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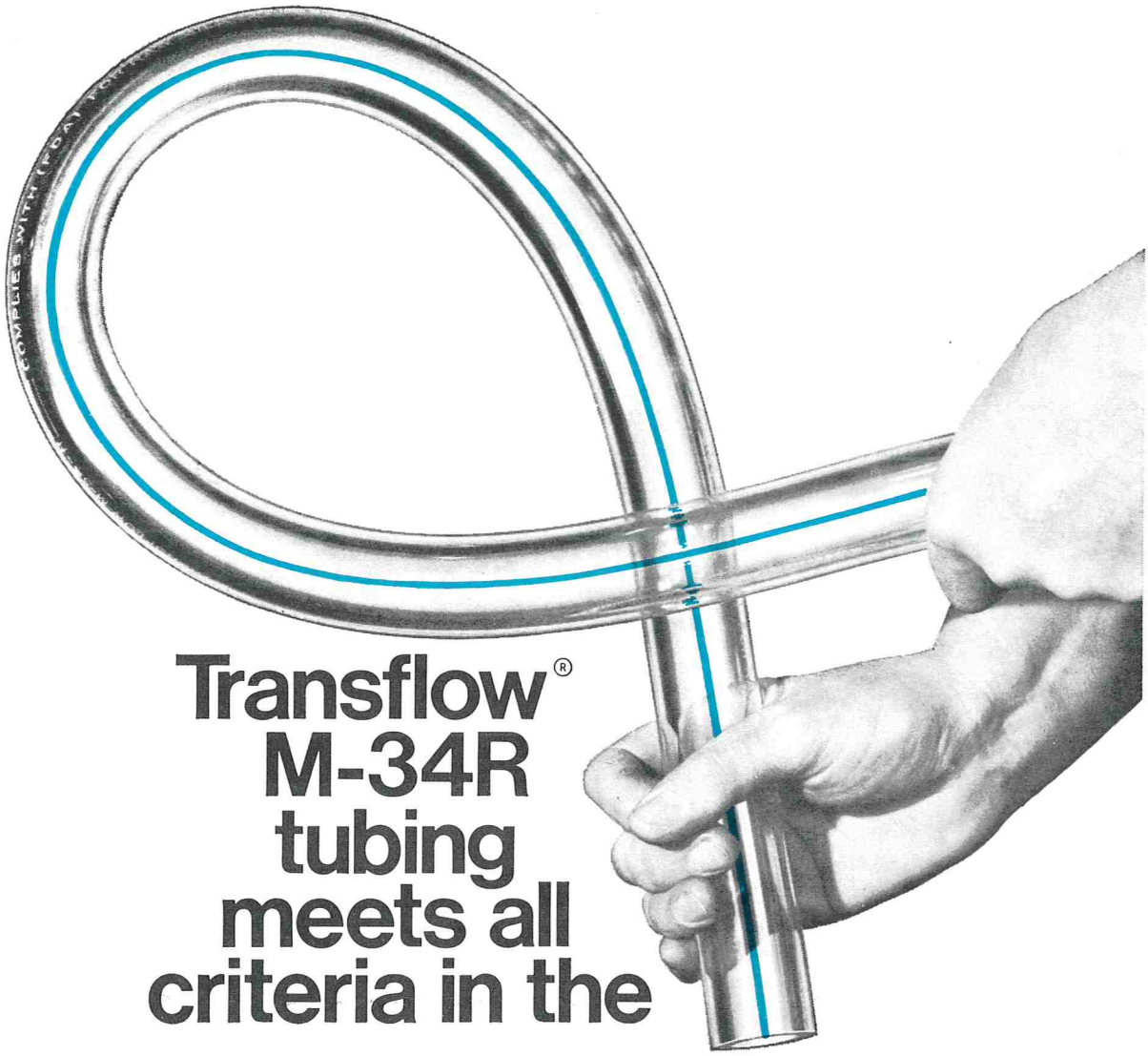

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Vol. 37 July, 1974 No. 7

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COMPILED AND EDITED BY

**J. C. FLAKE, A. E. PARKER, J. B. SMATHERS, A. K. SAUNDERS AND E. H. MARTH**



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# MICROBIOLOGICAL MONITORING OF THE FOOD PLANT: METHODS TO ASSESS BACTERIAL CONTAMINATION ON SURFACES[1]

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Abstract

Procedures for microbiological assessment of bacterial contamination on surfaces of equipment and foods are reviewed. The procedures for food-contact surfaces include the following categories: swab contact, surface rinse, direct contact, agar and impression methods, and vacuum probe. Three categories of methods to estimate bacterial numbers on food surfaces are discussed. The importance of knowledge about the various techniques, design of sampling, their limitations and variations are emphasized. Design of sampling and interpretation of results are also considered.

Detection and enumeration of microorganisms on environmental surfaces is of concern not only in the food industry but also in such areas as the space program, research laboratories, hospitals, and the like, *(5, 16, 17, 29)*. The ultimate specific use of the data obtained from surface sampling naturally depends on the industry involved. In general, the level and type of surface contamination is the desired information. The food industry puts such information to use in evaluating the efficiency of cleaning and sanitizing compounds and in assessing the overall sanitary condition of food processing areas.

The specific technique to use in sampling an environmental surface is a problem common to any industry or agency involved in contamination profiles. Within a single industry no one technique can be used for all situations; a choice must be made of the technique that will best solve the problem encountered. The sanitarian or investigator needs to know the available techniques of sampling so he can make the best choice by recognizing the pitfalls of the methodology. The choice will depend on three major factors: *(a)* what you hope to prove, *(b)* what effort you want to put into getting the answers with the available equipment, and *(c)* how precise or accurate you need to be.

The object of this paper is to review several of the common techniques available to the food industry for assessing the level of microbial contamination on surfaces. Also, several modifications of these techniques will be discussed along with certain methods of surface sampling used in other industries.

[1]Presented in part at the 60th Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, Rochester, New York, August 13-16, 1973.

## Surface Sampling Techniques

Most of the surface sampling techniques used to assay microbiological contamination fall into three categories based on methods of transport: *(a)* removal of contamination by direct contact with materials, e.g. contact agar plates, swab contact, etc.; *(b)* elution of the contamination by rinsing to allow a microbial assay of the resultant suspensions and *(c)* a combination of the first two methods as with the swab method. A fourth transport method utilizes vacuum and is based on air velocity impinging particulate contamination onto a membrane filter or growth supporting nutrient.

### *Swab method (Swab contact)*

The first report of the swab method was made in 1917 by Manheimer and Ybanez (cited in Favero et al. *(17)*. The recommended swab procedure of the APHA *(2)* is essentially the same as the original method. The method involves rubbing a moistened sterile cotton swab over the test surface and placing the swab in a dilution bottle to be subsequently diluted and plated on an appropriate medium. The swab method is adaptable to a variety of surfaces and can provide information on the number and/or type of microorganisms on an environmental surface. The swab contact technique has certain advantages and disadvantages as listed in Table 1B.

The swab method has been modified in many ways. One of the first modifications was the use of calcium alginate fiber to replace the cotton fiber *(23)*. Calcium alginate dissolves in Ringer's solution or sodium hexametaphosphate, and thus frees any microorganism entrapped and apparently produces higher counts than the cotton swab. However, there is contradictory evidence supporting higher counts by the use of calcium alginate swabs over cotton swabs *(17)*. Certain researchers contend that cotton is superior to alginate swabs *(17)*.

Greene and co-workers *(20)* reported a modified swab method that they call "Swabpression." The device consists of a replicating floc material (velveteen) mounted on a section of metal pipe or curved

section of metal. The floc is cut to a definite size and mounted by double stick tape. The sterilized unit is rolled over nutrient agar to moisten the floc and next rolled once over the test surface and finally rolled over a nutrient culture agar that is subsequent incubated. The "Swabpression" method is considered an improved swab technique and it can be used in lieu of the contact agar plate to give comparable counts in the evaluation of multi-shaped surfaces *(20)*.

Another modification of the calcium alginate swab has been presented in a report by Walter *(29)*. The method suggests that the dissolved calcium alginate swab sample be filtered through a membrane filter. The membrane filter is placed on a growth medium, incubated, strained, and observed by direct microscopic examination. An alternative incubation method involves incubating the membrane filter directly on a nutrient pad of differential growth medium and thus counting directly under a sterescopic microscope (10×) *(6)*. Numerous publications have described application of the membrane filter technique in surface sampling *(4, 18)*.

Higher recovery is obtained when an analyst swabs several similar sites with the same swab rather than swabbing a single site with a single swab. This will help average out variations of counts between similar areas with non-uniform distribution of bacterial cells. In addition, variation between similar areas is reduced by swabbing several large areas instead of smaller areas in localized sites *(4)*. Evaluation of the variation in the swab technique should include such variables as: *(a)* angle and pressure applied to the swab, *(b)* inability to swab equivalent areas on localized sites, *(c)* incomplete release of bacteria from the swab during shaking of the diluent, *(d)* error associated with pipetting and counting, and *(e)* porosity of the surface.

As a point of clarification, the swab contact method should not be confused with the "swab-smear" technique that is used in diagnostic bacteriology. The latter provides qualitative data and is not designed to deal with bacterial enumeration.

*Surface rinse method*

The rinse method is an older, somewhat standardized method used largely in the dairy industry *(2)*. The method involves taking a sterile fluid that is mechanically or manually agitated over an entire surface. The rinse fluid is then approapriately diluted and plated. Where applicable, the method is more precise and accurate than the swab method because the entire surface is tested. Sampling an entire area reduces error and yields higher recoveries by eliminating error introduced by inconsistent or non-uniform contamination. The method has application mainly in evaluating food containers, samilar utensils, or pipelines. The method is not applicable to surfaces of large stationary equipment where the test liquid cannot be contained. The rinse method can be used to good advantage and may become a method of choice for certain applications if accompanied by a scrubbing action over the entire surface (See Table 1A).

The surface rinse method is aided by the use of the membrane filter, especially when microbial counts are low (e.g., pipelines "cleaned in-place"). The membrane filter concentrates the microorganisms, thus increasing precision of the rinse technique. The membrane bearing organisms on its surface, can be incubated intact on a saturated pad containing about 2 ml of broth medium, usually double strength *(6)*. After 4-6 h of incubation, it is possible to stain and observe microcolonies on the filter using 80 to 100× maginification *(31)*.

Ultrasonic energy is becoming widely used as a technique to dislodge microorganisms from surface sampling devices. Heretofore, the application has been used primarily in analysis of space craft surfaces where component parts require sterilization *(17)*.

## CONTACT METHODS

The agar contact method is widely used. Many publications have referred to the method and its modification *(2, 5, 21, 22)*. The method is most useful on smooth or semi-smooth, flat surfaces. The agar surface is simply placed onto the test area, removed, covered, and incubated. A point of caution is that the contact plate should be pressed and not wiped on a surface to prevent distortion of the colony count. The method can be qualitative or quantative depending on the type of agar medium; however, it can only quantitate relatively low numbers. High levels of microorganisms will completely obscure any quantitative reading. Molds and spreaders present difficulty to the analyst in counting the colonies. The main drawback is that the contact plate cannot distinguish between single cells and/or clumps of cells on a surface. Quantitative data are not as accurate as from the swab method because the swab tends to break up clusters of cells, thus providing data more representative of a true cell count. Advantages and disadvantages are listed in Table 1A.

The most widely used name for the agar contact plate is the RODAC Plate (Replicate Organism Direct Agar Contact ) *(21)*. The RODAC plate is available commercially and is often used in field studies because of its simplicity and portability. Virtually no supporting materials or equipment are

TABLE 1A. A COMPARISON OF METHODS FOR THE ASSESSMENT OF MICROBIAL CONTAMINATION ON SURFACES

| | Rinse method | | Contact method | |
|---|---|---|---|---|
| | Surface rinse | Spray | Agar plate (RODAC) | Acetate tape and filter pad |
| Field application | Limited to container-type utensils and equipment | Very limited | Wide use, excellent method | Wide use |
| Recovery | Moderate (70%)[a]; improved by scrubbing action | High percentage (95%)[b] | | Low to medium percentage |
| Precision | Fair to Good (71-97%)[a] | Good | Fairly good to excellent (reproducibility) | Fairly good |
| Surface requirement | Restricted to horizontal enclosed surfaces (trays, bins, pipelines) | Restricted to horizontal surfaces; adapts to rough irregular surface, e.g., surface of meat cuts | Restricted to mostly flat, dry surfaces; adapts to use on food surfaces | Restricted to smooth, flat equipment surfaces; or meat or poultry surface (filter used as static swab test) |
| Recognized | APHA | Experimental or laboratory use | APHA (1972) standard method | As field procedure |
| Time | Considered rapid | Slow sampling | Rapid sampling | Rapid sampling |
| Convenience | Fairly simple; laboratory facilities needed minimal; works on heavily contaminated surfaces | Simple procedure; requires special equipment and laboratory backup; works on heavily contaminated surfaces | Simple; easy to use requires no dilution, equipment or laboratory (RODAC); permits large number of samples; constant test area, valuable teaching aid | Simple; easy to use; no special equipment; laboratory backup not required; large number of samples |
| Error | Low inherent error (entire area sampled) | Minimal | Minimal if large enough number of samples taken in localized area | Minimal; exact count not obtainable; high number not obtainable; error reduced by increasing number of samples for each test area |
| Modification | Use of membrane filter (excellent for low levels contamination and for food surface assay) | Use of special diluents to cope with physical and chemical nature of test area | Use of agar slice methods (syringe extrusion, agar sausage); use of selective and differential media | Use of selective and differential media |

[a]Percentages from Greene and Herman *(19)*
[b]Clark *(11)*

TABLE 1B. A COMPARISON OF METHODS FOR THE ASSEESSMENT OF MICROBIAL CONTAMINATION ON SURFACES

| | Swab contact method | Direct surface agar plate | Vacuum method |
|---|---|---|---|
| Field application | Wide use | Wide use | Limited |
| Recovery | Moderate 52-90%)[a] | High (80%)[a] | High percentage on dry smooth surfaces |
| Precision | Lacking (difficult to duplicate results) | Very good (87-98%)[a] | Good where sufficient number of cells present (ca. $10^4$/ sample) |
| Surface requirement | Adaptable to a variety of surfaces (best on smooth, flat, non-porous surfaces) | Restricted to smooth, horizontal equipment or utensil surfaces | Restricted to smooth dry surfaces |
| Recognized | APHA (1972) standard method (cotton swabs) | Field procedure | Space program, clean room work |
| Time | Considered rapid | Rapid sampling | Slow sampling |
| Convenience | Simple test and easy to use; minimal laboratory facilities needed; works on heavily contaminated surfaces | Simple; *in situ* sample; no special equipment required; laboratory backup not required but desirable | Tedious and cumbersome; sophisticated equipment and laboratory backup required in comparison to other methods |
| Error | Large: due to inherent variability in test, greasy or sticky surfaces swab retains some organisms, operator techniques vary | Exact counts not obtainable | Minimal in counts exceeding $1 \times 10^4$/ sample; dessiccation may reduce viability |
| Modification | Use of alginate, dacron, or rayon swabs (APHA 1972 supplemental method) | Use of selective and differential media | Use of selective media |

[a]Percentages from Greene and Herman *(19)*

required for this technique. Although it is considered to be an excellent method for certain field applications, its accuracy is low (about 50%); nevertheless, its precision is quite high *(5)*.

*Agar slice methods*

The agar syringe as proposed by Litsky [cited by Walter *(29)*] is the first agar slice method. A syringe-like apparatus is filled with melted agar medium. The solidified agar can be pushed out to be used to make contact with the test surface. The portion making contact is then sliced off with a sterile scalpel or wire and is placed in a culture plate and subsequently incubated. The agar syringe provides the same benefits as the agar contact plate while minimizing space requirements; however, it should be realized that the agar syringe method is not applicable to a wide variety of situations, only to those surfaces that are smooth, flat, or slightly rounded with a fairly uniform contamination level. In addition, agar slices will not give exact counts of microorganisms present on the surface because of bacterial clusters (micro-colonies) and non- adhering cells. However, if sufficient samples are taken, comparative values can be obtained because errors in the method are randomly distributed.

As a substitute for the syringe, artificial sausage casing will serve as a satisfactory container for cylinders of agar *(10, 28)*. The agar sausage method is highly regarded, especially in Europe. In the United States the method has not gained routine prominence as a quality control technique. It, like the other agar contact methods, provides a pictorial demonstration of the presence of bacteria on a variety of surfaces and can serve as a valuable teaching aid to food handlers. As with other agar contact methods, the agar sausage technique possesses simplicity, speed, and economy but its recovery rates are relatively low in comparison to other methods. Thus, practical experience is necessary for interpretation of results. Any agar contact method warrants consideration over the cotton swab technique in situations where routine replicate determinations are required.

*Impression methods*

The acetate adhesive tape method, described by Edwards and Hartman *(15)*, is a contact method devised to detect and identify molds present on surfaces. The exposed tape is stained and viewed microscopically. The method as described does not distinguish between living and dead cells and thus

will measure only gross contamination. A similar tape contact method, available commercially, is designed to contact a test surface and thus act as a replicator to transfer cells to a growth supporting nutrient agar. The agar plates are incubated and counts are determined after 24 to 48 h. This type of contact method simply gives a mirror image of the contamination and does not distinguish between particles of contamination containing one or more cells (Table 1A).

A plastic replica embedding method utilizing fingernail polish to form a peelable film has been devised *(25)*. The polish placed on the test surface forms a film that can be removed and stained for microscopic examination. The method does not distinguish between living and dead cells. Application is somewhat limited because a high level of contamination (ca. $10^5/cm^2$) is required before organisms can be located by optical scanning.

Another contact method reviewed by Walter *(29)* utilizes a membrane filter. The moistened or nutrient-soaked filter is placed on the test surface, removed, and incubated on an agar- or broth-saturated pad. After several hours, the filter is stained (0.5% malachite green; 5 sec), dried, and read under low power microscopy. A modified method by Förg [cited by Hartman *(22)*] uses paper strips impregnated with a moist agar medium. Strips have a plastic backing that is removed after handling. Strips are incubated in sterile envelopes. Tetrazolium is included in the medium and the dye induces production of red colonies and thus facilitates counting.

If any contact method is to be used to detect the efficacy of disinfestion then specific neutralizers should be present in the agar (See Table 2). Commercial media containing neutralizers such as Tween or lecithin are available. The mode of action of neutralizers added to agars has not been fully described *(2)*.

*Direct surface agar plate*

The direct surface agar plating (DSAP) procedure has been recognized as a valuable technique to examine microbial contamination on surfaces in situ *(3, 4)*. In sampling eating utensils, melted agar medium is poured into cups, plates, etc., is allowed to solidify; is removed aseptically; and is transferred to sterile culture plates. It is overlayed with agar and incubated. As an alternate method, the agar slab is protected by a suitable cover and left in place. Microbial counts are taken after the incubation period.

The direct surface plating method is fairly accurate on clean surfaces free of residual germicidal agents. A summary for the DSAP method is given in Table 1B. Residual sanitizers or disinfectants on the test surfaces will interfere with development of microbial growth and thus should be neutralized. The method is not reliable on heavily contaminated surfaces because resultant colonies will coalesce. If the antibacterial activity is eliminated, the method is valuable to the food industry especially to evaluate sanitizing materials and washing equipment (Table 2).

TABLE 2. NEUTRALIZERS FOR COMMON GERMICIDES[1]

| Agent | Neutralizer |
|---|---|
| Halogens | Sodium thiosulphate |
| Quaternary ammonium compounds | Lecithin |
| Hexachlorophene | Tween 80 |
| Phenolic disinfectants | Tween 80, charcoal, ferric chloride |
| Formaldehyde | Ammonium ions, Tween 80, sodium sulfite |
| Hydrogen peroxide | Catalase, sodium thiosulfate |
| Mercurials | Sodium trioglycollate, sodium sulfite |
| Ethanol | Lecithin in Tween 80 |

[1]Table from Favero et al. *(17)*.

## VACUUM METHOD

The vacuum method has been used experimentally to evaluate microbiological contamination in hospital carpeting *(17)*. A canula connected to a vacuum system is attached to either a slit sampler or to an all glass impringer. The vacuum removes particles, impinging them against an agar surface in the slit sampler or into a liquid medium in the impinger. The method is rather sophisticated and requires laboratory back-up.

The vacuum probe method to sample environmental surfaces was developed in the mid-1960's by the Sandia Corporation *(13, 14)* and later modified by Farmer and co-workers *(16)*. The vacuum probe has been used almost exclusively in the space research program. The device will pick up particulate contamination on relatively flat surfaces by means of a shock wave produced by the flow of air through a critically sized orifice. Particles on the surface are removed my vacuum and are captured on a membrane filter located inside of the probe cone. The method is useful to sample large areas with relative low contamination levels. Application to the public health and criminal investigation fields is suggested *(16)*.

The use of the vacuum probe in food processing plants has been investigated by Farmer and Pierson

(Virginia Branch of American Society for Microbiology Annual Meeting, September 20, 1973, NASA, Langley AFB, Hampton, Virginia). Its recovery is comparable to the swab method; however, the equipment requirements and complexity of this system make its use impractical in food processing plants (Table 1B).

## Estimation of Bacterial Numbers on the Surface of Food

Most methods for microbiological sampling of surfaces are designed for smooth, non-porous surfaces as on equipment or utensils. Methods to enumerate microorganisms on the surface of food are often destructive, that is, methodology utilizing maceration of a sample cut directly from the product in question. The swab method has been advocated for sampling the surface of various cuts of meat or poultry; although it is non-destructive in nature, its recovery of microorganisms is low and reproducible quantitative results are difficult to achieve.

*Rinsing the food surface*

The rinse method to assess the level of microorganisms on whole poultry or its pieces has been described *(1)*. Because of difficulty in relating microbial count to a given surface area, the worker must rely on a graph or table relating weight in grams (poultry part) to surface area ($cm^2$) *(1)*. Simonsen *(27)* has recommended shaking a whole chicken in a plastic bag containing approximately 1000 ml deluent. The container is shaken vigorously for 30 sec to give higher recovery. To convert the number of bacteria/ml to the number/$cm^2$ on the surface of a broiler, one uses: wt(g) + 500 = surface ($cm^2$) of skin *(27)*.

