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Vol. 40 March, 1977 No. 3

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Acceptability of a Whey-Based Quiescently Frozen Novelty

J. W. STULL1, R. R. TAYLOR1, R. C. ANGUS2, and T. C. DANIEL3

Department of Nutrition and Food Science, Department of Agricultural Economics, and Department of Psychology, The University of Arizona, Tucson, Arizona 85721

ABSTRACT

In one experiment, an observer panel of young people aged 6 to 14 years indicated no difference in preference for quiescently frozen novelties formulated with water or ones containing untreated cottage cheese whey as the entire formulation liquid. In another experiment, nine whey-based, orange flavored, novelties were evaluated by a panel of seven female and eight male children aged 10 to 12 years at triplicate sessions 2 to 4 days apart. Samples were coded differently at each session. Untreated cottage cheese whey supplied at the formulation liquid. Sucrose: dextrose (3:6:1) at total concentrations of 17, 23, or 29% and pH values of 3.2, 3.5, or 3.8 were evaluated on a 10-point degree of certainty scale from "like" to "do not like." Theory of signal detection (TSD) analysis shows that products with 23% total sweetener at pH 3.2 or 3.5 were rated equally acceptable. These products were rated significantly more preferable than the others. There were no significant differences in ratings made at each session or between individual panel members.

Use of fluid cottage cheese whey as a food ingredient has considerable interest. In addition to nutrient content, its biological oxygen demand (B.O.D.) contribution to industrial waste either is or will be subject to a disposal charge in most areas (1,3). Furthermore, its relatively high water content (ca. 94%) results in high cost handling, storage, transportation, and processing procedures (1).

While various processed forms of whey are developed or proposed as food ingredients (4), limited information concerning liquid whey use is available. Its use as an ingredient for flavored beverages has been investigated (5, 6). In these reports, a 100% substitution of cottage cheese whey for water as the liquid component resulted in equal acceptability. Demott (2) made a beverage with cottage cheese whey produced by the direct acidification process. It was concluded that such a whey drink might very well be marketable.

A formulation using acid whey as a base for salad dressings has been reported (7,1). Panelists scored these samples at 5 or above on a 7-point hedonic scale, where 7 represented a value of "like extremely."

The possible use of liquid cottage cheese whey in a quiescently frozen novelty formulation has not been previously reported and has interesting possibilities.

Since water ice type novelties are consumed to the greatest degree by young persons (12), it was considered important to evaluate certain composition variables relating to acceptability by this age group. Limited information is published concerning use of young persons in taste panel observations (4, 7, 10).

The present study reports the flavor acceptability of such novelties using either cottage cheese whey or water as the formulation liquid. A second objective was to evaluate the ability of preteenage young persons to evaluate the acceptability of whey-based novelties of various sweetener concentrations and pH values. Experiment 1 involves a 100% cottage cheese whey substitution for water in the ingredient array. Experiment 2 reports the observations concerning various sweetener concentrations and pH values.

MATERIALS AND METHODS

Novelties with either untreated liquid cottage cheese whey or water as the liquid ingredient were prepared (Table 1). The pH was adjusted after heat treatment by addition of 50% aqueous citric acid. After blending dry and liquid ingredients, heating to 71 C, and cooling to 4 C, the mixture was flavored with a natural, concentrated orange flavoring containing artificial coloring and added as recommended by the manufacturer. Fifty-ml aliquots were frozen on wooden color coded novelty sticks at -35 C. In preparation for serving, samples were tempered at -17 C.

In Experiment 1, the observer group was 779 persons (approximately an equal number of males and females) aged 6 to 14 years. In Experiment 2, the observer panel was eight male and seven female

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1Department of Nutrition and Food Science.
2Department of Agricultural Economics.
3Department of Psychology.
persons aged 10 to 12 years. The individuals were not aware of the composition characteristics or the variables in the samples. Following oral instructions regarding tasting procedures and decision recording, panel members were either seated in a cafeteria (Experiment 1) or in a large laboratory (Experiment 2) providing suitable spacing between individuals. Sufficient proctors were stationed to control distractions, maintain adherence to instructions, and provide general surveillance of sample serving.

In Experiment 1, the observers were presented two samples with random color coding on the novelty sticks. The observers reported their decisions on a form, “I liked the one with the (red, green) stick best.” In Experiment 2, the nine samples were presented in random order at a uniform interval of about one every 2 min. Every panel member made replicate evaluations of the nine samples at three sessions 2 to 4 days apart with different randomization and the numbering at the sessions. The panel reported their decisions in this trial on the basis of a 10-point scale from “absolutely certain that I like” to “absolutely certain I do not like.”

RESULTS AND DISCUSSION

Experiment 1

A preference selection for the product formulated with water (Sample 1) was made by 396 observers while the remainder (383) indicated a preference for the whey formulation (Sample 2). This indicates that, under the conditions of this observation, young people (aged 6 to 14 years) cannot detect the difference between water ice novelties formulated with water or ones containing untreated, liquid cottage cheese whey.

Experiment 2

The rating scores in this experiment were analyzed using a methodological advance based on the theory of signal detection (TSD) (3). The product with medium sweetener concentration and pH (23% and 3.5, respectively) was used as the standard of comparison in the TSD analysis. Mean ratings in the various observations were then used to calculate d_m values for all other samples. The d_m value indicates the effect of composition variables on the observer’s ability to distinguish between like vs. do not like decisions. The lower the d_m value, the greater the observers’ preference. Detailed explanations of the computational procedures for TSD are presented by several sources (3, 8, 9, 15).

Preference ratings for the nine samples indicate that products with 23% sweetener and either pH 3.2 or 3.5 were rated equally acceptable (Table 2). These two products were rated significantly more acceptable than the others (P < 0.05).

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<tr>
<td>29</td>
<td>4.2</td>
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At the 29% sweetener concentration, the effect of the pH variable on preference ratings was apparently masked to a degree as seen by the relatively narrow dispersion of the three d_m values. The panel rated the products with more sweetener higher than those at the 17% concentration (P < 0.05).

As compared to the significantly high preference for a low or medium pH in the products with 23% sweetener, the sample at the low pH with only 17% sweetener was the least preferred. At the lower sweetener level, a medium or high pH (3.5 or 3.8, respectively) resulted in a more preferable combination (P < 0.05).

This work indicates that a panel of preteenage children can make sensory preference discrimination decisions concerned with sweetener: pH variables in whey-based frozen novelties. Such a panel can make accurate evaluations in up to nine samples at a time as shown by TSD analysis.

ACKNOWLEDGMENTS

Technical Paper No. 2471 of the Arizona Agricultural Experiment Station. The authors express appreciation for the technical assistance of Mr. C. W. Braun and Dr. W. C. Brown.

REFERENCES

Persistence of *Mycobacterium bovis* BCG in Soil and on Vegetables Spray-Irrigated with Sewage Effluent and Sludge

DALE J. VAN DONSEL and EDWARD P. LARKIN

Department of Health, Education, and Welfare
Public Health Service, Food and Drug Administration
Bureau of Foods, Division of Microbiology
Virology Branch, Cincinnati, Ohio 45226

(Received for publication July 19, 1976)

**ABSTRACT**

Survival of *Mycobacterium bovis* BCG on garden plots spray-irrigated with sewage effluent or sludge was studied to determine persistence of mycobacteria under conditions approximating current practices. The D value (90% reduction time) in effluent-sprayed soil was 11 days, and in sludge-sprayed soil it was 8 days. On effluent-sprayed radishes, the D value was 6 days, and on sludge-sprayed radishes, 4 days; however, this difference was not significant. Isolation from lettuce was too erratic to determine a true survival rate, but the organism was isolated sporadically for up to 35 days.

**MATERIALS AND METHODS**

Vegetable plots

Vegetables were grown in plywood boxes 6 x 2 x 1 ft (1.8 x 0.6 x 0.3 m), lined with polyethylene sheeting and with bottom drains connected to a container to collect percolate. Each box was filled with 9 inches (23 cm) of fertile topsoil evenly distributed over 2 inches (5 cm) of field stone. The plots were exposed to the normal outdoor conditions of the Cincinnati area. Two weeks before the beginning of spraying, the plots were seeded with Black-seeded Simpson lettuce and Early Scarlet Globe radishes. Three rows of each were sown in each plot, in rows 10 inches (25 cm) apart. Plots were cultivated by hand when necessary.

**Bacterial inoculum**

*Mycobacterium bovis* BCG cultures were provided by the U.S.-Japan Cooperative Medical Science Program — National Institute of Allergy and Infectious Diseases. Strain 1029 (Phipps) was chosen because of its growth rate on the isolation medium and its distinctive colonial morphology. Cultures were grown in 800-ml bottles of Dubos broth at 35°C for 4 weeks. Sedimented growth was harvested by pipet, pooled, and homogenized by adding 0.5% Tween-80 and mixing for 1 h on a magnetic stirrer. Activated sludge and unchlorinated secondary effluent were obtained from the pilot plant at the Environmental Protection Agency, National Environmental Research Center, Cincinnati, Ohio, or from a municipal activated sludge plant. Approximately 200 ml of the cell suspension was added to each 20-liter Millipore pressure vessel of sewage effluent or sludge to yield an estimated 10⁶ colony-forming units (CFU) per ml, and the contents of the pressure vessels were mixed for an additional hour on magnetic stirrers. Pressure vessels were fitted with a nozzle that applied a gentle
spray of the inoculum to simulate existing spray irrigation equipment. The amount applied was equivalent to a 1-inch (2.5-cm) depth of liquid. Plots were sprayed four times at weekly intervals.

**Sampling**

Sampling was begun 1 day after the last spraying. Soil and vegetables were collected daily for the first week and twice weekly thereafter. About 50 g of soil was collected by taking a soil plug from a depth of 1 in. from a minimum of six points. Estimated 50-g samples of vegetables were collected. Radishes were pulled out and the clinging soil was knocked off. Lettuce was cut about an inch above soil level. After rainfalls, drainage in the collecting tanks was also sampled.

**Assay**

Five-gram portions of soil were diluted 1 to 10 in phosphate-buffered distilled water containing 0.1% Tween-80 and mixed by repeated shaking and vortexing. Whole vegetables were placed in plastic bags and weighed, and 100 ml of the same diluent was added. Bags were shaken and kneaded for 1 h to remove surface material, and the wash water was poured off for assay. Samples that could not be assayed immediately were stored at -70 C (11). The isolation medium was a modification of the selective medium of Mitchison et al. (4), utilizing the synergistic inhibitory effect of sulfa and trimethoprim (5). After cooling Middlebrook 7H10 oleic acid albumin agar to 45 C, the following were added: polymyxin B, 1,000 units per ml; penicillin G, 25 units per ml; novobiocin, 15 μg per ml; amphotericin B, 100 μg per ml; sulfapyridine, 50 μg per ml; and trimethoprim lactate, 30 μg per ml. Soil suspensions and vegetable washings were decontaminated by adding aqueous Zephiran to attain a concentration of 700 μg per ml. After 60 min, decontamination was halted by diluting the samples 1:10 in buffered distilled water containing 150 μg of lecithin (brain) per ml. Plating on the isolation medium was done with a semisolid overlay (15). An equal volume of 7H10 base medium diluted to 40% of the normal concentration, melted, and held at 46 C, was mixed with each dilution to be plated (2 ml of the mixture added per plate), and plates were quickly rotated to spread the inoculum before it hardened. A minimum of five plates were used per dilution. Plates were air-incubated at 35 C for 3 days to eliminate excess moisture, then reincubated in sealed plastic bags for minimum of 35 days.

**Colony verification**

Suspect colonies were picked to Dubos oleic agar and plain 7H10 agar. An isolate was regarded as the indicator mycobacterium only if it fulfilled all the following criteria: (a) possession of typical colonial morphology on the isolation medium, (b) failure to produce significant growth in 7 days on Dubos oleic agar and 7H10 agar, (c) production of typical colonies on these media in 14-21 days, and (d) growth from Dubos plates that possessed characteristic acid-fast morphology with Ziehl-Neelsen staining. Nontypical mycobacteria were not further identified.

**RESULTS AND DISCUSSION**

No organisms similar to BCG were found in soils, effluent, or sludge before inoculation. Counts of the indicator organism in inoculated effluent and sludge are shown in Table 1. BCG was isolated from soil until day 29: thereafter the recovery methods were inadequate to suppress the high levels of soil molds and bacteria. Isolation from radishes continued until day 13, and from lettuce until day 35, at which points the crops were exhausted because of the combined effects of sampling, insects, and disease. No insecticide or fungicide sprays had been used because of concern for their effects upon the indigenous soil flora as well as the mycobacteria.

Persistence in soil, plotted on least squares curves, is shown in Fig. 1. The D value (90% reduction time) in effluent-sprayed soil is 11 days, and in sludge-sprayed soil it is 8 days. This difference is significant, but no obvious reasons can be seen for the more rapid death rate in sludge. Intuitively, the converse would have seemed more likely because of the supposed protective effect of the sludge solids. Persistence on radishes is shown in Fig. 2. The D value on effluent-sprayed radishes is 6 days, and on sludge-sprayed radishes, 4 days; however, this difference is not significant. Persistence on lettuce is shown in Fig. 3. No survival rates were estimated because of the extreme scatter of points. Rainfall was probably responsible for washing surface inoculum from the leaves and causing the rapid initial loss, but the consistent, low-level isolation for 35 days indicated that a certain number of organisms had become firmly attached.

Washout of the inoculum from the plots by rainfall did not seem to be an important factor. The total number of mycobacteria found in the percolate tanks amounted to about 1% of the total applied to the plots.

![Figure 1. Recovery of BCG from effluent- and sludge-sprayed soil.](image-url)
The survival times indicated should be considered closer to minimum rather than general values. Heat was undoubtedly the most important single factor in death of the mycobacteria. The period allocated for this study was later than the normal growing season in the Cincinnati area, and summer temperatures were high. Soil surface temperature as high as 45°C was observed during afternoons.

All survival studies employing mycobacteria grown in vitro should be interpreted with caution. Segal and Bloch (16, 17) demonstrated differences between the same strain of M. tuberculosis grown in mouse lungs and in liquid culture. Cells grown in vitro exhibited higher hydrogen transfer capacity, substrate response, and immune response, despite the fact that cells grown in vivo were more virulent for mice. Kondo and Kanai (12) determined some of the reasons for these differences. Mycobacteria grown in vitro had much higher levels of the immunologically active chloroform-soluble waxes, although organisms grown in vivo had higher levels of other fats and waxes. These findings suggest the likelihood of real differences between mycobacteria cultured for use in survival studies and those occurring in sewage as a result of active human or bovine tuberculosis. These differences may also extend to resistance to environmental stress. BCG is especially open to criticism in this respect; the strain used in this study was derived from Calmette's original 1908 isolate and has been under laboratory conditions ever since. Despite this fact, it was chosen as an indicator organism in this study because any of the more pathogenic mycobacteria would have posed an intolerable infection hazard under the outdoor gardening conditions employed.

The numbers of tubercle bacilli occurring in "typical" urban sewage cannot really be estimated because of the difficulty of isolating them from material with very high levels of other organisms. The number varies, depending on the incidence of tuberculosis. By means of animal inoculation, Jensen (10) tried to recover tubercle bacilli from sewage influents, effluents, and sludges having their origin in populations with known tuberculosis cases. Ratios of cases to total population ranged from 1:3.6 to 1:2,000. Tubercle bacilli were found in all influents, effluents, and sludges up to a ratio of 1:600 (except one effluent from an activated sludge plant where the ratio was 1:460). It took 11½ to 15 months for sludge on drying beds to become free of the organisms. Using artificially inoculated sewage, Heukelekian and Albanese (8) found that effluents treated by means of chemical coagulation, intermittent sand filtration, and chlorina-

Figure 2. Recovery of BCG from effluent- and sludge-sprayed radishes.

Figure 3. Recovery of BCG from effluent- and sludge-sprayed lettuce.
tion were free of demonstrable tubercle bacilli, but there was a 13-fold increase of the organism in sludge after 6 h of settling and only an 85% reduction after digestion for 35 days. There was little, if any, decline after 25 days of drying. Weiser et al. (20) were able to isolate M. tuberculosis from sludge beds of a tuberculosis hospital, but not from treated and chlorinated effluent. Tison et al. (19) were able to isolate M. tuberculosis from the effluent samples from a sanitorium. The effluent had received full treatment, including chlorination.

Packing plant wastes are another potential source of mycobacteria. Bovine tuberculosis has been brought under nearly complete control in the United States, but occasional large outbreaks still occur (4). Although tuberculin reactors are slaughtered and the meat used only in cooked products, packing plant wastes often enter municipal treatment facilities.

ACKNOWLEDGMENTS

We thank Brenda K. Boutin for her excellent assistance in mycobacterial assays and James T. Peeler for providing statistical evaluations.

REFERENCES

Potential for Non-Specific Inhibition in the Sarcina lutea Test

R. A. LEDFORD and JANICE BROWN

Department of Food Science, Cornell University, Ithaca, New York 14853

(Received for publication June 1, 1976)

ABSTRACT

A survey of commercial milk samples for antibiotic residues, tested by the Sarcina lutea cylinder plate method, revealed a high incidence (72%) of non-specific inhibition. Penicillin was detected by penicillinase assay in 3.1% of the samples. The method was found to be non-quantitative for residual levels of sanitizers in protein media. Apparently, substances inhibitory to S. lutea develop normally in some milks during marketing.

Interest in the use of the Sarcina lutea cylinder plate (SLCP) method in milk quality control programs is increasing. Currently the Bacillus subtilis disc assay (BDSA) method is used routinely by industry and regulatory laboratories; however, the SLCP method, according to an FDA memorandum (4), is to be used for detection of penicillin in nonfat dry milk. In addition, in testing fluid milk, apparently the BDSA method will be used to screen milk supplies while the SLCP method will be used as the confirmatory procedure (3). During the last year, we have observed, in testing large numbers of commercial milk samples by the SLCP method, a high incidence of inhibitory zones which were not caused by penicillin. Other antibiotics and/or inhibitors could have been involved as the penicillinase assay is qualitative only for penicillin. We tested the response of S. lutea to different materials, especially to residual levels of detergents and sanitizers, to determine if non-antibiotic materials cause interference. If the S. lutea method is applied in finished product analysis as directed by the FDA, and understanding of the method limitations is required.

MATERIALS AND METHODS

Samples

During the past year on several occasions, a total of 234 commercial milk samples, obtained outlets throughout New York state and kept refrigerated were analyzed.

Sarcina lutea cylinder plate method

The procedure followed was published by the FDA (5). This is the same method outlined by Carter (2) as cited in Standard Methods for the Examination of Dairy Products (1).

Penicillinase assay

Samples positive for inhibitory substances were tested for the presence of penicillin by the penicillinase procedure of Standard Methods for the Examination of Dairy Products (1).

Sanitizer treatments

The four following commercial sanitizers were added to inhibitor-free milk at several levels considered to be in the possible residual range and assayed by the SLCP method: (a) Product A - a monosodium salt of dichloroisocyanuric acid sanitizer (b) Product B - a nonionic wetting agent plus iodine, (c) Product C - an acid anionic sanitizer, and (d) Product D - a chlorinated alkaline cleaner/sanitizer.

RESULTS AND DISCUSSION

In testing 234 commercial milk samples, most (72%) were found to contain substances inhibitory to S. lutea; penicillin was confirmed in only 3.1% of the positive samples. The non-specific zones of inhibition were somewhat atypical in that they were slightly hazy, unlike the clear zones caused by penicillin, but nevertheless confusing to an experienced technician.

The response of the SLCP method to residual levels of commercial sanitizers was tested to determine if residues of these compounds might be the cause of the high incidence of inhibitory substances noted in commercial milk, Table 1. Possible involvement of these compounds was suggested by Zall and Brown (7). Gross variations were noted among the minimum detectable levels of sanitizers in a series of experimental replicates. Research on the probable cause of these variations led to the finding that proteins interfere with the method in this particular application, Fig. 1. When proteins were removed from the system, by placing the sanitizers in water rather than in milk and by removing the assay medium proteins (S. lutea grew well on yeast extract and dextrose), the results were reproducible and positive correlations were obtained between concentrations of sanitizers and sizes of zones of growth inhibition. These
NON-SPECIFIC INHIBITION OF SARCINA LUTEA

Figure 1. Protein interference in the measurement of acid anionic sanitizer [AAS] by the SLCP method.

data indicate that the S. lutea method, which was developed with success for detection of penicillin, cannot be applied to monitor residual amounts of sanitizers in milk.

Apparently, substances causing non-specific zones develop during refrigerated storage of commercial milk samples to established sell-by dates, Table 2. Fifty-eight commercial milks were analyzed soon after sampling and again on sell-by dates. In most of the samples, increased concentrations of inhibitory substances were observed. Consequently, normal changes in milk occurring during the marketing process could cause non-specific inhibition in this sensitive antibiotic test.

Generally, induction of hydrolytic rancidity in milk samples to various acid degree values did not correlate with growth inhibition. Replicate testing led to the conclusion that false-positive zones are not caused by hydrolytic rancidity. Further limited study is underway on the identity of these growth inhibitors. Psychrotrophic proteolysis or production of antibiotics during refrigerated storage might be involved. Preliminary observations suggest that some peptides and/or proteins inhibit growth of S. lutea.

The S. lutea method was developed to detect penicillin residues in milk and dairy products; our observations over the past 12 years confirm its value in this particular application. However, if the method becomes widely used in milk quality control programs as anticipated, laboratory personnel should be aware of the potential for non-specific inhibition noted in our laboratory. The sensitivity of the SLCP method to low levels of penicillin might make the method attractive for use in quality control programs for monitoring other growth inhibitors.

ACKNOWLEDGMENTS

The cooperation and assistance of Eugene T. Wolff for the collection of samples and George Houghton in data analysis are appreciated. We thank Professor Robert Zall and David P. Brown for their interest and assistance in several aspects of the project.

REFERENCES

Selected Food Microbiological Data Collected through a Computerized Program

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ABSTRACT

Selected results from a Computerized Food Microbiological Data Collection program are presented. Data are generated by 11 Department of Defense (DoD) laboratories and forwarded to a central location, coded into computer language, and entered into an existing program. As designed, the program is quite flexible, and selected retrievals for special purposes are easily accomplished. The total file contains data from over 30,000 food samples. Presented in this report are data on 810 samples of comminuted beef products, 215 samples of luncheon meats, 653 prepared sandwiches, and 1,023 delicatessen salads. Data are compared with existing microbiological criteria, if applicable. The retrieval of data from the file on such products should be useful in establishing future standards or guidelines.

Use of computers can greatly assist in assimilation and analyses of the large volumes of data generated in food microbiology. Thus handled, these data can be the basis for establishing microbiological guidelines or standards for food products and for production guides in segments of the food industry. A Computerized Food Microbiological Data Collection system has been previously reported, together with selected food microbiological data (13). This report presents selected data from the 1973 file (14). Comparisons are made with existing food microbiological guidelines or standards, where applicable.

MATERIALS AND METHODS

Test data

During 1973, the Department of Defense (DoD) operated 11 official food testing laboratories located in the United States, Germany, and Japan. Copies of laboratory reports were forwarded to the Letterman Army Institute of Research, Presidio of San Francisco, California, coded, and then data entered into the computer file.

Microbiological methodology

If a military specification exists for the product being analyzed, official DoD laboratories are required to utilize microbiological procedures specified in that document. Military specifications (with microbiological criteria) do not exist for comminuted beef products, luncheon meats and sausages, or prepared sandwiches. Analyses of these products, therefore, were accomplished with procedures outlined in the Bacteriological Analytical Manual for Foods (1), the Microbiological Laboratory Guidebook (9), or the Official Methods of Analysis of the Association of Official Analytical Chemists (9), used according to the individual preference of each participating laboratory officer. Delicatessen salads, purchased under an Army and Air Force Exchange (AAFES) specification (2), were analyzed in accordance with specified procedures (17) outlined in a prior report (12).

