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Journal of Milk and Food Technology

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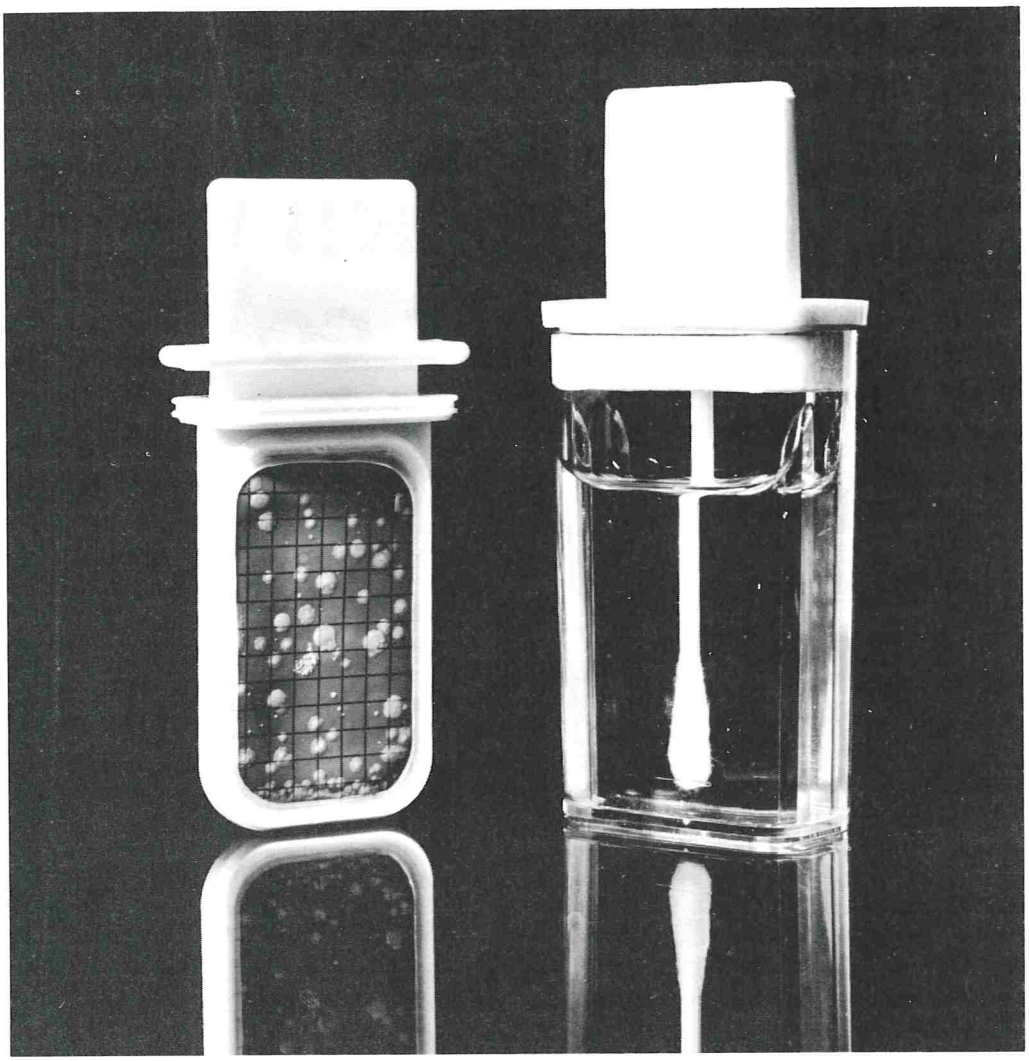

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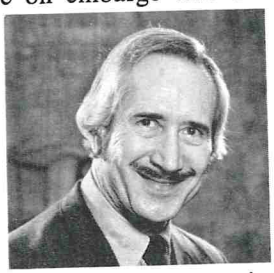
BEYOND WHICH WE WILL NOT GO

Plain, ordinary non-political citizens have recently opened a new chapter in American history. They have caused the cancellation of multi-billion dollar projects including the Florida barge canal, the supersonic transport and two huge jetports because these ventures threatened the *environment*. That was before the oil embargo and the ensuing recession. Today, in a 180° reversal, many of these same citizens are less concerned about auto emissions than they are about sticker prices. They are likely to vote "No" on higher taxes to pay for better sewage treatment and they will be up in arms if ecologists propose any pollution control measures that eliminate local jobs.



At NSF we are not alarmed but we are concerned about these shifts in public sentiment. We have had decades of experience in trade-offs which put environmental quality on one side of the scales and socioeconomic values on the other. We have helped to establish more than 50 national standards for products that affect public health or environmental safety, and each of these NSF standards is based on consensus agreements between representatices of *government, industry* and the *consuming public*.

Out of this experience we have learned one great lesson: In every environmental situation there is a cutoff point beyond which no responsible citizen or agency should go. At this point, executives from industry refuse to give further consideration to profits. Public officials turn their backs on powerful special interests. Consumers stand ready to express themselves in no uncertain terms at ballot boxes and cash registers.

How do these diverse groups within our society reach a point of decision and a basis for agreement? They do it with facts that take the place of partisan rhetoric. They rely on the impartial, scientific findings of environmental laboratories such as we maintain at NSF in Ann Arbor. Parties to environmental standards arrive at pragmatic agreements on specifics such as parts per million, pounds per square inch or gallons per hour.

As a citizen, you are going to be called upon in the next few years to make judgments about the environment. You will have to weigh the cost of each to you in terms of expense, inconvenience or diminished freedoms. Many of your decisions may have little effect on your well being today but they may irreversibly affect the kind of world you pass on to your children or grandchildren. That is why *we cannot afford to forsake environmental quality even in a recession.*

As environmentalists who have close ties to public health agencies, industry and consumer organizations we urge you to take an active interest in environmental issues. Insist on fact finding and be guided in your judgment by demonstrable human costs as well as dollars.

Today our nation is faced with health care expenses that are soaring out of sight. A routine stay of seven days in a hospital can now exceed $1,300. One of the best and surest ways of bringing the cost of health care under control lies in the *prevention* of disease and injury.

A quality environment is the most basic defense of all.

Robert W. Brown
President

NSF

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A Rapid Method for the Aseptic Collection of Tissue

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(Received for publication March 20, 1975)

ABSTRACT

The existing methods of collecting muscle tissue samples, with a low microbial level, have many disadvantages which make them impractical for many research facilities. A procedure is described for obtaining 100 g samples of tissue containing very low microbial levels. The procedure consists of using a sterile coring device to remove samples deep within the *longissimus dorsi* muscle. The deeper tissue contains little, if any, bacterial counts since air contamination has not occurred. Of the 48 pork samples collected, 34% were found to be sterile (< 1 organism/g) and 64% had a level of ≤ 5 microorganisms per gram. Of the 23 beef samples collected, 22% were sterile (< 1 organism/g) and 74% had a contamination of ≤5 microorganisms per gram. With this procedure, a researcher with modest facilities can examine changes that occur in muscle tissue caused by specific microorganisms, enzymes and other treatments.

Aseptic tissue for some time has been recognized as a valuable tool to the meat researcher. It provides a means of separating microbial deterioration from that which is chemical and/or enzymatic in nature (*2, 4, 5, 7, 8, 9, 11, 12, 13, 14, 15, 17*). It is generally agreed that, excluding the lymphatic system, most normal muscle tissue is sterile or relatively free of microorganisms, immediately post-mortem (*12, 16, 17*). The problem has been one of collecting this tissue without contamination. The four methods (*3, 7, 9, 12, 13, 17*) most commonly used are: the gnotobiotic method, surgical isolator method, V-trap method, and flaming method. These methods have produced tissue which is sterile or extremely low in bacterial numbers. The disadvantages of these techniques are: high cost, a need for special equipment, a need for special facilities, a large amount of time required to collect a limited amount of tissue, denaturization of tissue and oxidation of tissue due to chemicals. The following method has been developed to eliminate many of these disadvantages. Using this technique, it is possible for researchers to obtain aseptic tissue easily and inexpensively in their own laboratories.

MATERIALS AND METHODS

The sampling device was constructed from a widemouth, glass-stoppered, clear 500 ml bottle, and a metal cylindrical coring device. The instrument was made from an internally and externally chromed, brass "tail piece" with a diameter of 38.1 mm and a length of 609.6 mm, purchased from a plumbing supply house. One end of the instrument was flanged. This material was relatively soft and the non-flanged end was easily sharpened with a fine file and abrasive emery paper (1/0 fine). The edge was easily maintained with the use of a butcher's steel.

The flanged end of the coring device produced a snug fit when placed into the mouth of the bottle. The edge between the coring device and the mouth of the container was sealed with plastic tape (Fig. 1).

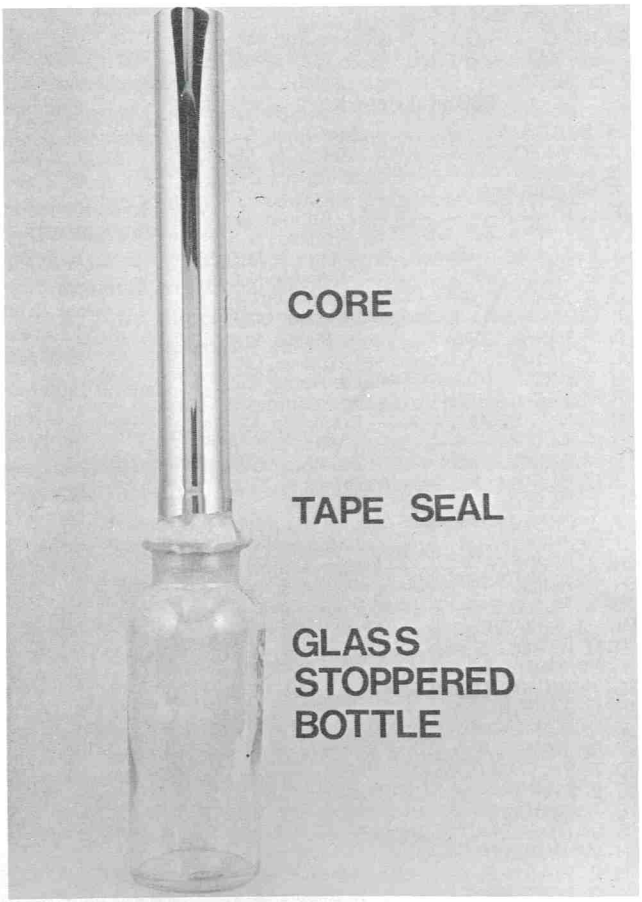

Figure 1. *Sampling unit.*

The following procedures were conducted on a stainless steel surface which was washed with a soap solution containing Wescodyne ("tamed iodine" detergent and germicide). This was followed by a flushing with sterile distilled water and final rinse with a 1:1250 solution of Roccal. The surface was allowed to air dry. The laboratory exhaust vents were shut down prior to the operations to prevent any unnecessary air movement. All equipment used for the removal of the samples was thoroughly washed with Alconox laboratory detergent, rinsed with distilled water, and placed in a 100 C Boekel Drying Oven. These items

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[2]*Approved as Journal Article No. 16-73 by the Associate Director of the Ohio Agricultural Research and Development Center, Wooster.*

were then wrapped in a double layer of paper to prevent contamination after sterilization. Paper caps were placed over the sharpened ends of the cores. All of the items were then transferred to a Barnstead Sterilizer and autoclaved for 15 min at 15 PSI and 121 C. Samples were removed from both pork and beef loins. The procedure used was basically the same for both species except one to three cores can be removed from the same area of one beef *longissimus dorsi,* and only one from pork. Pork will be used for the following discussion.

The pork loins were selected on the basis of physical appearance. Loins selected were firm, had a pinkish-red color, were free from bruises and defects, and had an unbroken pleura membrane. The loin was placed in such a position that the thoracic vertebra could be sawed. To sterilize the saw, the blade was dipped in a 95% ethyl alcohol solution and flamed. Two saw cuts were made—the first between the sixth and seventh ribs and a second between the eleventh and twelfth ribs. The saw was allowed to penetrate only deep enough to split the thoracic vertebrae without penetrating into the *longissimus dorsi* muscle. A sharpened knife, which was flamed, was slipped between the sawed vertebra in the sixth and seventh rib area and with a single cut, a three rib section was removed. The three rib section was held against the remainder of the loin so as not to expose the *longissimus* muscle to air. After removing the paper cap, the sampling unit immediately was placed against the *longissimus dorsi* muscle at the seventh rib. By the use of a twisting motion, the core was allowed to penetrate until it approached the sawed vertebrae at the eleventh rib. A second knife cut was made between the eleventh and twelfth ribs. The core was continued until it exited from freshly exposed surface. The sampling unit was placed upright, and the sample was pushed through the core into the glass container by the use of a sterile glass rod. The core and tape were separated from the glass-stoppered bottle. The stopper was dipped in 95% ethyl alcohol, flamed, and placed in the bottle. Plastic tape was used to seal the edges of the stopper. The sample size obtained was approximately 100 g.

RESULTS AND DISCUSSION

Quality of core method

The core technique was evaluated by collecting samples of beef and pork *longissimus dorsi* muscle, and measuring the amount of bacterial contamination. These samples were analyzed using the standard total plate count method (*1, 6, 10*).

Tryptone Glucose Extract agar was used as the medium with an incubation time of 48 h at 37 C (± 1 C). Since preliminary work showed the total number of microbes to be extremely low, a gram of tissue was plated on 10 plates, and the total count for the 10 plates was expressed as the count/g. A total of 48 pork samples and 23 beef samples was obtained by the method described. Tables 1 and 2 show the total bacterial counts of the samples.

TABLE 1. *Total aerobic bacterial counts of pork samples collected by the core technique*

Range of total bacterial count/g	Number of samples	Percent of total samples
0	17	35
1 - 5	14	29
6 - 10	6	13
11 - 30	6	13
31 - 50	3	6
51 - 110	2	4
Total	48	100

TABLE 2. *Total aerobic bacterial counts of beef samples collected by the core technique*

Range of total bacterial count/g	Number of samples	Percent total samples
0	5	22
1 - 5	12	52
6 - 10	4	17
11 - 30	1	4
31 - 50	1	4
Total	23	99

Of the 48 pork samples collected, 35% were aseptic. A total of 64% had a contamination of $\leq$5 microorganisms per g. Of the 23 beef samples collected, 22% were aseptic. A total of 74% had a contamination of $\leq$5 microorganisms per g.

Advantages of the core method

A number of advantages are evident in the use of the core method as a means of collecting aseptic tissue. Cost is a factor of great importance to most researchers. The core method was found to be an inexpensive method for tissue collection. The total cost for each sampling unit was under $3.00. This is far below the cost of a surgical isolator, a positive pressure pump, and chemical supplies needed for the isolator technique. The salvage value of the non-aseptic tissue was much higher for the core method. This method does not damage the remaining muscle tissue, allowing it to be further processed for human consumption. No special facilities, other than a room with little air movement, is required for the core method.

The amount of time necessary for tissue collection is greatly reduced by the core method. Techniques requiring an isolator have a 5 or 6 day sterilization period for the internal environment of the chamber. Sterilization of the core units require less than an hour. This suggests that if samples are to be collected over periods of time, the core method may be more advantageous.

A final advantage is that the samples collected are uniform in shape and weight. This makes dissection and/or inoculation of the tissue easier to perform.

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I certify that the statements made by my above are correct and complete.

Earl O. Wright, Managing Editor

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Further Studies on a Growth Medium for *Lactobacillus sanfrancisco*

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(Received for publication March 14, 1975)

ABSTRACT

The medium developed for the original isolation studies on *Lactobacillus sanfrancisco* has been further refined to provide a basis for development of media for commercial preparation of cell concentrates. Addition of salts, purines, pyrimidines, and optimization of casein hydrolysate and Tween 80[1] levels resulted in markedly improved growth but did not eliminate the need for freshly prepared yeast extractives (FYE). Commercial yeast, liver, corn steep, and protein hydrolysate preparations tested were inadequate substitutes for the FYE. High maltose levels appear to stabilize viable counts over longer incubation periods. Some differences between strains in growth requirements were observed. Strains L and T were the least fastidious nutritionally, appeared indifferent to oxygen, and had a minimal requirement for Tween 80, but a primary requirement for purines-pyrimidines. Strains B and C were inhibited by oxygen and had a primary requirement for trace element supplementation and Tween 80. Strain L which showed the most rapid and heaviest growth has been selected as the type strain and the one to be utilized in initial commercial tests as a starter culture preparation.

A recent report by the present authors (*2*) described the isolation and preliminary characterization of an apparently previously undescribed species of heterofermentative *Lactobacillus* from San Francisco sour doughs (used in preparation of French bread) for which we proposed the name *Lactobacillus sanfrancisco* pending confirmation by the techniques of genetic compositional analyses and hybridization. These latter studies, assigned by us under contract to another laboratory, are completed and have established that these sour dough isolates are, indeed, not related genetically to any other known species but are closely related to each other (*6*) thereby lending additional support to the validity of selecting a new species designation. Of the four strains or isolates studied (C, B, L, T), the L strain (more specifically L-12) grows out most readily on the artificial media devised and is designated as the type strain.

In the previous report, nutritional emphasis was on development of a medium which would permit successful isolation, and adequate outgrowth for enumeration purposes, of the sour dough lactobacillus from flour-containing systems. As pointed out in this previous report no growth was obtained on the usual agar media used for lactobacilli including APT, LBS, tomato juice, Elliker's, orange juice, thioglycolate, wort, plate count, as well as media used specifically for enumeration of lactic acid or other bacteria in flours and dough as tested both aerobically and anaerobically (*2*). The present investigation deals with refinement of the medium with regard to the levels and interrelationships of the various ingredients with a view to maximizing growth and to minimizing the need for freshly prepared yeast extractives previously reported to be required at substantial levels. The direction of this study was oriented towards providing a basis for development of a practical medium for production of *L. sanfrancisco* starter cultures rather than development of a synthetic medium with all essential components identified.

[1]*Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.*

MATERIALS AND METHODS

Organisms

The sour dough isolates used in these studies were those previously described by us (*2*) and are identical with the isolates used in the genetic analyses referred to above. They are on deposit in stock culture collections as follows: L : NRRL B-3934, ATCC 27651; T : NRRL B-3935, ATCC 27653; B : NRRL B-3932. ATCC 27652: C : NRRL B-3933.

Culture media

The basal broth medium used previously (*2*) served as a starting point for the present studies. This medium consisted of maltose, 2.0%; yeast extract (Difco), 0.3%; freshly prepared yeast extract solids (FYE), 0.5%; Trypticase (BBL), 0.6%; and Tween 80, 0.03%. The pH was adjusted to 5.6 with 1 N to 6 N HC1 before the medium was autoclaved. The FYE were prepared as previously described by autoclaving a 20% suspension of commercial compressed Bakers yeast in distilled water; the clarified supernatant was used directly or after freezing or freeze-drying. Supplementary solutions or ingredients used were as follows: Salts A: 25 g each of K_2HPO_4 and KH_2PO_4 per 250 ml of water. Ten milliliters of this solution used per liter = 0.1% of each in final medium. Salts B: $MgSo_4 \cdot 7\ H_2O$, 10.0 g; $MnSO_4 \cdot 2\ H_2O$, 2.0 g; $\pm\ FeSO_4 \cdot 7\ H_2O$, 0.5 g; per 250 ml of water. Five milliliters of this solution were used per liter of final medium to give the following concentrations in mg/liter (ppm): Fe, 2; Mn, 12; Mg, 20 ($FeSO_4$ was not included except where specifically noted). Purines-pyrimidines (PP): 0.20 g each of adenine sulfate, guanine hydrochloride, uracil, xanthine, and thymine per 100 ml of water (dissolved with the aid of 6 N NaOH). Ten milliliters of this solution were used per liter of final medium to give concentrations of 20 mg of each base per liter. Organic supplements: Nutritional Biochemicals Corporation, Cleveland, Ohio, (NBC): Lactalbumin Hydrolysate Enzymatic, Soy Hydrolysate, Casein Hydrolysate Enzymatic, Yeast Hydrolysate Enzymatic, Liver Extract Concentrate 1:20, Liver Fraction L, Liver Fraction S. BBL Division of Bioquest, Cockeysville, Maryland (BBL): Trypticase, Malt Extract, Yeast Extract. Difco Laboratories Inc., Detroit, Michigan, (Difco): Yeast Extract, Yeast Autolysate. Consolidated Lab., Inc., Chicago Heights, Illinois: Yeast Extract Oxoid L-20, Yeast Autolysate Colab

11-54F and 11-54K, Yeast Autolysates (partial autolysates) 11-54G and 11-54H. CPC International Inc., Industrial Division, Englewood Cliffs, New Jersey, (CPC): Corn Steep Water Concentrate E801, CPC Bioenhancer 1075A. Sheffield Farms Company, Inc., New York City, New York: NZ-Case (tryptic digested casein). A. E. Staley, Decatur, Illinois: Yeastamin 75N.

Method of cultivation

Growth studies were done by inoculating 100 ml of broth in 250 ml screw cap Erlenmeyer flasks with 1% of an actively growing 24-h broth culture and incubating at 30 to 31 C with mild rotatory agitation (100 excursions/min). Unless otherwise specified, flasks were gassed with CO_2 by evacuating the loosely-capped flasks in a vacuum desiccator to about 50 mm Hg followed by refilling with CO_2, repeating the process, and then tightening the screw cap before placing in the incubator. The pH of the cultures was not adjusted (i.e., not neutralized) during growth.

Growth measurements

Growth was estimated by measurement of turbidity (optical density at 525 nm in a Bausch & Lomb Spectronic 20) after first diluting the culture either 1:5 or 1:10 with distilled water and/or by determination of cell dry weight. For the latter, cells from 40 ml of culture were separated by centrifuging at 10,000 rpm in a Sorvall RC-2 refrigerated centrifuge, resuspending in 40 ml of distilled water, recentrifuging, and then transferring the cells with a minimal amount of water to a tared aluminum weighing dish and dried in a forced air oven at 105 C for 3 h. Cell dry weights are reported per 100 ml of culture. The correlation between cell dry weight and O.D. was excellent up to an O.D. of approximately 0.7; an O.D. of 0.52 ± 0.01 was equivalent to a cell dry weight of 0.10 g per 100 ml of culture. Optical densities of 0.8 and above, although reported, were not always quantitative indicators of growth due to inadequate dilutions in some instances and wherever applicable are supplemented with or replaced by dry weight measurements. Viable cell counts in these studies, where the pH was not controlled, were found to be unreliable indicators of growth generally peaking at 19 to 24 h (at up to 3×10^9 per ml) and dropping off markedly between 24 and 42 to 48 h.

RESULTS

Effect of fresh yeast extractives (FYE)

FYE is required by all strains for heavy growth in addition to the basal medium, which contains 0.3% of a commercial yeast extract (Table 1). These results also illustrate both the general differences between strains in growth response on the broth medium developed and that the L strain grows out most luxuriously, particularly at below minimal levels of FYE, followed by strains T, B, and C in that order. Addition of higher levels of commercial yeast extracts does not off-set the need for FYE for the growth obtained at the maximum level (2.0%) of commercial yeast extracts was much less than that obtained at the minimum level (0.25 - 0.50%) of FYE (cf. Table 12). Changes in pH are not generally shown in these and the following tables but usually decreased from 5.6 to 3.4 - 3.7 in 24 to 42 h with the final pH not always correlating with growth possibly due to variations in the buffering capacity of the medium with variable levels of organic supplements.

Effect of salts

In light of the known nutritional requirements of other lactobacilli (*1, 4, 5*) and the heat stable and dialyzable nature of the FYE factors, one obvious possibility to explore was the contribution of salts, particularly trace elements. A preliminary experiment done with only strains B and C, and in the absence of added FYE, did, indeed, show markedly growth-stimulatory effects (Table 2). Mn, tested alone, with virtually as effective as the combined salts B, although Mg, also tested alone, did exert a slight stimulatory effect. Fe appeared unessential and was not tested further. In another study Salts A (KH_2PO_4 and K_2HPO_4) were found to be non-stimulatory for all four strains either when used alone or in the presence of Salts B.