Mechanical shaking has added greatly to the effectiveness of the rinse method as reported by Pierson and co-workers *(26)*. In their method, meat cubes were placed in a jar with 100 ml of diluent and then placed on a reciprocating shaker for 5 min. The rinse solution was plated by appropriate microbiological procedures. Dimensions of the meat cube were measured, thus microbial counts were related to surface area. Addition of glass beads to a shaker vessel greatly enhanced recovery *(24)*.

The spray method is a nondestructive precedure, reported by Clark *(11, 12)*, that is designed to assay the number of microorganisms present on surface of meat, poultry, or other food. The method is based on a spray gun apparatus that empinges a washing solution under pressure against a circumscribed area of surface. While washing the surface, the rinsing solution is collected in a receiver tube and by subsequent plating of the resultant suspension the number of bacteria is determined. The method is excellent for studies involving food surfaces and has been shown to be superior to conventional methods. The drawback is that it can be used only for an approximately vertical surface (Table 1A). There is a question as to whether or not the method will successfully remove or dislodge bacteria from feather follicles. There is also a problem with the removal of *Acinetobacter* spp, (formerly called *Achromobacter*) some of which form gelatinous colonies *(9)*.

*Blender method*

The blender method for samples of skin or internal tissues is reported by Avens and Miller *(7)* to give higher recoveries of bacteria than other methods when used to test poultry skin. Maceration allows recovery of those bacteria inside feather follicles as well as those found on the surface. The difficulty comes in equating the sample to a surface area of approximately 50 $cm^2$ *(8)*. Skin of other species would have to be equated on a weight to surface area basis. Surface of internal tissue presents difficulties in equating numbers to area. A sample taken on a weight basis is generally preferred by most analysts. The proportion of exposed exterior surface to unexposed internal surface in a given sample greatly affects the bacterial counts. Thus, it is difficult to obtain a representative surface sample with proportionate internal tissue without macerating representative portions.

The use of mechanical shaking in lieu of the maceration method has the advantage of avoiding the necessity of sterilizing large number of blender jars and cutting devices. Mechanical shaking merely calls for sterile shaker jars containing sterile water and glass beads and this allows the handling of large numbers of samples. Consistently higher counts have been reported from samples shaken with glass beads than from samples macerated mechanically *(24)*.

Use of skin samples is destructive in nature and removal of skin tends to down-grade or deface the product. A way to prevent damage to the carcass is the use of neck flap skin *(27)*. Another non-destructive skin method proposed by Williams *(30)* involves use of a sterile tin can placed firmly against the surface of the carcass. Twenty five milliliters of diluent are placed in the can. The diluent is stirred while the skin is scraped, but not macerated, with a sterile spatula to release the maximum number of organisms. The method gives a 10-fold increase in count over the swab method *(30)* and does not tear or damage the skin.

*Contact methods*

The agar sausage has been reported to be useful to evaluate contamination on carcasses *(28)*. The method is non-destructive; a low correlation is expected between counts from the maceration method and the agar sausage method. However, there is great advantage in simplicity of use and equipment required. One of the main differences is the breaking of bacterial clumps by the mechanical process of macerating and this results in higher bacterial counts. The agar contact plate offers the same advantages and can be used as well in tracing a particular type of microorganism (e.g. fecal variety) through all phases of processing.

Walter *(29)* reviewed a non-destructive method for assaying microbiological contamination in carcass meat. The method features a nutrient-soaked filter pad placed on the test surface. The contacted pad is disintegrated by blending in a diluent following a routine bacteriological plating. This method is similar to a contact method described in the preceding section, the one where filter pad strips from test surfaces were incubated for several hours, stained, and viewed directly with low power microscopy.

## DESIGN OF SAMPLING

The first step in the sampling program design is to establish which points on the processing line constitute "direct" and/or "remote" contamination. Sampling should be oriented most heavily toward those areas that routinely contact the product (e.g., belts, chains, vats, etc.). Remote areas need not be sampled as frequently. As a suggestion, remote areas should be tested by a simple, time saving method, such as the RODAC Plate that determines gross contamination with the least amount of effort.

The sampling plan should be designed to cover the overall plant areas and to adequately cover localized areas along an individual processing line. Sampling should be based on sound statistical methods. At least three samples per localized area are needed to insure a high degree of confidence. Also, use of random samples is essential in a localized situation. In addition, the sampling plan will depend on the reasons why an operator is sampling in the first place. An operator may be attempting to detect a source of contamination that may call for a restricted sampling program. Another reason for sampling might be to develop a microbiological profile of a processing operation. Here a broad sampling approach is in order.

The size of an individual sampling area is important to the sampling program. When detection of small differences is important, one has to establish a sample size sufficiently large to give readable microbial counts. On a standard decimal dilution of 1/10 it is desirable to have an area sufficiently large to yield at least 30 colonies on a culture agar plate.

## INTERPRETATION OF RESULTS

To meaningfully interpret results the first consideration is the objective(s) of the experiment (what are we hoping to accomplish?) The type of sampling procedure is most important, since in interpretation of data, certain methods do not distinguish between contamination of single cells and that of a clump or micro-colony. Variables of the tests used should be analyzed. The technique with the least number of variables will detect smaller differences. The need to detect small differences is important especially in evaluation of cleansers and sanitizers.

In data reduction, it is necessary to calculate the mean count of several samples. When there is extreme variation in counts, or when counts are high, then a geometric mean should be used. Where there is small variation in counts or the counts are rather low, the arithmetic mean may be quite acceptable.

## CONCLUSIONS

There is a great deal of interest in the food industry in improved methods to control and monitor numbers of microorganisms. Contributing to this interest is the activity and movement toward microbiological standards and stricter sanitation regulations as implemented by regulatory agencies. Also, the increased use of aseptic packaging in food processing is demanding specific control of microorganisms. Improved microbial control needs to be monitored with approapriate surface sampling methods. Thus, sampling techniques are of greater importance not only to evaluate cleaning and sanitizing procedures, but to evaluate all sources of contamination, whether they be food contact surfaces, the food, or man.

Finally, it is hoped that this report will help those individuals concerned with evaluating and selecting the proper sampling technique to do the job in question. If one is aware of the advantages, disadvantages, and especially the limitations of the method, he will be better able to obtain meaningful results with the least expenditure of time, effort, and money.

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# COMPARISON OF *SALMONELLA* POLYVALENT H ANTISERA, DIRECT FLUORESCENT ANTIBODY, AND CULTURE PROCEDURES IN DETECTING SALMONELLAE FROM EXPERIMENTALLY CONTAMINATED GROUND BEEF UNDER FROZEN STORAGE

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Abstract

A direct fluorescent antibody (FA) test and a polyvalent H agglutination (poly H) test with separate gram-negative (GN) and tetrathionate (TT) broth enrichments were compared with culture methodology in detecting salmonellae from experimentally contaminated ground beef. The FA test had the most false-position reactions (28%, but concurrent culture results reduced these to 7%) and the lowest number of false-negative reactions (4%). The poly H test was more efficient when TT broth was used for selective enrichment. On the basis of fewer false-negative reactions and the greater number of false-positive reactions that were culturally confirmed to be salmonellae, the FA test would appear to be a more efficient screening test in detecting salmonellae from artificially contaminated ground beef held at frozen temperatures.

Current methods for detection and identification of salmonellae involve considerable time and effort. Due to the public health concern for foodborne outbreaks of salmonellosis much attention has been given to development of rapid, reliable tests to detect salmonellae contamination before a food commodity reaches the consuming public. Most studies on the use of the fluorescent antibody (FA) techniques have reported good correlation with conventional culture methods while the time involved was decreased by several days *(4, 7, 8, 9)*. Several investigators have reported polyvalent H serology to be as reliable as culture methods with considerably less time involved *(5, 10)*.

The purpose of the present study was to compare the efficiency of a *Salmonella* polyvalent H antisera tube agglutination (poly H) test with a direct fluorescent antibody test in detecting *Salmonella* serotypes from artificially contaminated ground beef held at −20 C.

## Materials and Methods

### *Samples*

Fifty-six 1 lb. samples of ground beef were obtained from a local retail establishment and examined for salmonellae *(1, 6)* by adding 25 g to 225 ml mannitol tergitol (MT) broth [1% mannitol in 0.3% nutrient broth (Difco) plus 3 ml of 0.6% Tergitol No. 7] and blending at high speed for 3 min in a 1-liter stainless steel Waring Blender. After incubating at 35 C for 18 to 24 h, 1 ml was transferred to each of 10 ml selenite cystine (SC) broth (BBL) and 10 ml tetrathionate (TT) broth (BBL) tubes. These were incubated at 35 C for 24 h. Using a 3-mm platinum wire inoculating loop, brilliant green sulfadazine (BGS) agar (BBL), MacConkey (MaC) agar (BBL), and bismuth sulfite agar (BBL) were streaked from each broth culture and incubated at 35 C for 24 h (bismuth sulfite for 48 h). Suspect *Salmonella* colonies were transferred to triple sugar iron (TSI) agar (BBL) and tested serologically with polyvalent 0 antiserum (Difco). If *Salmonella* was still suspected appropriate biochemical reactions *(3)* were used for confirmation.

### *Experimental inoculation*

Fourteen different *Salmonella serotypes*[1] were used as test organisms. The serotype to be inoculated was transferred to 10 ml brain heart infusion (BHI) broth (BBL) from trypticase soy agar (BBL), incubated at 35 C for 6 h, and diluted with sterile phosphate buffered water so that from 50 to 500 organisms were seeded per gram of ground beef. Inoculation was accomplished by adding 5 ml of the diluted culture to the 1-lb. sample, placing in a 4-liter stainless steel blender jar and blending at low speeds for three consecutive 5-sec periods. The contaminated ground beef was divided into three aliquots in sterile polyethylene bags and immediately placed at −20 C. The process was repeated with three additional 1-lb. samples, making a total of 12 aliquots contaminated with one specific salmonella serotype. Testing procedures began after 3 months at −20 C with 2 samples analyzed each month for 6 months so that a total of 8 samples containing each serotype were examined.

### *Fluorescent antibody method*

A 25-g portion from each contaminated sample was placed into 225 ml MT broth and blended in a 1-liter stainless steel Waring blender at high speed for 3 min. One milliliter of broth slurry was placed in 10 ml of gram-negative (GN) broth (BBL) and incubated at 35 C overnight. Fluorescent

[1]Cultures were obtained from Dr. J. Dizikes, USDA, Agricultural Marketing Service, 1819 Pershing Rd., Chicago, Il., and Dr. S. Kenzy, Dept. of Microbiology, College of Veterinary Medicine, Washington State University, Pullman, WA.

[2]Salmonella diagnostic sera, flagellar, formulated by Dr. E. M. Ellis, Chief, Diagnostic Bacteriology, and provided by the General Bacteriology Unit, Diagnostic Services, National Animal Disease Laboratory, Ames, Iowa.

[3]Recommended Method for Use of Salmonella H Polyvalent Antiserum, Lot 1. 1971. Biological Reagents Section, Center for Disease Control, Atlanta, GA.

[4]Salmonella H polyvalent rabbit antiserum, lot 1, provided by the Biological Reagents Section, Center for Disease Control, Atlanta, GA.

TABLE 1. COMPARISON OF THE FA TEST WITH THE POLY H TEST (WITH SEPARATE TT BROTH AND GN BROTH ENRICHMENTS) IN DETECTING SALMONELLAE FROM 112 SAMPLES OF EXPERIMENTALLY CONTAMINATED GROUND BEEF

| Test | % Detected | Follow-up culture | | | Concurrent culture | |
|---|---|---|---|---|---|---|
| | | % Agreement | % False negative | % False positive | % Agreement | % False positive |
| FA | 75 | 68 | 4 | 28 | 79 | 7 |
| poly H-TT[1] | 55 | 73 | 13 | 14 | 46 | 4 |
| poly H-GN[2] | 35 | 66 | 24 | 10 | 57 | 8 |

[1]With TT broth enrichment.
[2]With GN broth enrichment.

antibody staining was then done (2) using salmonella FA conjugate[2]. A V20-Fluorestar Microscope (American Optical Corp., Buffalo, New York) was used with a Schott OG-1 barrier filter and a Schott BG12 exciter filter.

Immediate culture follow-up was done by streaking from the overnight culture of GN broth onto BGS and MaC plates. After incubation at 35 C for 24 h, suspect *Salmonella* colonies were transferred to TSI agar and tested serologically with polyvalent 0 antiserum (Difco). If *Salmonella* was still suspected appropriate biochemical reactions (3) were used for confirmation.

*Polyvalent H antisera test*

Two 1-ml amounts from the original MT broth slurry were placed one each in 10 ml of GN broth and 10 ml of TT broth. These were incubated overnight at 35 C and 1 ml from each transferred to 10 ml BHI broth, respectively. After incubating for 6 h at 35 C, a tube agglutination test[3] was done using *Salmonella* H polyvalent antiserum[4]. Immediate culture follow-up was done by streaking from the overnight cultures of GN and TT broths onto BGS and MaC plates. After incubation at 35 C for 24 h, suspect *Salmonella* colonies were confirmed in the same manner as for the culture follow-up with the FA test.

*Concurrent culture methodology*

Three 10-ml amounts from the MT broth slurry, prepared as described above, were placed separately into each of three sterile tubes. The culture technique for *Salmonella* was done as already described. To determine the viability of the seeded salmonellae, one sample was tested 24 h after the inoculated samples were placed at −20 C and again after 1 and 2 months.

## RESULTS

No salmonellae were detected in the 65 1-lb. ground beef samples before experimental inoculations. All artificially contaminated samples checked culturally after 24 h at −20 C contained viable salmonellae.

Results of the comparison of the FA and poly H tests are summarized in Table 1. The FA test detected 75% of the samples as positive for *Salmonella* while the poly H test with TT broth enrichment (poly H-TT test) detected 55% and the poly H test with GN broth enrichment (poly H-GN test) 35%. The FA test agreed with follow-up culture results 68% of the time while the poly H-TT and poly H-GN tests agreed 37% and 66% of the time, respectively. The FA test showed the lowest number of "false-negatives" (negative test with a positive follow-up culture) with 4%. The poly H-TT and poly H-GN tests had 13% and 24% "false-negatives," respectively. The "false-positive" (positive test with a negative follow-up culture) results were reversed with the FA test showing 28%, the poly H-TT test 14%, and the poly H-GH test 10%. Concurrent culture results reduced the number of false-positive reactions to 7%, 4%, and 8%, respectively.

Table 2 compares the percentage of samples in which specific *Salmonella* serotypes were detected using the FA and poly H tests. A higher number of most serotypes was detected by the FA test than by either poly H test.

## DISCUSSION

The FA test detected salmonellae in the most number of samples (75%). The high percentage (28) of FA false-positive reactions was probably due to a failure of the culture follow-up procedure since GN broth is not highly selective for salmonellae allowing competitive growth of other bacteria. The follow-up culture also did not have the advantage of the preenrichment broth which has been indicated (6) as an aid in restoring stressed salmonellae to an active state. Concurrent culture results of 7% false positive reactions indicates viable salmonellae were actually present in many of the samples.

The poly H-TT test detected salmonellae in fewer samples and agreed with concurrent culture results less often than did the FA test. Fewer false-positive reactions occurred with the poly H-TT test but a lesser number of these were found to contain salmonellae by the concurrent culture method. Due to the lower percentage of false-negative reactions plus the greater number of false-positive samples found to contain salmonellae we conclude the FA technique would be a more desirable screening procedure for detecting salmonellae in artificially contaminated ground beef. To accurately evaluate the performance of the FA test in detecting salmonellae from ground beef, naturally contaminated samples would need to be analyzed.

TABLE 2. PERCENT SALMONELLA-POSITIVE SAMPLES DETECTED IN 112 EXPERIMENTALLY CONTAMINATED GROUND BEEF SAMPLES ACCORDING TO SEROTYPE USING THE FA AND POLYVALENT H AGGLUTINATION TESTS

| Serotype | FA | poly H-TT[1] | poly H-GN[2] |
|---|---|---|---|
| S. *typhimurium* | 87 | 25 | 50 |
| S. *newington* | 50 | 37 | 13 |
| S. *muenchen* | 87 | 63 | 50 |
| S. *heidleburg* | 100 | 37 | 87 |
| S. *minnesota* | 50 | 87 | 50 |
| S. *worthington* | 75 | 75 | 0 |
| S. *tennessee* | 87 | 50 | 13 |
| S. *cubana* | 60 | 50 | 30 |
| S. *eimsbuettel* | 90 | 30 | 10 |
| S. *anatum* | 60 | 30 | 30 |
| S. *infantis* | 90 | 60 | 60 |
| S. *taksony* | 90 | 75 | 17 |
| S. *senftenberg* | 100 | 83 | 90 |
| S. *paratyphi A* | 33 | 16 | 9 |

[1]With TT broth enrichment.
[2]With GN broth enrichment.

The poly H-GN test was not as efficient as either of the other two tests. Salmonellae were detected in only 35% of the samples. The test agreed with culture results the least percentage of time and had the highest number of false-negative reactions. The decreased efficiency was probably the result of the presence of competing microorganisms which were not inhibited by the GN broth. It is evident the more selective TT broth increased the efficiency of the poly H test.

The number of false-negative reactions that occurred with both tests was distrurbing. Perhaps competition from the natural microflora prevented adequate serological reactions with the seeded salmonellae. More likely, the stress caused by frozen storage for 3 to 6 months hindered antigenantibody complexing since the antisera were flagellar in nature and no preenrichment was used in an attempt to restore the *Salmonella* organisms to a more active state.

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# EFFECT OF MILK SAMPLE CONDITION ON REPLICATE MILKFAT TESTS ANALYZED BY A MILKO-TESTER MK II

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Abstract

Milk sample variables of homogeneity, age, temperature, pH, and fat level, as well as the fat level used to standardize the Milko-Tester MK II were studied in relation to repeatability of results. Fresh milk samples from individual cows as well as from a bulk tank of low (3.2%) and high (5.0%) fat levels were used for these studies. Milk was warmed to 38 C and subdivided into twenty 120-ml portions and placed in plastic sample bags before analysis. Half of the samples in addition to the normal mixing received mild agitation by hand-squeezing the bags during pipetting. The difference between the means of agitated, hand-squeezed, and non-agitated, not squeezed, samples was only +0.009%. The average deviations from the Babcock standards showed that when the Milko-Tester was standardized with low fat milk, the low fat samples deviated by +0.003% while the high fat samples deviated by +0.076%; with high fat standardization, the high fat samples deviated by −0.002% while low fat samples devioted by −0.058%. Temperatures of pipetting in Milko-Tester at 21, 31, and 39 C had no great effect on the difference between samples. Analysis by the Milko-Tester of samples preserved with potassium dichromate as well as non-preserved samples stored at 9 C and 21 C for 1-22 days showed a decrease in fat test when the pH decreased to 6.50.

The Babcock test for dairy products has been used for many years in the dairy industry. Recently electronic devices, namely, the Milko-Tester Mark II and III[1] have been adopted for DHI testing *(2, 10, 12, 13)* and in some markets *(7)* for fat tests involved in the purchase of milk. Currently two Mark III units have been officially approved by Louisiana for milk purchase, with the Babcock test being the basis of standardization. AOAC *(1)* has reported that if the standard deviation of the difference between the Milko-Tester results and the reference results exceeds 0.10% fat for individual cow samples, the Milko-Tester should be calibrated. Factors, such as preservative *(4, 5, 6, 8)*, calibration *(9)*, and sample age *(4, 8)* have been shown to influence accuracy of the method. Because sample handling methods vary, and also because the fat test of individual cows varies widely, the investigation reported here in was carried out. In this study, it was desired to determine the extremes of deviation in replicate analysis. The effect of milk fat level of samples used for equipment standardization, sample agitation, temperature, age, and pH of samples were examined.

## Experimental Methods

Milk from individual cows, was warmed to 38 C, mixed by continous manual stirring by hand for 10 sec and sub-sampled into twenty 5-oz. plastic bags, placing 120 ml in each bag. These sample plastic bags were filled to about two-thirds capacity and held at 4 C for no longer than 14 h. All samples were normally warmed to 38 C before sampling for either Babcock and Milko-Tester except in the temperature study. The plastic sample bags were partially opened to provide air space for thorough mixing by shaking 10 times through an arc of about 8 inches in approximately 5 sec. In addition, samples in plastic bags were alternately squeezed to provide mild agitation during pipetting into the Milko-Tester. These samples are termed "agitated." Unsqueezed samples are termed "non-agitated." Standard Babcock fat test procedures as outlined in AOAC *(1)* were followed in all Babcock tests. The Babcock bottles used in this study were made according to specifications of ADSA and AOAC and were labeled "sealed." Babcock readings were estimated to the nearest 0.025%. This was accomplished by using a 10× magnifying glass with test bottles in front of a frosted light panel. It was found that if these results were rounded to the nearest 0.05% level that the resulting means were essentially the same.