Computer program

The computer system utilized in this program was maintained by the Lawrence Berkeley Laboratory, Berkeley, California. Modifications made to the existing program are described elsewhere (4).

RESULTS AND DISCUSSION

The basic questions underlying the establishment of microbiological standards are exceedingly complex as well as controversial. In an excellent review, Elliot (11) distinguished between various microbiological criteria for food products and discussed methods for their establishment. The consuming public is often exposed to information concerning the microbial quality of food products and this information often can be misleading to the consumer as well as harmful to the food industry. Such information can destroy the trust and confidence that the public has in its public health and regulatory agencies. It is imperative that microbiological criteria be based on sound scientific data and applied with good judgement.

Recently, the subject of microbiological criteria for ground beef has received much attention (4-8, 16). Presently, Oregon is the only state in the U. S. employing microbiological standards for this product. (Other states have formulated proposed standards or guidelines). Not only have Oregon’s standards attracted much attention but they also have generated considerable controversy. Carl (10) has reported on Oregon’s experience with these standards, while Winslow (20) has discussed the same standards from the producers’ viewpoint. Westhoff and Feldstein (19) have summarized current U. S. microbiological criteria for ground beef, and Pivnic et al. (18) recently proposed standards for the same products in Canada.

Table 1 lists the standard plate counts (SPC) for 810 samples and the total coliform counts for 793 samples of comminuted beef collected from military sources and analyzed in DoD laboratories. In comparing the SPC data to the Oregon standard, we have found that only
68.3% of the samples tested had SPC’s less than this limit. Making the same comparisons with the proposed Maryland standard and with Virginia’s guideline of \(1.0 \times 10^7\) g, 80.4% of the samples would have complied with those limits. These data compare favorably with those of Westhoff and Feldstein (19), who found that 82% of their samples had SPC’s of less than \(1 \times 10^7\) g. It appears that the management of the processing facility from which samples were collected which had SPC’s in excess of \(1.0 \times 10^7\) g, should investigate and correct the cause of such high counts.

The total coliform counts of 793 samples, presented in Table 1, cannot be compared with Oregon’s standards since the microbiological criteria are not the same. This is unfortunate, and only serves to re-emphasize the need for standardized methodology and reporting methods for officials in food microbiology. Five states (19) (Georgia, Idaho, Massachusetts, Rhode Island, and Utah) base their criteria on total coliforms. Our data, however, do not compare favorably to their upper limits. Only 38.8% of the samples contained less than 300 coliforms/g, while 19.6% contained more than \(1.0 \times 10^4\) g.

The results of analyzing 215 samples of luncheon meats and sausages comprising 15 different types are presented in Table 2. Comparing these data to the Oregon SPC limit of \(1 \times 10^4\) g, it can be seen that 31.1% of these samples would not have complied with this limit. However, if salami, Kosher pastrami, and pepperoni are excluded from the calculations, only 21.1% of the samples exceeded the SPC limit of \(1 \times 10^4\) g. It should be mentioned that these samples were collected from retail outlets, and that the counts do not reflect the microbiological quality at the time of manufacture.

DoD has not established microbiological criteria for prepared sandwiches, nor are we aware of generally accepted criteria in any of the states. Table 3 lists the standard plate counts for 653 samples and the total coliform counts from 651 of these samples. These sandwiches were collected from vending machines, snack bars, and governmental preparation facilities, and are representative of 23 different types. It should be noted that an “unofficial guideline” of \(1 \times 10^4\) g for SPC

### Table 1. Standard plate counts and coliform counts of comminuted beef products

<table>
<thead>
<tr>
<th>Food item</th>
<th>Number of samples in SPC range x 10^7/g</th>
<th>Number of samples in coliform range x 10^9/g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; 3</td>
<td>10-50</td>
</tr>
<tr>
<td>Ground beef</td>
<td>419</td>
<td>68</td>
</tr>
<tr>
<td>Ground chuck</td>
<td>40</td>
<td>11</td>
</tr>
<tr>
<td>Ground round</td>
<td>113</td>
<td>41</td>
</tr>
<tr>
<td>Hamburger</td>
<td>84</td>
<td>19</td>
</tr>
<tr>
<td>Lean ground beef</td>
<td>154</td>
<td>41</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>810</td>
<td>180</td>
</tr>
</tbody>
</table>

Percent of samples within range: 22.2%
Cumulative percent: 22.2%

### Table 2. Standard plate counts and coliform counts of luncheon meats

<table>
<thead>
<tr>
<th>Food item</th>
<th>Number of samples in SPC range x 10^7/g</th>
<th>Number of samples in coliform range x 10^9/g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; 3</td>
<td>10-40</td>
</tr>
<tr>
<td>All beef bologna</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>All meat bologna</td>
<td>15</td>
<td>7</td>
</tr>
<tr>
<td>Bologna</td>
<td>24</td>
<td>2</td>
</tr>
<tr>
<td>Beef++ pork</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>BBQ loaf</td>
<td>15</td>
<td>4</td>
</tr>
<tr>
<td>Breast of turkey</td>
<td>19</td>
<td>1</td>
</tr>
<tr>
<td>Cooked salami</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Ham+ cheese loaf</td>
<td>19</td>
<td>15</td>
</tr>
<tr>
<td>Ham cooked</td>
<td>14</td>
<td>6</td>
</tr>
<tr>
<td>Hard salami</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td>Kosher pastrami</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>New England loaf</td>
<td>17</td>
<td>5</td>
</tr>
<tr>
<td>Peppered loaf</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>Pepperoni</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>Pickle + pimento loaf</td>
<td>17</td>
<td>15</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>215</td>
<td>59</td>
</tr>
</tbody>
</table>

Percent of samples within range: 27.4%
Cumulative percent: 27.4%
100/g for total coliforms is often used as a surveillance criterion in military establishments producing or handling prepared sandwiches. For comparison purposes only, data in Table 3 are discussed in relation to these “unofficial guidelines.” The data show that 82.8% of all sandwiches analyzed has SPC’s of less than $1.0 \times 10^3$/g. Of the 651 sandwiches analyzed for total coliforms, 70.8% contained less than 3/g while 90.2% contained less than 100/g. These data tend to substantiate the validity of the “unofficial guidelines.”

Table 4 is a tabulation of the SPC’s of 1,023 delicatessen salads, and the total coliforms from 1,021 salads. As previously stated, AFIFS has adopted microbiological specifications (2) for these products. These are:

**BACTERIOLOGICAL REQUIREMENTS:** Samples shall not exceed the following bacteriological limits:

<table>
<thead>
<tr>
<th>Standard Plate Count</th>
<th>NMT 100,000/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coliform</td>
<td>NMT 10/g</td>
</tr>
<tr>
<td>E. coli</td>
<td>NMT 10/g</td>
</tr>
<tr>
<td>Yeast and Mold</td>
<td>NMT 100/g</td>
</tr>
</tbody>
</table>

Contractor performance related to bacteriological requirements.

### TABLE 3. Standard plate counts and coliform counts of prepared sandwiches

<table>
<thead>
<tr>
<th>Food Item</th>
<th>N&lt;sup&gt;b&lt;/sup&gt;</th>
<th>&lt; 3</th>
<th>3-40</th>
<th>41-80</th>
<th>81-100</th>
<th>&gt; 100</th>
<th>N</th>
<th>&lt; 3</th>
<th>3-40</th>
<th>41-80</th>
<th>81-100</th>
<th>&gt; 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>BBQ</td>
<td>36</td>
<td>17</td>
<td>9</td>
<td>2</td>
<td>1</td>
<td>7</td>
<td>36</td>
<td>22</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Bologna</td>
<td>28</td>
<td>7</td>
<td>10</td>
<td>2</td>
<td>0</td>
<td>9</td>
<td>30</td>
<td>27</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bologna + cheese</td>
<td>12</td>
<td>4</td>
<td>6</td>
<td>2</td>
<td>0</td>
<td>12</td>
<td>023</td>
<td>17</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cheeseburger</td>
<td>35</td>
<td>16</td>
<td>16</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>35</td>
<td>31</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Chili dog</td>
<td>22</td>
<td>16</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>22</td>
<td>19</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Chicken</td>
<td>24</td>
<td>15</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>24</td>
<td>17</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Chicken salad</td>
<td>20</td>
<td>7</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>8</td>
<td>20</td>
<td>14</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Egg salad</td>
<td>35</td>
<td>11</td>
<td>11</td>
<td>1</td>
<td>5</td>
<td>6</td>
<td>35</td>
<td>15</td>
<td>14</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Ham</td>
<td>70</td>
<td>35</td>
<td>19</td>
<td>5</td>
<td>1</td>
<td>10</td>
<td>64</td>
<td>45</td>
<td>8</td>
<td>4</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Ham + cheese</td>
<td>69</td>
<td>26</td>
<td>18</td>
<td>3</td>
<td>2</td>
<td>20</td>
<td>68</td>
<td>48</td>
<td>9</td>
<td>3</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Ham + egg</td>
<td>20</td>
<td>8</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>5</td>
<td>20</td>
<td>11</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Ham + egg salad</td>
<td>16</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td>16</td>
<td>1</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Ham salad</td>
<td>19</td>
<td>9</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>19</td>
<td>15</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Hamburger</td>
<td>18</td>
<td>8</td>
<td>7</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>18</td>
<td>14</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Hoagie</td>
<td>19</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>19</td>
<td>3</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Hot Dog</td>
<td>42</td>
<td>24</td>
<td>11</td>
<td>4</td>
<td>0</td>
<td>2</td>
<td>41</td>
<td>35</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Pimento cheese</td>
<td>22</td>
<td>10</td>
<td>6</td>
<td>0</td>
<td>1</td>
<td>5</td>
<td>22</td>
<td>20</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Poor boy</td>
<td>12</td>
<td>3</td>
<td>7</td>
<td>2</td>
<td>0</td>
<td>15</td>
<td>15</td>
<td>12</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Roast beef</td>
<td>40</td>
<td>21</td>
<td>11</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>40</td>
<td>30</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Salami</td>
<td>17</td>
<td>3</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>17</td>
<td>16</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sloppy joe</td>
<td>12</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>11</td>
<td>10</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tuna salad</td>
<td>43</td>
<td>28</td>
<td>9</td>
<td>1</td>
<td>0</td>
<td>5</td>
<td>44</td>
<td>31</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Turkey</td>
<td>31</td>
<td>11</td>
<td>8</td>
<td>2</td>
<td>0</td>
<td>10</td>
<td>31</td>
<td>16</td>
<td>9</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>TOTAL</td>
<td>653</td>
<td>297</td>
<td>189</td>
<td>41</td>
<td>14</td>
<td>112</td>
<td>651</td>
<td>461</td>
<td>196</td>
<td>16</td>
<td>4</td>
<td>64</td>
</tr>
</tbody>
</table>

Percent of samples within range
Cumulative percent

### TABLE 4. Standard plate counts and coliform counts of delicatessen salads

<table>
<thead>
<tr>
<th>Food Item</th>
<th>N&lt;sup&gt;b&lt;/sup&gt;</th>
<th>&lt; 3</th>
<th>3-40</th>
<th>41-80</th>
<th>81-100</th>
<th>&gt; 100</th>
<th>N</th>
<th>&lt; 3</th>
<th>3-40</th>
<th>41-80</th>
<th>81-100</th>
<th>&gt; 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carrot + raisin</td>
<td>20</td>
<td>4</td>
<td>7</td>
<td>3</td>
<td>1</td>
<td>5</td>
<td>20</td>
<td>12</td>
<td>1</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chicken</td>
<td>138</td>
<td>49</td>
<td>55</td>
<td>12</td>
<td>2</td>
<td>20</td>
<td>139</td>
<td>80</td>
<td>14</td>
<td>7</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Cole slaw</td>
<td>164</td>
<td>33</td>
<td>70</td>
<td>15</td>
<td>1</td>
<td>45</td>
<td>164</td>
<td>107</td>
<td>14</td>
<td>8</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Egg</td>
<td>27</td>
<td>11</td>
<td>7</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>27</td>
<td>13</td>
<td>10</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Green</td>
<td>55</td>
<td>8</td>
<td>28</td>
<td>7</td>
<td>10</td>
<td>5</td>
<td>55</td>
<td>20</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Ham</td>
<td>91</td>
<td>52</td>
<td>32</td>
<td>0</td>
<td>7</td>
<td>90</td>
<td>91</td>
<td>66</td>
<td>12</td>
<td>4</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Macaroni</td>
<td>168</td>
<td>75</td>
<td>54</td>
<td>10</td>
<td>3</td>
<td>26</td>
<td>169</td>
<td>158</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Mixed green</td>
<td>56</td>
<td>9</td>
<td>28</td>
<td>4</td>
<td>3</td>
<td>12</td>
<td>56</td>
<td>12</td>
<td>2</td>
<td>5</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Pimento cheese</td>
<td>17</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>18</td>
<td>16</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Potato</td>
<td>255</td>
<td>97</td>
<td>95</td>
<td>19</td>
<td>7</td>
<td>37</td>
<td>252</td>
<td>236</td>
<td>8</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Tuna</td>
<td>32</td>
<td>23</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>32</td>
<td>22</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>TOTAL</td>
<td>1,023</td>
<td>366</td>
<td>287</td>
<td>75</td>
<td>24</td>
<td>171</td>
<td>1,021</td>
<td>742</td>
<td>78</td>
<td>44</td>
<td>20</td>
<td>10</td>
</tr>
</tbody>
</table>

Percent of samples within range
Cumulative percent

---

<sup>a</sup> Standard Plate Count
<sup>b</sup> Number
stated above shall be judged according to the three-out-of-five compliance procedures outlined on page 125 of the 1967 edition of Public Health Service Publication No. 229.

Using the AAFES criteria, 83.3% of the salads were in compliance with the established SPC. Of the remaining 16.7%, several more may have complied when the "three-out-of-five" rule was used. However, the computer system does not contain information pertinent to this stipulation.

The total coliform data (Table 4) shows that 80.3% of the samples the criteria of the AAFES specification, based on single comparison without application of the three-out-of-five rule. Three products (cole slaw, green, and mixed green) greatly influenced the microbiological picture of salads. These latter products, discussed in a previous report (15), should not be categorized as delicatessen salads. Even with their inclusion, however, the compliance of salads to the AAFES specification was quite good.

In presenting data on the four types of products, it was not our original intention to favor or to recommend tentative guidelines. It was instead to contribute to the data base in the area of food microbiology. However, in making comparisons to existing standards, guidelines, or specification, the data lead us to draw conclusions as to the feasibility of guidelines for certain products. Based on the data contained in this report (and other unpublished data in the computer file), the following conclusions are made:

(a) An SPC limit of $1 \times 10^7/g$ appears to be feasible and reasonable for ground beef. A total coliform count limit of $1 \times 10^4/g$ appears to be feasible.

(b) Little difficulty should be experienced with guidelines which restrict the SPC in luncheon meats to $1 \times 10^6/g$. Total coliforms do not appear to be a significant problem in these products.

(c) An SPC limit of $1 \times 10^5/g$ as well as a total coliform count limit of 100/g appear to be feasible as microbiological criteria for prepared sandwiches sampled at military facilities.

(d) The present AAFES specification for SPC and total coliforms for delicatessen salads appears to be feasible and workable.

The tabulation and analyses of data through use of a computer system has proven, in this laboratory, to be a useful tool in managing food microbiological data. In managing the file and in making comparisons with existing microbiological criteria, attention has been drawn to the urgent need for standardization in food microbiological methodology as well as in reporting systems.

This report presents only a part of the data tabulated in the computer file. Other data are available upon request from this laboratory.

REFERENCES


Trace Metal and Protein Concentrations in California Market Milks

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(Received for publication July 14, 1976)

ABSTRACT

Market milk samples (225) were collected in three major milk marketing areas in California. Samples represented four products; regular, extra-rich, non-fat, and low-fat milk. Concentrations of cadmium, copper, lead, zinc, and protein were measured in all samples. The concentration of selenium was measured in 103 of the samples. Mean contrations in all samples examined were: cadmium 5.0; copper, 41.0; lead, 60.3; and selenium, 24.4 μg/kg. No significant differences between market or product means were found for these four metals. Mean product concentrations for protein and zinc were: regular milk, 3.29% protein and 3.61 mg/kg zinc; extra-rich milk, 3.33% and 3.70 mg/kg; non-fat milk, 3.61% and 3.98 mg/kg; and low-fat milk, 3.82% and 4.18 mg/kg. Low-fat and nonfat milks had significantly (α = .05) greater concentrations of protein and zinc than did regular and extra-rich milks.

Contamination of our food supply by environmental pollutants is receiving increased attention (4). Attention has been focused on milk, particularly since it is the principal food of infant nutrition and can provide a major part of human nutrition. Surveys of California herd milk (4, 5) have established limiting and mean concentrations for copper, lead, and cadmium occurring in raw milk. The present survey of market milk was undertaken to determine what influences processing and product standardization would have on these trace elements.

The nutritionally important element zinc and protein were also determined in market milks. Selenium was also measured in some market milk because of the concern (9) expressed relating a selenium deficiency to the sudden infant death syndrome. Millar and Sheppard (8) have stated that the selenium concentration in cows' milk is significantly lower than in human milk. The importance of having background information on the selenium concentration in market milk was therefore warranted.

MATERIALS AND METHODS

Samples of four products; regular, extra-rich, non-fat, and low-fat milk, were collected from markets in the San Francisco Bay Area and Sacramento Valley (Northern California), and the Los Angeles Metropolitan Area (Southern California) and held at 4°C until analyzed.

TABLE 1. Composition standards for California market milk

<table>
<thead>
<tr>
<th>Component</th>
<th>Non-fat</th>
<th>Low-fat</th>
<th>Regular</th>
<th>Extra-rich</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat (%)</td>
<td>&lt;0.25</td>
<td>1.9-2.1</td>
<td>≥3.5</td>
<td>no standard8</td>
</tr>
<tr>
<td>Solids not fat (%)</td>
<td>≥9.0</td>
<td>≥10.0</td>
<td>≥8.7</td>
<td>no standard8</td>
</tr>
</tbody>
</table>

8Product must be clearly labelled "20% (or more) richer than regular milk."

The California product standards are given in Table 1. Once the carton had been opened, all samples required for the different analyses were prepared as quickly as possible, generally within 4 h. The remaining milk was frozen and stored.

Copper determinations were made by the procedure reported previously (6) with no modification. Lead and cadmium determinations were made by the procedure reported previously (5) except that absorption at 283 nm instead of 217 nm was used to quantitate lead.

Selenium determinations were made by dry ashing 100-g samples, dissolving the ash in 15 ml of 2.4 N HCl, diluting the dissolved ash in 100 ml of deionized water and aspirating the final solution into an atomic absorption spectrophotometer (1). Absorption measurements were made at 214 nm using an air-acetylene flame. Standards were prepared from a Fisher Certified 1000 ppm solution diluted with 0.36 N HCl to obtain concentrations in the range of 0.5 mg/kg (0.5 mg/kg increments). This procedure was tested for accuracy and precision before application to the samples. Recoveries of zinc added to milk in the range 0 to 1.25 mg/kg (0.25 mg/kg increments) averaged 98.9 ± 0.29% (mean ± standard deviation, six trials). The standard deviation of triplicate analyses ranged from 0.01 to 0.06 mg/kg.

Selenium determinations were made by the wet ashing-fluorometric procedure recommended by the Association of Official Analytical Chemists (7). Recoveries of selenium added to milk in the range 0 to 54 μg/l (9, 18, and 54) averaged 99.14%, with a standard deviation of 1.84%.

Protein determinations were made by the dye-binding procedure recommended by the Association of Official Analytic Chemists (2). This procedure is a better test for total protein than Kjeldahl since the latter includes non-protein nitrogen (17). Our research using 21 herd milk samples collected in the Sacramento Valley indicates that the correlation coefficient (r) exceeds 0.99. The mean difference between triplicate determinations by dye binding and Kjeldahl was 0.014% protein.

Statistical analyses were done according to Snedecor and Cochran (15). Levels of significance are all reported at α = 0.05.

RESULTS AND DISCUSSION

The results are summarized in Tables 2 and 3.

Copper

The mean copper concentration found in the 225 market milk samples was 41 μg/kg with a standard
significant differences between markets, none were found. When the data were examined for significant differences between products, none were found.

Our values for copper are consistent with those reported by Murthy et al. (12). Our range was from 23 to 115 μg/kg milk; they reported a range from 64 to 130 μg/l milk for samples collected in California during 1967 and 1968. They reported seasonal variations in the copper concentration of milk. Our samples were collected over 13 months, but we could not detect any seasonal variation in our sample.

Lead

The mean lead concentration found in the 225 market milk samples was 60 μg/kg with a standard deviation of 32 μg/kg. The frequency distribution of concentrations found (Fig. 2) is skewed toward the lower concentrations, just as reported for raw milks (5). In the market milk samples, 50% had less than 55 μg lead/kg, and 95% had less than 120 μg lead/kg. There is very little probability that one would find a market milk in California containing as much as 156 μg/kg.

Data on lead concentration were examined for significant market and/or product differences. None were found. The difference between markets was consistent, however, Northern California samples averaging about 23 μg/kg higher than those collected in Southern California.

Our lead values are similar to those reported by Murthy et al. (10). Our range was from 5 to 183 μg/kg milk, while they reported a range from 19 to 104 μg/kg milk for samples collected in California during 1965 and 1966.

Cadmium

The mean cadmium concentration found in 225 market milk samples was 5 μg/kg with a standard deviation of 3 μg/kg. The frequency distribution of concentrations found (Fig. 3) is again skewed toward low concentration, just as it was for data collected for raw milk (5). The cadmium concentrations found in market milk are the same as reported in raw milk. Fifty percent of the samples had less than 5 μg/kg milk, and 95% of the samples had less than 10 μg/kg. There is very little

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**TABLE 2. Mean concentrations of trace metals in California market milk**

<table>
<thead>
<tr>
<th>Product</th>
<th>Copper (μg/kg)</th>
<th>Lead (μg/kg)</th>
<th>Cadmium (μg/kg)</th>
<th>Selenium (μg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regular</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N. Calif.</td>
<td>38.8</td>
<td>69.5</td>
<td>5.4</td>
<td>20.9</td>
</tr>
<tr>
<td>S. Calif.</td>
<td>40.9</td>
<td>41.1</td>
<td>3.8</td>
<td>26.8</td>
</tr>
<tr>
<td>Statewide</td>
<td>39.5</td>
<td>58.1</td>
<td>4.8</td>
<td>19.7</td>
</tr>
<tr>
<td>Extra-rich</td>
<td>38.7</td>
<td>73.4</td>
<td>6.1</td>
<td>22.0</td>
</tr>
<tr>
<td>N. Calif.</td>
<td>40.4</td>
<td>47.7</td>
<td>4.5</td>
<td>24.3</td>
</tr>
<tr>
<td>S. Calif.</td>
<td>39.4</td>
<td>61.6</td>
<td>5.3</td>
<td>23.3</td>
</tr>
</tbody>
</table>

**Non-fata**

<table>
<thead>
<tr>
<th>Product</th>
<th>Protein (g/100g)</th>
<th>Zine (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regular</td>
<td>3.29 ± 0.08b</td>
<td>3.61 ± 0.32b</td>
</tr>
<tr>
<td>Extra-rich</td>
<td>3.33 ± 0.15</td>
<td>3.70 ± 0.35</td>
</tr>
<tr>
<td>Avg. R + X</td>
<td>3.31 ± 0.11</td>
<td>3.64 ± 0.33</td>
</tr>
<tr>
<td>Non-fat</td>
<td>3.61 ± 0.26</td>
<td>3.98 ± 0.37</td>
</tr>
<tr>
<td>Low-fat</td>
<td>3.82 ± 0.12</td>
<td>4.18 ± 0.31</td>
</tr>
<tr>
<td>Avg. N + L</td>
<td>3.71 ± 0.20</td>
<td>4.08 ± 0.34</td>
</tr>
<tr>
<td>Avg. all samples</td>
<td>3.52 ± 0.17</td>
<td>3.88 ± 0.34</td>
</tr>
</tbody>
</table>

---

aSee Table 1 for product standards.
bMean = Standard deviation.

deviation of 10 μg/kg. The frequency distribution of the copper concentrations (Fig. 1) is what was expected based on the data for herd milks reported previously (4). Of the market milk studies, 50% had copper concentrations below 40 μg/kg and 95% were below 56 μg/kg. There is very little probability that one would find a market milk in California containing as much as 71 μg/kg.

