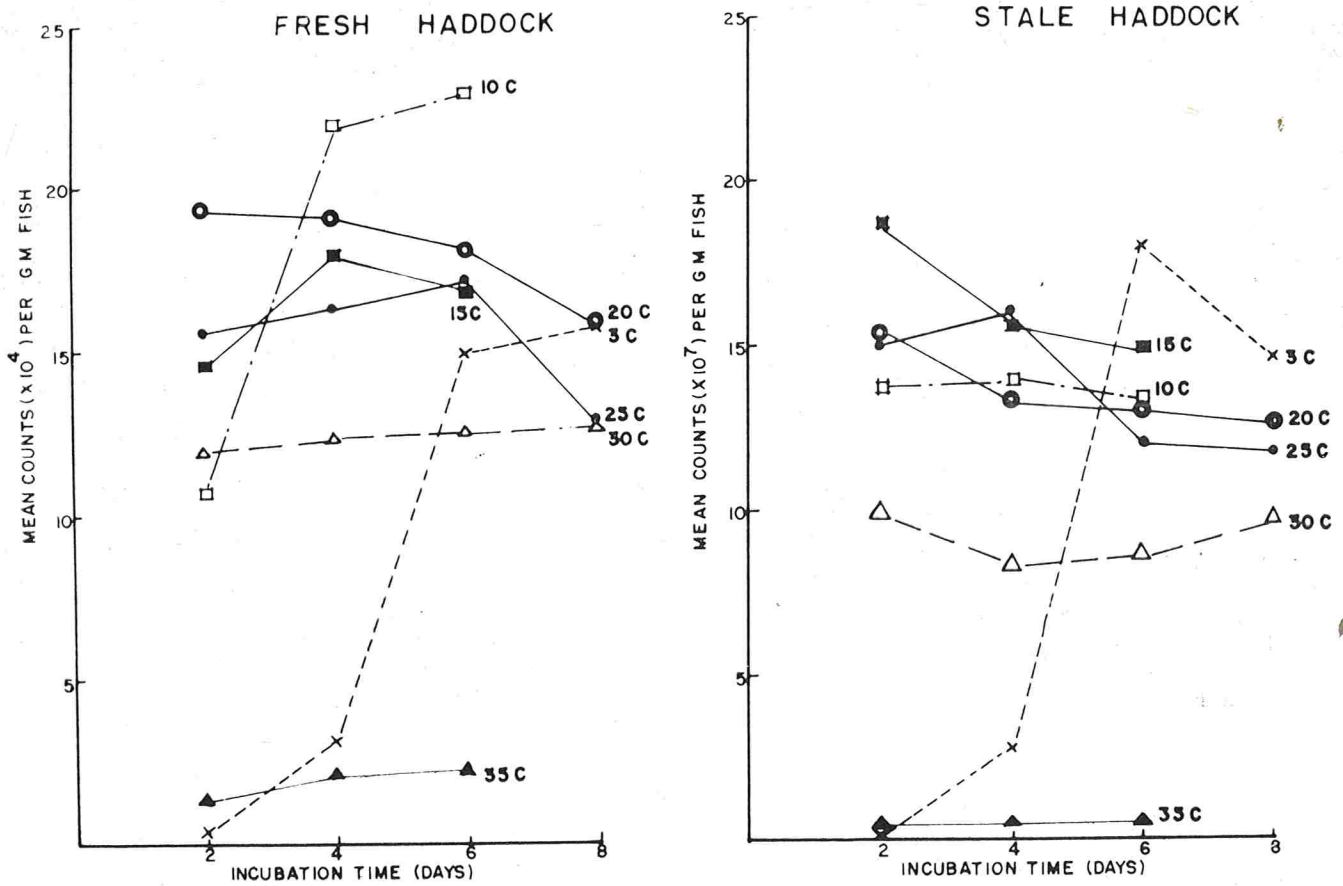


Figure 3. Effect of incubation temperatures on bacterial counts from fresh and stale haddock fillets. Plates were in-

cubated at the respective temperatures and the number of developed colonies plotted against time of incubation.