The Milko-Tester Mark II used in this study (purchased Dec., 1972) was standardized according to the recommendation of AOAC *(1)*. The amounts of fat were estimated to the nearest 0.025% on the read-out dial. Procedures for the variables studied were as follows: *(a) Agitation.* 20 replicate samples of milk from individual cows were normally mixed and tested in duplicate as follows: Ten of these samples received no further agitation and 10 samples were mildly agitated by squeezing during pipetting into the Milko-Tester. *(b) Standardization.* Milko-Tester was first standardized at a low milkfat level, 3.2% and 10 replicates or subsamples from individual cows of low and high milkfat test levels were tested in duplicate. The procedure was then repeated with the unit standardized at a high milkfat level of 5.2%. *(c) Temperature.* Samples were prepared from bulk tank milk and sub-sampled as previously mentioned, except that temperature was adjusted to 21, 31, and 39 C, respectively, for each of three 10 sub-sample groups, before being pipetted into the unit. Samples received normal agitation with hand-squeezing during pipetting into the Milko-Tester. *(d) Sample age and storage temperature.* Milk samples from a farm bulk tank were held at 9 C and 21 C for 1-22 days. Samples preserved with potassium dichromate (0.04 g $K_2Cr_2O_7$ per 100 ml), as well as non-preserved samples, were used for each temperature. Samples were

[1]Foss Electric Co., Hillerod, Denmark

TABLE 1. EFFECT OF MILK AGITATION DURING MILKO-TESTER PIPETTING ON THE REPEATABILITY OF MILKO-TESTER DUPLICATES AND SUB-SAMPLES RESULTS

| Trial | Treatment | Treatment mean[a] | Mean difference | Samples range High | Samples range Low | Duplicate range High | Duplicate range Low | S.D.[b] |
|---|---|---|---|---|---|---|---|---|
| | | | | (percent fat) | | | | |
| 1 | Agitated | 3.196 | 0.001 | 3.200 | 3.175 | 3.200 | 3.175 | 0.009 |
| | Non-agitated | 3.195 | | 3.200 | 3.175 | 3.200 | 3.175 | 0.010 |
| 2 | Agitated | 4.901**[c] | 0.001 | 4.925 | 4.875 | 4.900 | 4.875 | 0.001 |
| | Non-agitated | 4.902 | | 4.925 | 4.875 | 4.925 | 4.875 | 0.014 |
| 3 | Agitated | 3.051 | 0.001 | 3.075 | 3.025 | 3.075 | 3.050 | 0.001 |
| | Non-agitated | 3.050 | | 3.075 | 3.025 | 3.050 | 3.025 | 0.008 |
| 4 | Agitated | 5.064 | 0.008 | 5.075 | 5.050 | 5.075 | 5.050 | 0.013 |
| | Non-agitated | 5.056 | | 5.100 | 5.025 | 5.100 | 5.050 | 0.018 |
| 5 | Agitated | 5.018*[c] | 0.037**[d] | 5.050 | 5.000 | 5.025 | 5.000 | 0.016 |
| | Non-agitated | 4.981 | | 5.025 | 4.950 | 5.000 | 4.950 | 0.024 |
| 6 | Agitated | 3.940**[c] | 0.020*[d] | 3.975 | 3.900 | 3.950 | 3.925 | 0.022 |
| | Non-agitated | 3.920 | | 3.925 | 3.900 | 3.925 | 3.900 | 0.010 |
| Total | Agitated | 4.195[e] | .009 | | | | | 0.021[f] |
| | Non-agitated | 4.186[e] | | | | | | |

[a]Each mean includes ten samples tested in duplicate.
[b]Standard deviation.
*[c]Significant sample effect, $P < .05$.
**[c]Significant sample effect, $P < .01$.
[d]Significant treatment effect, $P < .05$.
**[d]Significant treatment effect, $P < .01$.
[e]Mean of 6 trials.
[f]Standard deviation of 240 analyses.

tested in duplicate by the Babcock and Milko-Tester procedures. The samples received normal agitation with hand-squeezing during pipetting into the Milko-Tester. All samples were tested according to the following testing schedule:

| *Temperature* | *Preserved* | *Non-preserved* |
|---|---|---|
| 9 C | Every other day | Daily |
| 21 C | Daily | Every 6 h |

The physical appearance and pH of the above samples were determined.

All results were analyzed statistically by the methods of Snedecor and Cochran *(12)* by an analysis of variance taken on a factorial arrangement.

## RESULTS AND DISCUSSION

In the operation of a Milko-Tester Mark II with samples contained in plastic bags it seemed possible that continuous mild agitation during pipetting would provide better repeatability than if no agitation were used.

Results reported in Table 1 include 6 trials involving two different sample treatments: 10 sub-samples or replicates in each treatment and duplicate tests of each sub-sample. When all results from the 6 trials were included in the analysis of variance, the mean difference of 0.009% between treatments was not significant. However, when the trials were analyzed individually the treatment effect was significant in Trials 5 ($P < .01$) and 6 ($P < .05$) as implied by the large mean differences.

Significant variations were noted between the agitated sub-samples of Trials 2 ($P < .01$), 5 ($P < .05$), and 6 ($P < .01$). For Trial 2 these results would seem to indicate that, even though there was no significant difference between treatments, milk agitation might enhance the possibility of detecting sample difference. In Trials 1 and 3 no significant variations were found and it is of interest that these were at the low fat level. The standard deviations associated with samples testing 4% or higher were generally larger than those of samples testing in the 3% range. In the agitated portion of Trial 6 the maximum standard deviation of 0.022% and the overall of 0.021% were well within the value of 0.069% reported by Shipe *(8)*. The maximum difference between the highest and lowest result in any sub-sample, agitated or non-agitated trial, was 0.075%. Within duplicates the range in the agitated treatment was 0.025% and in the non-agitated treatment it was 0.050%. Shannon et al. *(7)* have reported average variations in duplicates as high as 0.31% fat.

Data presented in Table 2 show the effect on test results of milkfat level of samples used in standardizing the Milko-Tester. The 10 sub-samples tested in duplicate were mildly agitated during pipetting. From the analysis of variance it was noted that standardization of the Milko-Tester at a fat level approximately 2.0% higher or lower than the milkfat level of test samples resulted in a statistically significant difference ($P < .01$) between Milko-Test-

TABLE 2. DIFFERENCE BETWEEN MILKO-TESTER AND BABCOCK TEST RESULTS WITH A MARK II MILKO-TESTER STANDARDIZED AT MILKFAT LEVELS SIGNIFICANTLY DIFFERENT FROM MILKFAT LEVELS OF TEST SAMPLES

| Trial | | Mkt. mean[a] | Babcock mean[a] | Mean diff. +Babcock | Range between samples: Milko-Tester High | Milko-Tester Low | Babcock High | Babcock Low | Range between duplicates: Milko-Tester High | Milko-Tester Low | Babcock High | Babcock Low |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | (Percent fat) | | | | | | | | | | |
| 1—Milko-Tester std.[b] 3.20% | A | 3.196 | 3.193 | +0.003 | 3.200 | 3.175 | 3.225 | 3.175 | 3.200 | 3.175 | 3.200 | 3.175 |
| | B | 4.899 | 4.823 | +0.076** | 4.925 | 4.875 | 4.875 | 4.800 | 4.925 | 4.900 | 4.850 | 4.800 |
| 2—Milko-Tester std. 5.52% | A | 5.064 | 5.066 | −0.002 | 5.075 | 5.050 | 5.100 | 5.025 | 5.075 | 5.050 | 5.075 | 5.050 |
| | B | 3.051 | 3.109 | −0.058** | 3.075 | 3.025 | 3.125 | 3.100 | 3.075 | 3.050 | 3.125 | 3.100 |
| 3—Milko-Tester std. 5.02% | A | 5.018 | 5.020 | −0.002 | 5.050 | 5.000 | 5.050 | 5.000 | 5.025 | 5.000 | 5.050 | 5.000 |
| | B | 3.940 | 3.944 | −0.004 | 3.975 | 3.900 | 3.975 | 3.900 | 3.950 | 3.925 | 3.950 | 3.900 |

[a]Each mean represents ten samples tested in duplicate.
[b]Milko-tester standardized at fat level of 3.20%.
**Significant difference, $P < 0.01$.

er and Babcock test results. This was observed in Trials 1B and 2B where the mean differences between the two methods were +0.076% and −0.058%, respectively. It has been recommended by Kristoffersen *(3)* that a difference of ±1.0% from a previous test result should be taken as cause for re-doing the sample and accepting results of the second test. When the milkfat content varied by approximately ±1.0% or less, mean differences varied from −0.004 to +0.003%. Variations between duplicates and/or sub-samples never exceeded 0.075% within the Milko-Tester or the Babcock procedures, both of which included 120 separate tests. The maximum difference between any Babcock test and any Milko-Tester test was 0.125%, as noted in Trial 1B. In Trials 2B and 3B the maximums were 0.10% and 0.075%, respectively. When the Milko-Tester was standardized near the fat level to be determined, as may be observed in Trials 1A, 2A, 3A, and 3B, the maximum mean difference did not exceed 0.004%.

Since milk samples are heated to 60 C in a water bath as they are pumped through the Milko-Tester, little attention has been given to sample temperature other than warming for satisfactory mixing. The authors found no reference in the literature to this factor. In this phase of work 60 samples were subdivided into three lots of 20 samples. In each lot 10 samples were analyzed by the Milko-Tester method, 10 by the Babcock method. For the Milko-Tester analyses, samples were tempered to 21, 31, and 39 C and agitated before pipetting. Samples for the Babcock tests were analyzed by the AOAC procedure. Results are in Table 3. There were significant differences between the higher temperature and each of the lower temperatures. While these differences were small, as indicated by the mean differences from Babcock tests, it does suggest that the better temperature for pipetting into the Milko-Tester may be 32-35 C.

Effects of preservative, sample age, and storage temperature on the fat analysis by the Milko-Tester are reported in Tables 4 through 7. In Table 4 results on non-preserved raw milk held at 21 C indicated that after between 3/4 and one-day of stor-

TABLE 3. EFFECT OF PIPETTING TEMPERATURE ON THE MILKFAT TESTS DETERMINED BY THE MILKO-TESTER

| Value | 21 C | 31 C | 39 C |
|---|---|---|---|
| | (Percent fat) | | |
| Mean Milko-Tester[a] | 4.510 | 4.527 | 4.520** |
| Range Milko-Tester | 4.525-4.500 | 4.500-4.550 | 4.525-4.500 |
| Mean Babcock[b] | 4.530 | 4.528 | 4.506 |
| Range Babcock | 4.500-4.550 | 4.500-4.550 | 4.500-4.525 |
| Mean Difference from Babcock | −0.020 | −0.001 | +0.014 |

[a]Milko-Tester standardized at 4.51% fat with each value the average of 10 replicate samples tested in duplicate.
[b]Each value is the average of 10 replicate samples tested in duplicate.
**Significantly different, $P < .01$.

TABLE 4. THE EFFECT OF AGE AND STORAGE TEMPERATURE AT 21 C, ON-NON-PRESERVED MILK SAMPLES FOR MILKFAT TESTS

| Time (days) | Babcock | Milko-Tester[a] | pH | Remarks |
|---|---|---|---|---|
| | —(Percent fat[b])— | | | |
| 0 | 4.800 | 4.825 | 6.70 | Normal |
| 1/4 | 4.800 | 4.825 | 6.65 | " |
| 1/2 | 4.800 | 4.825 | 6.58 | " |
| 3/4 | 4.825 | 4.800 | 6.50 | Curdy |
| 1 | 4.800 | 4.775 | 6.45 | Curdy |
| 1-1/4 | 4.775 | 4.750 | 6.10 | Curdy |
| 1-1/2 | — | — | — | Curdled |

[a]Milko-tester standardized at 4.8% fat.
[b]Average of duplicates.

TABLE 5. THE EFFECT OF AGE AND STORAGE TEMPERATURE AT 9 C, ON NON-PRESERVED MILK SAMPLES FOR MILKFAT TESTS

| Time (days) | Babcock | Milko-Tester[a] | pH | Remarks |
|---|---|---|---|---|
| | ——(Percent fat[b])—— | | | |
| 0 | 4.500 | 4.500 | 6.66 | Normal |
| 1 | 4.500 | 4.500 | 6.65 | " |
| 2 | 4.500 | 4.500 | 6.63 | " |
| 3 | 4.475 | 4.475 | 6.60 | " |
| 4 | 4.500 | 4.450 | 6.54 | " |
| 5 | 4.500 | 4.450 | 6.50 | " |
| 6 | 4.500 | 4.425 | 6.45 | Curdy |
| 7 | 4.450 | 4.425 | 6.30 | Curdy |
| 8 | 4.400 | 4.400 | 6.00 | Curdy |
| 9 | — | — | — | Curdled |

[a]Milko-tester standardized at 4.5% fat.
[b]Average of duplicates.

TABLE 6. THE EFFECT OF AGE AND STORAGE TEMPERATURE AT 21 C ON PRESERVED[a] SAMPLES FOR MILKFAT TESTS

| Time (days) | Babcock | Milko-Tester[b] | pH | Remarks |
|---|---|---|---|---|
| | ——(Percent fat[c])—— | | | |
| 0 | 5.250 | 5.275 | 6.68 | Normal yellow |
| 1 | 5.200 | 5.250 | 6.67 | " " |
| 2 | 5.200 | 5.250 | 6.65 | " " |
| 3 | 5.250 | 5.250 | 6.63 | " " |
| 4 | 5.200 | 5.200 | 6.50 | " " |
| 5 | 5.200 | 5.200 | 5.80 | Greyish-yellow |
| 6 | 5.200 | 5.150 | 5.68 | Greyish-yellow |
| 7 | 5.100 | 5.100 | 5.62 | Curdy and "oiled-off" |
| 8 | 5.100 | 5.100 | 5.50 | Curdy and "oiled-off" |
| 9 | 5.100 | 4.900 | 5.25 | Curdy and "oiled-off" |

[a]Potassium dichromate (0.04 g $K_2Cr_2O_7$ per 100 ml.)
[b]Milko-tester standardized at 4.8% fat.
[c]Average of duplicates.

age samples were unsatisfactory for testing. After one day's storage, fat values by both Babcock and Milko-Tester procedures were lower than the values at 3/4 day. The pH dropped to 6.50 – 6.45 and white particles which resembled curd appeared in the sample. These particles could not be dispersed with additional mixing. In Table 5 with storage at 9 C the same phenomena were noted between the 5th and 7th day. Similar data have been reported by Shipe *(8)*. On the 6th day the sample contained curd-like particles; the pH was 6.45, and the sample tested lower than on the 5th day. The Babcock test decreased on the 7th day. This suggests that a reduction in pH below 6.50 renders samples unsuitable for analysis. Visible observation of samples at this pH level indicated the presence of curd-like particles. This could serve as one method for determining acceptability of samples.

In Tables 6 and 7 results of similar observation on preserved samples are reported. At a storage temperature of 21 C obvious changes in the samples were noted on the 5th day, namely, a change from a normal yellow to grayish-yellow color. The pH decreased from 6.50 on the 4th day to 5.80 on the 5th day. The Milko-Tester fat test dropped to 5.20% on the 4th day and to 5.15% on the 6th day, while no apparent decrease occurred in samples tested by the Babcock method until the 7th day. A drop of 0.13% was also noted in similarly treated samples by Kroger *(4)* on the 5th day of storage at room temperatures. These data suggest that the Milko-Tester accuracy is more drastically influenced by poor milk quality than is the Babcock method. Curd-like particles, if present, tended to clog the screen of the pipette on the Milko-Tester and, in some instances, to clog the homogenizer as well. This is a technical problem that is important over and above the question of testing accuracy. Preserved samples stored at 9 C, as reported in Table 7, tested satisfactorily through 20 days with a slight drop noted in the Milko-Tester fat values on the 22nd day. During this time no changes were observed in Babcock test readings. Under similar conditions Shipe *(9)* reported decreases ranging from –0.05 to –0.07% within 14 days. Good quality preserved samples stored at cool temperatures utilized in a 15-day compositing sample program can, as indicated in Table 7, be tested with an assurance of fat test accuracy.

In viewing results of sample storage it appears that samples start to deteriorate at a pH of 6.50 with evidence of curd-like particles. Preserved samples

TABLE 7. THE EFFECT OF AGE AND STORAGE TEMPERATURE AT 9 C, ON PRESERVED[a] MILK SAMPLES FOR MILKFAT TESTS

| Time (days) | Babcock | Milko-Tester[b] | pH | Remarks |
|---|---|---|---|---|
| | ——(Percent fat[c])—— | | | |
| 0 | 4.500 | 4.500 | 6.68 | — |
| 2 | 4.500 | 4.500 | 6.67 | — |
| 4 | 4.500 | 4.500 | 6.65 | — |
| 6 | 4.500 | 4.475 | 6.62 | — |
| 8 | 4.500 | 4.500 | 6.60 | — |
| 10 | 4.500 | 4.500 | 6.60 | — |
| 12 | 4.500 | 4.475 | 6.58 | — |
| 15 | 4.500 | 4.500 | 6.55 | — |
| 18 | 4.500 | 4.475 | 6.53 | — |
| 20 | 4.500 | 4.450 | 6.52 | — |
| 22 | 4.500 | 4.425 | 6.50 | "oiled-off" |

[a]Potassium dichromate (0.04 g $K_2Cr_2O_7$ per 100 ml.)
[b]Milko-tester standardized at 4.5% fat.
[c]Average of duplicates.

may also show a change in color. These can serve as visual checks for sample quality.

## SUMMARY

Mild agitation of milk samples during pipetting did not have a great overall effect on Milko-Tester results. However, the influence, though small, appeared to be of greater magnitude in samples of higher milkfat test. The mean difference between the Milko-Tester and Babcock results was greatest when the difference between level of fat in standardization and the level in the samples to be tested was greater than ±1.0%. This suggests that the Milko-Tester MK II should be standardized as recommended over a range of fat tests, especially for DHI testing, where wide ranges in test values are to be expected. The sample tempering temperature at which the fat was in the liquid form gave Milko-Tester results that were nearer the Babcock tests than at lower temperatures. Milko-Tester accuracy is impaired at an earlier stage of sample deterioration than occurs when using the Babcock method. The critical pH level appeared to be at 6.50. Color change, "oiling-off," and curd-like particles were good visual indications of the deterioration of the samples.

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# TYRAMINE, HISTAMINE, AND TRYPTAMINE CONTENT OF CHEESE

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Abstract

Because of the increasing knowledge of the physiological importance of biologically active amines in man and the importance of the presence of these amines in cheese, this study was done to obtain quantitative information for tyramine, tryptamine, and histamine in cheese available in the United States. The tyramine, histamine, and tryptamine contents of 156 samples of cheese purchased at retail stores were quantitated by thin-layer chromatography and fluorescence measurements of NBD-chloride derivatives of the amines. Tyramine was found in 81 of 85 Cheddar cheese samples examined. Extra-sharp, sharp, and medium Cheddar cheese samples contained average tyramine values of 0.27, 0.21, and 0.24 mg/g, respectively. Average tyramine contents were lower in mild and processed Cheddar (0.09 and 0.11 mg/g, respectively). The highest Cheddar cheese tyramine content was 0.7 mg/g. Tyramine was consistently found in all cheeses except in unripened soft cheese (Cottage). Histamine concentrations varied from nondetectable amounts to 2.6 mg/g in a Sap-Sago cheese sample. Twenty-four Cheddar cheese samples contained histamine with the highest amount being 1.3 mg/g. A domestic Blue cheese contained 2.3 mg/g. Tryptamine was uniformly low or completely absent in the Cheddar cheese samples. The highest tryptamine concentration (1.1 mg/g) was detected in a Blue cheese.

The presence of biologically active amines in food and the ability of tyramine, a pressor amine, to produce hypertensive crises in patients treated with monamine oxidase inhibitors (MAO) has been well documented. While many foods contain biologically active amines, tyramine in Cheddar cheese has been implicated most often as the causative agent in reported attacks resulting in acute hypertensive reactions in patients receiving MAO inhibitors *(1, 2, 3, 13, 16)*. Hannington *(11)* has shown that tyramine in the quantities often reported in cheese produced migraine headaches in subjects prone to such attacks and postulated that such migraine sufferers may have genetic monamine oxidase deficiences.

The tyramine content of cheese is known to be variable, ranging from almost nil to amounts approaching 3.7 mg/g *(2, 3, 5, 13)*. Histamine, while not found as frequently as tyramine, has been reported in Cheddar *(4, 17, 19)* in amounts up to 10.5 mg/100g. Swiatek and Kisza *(18)* reported histamine amounts of 50-100 mg/100 g in samples of Trappisten, Tilsiter, and Roquefort cheese, and a Gouda cheese that produced histamine intoxication was found to contain 85 mg histamine/100 g *(9)*. Formation of histamine in the Gouda cheese was traced to the presence of an unknown histidine decarboxylating *Lactobacillus* contaminant in the rennet. Tryptamine has been reported to occur in various cheese varieties including Cheddar *(17)*, and has usually been found only at low levels.

Dahlberg and Kosikowski *(6, 7, 8)* have provided extensive information on conditions leading to tyramine formation in Cheddar cheese. However, many of the concentration values reported in the literature pertain to surveys comprising small numbers of samples or values derived from single samples. This is particularly true for tyramine values as many of the quantitations were completed because the cheese was implicated in a hypertensive crisis. Because of the increasing knowledge of the physiological importance of biologically active amines in man and the importance of the presence of these amines in cheese, this study was done to gain quantitative information for tyramine, histamine, and tryptamine in cheese available in the United States.

## Materials and Methods

### *Source of cheese*

All cheese samples (156) were randomly obtained from commercial sources in the South and Midwest. Most samples were of domestic origin, but some were imported. The samples were believed to be representative of cheese available to consumers throughout the United States. After purchase, the cheeses were sealed in polyethylene bags and stored at −30 C until assayed.

### *Amine extraction*

Tyramine, histamine, and tryptamine were extracted from the cheese samples by the method of Lovenburg and Engelman *(15)* and by the procedure of Blackwell and Mabbitt *(3)*. The extraction procedures were used interchangeably during the study and no differences were noted between the two. When both extraction procedures were used for a single sample, the amine contents were comparable (±10%). All reported values represent averages of four determinations. The procedure of Lovenburg and Engelman consisted of homogenizing 10 g of cheese in 20 ml of 0.1 N HCl in a glass homogenzier equipped with a motorized teflon coated stain-

TABLE 1. TYRAMINE CONTENTS OF VARIOUS CHEESES

| Cheese | | Tyramine (mg/g) Range | Average |
|---|---|---|---|
| Cheddar | | | |
| Extra-sharp | (11)[a] | 0.10 - 0.60 | 0.27 (10)[c] |
| Sharp | (34) | ND[b] - 0.50 | 0.21 (33) |
| Medium | (18) | ND - 0.70 | 0.24 (17) |
| Mild | (12) | ND - 0.50 | 0.09 (11) |
| Processed | ( 7) | ND - 0.22 | 0.11 ( 6) |
| Smoked | ( 3) | 0.07 - 0.21 | 0.12 ( 3) |
| Colby | ( 8) | 0.10 - 0.56 | 0.21 ( 8) |
| Edam | ( 2) | 0.30 - 0.32 | 0.31 ( 2) |
| Gouda | ( 6) | 0.08 - 0.67 | 0.29 ( 6) |
| California Jack | ( 1) | 0.13 | – |
| Swiss | ( 6) | ND - 1.80 | 0.41 ( 5) |
| Roquefort or Blue | ( 7) | 0.05 - 1.10 | 0.36 ( 7) |
| Camembert | ( 7) | 0.07 - 0.21 | 0.12 ( 7) |
| Limburger | ( 1) | 0.12 | – |
| Sap-Sago | ( 1) | 0.52 | – |
| Romano | ( 1) | 0.14 | – |
| Parmesan | ( 1) | 0.28 | – |
| Mozzarella | ( 1) | 0.16 | – |
| Fontinella | ( 1) | 0.10 | – |
| Cottage | ( 3) | ND | – |

[a]Number of samples examined

[b]ND = Not detectable at levels below 10 μg/g

[c]Number of positive samples

less steel pestle. The cheese slurry was centrifuged at 4 C at 12,000 × g for 10 min. The aqueous layer was decanted and adjusted to pH 10 with solid $Na_2CO_3$ and then saturated with excess NaCl. Fifteen milliliters of n-butanol were added and the mixture was agitated on a Genie Vortex mixer four times over a 10-min period. After centrifuging for 15 min (12,000 × g), the butanol layer was decanted and used for amine quantitation.