The survey samples represent three major milk markets in California. When the mean copper concentrations within markets were examined for significant differences between markets, none were detected. The variation in our sample is what was expected just as reported for raw milks (5).

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Figure 1. Frequency distribution of copper concentrations in California market milk.

Figure 2. Frequency distribution of lead concentrations in California market milk.
probability that one would find a market milk in California containing more than 14 μg cadmium/kg. There were no significant differences between products or markets with respect to cadmium concentration.

Compared to data reported by Murthy and Rhea (11) the values reported here are significantly lower. Our range was from 1 to 17 μg/kg milk, while they reported a range from 17 to 30 μg/kg nationally.

**Selenium**

The mean selenium concentration found in 103 samples of market milk was 24 μg/l with a standard deviation of 11 μg/l. The frequency distribution of concentrations found (Fig. 4) appears rather random.

Among the samples studied, 50% had less than 19 μg/l and 95% had less than 39 μg/l. There is very little probability that one would find a market milk in California containing more than 57 μg/l.

We found values that ranged between 7 and 54 μg/l; Millar et al. (8) reported 2.9 to 9.7 μg/l in New Zealand milks, and Bisbjerg et al. (3) reported an average value of 23 μg/l for Danish milk. We found no significant differences between products or marketing areas, though the samples collected in Southern California averaged about 5 μg/l higher than those from Northern California. It is quite possible that real regional and seasonal differences exist since selenium might be associated with the protein in milk (6).

**Protein and zinc**

Frequency distributions (Fig. 5 and 6) for protein and zinc concentrations found are complicated by the addition of non-fat dry milk solids to some of the extra-rich, non-fat, and low-fat milks. This is most apparent in the protein distribution, which is bimodal; there are two populations here, one centered about the mean value for regular and extra-rich milks, the other about the mean value for non-fat and low-fat milks. This is not so apparent for zinc because the zinc concentrations in each product are so variable.

Parkesh and Jenness (13) reported that about 88% of the zinc in milk is associated with the casein, so the relationship between zinc and protein concentrations found in California market milks is not surprising. The regular increase in zinc and protein concentrations from regular to low-fat milk is a consequence of the solids not fat standards for these products (Table 4). Non-fat and low-fat milk have significantly higher concentrations of zinc and protein than do regular and extra-rich milks, so a discussion of the probability of maximum or minimum values must be related to each individual product. Since the minimum value is more important nutritionally both...
TABLE 4. The effect of product standardization for solids not fat on the concentrations of zinc and protein in the products

<table>
<thead>
<tr>
<th>Component</th>
<th>Regular</th>
<th>Extra-rich</th>
<th>Non-fat</th>
<th>Low-fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNF, %&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.7</td>
<td>9.0</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td>Zinc, mg/kg&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.61</td>
<td>3.70</td>
<td>3.98</td>
<td>4.18</td>
</tr>
<tr>
<td>Protein, %&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.29</td>
<td>3.33</td>
<td>3.61</td>
<td>3.82</td>
</tr>
</tbody>
</table>

<sup>a</sup>State requirements.  
<sup>b</sup>Differences between means sharing the same underline are not significant, 0.05.  
<sup>c</sup>for protein and zinc, we can state that the minimum concentrations of protein and zinc likely to be encountered by the California consumer are in regular milk, and are 3.05 g of protein/100 g and 2.45 mg of zinc/kg.  

for protein and zinc, we can state that the minimum concentrations of protein and zinc likely to be encountered by the California consumer are in regular milk, and are 3.05 g of protein/100 g and 2.45 mg of zinc/kg. So a 100 g serving of regular milk provides at least 5.4% of the RDA for protein and 1.0% of the RDA for zinc.

ACKNOWLEDGMENTS

The authors are indebted to Terry Adams, Victoria Caballero, Frankie Chan, Lynne DeCouto, Chris Erwin, Kathy Hirsch, Dave Robin, Janet Williams, and Chris Yager for their technical assistance, and to Chris Lambert who helped with the statistical analyses and preparation of figures for publication. Research supported in part by a grant from the Dairy Council of California.

REFERENCES

Effect of Heat on Sarcoplasmic Proteins of Light and Dark Avian Muscle Tissue

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(Received for publication July 26, 1976)

ABSTRACT

Loss of solubility of sarcoplasmic proteins was used as an index for denaturation of leg and breast chicken muscle tissue water extracts heated to 40, 50, 60, 70, and 80 C. Proteins of the dark and light tissue proved to be different in heat sensitivity and electrophoresis patterns. After electrophoretic separation, some sarcoplasmic proteins from both leg and breast tissue yielded similar Rm (relative mobility) values but had different heat sensitivities. An electrophoresis band with the thermoinactivation characteristics of myoglobin was detected in the dark muscle tissue but not in light muscle.

In a domesticated chicken carcass it is possible to readily select muscles characteristic of the dark and light varieties. The leg muscle is typical of the dark tissue and the breast represents the light tissue, both visually characteristic of their respective class after cooking. This difference in color is due to their different biochemical constituents and the way they react upon heating. Much emphasis has been placed on organoleptic characteristics of these tissues after cooking (5, 11, 12, 15, 17) but little research has evaluated the influence of heat on the proteins responsible for tissue differences.

The purpose of this research is to evaluate the effect of heat on the sarcoplasmic protein fraction of dark and light chicken muscle tissue. Loss of solubility will be used as an index to denaturation as suggested by Hamm and Deatherage (4) and Disc Gel Electrophoresis will be used to observe the effect of heat on the sarcoplasmic proteins, as utilized by Laakkonen et al. (7).

MATERIALS AND METHODS

Six domesticated white leghorn chickens (one per trial) were slaughtered and leg and breast muscles were removed separately and ground. Twenty-four hours later, after rigor, the individually ground tissue was extracted (extraction temperature maintained below 20 C) with two volumes of cold (2 ± 2 C) water. The solubilized sarcoplasmic proteins were separated from the tissue by filtration. Four-ml aliquots of the extracts were heated in a water bath to 40, 50, 60, 70, and 80 C, held for 15 min, then cooled quickly in an ice bath. An unheated extract was used as a control.

The heat-precipitated denatured proteins were separated by centrifuging (3,000 × g) for 10 min. The remaining soluble protein solutions were used for nitrogen analysis and electrophoresis.

Soluble nitrogen was determined by a colorimetric (Nessler Reagent) method (6) and non-protein nitrogen (NPN) was determined after protein precipitation with trichloroacetic acid (10% by weight).

Extract samples of 30 µl for dark tissue and 15 µl for light tissue were required to yield 50 µg of protein nitrogen for electrophoretic analysis of control samples. This same volume was also used for samples after heat treatment. Electrophoretic separation was conducted on 6% polyacrylamide using the original system of Ornstein and Davis as described by Smith (14). A Cenco power source and equipment applied 5 mA per tube during separation.

RESULTS

Heating the extracted sarcoplasmic protein from both types of muscle to 40 C did not lower (P > .05) the quantity of soluble proteins when compared with the controls (Table 1). Heating to 50 C, however, yielded a precipitate (dark, P > .001; light, P > .01) when compared to samples heated to 40 C. The 50 C treatment resulted in a 31% precipitation of the sarcoplasmic proteins.

<table>
<thead>
<tr>
<th>Temperature of heating</th>
<th>Dark muscle mg N/ml</th>
<th>% of original nitrogen remaining in solution</th>
<th>Light muscle mg N/ml</th>
<th>% of original nitrogen remaining in solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.64 ± 0.15</td>
<td>100</td>
<td>3.30 ± 0.46</td>
<td>100</td>
</tr>
<tr>
<td>40 C</td>
<td>1.59 ± 0.14</td>
<td>97</td>
<td>3.16 ± 0.43</td>
<td>96</td>
</tr>
<tr>
<td>50 C</td>
<td>1.14 ± 0.09</td>
<td>69</td>
<td>2.16 ± 0.46</td>
<td>65</td>
</tr>
<tr>
<td>60 C</td>
<td>0.34 ± 0.03</td>
<td>21</td>
<td>0.36 ± 0.06</td>
<td>11</td>
</tr>
<tr>
<td>70 C</td>
<td>0.09 ± 0.01</td>
<td>5</td>
<td>0.07 ± 0.006</td>
<td>2</td>
</tr>
<tr>
<td>80 C</td>
<td>0.05 ± 0.003</td>
<td>3</td>
<td>0.06 ± 0.004</td>
<td>2</td>
</tr>
</tbody>
</table>

1Mean ± standard deviation of six determinations
2Different superscripts mean differences (P < .05) in column

1Research conducted in Cordoba with statistical analysis and translation done in Columbus.
2Approved as Journal Article No. 111-76 by the Director of The Ohio Agricultural Research and Development Center, Wooster.
compared with samples heated to 50°C and resulted in precipitation of 79% of sarcoplasmic protein of the leg muscle extracts and 89% of the breast muscle extracts. After heating to 70°C, only a small percentage (5% for dark tissue; 2% for light tissue) of the protein initially soluble remained in the solution, which differed from the results at 60°C (P < .001). The increase of temperature to 80°C yielded only slightly less soluble protein than 70°C.

Figures 1 and 2 illustrate typical densitometric curves corresponding to the gel patterns obtained from the soluble sarcoplasmic proteins after exposure to the different treatments. Relative mobility (Rm) calculations and labeling of peaks were accomplished according to Smith (14). Table 2 shows peak number identification and corresponding Rm values.

**TABLE 2.** Relative mobility (Rm) of electrophoretic bands of muscle tissue water extracts and their corresponding numbers that are used in the graphs and the discussion

<table>
<thead>
<tr>
<th>Peak number in graphs and discussion</th>
<th>Rm (peak travel/ maximum peak travel)</th>
<th>Peak number in graphs and discussion</th>
<th>Rm (peak travel/ maximum peak travel)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>6</td>
<td>24</td>
</tr>
<tr>
<td>2</td>
<td>69</td>
<td>7</td>
<td>18</td>
</tr>
<tr>
<td>3</td>
<td>56</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>4</td>
<td>41</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>29</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In the densitometric tracing (Fig. 1) of the control sample of dark muscle extract, six bands could be distinguished. In the corresponding tracing (Fig. 2) for the light muscle control sample, seven peaks could be distinguished. Four peaks (#1, 3, 4, and 9) had the same Rm values in both types of muscle.

In the dark muscle extract electrophoretic tracings (Fig. 1) there was no appreciable difference between the control pattern and the extract heated to 40°C. Upon heating to 50°C there was a reduction of intensity in bands of less mobility (Peaks 6 and 9). After the 60°C temperature treatment it was possible to still distinguish all bands; however, all bands were of reduced intensity except peak #3. With heating to 70°C, band #2 disappeared and the remaining bands were reduced in intensity. After treatment to 80°C only peaks #3, 6, and 9 could be slightly distinguished.

With light muscle extract (Fig. 2) there was no appreciable modification of band patterns between the control and heating to 40°C. Heating to 50°C caused band #3 to disappear and reduced the intensity of peaks #4, 5, and 7. After the 60°C treatment only four bands (Numbers 1, 4, 8, and 9) remained and they were at greatly reduced intensity. When extracts were heated to 70 and 80°C, no detectable peaks remained.

![Figure 1. Densitometric curves of electrophoretic patterns of water soluble proteins from chicken leg muscle (dark), heated to different temperatures for 15 min.](image-url)
DISCUSSION

The observed sensitivity of sarcoplasmic proteins to heat agrees with Hamm and Deatherage (4) who showed that globular proteins were very stable at 40 C; their solubility was greatly reduced between 40 and 60 C; and, heating from 60 to 80 C produced only a slight additional loss in solubility. Bendall (1) also showed that most of the sarcoplasmic proteins had been denatured when the temperature reached 62 C.

The number of electrophoretic peaks is similar to reports of Maier and Fisher (10), and Hoke et al. (5). These last workers also presented similar differences between red (dark) and white (light) muscle patterns in turkey muscle.

According to Scopes (13) the peak with the highest Rm value (band #1) would likely be myoalbumin and the next peak (band #2) would be myoglobin. This second identification is reinforced by the facts that this band appeared only in the dark muscle tissue extract; was at the level (13) of myoglobin (0.5 mg) to total sarcoplasmic fraction (55 mg); and, the intensity of this band was reduced by heating to 60 C and disappeared at 70 C, corresponding to the heat sensitivity of myoglobin (2, 3, 8, 9).

The heat sensitivity of band #3 differed between light and dark muscle extracts. For light muscle extract this band disappeared after heating to 50 C. In dark muscle extracts a band with the same Rm value remained in solution after heating to 80 C. This difference in heat sensitivity suggests that these bands are different proteins with same Rm value. Whitaker (16) indicated that Rm value alone may not identify protein from muscle of different species.

The bands of reduced mobility (bands 4 through 9) are probably glycolytic enzymes because they constitute the highest proportion of the sarcoplasmic fraction and only have a slight electrical charge at the pH used (pH 7.1). These proteins normally do not totally separate (13) under these conditions and in our research they present different mobilities and heat sensitivity for the two muscle groups which suggests different proteins. It is also possible that they are the same kind of proteins (with similar functions) and that they differ due to their adaptation to the specific metabolism of each type of muscle. In general these proteins were very sensitive to heat, which agrees with Laakkonen et al. (7) who pointed out that the bands with lower Rm values were the most heat-sensitive.

REFERENCES


Effect of Heating Avian Muscle Tissue on Solubility of Nitrogen Fractions and pH Values of Breast and Leg Muscle

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ABSTRACT

Sarcoplasmic, myofibrillar, and non-protein nitrogen fractions extracted from chicken leg and breast muscle tissue after heating to 40, 50, 60, 70, and 80 °C were compared with non-heated samples. The pH modifications that occurred during heating were also observed. The sarcoplasmic fraction was more resistant to heat denaturation than the sarcoplasmic proteins with a slight difference in sensitivity occurring between the leg and breast muscle tissues. Both of these fractions were only approximately 10% soluble after heating to 70 °C with only slight alterations caused by additional heating to 80 °C. The non-protein nitrogen fractions decreased slightly during the heating cycle and remained more than 90% soluble after heating to 80 °C. Initially, pH of breast muscle was lower (P < .05) than leg muscle and the breast sample increased in pH more rapidly than the leg sample during the heating cycle.

The effect of heat on muscle tissue is not uniform. This difference in behavior between muscles during heating is primarily due to differences in initial chemical composition. The proportion of connective tissue to other muscle components is fundamental to this non-uniformity (11) and the sarcoplasmic and myofibrillar fractions may also contribute to this variation by demonstrating different sensitivities to heat in different muscles.

In a previous study (13) it was observed that chicken leg and breast sarcoplasmic proteins in solution showed different resistance to heat denaturation. Hamm (6) has pointed out that the effect of heat on meat proteins is different when the proteins are in solution or when they are an integral part of the tissue structure. The objectives of this research were to evaluate the modifications induced by heating of the intact muscle on the amount of extractable sarcoplasmic and myofibrillar proteins. The pH alterations were also evaluated.

MATERIALS AND METHODS

Duplicate ground muscle (4 g) samples were individually taken from the leg and breast muscle tissue of six domesticated White Leghorn chickens (one per trial) at 24 h after slaughter (post-rigor). These muscle tissue samples were placed in tubes and heated for 30 min in water baths regulated at 40, 50, 60, 70, and 80 °C. Temperatures were rapidly lowered after the heating treatment by immersing tubes in an ice bath.

The method of Helander (7), as modified by Maxon and Marion (13), was used for extraction of sarcoplasmic and myofibrillar proteins. The non-protein nitrogen (NPN) fraction was determined from the sarcoplasmic fraction after separation of the proteins by precipitation with 10% (wt/vol) trichloroacetic acid. The nitrogen content of the extract was evaluated colorimetrically (9). The pH of tissue was determined on a meat-water (1:2) slurry with a Beckman Expandomatic pH meter.

RESULTS

As may be seen from data in Tables 1 and 2, the control breast muscle contained more sarcoplasmic (P < .01), myofibrillar (P < .05), and non-protein nitrogen (P < .001) than the control leg muscle. Heating to

### TABLE 1. Soluble nitrogen content of sarcoplasmic, myofibrillar, and non-protein (NPN) fractions of meat from avian breast muscle after heating

<table>
<thead>
<tr>
<th>Temperature of heating (°C)</th>
<th>Sarcoplasmic fraction</th>
<th>Myofibrillar fraction</th>
<th>NPN fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg N/g meat&lt;sup&gt;1&lt;/sup&gt;</td>
<td>%&lt;sup&gt;2&lt;/sup&gt;</td>
<td>mg N/g meat&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>9.68 ± 0.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100</td>
<td>12.84 ± 0.51&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>40</td>
<td>9.22 ± 0.66&lt;sup&gt;b&lt;/sup&gt;</td>
<td>95.2</td>
<td>13.34 ± 1.22&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>50</td>
<td>6.06 ± 1.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>62.6</td>
<td>2.83 ± 0.20&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>60</td>
<td>1.96 ± 0.25&lt;sup&gt;c&lt;/sup&gt;</td>
<td>20.2</td>
<td>1.24 ± 0.11&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>70</td>
<td>1.00 ± 0.13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.3</td>
<td>0.96 ± 0.19&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>80</td>
<td>0.94 ± 0.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.7</td>
<td>0.80 ± 0.20&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup> Mean of six trials ± standard deviation
<sup>2</sup> Percent of control values
<sup>a</sup>,<sup>b</sup>,<sup>c</sup>—Different superscripts mean differences (of at least P < .05) in a column.
CHANGES IN HEATED AVIAN MUSCLE

TABLE 2. Soluble nitrogen content of sarcoplasmic, myofibrillar and non-protein (NPN) fractions of meat from avian leg muscle after heating at different temperatures for 30 minutes

<table>
<thead>
<tr>
<th>Temperature of heating (°C)</th>
<th>Sarcoplasmic fraction</th>
<th>Myofibrillar fraction</th>
<th>NPN fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg N/g meat±</td>
<td>%±</td>
<td>mg N/g meat±</td>
</tr>
<tr>
<td>Control</td>
<td>6.93 ± 1.22</td>
<td>100</td>
<td>11.94 ± 0.74</td>
</tr>
<tr>
<td>40</td>
<td>6.93 ± 1.08</td>
<td>100</td>
<td>12.66 ± 1.29</td>
</tr>
<tr>
<td>50</td>
<td>6.48 ± 0.62</td>
<td>64.6</td>
<td>3.04 ± 0.29</td>
</tr>
<tr>
<td>60</td>
<td>2.22 ± 0.27</td>
<td>32.0</td>
<td>1.28 ± 0.02</td>
</tr>
<tr>
<td>70</td>
<td>0.68 ± 0.04</td>
<td>9.8</td>
<td>0.98 ± 0.10</td>
</tr>
<tr>
<td>80</td>
<td>0.64 ± 0.10</td>
<td>9.2</td>
<td>0.86 ± 0.09</td>
</tr>
</tbody>
</table>

1 Mean of six trials ± standard deviation
2 Percent of control values
a,b,c,...—Different superscripts mean differences (of at least P < .05) in a column.

40°C did not modify (P > .05) the amount of nitrogen in any fraction in either the leg or breast muscle. The apparent increase of myofibrillar solubility in both types of muscles was not significant (P > .05).

After heating to 50°C there was a reduction (P < .01) in the amount of soluble sarcoplasmic proteins in both types of muscle. The reduced solubility was more apparent in the myofibrillar fraction with precipitation of 78% of the originally soluble proteins in breast muscle (P < .001) and 74.5% in leg muscle (P < .001). There was no significant difference in NPN (non-protein nitrogen) between the control and samples heated to 50°C.

Heating to 60°C produced a reduction (P < .01) in solubility of sarcoplasmic proteins, with only 20% of the original amount of this fraction remaining in solution in the breast and 32% in the leg. Approximately 90% of the myofibrillar proteins were denatured in both types of meat at this temperature—an increase (P < .01) compared to the 50°C heat treatment. The quantity of NPN fraction was altered but not significantly (P > .05) by this increase in treatment temperature.

Sarcoplasmic and myofibrillar protein solubility was reduced by heating to 70°C (P < .01) compared to 60°C for both types of muscle. The 80°C heat treatment produced only a slight [three of four instances, non-significant (P > .05)] reduction in solubility of sarcoplasmic and myofibrillar proteins compared to 70°C.

The NPN of both types of muscle was not altered (P > .05) by either the 70- or 80°C treatment.

The relationships of pH and treatment temperatures are shown in Table 3. The pH values were lower (P < .01) in the control breast muscle than in the leg. The pH gradually increased in both types of muscle as treatment temperature was elevated. This increase was 0.31 pH unit higher (P < .05) than the control in the breast muscle and 0.19 pH unit higher (P < .05) in the leg muscle when the tissue was heated to 80°C. The rise of pH with temperature became significantly (P < .05) different from the control at the 70°C treatment in both types of muscle. The significant (P < .05) differences between pH value of the breast and leg muscle, noted in the control, were maintained after heating to 40, 50, 60, and 70°C, but after heating to 80°C the leg and breast muscles were not significantly (P > .05) different.

DISCUSSION

The quantity of materials found in the nitrogen fractions of the controls are within the normal ranges found in avian muscle. The existence of higher quantities of sarcoplasmic proteins, myofibrillar proteins, NPN, and lower pH values in the breast muscle compared to the leg are also typical for this type of tissue (12). The protein fractions are higher in breast muscle because the protein is higher in this tissue.

The stability of the sarcoplasmic fraction after heating to 40°C agrees with our previous study (13) in which the sarcoplasmic proteins were heated in solution rather than as intact muscle. Parkes and May (16) also reported stability of the sarcoplasmic protein fraction heated in solution to 37°C for 3 h.

After heating to 50°C a significant denaturation of proteins occurred with the myofibrillar fraction demonstrating greater heat sensitivity than the sarcoplasmic fraction. This agrees with Hamm and Deatherage (5) who stated that salt-soluble proteins were more sensitive to heat than water-soluble proteins. The large denaturing effect on the myofibrillar fraction from heating the tissue to 60°C agrees with Locker (14) and Bendall (3). Locker (14) indicated that beef actomyosin was completely denatured after heating to 55°C and Bendall (3) suggested that most of the myofibrillar proteins were precipitated after heating to 62°C.