TABLE 7. EVALUATION OF INCUBATION TEMPERATURES FOR BACTERIAL COUNTS FROM FRESH AND STALE HADDOCK FILLETS<sup>a</sup>

Fish	Incubation temperature (C°)	Mean counts (X10 <sup>5</sup> ) per g fish after incubation time (days) <sup>b</sup>			
		2	4	6	8
Fresh haddock	3	0.025	0.31	1.49	1.57
	10	1.07	2.18	2.28	
	15	1.45	1.79	1.67	
	20	1.92	1.91	1.81	1.58
	25	1.56	1.63	1.71	1.28
	30	1.19	1.23	1.25	1.26
	35	0.14	0.21	0.23	
Stale haddock	3	13	280	1700	1460
	10	1370	1380	1330	
	15	1870	1550	1480	
	20	1550	1330	1310	1260
	25	1510	1590	1200	1180
	30	990	840	870	970
	35	54	62	62	

<sup>a</sup>Each count is the average of 2 dilutions plated in 4 replicates. Decreased count at the end of incubation time is due to confluent overgrowth of colonies. The enumerating medium consisted of: 0.2% yeast extract, 0.2% tryptone, 0.2% sodium lactate, 0.25% sodium chloride, and 1.5% agar in distilled water at pH 7.0.

<sup>b</sup>Refers to days of incubation of plates at the respective temperatures.

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# STARTER CULTURES FOR CHEDDAR CHEESE<sup>1, 2, 3</sup>

E. R. VEDAMUTHU AND G. W. REINBOLD

*Department of Dairy and Food Industry,  
Iowa State University, Ames 50010*

(Received for publication May 1, 1967)

## SUMMARY

Starter bacteria play very important roles in the cheese vat including major contributions to flavor development and control of harmful microorganisms; hence, starter production is the single most important operation in Cheddar cheese production. Thus, the proper selection, blending, preservation, and packaging of starter strains by the culture suppliers and the adoption of strict sanitation, temperature control, and standardized procedures in cheese plants for bulk starter production would contribute toward ensuring a sound and un-failing starter program in the cheese industry.

Of the various ingredients that go into a cheese vat, none is more important than the cheese culture. This culture is appropriately called "starter" because it initiates (starts) most of the reactions and changes that take place in the vat during the manufacture of cheese, and the starter's effects extend into the ripening period as well.

In short, the starter culture to a large measure determines the final body, texture, and flavor of the cheese when it reaches the consumer. Because of the wide-ranging effects on the marketable quality of the final product, the type, quality, make-up, and preparation of the starter are of primary concern to the Cheddar cheese industry.

To fully appreciate these attributes, it is necessary to understand the over-all functions of the starter in the manufacture and ripening of Cheddar cheese. For the most part, these same activities are required in the manufacture of other varieties of natural cheese. The starter performs two important functions, (a) acid production in the vat at a rapid, but uniformly apposite, rate, and (b) development of flavor.

## ACID PRODUCTION

The need for carefully regulated acid-production rates is realized when the role of acidity in the suc-

cessive steps of Cheddar cheese manufacture is considered. For example, initial acid production in the cheese milk is needed for the coagulation of milk by rennet. Acid formation in milk releases free calcium ions from the bound state, which are necessary for the rennet to bring about the clotting of milk (10). Acid also weakens the dispersed phase of colloidal casein by neutralizing the electrical charges or Zeta potential (31). Further acid production beyond that formed up to the time of setting is necessary for the expulsion of moisture (or whey) from the curd cubes (13). Beyond this point, continued acid production during matting and cheddaring helps to control body and texture characteristics (13).

Such "programmed" acid production protects the product from growth and development of undesirable microorganisms. These include spoilage types of bacteria, such as pseudomonads, *Bacillus* species, and clostridia, that may cause gassiness, off-odors, off-flavors, discoloration, rust spots, gas blowing, putrefaction, etc., as well as disease-causing microorganisms, such as *Staphylococcus aureus*, *Salmonella*, and *Clostridium botulinum* (1, 8, 13). Relatively few outbreaks of food poisoning have been traced to cheese, and in cases where cheese has been incriminated, the lot of cheese in question invariably has had a history of insufficient acid production attributable to inactive or insufficiently active starters.

Finally, the acid as such, imparts a portion of the over-all characteristic flavor of Cheddar cheese, usually described as "the pleasant acid taste and sharpness" (12). This contribution will be considered at greater length.

It is no wonder that the progress of the entire cheese making operation is monitored by titratable acidity or pH measurement at various stages (see Table 1).

## FLAVOR DEVELOPMENT

The second important function of the starter is the role it plays in developing the characteristic flavor of Cheddar cheese. This is manifested in two ways, by direct contribution, as noted, or by indirect contribution to flavor.