TABLE 2. *Effects of salts B*

Supplement to base[1]	O.D. at 48 h (dil. 1:5) Strain C	B
None (base)	.01	.02
Salts B[2]	.35	.42
Fe only (2 ppm)	.01	.02
Mg only (20 ppm)	.10	.13
Mn only (12 ppm)	.31	.47

[1]Base = Same as Table 1
[2]Includes $FeSO_4$, $MnSO_4$, and $MgSO_4$.

TABLE 3. *Enhanced effects of salts B in presence of purine-pyrimidines (PP)*

Supplement to base[1]	O.D. at 42 h (dil. 1:5) Strain C	B	L	T
None (base)	.02	.03	.20	.09
PP	.02	.03	.18	.08
Salts B	.16	.44	.24	.22
PP + Salts B	.26	.55	.53	.57
0.5% FYE	.26	.34	.66	.52

[1]Base = Same as Table 1

As shown in Table 3, the stimulatory effect of Salts B on strains L and T is much less than that observed with strains B and C unless the base medium is also supplemented with a purine-pyrimidine mixture. These interdependencies will be subsequently further discussed below.

With regard to the efficacy of substituting known compounds for FYE the data thus far (Table 3) also indicate that addition of both Salts B and the purine-pyrimidine supplement resulted in a growth-stimulatory effect roughly equivalent to that obtained by

TABLE 1. *Stimulatory effects of fresh yeast extractives (FYE)*

FYE solids added to base[1] (%)	O.D. at 42 h (dil. 1:5) Strain C	B	L	T	Cell dry weight (g)/100 ml at 42 h Strain C	B	L	T
0	.02	.03	.19	.09	.005	.005	.071	.013
0.5	.24	.44	.73	.57	.045	.079	.165	.105
1.5	.66	.81	1.00	.95	.175	.202	.303	.238

[1]Base = Maltose, 2.0%; Trypticase, 0.6%; Difco Yeast Extract, 0.3%; Tween 80, 0.03%; pH 5.6.

addition of 0.5% FYE, at least for strains C, B and T.

The absolute requirement (i.e., in a purified medium) for Mn and Mg was not determined but effect of graded levels of each added to the base medium modified to include the purines-pyrimidine supplement is illustrated in Table 4. Generally it would appear that addition of 20 ppm Mg and 1 to 5 ppm of Mn suffice for maximum growth which is in accord with levels generally recommended for growth of other lactobacilli (*1*).

TABLE 4. *Varying levels of Mg and Mn*

Supplement to base[1]	O.D. at 42 h (dil. 1:5) Strain C	B	L	T
None (base)	.01	.02	.17	.06
10 ppm Mg	.03	.05	.18	.07
20 ppm Mg	.03	.09	.18	.08
40 ppm Mg	.04	.11	.21	.06
20 ppm Mg + 1 ppm Mn	.18	.33	.49	.54
20 ppm Mg + 5 ppm Mn	.23	.43	.47	.54
20 ppm Mg + 12 ppm Mn	.24	.47	.47	.56

[1]Base = Same as Table 1 but with addition of PP (Purines-Pyrimidines).

TABLE 5. *Effects of salts B in presence of FYE*

Medium	O.D. at 48 h (dil. 1:5) Strain C	B	L	T
Base[1] + 0.25 FYE	.05	.16	—	—
Base[1] + 0.25 FYE + Salts B	.46	.64	—	—
Base[1] + 0.25 FYE + Mn only	.42	.70	—	—
Base[1] + 0.25 FYE + Mg only	.22	.30	—	—
Base[1] + 0.50 FYE	.27	.43	.68	.61
Base[1] + 0.50 FYE + Salts B	.39	.83	.61	.63
Base[1] + 0.50 FYE + PP	.31	.36	.74	.67
Base[1] + 0.50 FYE + PP + Salts B	.47	.82	.75	.84

[1]Base = Same as Table 1.

Table 5 shows that the beneficial effect of Salts B on growth persisted even when FYE was included in the medium at a level of 0.5% either alone or in conjunction with added purines-pyrimidines. The effects of Salts B at higher levels of FYE was not examined.

Effect of purines and pyrimidines

As indicated in Table 3, addition of the PP mixture by itself, i.e., in the absence of Salts B, appeared to be without effect on growth of any of the strains but, in the presence of Salts B, was markedly stimulatory for all strains. The absence of PP appeared to be more of a limiting factor on the growth of strains L and T (as compared to C and B) and, consequently, the addition of PP was necessary to maximize the stimulatory effect of Salts B on strains L and T. Results in Table 6 show that the beneficial effects of PP on growth in the presence of Salts B persists even when FYE are included up to a level of 1.0% but appear to become unessential at an FYE level of 1.5%. In Table 7, various protein, liver, and yeast preparations were evaluated as possible sources of PP. The casein, soy, and lactalbumin hydrolysates appeared to be relatively poor sources as the addition of PP produced significant and roughly equivalent growth increments in these instances. However, both the yeast hydrolysate and the liver concentrate produced, with one exception (strain B, liver concentrate), growth responses that appeared indifferent to further supplementation with PP suggesting that these preparations might be useful as inexpensive sources of PP. This was further explored in a limited study shown in Table 8 where the effect of a relatively low level (0.1%) of yeast hydrolysate was found to be as beneficial as the addition of PP and superior to the effect of Trypticase added at the same level. These results are considered suggestive rather than conclusive but worthy of further exploration.

TABLE 6. *Effects of purine-pyrimidines in presence of FYE*

Supplement to base[1]	Cell dry weight (g)/100 ml at 42 h Strain C	B	L	T
Base + 0.5% FYE	.095	.182	.162	.115
Base + 0.5% FYE + PP	.157	.190	.206	.149
Base + 1.0% FYE	.144	.223	.217	.165
Base + 1.0% FYE + PP	.182	.239	.254	.190
Base + 1.5% FYE	.196	.253	.290	.204
Base + 1.5% FYE + PP	.196	.266	.281	.201

[1]Base = Same as Table 1 but Trypticase increase to 1.0% and Salts B included.

TABLE 7. *Effect of PP with various supplements*

Supplement to base[1]	O.D. at 42 h (dil. 1:5) Strain C	B	L	T
0.6% Trypticase	.11	.46	.27	.20
0.6% Trypticase + PP	.32	.54	.48	.55
0.6% Casein hydrol.	.14	.33	.32	.27
0.6% Casein hydrol. + PP	.24	.49	.48	.57
0.6% Soy hydrol.	.13	.41	.23	.23
0.6% Soy hydrol. + PP	.24	.49	.42	.55
0.6% Lactalb. hydrol.	.15	.20	.31	.26
0.6% Lactalb. hydrol. + PP	.24	.40	.47	.52
0.6% Yeast hydrol.	.18	.34	.28	.39
0.6% Yeast hydrol. + PP	.19	.37	.28	.36
0.6% Liver conc.	.18	.21	.40	.60
0.6% Liver conc. + PP	.25	.45	.48	.54

[1]Base = Same as Table 1 but Salts B added and Trypticase omitted.

TABLE 8. *Yeast hydrolysate as substitute for PP*

Supplement to base[1]	O.D. at 24 h (dil. 1:5) Strain L	T
None (base)	.31	.26
PP	.40	.37
0.1% Trypticase	.34	.32
0.1% Yeast hydrol.	.45	.40

[1]Base = Same as Table 1 with addition of Salts B.

Requirements for individual purines and pyrimidines were not studied. In one experiment the level of PP was doubled over that described under Methods without apparent beneficial or deleterious effect on any of the strains.

Effect of protein hydrolysate (Trypticase) level

As was noted from Table 7, comparable growth-stimulating effects were obtained with casein, soy, and lactablumin hydrolysates when compared at a level of 0.6% in a medium devoid of FYE. Results in Table 9 confirm that Trypticase as an example of an effective protein hydrolysate is virtually essential for good growth in the absence of FYE with the maximum effect

TABLE 9. *Determination of optimal trypticase level*

Trypticase added to base[1] (%)	O.D. at 42 h (dil. 1:5) Strain C	B	L	T	Cell dry weight (g)/100 ml at 42 h Strain C	B	L	T
A. (No FYE)								
0	.07	.06	.18	.23	—	—	—	—
0.5	.22	.52	.43	.49	—	—	—	—
1.0	.24	.56	.52	.56	—	—	—	—
1.5	.28	.68	.56	.62	—	—	—	—
2.0	.24	.67	.55	.70	—	—	—	—
B.[2] (+ 0.5 FYE)								
0	.64	.74	.72	.94	.073	.118	.147	.126
0.5	.68	.98	.98	1.04	.121	.172	.207	.176
1.0	.72	1.12	1.10	1.12	.123	.199	.247	.191
1.5	.58	1.16	1.04	1.08	.114	.202	.224	.199
2.0	.58	1.08	1.04	.98	.122	.198	.287	.198

[1]Base for A = Same as Table 1 but with Salts B and Purines-Pyrimidines (PP) added. Base for B = Same as A but also with addition of 0.5 Fresh Yeast Extract solids (FYE).
[2]Diluted 1:10 and O.D. values multiplied × 2 for comparative purposes.

obtained at a level of about 1.5%. In the presence of 0.5% FYE, Trypticase, while not as essential, was still markedly stimulatory with the effect leveling off at about 1.0%. However, the need for Trypticase, or other protein hydrolysate, at the higher FYE levels was not determined nor was the requirement for individual amino acids or peptides. Use of cell dry weights, as shown in Table 9, appear to be a more sensitive indicator of degree of growth particularly where heavy growth is involved.

Relative stimulatory effects of other organic supplements

Over a period of time, under various basal medium conditions, a number of organic supplements were tested as possible substitutes for FYE. These results are summarized in Table 10 relative to the effect of FYE given a value of 100. None of the supplements were equivalent to FYE when compared at the sensitive 0.5% level and, as subsequent results will show, increasing the level of one of the most promising materials (Yeastamin 75N) did not improve its relative value significantly.

TABLE 10. *Relative[1] stimulatory effect of various organic supplements compared at 0.5% level*

Compound added to base[2]	Deleted from base Salts B & PP	Trypticase	Difco YE
FYE	100	100	100
NZ case	8	—	—
Lactalb. hydrol.	41	41	—
Soy hydrol.	32	43	—
Trypticase	—	49	—
Casein hydrol.	19	48	—
Yeast hydrol.	—	22	55
Liver C	55	44	—
Liver L	40	—	—
Liver S	38	—	—
CPC bioenhancer	16	—	—
CPC corn steep conc.	—	50	—
Malt ext. (BBL)	0	12	—
Yeastamin 75N	47	—	75
Yeast ext. (BBL)	—	—	75
Yeast ext. (Difco)	—	—	43
Yeast ext. (Oxoid L-20)	—	—	26
Yeast autolys. (Colab-54F)	—	—	46
Yeast autolys. (Colab-54G)	—	—	52
Yeast autolys. (Colab-54H)	—	—	23
Yeast autolys. (Colab-54K)	—	—	19
Yeast autolys. (Difco)	—	—	52

[1]Average results obtained (O.D. and cell dry weight) for strains B, L, and T.
[2]Complete base = Maltose, 2.0%; Trypticase, 1.0%; Difco yeast extract, 0.3%; Tween 80, 0.03%; Salts B; PP; pH adjusted to 5.6.

Effects of FYE in the presence of Salts B and purines-pyrimidines

Before evaluating the effect of increasing the level of other supplements such as Yeastamin, it is seen in Table 11 that the stimulatory effects of FYE appear to continue to increase up to the maximum level tested of 2.0% even when other components are included at their optimum levels. Under the conditions of maximum nutrition the C strain appears to finally give heavy growth comparable at least to that of the B and T strains while the L strain continues to show the best growth in this particular experiment as well as the most consistantly heavy growth in most of the experiments.

TABLE 11. *Effects of fresh yeast extractives in presence of salts B and Purines-Pyrimidines (PP)*

FYE solids added to base[1] (%)	O.D. at 42 h (dil. 1:5)[2] Strain C	B	L	T	Cell dry weight (g)/100 ml at 42 h Strain C	B	L	T
0.5	.74	.92	.86	.68	.119	.148	.195	.108
1.0	.96	1.12	1.10	.76	.196	.215	.292	.154
1.5	1.16	1.20	1.24	.96	.236	.249	.411	.195
2.0	1.20	1.20	1.32	.92	.268	.276	.519	.216

[1]Base = Maltose, 2.0%; Trypticase, 1.0%; Tween 80, 0.03%; Salts B; PP; pH 5.6; (no Difco yeast extract).
[2]Diluted 1:10 and O.D. values multiplied × 2 for comparative purposes.

Increasing the level of other yeast supplements

Commercial yeast extract preparations while inferior to FYE are nevertheless, stimulatory and it remained to be determined whether they simply have a lower content of the same unidentified growth-promoting substances or indeed may supply only some of the essential substances contributed by FYE. Results in Tables 12 and 13 make it clear the latter is the case. In Table 12 it is seen that increasing the level of the Difco yeast extract over 0.5% is without further effect and that the maximum effectiveness of this additive (even at a level of 2.0%) is substantially less than that obtained at the lowest level (0.25%) of FYE. In the case of the Yeastamin its maximum stimulatory effect seemed to plateau at 1.0 to 1.5% at which point it was equivalent to between 0.25

TABLE 12. *Inability of commercial yeast extracts to substitute for FYE*

Yeast extract added to base[1] (%)	Cell dry weight (g)/100 ml at 42 h		
	Strain		
	B	L	T
None	.005	.032	.009
0.50 Difco	.107	—	.128
1.00 Difco	.100	—	.144
1.50 Difco	.108	—	.145
2.00 Difco	.104	—	.151
0.50 Yeastamin 75N	.137	.139	—
1.00 Yeastamin 75N	.143	.152	—
1.50 Yeastamin 75N	.149	.163	—
2.00 Yeastamin 75N	.153	.163	—
0.25 FYE	.132	.156	.168
0.50 FYE	.174	.182	.199
1.00 FYE	.220	.226	.243

[1]Base = Maltose, 2.0%; Trypticase, 1.5%; Tween 80, 0.03%; Salts B; PP; pH 5.6.

TABLE 13. *Inability of yeastamin to substitute for FYE*

Added to base[1]		Cell dry weight (g)/100 ml at 42 h			
FYE (%)	Yeastamin (%)	Strain C	B	L	T
0.5	—	.119	.148	.195	.108
1.0	—	.196	.215	.293	.154
1.5	—	.236	.249	.411	.195
2.0	—	.268	.276	.519	.216
0.5	1.0	.133	.145	.199	.125
1.0	1.0	.170	.186	.279	.159
1.5	1.0	.208	.216	.382	.194
2.0	1.0	.222	.234	.394	.206

[1]Base = Maltose, 2.0%; Trypticase, 1.0%; Tween 80, 0.03%; Salts B; PP; pH 5.6.

and 0.50% FYE. On the other hand, the growth-promoting properties of FYE continue to increase with increasing level added. The results in Table 13 further illustrate the futility of trying to substitute Yeastamin for FYE. Including Yeastamin at its maximum effective level of 1.0% is seen not to decrease the need for FYE, i.e., in this particular study, the use of the FYE at the lowest level tested (0.5%) made the addition of Yeastamin superfluous. In another study (results not shown) it was necessary to reduce the FYE supplement to 0.2% to obtain any significant growth-stimulation from Yeastamin. More recently other commercial yeast extracts of activity comparable to, or better than, Yeastamin have similarly been found to be grossly inadequate substitutes for FYE.

Maltose level

Studies to this point on factors affecting growth have been conducted without pH control and this includes the determination of optimal maltose levels of 1 to 2% (*2*). The experiment described in Table 14 was conducted with the pH being controlled at close to 4.7; without this control the pH would drop to 3.5 or less at the 2.0% maltose level. Increasing the maltose level to 3 to 4% is seen to, indeed, result in increased development of cell material (O.D.) and acidity production (KOH required for "neutralization"). However, as far as viable count is concerned, the main effect of the higher maltose levels appears to be in the maintenance of higher counts of viable cells over a longer incubation period, a factor, of course, of importance in the preparation of cell concentrates. In another study (data not shown) where slower growth was encountered a similar effect was observed: after 39 h incubation the viable counts at 1, 2, 3, and 4% maltose were $< 1 \times 10^6$, $< 1 \times 10^6$, 6.3×10^7 and 1.4×10^9 respectively.

It is noted that, with the optimal medium used, counts of close to 5×10^9 are obtainable in 14 h at 30 C using a 1% inoculum. The data also suggest that, for any particular maltose level, either the amount of KOH added or the optical density developed could be used to monitor growth and control harvest time or chilling the culture prior to harvest.

Miscellaneous

The four isolates, as previously reported (*2*), were obtained from the sour dough "starters" of four different bakeries in the San Francisco Bay Area. Although their growth and acid production appears comparable in dough systems (*3*), some consistent differences emerge with regard to their growth patterns and response on broth media. As noted earlier, the L strain grows best

TABLE 15. *Effect of Tween 80 level*

Tween 80 added to base[1] (%)	Cell dry weight (g)/100 ml at 42 h			
	Strain C	B	L	T
0	.012	.094	.166	.142
0.01	.100	.163	.240	.145
0.03	.138	.181	.214	.143
0.06	.138	.184	.202	.164
0.10	.139	.193	.229	.153

[1]Base = Maltose, 2.0%; Trypticase, 1.0%; Difco yeast extract, 0.3%; Salts B; PP; FYE, 0.5%; pH 5.6.

TABLE 14. *Effect of maltose level[1] with pH control[2] of medium (strain L)*

Incubation time at 30° C (hr)	O.D. at 525 nm[3] maltose level (%)				Cumulative 4N KOH added (ml per 200 ml medium) maltose level (%)				Viable count/ml[4] maltose level (%)			
	1	2	3	4	1	2	3	4	1	2	3	4
14	.84	1.14	1.15	1.18	1.40	2.48	3.15	2.85	3.3×10^9	4.5×10^9	4.6×10^9	3.8×10^9
16-½	.80	1.16	1.28	1.28	1.40	2.48	4.80	4.65	—	—	—	—
19	.83	1.12	1.36	1.31	1.40	2.48	5.85	6.40	1.2×10^9	2.9×10^9	3.9×10^9	4.5×10^9
21-½	.76	1.11	1.36	1.43	1.40	3.48	5.85	6.40	—	—	—	—
24	.76	1.11	1.36	1.43	2.10	4.73	7.50	9.20	$<1 \times 10^7$	4.0×10^8	2.2×10^9	1.7×10^9

[1]Base = FYE, 1.5%; Trypticase, 1.0%; Difco yeast extract, 0.3%; Tween 80, 0.03%; Salts B; PP; pH adjusted to 6.0.
[2]pH Adjusted at times indicated to 4.7 ± 0.1 with 4N KOH except at 24 h where final adjustment was to pH 5.0.
[3]Diluted 1:10 and O.D. values multiplied × 2 for comparative purposes.
[4]Zero time count = 1×10^7.

under sub-optimal nutritional conditions and C strain the poorest, and B and C strains are particularly responsive to Salts B whereas L and T strains are more responsive to the purine-pyrimidine mixture. These apparent pairings are also observed in other factors. Thus, the results of Table 15, illustrating the requirements of the different strains for Tween 80, show much greater response by B and C. The level of 0.03% Tween 80 generally used is supported by the data in this table. In previous work, particularly in marginal media used primarily for isolation purposes, CO_2 was found to be frequently, but variably, beneficial (*2*). In the study shown in Table 16 the results indicate that this effect

TABLE 16. *Effect of atmosphere*[1]

Atmosphere	O.D. at 42 h (dil. 1:5)			
	C	B	L	T
Air	.45	.43	.64	.52
CO_2	.52	.73	.64	.56
N_2	.52	.70	.62	.56

Base = Maltose, 2.0%, Trypticase, 0.6%; Difco yeast extract, 0.3%; Tween 80, 0.03%; Salts B; FYE; pH 5.6.
[1]For gassing methodology cf. (*3*).

may have been due, at least in part, to correcting the deleterious effect of oxygen, since N_2 appears as beneficial as CO_2. Again there is a suggestion of a pairing with the B and C strains being more sensitive to an air atmosphere than strains L and T.

DISCUSSION

Addition of Salts B and the purine-pyrimidine mixture and optimization of the Trypticase level (1.0%) and Tween 80 level (0.03%) give a good growth response in the absence of FYE as indicated by optical density values of 0.5 to 0.6 (diluted 1:5) and cell dry weight yields of 0.10 to 0.13 g per 100 ml of culture. This growth response is approximately 10 times the growth obtained on the basal medium without FYE and roughly equivalent to the growth stimulatory effect of 0.5% FYE solids. Addition of 0.5% FYE to this improved medium results in a further doubling of the growth. Further substantial growth increments are observed as the FYE level is increased to 1.5 to 2.0%. Accordingly, while the improvements described substitute partially for the contributions of FYE they, like the various organic supplements tested, are clearly incomplete substitutes, and further work is needed to elucidate the nature of the additional unidentified nutritional factors in freshly-prepared yeast extractives.

From a practical standpoint it is convenient to use the supernatant yeast water obtained in the preparation of FYE directly without dilution as the base for the media preparation as it uniformly contains 1.5% FYE solids. Supplementation of this undiluted yeast water (clarified, if desired) with the other recommended ingredients (cf. Table 14) yields rapid and heavy growth i.e., viable counts of close to 5×10^9 in 14 h at 30 C.

Although some differences were observed in the growth responses of the four strains studied, the Oregon State workers have, however, found these same four strains to have such a high degree of homology that a single species is indicated (*6*). The L strain has been selected by us as the type strain due to its greater ease of cultivation on artificial (nonflour-containing) media.

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Comparison of Bacterial Flora on Hands of Personnel Engaged in Non-Food and in Food Industries: A Study of Transient and Resident Bacteria

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ABSTRACT

Prevalence and level of coagulase negative and coagulase positive staphylococci, fecal coliforms and enterococci on hands of employees in non-food industries were compared with findings from five occupational groups, varying in their degree of food contact. Positive results increased significantly, parallel to the extent of food contact. A correlation was noted between the flora on the hands and that of the food contacted. Further evidence for transmission of bacteria from food to the hand emerged from comparison of the results before and during work in meat industries. The prevalence and level of coagulase negative staphylococci, fecal coliforms, and enterococci were significantly lower before work, although they were still higher than those in non-food employees. Coagulase positive staphylococci were present at the same rate before and during work. The conclusion was drawn that this organism and, to a limited extent, the other test bacteria had changed their status of transient bacteria and had become permanent residents on the skin. The complex etiological relationships in food production was described as a permanent interaction between three factors: food, environment, and foodhandler. Bacteria were considered ubiquitous and the foodhandler a small link in the multiphase process of food preparation. Strict implementation of hygienic measures in all stages of food preparation was urged to prevent bacteria from becoming a health hazard.

The role of the hand in transmission of disease-producing bacteria was most impressively demonstrated by Semmelweis 120 years ago. His then revolutionary plea for meticulous handwashing is today a basic tenet of personal hygiene. Since then the hand has generally been considered a contaminant and was shown to be so in numerous studies of nosocomical infections and food poisonings. Price (*8*) introduced a new concept. He distringuished between two types of skin bacteria, "residents" and "transients," the latter being collected from "extraneous sources." The U.S. Food Service Sanitation Manual (*3*) warns, "Hands often become soiled in the performance of routine duties . . . ." Further along this line of thought, Roskey and Hamdy (*9*) concluded that poultry bruises were a source of Staphylococcal infection among poultry workers and not the other way round, as might have been expected.

Contamination of food is usually explained by "unclean hands of foodhandlers." Yet, little is known about the bacterial flora of "clean" hands during work with food. To simply blame the foodhandler might not only insult him unjustly but might obstruct an objective investigation of other possible ways of food contamination and its prevention.

The purpose of this work was (*a*) to establish, by generally recognized parameters, a bacterial baseline for hands engaged in non-food occupations, and (*b*) to compare this baseline with the bacteriological findings on hands of workers engaged in food industries both before and during work.

MATERIALS AND METHODS

The examinees, adults only

According to the extent of contact with food, six occupational groups were formed. Much thought was given to the only partially measurable "extent of contact with food" and to the composition of the groups to present employees of various occupations. Criteria for formation of groups were as follows.