Increasing the treatment temperature to 70°C denatured 90% of the protein in both fractions for both types of muscle and an additional increase to 80°C did not have an appreciable effect. Huber and Stadelman (8) heated turkey breast muscle tissue to 85°C and also

TABLE 3. Values of pH in breast and leg avian muscle after treatment at different temperatures for 30 minutes

<table>
<thead>
<tr>
<th>Temperature of treatment (°C)</th>
<th>Breast muscle</th>
<th>Leg muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actual value ± standard deviation</td>
<td>Increase</td>
<td>Increase</td>
</tr>
<tr>
<td>Control</td>
<td>5.65 ± 0.22a</td>
<td>5.97 ± 0.15d</td>
</tr>
<tr>
<td>40</td>
<td>5.67 ± 0.24a,b</td>
<td>5.98 ± 0.14d</td>
</tr>
<tr>
<td>50</td>
<td>5.78 ± 0.22a,b,c</td>
<td>6.01 ± 0.13d</td>
</tr>
<tr>
<td>60</td>
<td>5.86 ± 0.24a,b,c</td>
<td>6.11 ± 0.13d,e</td>
</tr>
<tr>
<td>70</td>
<td>5.92 ± 0.24a,b,c</td>
<td>6.15 ± 0.13d,e</td>
</tr>
<tr>
<td>80</td>
<td>5.96 ± 0.24a,b,c</td>
<td>6.16 ± 0.13d,e</td>
</tr>
</tbody>
</table>

1 Mean ± standard deviation of six determinations
2 Difference with respect to control value
a,b,c,d,e—Different superscripts mean differences (of at least P < .05) in a column.
reduced soluble myofibrillar protein to less than 10% of the original values.

The protein solubility results of this research are slightly different from those presented by Acton (1). After heating poultry meat loaves to 43 C, Acton (1) reported a reduction of 25.9% in the solubility of the myofibrillar proteins. This compares with no significant changes by heating to 40 C in our study. In Acton's paper (1) this figure increased only to 60.9% after heating to 55 C as compared with 78% at 50 C in this report. When the temperature was raised to 70 C in Acton's (1) research, a reduction of 89% was achieved which was similar to the 92.5% achieved in this study. In both of these research works, increasing the temperature above 70 C did not yield significant changes. These differences noted may be explained by the differences in temperatures, sample size, methods of heating, and protein extraction procedures.

The stability to heat of the NPN content of meat agrees with the report of Bendall (2) who found no significant changes in the NPN fraction of beef tissue exposed to heating treatments. The non-significant reduction in the NPN fraction encountered in this study after heating might be attributed to the "trapping" of low molecular weight compounds by the precipitated proteins. Hamm and Deatherage (5) found a slight increase in the soluble NPN fraction upon heating to 80 C, but did not find an increase in the buffer capacity of this fraction. These authors (5) concluded that heating to 80 C did not increase the amount of low molecular weight nitrogen compounds which have a buffer capacity in the acid range of pH.

The increase in pH observed in chicken muscle with heating is similar to those previously observed in pork tissue (10), in rabbit muscle (17), in beef tissue (5), and in turkey breast muscle (1, 4).

The difference in pH between leg and breast muscle tissue at the lower treatment temperatures likely is due to the initially lower value present in the breast tissue. This lower pH is closer to the isoelectric point of the most important muscle proteins. Hamm and Deatherage (5) indicated that the increase in pH produced by heating depended on the initial pH of the meat and that the largest changes in pH occurred in tissue when the initial pH was close to the isoelectric point of the actomyosin system.

REFERENCES

Fish Peptones for Microbial Media Developed from Red Hake and from a Fishery By-Product

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ABSTRACT

Suitability of two soluble, heat stable fish peptones derived from Red Hake (Urophycis chuss.) and Atlantic menhaden (Brevoortia tyrannus), was explored as peptone constituents of microbiological media. Both processes for producing these fish peptones are described. Comparative growth curves of various microorganisms in peptone broth cultures were made by turbidity measurements. Recovery of fastidious lactobacilli and other microorganisms was compared on peptone agar media. The soluble fish peptones were usually equal to, if not better than, the commercially available peptones and beef extract. Fish represent a potentially new source of inexpensive and useful peptones for laboratory microbiological media and for commercial fermentations.

Use of soluble fish products or peptones (protein extracts and hydrolysates) in media for cultivation of microorganisms has been limited. There have been some reports in the literature about peptone-like preparations from fish (1, 9, 10, 11, 13, 14). The potential for new products for microbial culture from either fishery by-products or under-utilized species of fish presents a challenge to explore this area.

Previous studies at our facility included development and utilization of soluble fish peptones for the growth of microorganisms produced from fishery products and by-products, as described by Green et al. (3, 4, 5) and Hale (6, 7, 8). These fish peptones could serve as inexpensive peptones for microbial culture, and might prove to be advantageous in microbial growth or product formation by some species of microbes. This research report describes production of peptones from Atlantic menhaden (Brevoortia tyrannus) fish solubles, a fishery by-product, and from Red Hake (Urophycis chuss) and compares these peptones to commercially available peptones as to their ability to support microbial growth.

MATERIALS AND METHODS

Fish peptones

Two fish peptones were experimentally prepared.

(a) Soluble fish extract (SFE) was prepared indirectly from menhaden "stockwater." Stockwater, a by-product of the fish meal and oil industry, is the aqueous portion remaining after free oil has been removed by centrifugation of the press liquor and contains fish proteins, nonprotein nitrogen, minerals, residual lipid, and other materials. Stickwater is usually acidified with H₂SO₄ to a pH of approximately 5 or less and evaporated to a 1:1 (water-solids) slurry called "fish solubles." The characteristics of fish solubles, often used as a feed supplement, have been described (12).

The authors prepared SFE from fish solubles by blending with distilled water and hexane in the following ratio: 1:5:3 (wt/vol/vol). The hexane layer containing most of the fish oils was removed by siphon. The remaining dilute slurry was poured into 500-ml centrifuge cups and clarified by centrifugation at approximately 5 or less and evaporated to a 1:1 (water-solids) slurry called "fish solubles." The characteristics of fish solubles, often used as a feed supplement, have been described (12).

The authors prepared SFE from fish solubles by blending with distilled water and hexane in the following ratio: 1:5:3 (wt/vol/vol). The hexane layer containing most of the fish oils was removed by siphon. The remaining dilute slurry was poured into 500-ml centrifuge cups and clarified by centrifugation at 2,000 × g for 10-20 min to remove fines and other particulate matter. The clear supernatant fluid was collected, filtered on Whatman No. 1 paper, and freeze-dried for further use as SFE. The production of SFE is reminiscent of the old method of making infusion broth of which beef extract is an example.

(b) Soluble hake autolyte (SHA) was prepared from whole hake by autolytic digestion of the comminuted fish at 50-55 C followed by clarification and spray drying (7). We further purified SHA for media preparation by boiling a solution of SHA for 5 min, cooling, and removing a light haze by filtration through a Whatman No. 1 filter paper. Thus, SHA represents an enzyme hydrolysate and in this respect resembles other protein hydrolysates such as Bacto-Peptone (Difco).

Commercially available peptones and media

Other peptones used in these experiments were: Bacto-Peptone, Myosate (BBL), Trypticase (BBL), Phytone (BBL), and Beef Extract (Difco).

Prepared media formulations used in these experiments were: Nutrient Broth (Difco) Agar prepared from the commercial broth formulation by addition of 0.1% dextrose, or 0.3% dextrose in the case of lactobacilli tests, and 1.5% agar (Difco); Lactobacilli MRS Broth (Difco); Lactobacilli MRS Agar, the latter prepared from the broth formulation by the addition of 1.5% agar; Standard Methods Agar, SMA, (BBL); and Trypto-case-Soy, T-Soy, Broth (BBL). Media were prepared according to manufacturers' directions.

Peptone broth and agar media

Peptone broth media were prepared by the addition on a wt/vol basis of 0.25% each of anhydrous KH₂PO₄ and anhydrous Na₂HPO₄, and 0.2% dextrose to a 0.5% peptone or extract dissolved in distilled water. Dextrose (0.2%) was also added to the Nutrient Broth. The pH was adjusted to 7.0 ± 0.1 with either 1 N NaOH or 1 N H₃PO₄. 60-ml portions were placed in 250-ml Erlenmeyer flasks and autoclaved for 15 min at 121 C.

Peptone agar media were prepared by the addition on a wt/vol basis of 0.25% each of anhydrous KH₂PO₄ and anhydrous Na₂HPO₄, 0.1% dextrose 0.3% dextrose in the case of lactobacilli tests), and 1.5% agar to a 0.5% peptone or extract dissolved in distilled water. The pH was adjusted to 7.0 ± 0.1 (or 6.5 ± 0.1 in the case of lactobacilli tests) by
addition of either 1 M NaOH or 1 M H₃PO₄. Agar media were autoclaved for 15 min at 121°C.

Cultures
Lactobacillus casei ATCC 7469; Lactobacillus plantarum ATCC 8014; Pediococcus cerevisiae ATCC 8042; Pseudomonas aeruginosa ATCC 7700; Streptococcus lactis, Escherichia coli, and Staphylococcus aureus from the University of Maryland Microbiology Department; Proteus sp. and an Arizona group species from our laboratory collection; and Candida lipolytica 60-26 from the University of California, Davis, were used.

Culture and inoculum preparation
Lactobacillus species were maintained on MRS broth. All other species were maintained on T-Soy broth. Bacterial species to be tested were grown for 18-24 h in broth culture before experiments. All species were incubated at 35°C except P. cerevisiae and C. lipolytica which were incubated at 30°C.

Inocula for culture flasks used in turbidimetric studies were prepared by first washing cells from an 18-24-h culture by centrifugation 1,000 x g and resuspension in sterile distilled water. Cell suspensions of approximately 0.5-0.6 absorption at 625 nm (Spectronic-20, B&L) were prepared. Inocula for comparative total plate counts were serially diluted in distilled water blanks.

Growth curves by turbidity measurements
For a given experiment, three or four culture flasks of broth media were each inoculated with 1 ml of the washed cell suspension of a given species and then placed on a reciprocating shaker, set for 60 strokes/min housed in a BOD incubator set for 30 or 35°C. Periodically, 2.5 ml of culture were aseptically withdrawn from each shake culture, placed in a cuvet, and read. For each medium, the average of three or four readings per time was recorded.

Plating techniques
The following procedure applied to all plating techniques. For each species tested, either eight or nine different media were employed and six petri dishes per dilution for each medium were prepared. From the same 99-ml dilution blank, 1-ml aliquots were used to inoculate all plates of the same dilution for all of the different media being compared.

Statistical comparison
The Student t was used to compare the total plate count results of the commercially available and fish peptone agar media against a standard medium.

RESULTS
Turbidity measurements were made to determine the growth response of various microbial species in different peptone broths. In the initial attempts, static cultures resulted in slow and erratic outgrowth. Use of a slow shaker method resulted in faster and more uniform growth response among each of the three or four culture flasks in a set. Both SHA and SFE were compared to Bacto-Peptone and Beef Extract. We considered these latter two commercial peptones to be generally similar in preparation to the laboratory-produced fish peptones. Figures 1-4 show typical results. Growth responses of various species inoculated into SHA or SFE broths were usually equal to or better than those in Peptone broth and always superior to those in Beef Extract broth.

Growth curves by turbidity measurements
Figure 1. Growth responses of L. casei in different peptones. Shake culture method at 35°C was used.

The original objective and experimental design was to compare buffered peptone agars, void of other supplements, to a reference medium such as SMA or MRS Agar. Initial observations (data not shown) revealed that peptone agar media without sugar had poor recoveries often one or two log cycles below either SMA or MRS Agar. Therefore, for most species to be tested,
0.1% dextrose was added, the same concentration as in the SMA formulation, to all peptone agars. In the case of lactobacillus tests, we found it necessary to add at least 0.3% dextrose to get recoveries comparable to MRS Agar which contains 2% dextrose in its formulation.

The purpose of using an acceptable recovery medium such as SMA or MRS Agar was to have a point of reference which could be used from experiment to experiment. The commercially or experimentally produced peptones were statistically compared to this point of reference. SMA was the point of reference for all species except fastidious lactobacillus species which were compared to MRS Agar. Results of these comparisons are shown on Table 1.

The lack of colony formation (NG) of *L. plantarum* and the formation of microcolonies (MC) of *L. casei* in Beef Extract Agar compares with the results of the turbidity growth curves (Fig. 1 and 2) which show little growth in Beef Extract Broth. The microcolonies of *L. casei* could hardly be seen by the translucent light from a Quebec counter. Using indirect lighting, an observer could barely see "pin prick" sized colonies with the naked eye. Use of a biocular microscope at ×30 and ×80 magnification revealed that these small colonies contained an estimated 100-200 cells. A microscopic search
GROWTH CURVES
P. aeruginosa ATCC 7700

- - PEPTONE
○ ○ BEEF EXTRACT
× × SFE
△ △ SHA

Absorbance at 625 nm

2.0
1.0
0.0

0.6
0.4
0.2
0.1

0.06
0.04
0.02

0.08
0.06
0.04
0.02

0.1
0.2
0.4
0.6

Figure 4. Growth responses of P. aeruginosa in different peptones. Shake culture method at 35 C was used.

GROWTH CURVES
L. casei ATCC 7469

○ ○ NUTRIENT BROTH
× × SFE
△ △ SHA

Absorbance at 625 nm

0.6
0.4
0.2
0.1

0.06
0.04
0.02

0.08
0.06
0.04
0.02

0.1
0.2
0.4
0.6

Figure 5. Comparison of growth responses of L. casei in SFE, SHA and in Nutrient Broth. Shake culture method at 35 C was used.

responses for the organisms tested. In addition to the preparation of SHA and SFE described in this report, Hale (6, 7, 8) also described a variety of methods that can be used to prepare protein hydrolysates from different species of fish. This technology resembles the established hydrolysate processes used to produce peptones for use in microbiological media (2). Hale (8) estimates that the average cost of producing fish hydrolysates from whole fish would be 38.4 cents per pound based on 1972 production and supply cost information which is considerably less than the wholesale prices (1972) of other peptones. Chemical analysis and some other

DISCUSSION

These experiments indicate that both SHA and SFE can serve as the peptone, or nitrogen component, of microbiological media with good to excellent growth.

(×30 and ×80) of L. plantarum Beef Extract Agar plates revealed no visible colonies. Comparisons between the growth responses in broth (Fig. 1-6) and recoveries on corresponding peptone agar plates (Table 1) indicate other similar correlations. In general, growth responses of SHA and SFE were favorable or better when compared to most commercial peptones used in these experiments.
Figure 6. Comparisons of growth responses of various species in SHA and/or SFE and in Nutrient Broth. Shake culture method was used at 35°C except for C. lipolytica at 30°C.

Table 1. Recovery of different microorganisms using various agar media

<table>
<thead>
<tr>
<th>Test species</th>
<th>Standard media</th>
<th>Agar media use in recovery studies (Total plate counts)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dilution factor</td>
<td>MRS</td>
</tr>
<tr>
<td>L. casei</td>
<td>10^9</td>
<td>5.79</td>
</tr>
<tr>
<td>L. plantarum</td>
<td>10^9</td>
<td>3.67</td>
</tr>
<tr>
<td>P. cerevisiae</td>
<td>10^9</td>
<td>7.12</td>
</tr>
<tr>
<td>S. lactis</td>
<td>10^9</td>
<td>1.21</td>
</tr>
<tr>
<td>Arizona Sp.</td>
<td>10^9</td>
<td>1.88</td>
</tr>
<tr>
<td>E. coli</td>
<td>10^9</td>
<td>3.19</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>10^9</td>
<td>3.65</td>
</tr>
<tr>
<td>S. aureus</td>
<td>10^9</td>
<td>10.1</td>
</tr>
<tr>
<td>Proteus sp.</td>
<td>10^9</td>
<td>3.17</td>
</tr>
</tbody>
</table>

^aSignificant difference from standard P < 0.05.

^a*Very significant P < 0.01.

^aMC Microcolonies, barely visible to the naked eye (not easily counted).

^aNG No visible growth.

Characteristics of SHA and SFE, in comparison with other fish peptones, have been given in references 4 and 5.

In a previous report (4), we had described observed differences in colony characteristics (size, shape and pigmentation) of a given species growing on different peptone agar media. In this report, we had not measured the average colony sizes, but differences were apparent for some species growing on these simple peptone agars. The most obvious was no growth or microcolony formation by two species of lactobacilli growing on Beef Extract Agar. Perhaps the microbiologists' concept of "typical" colony characteristics for a given species
should be re-examined in terms of peptones used or nutrients available in the medium.

Experiments in previous reports (4, 5) also demonstrated that fish peptones could support good to excellent microbial growth. We have not necessarily demonstrated any special qualities for the isolation, growth or product formation of any particular microbe. However, data indicate that fish peptones can support the growth of fastidious bacteria such as the lactobacilli.

ACKNOWLEDGMENT

The supply of SHA by Malcolm B. Hale of our facility is greatly appreciated. We are indebted to Dennis J. Flynn for his assistance in performing these experiments.

REFERENCES

Isolation and Toxicity of Molds from Foods Stored in Homes

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(Received for publication August 4, 1976)

ABSTRACT

Penicillia (49% of 155 isolates) and aspergilli (38%) were the predominant molds isolated from food stored in private homes. Samples were taken from refrigerated and non-refrigerated food, and from refrigerator surfaces. Aflatoxin (9 isolates), kojic acid (3), ochratoxin A (3), penicillic acid (1), and patulin (1) were detected when culture extracts of isolates were screened for the presence of toxic mold metabolites.

Aflatoxigenic molds were first reported in 1963 as present in U.S. agricultural commodities used for human food (5). Further investigations demonstrated that aflatoxins and aflatoxigenic molds, primarily of the Aspergillus flavus group, were widely distributed in nature. A major concern was to evaluate the potential for aflatoxin production in food contaminated with aflatoxigenic molds and to determine the frequency and amounts of aflatoxin occurring naturally in food material. Improved methods of harvesting, drying, storing, and processing raw materials, and development of methods to detect aflatoxins in foodstuffs have reduced the likelihood that in the United States aflatoxin sufficient to cause acute or chronic effects might be consumed from the commercial food supply.

Mold contamination of food stored in the home and the possibility that these molds might produce mycotoxins, especially aflatoxin, have received only limited attention. Van Walbeek et al. (20) detected toxigenic molds in several foods involved in consumer complaints and human illnesses. From the 12 samples examined, seven molds were obtained that produced aflatoxins. This report describes the collection and partial identification of molds from home-stored foods and results of tests done to assess the potential of these molds to produce mycotoxins. A special effort was made to detect aflatoxigenic molds.

MATERIALS AND METHODS

Sources of samples

Samples were obtained from households participating in a nutrition education program directed by the University of Wisconsin Extension for a group of families whose economic and social history made them eligible for special assistance in food preparation and home management. Project assistants, who conducted home management training, had access to food stored in the home. The project included rural, low-income households in the Portage and Madison, Wisconsin, areas; and rural households of undetermined economic status from the Monticello, Wisconsin, area. Samples were obtained from 66 households.

Collection of samples

Training sessions were held for people aiding in the collection of sample collection was described. Participants were instructed to examine refrigerated and non-refrigerated food for molds and to place portions of suspected moldy foods in sterile polyethylene bags (Nasco, Fort Atkinson, Wis.). Samples from refrigerator surfaces (vegetable storage areas, door gaskets, light bulb fixtures, and soiled racks) were collected by rubbing the surface with a sterile, moist calcium alginate swab (Wilson Diagnostics, Inc., Glenwood, Ill.). The swab was then returned to a polyethylene bag for storage until contents were examined. Identification of the sample, its location in the home, cleanliness in the food storage area, and other remarks were recorded. Samples were refrigerated after collecting.

After the first samples had been received, additional meetings with extension staff members were held to answer questions and record information about food and food storage habits.

Isolation and purification of isolates

Portions of food samples were streaked in a crosshatched manner onto the surface of Potato Dextrose Agar, PDA (Difco), in plastic petri plates, and onto a glucose-salts silica gel (GS) medium in glass petri plates since plastic plates interfered with detection of fluorescence. In addition to tartaric acid and an alkaline silicate solution included to form a gel, the GS medium contained, per liter, 100 g of glucose, 3 g of (NH₄)₂SO₄, 0.5 g of KCl, 0.35 g of MgSO₄ • 7H₂O, 0.008 g of FeCl₃ • 4H₂O, 1.5 g of KH₂PO₄, and 0.004 g of ZnSO₄ • 7H₂O. The method for preparing this medium has been described (19). GS medium was developed to enhance the blue fluorescence associated with aflatoxin-producing molds, and to permit easy extraction of toxin from the medium.

Samples of spices and seasonings were sprinkled onto the GS medium with the aid of a sterile spatula. Swabs used to collect surface samples were dissolved in a sterile citric acid solution according to the manufacturer’s instructions, and a portion of that solution was streaked with an inoculating loop onto the surface of GS medium. Swabs were also incubated in the citric acid solution, and molds that developed on the surface of the solution were transferred to PDA or to GS medium. Plates were incubated at 25°C in a humidified chamber. After 3, 4, and 5 days of incubation, plates of GS medium were briefly placed right side up in a viewing cabinet (Ultra-Violet Products, Inc., San Gabriel, Calif.) and examined for fluorescence under long-wave ultraviolet (u.v.) light (320-380 nm, peak at 365 nm). If a fluorescent area appeared around a mold colony and other mold colonies threatened to overgrow it, then a portion of the colony responsible for the fluorescence was removed to fresh GS medium and incubated a second time. After 5 days of incubation, portions of all colonies that were visibly different were transferred to Mycological Agar (Difco) to obtain pure colonies. Stock cultures were prepared from the purified colonies. Mold colonies and surrounding medium that fluoresced under u.v. light were also excised with a spatula and transferred to 50-mm watch-glasses. The excised medium was dried, extracted, and analyzed for...
aflatoxin as described by Torrey and Marth (19).

Identification of isolates

Molds were identified according to descriptions and methods presented by Smith (16) and Raper and Fennell (10).

Screening isolates for toxic substances

Extracts of isolates were examined to detect biologically active substances. Extracts were prepared from cultures grown on silica gels containing Czapek Dox Broth (Difco) supplemented with 0.5% yeast extract and an additional 2% sucrose. Twenty ml of medium were prepared by mixing 10 ml of double strength broth and tartaric acid sufficient to gel the medium at pH 6.0, with 10 ml of alkaline silicate solution. After mixing, the medium was poured into glass petri plates and allowed to solidify. Preparing, incubating, drying, and extracting gels were done as described by Torrey and Marth (19), and these methods were adapted to this study in the following manner. Spores were spread over the entire medium surface, and plates were incubated at 28°C for 10 days. Once dried, gels were extracted with 40-50 ml of chloroform: methanol (7:3, vol/vol), followed by a second extraction of the same dried gel with hexane (b.p. 60-68°C). The extracts were concentrated, reduced to near dryness under N2 gas on a sand bath, and 1 ml of olive oil was added. Olive oil and the extract were mixed, and solvent was removed by evaporation under reduced pressure. Three mice were each given one intraperitoneal injection of 0.2 ml of oil containing the extract and observed for 4 days to detect any visible acute toxic response.

Screening for specific mycotoxins was done using the TLC method of Scott et al. (14). In addition to aflatoxin, standards were available for patulin, penicillic acid, and ochratoxin A; and these toxins were considered in the screening process. Kojic acid was presumptively detected by Rf value and color reaction with a reagent containing FeCl3.

RESULTS AND DISCUSSION

Members of the Extension staff reported that attitudes of program participants varied regarding moldy food and the value of refrigeration. An instance was reported in which a housekeeper believed it was not necessary to operate her refrigerator continuously. Some housewives expressed indignation when the possibility of moldy food in their households was considered, saying that molds represented filth and undesirable conditions. Many considered removal of food surfaces that contained mold, leaving the underlying food to be eaten, an acceptable practice.