The procedure of Blackwell and Mabbitt consisted of homogenizing 10 g of cheese with sufficient warm distilled water to bring the total volume to 40 ml. The mixture was boiled for 5 min in a water bath and centrifuged at 4 C for 15 min (12,000 × g). The aqueous extract was decanted and filtered through Whatman No. 42 filter paper. The extract was applied to a 1 × 10 cm bed of an Amberlite weak cation exchange resin (CGC - 270, 100-200 mesh) in the sodium form. After complete passage of the extract, the column was washed with 50 ml of deionized distilled water and 30 ml of 1 N HCl. The acid eluate containing the amines was freeze-dried and dissolved in 10 ml of water:acetone (2:1 v/v) in preparation for amine quantitation.

*Amine quantitation*

Glass plates were coated with a 250 μ layer of silica gel (Kieselgel G. Merck, Darmstadt, Brinkman Instruments, Westbury, N. Y.). The layer was applied as a slurry consisting of 30 g of silica gel suspended in 70 ml of distilled water. The plates were dried in an oven at 100 C for 1 to 2 h and kept in a desiccator until used. The plates were reactivated at 100 C for 30 min immediately before use. The amine extracts (10 to 50 μl) were applied 2 cm from the base of the plates with quantitative capillary pipets. The solvent system used throughout the study consisted of $CH_3Cl$:$CH_3OH$:$NH_4OH$ (12:7:1). This solvent system was chosen because of its ability to efficiently separate the three amines.

After development, the plates were air-dried and sprayed with a 0.2% NBC-Cl (7-chloro-4-nitribenzofurazan) - methanol solution and allowed to set for 24 h at 25 C *(20)*. The fluorescent amine spots were scraped from the plates and eluted from the silica gel with 5 ml of ethyl acetate. The silica gel-ethyl acetate mixture was agitated on a Genie Vortex mixer for four 30-sec intervals over a 10-min period and centrifuged for 15 min at 5000 × g to remove the suspended silica gel. Fluorescent measurements were determined on a Turner 110 Fluorometer with a primary filter No. 7-60 (365 nm) and a secondary filter No. 4 (465 nm) (G. K. Turner Assoc., Pala Alto, California). Amine contents were determined from standard curves prepared by adding known concentrations (0 - 2.5 mg/g) of tyramine, tryptamine, and histamine into a Cheddar cheese containing negligible amounts of the three amines and carrying out the extraction and the quantitation procedures as described above.

## RESULTS AND DISCUSSION

The tyramine contents of the various cheeses are given in Tables 1 and 2. A total of 85 Cheddar cheese samples of various degrees of flavor development as classified by label specifications were assayed. The constancy of tyramine formation in Cheddar cheese was demonstrated by its presence in measurable quantities in 81 of the 85 Cheddar cheese samples examined. The highest tyramine concentration (0.7 mg/g) was found in a medium Cheddar cheese. The average tyramine content was highest in the extra-sharp category (0.27 mg/g) and lowest in the mild category (0.09 mg/g). Average tyramine contents of the extra-sharp, sharp, and medium Cheddar categories were 0.27 mg/g, 0.21 mg/g, and 0.24 mg/g, respectively. Although label specifications do not always accurately describe the flavor intensity or indicate the age of the cheese, the small differences found in the above Cheddar cheeses would tend to support an observation by Bullock and Irvine *(5)* that tyramine contents of Cheddar cheese did not increase significantly after a 6-month aging period. Although differences in tyramine content of extra-sharp, sharp, and medium Cheddar cheeses were slight, the lower tyramine concentration observed in the mild Cheddar category compared to the concentrations in the Cheddar cheese samples with greater flavor development follows observations of Dahlberg and Kosikowski *(6)* that tyramine concentrations in Cheddar cheese increased as the intensity of flavor increased.

Table 1 also presents the data on tyramine contents of 46 cheese samples other than Cheddar categorized by variety. Tyramine was present in detectable quantities in 42 of the samples. Tyramine was present in all varieties except in Cottage cheese where amine build-up would not be expected to oc-

TABLE 2. TYRAMINE, HISTAMINE, AND TRYPTAMINE CONTENTS OF VARIOUS IMPORTED CHEESES

| Cheese | | Tyramine (mg/g) | Histamine (mg/g) | Tryptamine (mg/g) |
|---|---|---|---|---|
| Mimolette | (1)[a] | 0.28 | ND | ND |
| Rehmkase | (1) | 0.27 | ND | ND |
| Gourmandise (Fondu Blend) | (3) | 0.07-0.12 (3)[b] | ND-0.26 (2) | ND |
| Gjetost | (1) | 0.12 | ND | ND |
| German Blanco | (1) | 0.10 | 0.28 | ND |
| Cheurotin | (3) | ND[c]-0.36 (2) | ND-0.50 (1) | ND-0.23 (2) |
| Danbo | (1) | 0.62 | ND | 0.13 |
| Tybo | (1) | 0.66 | 0.98 | ND |
| Dofinio | (1) | 0.25 | ND | 0.14 |
| Graddoat | (1) | 0.12 | ND | ND |
| Norweigian Jarlsberg (Swiss Type) | (1) | ND | ND | ND |
| Port-Salut | (2) | 0.12-0.18 | ND | 0.12-0.28 |
| Reblochon | (1) | 0.22 | ND | 0.21 |
| Alpenjoi | (1) | 0.10 | ND | ND |
| Stilton | (1) | 0.46 | ND | ND |
| Muenster | (1) | 0.14 | ND | 0.06 |
| Boursault | (1) | 0.11 | ND | 0.06 |
| German Goldblock | (1) | 0.33 | ND | 0.10 |
| Brie | (2) | 0.04-0.26 (2) | ND | ND |

[a]Number of samples examined
[b]Number of positive samples
[c]ND = Not detectable at levels below 10 μg/g for tyramine and tryptamine and 50 μg/g for histamine.

cur. Colby, Edam, and Gouda cheese samples contained average tyramine concentrations of 0.21, 0.31, and 0.29 mg/g, respectively. These values approximate the average values found for the extra-sharp, sharp, and medium Cheddar cheese varieties. Average tyramine concentrations found for Swiss cheese and Blue and Roquefort cheese samples were 0.41 and 0.36 mg/g, respectively. The highest tyramine level found in the survey was 1.8 mg/g and was present in a Swiss cheese sample. A domestic Blue cheese contained 1.1 mg/g. All soft-ripened cheese samples (Camembert, Brie, and Limburger) contained tyramine but at levels lower than those noted in most other cheeses. Seven Camembert samples contained from 0.07 - 0.21 mg/g tyramine with an average content of 0.12 mg/g. A single Limburger sample contained 0.12 mg/g. Possible explanations for the lower levels of tyramine in soft-ripened cheese include the relatively short ripening period required for production and the rapid increase in pH of the cheese to levels above neutrality during ripening which would decrease tyrosine decarboxylase activity, since most microbial tyrosine decarboxylase enzymes demonstrate optimal activity at pH values lower than 6.0 *(10)*.

Table 2 presents data on the tyramine, histamine, and tryptamine concentrations found in several varieties of imported cheeses. For most of these varieties, the values are for single samples and were included in the study for comparative purposes. The tyramine levels observed in these cheeses were similar to the amounts found in the varieties listed in Table 1. None of the cheeses contained excessive amounts of tyramine, and the amine was present in measureable amounts in all but 2 of the samples assayed (23 out of 25).

The histamine contents of the cheese samples are given in Table 3. Histamine was present in measurable quantities in only 24 of the 85 Cheddar cheeses. The average histamine contents for the Cheddar cheese samples varied from 0.21 mg/g for the extra-sharp cheeses to non-detectable levels in the processed samples. The highest Cheddar cheese histamine content (1.3 mg/g) was found in both a sharp sample and in a mild sample. From the data, there appears to be no relationship between degree of flavor development and histamine content. It was evident that, in contrast to tyramine formation, histamine formation did not occur consistently in Cheddar cheese. It was interesting to note that none of the processed Cheddar cheeses contained histamine. Histamine has been reported to be partially heat-labile in food products *(14)* and possibly is destroyed through pasteurization of the processed cheese.

Measurable quantities of histamine were found in

TABLE 3. HISTAMINE CONTENTS OF VARIOUS CHEESES

| Cheese | | Histamine content (mg/g) Range | Average |
|---|---|---|---|
| Cheddar | | | |
| Extra-sharp | (11)[a] | ND[b] - 0.8 | 0.21 (5)[c] |
| Sharp | (34) | ND - 1.3 | 0.11 (9) |
| Medium | (18) | ND - 0.9 | 0.14 (6) |
| Mild | (12) | ND - 1.3 | 0.19 (4) |
| Processed | ( 7) | ND | – |
| Smoked | ( 3) | ND | – |
| Colby | ( 8) | ND - 0.5 | 0.07 (3) |
| Edam | ( 2) | ND | – |
| Gouda | ( 6) | ND - 0.45 | 0.075 (1) |
| California Jack | ( 1) | ND | – |
| Swiss | ( 6) | ND | – |
| Roquefort or Blue | ( 7) | ND - 2.3 | 0.5 (3) |
| Camembert | ( 7) | ND - 0.48 | 0.07 (1) |
| Limburger | ( 1) | ND | – |
| Sap-Sago | ( 1) | 2.6 | – |
| Romano | ( 1) | ND | – |
| Parmesan | ( 1) | ND | – |
| Mozzarella | ( 1) | ND | – |
| Fontinella | ( 1) | ND | – |
| Cottage | ( 3) | ND | – |

[a]Number of samples examined
[b]ND = Not detectable at levels below 50 μg/g
[c]Number of positive samples

only 9 of the 46 samples in Table 3 (not including Cheddar) examined in this study. The largest amount of histamine (2.6 mg/g) was found in a sample of Sap-Sago cheese. A domestic Blue cheese contained 2.3 mg/g and an imported sample of Danish Tybo cheese contained 0.98 mg/g (Table 2). Only 5 of the 25 cheeses in Table 2 contained histamine at detectable levels.

Tryptamine (Table 4) was uniformly low or completely absent in the Cheddar cheese samples. The highest level was 0.3 mg/g which was present in a sharp Cheddar cheese. The greatest tryptamine level found in this study (1.1 mg/g) was present in the domestic Blue cheese that contained 2.3 mg/g histamine. A Colby cheese sample contained 1.0 mg/g. Ten of the cheeses listed in Table 2 contained tryptamine, but the largest amount was only 0.28 mg/g which was present in a Port-Salut cheese sample.

The literature indicates that formation of biologically active amines in cheese appears to be dependent on several basic factors including sufficient ripening time for protein degradation to occur with the liberation of amino acids, and the presence of conditions necessary for formation and action of specific decarboxylases *(3, 7)*. This survey shows as previously reported by Dahlberg and Kosikowski *(6)* that tyramine concentrations generally increased with degree of flavor development in Cheddar cheese; whereas, formation of histamine and tryptamine occurred less frequently than tyramine in all of the cheese varieties examined. The tyramine levels found in Cheddar were lower than most reported earlier in the literature *(2, 3, 6, 13)* with the exception of values reported on individual Cheddar samples purchased at random by Blackwell and Mabbitt *(3)*. The highest tyramine content found in Cheddar cheese in this study was 0.7 mg/g which is somewhat lower than levels reported by Blackwell and Mabbitt *(3)* for a Cheddar cheese sample implicated in a hypertensive crisis (953 μg/g). It still must be emphasized that an individual susceptible to hypertensive crisis due to monoamine oxidase treatment or possible genetic lack of monoamine oxidase as suggested by Hannington *(11)* could be affected by ingesting cheese containing the lower levels of tyramine reported here. However, only 5 of the 85 Cheddar cheeses examined in this study contained over 0.5 mg tyramine/g cheese which was considered a dangerous dose by Blackwell and Mabbitt *(3)* for tyramine susceptible individuals consuming an average 50 g serving.

Only two samples of Cheddar cheese, one sample of Blue cheese, and the Sap-Sago cheese sample contained histamine at levels greater than 1.0 mg/g. Owing to individual differences in histamine metabolism, intake of 70-1000 mg is usually needed to cause moderate intoxication symptoms with small amounts leading to slight and often unnoticed re-

TABLE 4. TRYPTAMINE CONTENTS OF VARIOUS CHEESES

| Cheese | | Tryptamine (mg/g) Range | Average |
|---|---|---|---|
| Cheddar | | | |
| Extra-sharp | (11)[a] | ND[b] - 0.1 | 0.02 ( 2)[c] |
| Sharp | (34) | ND - 0.3 | 0.04 (12) |
| Medium | (18) | ND - 0.1 | 0.02 ( 6) |
| Mild | (12) | ND - 0.2 | 0.03 ( 3) |
| Processed | ( 7) | ND | – |
| Smoked | ( 3) | ND | – |
| Colby | ( 8) | ND - 1.0 | 0.13 ( 2) |
| Edam | ( 2) | ND - 0.16 | 0.08 ( 1) |
| Gouda | ( 6) | ND - 0.2 | 0.07 ( 3) |
| California Jack | ( 1) | ND | – |
| Swiss | ( 6) | ND - 0.16 | 0.19 ( 3) |
| Roquefort or Blue | ( 7) | ND - 1.1 | 0.20 ( 4) |
| Camembert | ( 7) | ND - 0.06 | 0.02 ( 2) |
| Limburger | ( 1) | 0.16 | – |
| Sap-Sago | ( 1) | 0.15 | – |
| Romano | ( 1) | ND | – |
| Parmesan | ( 1) | ND | – |
| Mozzarella | ( 1) | 0.10 | – |
| Fontinella | ( 1) | ND | – |
| Cottage | ( 3) | ND | – |

[a]Number of samples examined
[b]ND = Not detectable at levels below 10 μg/g
[c]Number of positive samples

actions *(12)*. With these dosage levels in mind and the reported intoxication of one individual resulting from ingestion of a Gouda cheese sample containing 85 mg/100 g, it appears that large portions of most cheese would be required to bring about histamine intoxication symptoms.

ACKNOWLEDGMENT

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## SEX ATTRACTANTS AND DISEASE COMBINATION MAY BE NEW INSECT CONTROL METHOD

An entirely new method of fighting insects is being tried out at the University of Wisconsin under a grant from the Rockefeller Foundation.

It consists of luring male insects to a central location with sex attractants given off by female insects, then infecting the males with a disease organism deadly for their species, and letting them wander back into the insect world to spread the disease to others of their type.

The researchers involved are G. Mallory Boush, entomologist in the College of Agricultural and Life Sciences at the University of Wisconsin-Madison, and Wendell Burkholder who holds a joint appointment with the College and with the Agricultural Research Service of the U. S. Dept. of Agriculture.

The general idea for the procedure has been worked out during many years of basic research and it seems practical, the researchers state. But considerable work yet needs to be done in working out details, putting various parts of the scheme together, and presenting evidence of effectiveness and safety of the procedure.

They're working with beetles which infest stored food products such as grains and dry milk. Beetles called *Trogoderma inclusum* and *Trogoderma glabrum* are the specific ones they'll use in the research.

Past research has pinned down the chemical identity of the sex attractants (called pheromones) for both of these beetles, and they can be made synthetically in the chemical laboratory. Plans are to use between 5 and 50 micrograms in each "trap." The researchers figure that one microgram is about equal to the peromone produced by one female insect. This material would be mixed with the disease spores and placed in small, simple, and inexpensive disposable devices made from corrugated paper similar to that used in packing cartons.

The researchers will use a disease agent called *Mattesia trogodermae*. The insects eat the spores of

(Continued on Page 386)

# DEVELOPMENT OF RESISTANCE TO HEAT AND SODIUM CHLORIDE IN *STREPTOCOCCUS FAECIUM* RECOVERING FROM THERMAL INJURY

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Abstract

Heating of *Streptococcus faecium* NRC 1261 at 55 C for 15 min produced injury. The injured population was sensitive to 2.5% NaCl but regained its tolerance when incubated in a recovery medium. Addition of penicillin and actinomycin D to the recovery medium showed that during recovery no cell division occurred and that this recovery was linked to RNA synthesis. Analysis of the fatty acid composition of uninjured and recovered cells by gas liquid chromatography showed an increased concentration of saturated fatty acids in recovered cells. A concurrent increase in resistance to heat shock at 55 C and salt tolerance in these recovered cells occurred with the change in fatty acid composition. It is suggested that a change in membrane structure as reflected by the modified fatty acid profile is related to an increased thermal resistance and salt tolerance.

When non-sporulating bacteria are subjected to a sub-lethal heat treatment, a variety of lesions are produced. The various manifestations of injury are impaired permeability *(2, 7, 8)*, an extended lag phase *(4, 6)*, degradation of ribosomal RNA *(10, 12, 13)*, some, but not extensive, metabolic damage *(3, 14)*, and an increase sensitivity to many selective agents *(4, 11)*. The injury is repairable, because the injured cells are able to recover when exposed to a suitable environment.

Although the literature is continuing to provide us with detailed information on the types of macromolecular syntheses necessary for recovery of a variety of different microorganisms, very limited studies of lipid biosynthesis during recovery have been reported *(9, 15)*. Also, little information is available on the subsequent behavior and characteristics of heat-injured cells once recovered.

It is hoped that the observations reported here regarding the effects of thermal stress on *Streptococcus faecium* NRC 1261, its lipid composition, and the relationship of these effects to the subsequent characteristics of such cells, will complement and extend the studies cited above.

## Methods

*Organism*

S. *faecium* NRC 1261 was obtained from the culture collection of the National Research Council, Ottawa, Canada.

*Growth media*

TYGA (tryptone 2%, yeast extract 0.5%, glucose 0.2%, $K_2HPO_4$ 0.4%, agar 1.5%) and TYGB, the broth equivalent of TYGA.

*Heat treatment*

Cells from exponential phase cultures of S. *faecium* NRC 1261 grown at 37 C in TYGB were harvested by centrifugation, washed twice in phosphate buffered saline (PBS-NaCl 0.8%, $K_2HPO_4$ 0.12%, $KH_2PO_4$ 0.034%, pH 7.2) and resuspended in 100 ml of the same buffer at a density of 2.0 to 4.0 $\times$ $10^8$ cells/ml. Five milliliters of this suspension were transferred to each of 20 sterile screw-capped test tubes (Kimble brand, 16 $\times$ 150 mm) and heated at 55 C for 15 min to induce injury, and for longer times when survivor curves were determined. The suspensions reached the desired temperature within 3 min. To minimize settling, tubes were agitated for 10 sec at 5-min intervals. After heating, suspensions were cooled by immersion in a waterbath maintained at 10-12 C.

Injury was assessed by pour-plating samples at regular intervals in TYGA and in TYGA containing 2.5% NaCl. Plates were counted after 48 h of incubation at 37 C and the degree of injury expressed as the difference between the counts on TYGA and TYGA-NaCl.

*Recovery*

Immediately after thermal treatment, cells were cooled to 10-12 C, harvested, washed, resuspended in 100 ml TYGB, and incubated at 37 C. Recovery of salt tolerance was determined by pour-plating in TYGA and TYGA-NaCl at regular time intervals.

*Effect of metabolic inhibitors on recovery*

Penicillin (100 $\mu$g/ml) and actinomycin D (1 $\mu$g/ml) were added to TYGB to study their effect on recovery from thermal stress.

*Lipid extraction*

Washed suspensions of uninjured, injured, and recovered cells were lyophylized. About 100 mg (dry weight) of cells were suspended in 5 ml of 95% ethanol containing 5% KOH and kept at 60 C for 18 h in a 20-ml heat-sealed ampoule. After cooling, 5 ml of distilled water were added and the nonsaponifiable material extracted into 10 ml of petroleum ether (b.p. 30 to 60 C). The aqueous mixture was acidified with 5% $H_2SO_4$ to pH 5.0 and the free fatty acids were extracted into three 5-ml portions of petroleum ether and evaporated to dryness in a flash evaporator.