The direct contribution is due to (a) the compounds

<sup>1</sup>Editorial Note: First of two papers dealing with two important aspects of cheese making technology having particular significance relative to the public health safety and consumer acceptance of cheese.

<sup>2</sup>Journal Paper No. J-5669 of the Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa. Project 1488.

<sup>3</sup>Presented at the Dairy Industry Conference, Iowa State University, Ames, Iowa, March 29, 1967.

TABLE I. CHANGES OCCURRING DURING THE MANUFACTURE OF CHEDDAR CHEESE

No.	Step in the manufacture of cheese	Duration of process	Conditions in the vat				Changes occurring in the cheese milk or cheese curd	Purpose of the step in the manufacturing process
			Temp.		pH/acidity			
			S <sup>a</sup>	E <sup>b</sup>	S <sup>a</sup>	E <sup>b</sup>		
1	Flash heating of cheese milk.	Held for 16 sec	147		6.6/ 0.16	6.6/ 0.16	Expulsion of dissolved gases and volatile odors. Partial destruction of microflora, and natural milk enzymes.	To eliminate undesirable flora such as coliforms, psychrophiles, yeasts, and staphylococci.
2	Ripening of milk after addition of 1% starter.	60 min	86	86	6.6/ 0.16	6.5/ 0.17	Increase in acidity and shift in salt balance. Growth of starter organisms.	To initiate rapid microbial growth. For liberation of soluble Ca and neutralization of Zeta potential. To facilitate rapid rennet coagulation.
3	"Setting"—Addition of rennet and allowing milk to coagulate.	20-30 (preferably 30) min	86	86	6.5/ 0.17	6.4/ 0.12	Formation of smooth shrinkable matrix due to uniform coagulation.	To rapidly coagulate milk to form a uniform, smooth coagulum.
4	Cutting of curd.	10 min <sup>c</sup>	86	86	6.4/ 0.12	6.4/ 0.12	Large mass of curd cut into small cubical or rectangular pieces with the liberation of whey.	To expel entrapped moisture as whey. Increase surface area for expulsion of whey as the curd matrix shrinks.
5	Cooking with agitation.	30-40 min <sup>d</sup>	86	104	6.4/ 0.12	6.2/ 0.13	Shrinkage and firming of the curd. Increase in whey acidity and temperature. Increase in microbial numbers.	To facilitate expulsion of whey from the curd caused by the shrinkage of curd matrix with increasing temperature and acidity.
6	"Dipping" or drainage of whey.	Starts 135 min after rennet	104	101	6.2/ 0.15	6.2/ 0.16	Removal of whey. Expulsion of moisture due to compaction as the curd settles. Slight cooling of curd.	To remove whey from the curd.
7	"Packing"—Matting of curd.	15 min	101	101	6.1/ 0.16	6.1/ -0.22	Further expulsion of whey due to compaction, fusion of curd particles.	To fuse curd particles into a solid mass to make convenient for handling.
8	"Cheddaring"—Turning and piling of cheese curd slabs.	105 min (varies)	99	97	6.1/ 0.18 -0.22	5.3/ 0.55	Further expulsion of whey due to pressure. Increase in acidity. Increase in microbial population. Changes in texture.	To expel whey and gas, and develop meaty body and close texture.
9	"Milling"—Cutting curd into small 2"x1"x1/2" strips.	10 min	97	95	5.1/ 0.55	5.1/ <sup>e</sup>	Reduction in the size of curd slabs. Slight cooling of curd. Increase in bacterial numbers.	To facilitate uniform salting of curd and cooling and further expulsion of whey. Cooling to prevent fat loss.
10	Salting (2.5% by weight of raw curd).	20 min	95	92	5.1/ <sup>e</sup>	5.1/ <sup>e</sup>	Further cooling of curd.	To allow dissolution of salt in the curd to improve flavor and to cool the curd further to prevent fat loss.
11	Hooping—Filling milled, salted curd into molds.	15 min	92	90	5.1/ <sup>e</sup>	5.1/ <sup>e</sup>		To pack the cheese curd into blocks for easy handling and marketing. Pressure applied to expel moisture and fuse curd pieces.
12	Dressing, etc.				5.0/ <sup>e</sup>	5.0/ <sup>e</sup>		To protect the cheese from molds etc. during curing period.