TABLE 1. Genera of molds isolated from refrigerated food and refrigerator surfaces

<table>
<thead>
<tr>
<th>Source</th>
<th>Number of Isolates</th>
<th>Aspergillus</th>
<th>Penicillium</th>
<th>Mucor</th>
<th>Cladosporium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food</td>
<td>Samples</td>
<td>isolated</td>
<td>isolated</td>
<td>isolated</td>
<td>isolated</td>
</tr>
<tr>
<td>Unprocessed fruits</td>
<td>23</td>
<td>19</td>
<td>2</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>and vegetablesb</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Home prepared:leftovers</td>
<td>18</td>
<td>11</td>
<td>1</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Meat/meat products</td>
<td>9</td>
<td>7</td>
<td>0</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Dairy/cheese</td>
<td>30</td>
<td>25</td>
<td>2</td>
<td>21</td>
<td>1</td>
</tr>
<tr>
<td>Jams/jellies</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bakery</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Surfaces</td>
<td>21</td>
<td>11</td>
<td>4</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

aAdditional isolate was Rhizopus, 1.
bAdditional isolate was unidentified, 1.
cAdditional isolate was Alternaria, 1.
dAdditional isolates were Rhizopus, 1; Sporendonema, 1.

Refrigerated foods and refrigerator surfaces

The genera of molds isolated from refrigerated foods and from refrigerator surfaces, and the category of foods from which they were obtained, are listed in Table 1. Refrigerated foods were generally sampled only when visibly moldy, and therefore the frequency of samples in a given food category reflects the frequency with which that food was observed to be moldy. Dairy products, unprocessed fruits and vegetables, and home-prepared food were the refrigerated materials most frequently sampled and provided most of the mold isolates from refrigerated food. Penicillia were the most frequently isolated molds from almost all food categories. These findings probably result from the ubiquity of molds in this genus, their ability to grow on diverse substrates, and the ability of many species of Penicillium to grow at refrigeration temperatures (11). Evidence of these attributes is provided in a study by Gunderson (8) in which 22 of 52 species of molds isolated from frozen convenience foods were species of Penicillium able to grow at 5°C and below. Molds representing five genera were detected on refrigerator surfaces (Table 1).

Non-refrigerated foods

Spices were the most frequently sampled non-refrigerated food (Table 2). The spices were collected by sampling convenience foods were species of Aspergillus and Penicillium. Penicillia occurred most frequently.

TABLE 2. Genera of molds isolated from non-refrigerated food

<table>
<thead>
<tr>
<th>Food</th>
<th>Number of Isolates</th>
<th>Aspergillus</th>
<th>Penicillium</th>
<th>Mucor</th>
<th>Cladosporium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unprocessed fruits</td>
<td>11</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>and vegetables</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Home prepared/leftovers</td>
<td>8</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Meat/meat products</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Jams/jellies</td>
<td>4</td>
<td>6</td>
<td>2</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Spices/seasoningsa</td>
<td>88</td>
<td>49</td>
<td>33</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Bakery</td>
<td>15</td>
<td>10</td>
<td>3</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>5</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

aAdditional isolates were Mucor, 4; Rhizopus, 3; Scopulariopsis, 1; Paecilomyces, 1; unidentified, 1.

without regard to appearance, while in most instances other non-refrigerated food samples were collected because of the presence of visible mold or because of composition, handling, or storage location. Non-refrigerated food, except spices, yielded only species of Aspergillus and Penicillium. Penicillia occurred most frequently.

Our data indicate that spices are contaminated with a variety of molds, but that aspergilli predominate. These data agree with results of Christensen et al. (9), who found that various spices were contaminated with an assortment of molds, predominantly Aspergillus spp., including some that produced substances lethal to ducklings.

Data in Table 3 indicate that nearly one-half of the isolates from all sources were species of Penicillium, and approximately one-third were Aspergillus spp. The remaining isolates were identified as belonging to seven other genera, and two isolates could not be identified.

Aflatoxin-producing isolates

About 200 of the 242 samples streaked onto the GS
that foods were contaminated during home storage, and
the extracts for aflatoxins by TLC were considered
the intitial contamination of food by aflatoxigenic molds
contain aflatoxigenic molds (fable 5).

took place before the food entered the home.
also account for seven of the nine samples found to
necessary to confirm the presence of aflatoxins.
from the above-listed foods comprised a large portion of
especially cheese (7,
commercial preparation or in marketing channels, and
fluorescent cultures were aspergilli and penicillia. Few of
produced substances that fluoresced under u.v. light
in other parts of the home environment. This suggestion
may result when food is eaten after visible mold is
and Marth (15), indicates the potential hazard that
remained after visible mold. Diffusion of mycotoxins into a substrate beneath the
mycelium, as described for aflatoxin by Frank (6) and
that conditions in the household favorable for mold
growth resulted in spoilage.

**Toxic extracts**

In addition to the primary screening done for aflatoxin-producing molds, extracts from 58 representa-
tive isolates grown on modified Czapek Dox medium were
examined for lethality to mice. The amount of material
obtained by extracting cultures with chloroform:
methanol, and hexane varied from 8 to 192 mg per petri
plate. Two extracts killed mice. Analysis of the lethal
extracts by TLC failed to reveal known mycotoxins for
which standards were available (patulin, penicillic acid,
ochratoxin A, and aflatoxins B₁, B₂, G₁, G₂). In some
instances, sufficient quantities of toxic material may not
have been present to kill mice. Although mice are
considered resistant to the toxic effects of some
mycotoxins (22), Saito and coworkers (12, 13) reported
that mold isolates from foodstuffs could be successfully
screened using mice.

Extracts from 87 isolates were examined by TLC.
Three contained kojic acid; three, ochratoxin A; and one
each contained patulin and penicillic acid (Table 6). No

**TABLE 3. Identity of molds isolated from food samples and refriger-
ator surfaces**

<table>
<thead>
<tr>
<th>Genus</th>
<th>Number of Isolates</th>
<th>Number of Fluorescent Cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillium</td>
<td>76</td>
<td>18</td>
</tr>
<tr>
<td>Aspergillus</td>
<td>54</td>
<td>18</td>
</tr>
<tr>
<td>Mucor</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Cladosporium</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Rhizopus</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Scopulariopsis</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Sporendonema</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Paecilomyces</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Alternaria</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Unidentified</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

aTotal isolates = 155.
bTotal fluorescent cultures = 40.

medium were also streaked onto PDA. Molds growing
on PDA also grew on GS medium. Of the 155 isolates, 40
produced substances that fluoresced under u.v. light
when grown on GS medium (Table 3). Most of the
fluorescent cultures were aspergilli and penicillia. Few of
the non-aflatoxigenic isolates produced fluorescence that
resembled that of the aflatoxins. However, extraction
from all fluorescent areas of GS medium and analysis of
the extracts for aflatoxins by TLC were considered
necessary to confirm the presence of aflatoxins.

Tables 4 and 5 indicate the sources of aflatoxin-
producing isolates. Six of the nine cultures that were
aflatoxigenic were from non-refrigerated food, with three
being recovered from bakery goods, two from spices, and
one from home-canned pumpkin. Refrigerated samples
that contained aflatoxin-producing isolates included
cheese, cottage cheese, and a cake of compressed yeast.

Bakery products (17), spices (3), and dairy products,
especially cheese (7, 20) have been examined during
commercial preparation or in marketing channels, and
aflatoxigenic molds have been isolated. Representatives
from the above-listed foods comprised a large portion of
the samples collected and examined in this study, and
also account for seven of the nine samples found to
contain aflatoxigenic molds (Table 5).

It was not possible in this study to determine whether
the intitial contamination of food by aflatoxigenic molds
occurred before the food entered the home. It is possible
that foods were contaminated during home storage, and

**TABLE 4. Sources of isolates including those producing aflatoxin**

<table>
<thead>
<tr>
<th>Source</th>
<th>Samples</th>
<th>Isolates</th>
<th>Isolates producing aflatoxin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refrigerator surfaces</td>
<td>21</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>Refrigerated food</td>
<td>87</td>
<td>68</td>
<td>3</td>
</tr>
<tr>
<td>Non-refrigerated food</td>
<td>134</td>
<td>76</td>
<td>6</td>
</tr>
</tbody>
</table>

**TABLE 5. Samples positive for molds producing aflatoxin**

<table>
<thead>
<tr>
<th>Food</th>
<th>Number of samples examined</th>
<th>Isolates producing aflatoxin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refrigerated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cheese</td>
<td>30</td>
<td>2</td>
</tr>
<tr>
<td>Yeast</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Non-refrigerated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bakery</td>
<td>15</td>
<td>3</td>
</tr>
<tr>
<td>Spices</td>
<td>88</td>
<td>2</td>
</tr>
<tr>
<td>Canned pumpkin</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

aAflatoxin isolates not tested further.

**TABLE 6. Specific toxina detected by screening culture extracts**

<table>
<thead>
<tr>
<th>Toxin detected</th>
<th>Source of isolate</th>
<th>Identity of isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ochratoxin A</td>
<td>Meat, refrigerated</td>
<td>Penicillium</td>
</tr>
<tr>
<td>Patulin</td>
<td>Refrigerator surface</td>
<td>Aspergillus</td>
</tr>
<tr>
<td>Penicillium</td>
<td>Refrigerator surface</td>
<td>Penicillium</td>
</tr>
<tr>
<td>Kojic acid</td>
<td>Butternut squash, unprocessed, not refrigerated</td>
<td>Aspergillus</td>
</tr>
<tr>
<td>Spice</td>
<td>Spice</td>
<td>Aspergillus</td>
</tr>
</tbody>
</table>

aAflatoxin isolates not tested further.

extract contained more than one detectable toxin.

Presence of toxigenic molds on home-stored foodstuffs
indicates a potential hazard to humans, the extent of
which is difficult to evaluate. Some food samples
collected in this study were obviously remnants and their
presence indicated poor management of food storage
areas. These food residues would probably not have been
eaten. Other samples collected were portions of food that
might have been eaten after removal of visible mold.
Presence of toxigenic molds on home-stored foodstuffs
indicates a potential hazard to humans, the extent of
which is difficult to evaluate. Some food samples
collected in this study were obviously remnants and their
presence indicated poor management of food storage
areas. These food residues would probably not have been
eaten. Other samples collected were portions of food that
might have been eaten after removal of visible mold. Diffusion of mycotoxins into a substrate beneath the
mycelium, as described for aflatoxin by Frank (6) and
Shih and Marth (15), indicates the potential hazard that
may result when food is eaten after visible mold is
removed. Van Walbeek et al. (21) suggest that aflatoxi-
genic fungi may grow on refrigerated food without
producing spores and the characteristic spore color;
thus, mold growth might be overlooked entirely. A flavus
isolates reportedly produce detectable aflatoxin in less
than one week when incubated at 7.5 C as broth and agar
cultures (27).

The ease with which mold spores are dispersed
suggests that molds contaminating foods are also present
in other parts of the home environment. This suggestion
is reinforced by the recovery of *A. flavus* and other molds from bedding materials (4), dust, and air in dwellings (18).

The possible relationship between occurrence in the home of mold species known to produce toxins and cases of neoplastic diseases including leukemia and other cancers has been investigated by Aleksandrowicz and Smyk (2). *A. flavus* and other molds with known oncogenic properties for animals were found more frequently in dwellings, food, and wall surfaces associated with patients suffering from neoplastic diseases, such as proliferation of the lymphoreticular system, than were identified in dwellings of control families not affected by the diseases (P<0.05). Positive serological tests for antigens of *A. flavus* were observed in tumor patients from homes found contaminated with *A. flavus*, while only weak or negative serological reactions were obtained from healthy persons (I).

Results of these investigations by Aleksandrowicz and associates (I, 2) offer strong circumstantial evidence for the relationship just described, especially with respect to *A. flavus*. The potential hazard presented by molds and by the toxins they produce merits further investigation. Molds that we isolated most frequently from food (penicillia and aspergilli) and that were found in several instances to produce mycotoxins are from genera in which mycotoxins commonly have been detected (9). A more serious attitude should be taken toward the presence of molds in home-stored foods.

**ACKNOWLEDGMENTS**

We thank Professor C. H. Dunn, Mrs. Janet Floeter, Mrs. Ethyl Waisman, Mrs. Margaret Patchett, the Reverend and Mrs. C. G. Workman, and members of Zwingli United Church of Christ, Monticello, Wisconsin, for their help in obtaining samples or for providing samples. This research was supported by the College of Agricultural and Life Sciences, University of Wisconsin-Madison, by Public Health Service Grants FD00143 and FD00009 from the Food and Drug Administration, and by the Food Research Institute, University of Wisconsin-Madison. One of us (G.S.T.) received an educational allowance from the U.S. Veterans Administration.

**REFERENCES**


Incidence of *Salmonella* in Retailed Raw Cut-Up Chicken

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(Received for publication July 20, 1976)

ABSTRACT

The incidence of *Salmonella* organisms in 69 packages of raw chicken pieces obtained from retail stores was 34.8%. Eleven different serotypes were isolated.

There have been several reports on the incidence of *Salmonella* in chicken carcasses. Percentages of contamination have ranged around 2.7% (1), 7.5% (6), 8.3% (3), 15% (8), 26% (5), and 27% (9). Packaged pieces (legs, wings, breasts) are a popular commercial item sold in almost every grocery store in Canada. Its wide distribution and the part poultry plays in the spread of infection (7) prompted this investigation into the incidence and type of *Salmonella* in retail poultry.

MATERIALS AND METHODS

A total of 69 packages each containing either four to five legs, wings, or breasts was purchased from five different retail outlets over a period of 9 weeks during the summer of 1975. The experimental procedure consisted of aseptically transferring the pieces to a sterile plastic bag and rinsing with 11 of lactose broth by shaking by hand for 3 min. The rinse was transferred to a sterile container for preenrichment at 41°C for 24 h. All media were obtained from Difco Laboratories, Detroit, Michigan. Suspicious colonies were selected for standard diagnostic examinations, and confirmed serologically.

RESULTS AND DISCUSSION

Twenty-four of the 69 samples of raw chicken pieces were found to contain *Salmonella* (Table 1). This represents an incidence of 34.8%, which is in the range previously reported (5, 9).

TABLE 1. *Salmonella* in retail packages of raw chicken.

<table>
<thead>
<tr>
<th>Retail outlet</th>
<th>Number and type of sample</th>
<th>Number of samples containing <em>Salmonella</em></th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5 (2)</td>
<td>4 (1)</td>
<td>28.6</td>
</tr>
<tr>
<td>B</td>
<td>5 (1)</td>
<td>5 (1)</td>
<td>21.4</td>
</tr>
<tr>
<td>C</td>
<td>5 (3)</td>
<td>8 (1)</td>
<td>61.5</td>
</tr>
<tr>
<td>D</td>
<td>5 (3)</td>
<td>6 (1)</td>
<td>42.8</td>
</tr>
<tr>
<td>E</td>
<td>5 (1)</td>
<td>3 (2)</td>
<td>21.4</td>
</tr>
</tbody>
</table>

Total 25 (11) 24 (10) 20 (3) 69 24 34.8

Eleven different serotypes of *Salmonella* were isolated (Table 2). *Salmonella muenchen* was the predominant organism and was isolated most often from the samples purchased from store C. This high incidence of *Salmonella* is not without concern, particularly with respect to possible contamination of utensils and personnel with *Salmonella* organisms due to handling the raw chicken pieces. *Salmonella anatum* has been reported to survive for 3 h on fingers of food plant personnel and a 15-sec hand wash with warm water did not completely remove the organisms (4). Domestic fowl have been recognized as a major source of salmonellae and *Salmonella* food poisoning (2). The present data further emphasize that retailed raw chicken pieces carrying *Salmonella* organisms are potentially dangerous in stores and kitchens.

TABLE 2. Summary of occurrence of different serotypes of *Salmonella* in samples of chicken pieces obtained from different retail stores.

<table>
<thead>
<tr>
<th>Salmonella serotypes</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. california</em></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><em>S. infantis</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td><em>S. muenchen</em></td>
<td>2</td>
<td>1</td>
<td>5</td>
<td>5</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td><em>S. newport</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>S. manhattan</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>S. heidelberg</em></td>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td><em>S. Thompson</em></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. senftenberg</em></td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. schwerengrund</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><em>S. typhimurium</em></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td><em>S. copenhagen</em></td>
<td></td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. saint paul</em></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
<td>3</td>
<td>8</td>
<td>6</td>
<td>3</td>
<td>24</td>
</tr>
</tbody>
</table>

ACKNOWLEDGMENTS

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

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A Research Note

Loss of Cured Pigment in Dehydrating Fermented Sausage

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(Received for publication July 19, 1976)

ABSTRACT

An increase (P < 0.01) in the content of total heme pigment found during dehydration of a fermented sausage resulted from a concentrating effect due to the moisture loss from sausages. The percent of the total pigment converted to the “cured” nitric oxide heme pigment decreased (P < 0.01) during dehydration, indicating possible nitric oxide dissociation from the heme pigment.

In the production of fermented dry sausage, sausage is generally held to dry for extended periods after processing to attain desired moisture levels, texture, flavor, and color. A brownish discoloration on the surface of hams and sausage has often been associated with dehydration (1,3). In fresh meats (5) dehydration increased the concentration of pigments at the meat surface. Changes in the percent of total heme pigment existing as the cured nitric oxide heme pigment in ham (6,9) and frankfurters (3,10) have been followed using Hornsey’s (4) analytical method.

The moisture content found among dried sausage products as a group varied from 58.9 to 26.4% and was dependent upon the specific type or style of sausage manufactured (1). Percent conversion of total heme pigments to nitric oxide heme pigments was greater in sausage products containing 45 to 60% moisture than in those containing 25 to 30% moisture (12). The objective of this study was to determine the stability of the nitric oxide heme pigment during the dehydration phase of fermented, dry sausage preparation.

MATERIALS AND METHODS

A sausage mix was prepared using a blend of fresh boneless beef and beef fat adjusted to approximately 25% fat. Ingredient quantities per kg meat were as follows: 0.078 g of NaNO₂, 0.156 g of NaNO₃, 0.47 g of sodium erythorbate, 30.0 g of NaC₁, 10.61 g of seasoning mix, 5.05 ml of Pediococcus acidilactici suspension (LACTACEL), 7.51 g of dextrose, and 25.0 ml of water. The starter culture yielded an approximate level of 2.5-h total. The sausage links (of approximately 125 g each) were cooled to 20 C by a cold water spray and placed in a 7.5 ± 2 C drying room having 20 to 25 air changes/h. Drying room humidity ranged from 80 to 85%. Two sausage links were removed at random for analysis over an 11-day period.

The methods for nitric oxide heme pigment and total heme pigments as described by Hornsey (4) were used with a modification in extraction technique. A Tri-R homogenizer with teflon pestle was used to homogenize 4 g of sample with an acetone-water volume calculated (including sample moisture) to yield 80% acetone in 20 ml of total extractant. Samples were homogenized for 2 min, kept in the dark for 13 min, and then filtered through Whatman #4 paper into 1-cm spectrophotometer tubes. The filtrates were collected and read immediately at 540 nm. The concentration of nitric oxide heme pigments was calculated using the absorption coefficient given by Hornsey (4).

Total heme pigments were determined using the above procedure except that 1 ml of concentrated HCl was substituted for 1 ml of water in the acetone-water extracting solution. After homogenization, samples were allowed to sit for 60 min before filtering. The filtrates were read at 640 nm and the total heme pigment concentration calculated from the absorption coefficient of Hornsey (4). Results were analyzed statistically by analysis of variance and the significance of means tested by the least significant difference (LSD) method (9).

RESULTS AND DISCUSSION

At the beginning of the dehydration phase fermented sausage contained 59.9% moisture (Table 1). Of the total heme pigment content, 79.3% existed as the nitric oxide heme pigment. This maximum conversion to the cured pigment form agrees well with findings for other cured sausages.

<table>
<thead>
<tr>
<th>Moisture in sausage (%)</th>
<th>Total heme pigment (ppm)</th>
<th>NO heme pigment (% of total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>59.9b</td>
<td>217.6a</td>
<td>79.3b</td>
</tr>
<tr>
<td>54.8b</td>
<td>251.6bc</td>
<td>72.3b</td>
</tr>
<tr>
<td>48.0c</td>
<td>265.9bc</td>
<td>70.8b</td>
</tr>
<tr>
<td>46.1d</td>
<td>278.1bc</td>
<td>66.2c</td>
</tr>
<tr>
<td>43.3e</td>
<td>290.4c</td>
<td>64.7d</td>
</tr>
<tr>
<td>34.5f</td>
<td>361.1d</td>
<td>56.9f</td>
</tr>
</tbody>
</table>

Means in columns having the same superscript(s) are not significantly different (P < 0.05).
products. Simon et al. (10) reported 71 to 76% pigment conversion in frankfurters formulated with 78 ppm of nitrite and 83 to 88% conversion when 156 ppm of nitrite was utilized. For frankfurters heated to 60 to 66 °C and processed with a combined cure of 154 ppm of nitrite and 1248 ppm of nitrate, Fox et al. (3) reported a conversion of 80 to 85%. A cure blend of 78 ppm of nitrite and 156 ppm of nitrate was used in the current study. The recommended level of nitrite for fermented sausage employing frozen concentrate starter cultures of \textit{P. acidilactici} is 78 ppm of nitrite with nitrate (up to 156 ppm) as an additional optional ingredient (7).

As the sausage moisture content began to decrease during drying, the quantity of total heme pigment increased \((P < 0.01)\) as a result of a concentrating effect. If calculated on a sample dry weight basis, the total heme pigment quantity remained relatively constant and within experimental error. Thus, no destruction of the total heme pigment was indicated nor was there any apparent decrease in the extraction efficiency for the total pigment. A highly significant \((P < 0.01)\) correlation coefficient \((r)\) between moisture content and total heme pigment was obtained.

Dissociation of the nitric oxide from the nitric oxide heme pigment may have occurred during dehydration. The percent of total pigment converted to the cured pigment form decreased \((P < 0.01)\) with moisture loss. The results in Table 1 are in agreement with Townsend’s (12) findings of lower conversion percentages for sausages of the lower moisture contents. Other studies (6, 11) have shown that the dissociation is light-accelerated. The dissociated nitric oxide may be oxidized by air and the free pigment oxidized by excess nitrite, if present, to form metmyoglobin (2). In addition the cured pigment, once formed, is more stable at higher pH values near 6.0 (2). The sausage fermented in this study had a pH of 4.7.

ACKNOWLEDGMENTS

The authors express their appreciation to the Merck Chemical Division of Merck and Company, Inc., Rahway, NJ for providing samples of LACTACEL, and to Griffith Laboratories, Lithonia, Georgia for supplies and seasoning ingredients.

Reference to a firm or trade name does not imply endorsement over firms or products not mentioned.

Technical contribution No. 1387 of the South Carolina Agricultural Experiment Station, Clemson University, Clemson, S.C. 29631.

REFERENCES

Change in WMT Score with Time of Milk Storage

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(Received for publication July 21, 1976)

Samples of milk to be tested by the Wisconsin Mastitis test (WMT) are to be held at 0-4.4 °C and tested within 36 h of collection, according to Standard Methods for the Examination of Dairy Products (1). Data reported below emphasize the importance of age of sample in interpretation of the Wisconsin Mastitis test. Kroger and Jasper (2) previously reported on effects of time and conditions of storage but did not report on changes in variance of test results with time.