*Gas-liquid chromatography (GLC)*

Fatty acid methyl esters were prepared for GLC according to the method of Agate and Vishniac *(1)* except that the methyl esters were reconstituted to 0.1 ml with hexane. Chromatograms were obtained with a Hewlett-Packard gas chromatograph 7620 A, equipped with a dual flame ionization detector and connected to a Spectra-Physics Autolab System 4 computer and electronic integrator. The GLC conditions were. 9 ft $\times$ 1/8 inch stainless-steel column pack-

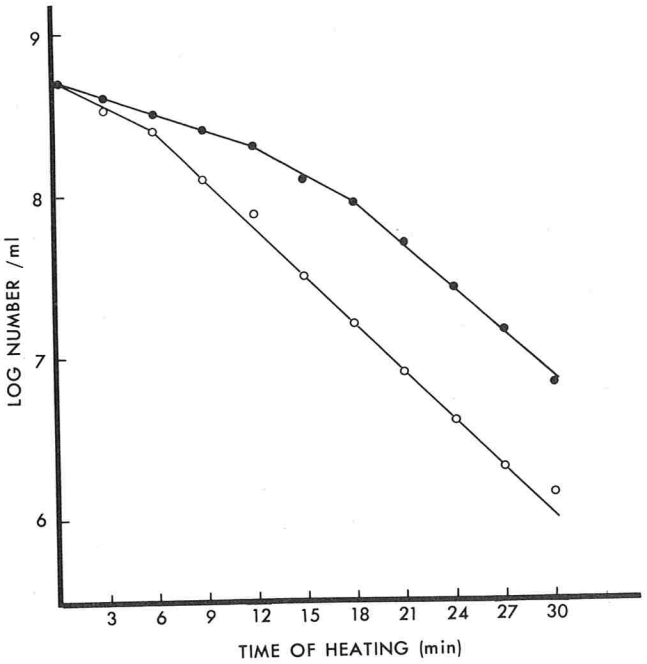


Figure 1. Survivor curve of *Streptococcus faecium* NRC 1261, heated at 55 C in phosphate buffered saline at pH 7.2 for 15 min and plated at intervals on TYGA and TYGA containing 2.5% NaCl. Incubation was at 37 C. Closed circles, TYGA; open circles, TYGA plus 2.5% NaCl.

ed with 15% diethylene glycol succinate (DEGS) on 80/100 mesh Chromosorb W-AW; injection port temperature 210 C; detector temperature programming (total time $10^4$ sec) 70 C to 140 C at 4 C/min, 140 C to 170 C at 0.5 C/min and hold at 170 C for 10 min, 170 C to 200 C at 0.5 C/min and hold at 200 C for remaining time; nitrogen gas flow rate, column A, rotameter setting 1.7, column B rotameter setting 1.4; range $10^2$; attenuation 1. Fatty acid methyl esters were identified by comparing their retention times with those of commercially available standards. (Applied Science Labs., Inc., State College, Pa.).

## Results and Discussion

Differences in survival were found when heated cells of *Streptococcus faecium* NRC 1261 were plated out in TYGA and TYGA-NaCl (Fig. 1). The total viable population, as represented by the counts in TYGA, decreased steadily after 3 min of heating, indicating that death occurred very early in the treatment. However after 15 min, significant injury, manifested as salt-sensitivity, was observed in the surviving population. Injury was further expressed by a continuous increase in the heating menstruum, with time, of compounds absorbing at 260 nm.

When the heat-stressed cells were transferred to a recovery medium (TYGB) and incubated at 37 C, an extended lag time was observed (Fig. 2, curve 3). During this period, recovery of the cells from thermal stress was demonstrated by a complete return of their competence to grow on TYGA-NaCl medium. The fact that this extended lag and the recovery of salt tolerance (curves 1, 2) occurred in the same time interval, reinforces the assumption that these are valid parameters for measuring heat-induced injury. It was observed that during recovery, an injured cell population acquired a salt tolerance somewhat higher than that of the original cells, which showed growth inhibition at 3% NaCl (Fig. 2, curve 1).

The shape of the recovery curve, the absence of an increase in the TYGA count during recovery, and the fact that recovery occurred in the presence of penicillin, indicate that this phenomenon was not the result of the multiplication of a few survivors (Fig. 3). Actinomycin D inhibited recovery of the injured cells completely, indicating participation of RNA synthesis in the repair process.

Since permeability control is mainly membrane associated, the phenomena of leakage, increased salt sensitivity, and an altered response of recovered cells to salt, suggested that the cellular defects responsible were located in the cell envelope.

Studies have indicated that changes in lipid composition play a major role in altered membrane characteristics of bacteria as a result of mutation or environmental challenges *(5, 16)*. Since the bulk of bacterial lipids is located in the cell envelope, differences in fatty acid profiles between untreated

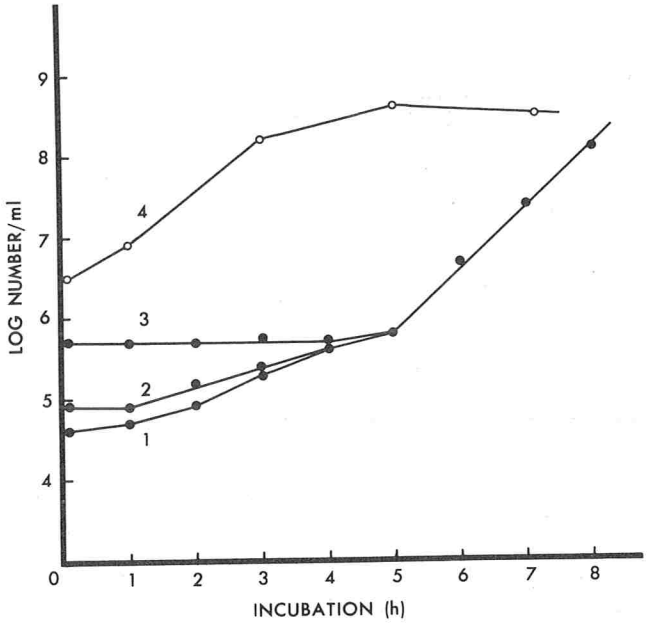


Figure 2. Growth and recovery of *Streptococcus faecium* NRC 1261 in TYGB at 37 C before and after sublethal heat treatment at 55 C for 15 min in phosphate buffered saline at pH 7.2. Open circles, no heat treatment (curve 4; plated on TYGA); Closed circles, heated: curve 1, plated on TYGA + 3.0% NaCl, curve 2, plated on TYGA + 2.5% NaCl, and curve 3, plated on TYGA

TABLE 1. FATTY ACID COMPOSITION OF LIPID EXTRACTED FROM UNINJURED, INJURED AND RECOVERED CELLS OF *Streptococcus faecium* NRC 1261

| Peak no. | Fatty acid | Uninjured | Injured | Recovered |
|---|---|---|---|---|
| | | (% Total peak area) | | |
| 1 | C12:0 | 0.12 | 0.66 | 2.22 |
| 2 | u.i.[a] | trace[b] | —[c] | ——— |
| 3 | C14:0 | 4.34 | 4.67 | 6.43 |
| 4 | C14:1 | 1.40 | 1.50 | 1.10 |
| 5 | u.i. | 0.11 | ——— | ——— |
| 6 | C16:0 | 12.84 | 13.64 | 14.78 |
| 7 | C16:1 | 19.26 | 15.85 | 13.88 |
| 8 | C18:0 | 1.36 | 2.80 | 2.21 |
| 9 | C18:1 | 55.14 | 56.39 | 54.95 |
| 10 | C18:2 | 0.96 | 1.39 | 0.73 |
| 11 | u.i. | 0.40 | ——— | ——— |
| 12 | u.i. | 2.36 | 1.44 | 1.40 |
| 13 | u.i. | 0.82 | 1.67 | 2.30 |
| 14 | u.i. | 0.38 | ——— | ——— |
| 15 | u.i. | 0.21 | ——— | ——— |
| 16 | u.i. | 0.24 | ——— | ——— |
| Saturated | | 18.54 | 21.77 | 24.93 |
| Unsaturated | | 76.76 | 75.13 | 68.72 |
| Unidentified | | 4.41 | 3.11 | 6.35 |

[a]u.i., unidentified [b]Trace indicates <0.1% of the total fatty acids [c]Dash indicates no detectable amount.

TABLE 2. EFFECT OF THERMAL STRESS ON SURVIVAL OF NON-RECOVERED AND RECOVERED CELLS OF *Streptococcus faecium* NRC 1261[a]

| Treatment | Non-recovered cells | Recovered cells |
|---|---|---|
| | (Viable cell count/ml) | |
| Unheated | $21 \times 10^7$ | $76 \times 10^6$ |
| Heated | $22 \times 10^5$ | $24 \times 10^6$ |
| % Killed | 98.9 | 68.4 |
| % Of survivors injured | 97.0 | 62.4 |

[a]The cells were heated in phosphate buffered saline (PBS), pH 7.2, at 55 C for 15 min. For recovery, cells were transferred to tryptone yeast extract glucose broth (TYGB) and incubated at 37 C for 5 h and again PBS heat-stressed. For each treatment, both types of cells were pour-plated in tryptone yeast extract glucose agar (TYGA) and in TYGA + 2.5% NaCl (TYGAS). The TYGA count gave a measure of all viable cells; the TYGAS gave an estimation of the uninjured cells.

and treated cells would be indicative of heat-induced effects at this locus. The observed phenomenon of heat-induced permeability impairment suggested the importance of an analysis of the fatty acid composition of uninjured, injured, and recovered cells.

Analysis of the methyl esters of fatty acids from normal cells by GLC gave 16 peaks of which 8 were unidentified. These eight components represented only 6.35% of the total amount of fatty acids present. The injured and recovered cells each gave 10 peaks of which 2 were unidentified, representing 3.11 and 3.70% of the total fatty acid fraction respectively (Table 1). There was a definite tendency for cells repairing their injury to synthesize more saturated fatty acids, in particular lauric ($C_{12}$), myristic ($C_{14}$), palmitic ($C_{16}$) and stearic ($C_{18}$) acid. The trend of the heat-induced alterations becomes quite clear if groups of individual fatty acids, arranged according to saturation and unsaturation, are compared.

Although imposition of heat-stress on cells caused only slight differences in the percentage distribution of some major fatty acids, some minor lipid components with chain lengths greater than $C_{18}$ had decreased or were not re-synthesized, while others increased two-fold from their original value. The shift from a predominance of unsaturated fatty acids in uninjured cells to an increase of saturated fatty acids in recovered cells is a definite indication of heat-induced lesions in cellular lipid-containing structure(s). The quantitative modification in the profile of recovered cells suggests not only that lipid synthesis occurs during recovery, but also that the repaired structure(s) is (are) different in composition from that of the undamaged cells.

The membrane, delimiting the cell, and presumably conferring to it the characteristic of selective permeability, is in large part constituted of lipid. Any alteration in the fatty acid profile taking place during recovery should be a valid indication of a change in the physical state and functional properties of the membrane. There was indeed ample evidence of thermally-induced membrane damage which was subsequently repaired, but why the exact quantitative levels of the original fatty acid species were not reconstituted in recovering cells is difficult to appreciate.

Very early in this study, (Fig. 2), it was observed that cells recovering from heat-induced injury acquired a higher salt tolerance than their untreated counterparts. It is tempting to speculate on a possible connection between this increased salt tolerance and the slight modification in lipid composition, thus possibly reflecting a change in the physical state and functional properties of the membrane of heat-injured but recovered cells. Indeed, recovered cells were able to tolerate 3.5% NaCl (1% more NaCl than the untreated population).

When an injured population, immediately after a 5 h recovery period, was again subjected to the thermal stress, a substantial increase in heat resistance and a concommittant decrease in the percentage of injury amongst the survivors occurred (Table 2). Therefore, it was of interest to know if the change in salt tolerance and heat resistance of recovered cells which accompanied the modification in fatty acid composition was temporary or if it had become a permanent characteristic. Consequently, recovering cells were allowed to proceed into the log phase of their growth

TABLE 3. HEAT RESISTANCE, SALT TOLERANCE, AND EXTENT OF INJURY OF NORMAL AND RECOVERED CELLS OF *Streptococcus faecium* NRC 1261[a]

| Treatment | Normal | Recovered subcultured once | Recovered subcultured 12 × |
|---|---|---|---|
| | (Viable cell count/ml) | | |
| *Unheated* | 19 × $10^7$ | 16 × $10^7$ | |
| *Heated* | | | |
| 0.0% NaCl | 17 × $10^5$ | 94 × $10^6$ | |
| 2.5% NaCl | 31 × $10^4$ | 89 × $10^6$ | |
| 3.0% NaCl | 8 × $10^3$ | 55 × $10^6$ | |
| % Killed | 99.1 | 41.2 | |
| % Of survivors injured | | | |
| 2.5% NaCl | 81.7 | 5.3 | |
| 3.0 NaCl | 99.5 | 41.5 | |
| *Unheated* | 12 × $10^7$ | | 90 × $10^6$ |
| *Heated* | | | |
| 0.0% NaCl | 24 × $10^5$ | | 66 × $10^6$ |
| 2.5% NaCl | 42 × $10^4$ | | 59 × $10^6$ |
| 3.0% NaCl | 16 × $10^3$ | | 48 × $10^6$ |
| % Killed | 98.0 | | 26.6 |
| % Of survivors injured | | | |
| 2.5% NaCl | 82.5 | | 10.3 |
| 3.0% NaCl | 99.3 | | 27.2 |

[a]Treatment as for Table 2

cycle. A subculture (log phase) of these cells was then again thermally stressed in phosphate buffer. The subsequently recovered cell population showed a marked increase in heat resistance and salt tolerance with a concommittant drastic decrease in extent of injury (Table 3). The augmented resistance of recovered cells observed earlier thus does not appear to be lost after one subculture. In fact when recovered cells were subcultured 12 times and then subjected to thermal stress, similar results were obtained (Table 3). In addition, the fatty acid profile of these cells showed a large increase in saturated fatty acids, particularly $C_{14}$ and $C_{16}$, as compared to that of the original untreated population (Table 4). These observations are at variance with the belief that when injured cells are reinoculated into a suitable recovery medium, they regain their normal characteristics during the extended lag time. This belief is based on the observation that upon emergence from the recovery period treated cells grow at the same rate as untreated cells and achieve a comparable total viable population. The assumption is made that complete repair of the heated cells and a return to the normal state has taken place.

The present study shows that selection for heat resistance and salt tolerance can occur very rapidly in sublethally-heated S. *faecium*. The fact that restoration of salt tolerance occurred concurrently with a change in fatty acid composition, reinforces the suggestion that membrane damage with a coincident degradation of RNA might be the major effect of thermal stress and that both lipid synthesis and RNA synthesis is involved in repair. Depletion of the intracellular pool through leakage may be a secondary but important contribution to irreversible death of the cell. For recovery to take place, the membrane must be repaired and the lost material re-synthesized and reconcentrated in the metabolic pool.

The permanent alteration in fatty acid composition which resulted, coincident with an augmented

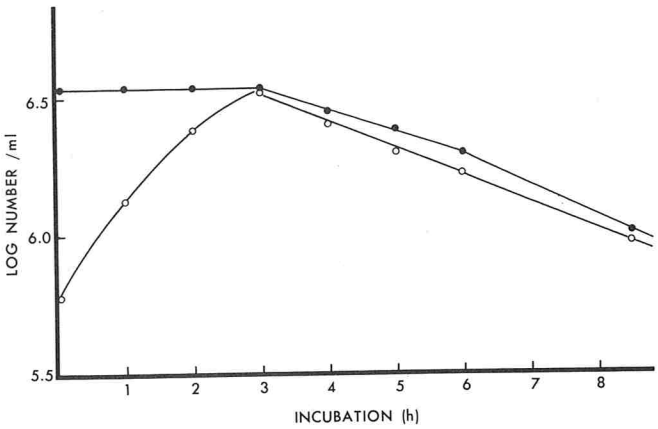


Figure 3. The effect of penicillin on the recovery of salt tolerance by heat-injured cells of *Streptococcus faecium* NRC 1261 when subsequently incubated at 37 C in TYGB containing 100 μg of penicillin per milliliter. Closed circles, TYGA; open circles, TYGA + 2.5% NaCl.

TABLE 4. FATTY ACID COMPOSITION OF HEAT-INJURED RECOVERED CELLS OF *Streptococcus faecium* NRC 1261, SUBCULTURED 12 TIMES

| Fatty acid | % Of total peak area |
|---|---|
| C10:0 | 0.369 |
| C12:0 | 1.187 |
| C14:0 | 9.366 |
| C14:1 | 0.931 |
| C16:0 | 25.949 |
| C16:1 | 14.519 |
| C18:0 | 1.297 |
| C18:1 | 41.065 |
| C18:2 | 0.510 |
| C18:3 | 0.215 |
| C20:0 + C21:1 | 0.154 |
| C22:0 + C22:1 | 1.368 |
| u.i.[a] (13 peaks) | 3.058 |
| Saturated | 38.168 |
| Unsaturated | 57.240 |
| C20:0 | |
| C21:1 | 1.522 |
| C22:0 | |
| C22:1 | |
| u.i. | 3.058 |

[a]u.i., unidentified

salt-and heat-resistance of recovered heat-injured cells, was not anticipated. We have no clear explanation for this result. The interpretation of this behavior in terms of the classical theories of mutation, adaptation, and selection is very difficult. The organisms were not "trained" to grow at higher temperatures, and neither were repeated heat treatments applied to select and build up the proportion of possibly resistant cells. The absence of multiplication during recovery would exclude the concept of mutation and selection.

If this is a general phenomenon and occurs in other organisms as well, some conditions of processing and subsequent handling of foods in the food industry may be such that progressively more organisms are "selected" for heat and salt-resistance.

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## SEX ATTRACTANTS AND DISEASE COMBINATION MAY BE NEW INSECT CONTROL METHOD

(Continued from page 381)

this protozoa and begin dying off after about nine days. It takes about a month before all infected insects die, so there's plenty of time for the infected beetles to carry the disease to other beetles. The disease organisms multiply within the infected insect, so there's a good chance of infection even if the insect originally eats only a few spores. Insects which die from the disease serve as sources of spores for use in the control programs.

Many laboratory and field studies still need to be done to work out the procedure. The researchers need to establish best levels of sex attractant materials and disease spore concentrations for use in the inoculation devices, as well as proper spacing of the devices in a plant or warehouse and the influences of low temperatures on inoculation effectiveness.

They also want to develop improved formulations of the disease spores for easier mass production, longer life of the inoculation devices, and improved pick-up by the insects. Finally, they need to try the technique in practical situations—feed mills, grain elevators, warehouses, and food processing plants.

The procedure has a lot of potential advantages for use around foods. It does not involve use of chemical insecticides which are difficult to apply and which might contaminate the food, beetles which have become resistant to insecticides can be controlled, and the inoculated insects themselves carry the control program to other insects which may be hidden, inaccessible to other control procedures, or not even hatched out.

# INHIBITION OF *CLOSTRIDIUM PERFRINGENS* BY *STREPTOCOCCUS FAECALIS* IN A MEDIUM CONTAINING CURING SALTS[1]

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(Received or publication February 11, 1974)

Abstract

Inhibition of two strains of *Clostridium perfringens* by *Streptococcus faecalis* in Cooked Meat Medium containing curing salts was investigated. Inhibitory effects were evaluated by growth-curve studies and by measurement of inhibition zones on agar. Both strains of *C. perfringens* were inhibited by S. *faecalis* in a medium containing glucose, although to different degrees. Addition of nitrite to the medium containing glucose increased the inhibitory effect of S. *faecalis* towards *C. perfringens*. Production of lactic acid by S. *faecalis* appears to play an important role in this inhibition of *C. perfringens*.

Sodium nitrate plays an important role in the preservation of cured meats. Several mechanisms have been proposed to explain the means by which nitrate prevents growth of food poisoning and food-spoilage organisms. It has been suggested that sodium nitrite, when heated in a laboratory medium, forms a bacterial inhibitor that is effective in preventing growth of several species of clostridia *(12, 13)*; such a mechanism, however, may not be active in cured meats *(8)*. Another role of sodium nitrite may be its ability to induce germination of spores of clostridia and thus render the spores susceptible to heat processing *(6)*. On the other hand, heat processing may damage the spores, and the role of the preservative action of nitrite may be to prevent outgrowth of these heat-damaged spores *(11)*. Another mechanism of inhibition by nitrite may be its possible interference with the normal metabolism of pyruvate *(4, 5)*.

Addition of nitrite to foods has become a controversial issue because nitrites may combine with secondary, tertiary, and quarternary amines under certain conditions to form nitrosamines, which are potential carcinogens *(15)*. Although control of *C. botulinum* in fish or fish products is accomplished by nitrite, nitrite may not be essential to control clostridia in canned hams *(9)*. Kafel and Ayres *(9)* observed that enterococci play a role in the preservation of canned hams by acting antagonistically on selected species of *Clostridium, Bacillus,* and *Lactobacillus*.

The objectives of this study were to determine conditions under which S. *faecalis* inhibits *C. perfringens* and to determine the nature of the inhibitory substance(s) produced during growth of S. *faecalis*.

## Materials and Methods

*Clostridium perfringens* type A, strain FD#1, was obtained from the Food and Drug Administration, Kansas City, Kansas. A second culture of *C. perfringens* (C46) and *Streptococcus faecalis* (C52) were isolated from a sample of ground beef in our laboratories. All cultures were maintained in Cooked Meat Medium (Difco), supplemented with 3.8% Reinforced Clostridial Medium (RCM) (Oxoid), and stored at room temperature.

The basal medium for growth was made by adding 200 ml of 3.8% RCM in distilled water to 16.6 g of Cooked Meat Medium. The medium, after standing for 10 min, was autoclaved at 121 C for 15 min. Curing salts were added singly or in various combinations to the basal medium at levels of 3.5% NaCl, 0.03% $NaNO_3$, and 0.02% $NaNO_2$ before sterilization *(2)*. A filter-sterilized solution of glucose was added to the sterilized medium to yield a final concentration of 2%. The basal medium was adjusted to various pH values before and after autoclaving with either sterile 1 N HCl or sterile 2.96 M L-lactic acid.

Each flask containing 200 ml of the test medium was inoculated with 1.0 ml of a diluted culture of *C. perfringens* grown for 24 h at 37 C, and/or with 1.0 ml of a diluted culture of S. *faecalis* grown for 48 h at 37 C, so that each flask contained approximately $10^4$ cells/ml. The cultures were diluted in 0.1% peptone water. Inoculated media were incubated at 37 or 25 C. Samples for enumeration of numbers of organisms were taken at intervals of 0, 6, 24, 30, 48, and 72 h.

For streak plates, APT agar was used either as obtained from Baltimore Biological Laboratories, Baltimore, Maryland, with or without curing salts added, or made up from its components, but omitting glucose. Fifteen milliliters of APT agar was poured into sterile petri dishes, and the surface dried for 24 h at 37 C before inoculation. The surface of the agar was streaked with a 48-h old culture of S. *faecalis* and incubated either aerobically or anaerobically for 48 h before streaking at right angles with a 24-h old culture of *C. perfringens (9)*. The inhibition zone was measured in millimeters with a vernier caliper.

*C. perfringens* was enumerated on SPS medium, suggested by Angelotti et al. *(1)*, incubated for 24 h at 37 C in an anaerobic incubator under a nitrogen atmosphere. S. *faecalis* was enumerated on KF agar *(10)* with incubation for 48 h at 37 C.