<sup>a</sup>Start of process; <sup>b</sup>End of process. (Temp. in °F)

<sup>c</sup>Depending upon moisture level desired in finished product, up to 30 min of stirring curd in whey may precede cooking.

<sup>d</sup>Following cooking, up to 45 min of stirring curd in whey may precede dipping.

<sup>e</sup>Titrate acidity measurements no longer necessary or applicable.

(such as lactic, acetic, and propionic acids) formed by metabolism of carbohydrates, (b) certain odoriferous and flavorful compounds, such as aldehydes and ketones (acetaldehyde, acetone, diacetyl) or their precursors such as pyruvic acid, and (c) certain compounds (alcohol, for instance), which in combination with others (fatty acids, for example), form important components, such as fatty esters, that contribute to Cheddar flavor (19, 24). To a small degree, the starter organisms also break down proteins to impart a distinctive mellowness of flavor and solubility of protein to the cured cheese (23, 24).

The indirect effect (or contribution) is related to the effect of starter bacteria on the other bacteria that grow in cheese. The population of this group (adventitious microorganisms introduced into the cheese from the hands or garments of the workers, atmosphere of the cheese make room, equipment, etc.) is controlled by the starter population and its activity. Certain types of these accidentally acquired bacteria (23) are considered very important in typical flavor development of cheese. The time of appearance, the proportion in the total population, and the extent of the metabolic activity of other bacteria have been used as indexes of flavor development (11, 22, 23).

#### MICROENVIRONMENT OF CHEESE DURING ITS MANUFACTURE

To understand and appreciate the desirable qualities required of cheese starters, it is necessary to examine the physical, chemical, and biological environment and the stresses under which starter bacteria have to perform their functions.

Although the cheese milk usually is heat processed before being pumped into the vats, this treatment is mainly intended to destroy the coliforms, psychrophiles, yeasts, and other heat-sensitive flora. The heat treatment by no means eliminates other heat-resistant flora, such as sporeformers, certain mesophiles, thermodurics, thermophiles, bacterial-, yeast-, and mold-spores. Thus, the starter flora must compete with these other microorganisms if the milk is heavily contaminated. Further contamination with extraneous microorganisms from the atmosphere, equipment, clothing, and the workers' bodies occurs during the complete manufacturing process in the open vats. It is obvious that the entire microbiological environment in the cheese vat is competitive.

During coagulation of the milk, the starter bacteria are held immobile in the thickening curd. They must be able to grow in this location to establish conditions favorable for further growth.

As the cooking of the curd continues, the starter bacteria are subjected to gradual heating up to a

maximum temperature of 104 F. (Some cheese manufacturers are known to cook to 106 F, but this practice is rarely encountered.) The starter microorganisms, therefore, have to be heat-tolerant to perform their functions. As the cheesemaking process continues, there also is an increase in acidity. The starter flora should be capable of withstanding this increased acidity. After the curd is salted, salt tolerance becomes important because the degree of salt tolerance will determine the numbers of viable starter bacteria in the fresh, uncured cheese (20, 36).

Heat, acid, and salt concentration (apart from being considered as separate entities affecting the population and activity of starter bacteria) also should be considered in terms of composite effect; i.e., the combined effect of increasing temperature and acid concentration and the combined effect of increased acid and salt concentrations (33).

Finally, bacteriophages pose a serious challenge to starter flora during cheese manufacture. Other bacterial inhibitors, such as residual antibiotics and quaternary ammonium compounds, also are important. Since it is easier to police improper usage of antibiotics and quaternary compounds, however, the main cause of trouble is bacteriophage (6).

#### DESIRABLE PROPERTIES IN STARTER CULTURES

Having considered the functions of starters in cheese and the conditions under which these properties are performed, one can now list the desirable properties needed in starter cultures.

The bacteria in starter cultures should be vigorous, active, and metabolically competitive to predominate the entire flora in the cheese vat. Also, they should be present in sufficient numbers at the onset of the make procedure. These qualities depend on the type or strains of bacteria used in the starters and on the manner in which the cultures were made, shipped, stored, and propagated. The starter microorganisms should be chosen on the basis of rate of acid production under the entire range of temperatures attained in the cheese vat. Activity tests to meet these criteria have been developed (15). Additionally, starter bacteria should be tested for acid and salt tolerances and capability to withstand the combined effect of heat and acid and salt concentrations (7, 33).