MATERIALS AND METHODS
Forty-seven samples of milk were collected in Whirl-pak bags directly from individual quarters of cows and from weigh jars containing the complete milking of individual cows. They were immediately cooled in an ice bath and were delivered to the laboratory within 2 h. Initial WMT Scores ranged from 15 to 37 mm. There were 24 samples in the 15-25 and 23 in the 26-37 mm ranges. The overall sample mean WMT score was 25.2 mm.

Wisconsin Mastitis tests were done in duplicate according to Standard Methods (1) on each sample within 6 h of collection and each 24 h thereafter for 96 h. Changes in WMT scores (mm) were calculated from averages of the two observations per time of storage.

RESULTS AND DISCUSSION
Reproducibility of the test was excellent. The standard deviation of the differences between means for duplicate samples in the 235 comparisons was .89 mm. Thus, the coefficient of variation based on the overall sample mean, 25.2 mm, and this standard deviation was only 3.5%. Differences between duplicate samples were

2 mm or less in 96% of the samples.

As expected, the milk/reagent mixture usually became less viscous with increasing age of samples. Decreases in quantities of sample retained in tubes averaged 2, 5, 7, and 10 mm after 24, 48, 72, and 96 h, respectively. There was little correlation between initial score and decrease in reactivity with age (line 7 of Table 1). There was a slight tendency for higher scoring samples to remain high in score during the first 48 h as evidenced by the correlation coefficients of .37 and .45 for storage times of 24 and 48 h, respectively.

More important, however, are the increases in the coefficient of variation of WMT scores with age. After 24-h storage, standard deviation was only 9% of the overall sample mean, but this percentage increased to 64 after storage for 72 h. Thus, an acceptable variance was experienced only during early storage. When it is recognized that about one-fourth the volume of most milk samples is at least 36-h-old at pickup, and that about three-fourths is at least 12-h-old, it becomes obvious that WMT testing should be done within not more than 36-h of milk collection. Furthermore, it would not be appropriate to employ a correction factor based on age of sample.

REFERENCES

TABLE 1. Means, standard deviations, ranges, correlation coefficients, and slopes of regression lines for changes in WMT score with time

<table>
<thead>
<tr>
<th>Time stored at 4 °C (h)</th>
<th>0</th>
<th>24</th>
<th>48</th>
<th>72</th>
<th>96</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall sample means (mm)</td>
<td>25.2</td>
<td>23.1</td>
<td>20.0</td>
<td>18.3</td>
<td>15.4</td>
</tr>
<tr>
<td>Mean change in score (mm)</td>
<td>-2.1</td>
<td>-5.2</td>
<td>-6.9</td>
<td>-9.8</td>
<td></td>
</tr>
<tr>
<td>Standard deviation (mm)</td>
<td>3.5a</td>
<td>2.6b</td>
<td>3.8b</td>
<td>3.7b</td>
<td>3.4b</td>
</tr>
<tr>
<td>Coefficient of variation (%)</td>
<td>7.8a</td>
<td>3.8b</td>
<td>26b</td>
<td>15b</td>
<td>9b</td>
</tr>
<tr>
<td>Range of changes in score (mm)</td>
<td>+2.5 to +1 to +5 to +1 to</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coefficient of correlation (initial score vs. change)</td>
<td>.37 .45 .12 .04</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope of regression line (initial score vs. change)</td>
<td>.15 .25 .06 .03</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

These values represent variation between duplicate determinations.

These values represent variation among averages of duplicate determinations.

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Foodservice Manager Certification—The NIFI Program

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(Received for publication August 10, 1976)

ABSTRACT

A trend toward tightening sanitation regulations is spreading across the nation. Supported by an increasing number of health jurisdictions at all levels (city, county, and state) and by the industry's customers in every state, new regulations are becoming a way of life for every restaurateur. Courses at all degrees of technical specificity, length and cost have appeared, culminating in what has been called "cross-country chaos."

The National Institute for the Food Service Industry (NIFI) course, "Applied Foodservice Sanitation," meets the FDA's recently-issued recommendations in every respect and is finding increased acceptance each day. It serves as the basis for the Illinois mandatory program and is accepted as one means of obtaining certification in the other two state mandatory programs (Florida and Washington, D.C.). Statewide voluntary programs are in full operation in Wisconsin, Michigan, Missouri, and Massachusetts and have started in Hawaii, South Carolina, Southern California, and Pennsylvania. Pilot programs are underway in Indiana and Kentucky and have been approved for early implementation in the state of Washington. Approximately 7,000 foodservice managers and supervisors have been certified by NIFI, and more than 17,000 textbooks are in use. NIFI has been chosen by the Food and Drug Administration to develop a plan for implementing a uniform national foodservice sanitation training and certification program.

The National Institute for the Foodservice Industry (NIFI) is supported by restaurateurs, associations, foodservice companies, distributors, and manufacturers. We have set as our goal nothing less than the professional development of foodservice management through education.

It is apparent that one of the biggest challenges facing the foodservice industry is insuring that the eating-out public is protected from the hazards of foodborne illness. We have always had this requirement. But a number of factors in today's world have sharpened the urgency of meeting it. Customers, more consumerist-minded than ever, want it. Environmentalists and sanitarians want it. Responsible industry leaders want it. And now, most importantly, public health departments, city councils, state legislatures and the federal government want it.

A trend toward strengthening sanitation regulations is spreading across the nation. Supported by an increasing number of health jurisdictions at all levels (city, county, and state) and by the industry's customers in every state, new regulations are becoming a way of life for every foodservice operator.

The major focus of most of the changing regulations is a new one and, in principle, a good one. They focus on management and the manager's professional qualifications to protect the public in a clean and sanitary operation. Going back to the recommendations of the 1971 National Conference on Food Protection, held in Denver under the sponsorship of the American Public Health Association and the FDA, they recognize that the manager is the key, that he must be trained and that his qualifications should be demonstrated.

FOODSERVICE INDUSTRY RESPONSIBLE FOR SANITATION

That conference in 1971 ended with the responsibility of sanitation being that of the foodservice industry. Foodservice management personnel must be more closely involved in sanitation. Manager certification is the way to accomplish this objective. Through foodservice manager certification we can provide foodservice management personnel with the education tools they need to enable them to self-inspect themselves and carry on the training of their employees. Secondly, maintenance of a high level of sanitation can be achieved in the foodservice operation with less governmental control. If the manager knows how and why sanitation is important a cleaner operation will exist. This objective can only help the sanitarian as he can communicate more effectively with the certified manager, making the sanitarian's job a little easier, and more importantly, more rewarding.

While sanitation is not the most exciting part of an industry which the public considers glamorous, it is being moved by events to the top of the business agenda. It could hardly not be so when essentially every state health department is looking at the subject with a new interest. In several cities (including Chicago), in dozens of counties, and in three states to date (Illinois, Florida, and the District of Columbia) mandatory manager certification has been enacted. Elsewhere, the existence or the threat of mandatory certification has resulted in establishment of voluntary certification programs. We in NIFI are cosponsors, with state restaurant associations and health departments, of statewide voluntary programs in 10 states.

But publicity has also played a part. Intense coverage by the news media in many cities is fanning the flames of public concern. Newspapers and television stations in New York, Washington, D.C., Chicago, Cleveland, and Boston have been quick to point out consumer interest in
FOODSERVICE MANAGER CERTIFICATION

Foodservice sanitation. In Miami, Wichita, South Bend, Los Angeles — all over the country — there have been restaurant kitchen exposes.

Some in the foodservice industry have shuddered at the fear generated by this publicity. Foodservice operators have nightmares about being put out of business by foodborne illness. Certainly, the operations whose unsanitary kitchens have appeared on television have suffered marked loss of customers, and in several instances, they have never recovered.

PROGRAM FOR EDUCATION

Yet, the most sensible reaction is not fear but an emphasis on and a professional program for education and training of foodservice managers. It takes careful, trained people to insure the prevention of foodborne illness.

Overall, results of this publicity have been varied, including establishment of firmer inspection procedures and heightened self-inspection programs by foodservice operators. But it has also brought the threat of proliferation of numerous and widely differing and expensive sanitation education programs.

Foodservice manager certification courses at all degrees of technical specificity, length, and cost have appeared, culminating in, what has been called "cross-country chaos." Some of the training programs are elementary food handler training courses. It is hard to imagine how some of these programs could meet the foodservice manager's needs for understandable operations-centered knowledge.

To help alleviate some of the confusion, the U.S. Food and Drug Administration has recently published a brochure, "Food Service Manager Training and Certification Program," containing recommendations for a training course to improve food protection practices in foodservice establishments. It is, of course, not directive to the states, but it will be highly influential. Included are FDA recommendations for implementation of a course, the course outline, tuition, facilities, course materials, instruction, and certification.

Specifically, the brochure says: "with the increased emphasis on training, consideration is being given that: (a) the manager who demonstrates sufficient competence in the sanitary operation of a foodservice establishment should be certified; (b) training and certification criteria should be uniform across the nation; and (c) uniform training and certification should result in reciprocity between governmental agencies and within industry."

THE NIFI COURSE

The NIFI course, "Applied Foodservice Sanitation" meets the FDA recommendations in every respect, and we are finding increased acceptance each day. It serves as the basis for the Illinois mandatory program and is accepted as a means of obtaining certification in the other two state mandatory programs (Florida and Washington, D.C.). Statewide voluntary programs are in full operation in Wisconsin, Michigan, Missouri, and Massachusetts and have started in Hawaii, Southern California, and Pennsylvania. Pilot programs have been conducted in Indiana and Kentucky, and have been approved for early implementation in the State of Washington. Approximately 7,000 foodservice managers and supervisors have been certified by NIFI, and more than 17,000 textbooks are in use — in these programs, in colleges, and in home study. And while mandatory certification and the threat of mandatory certification have resulted in some programs, we must certainly point out that some excellent voluntary certification programs have developed from genuine interest among industry leaders in upgrading foodservice through education. We believe that mandatory and voluntary training and certification programs can work together effectively if they are based on an agreed national standard uniformly applied. This will, in my opinion, make reciprocity possible and possession of a valid certificate an employment requirement in much of our industry, commercial and institutional.

The National Institute for the Foodservice Industry has been chosen by the Food and Drug Administration to develop a plan for implementing a uniform foodservice sanitation training and certification program. Currently, NIFI is engaged in gathering and assembling information for a "status report" on various types of foodservice owner/operator/manager training activities which lead to certification. After examination and evaluation of these programs it will be NIFI's responsibility to recommend to the Food and Drug Administration a national model program.

NIFI is in the progress of collecting information on foodservice management training programs now in existence in various parts of the United States, as well as throughout the world. The input of professional sanitarians is vital, for only through assistance and guidance of sanitarians will come a uniformity and consistency in certification programs. Cooperation is the key — industry, education, and sanitation.
Sanitation Training for Food Service Managers — A Must!

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(Received for publication June 10, 1976)

ABSTRACT

In the past two decades, growth of the foodservice industry has been sensational, and this means greater opportunities for consumers to become victims of foodborne illness. Safety of food is a responsibility of the industry, and establishment managers carry the burden of that responsibility. Sanitation training for foodservice managers offers great promise for improving food protection. Industry must assume the lead role in sanitation training, with cooperation and support of regulatory agencies.

At its annual Restaurant Show in Chicago during May, 1976 the National Restaurant Association announced some business achievements of the foodservice industry and some projects for the future of this dynamic industry. Gross sales for the industry reached 64 billion dollars in 1974. For 1975, gross sales are estimated at 72 billion dollars, a 12.5% increase in one year. Sales in 1976 are expected to reach 80 billion dollars; in 1980, 100 billion dollars.

In 1975, 40% of the family food budget was spent for food eaten away from home. By 1980, it is expected that one of every two dollars spent for food will be spent for food eaten away from home.

There are some 600,000 public and institutional food service establishments in this country, employing about 8 million full-time and part-time persons. The industry ranks first in employment, and if not already, will soon rank first in the U.S. in retail sales.

Industry growth in the past two decades has been sensational, and to those of us concerned with public health, this means increased opportunities for the consumer to become a victim of foodborne disease. Industry growth and greater public contact means that we must be more conscious of public health considerations.

RESPONSIBLE AGENCIES

Agencies responsible for overseeing sanitation in the diverse and entrepreneurial food service industry have a myriad of problems. Many operate with inadequate resources, or under laws that are virtually unenforceable, or in an environment of general apathy. One might conclude that inspection routines of many public health agencies serve only as an infrequent reminder to the restauranteur that he should indeed feel responsible for assuring safe food for his customer. My point is that even the best of regulatory agencies cannot assure safe food in eating establishments. This responsibility rests squarely on the shoulders of the industry, and industry management personnel must carry the burden.

INDUSTRY MUST CARRY BURDEN

Many persons will argue that growth of the food service industry heralds more problems in sanitation and greater risks of foodborne illness. I do not subscribe to this. The industry now attracts well-educated young people who already know, or can readily learn, the fundamentals of food protection. Much of the industry growth is accounted for in the emergence of multi-unit operations, or chains. Most of the large chains have quality control and sanitation staffs that develop procedures, provide training, and monitor operations to assure safe food-handling. Smaller chains may not be able to support a full-time professional staff, but can provide essential training and can monitor their operations to assure safe food handling. So I believe that, on the whole, industry is improving significantly its capability to provide safe food to the public.

It seems obvious to me that many regulatory agencies should modify their approach to regulating food service sanitation so as to maximize cooperation of the industry. By this I mean regulatory agencies should find time in their schedules to work with industry and individual firms toward improvement. There is a need for open and constructive dialogue. One medium for such dialogue is a cooperative effort to provide sanitation training for food service managers.

SANITATION TRAINING

We have taught various trade skills in classrooms and on-the-job for as long as public eating establishments have existed, but only in recent years have we become
seriously interested in teaching the fundamentals of food protection. A few years ago, we changed our emphasis in sanitation training from the food handlers to the owners, operators, and managers. This makes sense! The manager should know sanitation and proper food protection as well as he knows any other facet of his job. If he knows it, he can teach his work force, and integrate food safety considerations into his daily work routines. Having done this, he can do a bit of self-inspection each day to assure that what he taught is being done effectively. This is what manager training and certification is all about.

TRAINING AND CERTIFYING MANAGERS

For the past 4 years, the Food and Drug Administration has promoted the concept of training and certifying food service managers. Several good programs have already been initiated in various sections of the country, and more are starting each year. In February of 1976, the Food and Drug Administration announced its recommendations for a uniform training course. We now have entered into contract with an industry educational foundation to develop a national plan for implementing the training and certification of managers.

Do not be lulled into thinking that conducting a national program is easy. The thought of one to two million food service managers completing a course in sanitation is mind-boggling. When one considers certification of these managers, some questions arise. On what basis is he certified? For how long is he certified? If certification is good for a limited number of years, how is he recertified? Who maintains records of all these certifications? When certified in one jurisdiction, will the certification be recognized in other jurisdictions? These are hard questions that must be answered as we pursue this worthwhile program.

Despite any problems we foresee with it, I think this training is essential. Experience shows the training to be a real eye-opener to food service managers of long experience. It shows that many managers do not understand the potential within their own establishment for serving unsafe food. Teaching this awareness of the potential is a specific objective in training.

FDA recommends that sanitation training and certification programs be co-sponsored, as a minimum, by industry and the cognizant regulatory agency. We like to see others, such as educational institutions and consumer groups, involved in the program. A concerted effort is required to make a success of the program.

Earlier, I stated that FDA has entered into contract with an industry educational foundation to develop a national plan for training and certification of managers. In my judgement, this puts the program in proper perspective, with industry assuming a leadership role and government an assistance role. Industry has demonstrated sufficient capability and interest to successfully carry out the program. In so doing, industry is assuming its responsibility to assure that proper food protection practices are used universally, and that risk of foodborne illness is minimized. Public health officials should concurrently modify their approaches as necessary to provide all reasonable assistance to that end. While not a panacea, the training and certification of food service managers offers great promise for improving food protection in the food service industry.

ACKNOWLEDGMENT

In Defense of Technology

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ABSTRACT

High yields, efficient harvesting, sanitary and rapid processing, high quality distribution and retailing — factors which any country should be proud of. However, for some strange reason these are the very factors that are coming under attack by a vociferous group of individuals who seem bent on destruction of American Food Technology as we know it today. The reasons for the screams of outrage hurled at this Technology escape logical analysis. In this country today we have the potential to be the best fed people in the history of the world with the products at our disposal. Food is not only nutritious, but it is safe, convenient, of high quality, flavorful, and presented to the consumer at a cost, relative to earnings, which is competitive with any other country in the world at any other time in history, or the present. It is a gross injustice for consumers to have their confidence in the safety and nutritive value of their food supply destroyed without presenting them with all the facts. Perhaps it is necessary to present all the realities of nutrition to the consumer rather than simply to mention nutrients. Perhaps it is time for science and technology to present the facts of an overpopulated-underenergized world to the American consumers who take for granted the food supply at their disposal without an understanding of the technology it involves. It is ludicrous to believe in the natural way. A morally responsible usage of technology, not a profit-at-all-cost technology is the way to solve the hunger problems of the world. This information must be conveyed to the consuming public as a part of a nutrition education if we are to have a well fed and healthy nation without paranoia about its food supply.

In the past several years, technology has been under severe criticism, attack, and intuendos of corruption, and even perhaps criminality. The establishment, which in the food industry means the agricultural production divisions, food processors, food distributors, and even food retailers, is under this cloud. As a result the consumer is becoming biased on the basis of information which does not represent actual scientific fact but value judgements and opinions on “what might be the case” or on expressions of concern about “trends” which do not really exist. The net result being, that the consumer is becoming more confused, more paranoiac, and more mistrusting of the very food supply which provides the potential for and choice of the best diet which the world has ever known. Public relations and press releases seem to be replacing more conventional scientific publication and peer review systems. The consumer is being injected with scientific certainty when scientist themselves know that there are few absolutes in science and certainty is simply a statement of possible risk-benefit and not certainty at all.

On October 31, 1975, the Honorable Richard O. Simpson, chairman of the U.S. Consumer Product Safety Commission said in a speech that “many regulatory decisions as well as congressional decisions involve extremely complex technical questions with an attendant high degree of technical uncertainty. Complicating this process, is a growing phenomenon where some technical experts appear to have joined those in other professions who agree that the 'end justifies the means' and are making public pronouncements of technical certainty on many social issues. On closer examination much of the certainty disappears, but by then the technical issues are so intertwined with the social goals that both the public-at-large and governmental decision makers face an extremely complex and time consuming process to sort out technical facts. Public concern and confusion is often the by-products of these misstatements or overstatements.”

As a consequence of this general philosophy, government, industry, and science are taken to task, berated, pushed and pulled, and placed in an impossible situation. This situation demands scientific certainty where certainty can’t exist, it demands scientific answers for social, non-scientific questions and it demands decisions, restrictions, and impractical solutions on problems which have not been shown to actually exist. As Santayana has said, “trivial questions have easy solutions, but important questions may be insoluble.”

WHY THE ACCUSATIONS?

Why is this accusatory phenomenon taking place? It may be blamed on antiestablishment feelings, work of consumer activists, general mistrust of the food industry, mistrust in government regulatory agencies, and perhaps a general misunderstanding of what chemicals are. Certainly there is a fear of chemicals, a “chemophobia,” developing in our nation. This has led to the belief that any processed food is bad, that any food manipulated by man is bad, and that addition of any chemical to a food material is wrong. This concept is ludicrous at best because it must be recognized that foods themselves are simply chemicals, humans are simply chemicals, and that the only really scientific reason for eating is to replace the chemicals in the human body with chemicals from food. One can only conclude, therefore, that addition of chemicals to food material, as long as such
chemicals are safe, is at times a necessary and indeed fortuitous thing to do. At times, unfortunately, scientists proclaim that certain foods are bad for health and, therefore, the consumer should not eat them on the basis of very poor evidence. Generally, this involves a minority of the scientific community. Some consumer activists, certainly not all, claim that anything the food industry does is somewhat at fault for world starvation; a concept which escapes logical analysis.

THE SYMBOLIC BLAMEE

Dr. Richard K. Sparks in a recent article in the *Phi Kappa Phi Journal* (9) stated “there seems to be increasing reasons for believing that a little recognized socio-cultural phenomenon is evolving within our society which may have a seriously destructive impact on various forms of our institutional life, including those dedicated to the propositions of higher education. While the manifestations of this particular form of social pathology can be observed and its effects are already apparent in some areas, the phenomenon itself has yet to be clearly described. For the present, the phrase, ‘symbolic blamee syndrome,’ comes fairly close to stating the essential nature of the condition. What is involved is an apparent growing tendency within our society to seek out vaguely defined symbolic representations which can serve as blamees for many of the uncertainties, frustrations, and tarnished illusions which seem to confront us at every turn. The symbolic blamee is purposefully defined in the broadest possible terms and is always stated as a generality. This serves a number of purposes. First, by selecting a broadly defined blamee, it becomes almost impossible to determine exactly who is being blamed for precisely what. Second, by stating the blamee as a broad generality the possibility of a systematic response or retaliation is reduced to a minimum. Third, the use of a vague generally stated blamee injects an atmosphere of helplessness into a situation, relieves individual responsibility, and carries forward the implication that probably nothing can really be done anyway or, even if it can, someone else will have to do it. Some clear examples of the symbolic blamee syndrome are readily available. The dissident youth of the 1960’s used ‘the establishment’ as a convenient symbolic blamee without in fact, ever feeling any real need to define the term. The successful launching of a ‘movement’ often depends on the selection of an appropriate symbolic blamee. One current movement, for example, which enjoys considerable support has selected the entire male gender as a symbolic blamee. Political parties, ‘international money interests,’ the ‘corporate state,’ big labor, even ethnic groups — in one way or another, all broadly based socio-cultural institutions have been or are likely to become candidates for the role of symbolic blamee.”

I believe that this description of the symbolic blamee, is in part, the cause of the destructive criticism that technology is currently undergoing. The obvious question now arises as to how this kind of negativeness is to be handled. Certainly logical analysis of the problem is a little difficult because the symbolic blamee syndrome seeks to avoid logical and systematic analysis. However, technology cannot continue to be the “whipping-boy” for the activist in society.

TECHNOLOGY IS NEEDED

Clearly, the world would be in a very sorry state in its condition of overpopulation and underenergization if it were not for technology. We are faced, as scientists and technologists, with the problem of banishing hunger and malnutrition. It must be pointed out again and again with rational analysis that the solution to such a problem depends on how rapidly agricultural productivity can be increased, and how rapidly the technology involved with processing, storage, transportation, and marketing problems can be evolved. It must be emphasized that food processing has many goals, that perishable foods are preserved in a stable form that can be stored and shipped to distant markets all year round. It must also be pointed out that the basic preserving processes are canning, freezing, dehydration, salting, pickling, and freeze-drying. These are all processes which go on in the kitchen of the normal consumer. It must be pointed out that when a food material is cut, cooked, cooled, refrigerated, or handled in the kitchen, it is in fact being processed. Processing is simply a method to maintain food in an acceptable, safe, and nutritious form for an extended period. Therefore, when we speak of technology we speak of much more than just growing and eating food.

In this time of dramatic population increase and energy decrease the American public is living in the lap of luxury. Perhaps that is part of the problem of technology, perhaps scientists and technologists have done too good a job, perhaps the American public has come to expect luxury, a consistent and economical food supply never fearing for starvation or hunger. Foster and Foster (2) made a comment in a recent paper which I found most interesting: “I must admit that I, too, am disappointed, puzzled and sad. Why did we Americans become so complacent after World War II? Did we just get too fat and lazy? Because our way of life seemingly produced the highest standard of living for the greatest number of people in history we have assumed that everybody believes democracy and capitalism are the best systems in the world. But we have made very little effort to teach our young the basic principles of Americanism, either at home or in our schools. It comes as a shock to see on every hand the evidence that we have betrayed and neglected our children. Now we are seeing the result. A moral vacuum was there and those who would take over the system have been busy filling it.”