Lactic acid was estimated in the supernatant of the growth medium after diluting 1:10 with water. The method of Harper and Randolph *(7)* was used. Concentration of lactic acid was estimated from a standard curve prepared with the 1:10 diluted

[1]Journal Paper No. J-7798 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa. Project No. 1896. This investigation was supported in part by Public Health Service Grant No. FD 00444.

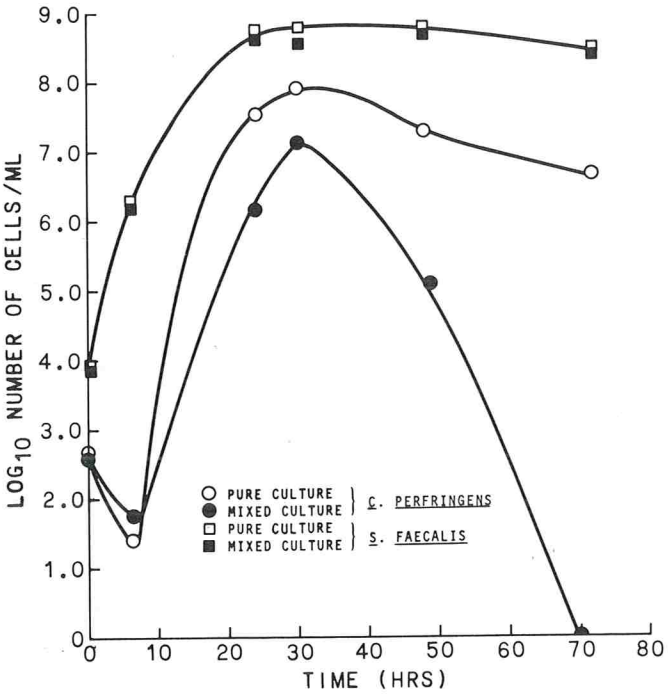


Figure 1. Growth of *C. perfringens* strain C46 and FD1 in the presence and absence of *S. faecalis* in Cooked Meat Medium containing 0.03% $NaNO_3$, 0.02% $NaNO_2$, 3.5% NaCl, and 2% glucose at 37 C. (Both strains of *C. perfringens* gave the same response.)

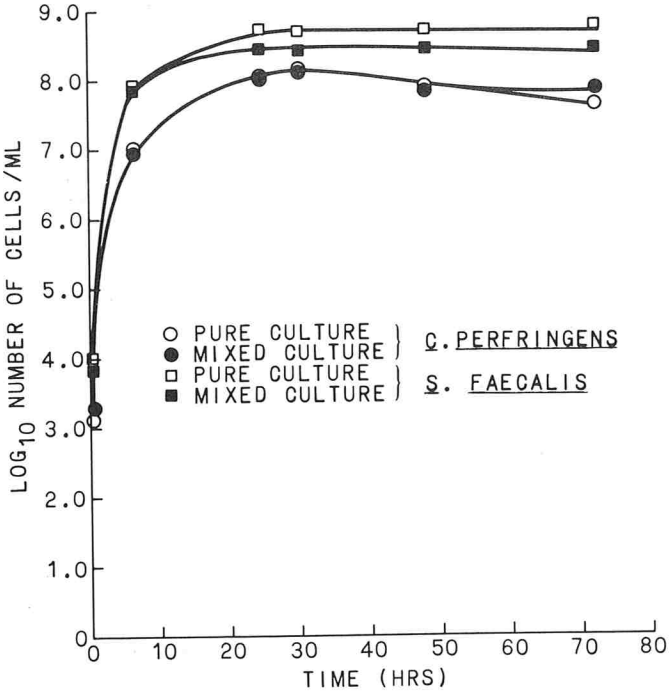


Figure 2. Growth of *C. perfringens* strains C46 and FD1 in the presence and absence of *S. faecalis* in Cooked Meat Medium at 37 C. (Both strains of *C. perfringens* gave the same response.)

growth medium containing known quantities of lactic acid.

## RESULTS

Effects of *S. faecalis* on *C. perfringens* were observed in Cooked Meat Medium with and without curing salts and in Cooked Meat Medium plus individual and various combinations of curing salts. Glucose is a common ingredient of such mixtures (2) and was included with the curing salts throughout this study.

When *S. faecalis* was grown in Cooked Meat Medium containing sodium nitrate, sodium nitrite, sodium chloride, and glucose, an inhibitory effect on growth of both strains of *C. perfringens* was evident (Fig. 1). This effect was observed 30 h after inoculation of the medium with *S. faecalis* and *C. perfringens; C. perfringens* was not recovered from cultures 72 h after inoculation. When *S. faecalis* was not present in the culture, and curing salts were present, *C. perfringens* continued to grow. In Cooked Meat Medium without curing salts, *S. faecalis* had no effect on the growth of *C. perfringens* strains tested (Fig. 2).

Neither sodium nitrite nor sodium chloride at levels used in curing mixes prevented growth of *C. perfringens* at 37 C. Sodium chloride, however, prolonged the lag phase of growth for both strains of *C. perfringens;* sodium nitrate caused little or no change in the growth patterns, and sodium nitrite was not inhibitory to growth of *C. perfringens* at

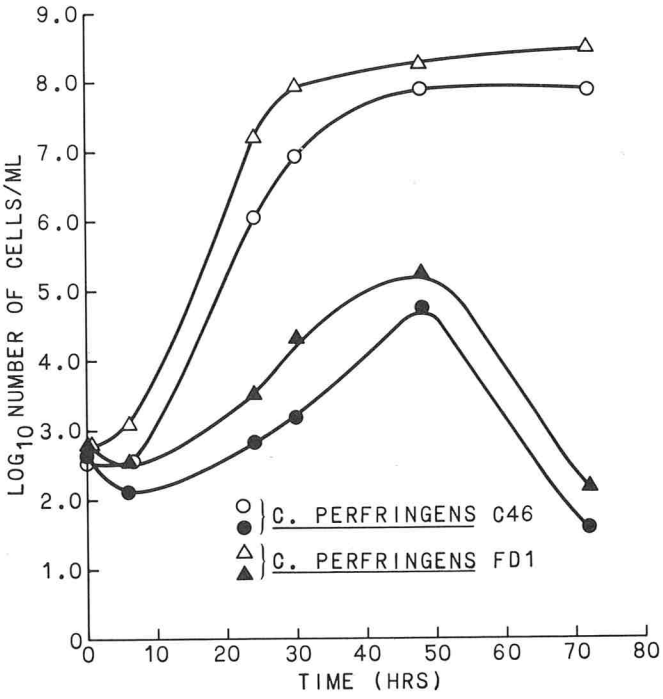


Figure 3. Effects of sodium chloride and sodium nitrite on growth of *C. perfringens* at 25 C. Open circles and triangles, 3.5% sodium chloride, closed circles and triangles, 3.5% sodium chloride and 0.02% sodium nitrite.

TABLE 1. INHIBITION OF *C. perfringens* BY S. *faecalis* ON APT AGAR STREAKPLATES

| Medium | Strain of *C. perfringens* | Inhibition zone in mm[a] | |
|---|---|---|---|
| | | *S. faecalis* grown[b] | |
| | | Aerobically | Anaerobically |
| APT[c] | FD#1 | 5.3 | 6.0 |
| | C46 | 2.5 | 6.1 |
| APT plus curing salts | FD#1 | 10.5 | 12.6 |
| | C46 | 12.8 | no growth |
| APT without dextrose | FD#1 | 0 | 0 |
| | C46 | 0 | 0 |

[a]Measurements are averages of 6 replicates.
[b]S. *faecalis* culture was streaked across the center of the plate, followed by incubation before streaking at right angles with *C. perfringens* culture.
[c]APT agar contains 1% dextrose.

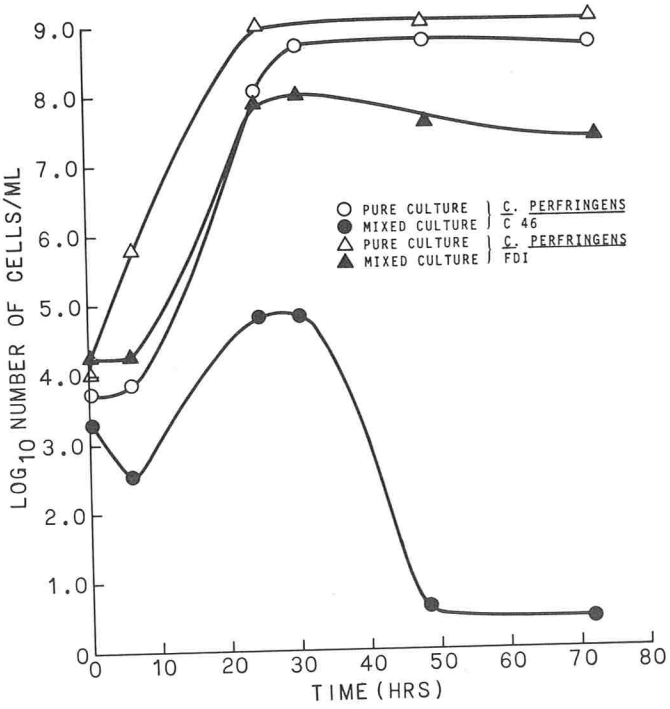


Figure 4. Growth of *C. perfringens* cultures in the presence of S. *faecalis* in a medium containing 2% glucose at 25 C.

37 C. In contrast to results from incubation at 37 C, sodium nitrite inhibited growth substantially at 25 C (Fig. 3). Most of these studies were, therefore, done at 25 C.

The inhibitory effect of S. *faecalis* on the growth of *C. perfringens* was observed only when the medium contained glucose (Fig. 4). The two strains of *C. perfringens* exhibited a difference in their sensitivity to the inhibitory agent produced by S. *faecalis* in the medium containing glucose. Strain FD1 was not as sensitive as strain C46 to the inhibitory agent produced by S. *faecalis*, but addition of nitrite to the medium containing glucose resulted in inhibition of growth of both strains (Fig. 5) when S. *faecalis* was present.

The inhibitory effect produced by S. *faecalis* with the complete mixture of curing salts was first noticed 30 h after simultaneous inoculation of S. *faecalis* and *C. perfringens* into the growth medium. This time coincided with a rapid decrease in pH and accumulation of lactic acid. S. *faecalis* produced a concentration of 200 mM lactic acid (pH 4.8-4.9) after 72 h in a medium containing 2% glucose; *C. perfringens* produced a concentration of 100-120 mM (pH 5.2-5.3) under the same conditions. Sensitivity of *C. perfringens* to lactic acid was tested in Cooked Meat Medium adjusted to various pH values with L-lactic acid. The results in Fig. 6 show that inhibition of growth of *C. perfringens* was greater in media containing lactic acid and no curing salts than in media adjusted to the same pH with HCl. In the absence of curing salts, little or no growth of *C. perfringens* was observed in media adjusted to pH 5.4 or below with L-lactic acid as compared with those media adjusted with HCl to the same pH. However, when curing salts were added, HCl was more inhibitory than L-lactic acid at the same pH. Media adjusted with L-lactic acid in the presence or absence of curing salts seemed to have about the same effect on the survival of *C. perfringens*.

Inhibition of *C. perfringens* by S. *faecalis* on APT agar followed the same general pattern as observed with the Cooked Meat Medium (Table 1). However, on APT agar with no curing salts added, inhibition

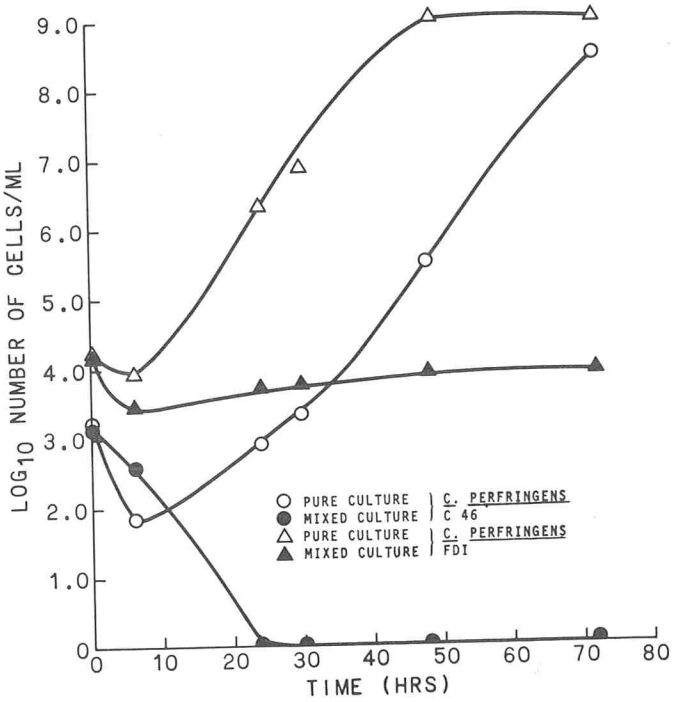


Figure 5. Growth of *C. perfringens* cultures in the presence of S. *faecalis* in a medium containing 2% glucose and 0.02% $NaNO_2$ at 25 C.

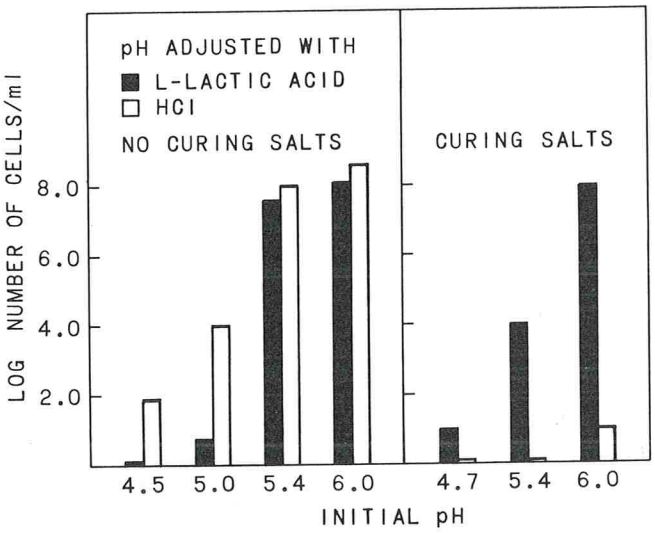


Figure 6. Extent of growth of *C. perfringens* C46 in growth media adjusted to various initial pH levels using lactic acid and hydrochloric acid in the presence and absence o curing salts.

of *C. perfringens* by S. *faecalis* was observed. When this agar was prepared from its constituents, but omitting glucose, no inhibition was observed. The inhibition zone was larger when the plates streaked with S. *faecalis* were previously incubated anaerobically, rather than aerobically, before counter-streaking with *C. perfringens*.

## Discussion

Kafel and Ayres *(9)* have presented evidence that inhibition of *C. perfringens* by *S. faecalis* in canned hams and on APT agar streak plates is caused by a metabolite that diffuses into the medium. The inhibitory agent that they observed was effective over a wide range of pH ,was not filterable, and was removed by autoclaving. Curing salts were not implicated in their work, but the primary reason for inhibition of *C. perfringens* was related to the presence of S. *faecalis*.

S. *faecalis* in the presence of glucose produces large quantities of lactic acid *(14)*. Lactic-acid producing organisms have been widely used in starter cultures for the preparation of milk products, in part to inhibit growth of undesirable bacteria. In the experiments presented here, S. *faecalis* produced almost twice as much lactic acid as *C. perfringens* in Cooked Meat Medium containing 2% glucose (200 mM and 100-120 mM, respectively, and corresponding to final pH values of 4.8-4.9 and 5.2-5.3). Results obtained from experiments with Cooked Meat Medium adjusted to various pH values with lactic acid or HCl indicated that lactic acid is inhibitory and that inhibition is not a matter of pH only. S. *faecalis* under anaerobic conditions produces larger quantities of lactic acid per unit of glucose than under aerobic conditions *(14)*. If it is assumed that lactic acid is the main inhibitory agent, then this would explain the greater inhibition observed on APT agar when S. *faecalis* is first incubated anaerobically rather than aerobically before inoculating with *C. perfringens*.

A second inhibitory effect on the growth of *C. perfringens* by S. *faecalis* is observed when nitrite is added to the medium containing glucose. This inhibitory agent is presumably nitrious acid formed from nitrite at low pH. Nitrous acid reacts with primary and secondary amines to form the corresponding nitrosamines and also could inactivate certain enzymes by deamination *(3)*. Another mechanism for inhibition by nitrous acid may be that suggested by Buchanan and Solberg *(4)*. These authors postulate that nitrite blocks the sulfhydryl sites of either $\alpha$-lipoic acid or coenzyme A, thus blocking normal pyruvate metabolism.

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## SELENIUM REDUCES MERCURY TOXICITY

Mercury in tuna is getting a second look from University of Wisconsin researchers because selenium, a second metal found in tuna apparently reduces mercury poisoning.

Poultry scientist M. L. Sunde and nutrition scientist H. E. Ganther, researchers in the College of Agricultural and Life Sciences, are studying mercury-selenium interactions. It appears that mercury and selenium interact or possibly combine, each reducing the harmful effects of the other when fed at other than low concentrations.

The researchers consistently found that animals raised on diets with high levels—6-20 parts per million (ppm)—of both metals were healthier than animals which received similar amounts of only one metal.

Tuna is naturally rich in selenium. As it accumulates mercury, it also accumulates selenium. The researchers say that if enough data can be gathered supporting the protective effect of selenium, it would perhaps be worth reconsidering the Food and Drug Administration's (FDA) established acceptable mercury level in tuna. The FDA bans the sale of tuna with more than .5 ppm of mercury.

The researchers have studied mercury-selenium interactions in poultry and rats.

Sunde raised seven generations of Japanese Quail on diets with selenium, mercury, and mercury and selenium. Tuna was the mercury source in some diets. The 17 percent tuna in the diet fed quail may be equated to a human being eating 40, 6 ½ oz. cans of tuna a week, Sunde says. Sunde observed no harmful effects from feeding this much tuna.

An additional 7 ppm of mercury was added to the tuna diet making the total mercury 15 times greater than the maximum allowable level. When parents were fed this diet, 48 percent of their offspring died within the first week of life. Ten ppm of mercury reduced fertility, and 20 ppm killed the birds sometime after four weeks. They survived that long, Sunde says, because the birds got rid of mercury in their feathers until the final set of feathers grew in when they were about 4-5 weeks old. Females survived better than males because they were able to deposit extra mercury in their eggs.

Regular corn-soy meal diets with various levels of these metals added were fed to other quail. Only 5 ppm of mercury in the maternal diet was necessary to kill 55 percent of offspring within one week of life. Birds with 20 ppm also began dieing at four weeks, but died before the tuna-fed birds.

The researchers found that mercury reduced the toxic effects of high levels of selenium. When fed 3 to 6 ppm of selenium, quail produced more abnormal embryos than birds fed a combination of mercury and selenium.

Rats with selenium and mercury in their diets survived better than rats with mercury alone. Ganther fed them 10 ppm of mercury in drinking water or 20 ppm of mercury in food, the minimum amounts for noticeable mercury poisoning. He added selenium to some of their diets.

Selenium doesn't seem to effect absorption or excretion of mercury. The researchers found that rats and quail fed a combination of selenium and mercury stored mercury in the same amounts and places as those fed only mercury. This means they combine in some way that doesn't decrease the amount stored in the body but does reduce toxicity. The researchers are planning more experiments to help determine how selenium and mercury protect against each other.

# SENSORY EVALUATION OF HEALTH FOODS – A COMPARISON WITH TRADITIONAL FOODS[1]

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Abstract

Twenty-five health foods were compared to traditional foods with respect to color, flavor, texture, odor, and overall acceptance. None of the health foods was found to be superior on the basis of overall acceptance, but three of the items were individually preferred for color or odor. Many traditional foods, however, were preferred for color, odor, flavor, and texture as well as overall acceptance.

There is a widespread and growing interest in the value and use of health foods *(2)*. Once use of health foods was restricted to a relatively small group of Americans who had to shop in specialty stores to find desired products. Today interest in health foods has spread to a much larger segment of our society. It is not uncommon to see new products appealing to consumers interested in natural or health foods in our supermarkets–breakfast cereals being a prime example.

Although many claims are made for the nutritional value and organoleptic quality of health foods, few objective data exist to support these claims *(4)*. In one of the few published reports, it was shown that certain health foods possess no more nutritional value than traditional foods *(1)*. The present study was undertaken to determine whether or not there is a subjective preference for health foods based on sensory qualities.

## Materials and Methods

A taste panel was set up to evaluate the sensory properties of health foods. Every attempt was made to make the taste panel as representative as possible within the limits of our academic community. The panel consisted of 20 members, male and female, from ages 18 to 60. Certain of the panel members indicated that they had consumed health foods in the past. The taste panel met three to four times a week for three months.

Foods were judged for odor, flavor, texture, color, and overall acceptance. Panelists were asked to rank each attribute on a 9-point hedonic scale. In addition, the health foods were paired as closely as possible with comparable traditional food items to see whether or not the health foods are more desirable. Results were statistically analyzed by analysis of variance and Duncan's Multiple Range Test *(3)*.

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## Results and Discussion

Of the 25 health food items judged, none were found to be significantly superior in overall acceptance to traditional food items (Table 1). Traditional dried apples, apple juice, apple sauce, cashews, cereal, Swiss cheese, coconut, corn chips, ice cream, mayonnaise with tomato, peanut butter, sesame chips, and tomato juice, however, were found to be significantly more acceptable overall than their health food counterparts. The only health food sensory attributes found to be superior to the corresponding attributes of traditional food were the odor of apple butter and pizza and the color of ketchup. Many traditional foods on the other hand were individually preferred for odor, color, texture, or flavor.

The marked preference for traditional foods can be due to a number of reasons. First, the American consumer is accustomed to products of high quality. Continued consumption of these products undoubtedly results in preferences. Secondly, most food scientists recognize that it is impossible to build in and maintain high standards of quality without the utilization of food additives. In addition, where quality control and modern technologies are used to produce a traditional food with natural or health characteristics–such as the traditional cereal used in this study –a most acceptable product is formulated at a lower cost.