Groups 1 and 2—No or indirect contact with food. Workers from various places and occupations were examined with the intention of minimizing behavioral, educational or other bias.

Groups 3 to 6—Direct contact with food. For these occupations we strove to select homogeneous groups. Obviously, to test the variable "contact with food" other factors had to be controlled as far as possible.

Composition of the occupational groups

Group 1—Non-food employment. 100 clerical employees from three regional towns, 50 workers in the metal industry, and 50 in the textile industry.

Group 2—Mechanized food industries. 89 employees in the chocolate industry, 28 workers engaged in bottling soft drinks, and ten workers from a mechanized bakery.

Group 3—Catering service. From each of 36 kibbutzim, five to six workers, occupied in the communal kitchen, were examined. Duties of the catering service include contact with diverse food categories in various stages from cleaning vegetables and handling raw meat to dispensing cooked, ready-to-serve dishes. Since only a few workers are employed even in large kitchens, workers from several establishments had to be examined. This might have introduced additional diversity. However, by taking our workers from kibbutzim, where equipment, facilities for food preparation and storage as well as working habits tend to be similar, this diversity was partially controlled.

Group 4—Bakeries. From three old-fashioned bakeries where most of the work was done by hand, seven, nine, and eleven workers respectively, were examined.

[1]*Haifa, Israel*
[2]*Afula, Israel*

Group 5—Cheese industry. 124 workers from one central dairy plant, engaged in cutting and wrapping ripened cheese. They had no contact with cheese preparation or with other dairy products.

Groups 6—Meat industry. 109 workers from three similarly equipped poultry processing plants and 20 from two similar factories for meat products. Initially workers from poultry plants and those from meat processing factories were listed separately, but as the results proved almost identical they were pooled for simplification.

Collection of samples

The palm of the right hand, the area between the fingers, the fingertips and the fingernails were swabbed by Public Health Inspectors. Sterile cotton swabs on wooden applicators in 4 ml phosphate buffer solution were supplied by the laboratory. The right hands of 814 workers were swabbed during working hours, between 9 to 11 A.M. and those of 118 workers before work started, between 6 and 7 A.M. Handwashing was not permitted before sampling. The swabs were returned to the laboratory under refrigeration and cultured immediately, 2 to 3 h after collection. At the time of swabbing, information relating to country of origin, general and professional education, work experience, etc. was collected in a questionnaire.

Bacteriological parameters

The following five bacteria were chosen: coagulase negative staphylococci, coagulase positive staphylococci, fecal coliforms, enterococci, and *Clostridium perfringens.*

Culture media and procedure

Staphylococci were isolated from Mannitol Salt Agar: 0.2 ml of the rinse solution (1/20 of the whole rinse) was spread on the surface of the medium. Plates were incubated at 35 C for 48 h. The growth of one colony was ignored. Two or three colonies were tested for coagulase production using human plasma from the local blood bank. Clotting was recorded after incubation for 4 h and, if negative, after 24 h. Negative tests were repeated with plasma from a different lot.

Enterococci were isolated from KF-Agar. Procedures for plating and incubating were as described for staphylococci. Enterococci were confirmed by catalase activity, survival at 60 C for 3 min, growth at 45 C, growth at pH 9.6 and in broth containing 6.5% NaCl.

Fecal coliforms were determined by the multiple tube method, planting portions of 1 ml, 0.1 ml, etc. The tubes were incubated in a waterbath at 44.5 C for 24 h and checked for fermentation (*4*). The Laboratory Manual by Theroux et al. (*11*) was used for calculation of the most probable number (MPN). For comparison with the actual count of the other test bacteria, the most probable numbers were adapted in consideration of their range: MPN up to 95 was transformed to 10^1, MPN between 96 to 950 to 10^2 and MPN 960 or more to 10^3. (Note: Preliminary tests with 0.2 ml of the rinse solutions spread on the surface of Violet-Red Bile Agar proved unsuitable.)

For determination of *C. perfringens* thioglycollate broth was inoculated in parallel cultures with 0.2 ml and with 0.4 ml, respectively, incubated at 35 C for 48 h and then streaked on Neomycin Blood Agar. Confirmation tests were performed according to the procedure of Seligmann (*10*). In preliminary examinations the swab itself was inserted into the thioglycollate for incubation.

RESULTS

Tables 1 and 2 deal with four test bacteria: coagulase negative staphylococci, coagulase positive staphylococci, fecal coliforms, and enterococci. The occupational groups are listed according to increasing contact with food.

Table 1 summarizes the populations in the six groups and the number of workers yielding positive cultures of one or more of the four test bacteria. The number of positive findings increases with the extent of food contact. While the difference between non-food and mechanized industries is only of borderline significance

TABLE 1. *No. of workers engaged in different occupations and positive cultures of coagulase negative and coagulase positive staphylococci, fecal coliforms and enterococci obtained from their hands*

Occupation	No. of workers examined	No. of workers w/pos. cultures	
		(No.)	(%)
Non-food employment	200	87	43.5
Mechanised food industry	127	68	53.5
Catering service	207	151	72.9
Bakeries	27	26	96.3
Ripened cheese industry	124	114	91.9
Meat industry	129	125	96.9

($\chi^2 = 3.14$ $0.05 > p > 0.02$, one sided) the successive differences between mechanised industries and catering services, catering and cheese cutting, catering and meat industries are all significant at the 0.001 level. There is no difference between cheese and meat industries. The small group from bakeries behaves like the latter two groups.

Table 2 details the prevalence and level of the four test bacteria obtained from the different occupational groups. Analysis of the prevalence rate by the chi-square test showed highly significant differences between the six groups for coagulase negative staphylococci ($\chi^2_{(5)} = 147.419$, $p < 0.001$), for fecal coliforms ($\chi^2_{(5)} = 70.219$, $p < 0.001$) and for enterococci ($\chi^2_{(5)} = 204.229$, $p < 0.001$). The prevalence of coagulase negative staphylococci rises directly with the extent of contact with food. The same was observed with fecal coliforms and enterococci with the remarkable exception in the cheese industry. Enterococci were more frequently isolated than fecal coliforms. The latter were absent in employees engaged in non-food industries. For each of these three bacteria the increase in the prevalence of particularly high levels (10^3 and 10^4) is parallel to the overall prevalence of positive findings.

The chi-square value for testing homogeneity with respect to coagulase positive staphylococci findings was 16.97, $p < 0.01$, an effect wholly due to the meat industry. The prevalence rate of coagulase positive staphylococci did not differ in groups 1-5. From 685 workers in these groups, 34 (5%) positive cultures were obtained (within the range of 2.5% - 7.1%), ($\chi^2_{(4)} = 4.5$, $0.5 > p > 0.3$). The percent rate in the meat industry was 14 as against an average 5 in the other groups, this difference is highly significant ($\chi^2_{(1)} = 11.925$, $p < 0.001$).

The level of coagulase negative staphylococci, fecal coliforms and enterococci is associated with the prevalence rate. This association is most strikingly illustrated by the results of coliforms and enterococci in the cheese industry. Coagulase positive staphylococci do not seem to conform with this trend but the number of positive findings is small and therefore inconclusive. However, in meat industries, with 18 positive cultures, 12 (67%) reached a level of 10^3 bacteria.

The association between positive findings and contact with food was further tested by handswabbing before and during work. Meat industries were chosen for this

TABLE 2. *Prevalence and level of coagulase negative and coagulase positive staphylococci, fecal coliforms and enterococci in hands engaged in different occupations*

Occupation	No. of workers examined	Coagulase positive staphyloc.						Coagulase negative staphyloc.								Coliforms						Enterococci					
		Positive findings		10^2[a]		10^3[b]		Positive findings		$10^1 + 10^2$		10^3		10^4		Positive findings		$10^1 + 10^2$		10^3[b]		Positive findings		$10^1 + 10^2$		10^3[b]	
		(No.)	(%)	(No.)	(%)	(No.)	(%)	(No.)	(%)	(No.)	(%)	(No.)	(%)	(No.)	(%)	(No.)	(%)	(No.)	(%)	(No.)	(%)	(No.)	(%)	(No.)	(%)	(No.)	(%)
Non-food employment	200	5	2.5	4	2.0	1	0.5	80	40.0	70	35.0	10	5.0	0		0		0		0		9	4.5	9	4.5	0	
Mechanised food industry	127	9	7.1	8	6.3	1	0.8	53	41.7	45	35.4	8	6.3	0		11	8.7	9	7.1	2	1.6	8	6.3	8	6.3	0	
Catering service	207	10	4.8	7	3.4	3	1.4	132	63.8	97	46.8	30	14.5	5	2.4	38	18.4	21	10.1	17	8.2	46	22.2	33	19.4	13	6.3
Bakeries	27	2	7.4	1	3.7	1	3.7	21	77.8	7	25.9	13	48.1	1	3.7	3	11.1	0		3	11.1	15	55.6	10	37.1	5	18.5
Ripened cheese industry	124	8	6.5	6	4.8	2	1.6	110	88.7	38	30.6	42	33.9	30	24.2	3	2.4	3	2.4	0		24	19.4	23	18.6	1	0.8
Meat industry	129	18	14.0	6	4.7	12	9.3	119	92.2	30	23.3	63	48.8	27	20.9	50	38.8	15	11.7	35	27.1	86	66.7	60	46.5	26	20.2

[a]Includes 1 finding of less than 10^2 organisms, it was encountered in non-food employment.

[b]Highest number of bacterial obtained.

TABLE 3. *Prevalence and level of coagulase negative and coagulase positive staphylococci, coliforms and enterococci; workers in meat industry before and during work compared with the non-food baselines*

Occupation	No. of workers examined	Coagulase positive staphyloc.						Coagulase negative staphyloc.								Coliforms						Enterococci					
		Positive findings		10^2		10^3[a]		Positive findings		$10^1 - 10^2$		10^3		10^4		Positive findings		10^2		10^3[a]		Positive findings		$10^1 - 10^2$		10^3[a]	
		(No.)	(%)	(No.)	(%)	(No.)	(%)	(No.)	(%)	(No.)	(%)	(No.)	(%)	(No.)	(%)	(No.)	(%)	(No.)	(%)	(No.)	(%)	(No.)	(%)	(No.)	(%)	(No.)	(%)
Meat industry:																											
before work	118	16	13.6	10	8.5	6	5.1	96	81.4	49	41.5	40	33.9	7	5.9	11	9.3	6	5.1	5	4.2	13	11.0	9	7.6	4	3.4
during work	129	18	14.0	6	4.7	12	9.3	119	92.2	30	23.3	63	48.8	27	20.9	50	38.8	15	11.7	35	27.7	86	66.7	60	46.5	26	20.2
Non-food employment	200	5	2.5	4	2.0	1	0.5	80	40.0	70	35.0	10	5.0	0		0		0		0		9	4.5	9	4.5	0	

[a]Highest number of bacteria obtained.

investigation because most positive findings were expected from this occupational group. Table 3 compares the results obtained before and during work. Fisher's exact test calculated highly significant values for fecal coliforms: $z = 5.359$, $p < 0.0001$ and for enterococci: $z = 8.9145$, $p < 0.0001$. It showed a smaller but still significant difference for coagulase negative staphylococci: $z = 2.5463$, $p < 0.01$ and none for coagulase positive staphylococci: $z = 0.898$. Notwithstanding the significantly lower prevalence and level of coagulase negative staphylococci, fecal coliforms and enterococci on the hand before work, they were still significantly higher than those in non-food employment. The chi-square test calculated for the prevalence rate of coagulase negative staphylococci, $\chi^2 = 50.55$, $p < 0.0001$, of coliforms $\chi^2 = 19.22$, $p < 0.001$ and of enterococci, $\chi^2 = 4.88$, $p < 0.01$. For coagulase positive staphylococci $\chi^2_{(1)} = 14.67$, $p < 0.001$.

The almost identical results of coagulase positive staphylococci on the hand before and during work prompted us to examine the nasal carrier rate of this organism among workers in meat industries for comparison with employees without food contact. Table 4 demonstrates the same nasal carrier rate of coagulase positive staphylococci in both groups (32%).

TABLE 4. *Nasal carrier rate of coagulase positive staphylococci-workers in non-food and in meat industries*

Occupation	No. of workers examined	Positive findings (No.)	(%)
Non-food employment	154	48	31.2
Meat industries	114	36	31.7

TABLE 5. *Prevalence and level of C. perfringens in non-food employment*

No. of workers examined	Positive findings of C. perfringens: Qualitative[a] (No.)	(%)	Level of organisms: 10 (No.)	(%)	20 (No.)	(%)	Total (No.)	(%)
90	35	38.9	—[b]		—[b]		35	38.9
180			18	10	10	5.6	28	15.6

[a]Swab culture.
[b]Not done.

The results of *C. perfringens* are outlined in Table 5. Inoculation of the swab itself yielding 38.9% positive results proves again the ubiquity of this organism in our environment but lacks quantitative meaning. Measured inocula (0.4 ml and 0.2 ml of the 4 ml rinse solution) indicated the presence of the organism at the low level of 10 or 20 bacteria on the hand in 15.6% of 180 workers in non-food industries.

DISCUSSION

Of the five test bacteria studies, coagulase negative staphylococci are commonly found on the skin. They are true residents according to Price (*8*). Welch, almost 100 years ago, named them *Staphylococcus epidermis* to express their affinity for the skin. To avoid taxonomic controversy, the simple descriptive term "coagulase negative staphylococci" was used in this work. Coagulase positive staphylococci, although particularly colonized in the nose, are known to be present on the skin. Hand carrier rates of 10-14% were reported (*12*). Coliforms are usually reported absent from the hand except in special studies of foodhandlers (*7*). These bacteria are transients and disappear from the hand after a short time (*5, 7, 8*). The presence of enterococci and of *C. perfringens* on the skin has not received much study. Both are absent from the list of indigenous microorganisms on the skin, detailed by Dubos (*1*). Hellat (*5*) found that hemolytic streptococci die within a few days and that enterococci survive for "prolonged" periods. *C. perfringens* were not isolated in anaerobic cultures from skin scrapings (*2*).

The examination of 200 workers not in contact with food—our occupational group 1—revealed the presence of one or more test bacteria on the hands of 87 (43.7%) (Table 1). Coagulase negative staphylococci were isolated from 40% of the workers, coagulase positive staphylococci from 2.5% and enterococci from 4.5%. Fecal coliforms were not detected (Table 2). In our procedure, a positive finding indicated the presence of at least 40 bacteria. In most of the cultures 10^2 bacteria were enumerated.

C. perfringens was studied in another series of 180 non-food workers and 15.6% positive findings were obtained (Table 5). Unfortunately, this was not followed up in the examination of workers in food industries.

Personal data relating to age, origin, education, etc. recorded at the time of sampling showed the group of 200 workers, engaged in non-food employment, to be comparable to the populations in the other occuptional groups. We, therefore, assume the findings of the five test bacteria to represent a normal bacterial profile of the active hand in an average group of employees in local conditions (habits, climate, etc. in Israel). This was accepted as a baseline for comparison with findings from occupational groups with food contact.

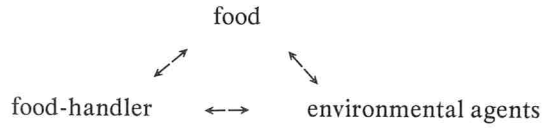
With food contact, significant changes of this baseline were observed, both as regards the number of workers affected and the prevalence and level of bacteria. Whereas 44% of workers in non-food employment yielded positive cultures, 73-97% of workers in food industries were positive (Table 1). Coagulase negative staphylococci increased directly with the extent of food contact. Coagulase positive staphylococci increased only on the hands of workers in meat industries. Fecal coliforms were present in all groups and increased with the extent of food contact except in ripened cheese plants. Enterococci reacted the same as fecal coliforms (Table 2).

Further evidence for transmission of bacteria from food to the hand and corresponding change of the skin flora was provided by comparison of bacterial findings before and during work in meat industries (Table 3). Prevalence and level of coagulase negative staphylococci, fecal coliforms, and enterococci were significantly lower

before work, at 6 A.M., yet they were still higher than in non-food employment. Coagulase positive staphylococci were found to have the same high rate before and during work, which was much higher than the baseline. The nasal carrier rate was not affected. It was found to be identical with that in non-food employees, 31-32% (Table 4). This rejects the hypothesis that the group in meat industries might represent an unusual population with a high nasal carriage of staphylococci and a corresponding high rate on the hand.

It follows that coagulase positive staphylococci and, to a limited extent, the other three test bacteria had changed their status of transients and had become permanent residents. A similar process was described in hospitals by Price (*8*) and Petersen et al. (*6*). The latter reported coliforms on washed hands of approximately 33% of personnel in nurseries and of 56% employed in a burn unit. With food contact, illustrated in our case in meat industries, the resident skin flora on the hand was modified and significantly different from the bacterial profile of the hand described as the baseline (workers in non-food employment). This is an important result because resident bacteria form a comparatively stable population and are rather resistant to either detergents or germicides. Even when the skin was "degermed" the resident flora was reestablished after about one week (*8*).

Purposely, we refrained from using the term "contamination" in this work. Its rigid, one-way concept of the accusative case (A contaminates B) seemed too simple for the complex etiological relationship in food production and handling. This relationship is a closed, constant interaction, that might be expressed diagramatically:



Foodhandlers were often suspected and sometimes "proved" to be the "source" of contaminating bacteria in food. Our results might be interpreted from the opposite point of view. The foodhandler is one link in the complex multiphase process of contaminated food—infection—enteric disease. Personal hygiene and frequent thorough handwashing is mandatory for the foodhandler. However, the presence of contaminating bacteria on his hands does not automatically implicate him as "source" of contaminants in food.

Bacteria, including pathogens, are part of our world. The mechanisms of ecology will not enable us to eliminate them. The acquisition of transient bacteria and their colonization as residents on the skin is just one aspect of this. Strict implementation of technological knowledge concerning food processing, storing, and marketing, with enforced hygienic measures to control sanitation in all stages, are the problems that must be solved. These might not prevent the presence of bacteria in the process of food production but could prevent them from becoming a health hazard.

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

I. Effects of moist heat, age of conidia, and sporulation medium

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ABSTRACT

Thermal resistance at 45, 50, 55, and 60 C of conidia from various strains of *Aspergillus flavus* and *Aspergillus parasiticus* was determined using a heating menstruum buffered at pH 7.0 with KH_2PO_4-NaOH. Heat resistance of conidia from both molds was strain-dependent. With moist heat at 45 C, D-values for conidia from various strains ranged from 14 to >161 h, whereas at 60 C the range was from 8 to 59 sec. At 50 and 55 C, D values ranged from 16 to 987 and 3 to 29 min, respectively. There appeared to be a positive relationship between the degree of heat resistance of conidia and the amount of aflatoxin produced by the different aspergilli. Conidia that were 15 and 20 days old were less resistant to heat than when they were 7 or 10 days old. Conidia that were produced on a substrate low in protein and high in glucose were more heat resistant than were those produced on a more proteinaceous substrate that contained little glucose.

Much work has been done on conditions that influence thermal inactivation of bacterial spores. In contrast to this, only minimal research efforts have been devoted to establishing the parameters that govern thermal inactivation of fungal conidiospores.

The conidia of *Aspergillus flavus* and *Aspergillus parasiticus* are of particular interest because these molds can produce aflatoxin. Aflatoxin is a secondary metabolite which, when ingested by various animals, causes such conditions as lack of appetite, reduced alertness, a staggering gait, and death (*3*). In addition, exposure to aflatoxin for extended periods often induces malignant liver tumors in virtually all experimental animals and probably also in man.

It is clearly evident that there is a need to control contamination of foods and feeds by aflatoxigenic molds. One method to control such contamination is to inactivate the conidia that give rise to the mold mycelium since growth of the mold is necessary for synthesis of aflatoxin. A common treatment in processing foods and feeds is the application of heat. Although it is generally accepted that mold spores are considerably less heat resistant than bacterial spores, specific information on the thermal sensitivity of conidia from toxigenic aspergilli is lacking. Consequently, studies to be reported in this and a subsequent paper (*1*) were done to provide some of the missing data.

MATERIALS AND METHODS

Three series of experiments were done. The first dealt with the resistance and/or sensitivity to moist heat of conidia produced by various strains of *A. flavus* and *A. parasiticus*. The strains to be tested were selected on the basis of a study by Hesseltine et al. (*4*) in which 67 strains from the *A. flavus* series were grown on three different

TABLE 1. *Strains of aspergilli that were selected for studies on inactivation of conidiospores (4)*

Taxonomic classification	Collection number	Medium	Aflatoxin (ug/g in R & W, mg/1 in L)[a] B_1	G_1	M
Aspergillus parasiticus	NRRL 2999	R	467	109	2.2
		W	930	415	3.4
		L	21	3	0.07
Aspergillus parasiticus	NRRL 3315 A-13360	R	50	54	0.6
		W	201	193	2
		L	0.2	0.1	0.001
Strain intermediate between *Aspergillus parasiticus* and *Aspergillus flavus*	NRRL 3161	R	248	210	2.4
		W	82	107	1.2
		L	18	8.4	0.10
Aspergillus flavus	NRRL 482	R	ND[b]	ND	ND
		W	ND	ND	ND
		L	ND	ND	ND
New taxon (originally *Aspergillus flavus*)	NRRL 3251	R	1,257	ND	6
		W	1,188	ND	4
		L	22	ND	0.06
Aspergillus flavus from alkali bees	NRRL 3353	R	19	5	0.2
		W	56	62	0.8
		L	106	35	0.7

[a]R = rice; W = wheat; L = liquid medium (10 g of rice flour to 200 ml of tap water;
[b]ND = none detectable.

substrates under two different fermentation conditions and yields of aflatoxins B_1, G_1, and M were determined. Furthermore, Hesseltine et al. subdivided strains of the *A. flavus* series into more specific groups. For the present study, one representative strain was selected from each subgroup of *A. flavus* or *A. parasiticus* with preference given to those organisms in each taxonomic grouping that produced above average, if not the greatest, amounts of aflatoxin. Table 1 identifies each organism selected, indicating its taxonomic classification, its collection number, and the amounts of aflatoxins B_1, G_1, and M produced on the various substrates. All cultures were obtained from the Northern Regional Research Laboratory, U.S. Department of Agriculture, Peoria, Illinois.

The second series of experiments dealt with the effects that moist heat had on 7-, 10-, 15-, and 20-day old conidia produced by *A. flavus* and *A. parasiticus*. The age of conidia was determined by measuring the time from when a medium was inoculated with spores until the day the new spore crop was harvested.

The third series of experiments dealt with the effects that moist heat had on conidia produced by *A. flavus* and *A. parasiticus* during growth on different media. The substrates used for growth included Czapek's Solution Agar (Difco), Y-M Agar (Difco), Potato Dextrose Agar (Difco), Mycological Agar (Difco), and a modified version of Moyer's medium (*5*). Differences between the original Moyer's medium and the modified version are that the latter did not contain 0.04 g of Fe tartrate, that it contained 15 g rather than 0.25 g agar, and that 10 g of yeast extract were added.

Spore suspensions

An inoculum of 0.5 ml of a suspension that contained, per milliliter, approximately 10^7 conidia of the *A. flavus* or *A. parasiticus* strains previously described was surface plated on modified Moyer's, Czapek's, Y-M, Potato Dextrose, and Mycological agar slants in 250-ml dilution bottles. Cultures were grown for 7, 10, 15, and 20 days at 28 C. Conidia were harvested by adding sterile distilled water to the surface growth and gently brushing the conidial chains with an inoculating loop. Clumping of spores was eliminated by filtering and refiltering the suspension through several layers of sterile cheese cloth. Microscopic observations confirmed that clumps were broken up. Spores were washed three times with sterile distilled water to remove any contaminating nutrients. Suspensions were adjusted to approximately 10^7 spores/ml by removal or addition of sterile water. Suspensions were then stored at 1-2 C.