Most bacteriophages attack only one specific type of bacteria. So by having more than one type of bacteria in the starter, one can insure against complete failure of the starter in the cheese vat. Hence, the mixed-strain starter should consist of bacterial types of unrelated phage-susceptibilities (6).

In addition to these considerations, there are other desirable properties.

Some strains of bacteria commonly used in cheese starters produce enormous amounts of gas (27). Such types cause floating curd in the vat (25) and severe gas-induced openness and splitting in the finished cheese (33). These types should be eliminated from the culture mixtures compounded for cheesemaking.

Certain varieties of lactic-acid bacteria used in starter cultures produce antibiotic substances against other strains, and certain others exhibit "antagonism" and "dominance" in mixtures. Therefore, strains to be used for making up composite cultures should be screened for "compatibility" (3, 5, 35).

Studies made in Canada (9) showed that certain types of starter bacteria that lacked good protein-splitting ability produced bitter cheese in repeated trials. The bitterness was due to incomplete breakdown of proteins. Some dipeptides and polypeptides have a bitter taste. Thus, the selection of strains that do not produce bitter flavors (presumably, strains with sufficient proteolytic ability) also is important.

Work done in Australia (7), New Zealand (22), and in the U. S. (35) has shown that certain types of starter bacteria predominate in the cheese during ripening for prolonged periods and thus suppress the appearance, growth, and metabolic activity of the adventitious bacteria considered necessary for normal flavor development in the cheese. Such types should be eliminated from the starter mixtures to prevent abnormal flavors or lack of full flavor in the final product.

Recent investigations have shown that there is a definite relationship between the use of certain strains in starter mixtures and the development of fruity flavor in Cheddar cheese (33). Such strains accumulate high concentrations of certain compounds (aldehydes, ketones, and their precursors) that, by chemical combination with or by blending with other compounds in the cheese, are considered to impart the fruity off-flavor (34). A chemical test on milk cultures has been developed for screening out these undesirable bacteria. Certain other varieties of the commonly used starter bacteria produce "malty" flavor, and these strains also should be avoided in starter mixtures for cheese (17).

#### STARTER FLORA AND HANDLING

The types of bacteria commonly found in cheese starters belong to the lactic group of the genus *Streptococcus*. This group comprises the species *Streptococcus cremoris*, *Streptococcus lactis*, and *Streptococcus diacetylactis*. Some commercial mixtures also contain microorganisms belonging to the genus *Leuconostoc*; namely *Leuconostoc citrovorum* and *Leuconostoc dextranicum*. These organisms can be differentiated by simple tests described by San-

TABLE 2. TAXONOMIC TEST FOR DIFFERENTIATING TYPES OF BACTERIA IN CHEESE STARTERS (28)

Organism	Coagulation of milk in 48 hrs at 30 C	Diacetyl production	NH <sub>3</sub> from arginine	Dextran from sucrose	Preparation with Group N antiserum
<i>S. lactis</i>	+	-	+	-	+
<i>S. cremoris</i>	+	-	-	-	+
<i>S. diacetylactis</i>	+	+	+ <sup>a</sup>	-	+
<i>L. citrovorum</i>	-	+ <sup>b</sup>	-	-	-
<i>L. dextranicum</i>	-	+ <sup>b</sup>	-	+	-

<sup>a</sup>A few strains do not produce ammonia from arginine.

<sup>b</sup>Only in association with *S. lactis* or *S. cremoris*.

dine (28) and shown in Table 2. *Streptococcus diacetylactis* and the *Leuconostoc* species are heterofermentative and use lactose and citrate in milk to produce appreciable amounts of metabolic by-products (such as acetic acid, acetaldehyde, alcohol, and carbon dioxide) other than lactic acid. *Streptococcus lactis* and *Streptococcus cremoris* are generally considered homofermentative, although there are a few exceptions (32). In recent investigations, *Streptococcus cremoris* strains have been found suitable for consistent production of good quality cheese; certain strains of *Streptococcus lactis* and the heterofermentative species have been determined to cause fruity flavor in the cheese in repeated trials (33).