WE MUST SPEAK OUT

Personally I have a great deal of faith in our young. I believe that a great part of the problem has come from the desires of scientists and technologists to keep silent.
about the issues. It is easier for most of us to stay in the laboratory, to stay with students, and not to speak out. However, the time has come, when for the sake of continuing technology and the furtherance of plenty in our land, we must speak out, and we must be heard.

The Honorable W. S. Stuckey, Jr. said in a speech in June of this year (10) that "living in America is still the best bargain on earth. Between 1960 and 1973, the number of Americans living below the government's official poverty line dropped from 40 million down to 23 million!"

"Today, poverty-line income in this country is pegged at $5,000 for a non-farm family of four. How many realize this is higher than the average income in the Soviet Union!"

"The median income in America is over $12,000 a year. In terms of purchasing power per family, we are the number one nation in the world."

"Why does our country enjoy this level of affluence? I believe the answer to that question is directly related to something called free enterprise."

"In 1915, a phone call from San Francisco to New York cost $20. Today, it costs only $1.36. In 1915, $20 would have mailed 1,000 letters; but today it mails only 153. Private enterprise runs the telephone company; the government runs the postal service."

"The general accounting office recently concluded that it costs the Social Security Administration nearly twice as much to process a claim as it costs privately-owned companies to process a medicare claim."

"Garbage collection costs the average municipal agency nearly 70% more than it costs the average private firm to do the job."

"You have heard a thousand times that government can do it cheaper than private enterprise because the government doesn't make a profit, but that was the logic used by the man who killed the goose which laid the golden egg."

Congressman Stuckey then went on to quote a bumper sticker which he recently saw, "I saw a bumper sticker the other day which read: if you like the U.S. Postal Service, you'll love nationalized oil."

DECISIONS ABOUT TECHNOLOGY

Unfortunately, decisions about technology are not made today between industry and government agencies as they were 10 or 15 years ago. Today, we have an entirely new concept which pervades. That concept is predicated upon the voice of the crowd, the interpretation of scientific data by public relations and not scientists. As a result of this we are seeing social decisions that are being made by distorting science and an ever increasing growth of regulatory agencies to make these decisions. To again quote Congressman Stuckey "10 years ago, the federal regulatory establishment was composed of 12 agencies which employed 60,000 persons and spent $860 million. But now there are 24 agencies which employ nearly 110,000 persons and spends nearly $4 billion."

"While Congress was enacting 404 laws in 1974, the federal bureaucracy turned out 7,496 new or amended regulations. This is 1/8 for every law...and they are just as binding as any law adopted by Congress. Many were for good and worthy purposes. But is concerns me that we have created in our government a branch which is beyond the reach of the ballot box...beyond the reach of the people."

I believe that there is a definite necessity for our regulatory agencies. I also believe that the regulatory agencies do their job as it is outlined. In fact, the regulatory agencies carry out the law of the land, which indeed is their charge. The confusion results from how the law of the land is currently decided in the area of technology and, in particular, food technology. We are seeing a vast public relations effort, not by science but by letters to the editor, by charges in the press inspired by editorial writers who know very little about science but are inspired by consumer activists who, in my opinion, do not in fact help consumers but hinder them. It seems to me that many of the regulations which are promulgated due to pressures from those who decry technology end up aiding the consumer activists because it gives them a purpose in life; indeed often creating new jobs in industry; indeed raising prices; indeed creating new grants for the researcher. The only person who suffers and suffers most from such a point of view is the consumer. The consumers who I referred to earlier as being confused and paranoiac about the foods they eat.

Josephson (5) has summarized a rather dramatic account of over-regulation. In August 1973 the U.S. Consumer Product Safety Commission announced that research had shown that there was an association between some spray adhesive and chromosomal breakage and birth defects in humans; they then immediately banned and recalled the products from the market. The commission widely publicized a warning and asked those exposed to consult a physician. Members of the New York State Department of Health have recently surveyed medical genetic centers around the country and found that there were more than 1,100 inquiries at these centers alone and that more than 1,200 working days were lost because of the issue. More seriously, they found that nine pregnant women who were exposed to the spray adhesives elected to abort, and eight did so without undergoing diagnostic test.

However, something has been left out of this story that is very important. Six months after the warning, the commission withdrew its ban because, in fact, it was unable to confirm the alleged teratogenic or mutagenic effects or any toxicity of these substances. This is where public relations and media pressures can preclude science. Science should not proceed via the press conference but must evolve through publication in scientific journals which have undergone the scrutiny of peer review.
TOO MANY PROMISES?

Perhaps science has made too many promises, perhaps we in the field of technology have allowed the consumer to believe that technology can solve all problems. If this is indeed true then we must let the consumer know just what it is that technology can accomplish and what it cannot accomplish. We obviously must have tests for safety, we obviously must proceed with good manufacturing processes while utilizing food additives in a sane, moral, and rational sense. We must never forget the lessons of thalidomide. However, we must do this based on factual evidence and not on suspicion.

Perhaps we should make it plain to the consumer that it is a fact that most nutritious foods are worthless if they are not maintained in an acceptable form. Nutrition, per se, is not the most important factor to the consumer and cannot even be considered as a single entity. The consumer must become aware of the realities of food production and the fact that nutrition must be considered within the framework of many other factors such as cost, acceptability, safety, availability, convenience, and quality.

It is true that nutrition as a generalized concept, is important to the consumer, however, it maintains this preeminent position only up to the point of purchase. At purchase, the other factors mentioned previously loom into the overall picture and predominate in the minds of most consumers. At this time of economic turmoil, who would argue the importance of cost. At this time of mistrust, who would argue the importance of safety. The psychological values in our society imply a demand for good flavor, acceptability, safety, availability, convenience, and quality.

At this time of struggling for individual rights for the role of women in our society, who would argue the importance of safety. The psychological values in our society imply a demand for good flavor, acceptability, safety, availability, convenience, and quality.

Consider first acceptability, availability, and quality: in the days of the Declaration of Independence, Count Volney, and outspoken Frenchman, tells of “writhing under the effects of a breakfast of hot bread, half baked and soaked in melted butter, served with the grossest cheese and hung beef, pickled pork, and fish. At dinner they have boiled paste under the name of puddings, and the fattest are esteemed the most delicious; all their sauces, even for roast beef, are melted butter, or fat; under the name of pie or pumpkin, their pastry is nothing but a greasy paste never sufficiently baked. To digest these viscous substances they take tea bitter to the taste, in which state it affects the nerves so powerfully that even the English find it brings on a more obstinate restlessness than coffee” (II). Further, Count Volney stated that “for the good of the country, the government should launch an educational campaign for the improvement of eating habits” (II).

Consider the position today: “we tend to take many of the positive elements of our advanced state of technology for granted — and even make them the subject of criticism. We routinely expect that we can come home at night after a day’s work, walk into a well-stocked pantry, pick and choose exactly what we will have for dinner from the refrigerator, and have it ready in less than an hour. We naturally assume the courses we prepare and eat will be nutritious, attractive, and enticing to our taste buds. We expect that our meals will be enjoyable occasions, not just routine periods for satisfying biological needs” [Whelan and Stare, (12)]. You know it wasn’t always that way.

Convenience and cost.

Consumers today are insisting on being able to buy “instant,” “heat and serve,” and “ready-to-cook” convenience foods that grandmother never dreamed of. One of the reasons is the struggle for individual rights and the consequent fact that more women are taking jobs — 74 million of them today, and a projected 135 million in 2,000 A.D. (7). As a result they’re willing to pay for convenience built into the food rather than domestic help. This kind of convenience allows a household to eat a varied, nutritional, high quality diet. A result of technology.

Technology saves a tremendous amount of money.

For those who wish to go back to nature and sell natural rather than additive bread, I would recommend an examination of Table 1 (7). Without reservations,

<table>
<thead>
<tr>
<th>TABLE 1. Impact of removing additives on the cost of white bread</th>
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<tbody>
<tr>
<td>Cost factor</td>
</tr>
<tr>
<td>Ingredients</td>
</tr>
<tr>
<td>Manufacturing costs</td>
</tr>
<tr>
<td>Selling and distribution expense</td>
</tr>
<tr>
<td>Grocer’s margin</td>
</tr>
<tr>
<td>Baker’s margin</td>
</tr>
<tr>
<td>Retail price</td>
</tr>
<tr>
<td>Difference</td>
</tr>
<tr>
<td>Additional cost per loaf to consumer</td>
</tr>
<tr>
<td>Additional cost to consumer nation-wide</td>
</tr>
</tbody>
</table>

²From Melnick, (7)

leavening agents, yeast foods, and dough conditioners, there would be no white pan-bread industry as we know it today. These additives amount to 70 million pounds, valued at $15 million and are used to produce 24.5 billion loaves valued at $6 million retail. Without these additives, bread would require slower materials handling and daily distribution throughout thousands of outlets and daily wipeout of unsold inventories of still nutritious bread. The total effect would be an additional cost to American consumers of 1.1 billion dollars per year. Consider the case 200 years ago (II) “apples were the most popular fruit because of their keeping qualities and cider potential” (note the importance of preservation or processing which is too often criticized today) “peaches,
scientists and non-scientists.

TABLE 2. Commonly used mistaken synonyms

<table>
<thead>
<tr>
<th>Scientists</th>
<th>Non-scientists</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypothesis</td>
<td>Facts</td>
</tr>
<tr>
<td>Theory</td>
<td>Discovery</td>
</tr>
<tr>
<td>Implications</td>
<td>Results</td>
</tr>
<tr>
<td>Suggests</td>
<td>Proves</td>
</tr>
<tr>
<td>Causes</td>
<td></td>
</tr>
</tbody>
</table>

From Nutrition and Food Choices by Kristen W. McNutt and David R. McNutt, SRA (in press).

Consider the case 200 years ago (11) "tomatoes were raised as a garden ornament but seldom eaten as many considered them to be poisonous. Potatoes were known but not widely cultivated...it was believed that if a man ate potatoes every day, he could not live 7 years." As well as these misbeliefs we all know that there was some truth in these beliefs. Potatoes containing large amounts of solanine could be dangerous and of course tomatoes were thought to belong to the family "deadly nightshade." But there were other problems: food poisoning was rampant, malnutrition was rampant, people starved to death. They do not do that today. The gross deficiency diseases which we were so familiar with have all but disappeared. We don’t even think or hear about them in North America today. Scurvy, rickets, beriberi, pellagra — who suffers from these today in America? It’s interesting that just recently as 1955 a Canadian textbook written by Hitzls (4) stated “in many parts of Canada one child in every nine shows evidence of having had rickets. This is true notwithstanding the fact that prevention and care are so simple. There is insufficient sunshine in Canada to prevent rickets in children so it should be standard practice to give them daily some form of liver oil or concentrate.” I remember, with fond distaste, the cod-liver oil pill that I received, how much more safer and more desirable to receive this in a staple food product like milk. This is due to fortification, this is due to technology.

Fortification of food with nutrients is probably the area which would be singled out by most as having had the greatest impact on the health of the consumer. However, it is clear that technology has provided many other innovations for the nutritional benefit of the consumer.

The concept of fortifying food and/or water with nutritional chemicals may be said to have had its beginning in 1833 in South America when the French chemist Boussingault recommended addition of iodine to table salt to prevent goiter. This practice has been adopted widely in Europe and North America with tremendous success since that time. The history of fortification is impressive. Margarine fortification with vitamin A; vitamin D fortification in milk; the enrichment of bakery-produced white bread and family-used flour by addition of thiamine, riboflavin, niacin, and iron. Enrichment of degerminated cornmeal, corn grits, whole grain cornmeal, rice, pasta products, and, of course, fortification of cereals. Ascorbic acid fortification of many, but not all, fruit beverages and non-citrus juices proceeding. More recently, addition of the vitamins A and D to fluid skim milk, fluid low-fat milk, and non-fat dry milk has been initiated. There can be no question as to the beneficial effects of this technology.

MARKETING MECHANISMS

These are the promises of technology. Technology and science, however, cannot practice or promise absolute safety, they cannot promise magic from food, they cannot promise strength and vigor from eating certain food materials to the exclusion of others. Perhaps it is time for the scientists and technologist to come grips with some of the marketing mechanisms used in this country. Certainly, food advertising, by and large, uses a very sensational technology. Perhaps it is time for the scientists and technologist to come grips with some of the marketing mechanisms used in this country. Certainly, food advertising, by and large, uses a very sensational approach in communicating with the consumer. However, there is probably about 2% of the total advertising which could do with some cleaning up. This 2% unfortunately, gives all food advertising a bad name. We should do away with it!

We should do away with the concept of industry promoting natural over synthetic. This gives an immediate economic advantage to a company but an immediate economic disadvantage to the consumer. It seems to stress that natural is somehow better than synthetic and seems to imply that what we are doing is somehow wrong. This must stop as well.

Consumers must be made aware of the positive aspects
of technology and the grammatical sleight-of-hand used
do to disclaim it. Table 3 shows a commonly used ploy, that
table of juxtapositioning sentences, to prove a point. This kind
of writing must be logically analyzed by consumers and in
fact, scientists must point out the existence of this kind of
rhetoric at every opportunity. Table 4 is another example

of juxtapositioning sentences to create the illusion of logic.

Juxtaposition of sentences:

"The American diet in 1942 provided half as much meat as does
the diet in 1976. Many recruits in World War II were found to be in poor
health."

"Cholesterol contributes to heart attacks. This product is lower in
cholesterol than the competitor's product."

a From Nutrition and Food Choices by Kristen W. McNutt and David
R. McNutt, SRA (in press).

or technology can be totally misrepresented by simply
changing the connotation. We must convince consumers
of the use of words to convey misinformation. The
examples in Table 4 clearly show that the desired end of
writing must be logically analyzed by consumers and in
fact, scientists must point out the existence of this kind of
rhetoric at every opportunity. Table 4 is another example

of a simple change in connotation can make a dramatic
change in meaning.

Table 4. A simple change in connotation can make a dramatic
change in meaning.

<table>
<thead>
<tr>
<th>Connotation comparison</th>
<th>Full of calories</th>
<th>Full of energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coarse</td>
<td>Textured</td>
<td></td>
</tr>
<tr>
<td>Slippery</td>
<td>Smooth</td>
<td></td>
</tr>
<tr>
<td>Manipulate</td>
<td>Alter</td>
<td></td>
</tr>
<tr>
<td>Filler</td>
<td>Carrier</td>
<td></td>
</tr>
<tr>
<td>Dissect</td>
<td>Separate</td>
<td></td>
</tr>
<tr>
<td>Stiffen</td>
<td>Stabilize</td>
<td></td>
</tr>
<tr>
<td>Synthetic</td>
<td>Formulated</td>
<td></td>
</tr>
<tr>
<td>Cheap</td>
<td>Economical</td>
<td></td>
</tr>
</tbody>
</table>

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R. McNutt, SRA (in press).

of the use of words to convey misinformation. The
table 4 clearly show that the desired end of
technology can be totally misrepresented by simply
changing the connotation. We must convince consumers
to read analytically. We must ask them to demand from
the media such information as: What dosage? What
human equivalent? How long a period? What kind
of animal used? This might stop some of the problems
that arise from banner headlines such as "Compound
X Causes Cancer."

Dr. Thomas Jukes, in a recent article, in Nature (6)
discussed DES and its risk-benefit. "Indeed, folic acid
was discovered as a result of the anemia in pregnant
women caused by its deficiency, by Dr. Lucy Wills in
1931. The deficiency is still prevalent throughout the
world, as noted by the World Food Congress in Rome,
in 1973. A continuation of the dietary lack of folic acid in
pregnancy will be aided by NCT's recommendation
against consuming beef liver. The protective effect
against cancer of this recommendation seems dubious."

This is a case where a consumer would have to eat
tonnage ranges of liver over a lifetime to simulate the
studies done with DES. The trade-off is perhaps a
deficiency in folic acid. This must be pointed out to the
consumer and the consumer must choose.

TECHNOLOGY MUST GO?

In the recent 1976 W. O. Atwater Memorial Lecture,
Dr. Emil M. Mrak, Chancellor Emeritus of the
University of California at Davis, made a striking plea
to scientists to speak out, to talk to consumers, and to
defend the technological way. It is critical that this be
done. We are facing a time of gigantic upheaval. We
have tried to please everyone and have pleased only a few.
Rebellion seems to have run its course in some areas, but
a far more serious rebellion is underway — the powerful
upwelling of the spirit of the counter-culture. This
includes not only anti-scientific, and anti-technological
forces, but total anti-intellectual forces as well.

One answer to this would be to remove from the
marketplace all convenience foods, bacon, ham, sausage,
all processed foods including those canned and frozen.
Ask the consumer to get back to nature and grow a vine
ripened tomato in 2 ft of snow in the midwest or
northeast. This is not the answer. We cannot push
technology back because we have an overpopulated
planet that would starve. Scientist and technologist have
always had a human, moral spirit which has been used
to produce benefits for human kind. However, it must be
remembered that science and technology cannot solve
all problems. It cannot promise the world and keep its
promise, however, it can indeed contribute to feeding
and increasing the comfort of a vast mass of humanity
whom, without technology, would face the four horsemen
of Apocalypse — death, famine, war, and pestilence.

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Preventing Addition of Water to Milk on the Farm

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ABSTRACT

Milk is adulterated when water is added. A simple, inexpensive, and effective method to control this offense at the farm level has been in operation since 1965 in Colorado. It consists of routine freezing point testing by the dairy industry combined with a “monetary penalty” system. The origin, development, administration and results are summarized.

A universally recognized fact is that adding water to the milk supply at any point during its journey to the consumer constitutes adulteration and alters its quality and consumer acceptance. It is generally conceded that the practice exists to a greater extent than most of us would like to admit. Those experienced in attempting to eliminate the practice or reduce it to a minimum agree this cannot be done without some type of penalty. To be effective, the penalty system should be simple and must be applied continuously.

The only reliable testing method to determine if water is added to milk is through the use of the cryoscope to ascertain the freezing point. Since this procedure is well known, we will not discuss the technical aspects of the test. Our purpose is to examine the method and results of one penalty program. This program has been in continuous use on the Colorado market for 11 years. Others may find the method applicable to their operations. The program controls added water at the farm level. It does not include any infractions beyond the bulk tank.

ORIGIN

The program had its origin in early 1965 when the Sanitation Division of the Denver Department of Health and Hospitals became disturbed over the extent of added water in producers’ samples of milk and in tankers of milk. Tabulation of test results showed that 25% of the dairymen were adulterating their milk occasionally or regularly, either through carelessness or by deliberate use of water. Coincidentally, this was at the time of extensive installations of bulk tanks and pipe-line milkers.

Added water ranged from a trace to 20% Milk tankers contained as much as 7% added water. Table 1 shows the average range of January and February, 1965, freezing points. The Health Department called a meeting with dairy industry representatives to review the situation. All agreed that the situation was serious and a positive approach was needed. Management of the Mountain Empire Dairymen’s Association (MEDA), the principal representative of milk producers in the area, proposed that it assume major responsibility for correcting the situation.

Table 1. Freezing point of milk before and after the control program was instituted

<table>
<thead>
<tr>
<th>Date</th>
<th>Number of samples</th>
<th>Mean freezing point (°C)</th>
<th>Range in freezing point °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tanker samples Jan.-Feb., 1965</td>
<td>107</td>
<td>-.531</td>
<td>-.510 to -.543</td>
</tr>
<tr>
<td>Milking time inspection samples</td>
<td>68</td>
<td>-.535</td>
<td>-.528 to -.555</td>
</tr>
<tr>
<td>Tanker samples June, 1965</td>
<td>47</td>
<td>-.536</td>
<td>-.528 to -.543</td>
</tr>
<tr>
<td>Tanker samples January, 1969</td>
<td>28</td>
<td>-.541</td>
<td>-.526 to -.545</td>
</tr>
</tbody>
</table>

PROPOSAL

Its proposal was to add routine freezing point tests to the established monthly quality control tests and adopt a monetary penalty program to apply to those found adding water to their supply. The Health Department’s role was to help establish a testing program and then routinely monitor its effectiveness through random sampling of producers’ samples of milk and tankers of milk. Milking time samples were collected from 68 herds to establish a basic freezing point for the area. The freezing points ranged from -.555 C to -.528 C with a mean of -.535 C.

PENALTY PROGRAM

In March 1965, the actual penalty program began. It has continued uninterrupted to the present. Since the “Universal Sample” collection method had been in operation on the market for several years, no change was necessary in this chore. The penalty program was administered by MEDA.

Following the first added water offense by a milk producer, a fieldman visits the producer to determine the cause and explain the seriousness of the offense. A warning is given. If, in a following unannounced test or later tests, the problem is not corrected, the percentage of added water found is deducted from the offender’s total milk weight and he is paid accordingly.

For example, if a dairymen delivers 150,000 lb. during the month in which a 5% added water offense is detected, 750 lb. is deducted from the weight shipped and he receives payment accordingly.

RESULTS

Four months after the program was put into effect 47 tankers of milk were sampled at random. The freezing point ranged from -.528 to -.543 C with a mean of -.536 C. This compares with -.510 to -.543 C and a mean of -.531 C 4 months earlier. After nearly 4 years of continuous operation of the plan, milk from 28
tankers examined in January, 1969, showed a range of -0.526 to -0.545 C and a mean of -0.541 C. Compared with February, 1965, this shows a mean difference of 0.010 C on the plus side. This is a substantial gain in remedying a serious practice.

Up to date results show that in 1972 only 1.57% of all samples collected showed any evidence of added water. In 1975 the figure was 1.5%. Serious consideration is now being given to raising the base from -0.530 to -0.535 C.

The program's success can be traced to three important factors -- its simplicity and ease of administering, routine and continuous application, and coordination of the whole operation between the dairy industry and the enforcement agency.

---

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Supplement No. 1
To the 3-A Sanitary Standards for Fittings Used on Milk and Milk Products Equipment and Used on Sanitary Lines Conducting Milk and Milk Products, Revised

Number 08-17A

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

This supplement adds the criteria for tank outlet valves to Section E. SPECIAL CONSIDERATIONS of the "3-A Sanitary Standards for Fittings Used on Milk and Milk Products Equipment and Used on Sanitary Lines Conducting Milk and Milk Products, Revised, Number 08-17." (Reference 3-A Drawings No. 3A-100-39, No. 3A-100-40, No. 3A-100-41 and No. 3A-100-42.)

E.10
These valves shall comply with the applicable provisions of this standard and the following:

E.10.1
The valve assembly shall consist of a flange, flange gasket, body with a helical slot, outlet, boot, poppet with a stud and ball, U clamp and O-Rings. In addition the assembly may include an end cap, dust cover and lock nut.

E.10.2
All product contact surfaces and surfaces which will become product contact surfaces if the boot or an O-Ring(s) fails in service shall be readily accessible for cleaning and inspection.

E.10.3
The end of the poppet covered by the boot shall have one or more 3/32 inch holes for detection of leakage. E.10.4
Rubber and rubber-like materials may be used for boots and in sealing applications.

E.10.5
Plastic materials may be used for poppets, end caps, dust covers, lock nuts and in sealing applications.