Heating apparatus

A 2-liter, jacketed, three-necked flask (ST 24/40 joints) was used to heat treat the spores. One neck of the flask was equipped with a thermometer, one with a stirring rod, and the third with a ground glass stopper to permit aseptic sampling of the contents. The stirrer was driven by a 55-watt Talboy T-line motor (Talboys Instrument Co., Emerson, New Jersey) which was connected to a rheostat (Statco, Inc., Dayton, Ohio). Stirring speeds were regulated by controlling the amperage output from the rheostat. Temperature-controlled water was provided by a Blue M Microtrol (Blue M Electric Company, Blue Island, Illinois) water bath. Water was circulated to the flask through 0.5-inch (I.D.) Tygon tubing connected to a 1/15-hp centrifugal pump (Little Giant, Oklahoma City, Oklahoma). For replication, two flasks were connected in series and duplicate measurements were made. Since circulation of the water was rapid, there was no detectable difference in the temperature of the menstruum in the two flasks.

Heating menstruum and heat treatments

Conidia were heated at 45, 50, 55, 60, and 65 C. Inactivation at 65 C was very rapid and reliable data could not be obtained by methods used in these experiments. Consequently results to be reported were obtained at the four lower temperatures. To enhance even distribution of heat and of spores, a stirring rod rotating at 700 rpm was used to agitate the menstruum during heat treatment. The heating menstruum consisted of 999 ml of sterile distilled water which was buffered to pH 7.0 using 0.1 *M* KH_2PO_4 – 0.1 *M* NaOH buffer. The liquid was heated to the appropriate temperature while under constant agitation.

An inoculum of 1.0 ml of a suspension containing approximately 10^7 spores/ml was introduced into the 999 ml of heating menstruum, giving a final spore concentration of approximately 10^4/ml. Spores were examined microscopically for swelling and excessive clumping. Because of the great difference between the amount of inoculum added and the amount of preheated liquid making up the heating menstruum, heating of spores to the desired temperature was instantaneous. Therefore, the usual come up time involved with most heat processes could be ignored.

Sampling and cooling of samples

A 3- to 5-ml sample of the heat treated spore suspension was aseptically withdrawn from the flask using a wide bore, rapid delivery pipette. The sample was immediately delivered to a precooled 12.5-cm × 1.5-cm screw-cap test tube. The test tube was precooled to 0.5 C by immersing it in ice water for several minutes.

Enumeration of survivors

The heat treated sample was diluted in sterile 0.1% peptone (Difco) water. One milliliter of each dilution was surface plated in duplicate onto Y-M agar in petri dishes. Petri dishes were swirled so that the inoculum covered the entire surface of the agar. All plates were incubated at 28 C and counted after 2 days.

Analysis of results

The best straight line fit for data in the survivor curves was determined using the least squares method (*6*). Results are also given in terms of D and z values. Derivations of these values are outlined by Stumbo (*7*).

RESULTS AND DISCUSSION

Effects of moist heat

Ten-day-old conidiospores were inactivated at constant temperatures of 45, 50, 55, and 60 C. Figure 1

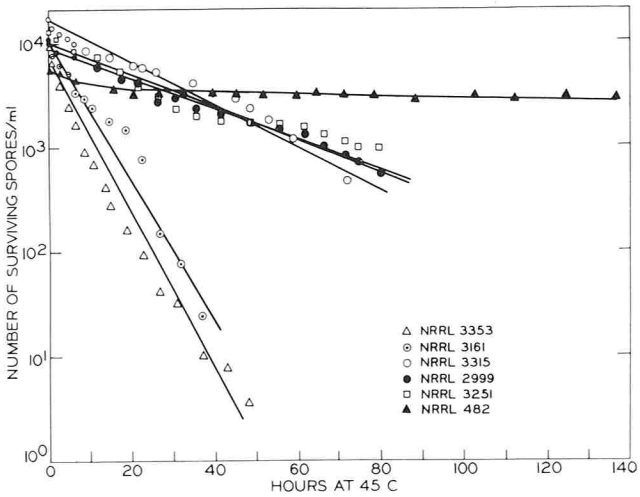

Figure 1. *Heat (moist) inactivation at 45 C of 10-day-old conidiospores from selected strains of* A. flavus *and* A. parasiticus. *Conidiospores were produced on Moyer's medium and were heated in a distilled water menstruum which was buffered at pH 7.0 using* KH_2PO_4-*NaOH.*

graphically illustrates the comparative rates for thermal inactivation of aspergilli at 45 C. Each point plotted in the figure (and in all other figures) represents the average of duplicate trials. Figure 2 illustrates the thermal inactivation rates for each strain of aspergillus at 50 C. Figure 3 provides data that were obtained when heating was at 55 and 60 C. In all instances, the thermal resistance of the six strains was consistent; i.e., NRRL 3353 was always the most sensitive, followed in order by NRRL 3161, NRRL 3315, NRRL 2999, NRRL 3251, and finally NRRL 482 which was the most resistant. Results, reported as D and z values, are in Table 2. The z values

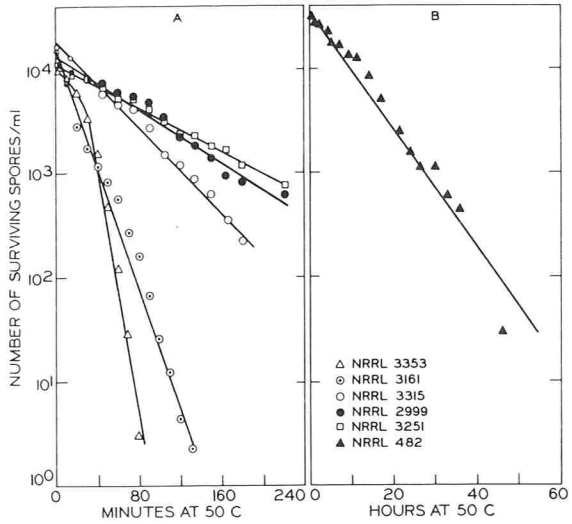

Figure 2. *Heat (moist) inactivation at 50 C of 10-day-old conidiospores from selected strains of* A. flavus *and* A. parasiticus. *Conidiospores were produced on Moyer's medium and were heated in a distilled water menstruum which was buffered at pH 7.0 using* KH_2PO_4-*NaOH.*

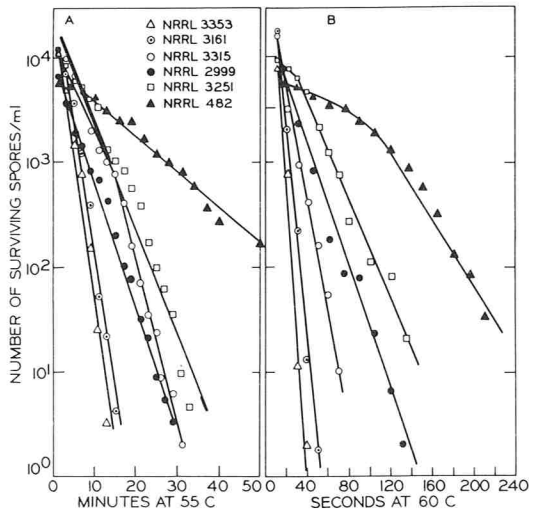

Figure 3. *A. Heat (moist) inactivation at 55 C of 10-day-old conidiospores from selected strains of* A. flavus *and* A. parasiticus. *B. Heat (moist) inactivation at 60 C of 10-day-old conidiospores from selected strains of* A. flavus *and* A. parasiticus. *Conidiospores were produced on Moyer's medium and were heated in a distilled water menstruum which was buffered at pH 7.0 using* KH_2PO_4-*NaOH.*

ranged from 3.3 to 4.1 C. This range is similar to that of yeasts (3-5 C), many non-sporing bacteria (4-6 C), and conidia of *Aspergillus niger* (5 C) (*2*).

As evidenced by these data, there is an appreciable difference in the thermal resistance of conidiospores produced by different strains of *A. flavus* and *A. parasiticus.* There is also a marked difference in the time required for thermal inactivation of the conidiospores at the various temperatures. At 45 C, for example, the time required to inactivate 90% of the conidia ranged from several hours to days, depending on the strain, while at 60 C, the time required to inactivate 90% of the conidia ranged in seconds. At 65 C and above, thermal inactivation was so rapid that it could not be measured accurately.

TABLE 2. *D and z values for conidiospores of* A. flavus *and* A. parasiticus *as determined at various temperatures*

Strain	D value at 45 C (h)	D value at 50 C (min)	D value at 55 C (min)	D value at 60 C (sec)	z value (C)
NRRL 3353	13.97	16.2	3.1	7.7	4.0
NRRL 3161	14.65	34.8	3.8	9.8	4.1
NRRL 3315	52.62	98.2	6.3	19.9	3.8
NRRL 2999	67.28	155.6	8.4	34.8	3.9
NRRL 3251	69.11	188.8	9.5	42.0	4.0
NRRL 482	>161	986.8	28.9	58.8	3.3

A comparison of the thermal resistance of conidia from each strain with amounts of aflatoxin produced by the strains (Table 1) suggests that a relationship exists between the amount of aflatoxin produced and the degree of thermal resistance. The greater the amount of aflatoxin produced by a particular strain (using wheat as a substrate), the greater its thermal resistance. For example, of the strains that were studied, NRRL 3251 was able to produce the greatest amount of aflatoxin on the wheat medium and also had the most heat-resistant conidia. On the other hand, strain NRRL 3353 produced the least amount of aflatoxin (of the aflatoxin producers) on a wheat substrate and also formed conidia that were least resistant to heat. However, this relationship appears to be true only for aflatoxin producers, since the non-aflatoxin producing strain, NRRL 482, had conidia that were more heat resistant than were those of any of the aflatoxin producing aspergilli. To further verify this observation, additional strains of aflatoxigenic *A. flavus* and *A. parasiticus* must be tested.

It should be noted that there were instances in these studies where clumping of conidia occurred. Filtration and shaking of spore suspensions were used to circumvent the problem but the treatments were not always sufficient. An example of this is illustrated by a curve in Fig. 2. The inactivation curve for strain NRRL 3353 is not entirely a straight line, but is curvilinear during the early stage of heating. This was caused by clumping of the conidia, as explained by Stumbo (*7*). The D values for such curves were determined from the straight-line portion of the curve that resulted from data obtained after spores were no longer clumped. Presence or absence of clumped conidia was confirmed by microscopic examination.

Effects of age of conidia

Experiments were done to see what effect the age of conidia might have on their thermal resistance. Three selected strains of aspergilli, NRRL 3353, NRRL 3315, and NRRL 2999, were grown on modified Moyer's medium at 28 C and conidia were harvested after 7, 10, 15, and 20 days of incubation. They were heat treated at 55 C in deionized distilled water that was buffered to pH 7.0 with 0.1 *M* KH_2PO_4-0.1 *M* NaOH. Results obtained from the experiments are summarized in Fig. 4. D-values calculated from these data are in Table 3.

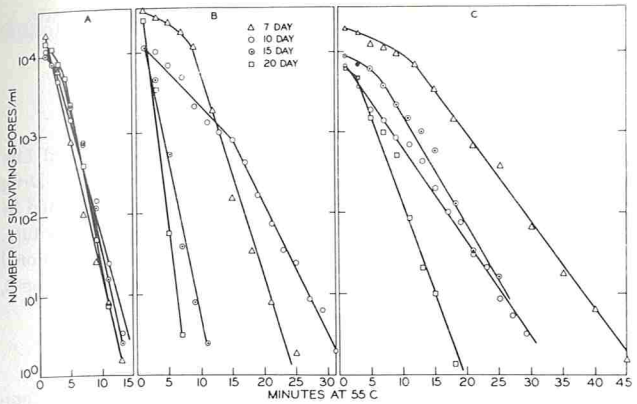

Figure 4. *Heat (moist) inactivation at 55 C of 7-, 10-, 15-, and 20-day-old conidiospores of aspergilli. A,* A. flavus *NRRL 3353; B,* A. parasiticus *NRRL 3315; C,* A. parasiticus *NRRL 2999. Conidiospores were produced on Moyer's medium and were heated in a distilled water menstruum that was buffered at pH 7.0 using KH_2PO_4-NaOH.*

TABLE 3. *D values determined at 55 C for 7-, 10-, 15-, and 20-day-old conidiospores produced on Moyer's medium by selected strains of* A. flavus *and* A. parasiticus

Strain	D Value (min) for conidiospores of different ages			
	7 days	10 days	15 days	20 days
NRRL 3353	2.8	3.1	2.8	2.4
NRRL 3315	4.1	6.3	2.5	1.5
NRRL 2999	9.0	8.4	7.2	4.1

It was observed that 20-day-old conidia from strains NRRL 3353 and NRRL 3315 and 7-day-old conidia of strain NRRL 2999 were most heat resistant of the conidia that were studied. Strain NRRL 2999 grew more rapidly and sporulated sooner on modified Moyer's medium than did strains NRRL 3353 and NRRL 3315; therefore, at 7 days its conidia were mature while those of the other two strains did not reach maturity until after 10 days. This may explain why conidia from one strain were more resistant than those of equivalent age from different strains. In addition it was found that as conidia became older their resistance to moist heat decreased. Without knowing the chemical composition, physiological characteristics, and other traits of the *A. flavus* and *A. parasiticus* conidia, it is difficult to explain why their thermal resistance decreased with age. Perhaps it was caused by release of substances such as enzymes or cations that contribute to heat resistance, structural changes, or changes in permeability of the spore. Additional investigation is needed to explain this unusual phenomenon.

Effects of growth substrate

The three selected strains of *A. flavus* and *A. parasiticus* were grown on five different substrates (previously described) for 10 days at 28 C, after which their conidia were harvested. The conidia were then thermally inactivated at 55 C in a heating menstruum adjusted to pH 7.0 with a buffer consisting of 0.1 *M* KH_2PO_4-0.1 *M* NaOH. A graphical representation of these results is presented in Fig. 5. D-values calculated from the data are in Table 4. Generally conidia were

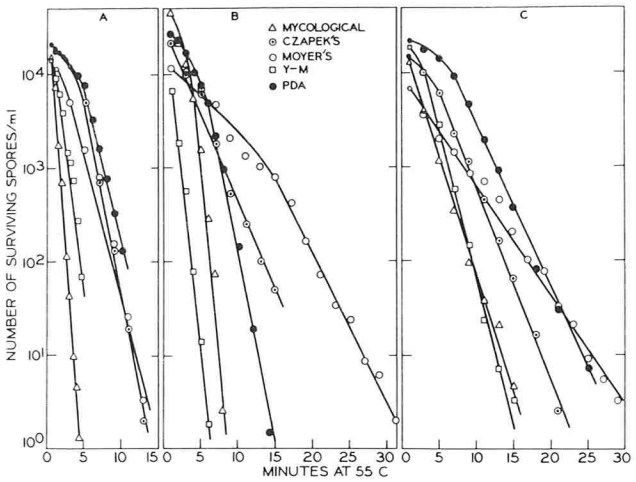

Figure 5. *Heat (moist) inactivation at 55 C of 10-day-old conidiospores of aspergilli. A,* A. flavus *NRRL 3353; B,* A. parasiticus *NRRL 3315; C,* A. parasiticus *NRRL 2999. Conidiospores were produced on various media and were heated in a distilled water menstruum that was buffered at pH 7.0 using KH_2PO_4-NaOH.*

TABLE 4. *D values at 55 C for 10-day-old condiospores produced on various media by* A. flavus *and* A. parasiticus

Strain	D values (min) of conidia produced on				
	Mycological	Czapek's	Moyer's	Y-M	PDA
NRRL 3353	1.0	2.6	3.1	1.8	2.9
NRRL 3315	1.3	5.1	6.3	1.4	2.3
NRRL 2999	4.1	4.8	8.4	3.3	5.6

most heat resistant when they were produced on the modified Moyer's medium. Moyer's medium is a very nutritious substrate containing vitamins, specific trace salts, limited amounts of amino acids and protein in the form of peptone, and glucose as a carbohydrate source. This medium has a relatively high osmotic pressure since it contains 165 g of glucose per liter of distilled water. Organisms growing on this medium sporulated rapidly, often within 7 days, and produced an abundance of mature conidia. Spores produced on Mycological agar and on Y-M agar were least resistant to heat. These media contain large amounts of amino acids and protein and relatively small amounts of glucose and polysaccharides such as starch. The thermal resistance of conidia grown on Czapek Solution agar and Potato Dextrose agar ranged between that of those grown on modified Moyer's medium and those grown on Mycological and Y-M agar. Czapek Solution agar contains several trace salts and three times as much sugar as is present in Y-M and Mycological agars; however, the sugar in Czapek's agar is sucrose rather than glucose. Potato Dextrose agar contains infusion from potatoes which contains several trace salts and large amounts of polysaccharides largely in the form of starch. In addition, it contains twice as much glucose as Y-M and Mycological agar.

Absolute differences are not apparent when the degree of heat resistance is related to the different media on which the various conidia were produced. Each situation appears to be strain dependent; however, trends do exist.

The data suggest that conidia which were produced when the mold grew on a medium with a substantial amount of sugar were more heat resistant than those formed when the amount of sugar was less. In addition, there may be a relation between heat resistance of spores and the amount of protein, possibly even specific amino acids, in the sporulation medium. Conidia which were produced on media that contained a relatively large amount of protein and amino acids, such as Y-M and Mycological agar, were less heat resistant than were those conidia produced without any or with much smaller amounts of exogenous protein present in the medium.

To determine the exact compounds that enhance or reduce heat resistance of conidia, further studies should be made using more carefully defined media and paying particular attention to the kinds and amounts of sugars and amino acids present in the substrates. Other substances such as trace salts and vitamins might also be tested to see what influence they have on heat resistance of conidia.

It should be stressed that thermal resistance of conidia from *A. flavus* and *A. parasiticus* is strain dependent. This may be true because some strains sporulate better, i.e., under certain conditions they produce more spores that are mature, while other strains sporulate better under other conditions. Perhaps this is true because different strains assimilate or utilize compounds differently, or because the strains are structurally dissimilar. Hence data obtained in this study, permit us to identify trends but additional investigations are needed to focus on the specific factors that affect heat resistance of conidiospores.

ACKNOWLEDGMENTS

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Microbiology of Processed Spices

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ABSTRACT

The microbiology of spices procured by the Army, Navy, Marines and Air Force was determined. The incidence of *Clostridium perfringens* in the spices analyzed was 15%. The organism was found in 4 out of 7 types of spice and in 53% of the oregano samples. No other bacteria of public health significance were found. The microflora of spices from 10 different brands varied widely.

Some spices harbor great numbers of microorganisms (*3*) including sporeforming bacteria and spoilage organisms (*7*). Unless spices are treated they may contaminate menu items and cause spoilage and may even introduce harmful organisms (*11*). While the bacteriological safety of spices can effectively be achieved by several fumigation practices (ethylene oxide, propylene oxide, microwave and gamma irradiation), the methods are limited by cost, time required to completely destroy microorganisms and their effect on flavor and color of some spices (*12*). Furthermore, the amount of residual ethylene oxide and propylene oxide in spices is limited by the Food and Drug Administration (FDA). Although gamma irradiation was found to be more effective than ethylene oxide in reducing the bacterial population of spices (*12*), irradiation of spices is not permitted by the FDA.

Because of the aforementioned limitations spices are produced with various microbial loads. Although a few studies have been done to determine the microflora of black and red pepper (*3*), some untreated spices (*8*) and selected spices and herbs at import (*7*) there is still little information regarding the microbiology of processed spices commonly used by the ordinary consumer including the military forces.

The purpose of this investigation was to determine if the military is receiving satisfactory products and whether or not a potential health problem is associated with spices procured by the military.

MATERIALS AND METHODS

Number and source of samples

A total of 114 samples of seven types of spices and herbs were received from 16 military bases. Army, Navy, Marine, and Air Force bases located in different geographical areas of the United States were represented. Spices were procured locally by each base and represented 10 different processors. With the exception of bay leaves all spices were ground or powdered. Upon receipt all samples were stored in their sealed containers at 23 C until analyzed.

Preparation of sample

Ten grams of each spice, including herbs, were weighed into a sterile, tared blendor jar, and blended for 1 to 2 min in 90 ml of sterile phosphate buffered water (SBW) (*1, 2*). Dilutions were made from the initial blend (1:10) by transferring 10 ml into 90 ml SBW (*2, 6*). The lowest dilution cultured was 1:100 to avoid possible inhibition of growth by bactericidal or bacteriostatic properties of some spices (*5, 8*). For *Clostridium perfringens* counts, this dilution was achieved by spreading 0.1 ml of 1:10 dilution on duplicate plates of Shahidi Ferguson Perfringens (SFP) agar.

Inhibition of bacterial growth by spices

To determine if the spices tested would inhibit bacterial growth, *Escherichia coli* and *Staphylococcus aureus* were inoculated into tubes of Lauryl Sulfate Tryptose (LST) broth and Trypticase Soy Broth (TSB) containing 10% sodium chloride (NaC1), respectively, to which was added 1 ml of 1:10 dilution of each spice. Excellent growth of both organisms was achieved in their respective media in less than 24 h at 35 C, indicating that the spices were not inhibitory at the concentration tested. Subsequent recovery of both organisms by Standard Methods described below also was achieved. To allow a margin of safety the lowest "in-use" dilution of spices for all counting procedures was 1:100.

Media

All media were purchased from Difco Laboratories, Detroit, Michigan.

Aerobic plate count

One milliliter of dilutions ranging from 10^{-2} to 10^{-5} was pipetted into duplicate petri plates, poured with Plate Count Agar and incubated at 35 C for 48 h.

Yeast and mold count

Dilutions prepared for aerobic plate counts also were used for making yeast and mold counts. One milliliter of each dilution was added to petri plates and the plates were poured with Potato Dextrose agar (PDA), acidified to pH 3.5. Plates were incubated at 23 C for 5 days before counting.

Coliform and fecal coliform count

Coliforms were estimated by a 3-tube most probable number (MPN) determination in Lauryl Sulfate Tryptose (LST) broth by Standard Methods (*2*). Gas producing LST tubes were confirmed by use of Brilliant Green Lactose Bile (2%) broth (BGLB). Fecal coliforms were estimated by transferring growth from positive LST tubes to EC broth and incubating at 45.5 C for 48 h (*2*). Counts determined from MPN tables are presented as <30/g when all tubes at the 1:100 dilution are negative.

Coagulase positive staphylococci

Coagulase positive staphylococci were estimated by a modification of the 3-tube MPN procedure, using the same dilutions as for the coliform count. To allow for recovery of injured cells, 1 ml of appropriate dilutions was added to 5 ml of TSB and incubated at 35 C for 3 h. An equal volume of TSB containing 19.5% NaC1 was then added to each tube to yield a final salt concentration of 10% (*6, 9*). After incubation at 35 C for 48 h, 0.1 ml of the TSB-NaC1 culture was spread on each of

TABLE 1. *Microbiology of processed spices used by the Armed Forces*

Spice	No. of samples	Range of counts/g of spice					
		APC	Yeast and molds	Coliforms (MPN)	Fecal coliforms	Coagulase positive staphylococci (MPN)	*Clostridium perfringens*
Bay leaves	16	<100 to 30,000	<100 to 670,000	<30	<30	<30	<100 to 500
Cayenne pepper	15	3400 to 9,100,000	<100 to 20,000	<30 to 2,400	<30	<30	<100 to 250
Chili powder	18	100 to 540,000	<100 to 8,100	<30	<30	<30	<100
Cinnamon	18	10,700 to 780,000	<100 to 4,600	<30 to 230	<30	<30	50 to 300
Garlic powder	16	3700 to 980,000	<100 to 500	<30 to 230	<30	<30	<100
Mustard powder	16	500 to 5300	<100 to 2,200	<30	<30	<30	<100
Oregano	15	1200 to 3,600,000	<100 to 27,000	<30	<30	<30 to 30	50 to 2850

two plates of Vogel Johnson Agar. Plates were incubated at 35 C and examined after 24 and 48 h for the presence of smooth black colonies with yellow zones. Two or more typical representative colonies were transferred to tubes of Brain Heart Infusion (BHI) broth and tested for coagulase production according to standard procedures (*2*). Counts, determined from MPN tables, are presented as <30/g when all tubes at the 1:100 dilution were negative.