The proper selection and blending of bacterial types in mixed strain starters concern primarily the culture manufacturing laboratories. The commercial culture suppliers have made serious attempts to incorporate many of the research findings in their manufacturing processes. Many of the suppliers screen the strains that go into the starter mixtures for gas production (26), rate of acid production (8), and compatibility and phage susceptibilities (5). Some starter manufacturers blend only pretested strains to eliminate those that accumulate high concentrations of a certain class of chemical compounds (carbonyl compounds) found to have an indirect relationship to off-flavors in the finished cheese (33). Several manufacturers include nutritive additives, such as various protein hydrolysates or pancreas extracts, in starter mixtures to ensure rapid growth and vigorous activity (4, 30). Innovations, such as quick freezing in liquid nitrogen (at -196 C or -321 F) and shipping in cartridges immersed in liquid nitrogen, have been introduced to provide maximum viability and vigor of starter cultures (2, 18).

With the introduction of phage-inhibition media, further screening of strains for adequate growth and activity in the specific medium recommended also

has been adopted by culture suppliers. In general, most of the types used in starter cultures, except citrate-fermenting bacteria (or flavor bacteria), adapt themselves to one brand of phage-inhibition medium used extensively in the U. S. (14, 21). For Cheddar cheese manufacture, citrate-fermenting (or flavor bacteria) organisms are considered unnecessary and oftentimes undesirable (33). Further developments predicted in the culture manufacturing technology include frozen concentrated cultures in droplet forms, which could be used for direct setting of bulk starters (2).

A successful culture program for cheese manufacture does not end with the acquisition of starter cultures from a reputable culture supplier. It may be no exaggeration to state that most of the problems with starters arise from improper storage, propagation, and handling of the cultures in the cheese plants.

In storing cultures, it is best to follow the instructions of the supplier. Where no instructions are available, a good practice would be to hold them in the freezing compartment of a refrigerator or freezer until use. This applies to frozen liquid cultures and lyophilized powders. The cultures fast-frozen in liquid nitrogen should be kept in the receptacle provided by the supplier. When stored this way, all cultures once thawed and opened should be used completely and immediately. With frozen cultures, rapid thawing is most desirable (2). Where culture rotation is used to insure against starter failure due to bacteriophage (38), a suitable program should be arranged in consultation with the starter supplier.

In the preparation of propagation media using non-fat dry milk solids pretested for the complete absence of antibiotics, or phage-inhibition media, best results are obtained by following the manufacturer's instructions (14).

Recent work has clearly shown that, for consistent good results, there should be a minimum of transfers from the mother-culture stage to the bulk starter. Such a practice, not only reduces the chances of accidental contamination, but also helps to maintain the proper balance between strains in the mixture (35).

In making up bulk starter, after heat treatment at 180 to 200 F for 45 to 60 min, the medium should be rapidly cooled to the setting temperature of 72 F (13) and immediately inoculated. If held for long periods at 72 F before inoculation, survivors of the heat treatment (*Microbacterium lacticum*, certain *Bacillus* species, spores of bacteria, yeasts and molds, etc.) and certain contaminants that might have accidentally gained entrance, would have ideal conditions in which to rapidly develop and increase in numbers. This situation would permit the foreign microorganisms to effectively compete with the seed bacteria

added as the inoculum. In many cheese plants, where bulk cans are used for setting up bulk starter, often the temperature of the milk in the individual cans is not checked before inoculation. Milk in a bulk can cools very slowly, and unless the can is thoroughly stirred before checking the temperature, the inoculum may be added before the interior milk has sufficiently cooled. This being the case, a high proportion of the strains in the starter mixture could be injured or destroyed by heat, resulting in an inactive or poorly active culture. Very good activity is obtained when the culture is ripened up to 0.75 to 0.85% titratable acidity, rapidly cooled after the desirable level of acidity is reached, and held at 38 to 40 F until use.

A separate room, well protected from drafts from the rest of the plant (38), provided with positive pressure and (or) suitable air filters (37), and fitted with chlorine-fogging facilities (29), is highly important. Such an arrangement affords good protection to the starter culture from contamination with undesirable bacteria, bacteriophage, and other microflora. A recent cost survey of starter production in Cheddar cheese plants in Iowa with and without separate starter rooms has shown that the provision and operation of a well-designed and well-equipped separate starter room amounts to only a very small fraction of the total cost of starter production for day-to-day operation (16). In plants with a large volume, assignment of a separate labor crew for starter room work alone, with separate locker facilities, clothing, and footwear would be a worthwhile investment.

Above everything else, strict sanitation in the plant and the laboratory is the key to a successful starter program.

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