These results raise the issue as to whether or not health foods produced without the benefits of modern food technology are worth their additional cost (Table 1). Twenty-three of the 25 traditional foods were equal to or substantially less in price than the health foods to which they were compared. One health food product, apple butter, was six times the cost of the traditional food. The higher cost of the health foods was not reflected by any general preference for the sensory attributes of these products. When one considers this along with the results of our previous study which showed no inherent nutritional differences between the two groups of foods *(1)* it is difficult to justify the increasing demand for health foods unless it is based on a yet unfounded fear of

TABLE 1. SENSORY EVALUATION OF HEALTH AND TRADITIONAL FOODS[1]

| Product | | Odor | Color | Texture | Flavor | Overall acceptance | cost/oz |
|---|---|---|---|---|---|---|---|
| Apple butter | Traditional | 6.8b | 6.6a | 5.7a | 6.1a | 6.1a | $ .016 |
| | Health | 7.1a | 6.7a | 6.2a | 6.4a | 6.2a | .097 |
| | Traditional | 5.4b | 6.2a | 6.0a | 4.9a | 4.8a | .014 |
| Dried apples | Traditional | 6.1a | 6.4a | 6.2a | 6.6a | 6.8a | .082 |
| | Health | 4.8b | 4.4b | 4.4b | 5.6a | 5.0b | .108 |
| Apple juice | Traditional | 7.2a | 7.0a | 7.4a | 7.2a | 7.4a | .012 |
| | Health | 6.0b | 3.8c | 4.3c | 4.6c | 4.2c | .033 |
| | Health | 6.6ab | 5.1b | 5.9b | 6.0b | 5.6b | .028 |
| Apple Sauce | Health | 6.8a | 5.8b | 5.7b | 4.4b | 4.6a | .050 |
| | Traditional | 6.6a | 7.6a | 7.3a | 7.9a | 8.0b | .024 |
| Beans/ tomato sauce | Traditional | 7.0a | 7.4a | 7.3a | 7.2a | 7.2a | .015 |
| | Health | 7.2a | 7.3a | 7.4a | 6.7a | 6.8a | .023 |
| Bread whole wheat | Traditional | 7.2a | 6.9a | 6.8b | 6.9a | 6.9a | .028 |
| | Health | 6.9a | 7.4a | 6.8ab | 6.9a | 7.1a | .031 |
| | Traditional | 7.0a | 7.4a | 7.5a | 7.0a | 7.2a | .024 |
| Cashews | Health | 5.5b | 5.2b | 6.4a | 6.0b | 6.1b | .097 |
| | Traditional | 6.6a | 6.8a | 7.3a | 7.8a | 7.8a | .097 |
| Cereal | Traditional | 7.0a | 6.8a | 5.6a | 7.1a | 7.1a | .041 |
| | Health | 5.3b | 6.2a | 5.4a | 5.5b | 5.9b | .049 |
| Mild Cheddar cheese | Health | 6.5a | 5.1b | 6.5a | 6.8a | 6.7a | .120 |
| | Traditional | 6.2a | 7.5a | 6.7a | 6.5a | 6.8a | .076 |
| | Traditional | 5.8a | 7.1a | 7.0a | 6.4a | 6.6a | .085 |
| Swiss cheese | Traditional | 7.1a | 7.1a | 6.7a | 6.6a | 6.8a | .091 |
| | Traditional | 6.6a | 7.2a | 6.4a | 6.6a | 6.4a | .115 |
| | Health | 6.7a | 6.7a | 5.6a | 5.2b | 5.2b | .134 |
| Coconut | Health | 6.7a | 7.8a | 5.2b | 4.6b | 4.9b | .034 |
| | Traditional | 7.8a | 8.0a | 8.1a | 8.1a | 8.1a | .070 |
| Corn chips | Health | 4.8b | 5.3b | 5.6b | 5.0b | 5.2b | .122 |
| | Traditional | 7.0a | 7.4a | 7.3a | 7.4a | 7.7a | .058 |
| | Traditional | 6.0a | 7.1a | 6.9a | 7.0a | 7.0a | .045 |
| Dates | Traditional | 6.3a | 6.6a | 6.5a | 6.8a | 6.7a | .049 |
| | Health | 6.0a | 6.4a | 6.8a | 7.1a | 7.1a | .061 |
| Fig bars | Health | 7.0a | 6.5a | 7.0a | 7.0a | 7.0a | .044 |
| | Traditional | 7.8a | 7.4a | 7.0a | 7.2a | 7.4a | .044 |
| | Traditional | 6.2b | 7.0a | 6.2a | 6.7a | 6.6a | .022 |
| Orange blossom honey | Traditional | 6.2a | 8.4a | 7.4a | 6.8a | 7.0a | .043 |
| | Health | 6.3a | 7.0a | 7.2a | 6.2a | 6.8a | .056 |
| | Traditional | 6.8a | 7.2a | 7.5a | 7.2a | 7.4a | .042 |
| Ice cream | Health | 6.2b | 6.6b | 7.5b | 6.7b | 6.7b | .035 |
| | Traditional | 7.6a | 7.9a | 8.2a | 8.3a | 8.4a | .015 |
| Ketchup | Health | 7.0a | 7.9a | 7.6a | 7.7a | 7.6a | .046 |
| | Traditional | 7.2a | 7.0b | 7.0a | 7.4a | 7.2a | .024 |
| Mayonnaise/ potatoes | Health | 6.8a | 6.8a | 7.0a | 6.6a | 6.8a | .074 |
| | Traditional | 6.8a | 6.8a | 6.6a | 6.4a | 6.5a | .034 |
| Mayonnaise/ tomato | Health | 7.0a | 7.6a | 7.4a | 6.0b | 5.8b | .074 |
| | Traditional | 7.2a | 7.6a | 7.0a | 7.6a | 7.4a | .034 |
| Powdered milk | Health | 5.7a | 6.8a | 6.6a | 5.8a | 5.7a | .056 |
| | Traditional | 5.9a | 6.6a | 6.8a | 5.8a | 5.9a | .060 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Peanut butter | Health | 6.6a | 6.1b | 4.2b | 5.3b | 4.8b | .049 |
| | Traditional | 7.7a | 7.3a | 7.4a | 7.3a | 7.2a | .040 |
| | Health | 6.4a | 5.8b | 4.5b | 3.9c | 4.0b | .095 |
| | Traditional | 6.9a | 7.7a | 7.6a | 6.3b | 6.4a | .040 |
| Cheese pizza | Traditional | 6.4b | 7.4a | 6.3a | 6.4a | 6.5a | 1.110 |
| 8" | Health | 7.4a | 5.8b | 6.4a | 6.6a | 6.7a | 1.250 |
| Rice | Health | 6.8a | 5.4b | 5.5b | 5.9a | 5.9a | .026 |
| long grain | Traditional | 7.1a | 7.0a | 7.0a | 6.0a | 6.2a | .026 |
| Sesame chips | Health | 5.7a | 6.8a | 6.0b | 6.1b | 6.2b | .098 |
| | Traditional | 6.8a | 7.0a | 7.4a | 7.2a | 7.0a | .078 |
| Tomato juice | Traditional | 7.4a | 7.4a | 6.9a | 7.1a | 7.2a | .093 |
| | Health | 5.4b | 5.8b | 5.8b | 2.8b | 3.0b | .225 |

[1]Scale = 9-point hedonic with 1 = dislike extremely to 9 = like extremely. Values for each food product attribute which are followed by the same letter are not different at the 95% level of significance.

modern technology and distrust of the safety of our food supply.

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# PRODUCTION OF AFLATOXIN IN CONCENTRATED AND DILUTED GRAPEFRUIT JUICE[1]

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Abstract

Commercial concentrated and diluted (1:1, 1:2, 1:3; juice: water) steamed grapefruit juice was inoculated with known aflatoxigenic aspergilli and sampled after 10 and 14 days of incubation at 28 C. When *Aspergillus flavus* grew in juice, most aflatoxin $B_1$ (0.211 μg/ml appeared in concentrated juice and least (0.013 μg/ml) in single strength juice. Juices diluted 1:1 and 1:2 yielded 0.078 and 0.020 μg $B_1$/ml, respectively. Results were more striking when *Aspergillus parasiticus* grew in samples of juice. After 10 days, amounts of aflatoxin $B_1$ in concentrated juice and in concentrated juice diluted 1:1, 1:2, and 1:3 were 7.5, 1.59, 0.69, and 0.56 μg/ml, respectively. Aflatoxins $B_2$, $G_1$, and $G_2$ were also produced and greatest amounts also developed in concentrated juice. Amounts of these toxins decreased markedly when the percentage of soluble solids in the juices decreased. Fourteen instead of 10 days of incubation resulted in increases in the amount of each toxin in concentrated juice and in concentrated juice diluted 1:1. Although the greatest amount of aflatoxin occurred in concentrated juice, appearance of visible growth and onset of sporulation by the molds was slower in this than in diluted juices. The pH of the concentrated juice did not change appreciably after 10 and 14 days of incubation, but the pH of diluted juices rose progressively from the initial value as the percent soluble solids content in the juice decreased.

It is well established that aflatoxins are the most potent naturally occurring mycotoxins that are carcinogenic for many animal species, including man *(3, 25)*. Aflatoxins $B_1$, $B_2$, $G_1$, and $G_2$ are the most commonly encountered forms, with the former being the most potent and exhibiting $LD_{50}$ values in the range of 0.5-1.0 mg toxin/kg body weight for such animals as the duckling, guinea pig, rabbit, and dog *(15)*. When ingested at lower concentrations, aflatoxins elicit a variety of pathological liver lesions *(15)*. Aflatoxigenic strains of *Aspergillus flavus* and *Aspergillus parasiticus* have been frequently recovered from many commodities intended for animal and human consumption *(9)*. Growth of these fungi can occur on any agricultural commodity, provided that temperature, moisture, and aeration are adequate *(12)*. Aflatoxins have also been experimentally produced on a large variety of foods and laboratory media *(13)*.

Citrus products are prone to fungal spoilage in the field, after harvest, in transit and storage, during processing and marketing, and in the home kitchen *(8)*. *A. flavus* and/or aflatoxin have been found on or in citrus fruit *(10, 23)* and molds, including aspergilli, have been isolated from raw orange and grapefruit juices *(18)*. During processing, some fraction of the abundant microflora of the citrus peel inevitably finds its way into the juice *(5)*. Plate type heat exchangers can be a source of such contamination *(17)* and an accumulation of static citrus solids in an evaporator can supply nutrients for spoilage organisms *(11)*. This spoiled material could later drop or be flushed by circulation or ebullition into the body of the concentrate *(11)*.

Since *(a)* toxigenic aspergilli are ubiquitous in nature, *(b)* citrus fruits are often contaminated with mold, *(c)* fungi can grow on citrus products, and *(d)* aflatoxigenic aspergilli can grow and produce aflatoxin in single strength grapefruit juice *(2)*, experiments were done to study growth of and toxin formation by *A. flavus* and *A. parasiticus* in concentrated and diluted grapefruit juice.

## Materials and Methods

### *Organisms*

*A. flavus* CMI 89717 and *A. parasiticus* NRRL 2999, known toxigenic strains, were obtained from the Commonwealth Mycological Institute, Kew, Surrey, England and the Northern Regional Research Laboratory, Peoria, Illinois, respectively. Stock cultures were maintained at 5 C on slants of mycological agar (Difco).

### *Preparation of spore suspension*

The mold was grown on mycological agar (Difco) slants for 7-10 days at 28 C. Spores were harvested by adding sterile distilled water and a drop of Leconal wetting agent (Laboratory Equipment Co., St. Joseph, Michigan) to the slants. An inoculum of 0.2 ml of the heavy spore suspension was aseptically added to each flask of grapefruit juice.

### *Preparation and incubation of juice*

Frozen concentrated grapefruit juice (unsweetened) was purchased locally and was used undiluted or diluted one, two, or three times (single strength) with an equal volume of sterile tap water. One-hundred thirty milliliters of juice were added to 300-ml Erlenmeyer flasks and placed under

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TABLE 1. AFLATOXIN PRODUCED IN CONCENTRATED AND DILUTED GRAPEFRUIT JUICE INOCULATED WITH *Aspergillus flavus* AND HELD AT 28 C FOR 14 DAYS[1,2]

| Soluble solids in juice | Aflatoxin $B_1$ | |
|---|---|---|
| | Trial 1[3] | Trial 2[4] |
| (%) | ($\mu$g/ml) | |
| 37.5 | 0.124 | 0.211 |
| 20.0 | 0.075 | 0.078 |
| 13.0 | 0.034 | 0.020 |
| 9.0[5] | 0.028 | 0.013 |

[1]Only aflatoxin $B_1$ was measured.
[2]Results are averages of duplicate samples.
[3]Chloroform-methanol development system.
[4]Benzine-formamide development system.
[5]Single-strength juice.

flowing steam for 10 min before cooling and inoculation. Incubation was stationary at 28 C for 10 and 14 days.

*Determination of soluble solids*

The soluble solids content of the thawed grapefruit juice concentrate and diluted samples were measured with a hand refractometer (Carl Zeiss, Germany).

*Aflatoxin analysis*

The sample (50 ml of juice) was blended with 100 ml of hexane (Skelly B) and a mixture of 250 ml methanol : water (55:45, vol/vol) in a Waring Blender for 3 min at high speed. The mixture was then dispensed into 250-ml centrifuge bottles and was centrifuged at 1000 rpm (International Centrifuge Model K, International Equipment Company, Needham Heights, Mass.). Fifty milliliters from the methanol phase were mixed in a beaker with 45 g of Celite 545 (Sargent-Welch, Skokie, Ill.).

A chromatographic tube (38 × 330 mm) was packed with the celite-sample mixture to a final column height of 170 mm. The column was washed with 400 ml of hexane and aflatoxin was eluted with 500 ml of a chloroform : hexane (50:50, vol/vol) solvent mixture. The eluate was evaporated on a steam bath and the residue in the boiling flask (about 2 ml) diluted to 10 ml with chloroform. Thin-layer chromatoplates were prepared with Absorbosil-5 (0.25 mm thick) (Applied Science Laboratories, Inc., State College, Pa.). Plates were air dried and then heat-activated for 2 h at 110 C. Aflatoxins were resolved by thin-layer chromatography, the chromatograms being developed with chloroform : methanol (99:1, vol/vol) or benzene saturated with formamide *(1)*. Aflatoxins were quantitated by fluorometry *(21)* using a Turner fluorometer (G. K. Turner Associates, Palo Alto, Calif.) equipped with a plate scanner stage and a recorder (Leeds and Northrup Speedomax-H, Philadelphia, Pa.). Aflatoxin concentration was measured as recorder response and was calculated from a standard curve, taking into account the dilutions involved.

## RESULTS AND DISCUSSION

Citrus fruit juices have been shown to support growth of and aflatoxin formation by *A. flavus* and *A. parasiticus (2)*, although single strength grapefruit juice is a poor substrate for toxin production since it is almost devoid of nitrogenous materials and contains only 0.092 g carbohydrate/ml *(24)*. Mateles and Adye *(16)* demonstrated that 1% concentrations of sucrose, glucose, or fructose (those sugars present in grapefruit juice) supported mold growth but were suboptimal for aflatoxin formation, while Davis et al. *(7)* reported that a 20% concentration of sucrose was needed to support maximal production of aflatoxin. The amount of sugar in single-strength grapefruit juice lies between these extremes.

When *A. flavus* was inoculated into grapefruit juice and incubated 14 days (Table 1), the greatest amount of aflatoxin $B_1$ (0.211 $\mu$g/ml) appeared in the juice with the most soluble solids—the concentrated juice. The least aflatoxin $B_1$ (0.013 $\mu$g/ml) occurred in the single-strength juice (9.0% soluble solids). Data in Table 1 clearly show that for each trial, as the percentage of soluble solids in juice increased, more aflatoxin $B_1$ was produced by *A. flavus*.

When *A. paraciticus* served as the inoculum (Table 2), results were even more striking. This organism produced aflatoxins $B_1$, $B_2$, $G_1$, and $G_2$ in progressively greater amounts as the percentage of soluble solids in the juice increased. Four additional days (14 instead of 10) of incubation resulted in increases in the amounts of all aflatoxins in the concentrated juice and in its 1:1 dilution. Data in Table 2 also show that 10 times more aflatoxin was produced in concentrated juice than in single-strength juice, where-

TABLE 2. AFLATOXIN PRODUCED IN CONCENTRATED AND DILUTED GRAPEFRUIT JUICE INOCULATED WITH *Aspergillus parasiticus* AND HELD AT 28 C FOR 10 AND 14 DAYS[1,2]

| Soluble solids in juice | Aflatoxins | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | $B_1$ | | $B_2$ | | $G_1$ | | $G_2$ | |
| | 10 days | 14 days | 10 days | 14 days | 10 days | 14 days | 10 days | 14 days |
| (%) | ($\mu$g/ml) | | | | | | | |
| 38.5 | 7.5 | 11.58 | 0.20 | 0.31 | 2.99 | 4.25 | 0.094 | 0.16 |
| 20.0 | 1.59 | 2.32 | 0.054 | 0.064 | 0.68 | 1.24 | 0.025 | 0.075 |
| 12.3 | 0.69 | 0.38 | 0.06 | 0.01 | 0.64 | 0.54 | 0.014 | 0.008 |
| 9.5[3] | 0.56 | 0.24 | 0.02 | 0.014 | 0.26 | 0.12 | 0.008 | 0.006 |

[1]Results are averages of duplicate samples.
[2]Benzine-formamide development system.
[3]Single-strength juice.

TABLE 3. THE pH CHANGES IN GRAPEFRUIT JUICE INOCULATED WITH *Aspergillus parasiticus* AND HELD AT 28 C FOR 10 AND 14 DAYS

| Soluble solids | Initial | 10 days | 14 days |
|---|---|---|---|
| (%) | | (pH) | |
| 38.5 | 3.25 | 3.49 | 3.43 |
| 20.0 | 3.35 | 4.30 | 4.80 |
| 12.3 | 3.40 | 4.73 | 5.95 |
| 9.5[2] | 3.40 | 5.85 | 6.42 |

[1]Results are averages of duplicate samples.
[2]Single-strength juice.

as the amount of soluble solids was only four times greater in concentrated than in single strength juice. Schroeder *(20)* doubled the solids content of Czapek's broth by adding more corn steep liquor and found this increased the yield of aflatoxin by more than 2000-fold. When the concentration of sucrose in a semisynthetic medium was increased from 5 to 10% and from 10 to 20% *(7)*, the yield of aflatoxin also doubled. Shih and Marth *(22)* grew *A. parasiticus* in a glucose-salts medium and reported that increasing the initial concentration of glucose from 0.5% to 30% regularly resulted in production of more aflatoxin and lipid. The highest yields of aflatoxin and lipid were obtained with 30% glucose, whereas growth of the mold was optimal when the medium contained 10% glucose. Shih and Marth *(22)* suggested that with increased amounts of glucose, the mold developed an anaerobic mode of metabolism resulting in formation of more lipid and aflatoxin, and that a close relationship exists between aflatoxin and lipid synthesis. In the present study, it was noted repeatedly that appearance of visible mold growth and onset of sporulation by the mold was markedly slower in the concentrated than in the diluted juices. Other workers *(4, 19)* have reported that optimal mold growth does not necessarily coincide with maximum aflatoxin production and that the concentration of substrate affects the growth rate of *A. flavus*. Ciegler et al. *(6)* observed that in submerged culture, once the mold is committed to conidia production, little or no aflatoxin is synthesized.

Data in Table 3 show the pH changes in juice inoculated with *A. parasiticus*. As previously reported *(2)*, single strength grapefruit juice increased in pH with time of incubation. It is noteworthy that concentrated juice did not change appreciably in pH after 10 or 14 days of incubation. Furthermore, the increase in pH from the initial value was progressively greater as the soluble solids content of the juice decreased. Apparently, the mold exhausted available carbohydrates sooner in samples containing less soluble solids and then utilized (removed) citric acid and possibly released $NH_3$ from the small amount of protein and free amino acids present in the juice *(14, 24)*. These events may account for the increase in pH values.

The findings just described do show that aflatoxigenic fungi can grow and produce toxin in grapefruit juice concentrate as well as in diluted juices. Although mold growth is comparatively slower in concentrated juice, appreciably more aflatoxin was produced than in diluted juice. In view of these results, the citrus processing plant must maintain an excellent plant sanitation program that includes inspection and sanitization of incoming fruit, line checks, and regular cleanup of all equipment so that contamination of finished products by molds is minimized. Furthermore, handling of finished products must be done to preclude mold growth.

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## SANITARIAN'S CITATION AWARD OF THE MISSOURI ASSOCIATION OF MILK AND FOOD SANITARIANS—1973-74

The 1974 recipient of the Sanitation Citation Award began his public health career soon after World War II. He was first classified as a Sanitarian I in Environmental Services and after two years became a dairy inspector. After only 5 years in the sanitation profession, he was made Field Supervisor for milk inspection. After 5 more years, he became Chief of his department's dairy section.

The awardee has an enviable record in administration of his cities' milk inspection program. In evaluations by state and federal agencies, the milk supply has consistently been rated above 90% when measured according to procedures outlined in the Interstate Milk Shipper's Program. While complaints regarding violations or concerns about milk safety have been minimal, our honored sanitarian has had the policy of giving prompt and careful attention to each that was lodged. In fact, he has been careful to con-

The recipient of the 1974 Sanitarians' Award. Mr. Floyd Copenhaver (right), Chief of Milk Sanitation, Kansas City Health Dept., is receiving the award from Mr. Harold Bengsch (left), President of the Misouri Association of Milk, Food and Environmental Sanitarians for the 1973-74 membership year.

The officers for the 74-75 membership year. Left to right: Harold Bengsch (outgoing president), Superintendent of Sanitation, Springfield City Health Dept., Springfield, Mo.; Jerry Burns (president), Acting Chief of Milk Sanitation, Kansas City Health Dept., Kansas City, Mo.; Michael Sanford (1st vice-president), Chief Sanitarian, Columbia City Health Dept., Columbia, Mo.; Erwin Gadd (secretary-treasurer), Director, Bureau of Community Sanitation, Missouri Division of Health, Jefferson City, Mo.; and James Jamison (2nd vice-president), Farm Sanitation Supervisor, St. Louis County Health Dept., Clayton, Mo.

sider the interests and well being of all parties involved in production, processing, distribution, and consumption of milk in his area.