Clostridium perfringens

Clostridium perfringens was counted by spreading 0.1 ml of 1:10 and 1:100 dilutions onto duplicate plates of Shahidi-Ferguson Perfringens (SFP) agar (*10*). Plates were overlayed with 10 ml of SFP overlay agar (egg yolk omitted), placed into gaspak anaerobic jars (BBL) and incubated at 35 C for 24 h. Black colonies surrounded by a zone of precipitate were counted and confirmed in Lactose Motility (LM) agar. Lactose Motility agar was steamed for 10 min and cooled immediately before use. Inoculated tubes were incubated at 35 C for 24 h. Non-motile, lactose positive cultures in LM agar, exhibiting typical microscopic morphology, were considered to be *C. perfringens* (*10*). Counts are presented as 50/g when one colony was confirmed from 0.1 ml of a 1:10 dilution. When no colonies were observed, counts are presented as <100/g since the lowest dilution per gram was 1:100.

RESULTS

Bay leaves

Sixteen samples of bay leaves were analyzed. The APC range from <100/g to 30,000/g (Table 1). Yeast and mold counts were less than 10,000/g (Table 2) except for one sample of brand 9, which had a count of 670,000/g (Tables 1 and 3). *C. perfringens* (Tables 3 and 4) was found in two (12%) of the samples of bay leaves, with counts of 300/g and 500/g. Both were from brand 3 (Table 5). No coliforms, fecal coliforms, or coagulase positive staphylococci were found at the lowest dilutions tested and are reported as <30/g (Tables 1, 4, and 6).

Cayenne pepper

Fifteen samples of ground cayenne pepper were analyzed. The APC ranged from 3400/g to 9,100,000/g (Table 1). Thirteen samples had APC's greater than 100,000/g and nine had counts greater than 1 million/g (Table 7). Yeast and mold counts ranged from <100/g to 20,000/g (Tables 1 and 2).

TABLE 2. *Yeast and mold count of processed spices used by the* Armed Forces

Spice	Total No. of samples	Number of samples containing (per gram)				
		<100	101 to 1000	1001 to 10,000	10,001 to 30,000	>30,000
Bay leaves	16	7	3	5	0	1
Cayenne pepper	15	8	2	3	2	0
Chili powder	18	17	0	1	0	0
Cinnamon	18	3	9	6	0	0
Garlic powder	16	11	5	0	0	0
Mustard powder	16	12	3	1	0	0
Oregano	15	2	3	8	2	0
TOTAL	114	60	25	24	4	1

TABLE 3. Clostridium perfringens *count in processed spices used by the Armed Forces*

Spices	No. of samples	Number of samples containing (per gram)			
		<100	100 to 500	501 to 1000	1001 to 3000
Bay leaves	16	14	2	0	0
Cayenne pepper	15	13	2	0	0
Chili powder	18	18	0	0	0
Cinnamon	18	15[a]	3	0	0
Garlic powder	16	16	0	0	0
Mustard powder	16	16	0	0	0
Oregano	15	9[a]	2	2	2
TOTAL	114	101	9	2	2

[a]Two samples had 50/g

C. perfringens counts of 100/g and 250/g were found in two samples of cayenne pepper (Tables 1 and 3) from brands 2 and 3 (Table 5). Coliforms ranging from 40/g to 2400/g were found in four (27%) samples (Tables 4 and 6) from brands, 2, 3, and 9 (Table 5). No fecal coliforms or coagulase positive staphylococci were found at the lowest dilution tested and are reported as <30/g (Table 1).

TABLE 4. *Spices containing food poisoning bacteria and coliforms*

Spices	No. of samples	Percentage of spices containing *C perfringens*	Coagulase positive staphylococci	Coliforms
Bay leaves	16	12	0	0
Cayenne pepper	15	20	0	27[a]
Chili powder	18	0	0	0
Cinnamon	18	28	0	22[a]
Garlic powder	16	0	0	19[a]
Mustard powder	16	0	0	0
Oregano	15	53	7	0

[a]Fecal coliforms were negative (< 30/g)

Chili powder

Eighteen samples of chili powder were analyzed. The APC ranged from 100/g to 540,000/g (Table 1), but only three samples had APC's greater than 100,000/g (Table 7). The yeast and mold count was less than 100/g in all samples except one which was 8100/g (Tables 1 and 2). None of the samples contained *C. perfringens,* coliforms, or coagulase positive staphylococci at the lowest dilution tested and are reported as <100/g or <30/g respectively (Table 1).

Cinnamon

Eighteen samples of ground cinnamon were analyzed. The APC ranged from 10,700/g to 780,000/g (Table 1), but only six samples had APC's greater than 100,000/g (Table 7). The yeast and mold count ranged from <100/g to 4600/g (Table 1) and half of the samples had counts between 100/g and 1000/g (Table 2). *C. perfringens* counts from 50/g to 300/g (Table 1) were found in five (28%) samples of cinnamon (Tables 3 and 4) from brands 1, 2 and 3 (Table 5). Coliforms ranging from 40/g to 230/g (Table 1) were found in four (22%) samples (Tables 4 and 6) of brands 2 and 3 (Table 5). No fecal coliforms or coagulase positive staphylococci were found at the lowest dilution tested and are reported as <30/g (Table 1 and 4).

Garlic powder

Sixteen samples of garlic powder were analyzed. The APC ranged from 3700/g to 980,000/g (Table 1) and half of the samples had APC's greater than 100,000/g (Table 7). Yeast and mold counts were less than 500/g (Table 1) and most were less than 100/g (Table 2). Coliforms were found in two (19%) of the samples (Tables 4 and 6) of brand 3 (Table 5) with counts of 40/g and 230/g (Table 1). No *C. perfringens,* fecal coliforms, or coagulase positive staphylococci were found at the lowest dilution tested and are reported as <100/g and <30/g respectively (Table 1 and 4).

Mustard powder

Sixteen samples of mustard powder were analyzed. The APC was the lowest of all the spices, ranging from 500/g to 5300/g (Table 1). Yeast and mold counts were <100/g for 12 samples (Table 2) and ranged to only 2200/g (Table 1). No pathogens or coliforms were found in any of the samples of mustard powder at the lowest dilution tested and are reported as <100/g or <30/g as appropriate (Table 1).

Oregano

Fifteen samples of ground oregano were analyzed. The APC ranged from 1200/g to 3,600,000/g (Table 1). More than half of the samples had APC's less than 100,000/g (Table 7). The yeast and mold counts ranged from <100/g to 27,000/g (Table 1) and more than half of the samples had less than 10,000/g (Table 2). *C. perfringens* ranging from 50/g to 2850/g (Table 1) were found in 8 (53%) of the samples (Tables 3 and 4) from 4 different brands (Table 5). Thirty-three percent of the oregano samples containing *C. perfringens* were from brand 3 (Table 5). Coagulase positive staphylococci (30/g) were found in 1 (7%) sample of oregano (Tables 1 and 4) from brand 8 (Table 5). No coliforms were found in any of the oregano samples at the lowest dilution tested and are reported as <30/g (Table 1 and 4).

DISCUSSION

Spices procured from the local supermarket by the Military and other consumers may contain as high as 9,100,000 bacteria (APC) per gram (Table 1). The APC varied widely between most samples of the same spice and between different spices (Table 7). However, with the exception of cayenne pepper, half or more of all the spice samples had APC's less than 100,000/g (Table 7). The APC may have no overt public health significance, but it may contribute to some degree toward spoilage of a

TABLE 5. *Food poisoning bacteria and coliforms found in different brands of spice*

Brand	Percentage of spices containing: Bay leaves C. perf[a]	Bay leaves Coli.[b]	Cayenne pepper C. perf.	Cayenne pepper Coli.	Cinnamon C. perf.	Cinnamon Coli.	Garlic powder C. perf.	Garlic powder Coli.	Oregano C. perf.	Oregano Coli.	Oregano C. P. Staph.
1	0	0	0	0	11	0	0	0	0	0	0
2	0	0	7	7	5.5	11	0	0	7		0
3	6	0	13	13	11	5.5	0	19	33	0	0
4	0	0	—	—	—	—	—	—	—	—	—
5	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	—	—	—
7	—	—	—	—	—	—	—	—	7	0	0
8	—	—	—	—	—	—	—	—	7	0	7
9	0	0	0	7	0	0	0	0	—	—	—
10	—	—	—	—	0	0	—	—	0	0	0

[a]*C. perfringens*
[b]Coliforms

TABLE 6. *Coliform count of processed spices used by the Armed Forces*

Spices	No. of samples	Number of samples containing (MPN per gram) <30	30 to 100	101 to 230	>230
Bay leaves	16	16	0	0	0
Cayenne pepper	15	11	3	0	1[a]
Chili powder	18	18	0	0	0
Cinnamon	18	14	3	1	0
Garlic powder	16	14	1	1	0
Mustard powder	16	16	0	0	0
Oregano	15	15	0	0	0
TOTAL	114	104	7	2	1

[a] 2400/g

product in which a spice is used (*7*). The APC may also indicate to what extent the finished spice has been cleaned and whether or not it has been mishandled. There was no correlation between APC and yeast and mold counts.

Only five samples of spice, representing three different kinds, had yeast and mold counts greater than 10,000/g (Table 2). These counts were considerably lower than found in black and red pepper (*3*). An exception was one sample of bay leaves which had a yeast and mold count of 670,000/g (Tables 1 and 2). Whether such a spice, which appears to be an exception, poses a health hazard is a question since aflatoxin has not been demonstrated in spices. However, it cannot be considered wholesome and large numbers of toxigenic molds have been found in spices (*3, 4*).

Low numbers of coliforms were found in cayenne pepper, cinnamon, and garlic (Table 1 and 6), representing 9% of the samples analyzed. The counts equalled or exceeded 100/g in only three samples (one of each kind). Their presence does not indicate a health hazard since fecal coliforms were not found at the lowest dilution tested. However, coliforms are not a necessary ingredient of spices since they were found in only three kinds of spice (Table 4 and 6) and in only two brands out of 10 (Table 5). The occurrence of *E. coli* in spices is apparently rare and very sporadic (*7*). Preliminary studies (see Materials and Methods) showed that growth of *E. coli* was not inhibited by the concentration of spices tested, or by the procedures employed.

Since salmonellae and shigella are rarely, if ever, found in spices (*3, 7*) the only pathogens searched for in this study were *Staphylococcus aureus* and *C. perfringens*. Coagulase positive staphylococci were found in only one sample of spice (oregano) and at a very low level (Table 1 and 4). Their low incidence and low numbers in the spices analyzed indicate that they do not pose a health hazard and are not commonly found in spices. Preliminary studies (see Materials and Methods) showed that growth of *S. aureus* was not inhibited by the spice concentration tested or by the procedures employed. The modified MPN procedure employed favored the recovery of injured staphylococci, so it is not likely that viable staphylococci were present, but were unable to grow.

The incidence of *C. perfringens* in the spices analyzed was 15% (17 samples) as opposed to 6% or less in other American foods and pepper (*11*). The counts ranged from 50/g to 2850/g (Table 1). *C. perfringens* was found in four out of seven types of spices (Table 4), but in only two brands (Table 5). *C. perfringens* was found in more than half of the oregano samples analyzed, but in 33% of oregano from brand 3 (Table 5). Since *C. perfringens* in a food poisoning organism, its presence in spices is potentially hazardous to health. The spores may survive cooking temperatures and will grow in foods at temperatures between 20-50 C (68-122 F). It is also an indicator of fecal pollution in some European countries (*8*).

Except for the relatively high incidence of *C. perfringens* found in some spices, oregano in particular, this study would support the conclusion of a recent investigation that there was no potential health hazard associated with spices and herbs (*6*). However, the investigators did not search for *C. perfringens*.

Spices harboring *C. perfringens* must be considered a potential health hazard because they (and other pathogens) may grow in foods which are seasoned or garnished with spice if the food is not adequately cooked or properly refrigerated.

The support by others of the reported demand by the Consumer Union (*4*) for rigorous standards for cleanliness of spices is probably justified. Although many industries police their own products, apparently some do not (Table 5).

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TABLE 7. *Aerobic plate count (APC) of processed spices used by the military*

Spices	No. of samples	Number of samples containing (per gram) <100	100 to 1000	1001 to 10,000	10,001 to 50,000	50,001 to 100,000	100,001 to 500,000	500,001 to 1,000,000	1,000,001 to 5,000,000	5,000,001 to 10,000,000
Bay leaves	16	1	0	7	8	0	0	0	0	0
Cayenne pepper	15	0	0	1	1	0	3	1	4	5
Chili powder	18	0	6	3	4	2	2	1	0	0
Cinnamon	18	0	0	0	9	3	4	2	0	0
Garlic powder	16	0	0	4	2	2	5	3	0	0
Mustard powder	16	1	6	9	0	0	0	0	0	0
Oregano	15	0	0	5	4	2	1	2	1	0
TOTAL	114	2	12	29	28	9	15	9	5	5

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High Solids Cabbage for Sauerkraut Production: Its Effects upon Brine Reduction and Yield of Product[1]

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ABSTRACT

Experimental cabbage hybrids, especially selected for increased dry matter content, were examined for their abilities to increase the yields of finished product (sauerkraut) and concomitantly decrease the volumes of extraneous brine. The new hybrids, containing 20% more dry matter than the commercial control (Roundup), reduced the amount of final brine 57% or 388 lbs per ton of shredded cabbage. When fermented and packaged under commercial processing conditions, Roundup produced 1928 lbs, whereas the new varieties provided 2728 lbs of sauerkraut per ton of shredded cabbage. High-solids cabbage containing greater amounts of dry matter increased the product yield 41% or 800 lbs per ton of shredded cabbage.

In the sauerkraut fermentation, brine is generated by the addition of granular salt to shredded cabbage. As a result of this salting step and because of the inherently high moisture content of cabbage (92 to 94%), the volumes of brine generated during the fermentation may be far in excess of usage. For example, in the commercial manufacture of sauerkraut, 29% (w/w) of the shredded cabbage used in the filling operation, is discarded as extraneous brine (*2*). This excess sauerkraut juice, in addition to contributing to substantial losses in processing yields (nearly 500 lbs or 60 gal per ton), also possesses objectionable chemical properties which are not readily amenable to waste treatment by conventional means (*3*). Therefore, a reduction in superfluous brine formation would serve a two-fold purpose; namely, to increase the yield of finished product and reduce the volume of liquid waste.

In previous studies (*1*), it was shown that the amount of brine released during fermentation was inversely related to the dry weight content of the cultivar, thus those lines containing greater quantities of dry matter produced lesser amounts of "free" brine. These observations suggested that brine formation could be controlled or regulated by developing special varieties which inherently contained greater amounts of dry matter (less moisture). Newly developed lines, referred to as "high-solids" cabbage, were derived by crossing cabbage inbreds with Savoy cabbage, brussels sprouts, and kale. These latter species containing 10 to 18% dry matter, provided progenies with dry weight values of seven to 10%, whereas the commercial varieties (Roundup and King Cole) contained 5.5 to 8%. The objective of this paper is to describe the beneficial effects that "high-solids" varieties of cabbage have upon reducing brine formation while concomitantly increasing the production yield of finished product.

MATERIALS AND METHODS

Varietal selection by small-scale fermentation studies

Immediately after harvest of the selected hybrid, the outer wrapper leaves were removed, and the heads cored and shredded. The shredded cabbage (22.0 lb) was salted with sodium chloride (2.25% w/w), packed into plastic containers (14 in × 10 in) then capped with a water seal (4 in in depth) to assure anaerobiosis. After 6 weeks of incubation at 20 C, each variety, prepared in duplicate, was analyzed for salt, total acidity (expressed as percent lactic acid), and drained solids content as previously described (*1*). Although these latter analyses served as the major indices for selecting new cultivars, many lines were subsequently rejected because of unfavorable flavor characteristics (overtones of kale or brussel sprouts) and extensive discoloration (browning).

In-plant fermentation and recovery studies

During the 1974 processing season, one-ton lots of the high-solids composite and Roundup were processed at a local sauerkraut factory using standard commercial procedures. The high-solids sample was comprised of mixtures of seven hybrids averaging 8% dry matter, whereas the Roundup (control) contained 6.6% dry matter.

After eight weeks of incubation at ambient temperatures, the brines were separated from the solids by gravity drainage, and their respective solids to brine distributions were determined by weight.

Following drainage, 24 oz of drained sauerkraut was added to plastic bags of two lb capacities. Covering brine (10.5 oz per bag), was added by automatic dispenser immediately prior to bag closure. The brine components (water, sodium benzoate, and potassium metabisulfite) were present in concentrations to conform to the established practices of the processor.

RESULTS AND DISCUSSION

TABLE 1. *Effects of the dry matter of cabbage cultivars upon bulk sauerkraut yield*

	Dry matter (%)	Amount fermented (lbs)	Drained solids recovered (lbs)		Brine (lbs)	
		Actual	Actual	per ton[a]	Actual	per ton[a]
Roundup	6.6	1910	1266	1326	644	674
High-solids	8.0	1470	1259	1714	211	286

[a]Calculated, assuming no loss

[1]*Approved by the Director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 2190, April 1, 1975.*

[2]*Associate Professors of Food Science and Vegetable Crops, respectively.*

As shown in Table 1, the dry matter contents markedly influenced the quantity of brine released. In the case of Roundup (dry weight, 6.6%), 674 lbs of brine were produced per ton of shredded cabbage, whereas the high-solids fermentation (dry weight, 8.0%) generated only 286 lbs. Since the high-solids material generated a brine level sufficient to cover the shredded cabbage, that amount of kraut juice produced in excess of 386 lbs per ton was superfluous liquid waste and subject to discharge. Thus, a 20% increase in dry matter reduced the brine content 57%, or 388 lbs per ton.

In addition to reducing brine production, the high-solids varieties markedly increased the yield of drained solids. As shown in Table 1, Roundup provided 1326 lbs drained solids, whereas the high-solids material produced 1714 lbs per ton of shredded cabbage. Therefore, the high-solids material produced an additional 388 lbs of total drained solids, an increase of 29%.

Although the amounts of total drained solids recovered are potentially available for processing, complete recovery and utilization of the solids is rarely achieved. This loss frequently arises as a result of discarding that kraut which has undergone extensive surface spoilage. The losses due to such spoilage are evident by comparing the recoverable drained solids, Table 1, to that of utilizable solids, Table 2. In the case of Roundup, the drained solids (1266 lbs) were reduced to 1145 lbs; providing a loss of 121 lbs. On the other hand, the high-solids produced a loss of 11 lbs (1259 vs 1248 lbs). The high loss observed in the Roundup fermentation was apparently caused by an ineffective water seal.

The failure to gain an adequate seal is attributed to the poor compressibility of the cabbage mass in the presence of excessive amounts of brine. In commercial practice this "floating" response is avoided by reducing the brine to a level which provides sufficient coverage of the shredded product. However, in this experiment we did not decrease the brine levels because we wished to obtain material balances under undisturbed conditions of fermentation. Therefore, the heavy loss in drained solids displayed by Roundup (128 lbs per ton) might have been reduced by adjusting the brine levels as described above.

The effects of dry matter content upon the yields of finished product (kraut) are shown in Table 2. Upon filling and the addition of brine to the package, the utilizable solids of Roundup (1145 lbs) and high-solids (1248 lbs) yielded 1662 lbs (762 bags) and 1987 lbs (924 bags) respectively. When calculated on a per ton basis, Roundup yielded 1928 lbs (884 bags) compared to the high-solids yield of 2728 lbs (1269 bags). Therefore, the high-solids varieties increased the yield 41% or 800 lbs per ton of shredded cabbage. Of this latter amount, 388 lbs were gained as a result of the increase in drained solids yield. The remainder, more than 400 lbs, is that amount of brine required for hydrating and packaging the increased solids yield.

The influence of high-solids material upon hydration was particularly evident during the mixing (pre-fill) operation. For example, the drained high-solids kraut in contrast to Roundup, contained an insufficient amount of native brine to permit adequate mixing prior to packaging. Therefore, to facilitate handling, 2.4 oz of brine were added to 21.6 oz of drained solids. This amount of brine was rapidly absorbed by the shreds and provided a product with handling characterisitcs similar to that of 24 oz of Roundup. Thus, hydration of high-solids prior to final brining contributed 190 lbs per ton of shredded cabbage.

Prior to bag closure, 10.5 oz of brine were added to 24 oz of drained solids. Following eight days storage at 4 C, ten bags of each product were randomly selected for drained weight and chemical analyses. The distribution of solids to brine in the finished product (avg net wt 34.7 oz/bag) showed that Roundup yielded 25.4 oz and high-solids 24.9 oz of drained solids per bag. Since the increase in drained solids following short-term storage was quite low, less than six %, the significance of in-container hydration is inconclusive.

Chemical analyses of the stored kraut showed that Roundup contained 1.3% acid (expressed as lactic) and 1.80% salt, whereas the high-solids levels were 1.6% and 2.20% respectively. These comparative results show that each cabbage supported an adequate fermentation and likewise, produced sauerkrauts which conformed to the established standard of product identity (*4*).

When graded for flavor and textural characterisitcs by persons knowledgeable with kraut evaluations, the two samples were judged to be indistinguishable and of excellent qualities.

Although the high-solids materials showed exceptional promise under the experimental conditions described, their contribution to enhancing production efficiency under commercial conditions remain to be explored. Therefore, to encourage acceptance, five experimental lines will be released shortly for commercial use.

TABLE 2. *Effects of cabbage varieties on yield of finished product (sauerkraut)*

	Amount fermented (lbs)	Amount of utilizable drained solids (lbs)		Yield			
				Product (lbs)		Bags[a]	
	Actual	Actual	per ton[b]	Actual	per ton[b]	Actual	per ton[b]
Roundup	1910	1145	1326	1662	1928	762	884
High-solids	1470	1248	1714	1987	2728	924	1269

[a]Net weight, 34.7 oz per bag
[b]Calculated, assuming no loss

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A Research Note

Acceptability of Flavored Drinks Made With Cottage Cheese Whey Produced by the Direct Acidification Process

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ABSTRACT

An orange-flavored and lemon-lime-flavored drink has been formulated using whey from cottage cheese made by the direct acidification method. Two levels of sweetness were used in each flavored drink. A group of 21 persons familiar with taste panel procedures scored the drinks on a 6-point hedonic scale. The panel preferred the orange-flavored drink over the lemon-lime ($P < .05$), describing the orange-flavored drink as "like moderately" and the lemon-lime as "like slightly."

Cheddar cheese plants produce sweet whey (pH approximately 6.5) in large volumes and often arrange for the whey to be converted to a useable form. However, cottage cheese plants, frequently associated with a Grade A fluid milk operation, usually do not have sufficient volume of whey to economically justify its further processing. Thus most of the cottage cheese plants merely pay the extra disposal cost and permit the whey to go into the city disposal system. Cottage cheese whey is acid (pH approximately 4.7) and will not withstand heating and other processes as easily as will sweet whey.

The conventional method of making cottage cheese is to permit growth of lactic acid-producing bacteria causing a reduction in pH and thus aiding in the coagulation of casein. The principle of direct acidification of skim milk to the isoelectric point of casein has been used, and a definite procedure was recently published in a trade journal (*1*). Such whey does not have the characteristic "whey taint" taste as is present in whey from cottage cheese manufactured by the culture method. The pH of both types of whey is about 4.7.

Nelson and Brown (*6*) produced a cottage cheese whey based drink which was found to be acceptable, and Shenkenberg et al (*7*) developed a drink containing a mixture of milk and orange juice. Holsinger (*4*) has reviewed some of the history and problems related to the use of acid whey and its components in the fortification of soft drinks, and has presented data helpful to manufacturers of soft drinks who might be considering such action.

Development of a suitable drink for cottage cheese whey has been hampered somewhat by the carry-over of the culture flavor into the drink (*2, 3*). By use of whey from the direct acid method, this objection is avoided, yet the whey has approximately the same composition.

The objective of the present investigation was to make a whey drink from cottage cheese whey obtained from a commercial plant using the direct acidification process for cottage cheese manufacture.