Add the following to the list of drawings in APPENDIX, Section 1 in Part 2 of this standard:

<table>
<thead>
<tr>
<th>Fitting Name</th>
<th>3-A Drawing Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tank Outlet Valve</td>
<td>3A-100-39</td>
</tr>
<tr>
<td>Tank Outlet Valve</td>
<td>3A-100-40</td>
</tr>
<tr>
<td>Tank Outlet Valve</td>
<td>3A-100-41</td>
</tr>
<tr>
<td>Tank Outlet Valve</td>
<td>3A-100-42</td>
</tr>
</tbody>
</table>

This supplement is effective May 1, 1977.
The z-value is more than a number

DEAR SIR:

This letter is in regard to the z-value reported in the article entitled "Bacterial Spoilage of Citrus Products at pH Lower than 3.5" by B. J. Juven that appeared in the Journal of Milk and Food Technology, Volume 39, No. 12, pages 819 to 822.

We note that in the abstract in the caption to Fig 3 and in the text adjacent to Fig 3, the author reports a z-value of 8.3 with no unit listed with this value. We believe that the average reader is going to assume that the z-value is 8.3°C since we do not believe that there is a single Fahrenheit temperature in the entire manuscript.

The z-value is not a unitless number but is a measurement in degrees of temperature. It should have units either of F or C. We scaled Fig 3 and calculated a z-value of 4.5 to 4.7°C (temperatures on the graph are in °C). On the basis of this analysis, we assume that the author meant for the z-value to be 8.3°F since 4.6°C is equivalent to 8.3°F.

In any article on thermal destruction of microorganisms we feel the temperature unit for the z-value should be included with the z-value, and the temperature unit of the z-value should be consistent with the temperature unit used in preparing the thermal resistance curve.

We hope that these suggestions will be helpful to those preparing and reading reports that include z-value data.

THERON E. ODLAUG
Environmental Sterilization Laboratory
Department of Food Science and Nutrition
University of Minnesota
St. Paul, Minnesota 55108

Yes, a unit goes with the z-value

DEAR SIR:

Dr. Odlaug's comments are received with thanks.

The z-value is defined as: "The number of °F required for the thermal-death-curve to traverse one log cycle. It may be said that z characterizes an organism with respect to its relative resistance to different temperatures" (Stumbo, Thermobacteriology in Food Processing, 2nd ed. p. 147, Academic Press, 1973).

"The numerical value obtained by measuring the number of degrees Fahrenheit required for the thermal-death-curve to pass over one log cycle" (Hersom & Hulland, Canned Foods p. 124, J.&A. Churchill Ltd., 1969).


Letters to the Editor

This definition of z is also found in other textbooks of Food Microbiology: (1) Frazier, 1970, Food Microbiology, 2nd ed., p. 91; (2) Jay, Modern Food Microbiology, 1970, p. 158; (3) Nickerson & Sinskey, Microbiology of Foods and Food Processing, 1972, p. 37.

From these definitions it may be concluded: (a) that z is indeed an absolute value, based on measurements in degrees Fahrenheit; (b) when z-values appear without any indication of a temperature scale it refers to °F.


Nevertheless it is true that presentation of z-values as °C has recently become common when temperatures are reported as °C. However z-values are still very often given on a Fahrenheit basis.

With this situation we could agree with Dr. Odlaug that misunderstanding may arise from reporting z values without making clear in what temperature scale the calculation has been done.

This possibility occurred to me a few days after I returned the revised manuscript of my paper (27th April). On the 2nd May, therefore, I wrote suggesting that, since the z value (8.3) reported in my paper corresponds to °F, it might be advisable to so indicate (the corresponding value in °C is 4.6).

BENJAMIN J. JUVEN
Division of Food Technology
Institute for Technology and Storage of Agricultural Products
Agricultural Research Organization
The Volcani Center
P.O. Box 6
Bet-Dagan, Israel

Editor's Note: The letter mentioned by Dr. Juven arrived after the manuscript had been sent to the printer. A change could have been made in proofs but probably was overlooked.

Iodide in Australian milk

DEAR SIR:

In a recent U. Milk Food Technol. 39:544, 1976 article, Cantor and Most reported that "the use of properly formulated teat dips would seem of limited significance in the total human dietary intake" (of iodide). However, studies in Australia, where iodophor teat dips and sanitizers are widely used, led to somewhat different conclusions. In their report to the Australian National Health and Medical Research Council, Dunsmore and Luckhurst revealed that in New South Wales over a 12-month period the milk iodide content averaged 76 μg/100 ml, well above the 50μg/100 ml set by the Dairy Industry Association of that state. They found that with bad practice the teat dip (at one-half the strength used by Cantor and Most) added 19.7 μg/100 ml (19.2%) of total iodide, while with good practice it was responsible for 5.7 μg/100 ml (37%). Furthermore, they found the milking machine responsible for 24.8 μg/100 ml (24.2%) with bad practice, and for 9.5 μg/100 ml (61.7%) with good practice. And the bulk tank, which like the milking machine had been sanitized with an iodophor solution, added 29.6 μg/100 ml (28.9%) with bad practice, but only 0.2 μg/100 ml (0.3%) with good practice. (The difference here may be due to greater adsorption of iodide on rubber surfaces.) All of which suggests that producers should be more careful to wash udders thoroughly, make up sanitizing solutions according to directions, and drain equipment thoroughly to keep contamination of milk with iodide to a minimum. Dunsmore suggests using a teat dip formulation that is easily removed, thorough washing and drying of udders, using low-foam iodophor sanitizing solutions and following with an "air sweep," careful assembly of milking machines, and maximum drainage of milking machines and bulk tanks before use.

C. K. JOHNS
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Affiliate Meetings


MINNESOTA—April 19 and April 21, 1977. Outstate educational meetings at Alexandria and Albert Lea.

MISSOURI—April 4, 5 and 6, 1977. Ramada Inn, Columbia.


Calendar of Events

March 28-April 1, 1977. SHORT COURSE ON MAXIMIZING CONSUMER SATISFACTION BY COMPUTERIZED FOOD MANAGEMENT AND DIET CONTROL. For food service and dietary executives. For information contact: Dr. Joseph L. Balintfy, College of Business and Management, University of Maryland, College Park, MD 20742.

March 29-31, 1977. WESTERN FOOD INDUSTRY CONFERENCE. Freeborn Hall, University of California at Davis. Registration fee: $18.00. For information contact: Robert C. Pearl, Food Science & Technology Department, University of California, Davis, CA 95616 (916) 752-0980.


May 10-12, 1977. SECOND INTERNATIONAL POWDER & BULK SOLIDS HANDLING & PROCESSING SHOW. O'Hare International Trade & Exposition Center and the Regency O'Hare, Rosemont, Illinois. For information contact: Aaron Kozlov, Industrial & Scientific Conference Management, Inc., 222 West Adams St., Chicago, IL 60606 (312) 263-4866.

May 21-25, 1977. NRA RESTAURANT HOTEL - MOTEL SHOW. McCormick Place, Chicago, IL.

May 22-26, 1977. NATIONAL CONFERENCE ON INTERSTATE MILK SHIPMENTS. Stouffer's Inn, Cincinnati, Ohio. For information contact H. H. Vaux, Indiana State Board of Health, 1330 W. Michigan St., Indianapolis, IN 46206.

June 5-8, 1977. 37TH ANNUAL MEETING AND FOOD EXPO, INSTITUTE OF FOOD TECHNOLOGISTS. Philadelphia Civic Center, Philadelphia, Pennsylvania. More information is available from Dan Weber, Director of Convention Services, Institute of Food Technologists, 221 N. LaSalle St., Chicago, IL 60601.

June 9-11, 1977. INTERNATIONAL SYMPOSIUM ON SALMONELLA IN POULTRY AND PROSPECTS FOR CONTROL. University of Guelph, Guelph, Ontario, Canada. For further information contact: Office of Continuing Education, Johnston Hall, University of Guelph, Guelph, Ontario N1G 2W1, Canada.


June 27-30, 1977. FOOD PROCESSORS BASIC MICROBIOLOGY SHORT COURSE. Sponsored by the University of California, University Extension. Location: Cruess Hall, UC Davis Campus. Registration fee: $100. For further information contact: Dr. Robert J. Price, Food Science & Technology Dept., University of California, Davis, CA 95616 (916) 752-2193.

October 5-7, 1977. SOUTHEASTERN NATIONAL FOODSERVICE SHOW. Atlanta Marriott Hotel, Atlanta, GA.

News and Events

Portable Mini-Mizer Chiller Offers Efficient, Reliable Operation

Efficient, reliable cooling in small load applications (1/4 ton to 2 tons) can now be obtained with the compact, portable, solidly constructed Mini-Mizer Chiller from Mayer.

The Mini-Mizer is a self-contained, air-cooled unit which utilizes a closed system for recirculating refrigerated water. Mounted on heavy-duty casters for completely portable, plug-in operation, the Mini-Mizer is available in sizes designed to fit the smallest areas: the smallest model measures less than 25" long by 18" wide by 32" high but it can provide reliable, efficient, round-the-clock cooling in temperature ranges from 20°F (-6°C) to 60°F (15°C).

Mayer Refrigerating Engineers manufacture a broad line of conveyor systems, chillers, water economizers, and special cooling equipment for industrial process cooling applications.

For additional information on the new Mini-Mizer, contact: Mayer Refrigerating Engineers, Inc., 600 Winters Avenue, Paramus, NJ 07652.
Food Processors Basic Microbiology Short Course

A basic microbiology short course for food processors will be offered at the University of California at Davis during June 27-30, 1977. This short course is designed for food industry personnel who are new to the field of food microbiology or would like a review of basic food microbiology and microbiological techniques. There are no prerequisites to this course. Instructors will assume that students have had no formal training in biology or chemistry.

The objectives of the course are to develop basic skills in and an understanding of food microbiology. Topics to be covered include use of a microscope; basic laboratory skills; isolation, enumeration, and identification of microbes in processed foods. The short course will consist of lectures, laboratories, and evening discussions. Certificates of course completion will be issued.

Enrollment is limited to 32 students. The registration fee of $100 includes laboratory manual and fees, first evening's social, and last evening dinner. For registration information and further details contact: Dr. Robert J. Price, Department of Food Science and Technology, Crue Hall, University of California, Davis, CA 95616. Phone (916) 752-2193.

American Dry Milk Institute to Hold Meeting

The 52nd Annual Meeting of the American Dry Milk Institute and the 6th Annual Meeting of the Whey Products Institute will be held jointly at the Marriott Motor Hotel, Chicago, on April 27, 28 and 29, 1977, it has been announced by Dr. Warren S. Clark, Jr., Executive Director of both organizations.

All dry milk and whey product manufacturers, allied industry friends interested in processing and marketing of these products, and representatives and government and universities are cordially invited to attend the meetings.

The General Sessions program will present knowledgeable speakers from industry, government, education and the Institute's staff, who will discuss topics of current interest to manufacturers and users of dry milk and whey products.

Continuous, In-Container Product Sterilization Available with Hydromatic Sterilizer

High quality in-container product sterilization and pasteurization for food, dairy and pharmaceutical products can be accomplished efficiently and economically with the Hydromatic® sterilizer, available with Stork Food Machinery, Somerville, N.J.

Featuring continuous operation, with speeds ranging from 60 to 1200 containers per minute, the Stork-Hydromatic is designed to accommodate a wide range of containers reliably, efficiently and with uniform product quality. Its gentle operation can significantly reduce packaging costs by permitting the use of thinner, less expensive packaging material. It works on the hydrostatic principle which facilitates temperature changes and therefore, product variations.

Designed to be easily integrated into existing automatic production lines, the Hydromatic can process different products and different containers simultaneously. Rugged, high-grade construction permits outdoor installation while the compact design of this equipment calls for a minimum of space and little supervision.

Further information on the Stork-Hydromatic sterilizer, contact: W. Kornmann, Stork Food Machinery, Inc., P.O. Box 816, Somerville, New Jersey 08876.

New Directory of Food Labs Available

A directory listing more than 400 food testing laboratories and consulting organizations, including a description of their capabilities and areas of expertise, has just been made available by the Institute of Food Technologists here.

The new IFT "Regional Guide of Food Testing Laboratories and Consultants" is organized according to the six U.S. Census regions, to make it easier to locate needed services in a given part of the country. In addition to brief descriptions of testing capabilities and consulting services for each organization, the directory carries full addresses and the name and phone number of the director of each organization.

The 36-page directory is available from IFT Regional Guide, Institute of Food Technologists, Suite 2120, 221 N. LaSalle St., Chicago, IL 60601 for $5.00 per copy, postpaid.

New Lubricant Guide for Food Processors Offered By Pennwalt

A new lubricant selector guide for the food processing industries has been published by Pennwalt's Keystone Division.

The 16-page manual reviews properties and recommends specific applications for Keystone's Nevastone® line of 18 different greases, oils, and sprays specially compounded for food processors. Included are lubricants, release agents and anti-rust films, acceptable for use where there is contact with product (U.S.D.A. "AA") and where there is no direct contact ("BB").

A detailed table of recommendations indicates the proper lubricant, by type of equipment, for various segments of the food processing industries: bakeries, breweries, dairies, fruit and vegetable processing and soft drink plants, plus a section on general plant lubrication for all of the above.

A copy of the "Food Processing Lubricant Selector Guide" can be obtained from the Keystone Division, Pennwalt Corporation, 21st and Lippincott Sts., Philadelphia, Pa., 19132.

Pennwalt Corporation, headquartered in Philadelphia, produces chemicals, health products (dental and pharmaceutical), and precision equipment.
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North Texas Sponsors Winter Conference

More than 100 area dairymen and sanitarians met at the Sheraton Inn-Oak Cliff in Dallas for the 1st Annual NTAMFES Winter Conference.

The first annual Winter Conference of the North Texas Association of Milk, Food and Environmental Sanitarians was held on January 28 at Dallas.

More than 100 persons heard Dr. Nelson Philpot, the featured speaker, discuss "Control Methods for the Sanitarian, Fieldman, Dairy Farmer, and Veterinarian." Mr. Gary Douglas reviewed the "Rapid Detection of Antibiotics in Milk" thru use of the Delvotest. Mr. H. A. Turney, area entomologist, spoke during the afternoon session on "The Requirements for Pesticide Applicators" in Texas. The panel discussion on "New Legislation Affecting the Food Industry" was presented by Mr. Dennis Baker, Texas State Department of Health, Mr. Jim Blankinship, City of Dallas Health Department, and Mr. Joe Mashburn, 1977 NTAMFES Vice-President and Supervisor for Product Safety & Sanitation, Anderson-Clayton Foods.

Iowa Affiliate Plans for 1977 Annual Meeting

Local Arrangements Committee members discussed their plans with members of the Board. Standing (left to right) are Dr. H. V. Atherton, President, IAMFES, Richard Lane, LAC member, David Kreich, LAC member, Jim Burkett, LAC member. Seated are Dwane Hagedon, (left) chairperson LAC, and David Fry, IAMFES President-elect.
Association Affairs
Texas Association of Milk, Food and Environmental Protection Organizes

TAMFEP officers and (left to right): Del Madkins, board representative, Clair Gothard, president, Janet Greenwood, archivist, and Joe Klinger, vice-president. Not pictured were Dr. C. W. Dill, president-elect and Dr. Ranze Nickelson, II, secretary-treasurer.

A group of food sanitarians and other interested persons met January 10, 1977 at Texas A&M University to form an organization to be affiliated with the International Association of Milk, Food and Environmental Sanitarians. The group adopted a constitution and by-laws and elected a temporary board of directors.
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**Program**

**Association Affairs**

**PROGRAM**

Florida Association of Milk, Food and Environmental Sanitarians

Annual Meeting
March 15-17, 1977

Tuesday March 15, 1977

9:00 a.m.-1:00 p.m. Registration, committee meetings.

**PRESIDING:** Dave Fry, T. G. Lee Foods, Orlando, Florida

1:00 p.m. Welcome—Donald L. Crenshaw, Commissioner, City of Orlando

1:05 p.m. Welcome—Jay Boosinger, FAMFES President

1:15 p.m. Overview of Pest Control in the Food Manufacturing Plant, Ronald Snyder, Southern Milk Creek Products, Inc.

2:15 p.m. Milk Break

2:45 p.m. Determining and Controlling Microbe Contamination in the Food Plant—B. Sohn and J. Peters, Millipore Corporation

3:15 p.m. Exotic Fermented Dairy Foods—Dr. Ebenezer Vedamuthu, Microlife Technics

3:45 p.m. Environmental Radiation Monitoring—Dr. Wallace B. Johnson

4:15 p.m. Report to the Affiliate on Activities of the International Dave Fry, International President-Elect

**Wednesday, March 16, 1977**

**PRESIDING:** Dr. L. A. Scribner, Orange Co. Health Department, Orlando, Florida

8:45 a.m. The Quality Assurance Program at Burger King—W. D. Trebbi, Director, Product Quality

9:15 a.m. The Taylor Soft Serve Freezer—Actual Freezing of Samples—Bill Cooper, Edwards Equipment Company of Florida

9:45 a.m. Milk Break

10:15 a.m. Continuation of Taylor Freezer—Cleaning—Sanitizing—Maintenance

**Wednesday, March 16, 1977**

Laboratory Session

**PRESIDING:** Lupe Wiltsie, Borden, Inc. Miami, Florida

8:45 a.m. Methods of Determining Viable Lactobacillus Acidophilus Organisms in Pasteurized Milk—Dr. Eb Vedamuthu, Microlife Technics

9:15 a.m. Rapid Determination of Psychrotrophics—Bill Thornhill, Dairy Division, Florida Department of Agriculture and Consumer Services

9:45 a.m. Milk Break

10:15 a.m. Protein Determination in Frozen Dairy Foods—Don Lehman, Foss America Corporation

10:45 a.m. Entrance of Pesticides into the Food Chain—W. B. Wheeler, University of Florida

**Wednesday, March 16, 1977**

**PRESIDING:** Joe Hays, Food Equipment and Supply, Tampa, Florida

1:15 p.m. Clean Rooms and Plant Air for Sterile Processing—Steven Foodfellow, ABC Research Corporation

1:45 p.m. Sterile Processing Techniques—Joe Mitzan, DeLaval Separator Company

2:30 p.m. Milk Break

3:00 p.m. Sterile Packaging Techniques—Said Farahnik, Ex-Cell-O Corporation

3:30 p.m. Cleaning and Sanitizing Sterile Equipment—Jeff Malone, Economics Laboratory, Inc.

**NOTICE**

Second Printing

"PROCEDURES TO INVESTIGATE FOODBORNE ILLNESS"

The overwhelming response to the publication of the 3rd Edition of "Procedures to Investigate Foodborne Illness" has resulted in a temporary shortage of the publication. The 3rd Edition's second printing will be off the press March 1, 1977, and orders for the publication will be filled and processed as soon as possible after that time. The prices quoted for "Procedures" in the first printing will remain in effect for the second printing. However, the cost of pads of investigational forms has been lowered as follows:

Forms A, D, E, G and H—$2.35 per pad of 100

Forms B, C and F—$3.25 per pad of 100

Wednesday, March 16, 1977

**PRESIDING:** Dick Jolley, Suncoast Producers Coop, Tampa, Florida

1:15 p.m. Microbiology of Pop-top Cans—J. E. Kennedy, Jr., and J. L. Oblinger, University of Florida

1:35 p.m. Quality Control Program at Anheuser-Busch Brewery—Bohdan V. Bemko

2:05 p.m. Quality of Florida's Natural Water Bodies—Dr. Tim Stuart, Department of Environmental Regulation

2:35 p.m. Milk Break

3:05 p.m. Florida's Certified Food Manager Program—A. W. Morrison, Environmental Health Program, Florida Department of Health & Rehabilitative Services

3:35 p.m. Microbiology of Soy Supplemented Ground Meat—B. A. Masters and J. L. Oblinger, University of Florida

4:00 p.m. Business Meeting

6:30 p.m. Social Hour

7:30 p.m. Awards Banquet

**Thursday, March 17, 1977**

**PRESIDING:** Tom Hart, Hart's Dairy, Fort Myers, Florida

8:45 a.m. Dairy Plant Waste Monitoring—John Bechtol, Borden, Inc., Orlando, Florida

9:15 a.m. Foodborne Illnesses, Sources and Symptoms—Dr. Ken Smith, University of Florida

9:45 a.m. Milk Break

10:15 a.m. Update on Inhibitor Testing—Gary Douglas, Enzyme Development Corporation

10:45 a.m. Problems with Florida's School Milk—Bill Brown, Dairy Division, Florida Department of Agriculture and Consumer Services
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Dairy authorities speak out on better cow milking

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

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

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

LABOR TAKES A BIG BITE

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

With an investment to modernize milking parlors, including unit take-off, it is not unusual to substantially lower the labor costs of producing milk.

Many of the milking chores are repetitious and result in drudgery. According to our studies, 5 to 10 percent of the milker’s chore time is spent removing the milking unit. On top of that, the typical milker spends from 12 to 30 percent of his time machine- striping cows.

THE OPERATOR IS A BUSY MAN

Proper stimulation of cows in a milking parlor is important to good milk letdown. Recent New Zealand work shows that there is a loss of up to 1,000 pounds of milk per cow yearly when cows are not properly stimulated. In many barns the milker cannot effectively handle as many milking units as today’s economy demands. Frequently, washing and stimulation time is limited to less than 15 seconds per cow because the milker is too “busy” with machine stripping or handling other units. The result is slow milking combined with considerable overmilking. Automatic unit take-off should improve this situation. Addition of automated prep stalls will help even more, provided they function properly.

SOME RESEARCH RESULTS

Research studies comparing automatic take-off and conventional milking units involving 550 cows in a Louisiana herd resulted in these conclusions:

1. Automatic unit take-off significantly reduced the number of quarters infected with mastitis.
2. Automatic unit take-off reduced udder irritation as evidenced by lower CMT scores.
3. The men operating the automatic take-off units reduced their walking distance in the parlor by more than 25%.

Dr. Nelson Philpot, leader of this study, says one should not expect miracles. Automatic take-off units do not make a poor operator better. They do, however, allow a conscientious operator to do an even better job on more cows.

According to dairymen using this equipment, proper maintenance and proper operation of equipment is even more important than automated systems. The ability, cooperative attitude and location of your local serviceman should become a primary factor in deciding whether to install this more sophisticated and expensive equipment.

MASTITIS PREVENTION NOT SIMPLE

Dairymen should not necessarily expect a reduction in the number of cows requiring treatment for clinical mastitis (gagart). In a marginal system, more cases may result because the significance of a single variable is not the same in every milking system or in every situation.

Frank Smith, California milking system specialist, illustrates this point well. He indicates that too many researchers and educators have attempted to oversimplify the cause of mastitis. In turn, they have over-simplified its prevention. The concept of a direct, independent relationship shown in Figure 1 is incorrect. Figure 2 arranges these same variables in a manner which is sequential, additive, and interdependent.

As mentioned earlier, installing automatic unit take-off may allow one to milk cows faster and reduce overmilking. However, if such a change resulted in flooded milk lines and fluctuating vacuum, the incidence of mastitis might increase rather than decrease. Providing all other deficiencies in the system were corrected, automatic take-off would prove highly beneficial.

AUTOMATIC TAKE-OFFS A COMMON SIGHT?

Where cost of this mechanization is not excessive and such installations prove to be reasonably trouble-free over time, I’m sure that automatic take-off units will become an increasingly more common sight on dairy farms.

*For our purposes, the labor figures include all dairy chore labor, feeding labor, and the raising of offspring. Field labor isn’t included.

**In 250-cow and 500-cow herds, we assume the existence of a parlor and a free-stall barn with mechanized feeding and waste handling.

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This is one of a series of topics developed by noted Dairy authorities. For a complete set write for a free booklet.