Our citation winner has been a reliable leader and worker in statewide activities. He has been president of both the Missouri Association of Milk and Food Sanitarians, and of the local chapter of the National Association of Sanitarians. He has served numerous times on the program planning committee for this conference, was both president and secretary of his area Dairy Technology Society, served as representative of sanitarians on the Missouri Mastitis Council, and currently serves on a state legislative committee on public health.

He has a record of social and religious affiliations and activities, has been cited for honorable service by two mayors and the City Council of his city, and served his country as a member of the active Army Reserve until his retirement as Lieutenant Colonel in 1971.

Perhaps of greatest merit are the traits cited by his nominators which are typified by the following quotation: "His is an example of a dedicated individual who has quietly performed outstanding service with no intention of bringing credit or recognition to himself. He has always felt that such performance is merely part of the job."

The 1974 Sanitarian's Citation Award winner is Mr. Floyd M. Copenhaver, Sanitarian III, and Chief of the Dairy Section, Kansas City, Missouri. Mr. Copenhaver has just retired from that office and it is fortunate that this Association can honor him in this way on this occasion.

## 1974 PENNSYLVANIA DAIRY FIELDMEN'S CONFERENCE

3A Standards For Milking Equipment was the topic for the Monday evening session on June 10. More than 150 persons heard the four speakers review the highlights of talks given at a winter series of meetings for milking machine dealers. Speakers included A. Bizzarro, S. Spencer, G. Fouse and D. Evans. Manure handling systems also were discussed by R. Grout.

Mr. G. Fouse outlined the new milk regulations while Dr. S. Guss reviewed the Pennsylvania mastitis program to start Tuesday's session. R. Stevens and R. Belknap of FDA outlined the progress of the PMO revision and the program for hauler training and certification.

A panel composed of T. Balliett, A. Nixon and J. Walker reviewed milk procurement and quality control programs of two cooperatives and an independent dairy. D. Daum outlined the OSHA regulations for farms while C. Hoglund reviewed the economics of farm milk production.

B. Hinish, President of the Pennsylvania Dairy Sanitarians Association conducted the business meeting. All committee reports were included in the conference abstracts. J. Boore was elected president for 1974-75.

C. Roach received the 1974 Dairy Sanitarians Award. He is a field representative for Interstate Milk Producers Co-op., following more than 30 years with Sealtest. The banquet speaker was Dr. G.

Brandow who spoke on the world food supply.

More than 260 persons participated in the 32nd Annual Pennsylvania Dairy Fieldmen's Conference. It was held at the Keller Conference Center on the University Park Campus of the Pennsylvania State University on June 10-12, 1974. Farm inspectors, dairy processors, regulatory representatives and allied industry personnel participated.

The program on Wednesday featured panel on energy conservation on the farm with R. Hamsher and Dr. D. Price. They reviewed the situation with both oil and electricity.

J. Adams outlined present federal pollution regulations and how they will affect dairy farmers. R. March confirmed that dairy imports have had some impact on prices dairy farmers receive for milk. R. Roper reviewed the procedure used by Order No. 36 for checking bulk tank calibration with truck mounted meters.

The 1975 Dairy Fieldmen's Conference will be held June 9-11, 1975 in State College starting with a Monday evening program of the sanitarians association.

SIDNEY E. BARNARD
*Department of Dairy Science*
*The Pennsylvania State University*
*University Park, Pennsylvania*

## CARTOON FOOD SERVICE MANUAL

A cartoon training manual for food service employees is being offered by the Branch-Hillsdale-St. Joseph District Health Department for printing and shipping costs only. This new publication is entitled "What The Health Department Expects Of You." Send 50¢ for a copy and more information. Noel H. Wiley, R.S., M.P.H., Director of Environmental Health, Branch-Hillsdale-St. Joseph, District Health Department, Centreville, Michigan.

## SOUTH DAKOTA AFFILIATE MEETING

The South Dakota Association held their annual meeting on May 22-24. This was the 26th Annual Conference which was held at Brookings. It was well received with nearly 100 per cent of all sanitarians in the state attending.

Mr. Howard Froiland received a special plaque from President Robert Wermers. Mr. Froiland was honored for 30 years of continued and outstanding service as a milk and food sanitarian for the state. He has served the City of Aberdeen since 1948. Mr. Froiland will retire on December 31, 1974. He has been well known for his leadership in milk sanitation work as well as serving on several IAMFES Committees.

## UNIQUE TRAINING PROGRAM FOR COUNTY SANITARIANS

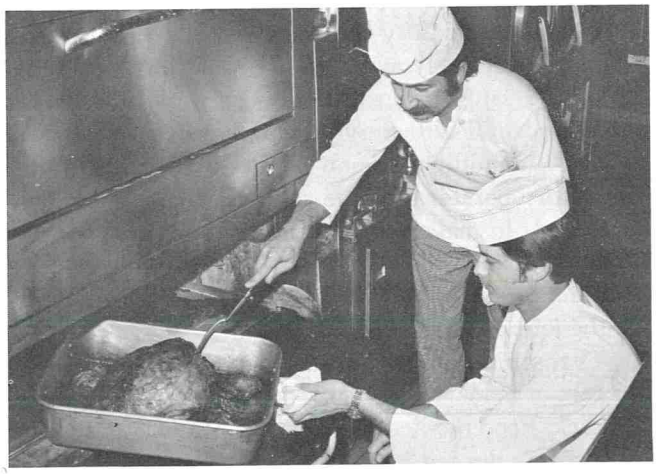
When a San Bernardino County Environmental Sanitarian makes an inspection, chances are excellent that he is not only familiar with health regulations, but, unlike most so called "health inspectors," also has a thorough knowledge of the business or operation involved.

This valuable background is the result of a unique training program developed by the County Department of Environmental Health Services one year ago to fully acquaint new sanitarians with all phases of their job before they actually begin work.

The new program centers around approximately eight weeks of on-the-job training, working with other County departments closely related to Environmental Health and with the businesses and industries inspected by sanitarians.

In the course of this training, the new sanitarian functions as a regular employee of businesses ranging from restaurants to sewage contractors, gaining insight into the operation because as one sanitarian put it "if you don't understand the problem, you can't help."

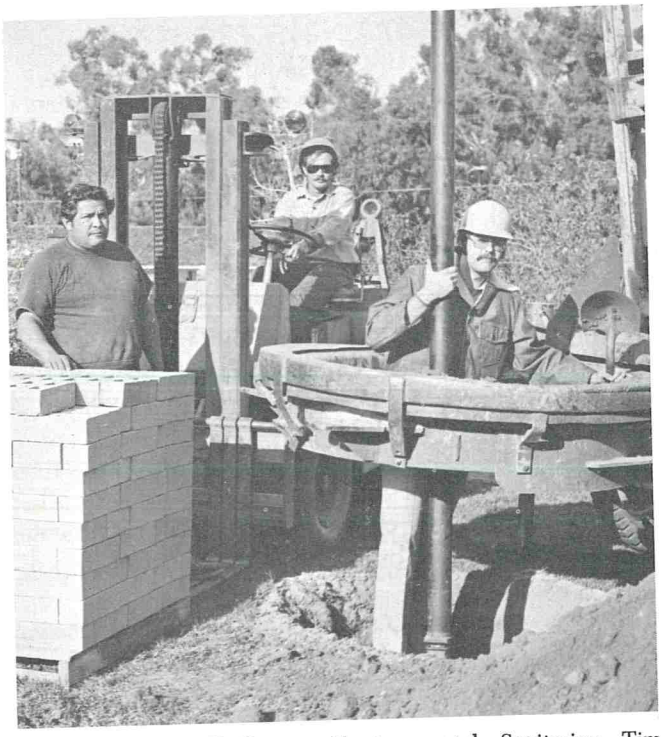
Also included is work with a sewage pumper, plumbing contractor, sewage treatment plant, well driller, water company, restaurant supply company, industrial plant, pool maintenance service, pest control operator, and mobilehome installer.



JUST ABOUT DONE—Sanitarian Trainee Dan Welebir (right) and Delm Jimenez, chief chef for May Co., check the progress of meat during the restaurant phase of Welebir's training.

But in each case, the sanitarian trainee works side-by-side with the firm's employees to the point of wearing clothing designed for the job, whether it be in the preparation of food at a restaurant or in digging a new sanitation system.

"If, for example, one of our men finds dirty dishes



LOWER AWAY—County Environmental Sanitarian Tim Mulligan (right) is about to be lowered down shaft as he assists in the construction of a septic tank during new on-the-job training program. Others (l-r) are Eddie Aldman and Tom Shuey, both of Joe L. Fernandez Co. Inc., a drill rig operation participating in the program.

in a restaurant, as a result of his training he should have sufficient knowledge of commercial dishwashing equipment to identify the source of the problem and estimate accurately what is necessary to correct the situation," said Richard L. Roberts, Director of Environmental Health Services.

"And, in addition, knowledge on the part of the owner or operator that the sanitarian knows his business tends to make him that much more cooperative," Roberts said.

The business community, too, is pleased with the on-the-job training concept.

"The sanitarian can speak with experience when he tells a manager he must do something," said Ken Riddick, Food Service Manager for May Co. "In other words, he's done it."

Drilling operator Joe Fernandez agrees, terming the program "a grand idea resulting in pratical and to-the-point recommendations from the sanitarians."

The other main portion of the on-the-job training is work with county personnel such as building inspectors, zoning enforcement officers, air pollution control investigators, public health nurses, animal disease control officers, and others whose responsibilities must be well-known to the sanitarian.

Experienced sanitarians point out that in the course of their inspections they often encounter problems outside their authority requiring that they be able to recognize the difficulty and know who to contact.

It is also noted that some situations involve action by several agencies such as the Environmental Health Department ordering the elimination of rats by burning which would require a permit from the Air Pollution Control District thus drawing attention to the need for the interdepartmental cooperation fostered by the program.

In addition to the field work, the trainee spends one day each week with his supervisor to answer any questions that may have arisen and, of course, instruction is given in departmental policies, procedures, and interpretation of codes and regulations.

The new program is in sharp contrast with training in other counties which, as one sanitarian summarized it, "consists of two hours of orientation, a grab by the collar, and a toss into the field. The training manual is thrown on the desk with instructions to read it.

"The program here is a dramatic presentation of what's in that book."

County officials indicate that the sanitarian training program has drawn attention to many common goals of both the private sector and government, and has been extremely effective in opening channels of communication between the industrial operator and the public health worker.

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## BURKHARDT NAMED TOP SANITARIAN

Robert C. Burkhardt of Huntsville has been cited as "Sanitarian of the Year" by the Alabama Environmental Health Association.

Meeting recently in Montgomery in conjunction with the Alabama Public Health Association, the organization honored Burkhardt, who has been active in environmental programs of the Alabama Department of Public Health for the past 35 years, especially in food and milk controls.

The award was presented by the association president, Charles R. Sutton of Decatur.

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## MILK PROCESSORS WORKSHOP

A workshop for milk processing personnel has been scheduled for November 4-15, 1974, at The Pennsylvania State University. It is conducted by the staff of the Food Science Department.

Subjects to be covered will include composition and properties and processing of fluid milk products, buttermilk, sour cream and cottage cheese. Quality

control procedures will be taught including basic fat tests, cryoscopy and flavor evaluation. Other subjects will include labeling requirements, basic dairy arithmetic and cleaning and sanitizing of dairy equipment. The workshop will be directed to dairy plant processing personnel.

For additional information and copy of the program brochure contact: Agricultural Conference Coordinator, Room 410, J. O. Keller Building, University Park, Pennsylvania 16802.

## JOHN HARVEY BRYANT

A leading Australian and world renowned dairy technologist, John Harvey Bryant, died suddenly at his home in Sydney in January.

Born in 1903, he was a pioneer of many new processes and products in the Australian dairy industry, particularly in the pasteurization of milk supply.

He travelled abroad extensively and represented Australia at many international dairy exhibitions and conventions in Britain, throughout Europe and in the United States of America.

Mr. Bryant wes educated at Sydney Grammar School and graduated from the Hawkesbury Agricultural College, New South Wales, with a Diploma in Agriculture with the College Honours Medal in 1922.

He was member of the first Milk Board of New South Wales from 1929-31 and was a past President of the Milk Market Federation of New South Wales and the Dairy Equipment Manufacturers of Australia.

Mr. Bryant was founder, past Federal and New South Wales President of the Australian Society of Dairy Technology, and received its Gold Medal in 1955. From the mid-1950's he was Vice-President of the Dairy Society International, headquarters in Washington, U.S.A. for about eight years.

He was also a foundation member of the Society of Dairy Technology, England, Vice-President of the Australian National Dairy Committee, and in 1970 was Vice-President of the 18th International Dairy Congress in Sydney.

In 1937 he commenced business as a Dairy Engineer with a small office of three people which has grown to what is now the Bell Bryant Group of Companies with activities throughout Australia, New Zealand and South East Asia.

At the time of his death, Mr. Bryant was Chairman of Directors of Bell Bryant Limited, Express Dairy Company (Australia) Pty. Ltd., A.P.V. (Australia) Pty. Ltd., Q.U.F. Industries Ltd. and a Director of Diversey (Australia) Pty. Ltd.

## DR. J. B. STINE PRESENTED AWARD

T. M. Minogue, Land O'Lakes, Inc. Pres. - ABI; K. W. Royer, Purity Cheese Co., Pres. - NCI; J. B. Stine, Kraft Foods; and George M. Burditt, of the law firm Burditt & Calkins.

Presentation of the "Silver Horn" Award to Dr. J. B. Stine, Vice President of Quality Standards and Regulatory Compliance, Kraft Foods Company. "Doc" Stine is chairman of the National Cheese Institute Research Committee and NCI President Ken Royer presented the Award to "Doc" in recognition of his distinguished service to the cheese industry. Dr. Stine received his B.S. in dairy science at Texas Tech. in 1933, and his M.S. in 1935 and Ph.D. in 1936 at Iowa State University.

The occasion was the Annual Banquet of the National Cheese Institute and American Butter Institute on April 16, 1974.

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## NEW CATALOG ON SIGHT GLASS ASSEMBLY

The Ladish Co., Tri-Clover Division announces the availability of Bulletin SG-74 on the Tri-Flo$^{R}$ Sight Glass Assembly for sanitary and general service applications.

According to Tri-Clover the sight glass assembly provides for immediate observation of product flow in a pipeline and can be installed in practically any critical area of a processing system—feed or discharge lines, filler lines or in any line where inspection of product flow is desired.

The Bulletin explains that the sight glass is available in sizes from 1-1/2" to 4", is easy to install and can be interchanged with a standard Tri-Clamp Tee or Cross in an existing line.

Materials for the sight glass assembly include: Body Assembly, 304 or 316ss; Clamps, 304ss; Glass, Tempered Pyrex® and Gasket, Buna N.

For a copy of Bulletin SG-74 write: Ladish Co., Tri-Clover Division, 9201 Wilmot Road, Kenosha, Wisconsin 53140. Attn: R. P. Jones, Marketing Department.

## NEW TYPE GENERAL CLEANER

Klenzade Redi-Kleen is a concentrated liquid alkaline multi-purpose detergent which can be used effectively in high pressure, foam, override or manual cleaning techniques. Its unique wetting agents and water conditioners plus a balanced alkalinity provide the right combination to remove varied types of soils. Redi-Kleen is non-corrosive to aluminum, mild or stainless steels, and is not harmful in proper use dilutions. This versatile detergent can be used effectively in a wide range of applications because of its ease of automatic dispensing, safety in use as a straight manual brushwashing cleaner, and capability of producing a high stable foam when desired for extended cleaning solution surface contact. It offers many properties of higher priced "built" detergents at a lower use dilution cost with trial offer performance assured.

## EXPO '74 DIRECTORY: A VALUABLE REFERENCE

Looking for a current listing of equipment fabricators and suppliers of products and services to the food and dairy industry?

Visitors to Food and Dairy Expo '74 at Dallas Oct. 20-24, 1974, will find the Expo Directory a valuable reference source for future use.

The Directory will include:

Listing of all exhibitors, with booth numbers, addresses, description of products displayed and all languages spoken, all nonexhibiting member companies of Dairy and Food Industries Supply Association, and all noncommercial exhibitors.

Classified Products Index, in which displayed products are itemized by major product or service headings.

Index of 3-A and E-3-A Sanitary Standards and Accepted Practices for cleanability of dairy processing and egg processing equipment.

The directory will also include a calendar of concurrent conventions of the Milk Industry Foundation, International Association of Ice Cream Manufacturers, National Ice Cream Retailers Association, National Ice Cream Mix Association, and Dairy Society International, and the program of the sixth Food Engineering Forum. Information on entertainment, Expo hours, admissions . . . . will be included.



## NOTICE TO IAMFES MEMBERS

There will be open discussion of Articles II of the By-Laws at the annual meeting at the Hilton Hotel, St. Petersburg, Florida on Wednesday, August 14, 1974 at 10:20 a.m.

Earl O Wright, President

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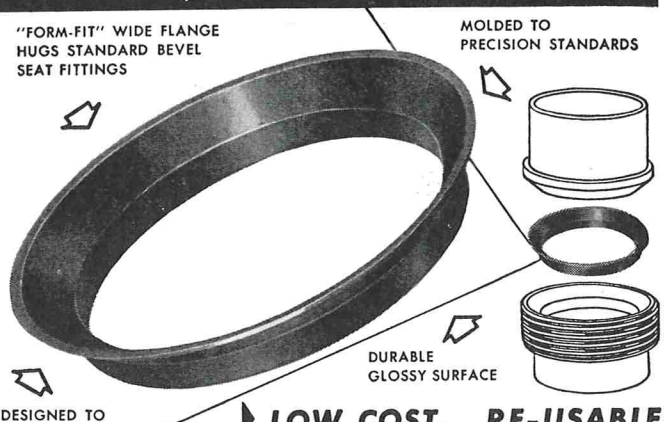
# HAYNES SNAP-TITE GASKETS




**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**

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# 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

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## A HEAVY DUTY SANITARY LUBRICANT


*Available in both*

**SPRAY AND TUBE**

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

ESPECIALLY DEVELOPED FOR LUBRICATION OF FOOD PROCESSING AND PACKAGING EQUIPMENT

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

**SANITARY • NON TOXIC • ODORLESS • TASTELESS**

SPRAY — PACKED 6 — 16 OZ. CANS PER CARTON
TUBES — PACKED 12 — 4 OZ. TUBES PER CARTON

**THE HAYNES MANUFACTURING CO.**
CLEVELAND, OHIO 44113

# Dairy authorities speak out on better cow milking

William L. Arledge
Director of Quality Control
Dairymen, Inc.

# Quality milk makes consumers happy and helps dairymen prosper.

Dairy farmers are unique. In most cases their primary source of income is their milk check. A major influence on that milk check is quality, and it has been shown that, as the quality of milk in a given market improves, so does consumer demand, and so does income to the dairy farmer.

High quality milk starts with the cow but it must be continued each step of the way until the milk is purchased and consumed.

## TASTE MAKES FRIENDS

The nutritional value of milk to the human diet is never disputed. The relative dollar value of these nutrients, particularly protein in relation to other food costs, is also not disputed. However, consumers could care less about protein and nutritional value if the milk does not taste good due to poor quality control practices somewhere between the cow and the consumer. People simply will not buy poor quality milk.

Over 90% of consumer complaints are the result of post-pasteurization contamination and improper temperature control of the bottled product after pasteurization. This is dramatized by the fact that seven Grade A dairy plants recently increased the shelf life of their total packaged products from an average of 6 days to more than 21 days by improving in-plant controls over post-pasteurization contamination. Little or no change was made in the quality of the raw milk coming into the plant. (This shows that significant improvements can be made in dairy plants as well as on farms.)

## REVIEW YOUR EFFORTS

Your role of producing quality milk daily can be as simple as you desire to make it.

It has been proven many times that it is cheaper to spend whatever is necessary to thoroughly clean your bulk tank, pipeline, or bucket milkers (all milk contact surfaces) than it is to do an improper job.

Follow these recommendations:

1. Read the label of your chlorinated cleaner detergent and *measure* the correct amount of water to be added to the correct amount of cleaner.
2. Be sure when circulating cleaning systems to start your wash cycle with 160° F. water and *stop* circulating when the temperature drops to 110° F. (If you don't, you will redeposit soil and fat.)
3. In colder areas, the use of a heating element in the wash tank is recommended to help maintain wash temperatures above 110° F., preferably 130-140° F. (Six to ten minutes is usually enough*).
4. Many dairy barns do not have hot water heaters that will deliver water at 160-180° F. and even if they do—the recovery of temperature in the heater is too slow and you end up trying to wash in lukewarm to cool water.
5. To prevent loss of *hot* water for cleanup, many dairymen install a second hot water heater and set the regulator at around 110-120° F. for use in hand washing of cows' teats and udders prior to milking as well as other manual cleaning chores. This leaves your other hot water heater available for the important job of cleaning equipment.
6. If you have an electric hot water heater, install fast recovery heating elements to prevent cool down of your hot water. For safety make sure all heaters are equipped with an approved *pressure* and *temperature* relief valve.
7. After thorough washing of equipment, rinse in an acid rinse and then immediately prior to milking always *sanitize* all milk contact surfaces with an approved sanitizer.

## CARELESSNESS IS A LUXURY

As surprising as it may seem, we still find some people rinsing equipment with plain water after sanitizing. This causes great problems affecting milk quality since all water supplies contain from a few to large numbers of the "cold loving" psychrothrophic bacteria that can and do cause some very undesirable off-flavors in milk. Granted these organisms are killed by pasteurization but, by carelessness, dairymen can alter the flavor of milk prior to its leaving the farm by 1) lack of sanitizing, or 2) rinsing equipment rather than washing, or by 3) only rinsing a bulk tank.

Too many times we see dairymen doing a good job in their milking management practices, but disregarding their water supply, temperature of cleaning solutions and sanitization, thus nullifying all other good practices.

Quality of your *only* product affects *your only* income; your milk check. You can do more concerning the quality of your milk! Follow the routine and procedures you know to be correct and with these few quality tips, you may prevent the shipment of a tank of less-than-superior-quality milk.

We must all relate to the consumer. Do a quality job in your personal operation and expect the same throughout the chain of events to the consumer. You will reap the benefits through personal pride "all the way to the bank".

*Refer to local Health Department regulations