MATERIALS AND METHODS

Direct acidification cottage cheese whey was taken from the cheese vat at draining time and divided into four lots. To the four portions were added the ingredients listed in Table 1. The drinks were held overnight at about 4 C and then examined by a panel of 21 people. Not all of the panelists were experts in the subject area of food technology, but all had previously served on panels evaluating various food products. No special effort was made to inform the panel members that the product was made from whey, but this fact was known to most panelists. Several of these persons had served on previous taste panels on whey drinks. The samples were put into paper cups, placed on a tray which was then placed before the panel members. Most persons were seated at an individual small table, but a few were seated at the ends of a long table. Panelists were cautioned to refrain from talking or making facial expressions during the examination. Each person evaluated each sample by checking one of the following: (*a*) Like Very Much, (*b*) Like Moderately, (*c*) Like Slightly, (*d*) Dislike Slightly, (*e*) Dislike Moderately, or (*f*) Dislike Very Much. The data were then analyzed by analysis of variance, and differences between individual samples were determined by Duncan's Multiple Range Test as explained by Larmond (*5*).

TABLE 1. *Composition of experimental whey drinks*

Sample	Quantity of whey	Orange concentrate	Lemon-Lime concentrate	Sugar	Sodium saccharin
	(gal)	(ml)	(ml)	(lb)	(ml)
1	5	390	0	2½	6½
2	5	390	0	2½	8½
3	2½	0	200	1¼	3
4	2½	0	200	1¼	2

RESULTS AND DISCUSSION

Data on the flavor socres on the whey drinks examined in these trials are listed in Table 2. The orange flavored drinks were more acceptable ($P < .05$) than the lemon-lime flavored drinks, although there also were variations among the judges. The standard deviations noted in Table 2 is an indication of the range of scores put on the samples by the judges. Regardless of the flavor (orange or lemon-lime as noted in Table 1), those samples containing more sweetner (samples 2 and 3) scored slightly higher than the less sweet samples. This however, could not be shown statistically to be of significance.

TABLE 2. *Flavor scores of whey drink*

Sample	Mean* flavor score		Standard deviation
1[a]	2.38**	±	1.16
2[a]	2.14	±	1.01
3[b]	3.00	±	1.45
4[b]	3.38	±	1.47

* 21 observations on each sample.
* * 1 = like very much
6 = dislike very much
[a]Samples denoted by the same letter did not have significantly different flavor scores.

Orange flavored drink made from cottage cheese whey manufactured by the conventional culture process (*2, 3*) was sometimes criticized by experts for having a "whey taint," and by consumer groups as "sort of like buttermilk," "too bitter," or "not like regular orange juice." Though many persons liked the drink made from ordinary cottage cheese whey, the drink made by the direct acid method did not receive the criticisms which were apparently due to bacterial growth.

The results of this investigation suggest that a whey drink might very well be marketed in those areas of the country which are faced with a problem of whey disposal and where the cottage cheese is being manufactured by the direct acidification method. The drink, as used in these trials, contained about 1% protein, 11.5% carbohydrate, and 50 calories per 100 g.

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A Research Note

Atomic Spectroscopic Determination of the Sodium Content of Cheese Solubilized with a Mixture of Ammonium Hydroxide and Methyl-Iso-Butyl Ketone

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ABSTRACT

A study was done to investigate the usefullness of a rapid digestion procedure for the determination of the salt content in cheese. This procedure compared favorably with values obtained from wet-ashed samples. The coefficient of variation of the suspension procedure was 3.3% and the mean recovery of added salt solutions was 100.7 ± 4.5%.

The sodium chloride content plays an important role in the ripening of cheese and in flavor development. The uptake of salt during brining and the distribution within the cheese needs to be controlled to maintain quality. Sodium chloride is usually determined by measuring the chloride present in cheese and calculating the sodium chloride content from these data (*1*). This method is laborios. The purpose of this work was to see if sodium could be determined in cheese with atomic absorption spectrophotometry using the digestion procedure previously described (*3*).

MATERIALS AND METHODS

Apparatus

A Perkin Elmer Atomic Absorption Spectrophotometer 303 equipped with a Deuterium Background Corrector was used. Signals were recorded on a Perkin Elmer Model 56 Recorder. Operating conditions were following: wavelength 330 nm, slit width: 0.7 nm, lamp current: 10 mA, burner: Boling, flame type: C_2H_2/air, oxidizing, time constant: 15 sec.

Reagents

All reagents used were analytical grade. Redistilled water was used throughout. A 1000 ppm sodium stock solution was prepared by dissolving 2.54 g of dry sodium chloride in redistilled water and making up to 1 l.

Cleaning

Pipettes and 50-ml polypropylene bottles with plastic caps were immersed in warm, dilute nitric acid (1:3 v/v) and held for several hours. After the acid treatment the objects were rinsed twice with redistilled water.

Sampling

Samples of various cheese varieties (Swiss, Gouda, Tilsit, Camembert) were obtained from different plants.

Sample preparation

Dissolution with ammonium hydroxide and MIBK. Approximately 0.5 - 1.0 g of ground cheese was weighed into a 50-ml polypropylene bottle. Twenty-four milliliters of an ammonium hydroxide solution (8%, Merck) and 1 ml of MIBK (Merck) were added. The bottles were closed with plastic caps. The dissolution temperature was 60 - 65 C. Usually after 10 - 15 min the cheese was dissolved. Mixing on a Vortex Genie mixer (Scientific Industries) reduced the dissolution time.

Wet-ashing with perchloric acid. Similar amounts of ground cheese were weighed into 50-ml Kjeldahl flasks, 5 ml of concentrated 65% nitric acid (Merck), and 4 ml of 70% perchloric acid (Merck) were added. The flask was heated to about 130 C on a Kjeldahl digestion apparatus. This temperature was found suitable for a slow digestion of the organic matter without causing violent reactions of the perchloric acid. Usually after 1 to 2 h the oxidation was complete as shown by the appearance of white fumes and a clear reaction mixture. The temperature was then raised to about 250 C to evaporate the perchloric acid. The non-volatile salts were taken up in redistilled water and transferred to a 25-ml volumetric flask. The Kjeldahl flask was rinsed three times and the total was made to volume with redistilled water.

RESULTS AND DISCUSSION

Preliminary studies showed that addition of 1000 ppm cesium did not increase absorption of sodium at this wavelength (*2*). The method of standard additions was applied to correct for differences in the viscosity of samples (*2*). For each cheese variety a regression line was calculated using least square analysis. The means of wet-ashed samples and those dissolved with ammonium hydroxide—MIBK mixture were statistically analyzed using the t-test (*4*). There were no significant differences. The coefficient of variation of 11 replicate determinations of two different samples was 3.3%. The accuracy of the procedure was verified by adding known amounts of sodium to the cheese before dissolution. The mean recovery of seven different additions was 100.7 ± 4.5%. At 330 nm as little as 5 ppm of sodium can be detected. This procedure should enable automation of the salt determination in cheese. Furthermore, a larger number of samples can be handled using atomic absorption than with the conventional titration method.

ACKNOWLEDGMENTS

I thank Dr. E. G. Hammond for his helpful advice in correcting this manuscript.

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J. Milk Food Technol. Vol. 38, No. 11, Pages 695-705 (November, 1975)

Patulin: A Mycotoxin of Potential Concern in Foods[1]

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(Received for publication April 21, 1975)

ABSTRACT

Patulin is a metabolite of numerous *Aspergillus* and *Penicillium* species. It is toxic to a wide range of biological systems including microorganisms, plants and animals. Patulin has also been shown to be carcinogenic to mice. The susceptibility of man to this toxin is unknown at this time. However, when the numerous biological systems affected by patulin are considered, it is likely that man would also be susceptible to the toxic effects of this compound in some manner. Thus, patulin must be considered a potential health hazard.

The fact that patulin can be produced by a number of penicillia at refrigeration temperatures is a source of possible concern. Many of these molds are found on refrigerated food products and the possibility that patulin production could occur during refrigerated storage is very real. However, the reactivity of patulin with sulfhydryl groups and other food components may cause its apparent disappearance from a food product. Whether the compound breaks down and disappears or is simply chemically altered to a non-detectable form is not known. Apparently toxicity is lessened, though it is not known if low pH or reactions with digestive enzymes might reverse these reactions or liberate free patulin in the gut. Certain foods such as cheeses and cured meats which are low in carbohydrates and high in protein do not appear to support patulin production to any great extent. This combined with the reactivity of patulin with sulfhydryl groups seems to lessen the danger of patulin occurrence in these foods. However, apple products and grain based products may pose more of a potential problem. Apple products are of particular concern since common apple rotting molds are frequent producers of patulin and because of the stability of patulin in these products.

Further work is necessary to determine more completely the foods most likely to be contaminated with patulin. Presently, foods high in carbohydrates would appear to be of greater concern than foods low in carbohydrates and high in protein. Refrigerated storage of foods will not necessarily guarantee that they will remain free of patulin since many molds capable of producing patulin can do so at low temperatures. Further work is also needed to determine the fate of patulin as it undergoes reactions with sulfhydryl groups and other food components to determine if there are conditions under which patulin can be regenerated or recovered in a toxic form.

Mold growth on foods and feeds is a common occurrence which may result in organoleptic deterioration of products, economic losses, and potential health hazards through production of toxic mold metabolites known as "mycotoxins." Diseases caused by poisoning of animals or humans with preformed toxic metabolites produced by molds growing in food products are referred to as mycotoxicoses. Animal mycotoxicoses have occurred throughout history; the most recent major outbreak occurred in England in 1960, and led to the discovery of aflatoxins (*82*). Human mycotoxicoses have also been recorded. "St. Anthony's Fire" of the middle ages was caused by ingestion of sclerotia of *Claviceps purpurea* in rye grain infested by Ergot. In Russia in the mid 1940's, as much as 10% of the population was affected by a disease known as Alimentary Toxic Aleukia (ATA), with an accompanying high mortality rate (*61*). This disease was caused by the consumption of overwintered grains that were molded by *Fusarium* and *Cladosporium* species. More recently, dietary intake of aflatoxins has been linked to the high incidence of liver disease in Uganda and Thailand (*3, 116*). Another mold metabolite, patulin, is causing increasing concern as a potential toxic fungal contaminant of food and feed products.

HISTORY

Discovery

Patulin was first described as an antibiotic during scientific endeavors in the 1940's to find additional antibiotics following the discovery of penicillin by Fleming in 1929 (*43*). Patulin was isolated by several workers from a variety of molds which gave rise to several synonyms for the compound. Table 1 lists the discoveries of various molds that produce patulin and the different names given the compound.

By 1944 comparisons were being made of these variously reported antibiotics and it was found that they all appeared to be the same compound. Bergel et al. (*15*) claimed clavatin, claviformin, and patulin were identical. Chain et al. (*32*) supported this claim, and found claviformin and patulin to be identical compounds. Anslow et al. (*6*) believed expansin to be patulin; Oosterhuis and Luyken (*89*) confirmed this finding and showed that expansin and claviformin were identical. Clavacin and patulin were reported by Hooper et al. (*56*), and Katzman et al. (*64*) to be alike, and the School of Pathology in Oxford, England, reported expansin to be identical with patulin (*2*). Lastly, gigantic acid was determined to be claviformin by Florey et al. (*44*).

Therapeutic potential

Possible therapeutic uses of patulin for combating human diseases were investigated soon after its discovery. One worker observed personal relief from a "common cold" by nasal douching of a 0.01% aqueous solution of patulin (*98*). He, and serveral of his

[1]*Published as Paper No. 3983, Journal Series, Nebraska Agricultural Experiment Station, Lincoln. Research was conducted under Project No. 16-22 and was supported by Public Health Research Grant No. CA14260 from the National Cancer Institute.*

TABLE 1. *Summary of patulin producing molds and synonyms for patulin*

Species of fungus	Reference	Name given substance
Penicillium expansum	Van Luijk (*130*)	expansine
	Anslow et al. (*6*)	clavacin
	Kent and Heatley (*67*)	clavacin
Penicillium sp.	Atkinson (*10*)	penicidin
P. claviforme	Chain et al. (*31*)	claviformin
	Bergel et al. (*15*)	clavatin
P. patulum	Raistrick et al. (*98*)	patulin
	Bergel et al. (*15*)	clavatin
P. melinii	Karow and Foster (*63*)	clavacin
P. urticae	Kent and Heatley (*67*)	clavacin
P. equinum and *P. novae-zeelanlial*	Burton and Pausacker (*26*)	
P. leucopus	Umezawa et al. (*129*)	patulin
P. cyclopium, *P. griseofulvum*, *P. equinum*, *P. divergens* and *P. lapidosum*	Singh (*117*)	patulin
Aspergillus clavatus	Wiesner (*137*)	clavacin
	Waksman et al. (*134*)	clavacin
	Umezawa et al. (*129*)	Patulin
	Bergel et al. (*15*)	clavatin
A. giganteus	Philpot (*93*)	gigantic acid
	Florey et al. (*44*)	claviformin
A. terreus	Kent and Heatley (*67*)	clavacin
Byssochlamys nivae	Karow and Foster (*63*)	clavacin
Mold sp.	DeRosnay et al. (*37*)	myocin c

colleagues, reported an immediate clearing of the nasal passages and cessation of all symptoms within 24 h. In a study involving application of a patulin solution to a person with early cold symptoms, no cure was evident but nasal congestion was relieved. In a much larger study involving 300 naval men with common colds, successful results were reported using a nasal spray containing patulin (*98*). Patulin concentrations ranging from 0.02 to 0.005% gave up to a 58% cure rate compared to 9.4% for controls. However, these results were not supported by findings of an extensive Army trial when compared to the natural progression of the disease (*120*). In addition, a clinical trial on the therapeutic value of patulin held in 1944 found it to be of little or no value as a cold cure (*77*).

Several studies involving the use of patulin as a treatment for dermal infections have also been reported. Jennings (*60*) utilized vaseline preparations of patulin in skin tests, and found that a 0.1% preparation caused no reaction, but a 1.0% preparation produced edema, redness, and a roughness of the skin which lasted up to two weeks. deWit (*38*) used etheral and ointment patulin preparations to combat fungal skin infections. All etheral solutions of from 0.025 to 0.005% produced severe irritation while less than 0.005% ointments were tolerated and produced a regression of the infections. However, use of patulin as a fungicidal agent was questioned by Hopkins et al. (*57*) who found it no better than other agents in use at the time.

CHEMISTRY, TOXICITY AND BIOCHEMICAL ACTIVITY

Chemical nature

The chemical nature of patulin has been thoroughly investigated. It can be isolated as colorless to white crystals from etheral extracts which have no optical activity. Patulin has a melting point of 110.5 C, and absorption maxima in the ultraviolet region have been reported at 275 (*36*), 277 (*94*) and 276 nm (*64, 139*). An infrared scan of patulin has absorption bands in the double bond region of 5.6, 5.9 and 6.1 microns (*36, 139*). Patulin is unstable in alkali and loses its biological activity (*10, 31, 56, 63*). It is also unstable in contact with SO_2 (*94*), but is stable in acid (*31, 59*). It is soluble in ether, chloroform, ethyl acetate, and ethanol (*133*).

Woodward and Singh (*139, 140*) are credited with the elucidation of the structure of patulin (Fig. 1). Patulin is

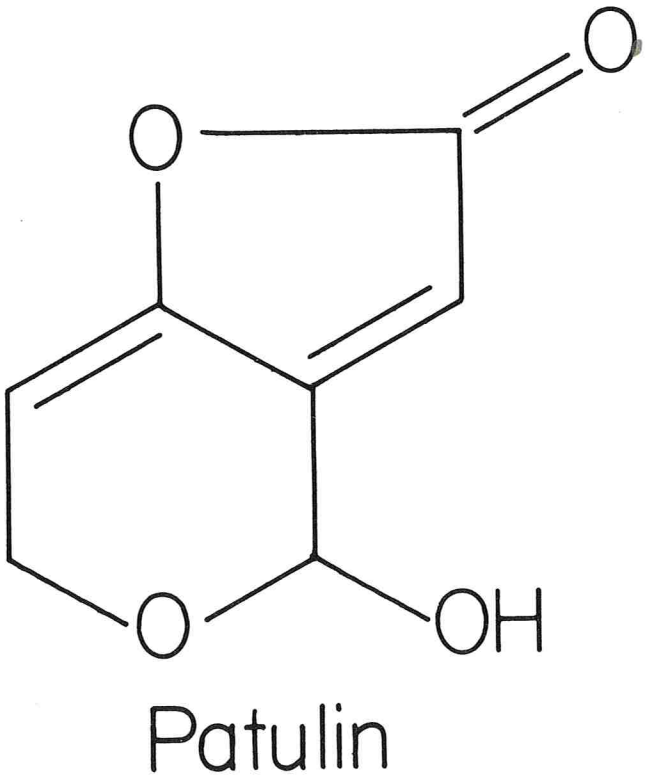

Figure 1. *Structure of patulin.*

an unsaturated lactone and has the empirical formula of $C_7H_6O_4$ with a molecular weight of 154. Patulin has been given the name 4-hydroxy-4H-furo [3, 2c] pyran-2(6H)-one.

Toxicity

Patulin is toxic to many biological systems. All bacterial species tested have been found to be sensitive to this antibiotic to some degree, irrespective of Gram type. The inhibitory effect of patulin on several human pathogens was quantitated by Chain et al. (*31*). *Bacterium coli* (*Escherichia coli*) and *Staphylococcus aureus* were completely inhibited by a 0.1%

concentration of patulin in 10 min. Chain et al. and Waksman et al. (*133, 134*) reported that patulin was a bactericidal compound. Indeed, over 75 species of bacteria have been demonstrated to be sensitive to patulin by several workers (*33*).

Patulin has also been shown to be an active yeast and mold antagonist, and can inhibit germination of mold spores (*102*). Several *Pythium* sp. have been oberved to be quite sensitive to patulin, even at concentrations as low as 0.00025% (*6, 130*). Katzman et al. (*64*) noted a strong fungistatic activity by patulin against *Rhizopus nigricans, Monilia albicans, Saccharomyces cerevisiae,* and *Sporotrichium schenkii* but no effect upon *Aspergillus clavatus,* a patulin producer, even at concentrations up to 1 mg patulin/ml. These results were also found by Sanders (*107*) who observed several human pathogenic fungi to be sensitive to concentrations of 0.01 to 0.0006% of patulin. Patulin has also been evaluated for control of several plant pathogens, including downy mildew of cucumbers (*7*), damping off of safflower (*47*), crown gall (*68*), and loose smut of wheat (*127*).

Acute toxicity of patulin to cells in vitro has been observed by many workers. Toxicity to leucocytes at concentrations ranging from 0.1 to 0.00012% has been reported (*31, 98, 132*). Rabbit corneal cells have also been reported to be inhibited by patulin in concentrations of 0.02% (*132*). Some stimulatory activity has also been noted. Mouse leucocytes and rabbit epithelium cultures were stimulated at 20-40 μg of patulin/ml but inhibited at 100-200 μg patulin/ml, and a 50% inhibition of the multiplication of rat and mouse fibroblasts in culture has been noted by as little as 154 ng of patulin/ml (*90, 97*). Chick fibroblasts and heart cultures were inhibited by 100 and 10 μg patulin/ml, respectively, in another study (*2*). No effect on any particular phase of replication of HeLa cells treated with patulin was noted by Kawasaki et al. (*66*), but rather a slowing of the entire cell cycle. Cancer cells may also be affected by patulin. Ehrlich carcinoma cells and mouse ascites tumor cells were reportedly inhibited by 20-40 and 60 μg patulin/ml, respectively (*70*). Tissues described as malignant have also been observed to be inhibited by a 0.004% concentration of patulin (*132*).

Patulin is also toxic to higher plants. Iyengar and Starky (*58*) reported that cucumber seeds were sensitive to patulin solutions and were unable to germinate or were stunted in root and stem length after exposure to patulin. Berestets'kyi and Synyts'kyi (*14*) have found 90% of culture filtrates of 85 strains of *Penicillium urticae* Banier to be toxic to seedlings of maize, peas, and flax, and patulin at 1 ppm to be toxic to sugar beets. Duckweed plant (*Lemna minor*) was inhibited by less than 1 μg patulin/g (*83*). Patulin at 50μg/g wilted safflower seedlings (*47*), and at 15 μg/g inhibited pea (*Ascochyta pisi*) seed germination (*135*), tomato seedlings (*78*), and germinated corn (*84*). But perhaps the most significant observation of phytotoxicity was that exhibited upon wheat. It was shown that stubble mulching of fields supported extensive growth of *P. urticae,* which produced patulin, causing reduced wheat seed germination rate and plant size (*85*). Indeed, wheat seeds and seedlings have been shown to be sensitive to as little as 20 ppm patulin (*40, 84, 127*).

Patulin has also been shown to be toxic to animal systems. Intravenous injection of patulin into mice and rats gave LD_{50} values varying from 0.3-0.7 mg patulin/20 g body weight, and 1 mg per mouse was always lethal (*17, 31, 98, 120*). Lethal dose for mice by intraperitoneal injection was reported by Lochhead et al. (*71*) to be 0.1-0.2 mg patulin, and by Stansfeld et al. (*120*) to be 0.25 mg patulin per mouse. Similar amounts as used for intravenous injection have produced death in mice and rats by subcutaneous injection (*71*). The LD_{50} by oral administration to mice is 0.7 mg patulin, and 2.5 mg patulin was always fatal (*17*). Rats have given comparable oral responses; death took up to 4 days in these studies, and if not immediate resulted in severe pathological changes in animals that did not die. Pathological changes reported were lungs edematous with hemorrhaging; capillary damage in the liver, spleen, and kidney; and edema of the brain (*2, 64*).

Several other animals have also been observed to be sensitive to patulin. Studies have found 10-15 mg patulin/kg fatal for cats, rabbits, and mice (*17, 41*). Chicken embryos (*55, 79, 80, 81*), chickens and quail (*74, 79, 80*), rabbit skin (*55*), guppies (*64*), the crustacean *Cyclops fuscus* (*100*), brine shrimp (*101*), and zebra fish larvae (*1*) have also been found to be sensitive to patulin in various degrees. An insecticidal activity of patulin has also been noted (*34*).

Besides the overt toxicity demonstrated by patulin to animals, carcinogenic activity has been observed with sublethal dosages. Mice, when repeatedly injected with sublethal doses of patulin, were observed to form localized tumors after 15 months (*39*). Thus it was concluded that patulin is also a potential carcinogen to animals.

Biochemical activity

Much work has been done to determine the precise mechanism of the toxic activity of patulin. Inhibition of aerobic respiration by patulin in several systems has been observed and reviewed by Singh (*118*). Bacteria, fungi, guinea pig kidney slices, brain homogenates, and phagocytic cells have all been found susceptible to respiration inhibition by patulin solutions. Cell free extracts of *Claviceps purpurea* were found to be inhibited much more rapidly (40 min) than complete mycelia (3-6 h) suggesting that a membrane barrier to patulin was present in whole cultures.

The effect of patulin on semipermiability of cell membranes has been studied. Potassium ion absorption by erythrocytes (*62*) and glucose uptake by fungal mycelia (*117*) have been shown to be interrupted by patulin. However, no leakage of metabolites such as inorganic phosphorous, carbohydrates, amino acids, etc., from *C. purpurea* mycelium or of hemoglobin from bovine erythrocytes was noted in preparations treated

with patulin (*49*). It has been pointed out by Singh (*118*) that inhibition of respiration would halt the natural uptake of several necessary substrates. The lack of metabolite leakage in treated preparations was suggested by Singh (*118*) to indicate that no altering of membrane transport systems occurs.

Inhibition of protein and RNA syntheses were not observed in the multiplication of influenza virus in mice treated with patulin (*106*). In another study, total lipids, carbohydrates, RNA, DNA, proteins, and chitin analyses of *C. purpurea* cultures treated with patulin showed no differences from controls, even though generation times were slowed (*117*). It has been noted, however, that patulin plus penicillic acid may induce DNA strand breakage in HeLa cells after 1 h of incubation (*128*).

Since the effects of patulin on respiration appear to be critical to its toxic activity, enzyme systems associated with respiration have been investigated. These data were reviewed by Singh (*118*). Dehydrogenase activities in mouse ascites tumor cells were found to be inhibited at 20 μg patulin/ml in one study and unaffected by 30-120 μg patulin/ml in another. *C. purpurea* succinate oxidase and dehydrogenase were inhibited up to 90% by 1,155 μg patulin/mg protein. NADH oxidase, succinate cytochrome C reductase, and cytochrome oxidase were inhibited by approximately 30% at a much higher concentration, 7,000 μg patulin/mg protein. Rabbit muscle aldolase was inhibited by patulin with an inhibition constant (K_i) of 1.3×10^{-5} M (*8*), while lactic dehydrogenase was inhibited with a K_i of 6.2×10^{-6} M (*9*). Adenosine triphosphatase (ATPase) from human erythrocytes has also been shown to be inhibited by patulin (*4*). Conversely, glucose oxidase and glyceraldehyde-3-phosphate dehydrogenase were not inhibited by patulin (*117*). Singh (*118*) has reasoned that because of the relative insensitivity of the terminal electron transport enzymes (NADH oxidase, cytochrome C reductase, and oxidase), the sensitivity of anaerobic bacteria to patulin (*37*), and the sensitive nature of oxygen consumption to patulin, the site of action of patulin in biological systems is before the terminal stages of respiration.

One suggested mode of action of patulin, once at the site of activity, has been the reported reactability of patulin with sulfhydryl groups (*13, 28, 29, 30, 48, 105*). Patulin has been observed to react with cysteine, glutathionine, thioglycolate, and dimercaptopropanol (*12, 13, 27, 37, 48, 55, 78, 105*). It has been theorized that by reacting with critical sulfhydryl groups in the active sites of enzymes a toxic activity would be exhibited. Conversely, an excess of sulfhydryl groups would detoxify patulin, supposedly by binding all of the patulin molecules before they could react with a vital group. Andraud and Andraud (*4*) attributed the loss of ATPase activity to this reaction and mixtures of cysteine and patulin were not inhibitory to lactic dehydrogenase (*9*). Cysteine but not glutathione or thioglycolate was observed to inhibit patulin by Cavallito and Bailey (*28*). In another study the reaction with cysteine was reportedly slow although reducing the UV absorption of patulin (*117*). The reaction of patulin with glutathione was observed to be pH dependent to some extent, with the reaction being slower at pH 5.0 than at 7.0 (*55*). Products of this reaction were less toxic to mice, rabbit skin, and chicken embryos. This theory has been challenged, however, by several findings. Ashoor and Chu (*8*) showed that patulin, bonded to cysteine, could still bond and inhibit muscle aldolase, and Singh (*117*) observed that patulin did not react with glyceraldehyde-3-phosphate dehydrogenase, an enzyme with sulfhydryl groups in its active site. Singh (*118*) has reported that the cysteine-patulin reaction is a very slow one, even at high cysteine concentrations and has suggested that a modified form of patulin may be the toxic form of the compound.

PRODUCTION AND BIOSYNTHESIS

Production conditions

Production of patulin by mold cultures in laboratory media has been investigated by numerous workers. Czapeks-Dox broth, at pH 6.9, has been utilized to produce patulin in still cultures of *A. clavatus, Aspergillus terreus* and *Penicillium patulum* incubated in the dark at 25 C for 2 weeks (*31, 72, 137*). Variations of this medium have included addition of 3% corn steep liquor (*63*) and use of maltose or glucose as the carbohydrate source (*142*).

Successful production of patulin by several *Aspergillus*

TABLE 2. *Summary of substrates utilized for the production of patulin by* Penicillium *species*

Medium	Species	Incubation temperature (°C)	Time (days)	Patulin (mg/ml)	References
Raulin-Thom	*P. patulum*	24	12-14	0.7 -1.26	(*98*)
	P. patulum + *P. expansum*	24	14	0.02-0.37	(*6*)
	P. urticae	25	14	0.1	(*88*)
Czapeks-Dox	*P. patulum*	25	14	1.37	(*123*)
+ 2.5% potato extract	*Penicillium sp.*	27-28	8-10	1.33	(*127*)
Yeast extract-sucrose	*P. expansum*	25	14	0.48	(*81*)
Potato-dextrose[a]	*P. expansum*	22-24	21	0.95	(*119*)
	P. urticae	25	14	2.7	(*88*)
	P. patulum	25	14	2.8	(*123*)

[a]Prepared from raw potatoes according to the method of Norstadt and McCalla (*86*)

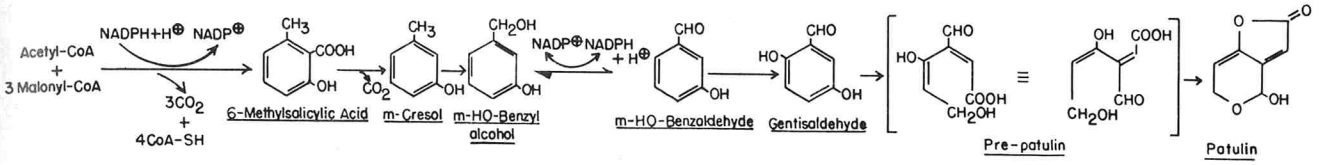

Figure 2. *The major pathway for patulin biosynthesis in* Penicillium urticae *NRRL 2159A (45).*

sp. has been reported using glucose-nitrate-trace mineral broth. *Aspergillus fumigatus* and *A. clavatus* produced maximum amounts of patulin in still cultures at 28 C after 8 and 6 days, respectively, as measured by antibiotic activities of the filtrates. Variations have included use of brown sugar instead of glucose and incubation for 7 to 10 days at 22 to 28 C in shallow cultures (*35*) and addition of 0.2% corn steep liquor or 1% yeast extract, at pH 4.5, with 5 to 11 days incubation at 24 C (*64*).

Several substrates have also been utilized to produce patulin by *Penicillium* spp. (Table 2). Studies of optimum production of patulin by penicillia have shown that with inorganic salts media, glucose and iron salts stimulated patulin production while most organic materials such as yeast extract, corn steep liquor, and peptone depressed toxin production (*71, 127*). However, potato extract prepared from raw potatoes resulted in very high yields of patulin from *P. urticae* (*86, 88*). In general, glucose has been found to be the most suitable carbohydrate source for patulin production by *Penicillium* spp., with sucrose, lactose, dextrin, and starch about half as effective. Greater yields of patulin have been obtained with stationary than with submerged cultures, and the optimum temperature for patulin production has been reported to be 20 to 25 C, though lesser amounts can be produced at 5 C. At 25 C highest yields of patulin have been obtained in 12 to 14 days of incubation.

More recently Stott and Bullerman (*123*) found that patulin production by *P. patulum* followed a similar pattern. Glucose supported higher yields of patulin than lactose in a salts medium, and more patulin was produced at 25 than at 5 C. An inorganic nitrogen source supported more patulin production at 25 C than organic nitrogren from milk, but the organic nitrogen supported more toxin production at 5 C. Growth of the fungus on broth containing organic nitrogen was always very extensive when compared to growth on broth containing inorganic nitrogen or potato dextrose broth, but toxin production was less. Patulin was produced on a broth medium containing 1% casein, but was not detectable in a 25% slurry of casein.

Biosynthesis

The biosynthetic pathway of patulin from 6-methylsalicylic acid (6-MS) was elucidated by Bu'Lock and Ryan (*22*) using [^{14}C] glucose. Forrester and Gaucher (*45*) and Scott et al. (*109*) confirmed these results and also reported a number of side reactions which could occur. In the synthesis of patulin one acetyl-CoA molecule plus three malonyl-CoA molecules combine, undergo reduction and decarboxylation, to produce 6-MS (Fig. 2). Upon further decarboxylation and oxidation, the 6-MS molecule is converted to m-OH-benzaldehyde which then undergoes a rearrangement resulting in one molecule of patulin.

Bu'Lock et al. (*23*) further characterized the biosynthesis of patulin as the result of secondary metabolism. Growth of *P. urticae* was explained in terms of two phases: (*a*) tropophase, in which mycelial nitrogen and phsophorous uptake occurs, and RNA and sulfhydryl containing compounds appear with the rapid utilization of glucose, primarily by the hexose monophosphate pathway; and (*b*) idiophase, in which a reduced assimilation of nitrogen and phosphorous occurs, RNA and sulfhydryl levels are lower, glucose oxidation slows and occurs primarily by glycolysis, fatty acids begin to accumulate, and special phenolic metabolites derived from 6-MS appear. The transition between phases is sharp, and occurs between 24 and 36 h of growth, at which time 6-MS appears. Bu'Lock et al. (*25*) have shown by selective protein synthesis inhibition that the formation of 6-MS is due to a 6-MS synthetase enzyme system which is metabolically stable. This enzyme is formed during replicatory growth and activated later. Feedback control of this enzyme by 6-MS has also been noted (*24*).

The conversion of 6-MS to gentisaldehyde and to patulin was observed to involve metabolically labile enzymes formed later in culture development and which were regulatable by both induction in the presence of substrate and repression by high nutrient levels (*25*). A key enzyme in this conversion has been identified as m-OH-benzyl alcohol dehydrogenase which requires $NADP^+$ cofactor and catalyzes the oxidation of m-OH-benzyl alcohol to m-OH-benzaldehyde (*46*). A possible feedback inhibitor of this enzyme was thought to be gentisaldehyde. Scott and Beadling (*110*) also reported the activity of m-OH-benzyl alcohol dehydrogenase and observed patulin production from gentisaldehyde in cell free systems from *P. patulum*. It was suggested that a dehydrogenase system is involved in the synthesis of patulin from gentisaldehyde.

DETECTION AND ANALYSES

Physicochemical methods

Many methods have been reported for extraction, identification, quantitation, and bioassay of patulin from laboratory media as well as some food products. Early methods of extracting patulin from liquid culture media involved the use of a norite absorption (1%) with acetone

elution giving crystalline patulin (*63*). Present methods simply use ethyl acetate extraction and concentration (*73, 84, 119*), or diethyl ether extraction and alumina (80-200 mesh) column clean up (*86*). Extraction methods from grains have involved the use of acetonitrile-water (9 + 1) combined with defatting using isooctane (*121*), or acetonitrile-hexane (4 + 1) extraction with preparative TLC clean up (*95*). Tauchmann et al. (*126*) reported a method for the extraction of patulin from sausages using acetonitrile-hexane (20 + 9) as the extracting solvent. Extracts were put on a celite column, defatted with hexane, and eluted with ethyl acetate. Extraction from several flours and fruit juices using ethyl acetate extraction with silica gel (G-60) column clean up with benzene-ethyl acetate (3 + 1) (*115*) or ethyl acetate elution (*112*) has been reported. Direct liquid-liquid extraction from apple cider with ethyl acetate has also been reported (*96*).

Identification and quantitation of patulin in food and culture extracts has been reported using several techniques. Patulin can be identified using gas-liquid chromatography of the patulin silyl ether, acetate, and chloroacetate derivatives which have lower detection limits of 100, 60, and 40 ng, respectively (*96*). This method can be used to quantitate patulin by preparing a standard curve. With this method, recovery of patulin from apple juice was found to have a lower detection limit of 0.7 μg/ml of juice. Gas chromatography (GC) of patulin without derivatization has also been reported (*114*). Although no detection limits were given, 1 ppm of patulin recovered from apple juice was detectable by this method. Pero et al. (*91*) and Pero and Harvan (*92*) described GC methods for patulin along with penicillic acid from corn and patulin alone as a trimethylsilyl ether for qualitative data in corn. Gas chromatography of patulin and its derivatives has been applied to the quantitation of patulin extracted from rice (*125*). Use of high pressure liquid chromatography (HPLC) for isolation of patulin, combined with detection using ultraviolet adsorption of 254 nm has also been reported (*136*). Using this method, levels of patulin as low as 11 μg/liter of apple juice could be detected with a lower detection limit of 4 ng.

Mass spectrometry was used to confirm the recovery of patulin from apple juice by Scott et al. (*114*). The maximum UV absorption of patulin has been utilized by Pohland and Allen (*94*) and Singh (*117*) to study the stability of patulin and to help identify it (*48*). Infrared (IR) scans of patulin has been reported and used to verify that patulin was recovered from liquid media (*84, 87*).

The most widely used quantitative tool for patulin determination has been thin layer chromatography with various solvent combinations and derivatizations of the chromatographed patulin. Several derivatizing agents have been reported which have quantitative value when proper reactions at correct R_f values are observed. Those reported with detection limits for quantitation of purified patulin include 0-dianisidine, 0.2 μg (*99*), acidic ethanolic p-anisaldehyde, o.1 μg (*113*), n-methylbenzthiazolone-(2)-hydrazone, 0.06 μg (*103*), and fluorescence quenching on fluorescent silica gel, 0.04 μg (*95*). Patulin recovered from flour and fruit juice has been quantitated by derivatization with phenylhydrazine (*114*), and 3-methyl-2-benzothiazolinone hydrazone hydrachloride (*115*), from meat with 1% diphenylborinic acid-B amino-ethyl ester (*126*), and from grain by 3% ammonium hydroxide then 4% phenylhydrazine (*95*). Detection limits reported with these methods were 0.02-0.05, 0.01, 0.1, and 0.12 μg patulin, respectively.

Bioassay methods

In addition to chemical means of analysis, several biological methods have been reported for the observance of the toxicity of patulin, but few quantitative methods exist. Chicken embryos have been widely utilized to test a variety of compounds, including mycotoxins, for toxicity. Potentially toxic compounds in aqueous or certain organic test solutions can be injected into the yolk or air sac (*131*). Chicken embryos have been found sensitive to at least 8μg of patulin and have been used to detect patulin and measure its biological activity (*20, 21, 55, 72, 79, 80, 81, 141*). Feeding of patulin to mice and ducklings was used in another study to measure toxicity (*111*). Rabbit skin, quail, mice, white leghorn cockrels, and brine shrimp have also been used by numerous workers to demonstrate toxicity due to their sensitivities to patulin as previously discussed.

A quantitative bioassay using germination of wheat seeds to measure the toxicity of patulin was reported by Norstadt and McCalla (*88*). The Cheyenne strain of wheat seed was found to be sensitive to about 20 μg patulin/ml. Patulin has an LD_{50} of 18μg/ml to zebra fish larvae, and this system has also been proposed as an assay system for patulin (*1*). Brine shrimp were found sensitive to as little as 10 μg patulin and might therefore also have potential application as a bioassay system (*51*).

Several bacterial systems have been utilized to assay patulin. These have been reported using the streak plate method with *Bacillus subtilis, Escherichia coli,* and *S. aureus* (*48, 64*), and the cylinder plate method with *S. aureus* and *E. coli* (*54, 127*). With these methods the potency of the sample was recorded as the highest dilution of the substance which prevented growth. Quantitation of patulin in the range of 2-4 μg/ml has been reported for *Agrobacterium tumefaciens* (*68*) and to 4μg/ml for *B. subtilis* (*65*) using turbidimetric and dilution methods, respectively. Disc assays using *Bacillus megaterium* have been reported with sensitivities to patulin as low as 1.0 μg and 1.7 μg (*119, 122*). The method of Stott and Bullerman (*122*) was shown to correlate well with TLC and spectrophotometric assays in terms of accuracy, but with less sensitivity. The method was applied to culture media, apple juice, and cornmeal, and could be applied to other products upon the demonstration of the absence of other inhibitory compounds or by their removal using a clean up step.

OCCURRENCE IN FOODS

Reactions of patulin with food components

Several compounds common to certain foods, have been observed to affect patulin, though not all contain sulfhydryl groups. Peptone, casein hydrolysate, serum, liver infusion, glycine, methionine, asparagine, p-amino benzoic acid (*5, 12, 32, 37, 64, 134*), sodium sulfate, sodium thiosulfate, and sodium pyrosulfate (*78*) display an inhibitory effect on patulin. Opposed to these are several compounds which are reported to enhance toxicity. These are tryptophan, urea, and thiourea (*37*).

Stability of patulin in food products

The stability of patulin in several food systems has been observed, relative to time and temperature. Pohland and Allen (*94*) have observed the stability of patulin in apple juice and in several grain systems (Fig. 3). At 4-8 μg patulin per gram, patulin was stable in

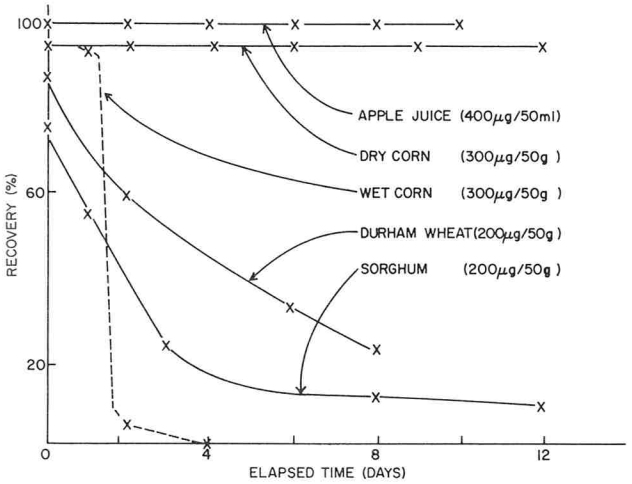

Figure 3. *Recovery of patulin from various commodities* (*94*).

apple juice and dry corn up to 14 days. Spiked Durham wheat, wet corn, and sorghum gave an 80% decrease in detectable toxin after 14 days. Timonin (*127*) has shown that patulin is also unstable in wheat flour. Scott and Somers (*112*) investigated the stability of patulin in several fruit juices at 22 and 80 C. At 4 ppm, patulin was observed to be stable in apple and grape juices up to 3 weeks at 22 C. Greater than 50% of the patulin in apple and grape juices was detectable after 10 min at 80 C, and 45% after 20 min. Harwig et al. (*53*) reported that patulin disappeared more rapidly in apple juice undergoing fermentation by *Saccharomyces cerevisiae* and *Saccharomyces ellipsoides* than in non-fermented juice. However, the disappearance could not specifically be related to cell growth or ractions with yeast metabolites, though these were given as possible explanations. The authors concluded that it would be unlikely for patulin to be detectable in the fermented type of apple cider they had studied.

The thermal stability of patulin has been studied in pure systems. Wiesner (*137*) noted the resistance of patulin to boiling and Heatley and Philpot (*54*) reported patulin to be stable at 100 C for 15 min. The effect of pH on stability of patulin was investigated by Lovett and Peeler (*75*). Patulin was found to be resistant to thermal destruction at pH 3.5, 4.5, and 5.5. Both D and Z values increased as the pH decreased indicating increased heat stability at acidic pH values.

Stott and Bullerman (*124*) found that when pure patulin was added to Cheddar cheese, there was an immediate decrease in the amount of patulin that could be recovered by solvent extraction. The decrease was the greatest during the first 3 h. From 12 to 48 h there was no further substantial decrease in the percent of patulin recovered. Neither storage temperature nor heat sterilization of the cheese had any effect on recovery of the patulin. Disappearance of patulin was attributed to chemical reaction with the cheese.

Contamination of foods with patulin producing molds

Toxigenic molds capable of producing patulin have been isolated from a variety of commercial food products and from poultry feed. Flour has yielded *A. terreus, A. clavatus,* and *P. urticae* contaminants (*50*). *P. expansum, A. clavatus, A. terreus,* and *P. urticae* have been isolated from cereal grains and legumes (*111*). *P. expansum* has also been isolated from inshell pecans (*108*); from apricots, crab apples, and persimmons (*119*); from pears and grapes (*6, 119*); and from apples (*6, 52, 119, 138*). *Byssochlamys* species are heat resistant contaminants of fruit juices, apples and grapes (*143*), and may represent a hazard since *Byssochlamys nivea* has been identified as the *Gymnoascus* species reported by Karow and Foster (*63*) and Kuehn (*69*) to produce patulin. Atkinson (*11*) reported that patulin producing penicillia came mainly from fruits and vegetables. But more recently, dried sausages were found to be contaminated with *P. expansum, P. urticae,* and *P. melinii* (*79, 80, 81, 126*). *P. expansum* has been isolated from aged, cured hams (*141*) and other meats (*42*). Lovett (*72*) has reported the isolation of *A. terreus* and *P. patulum* from poultry feed.

Patulin producing strains of molds isolated from foods generally make up a low percentage of the total isolates. Only 1% of the penicillia isolated from flour and bread by Bullerman and Hartung (*19*) were able to produce chemically detectable patulin in vitro. Six of 422 (1.4%) of the total molds isolated from European style dry sausages were observed by Mintzlaff et al. (*81*) to be capable of producing 0.02 to 0.48 mg patulin/ml of laboratory media. Of the 116 molds isolated from cornmeal only 0.9% were reported to produce patulin by Bullerman et al. (*21*). Thirteen of 349 (4%) molds isolated from Cheddar cheeses were found to produce patulin, but no patulin producing isolates were found in a survey of Swiss cheese (*18, 20*). However, 42 of 61, or 66%, of naturally rotted apples yielded *P. expansum* isolates which were observed to produce patulin (*52*). Thus, apple products appear to be the foods most likely to be contaminated with patulin with a lesser chance of contamination existing for grain products and

refrigerated food products such as cheese and cured meats.

Patulin production at refrigerated temperatures

Several molds isolated from foods have been reported to produce patulin at refrigerated temperature in laboratory media (*76*). Three strains of *A. clavatus,* and two each of *P. expansum* and *P. patulum* produced in excess of 400 μg patulin/ml media after 100 days of incubation at 1.7 C. Patulin production in excess of 70 μg/ml was observed from *P. claviforme* at 7.2 C and *P. griseofulvin* at 12.8 C after 55 days incubation. *P. claviforme* and *P. expansum* produced more patulin at 7.2 C than at 12.8 C, and detectable toxin was observed by 10 days at 1.7 C, 7 days at 7.2 C, and 5 days at 12.8 C. *P. urticae* has been reported capable of producing up to 630 μg patulin/ml liquid medium in 3 weeks at 10 C and up to 250 μg/ml at 5 C. Levels of 60 μg and 43 μg of the toxin was observed per milliliter of medium within 4 days at these temperatures, respectively (*88*). In a similar study using *P. expansum* in liquid media, maximum patulin production of 0.6 mg/ml media was observed after 5-7 weeks incubation at 10 C with 0.15 mg/ml produced within 2 weeks. At 0 C approximately 0.02 mg patulin/ml of media was noted after 2 weeks, 0.6 mg/ml after 12 weeks, and a maximum amountof 0.7 mg/ml media after 18 weeks incubation (*119*). Stott and Bullerman (*123*) found that *P. patulum* produced up to 674 μg patulin/ml of potato dextrose broth at 5 C when incubated for 6 weeks.

Natural occurrence of patulin in foods

Though patulin producing molds have been found on a variety of foods and wide temperature ranges, only apple sap, apple juice, and apple cider have been found to be naturally contaminated by this toxin. Brian et al. (*16*) discovered patulin in excess of 1000 ppm and Harwig et al. (*52*) found 0.02 to 17.7 mg per apple from the sap of apples rotted by *P. expansum.* Apple tissue was found to contain up to 125 μg patulin/g of tissue molded by *P. expansum,* but this was substantially lowered when incubated in atmospheres modified to 2% 0_2 or 7.5% CO_2 (*119*). Scott et al. (*114*) isolated 1 ppm patulin from one of 12 commercially available "Sweet Apple Cider" samples analyzed. Wilson and Nuovo (*138*) reported finding up to 45 ppm patulin from "Organic Apple Cider." In the Washington, D.C. area levels of 49 to 309 ppb patulin were found in eight of 13 commercial apple cider samples analyzed in another study (*136*).

Despite these findings, there are not reports of patulin being present in foodstuffs other than apple juice. This is true even though extensive growth of patulin producing fungi has taken place upon the product. Mintzlaff et al. (*81*) could find no chemically detectable patulin in fermented dried sausages even after extensive growth of *P. expansum* had occurred. *P. expansum* is a known patulin producing mold often isolated from this type of sausage and has even been recommended as a starter for mold fermented sausages in Europe. In another study, meat samples molded by *P. expansum* gave no detectable patulin (*42*). Finally, whole meal wheat-germ and linseed breads contaminated with *P. expansum* and analyzed for patulin after growth showed that the toxin could be detected after 10 days of growth but not after 20 days (*104*). The lack of or decrease in patulin evidenced in these studies was attributed to the reaction of the toxin with sulfhydryl groups present in the foods making it chemically undetectable and of lessened toxicity. Stott and Bullerman (*124*) found that when *P. patulum* was grown on Cheddar cheese, patulin was detected in the mold mycelia and the first 2-3 mm of cheese in samples stored at 25 C for 2 weeks. The amounts of patulin found ranged from 1.3 to 0.2 μg patulin/g of mycelia and cheese. However, no patulin could be detected in mycelia or cheese in samples stored at 5 C for 6 weeks.

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Indices of Water Content in Gaseous Systems, Their Measurement, and Relationship to Each Other[1,2]

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ABSTRACT

The quantity of water available to microorganisms influences both their growth and death kinetics. The water in microbial cells cannot be measured directly. The equilibrium atmospheric water conditions are usually measured and reported. The attributes of the several measuring units that can be used to indicate the water content in a gaseous atmosphere are described. Equations for their calculation and for converting from the several measurement units described to relative humidity at test temperature are presented.

The survival and growth of microorganisms requires adequate availability of water. Preservation of biological material by drying is accomplished because microorganisms cannot grow and reproduce under conditions of low water availability. Mossel and Ingram (*6*) in discussing the physiology of microbial spoilage of food list the "physical state of the food" as one of the important intrinsic factors affecting spoilage. They discuss the role of water vapor pressure and then specify the lowest equilibrium relative humidity (ERH) permitting development of spoilage organisms and list the data sources. Minimum ERH values are:

Organism	*Minimum ERH*
normal bacterial	0.91
normal yeasts	0.88
normal molds	0.80
halophilic bacteria	0.75
xerophilic fungi	0.65
osmophilic yeasts	0.60

They also list "alarm water contents" of some common "dry" foods assuming an RH of 0.70 and temperature of 20 C. For most dry food products the "alarm water content" is 13 to 15%.

Water availability is of critical importance in the preservation of "intermediate moisture foods" (IMF). These are foods that have a water content usually greater than 18% and an ERH of 0.60 to 0.85. The percent water is above the "alarm water content;" therefore, IMF's are not preserved by the reduced ERH alone. Intermediate moisture foods are preserved by a combination of the bacteriostatic effects of reduced ERH, reduced pH, and the addition of chemical preservatives.

The critical importance of water on microbial growth is universally recognized; however, there is little agreement regarding how to measure and report water conditions.

Bone (*3*) states that "Water activity is the basic control factor in preservation of dry foods, salted foods, mincemeats, jellies, etc." At a later point in his paper Bone states that "Water activity of a food can be defined as the ratio of the water vapor pressure above the food to the vapor pressure of pure water. A thermodynamic principle says that at equilibrium, the activity of a vapor equals the activity of the corresponding liquid phase. Thus, the measurement of the equilibrium relative humidity of a food is also a measure of its water activity."

The dry heat destruction of bacterial spores is a function of the water content of the spore during heating. Therefore, the D-value or other microbial destruction rate parameter must be related to a water content parameter—either water content itself or the relative vapor pressure of the water in the spore. For a specific spore prepared in a specific manner it is assumed that there is a fixed relationship between spore water content and the relative humidity or other measure of the water in the atmosphere adjacent to the spore. Some systems of water measure are conservative while other systems are not. (Conservative in the context we are using it means that the physical conditions of temperature and pressure of the system can change but the measure will not change.)

Several different indices—relative humidity at test temperature, relative humidity at ambient temperature, parts per million water, absolute humidity and dew point—are being used in the NASA Planetary Quarantine program to quantify water vapor in dry heat microbial destruction systems. To compare dry heat destruction data, where the concentration of water in the atmosphere surrounding the spores has an effect on the spore destruction rate, it is necessary that all the data be in terms of a single water measurement index. Since humidity is a material variable, it cannot be linearly

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converted from one form to another as can physical variables such as temperature and pressure. Furthermore, it is sometimes necessary to specify additional physical parameters before such a conversion can be made.

In this report we will discuss measures of atmospheric water which can be used to describe the equilibrium conditions and indirectly the water availability when microorganisms are dry, are in the intermediate moisture range, or are in the growth range. We will try to relate the different measure of atmospheric water and show how our formula and tables can be used.

DESCRIPTION OF WATER IN BACTERIAL SPORES

Scott (*8*) used the term "water activity" to describe the status of the water in a solution or substrate. He defined water activity (a_W) as the ratio of the apparent vapor pressure of the solution or substrate to the saturated vapor pressure at a given temperature. In his examples, the a_W of solutes in aqueous solutions are calculated using the osmotic coefficient, the molal concentration of the solute and the number of ions generated by each molecule of solute. Theoretically, under equilibrium conditions the water vapor pressure in the gas above and in the solution will be equal.

The water vapor pressure in the gas can be measured by psychrometric methods. The ratio of the water vapor pressure in the headspace to the saturated water vapor pressure is the relative humidity and, at equilibrium, is theoretically equal to the water activity of the solution.

The concept of water activity of solutions adopted by Scott (*8*) has been extended to microbial cells and spores. Since it is possible to confuse terms, the following are suggested to clarify the description of water in microbial systems: Relative humidity is a real, engineering unit that is the ratio of two measured quantities—the actual water vapor pressure in a system and the saturated water vapor pressure at the same temperature. It is used with gaseous systems, for example, to describe the water condition in the atmosphere surrounding bacterial cells or spores. Water activity, a_W, is a term used to describe the relative water availability inside a microbial cell or spore. Water activity is a theoretical term that cannot be directly measured. If the cell or spore is in equilibrium with the surrounding atmosphere, theoretically the water activity of the spore is equal to the external relative humidity.

When dry microbial cells are heated, the rate of destruction of the cells is influenced by the test system. An "open system" is open to the atmosphere; the ambient atmospheric water vapor pressure conditions determine cell-water content. In a "closed system" the microorganisms are in a fixed volume hermetic container where the total water present initially will be present throughout the test.

In reporting dry heat research data, if in a microbial destruction rate test the relative humidity is measured and controlled, then the results should be reported as a function of relative humidity, not water activity. Whenever a relative humidity value is reported, the temperature at which the relative humidity was measured should be included; for example, 0.2% RH (110 C).

PROPERTIES OF GAS MIXTURES

Before outlining the terms used to describe water content, the physical laws that describe the behavior of gas mixtures will be reviewed.

The physical state of a gas can many times be described by the Ideal Gas Law. The equation of the state of an ideal gas is expressed by the following relationship:

$$PV = nRT \qquad [1]$$

P = pressure exerted by the gas
T = absolute temperature
R = ideal gas proportionality constant
n = number of moles of gas
V = volume occupied by the n moles

In a mixture of gases, the molecules of each component gas are distributed throughout the containing vessel. The total pressure is the sum of the pressures exerted by the molecules of the component gases. By definition, the *partial pressure* of any one component gas is the pressure that the component gas will exert when it is alone in the same volume at the same temperature. Also by definition, the *pure-component volume* of a constituent gas is the volume that the component gas alone will occupy at the system pressure and temperature.

For an ideal gas, many properties are additive. The total pressure (P) is equal to the sum of the partial pressures.

$$P = p_a + p_b + p_c + \ldots \qquad [2]$$

p_a, p_b, p_c, etc. are the partial pressures of component gases a, b, c, etc.

This is a statement of Dalton's Law. In the same fashion, the total volume is equal to the sum of the pure-component volumes.

$$V = V_a + V_b + V_c + \ldots \qquad [3]$$

V_a, V_b, V_c, etc. are pure-component volumes of gases a, b, c, etc.

Equation 3 is many times referred to as the Law of AMAGAT or Leduc's Law.

An understanding of these relationships is required to understand the interrelationships between different methods of measuring water in an air atmosphere. For analytical purposes, the mixture of water and air is usually represented as a bi-component mixture of dry air and water vapor where the dry gas is a complex mixture of gases including nitrogen, oxygen, carbon dioxide, and

traces of the other gases found in the atmosphere. The molecular weight of the dry gas is taken to be 28.967.

While dry air exhibits the characteristics of an ideal gas, water vapor deviates somewhat from the Ideal Gas Law. However, this deviation is second order in effect and can be neglected.

DESCRIPTION OF WATER IN GAS MIXTURES (Humidity)[4]

There are many distinct humidity forms in use today. We shall describe eight forms that are used to describe the water vapor condition in dry heat or food storage atmospheres. All forms can be divided into two groups:

I. Expressions based on water substance
   - A. Vapor density
   - B. Relative humidity
   - C. Humidity to gas ratios
     1. Specific humidity (mixing ratio)
     2. Mole fraction
     3. Parts per million

II. Phenomenological forms
   - A. Vapor pressure
   - B. Dew point
   - C. Wet and dry bulb psychrometry

Vapor density

The gravimetric determination of water vapor in a gas yields data in the form "weight of water per volume of gas" at the temperature of the experiment.

Vapor density is useful because rates of evaporation, diffusion, and reaction are related to differences in concentration of the constituents of a system; also because it directly states the quantity of water vapor by weight in a given volume, rather than as a ratio involving other data.

The numerical value of the vapor density for a particular sample varies with both temperature and pressure in open systems such as we ordinarily encounter in most humidity problems. Therefore, it is sometimes cumbersome to handle in computations.

In a closed system the vapor density method is a conservative form maintaining the same value with changes in temperature and pressure. In the dry heat studies carried out at the FDA-Cincinnati Laboratories, humidity data has been reported using this form (*4*).

Relative humidity

The relative humidity form is widely used by the comfort air-conditioning industry and by persons involved in the storage and processing of materials. It is unique among the humidity reporting forms because the moisture content of many materials is a direct function of relative humidity; therefore, changes in water content are directly proportional to changes in RH.

Relatve humidity is defined in ASHRAE (*2*) as the ratio of the mole fraction of water vapor present in the air to the mole fraction of water vapor present in saturated air at the same temperature and barometric pressure. It approximately equals the ratio of the partial pressure or density of the water vapor in the air to the saturation pressure or density, respectively, of water vapor at the same temperature.

The relative humidity form is not conservative with respect to either temperature or pressure in closed or open systems. Since the relative humidity may be defined as the ratio of the moisture content of a space to the saturation moisture content, it is affected by temperature (the reference saturation moisture content is temperature dependent). Changes in the pressure of a given sample affect the relative humidity. When there is a change in temperature and/or pressure, the corresponding change in relative humidity can be determined by, (*a*) converting relative humidity to the defining equation, (*b*) adjustment of the individual terms, and (*c*) recombination to determine the new relative humidity.

Humidity to gas ratios

Three methods of expressing humidity as a ratio will be considered in this discussion:

$$\text{Specific humidity} = \frac{\text{Weight of water vapor}}{\text{Weight of dry gas}}$$

$$\text{Mole fraction} = \frac{\text{Moles water vapor}}{\text{Total moles wet gas}}$$

$$\text{Parts per million} = \frac{\text{Volume of water vapor}}{\text{Million } (10^6) \text{ volumes of wet gas}}$$

Specific humidity, humidity ratio (*2*), humidity (*9*), mixing ratio, and absolute humidity (*7*) are all terms used to describe the ratio of the weight of water vapor to a unit weight of coexisting dry gas. The dry basis ratio is used principally because it is a completely conservative form and lends itself readily to computations where the dry gas fraction does not change.

Mole fraction is the moles of water vapor to the total moles of wet gas. It is an equally conservative form.

Parts per million (ppm) is the ratio of the volume of the water vapor to a million volumetric parts of total gas. Using the Ideal Gas Law, the ppm of water can be related to the partial pressure of water vapor (p_w) in the following manner:

$$\text{ppm} = \frac{10^6\, p_w}{P} \qquad [4]$$

p_w = partial pressure of water vapor
P = total pressure

Since it is based on pure-component volume, it also represents moles of water per million total moles of gas and, therefore, a million-fold measure of the mole fraction of water. This measure is also independent of temperature and total pressure barring any phase change. Although this measure is usually employed to

[4] *In preparing this section of the report, "Humidity Reporting Forms and Humidity Sensors" by Elias J. Amdur (1) has been used extensively.*

indicate the water content of relatively dry gases, it can be used at all water levels up to saturation.

Vapor pressure

The vapor pressure of water is an absolute humidity form and the rate of evaporation, diffusion, and reaction are proportional to vapor pressure differences as well as to vapor density. The vapor pressure is, in fact, the preferred form for most purposes, largely because it is more conservative. A numerical value for the vapor pressure varies with total pressure in open systems but is not affected by temperature. In closed systems, the value of the vapor pressure depends on both total pressure and temperature.

Dew point

The water vapor pressure in a system consisting of a vapor space and a plane water surface in isothermal equilibrium is known as the "saturated" vapor pressure of water at that temperature. The saturation vapor pressure of water as a function of temperature is very accurately known. Because of this relationship, it is theoretically possible to determine the vapor pressure in a space by determining the temperature of a plane water surface which is in equilibrium with it.

The dew point determination, however conducted, is an experiment designed to approximate the saturation temperature of the vapor in a space. In the classical procedure one cools a surface until dew forms on it and determines the temperatures at which the dew just forms and just evaporates from the surface. The mean of these two temperatures is regarded as the dew point.

In spite of theoretical difficulties which prevent attainment of high precision, the dew point measurement yields a useful approximation of the saturation temperature and, therefore, the dew point form is a useful alternative method of expressing the absolute humidity. It is also very useful in itself because it indicates the temperature at which moisture will condense on surfaces or clouds will form in a space. This is a uniquely useful property of this reporting form.

Since the classical dew point determination involves bringing a surface to a "dew point" temperature, this type of measurement is independent of the original temperature of the gas being investigated. Since compression or expansion of a gas mixture affects the water vapor pressure, the dew point is also affected by total pressure changes. The dew point and vapor pressure are therefore equally conservative.

Wet and dry bulb psychrometry

The wet and dry bulb psychrometric instrument in the form of the sling psychrometer is probably the most widely used humidity measuring instrument. It is ordinarily considered to be a relative humidity instrument, but examination of the simplified basic equation for this instrument indicates that it yields absolute humidity:

$$(e_w - e_a) = C(T_a - T_w) \quad [5]$$

e_w = the vapor pressure of the water on the wick
e_a = the unknown ambient vapor pressure
T_a = the dry bulb temperature
T_w = the wet bulb temperature

The two temperatures, T_a and T_w, are read from the instrument. The value of e_w can be determined from a vapor pressure table knowing T_w. Thus, e_a is the only unknown variable. The percent relative humidity may be obtained by dividing e_a by e_w and multiplying by 100. In practice, tables, charts, or accepted empirical equations of RH vs. the wet and dry bulb are generally used.

The constant C is the ratio of the sensible heat transfer coefficient in the particular bulb design used to the vapor transfer coefficient converted to its equivalent value in terms of heat of evaporation. Since this ratio is not a constant unless the air velocity exceeds 900 ft/sec, a motorized blower drawing the air sample over the bulbs yields more satisfactory data than are obtained with a sling instrument.

It should also be noted that C is proportional to the total pressure as found from both theoretical and experimental considerations. Thus for measurements made at pressures significantly different than one atmosphere, normal psychrometric charts should not be used. Special nomographic charts for the appropriate pressure should be consulted (*9*).

Wet and dry bulb psychrometry is primarily used as a method of measurement rather than as a reporting form for water vapor in air. Since this method is a measure of the vapor pressure of water, it retains the conservative qualities of vapor pressure measurements.

WATER MEASUREMENT CONVERSION TO RELATIVE HUMIDITY

Relative humidity is the form of choice for reporting the water vapor characteristics of the gaseous environments surrounding microorganisms during dry heat testing or food product storage, since it is equal to the water activity when the cell and surrounding atmosphere are in equilibrium. During non-equilibrium periods, relative humidity again appears to be the proper choice from a diffusional transport viewpoint. Thus, it is desirable to have equations at hand which relate other water measurements to relative humidity.

Our general plan is to discuss water measurement conversion in the same order that we discussed the measurement forms. However, since vapor pressure is basic to other calculations we shall discuss it first, followed by a short discussion of the effect of temperature on relative humidity, before proceeding to the other forms.

Vapor pressure to relative humidity

The ratio of the partial pressure of the water vapor to the saturated water vapor pressure at the same temperature is the *relative humidity* of the gas. Relative humidity is thus expressed as

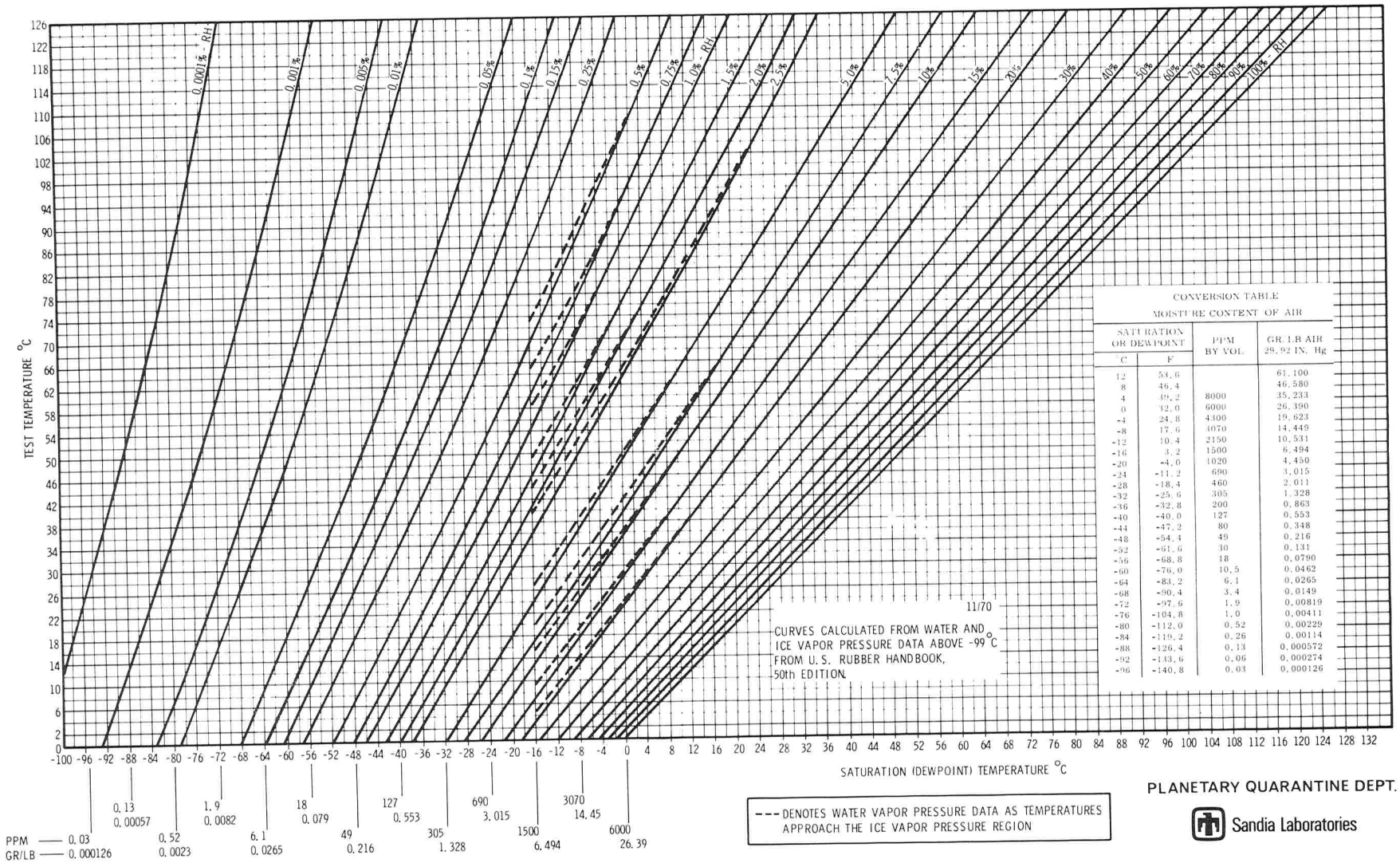

Figure 1. *Relative humidity conversion graph* (5).

$$RH = \frac{p_w}{p_s} \quad [6]$$

p_w = partial pressure of water vapor
p_s = saturated vapor pressure

The relative humidity of a particular gas sample will be sensitive to temperature and pressure changes. First of all, the partial pressure of the water vapor of a particular gas mixture is dependent on both temperature and pressure. From Dalton's Law and the Ideal Gas Law, we recognize that a change in total pressure of a gas sample effects a proportionate change in partial pressure of the water vapor. From the Ideal Gas Law, we see that a change in temperature changes the partial pressure of water in a closed system (the volume of the gas sample is constant). In an open system a change in temperature does not affect the partial pressure of the water vapor. Lastly, a change in temperature affects the value of the reference saturation vapor pressure used as the denominator of Equation 6.

Ambient relative humidity to treatment RH

When dry heat testing is done using a hot plate system or an air oven through which ambient air circulates, it is often desirable to know the relative humidity on the basis of the treatment temperature when we know the relative humidity of the atmosphere surrounding the hot plate or circulating through the oven. The equation relating relative humidity at ambient temperature, $RH_{(a)}$, to the relative humidity at test temperature, $RH_{(T)}$, is

$$RH_{(T)} = \frac{RH_{(a)}\, p_{s(a)}}{p_{s(T)}} \quad [7]$$

$p_{s(a)}$ = saturation pressure at ambient temperature

This equation holds as long as the total pressure at the ambient condition and the total pressure at the test condition are equal. Garst and Lindell (5) have developed a chart for the conversion from relative humidity at one condition to relative humidity at another condition. Their chart is reproduced as Fig. 1.

When the ambient air is contained in a closed system as the gaseous environment to be heated to test temperature T without any other source of water, the relationship between ambient and treatment relative humidities will be

$$RH_{(T)} = RH_{(a)} \frac{p_{s(a)}}{p_{s(T)}} \frac{T}{T_a} \quad [8]$$

T_a is the temperature of the ambient

Vapor density to relative humidity

From the Ideal Gas Law the partial pressure of water vapor behaves according to the equation

$$p_w = \frac{n_w}{V} RT \quad [9]$$

Thus, percent relative humidity can be linearly related to a given concentration measure:

$$\%RH_{(T)} = \frac{p_w}{p_{s(T)}} \times 100\% = \frac{n}{V} \frac{RT}{p_{s(T)}} 100\% \quad [10]$$

or more generally

$$\%RH_{(T)} = C \frac{T}{p_{s(T)}} \delta \quad [11]$$

C = concentration of water (units specified in Table 1)
δ = constant of proportionality which depends on units of C (see Table 1)
$p_{s(T)}$ = saturation pressure of water at temperature T in mm Hg
T = temperature in degrees Kelvin (°K)

TABLE 1. *Definition of Values for Equation 11*

Units of C	δ
$\frac{\text{moles}}{\text{liter}}$	$62.37 \frac{\text{mm Hg}}{\text{°C}} \frac{\text{liter}}{\text{g-mole}}$
$\frac{\text{g}}{\text{ml}}$	$3.46 \times 10^3 \frac{\text{mm Hg}}{\text{°K}} \frac{\text{cm}^3}{\text{g}}$
$\frac{\text{lb}_m}{\text{ft}^3}$	$9.59 \times 10^4 \frac{\text{mm Hg}}{\text{°K}} \frac{\text{ft}^3}{\text{lb}_m}$

Specific humidity to relative humidity

The absolute humidity, w, can be calculated from

$$w = \frac{w_{water}}{w_{air}} = \frac{18.016\, p_w}{28.967(P - p_w)} \quad [12]$$

w_{water} = weight of water vapor per unit volume
w_{air} = weight of dry air per unit volume
p_w = partial pressure of water vapor
P = total pressure
18.016 = molecular weight of water vapor
28.967 = molecular weight of dry air

If the water content is given in terms of absolute humidity, w, we can calculate $RH_{(T)}$ using the following equation:

$$RH_{(T)} = \frac{28.967\, w\, P}{p_{s(T)}\ (18.016 + 28.967w)} \quad [13]$$

w = humidity as lb of water vapor per lb of dry air

Parts per million to relative humidity

An equation relating water content expressed as ppm of water to relative humidity at a temperature can be obtained by combining equations 4 and 6. The resulting equation is

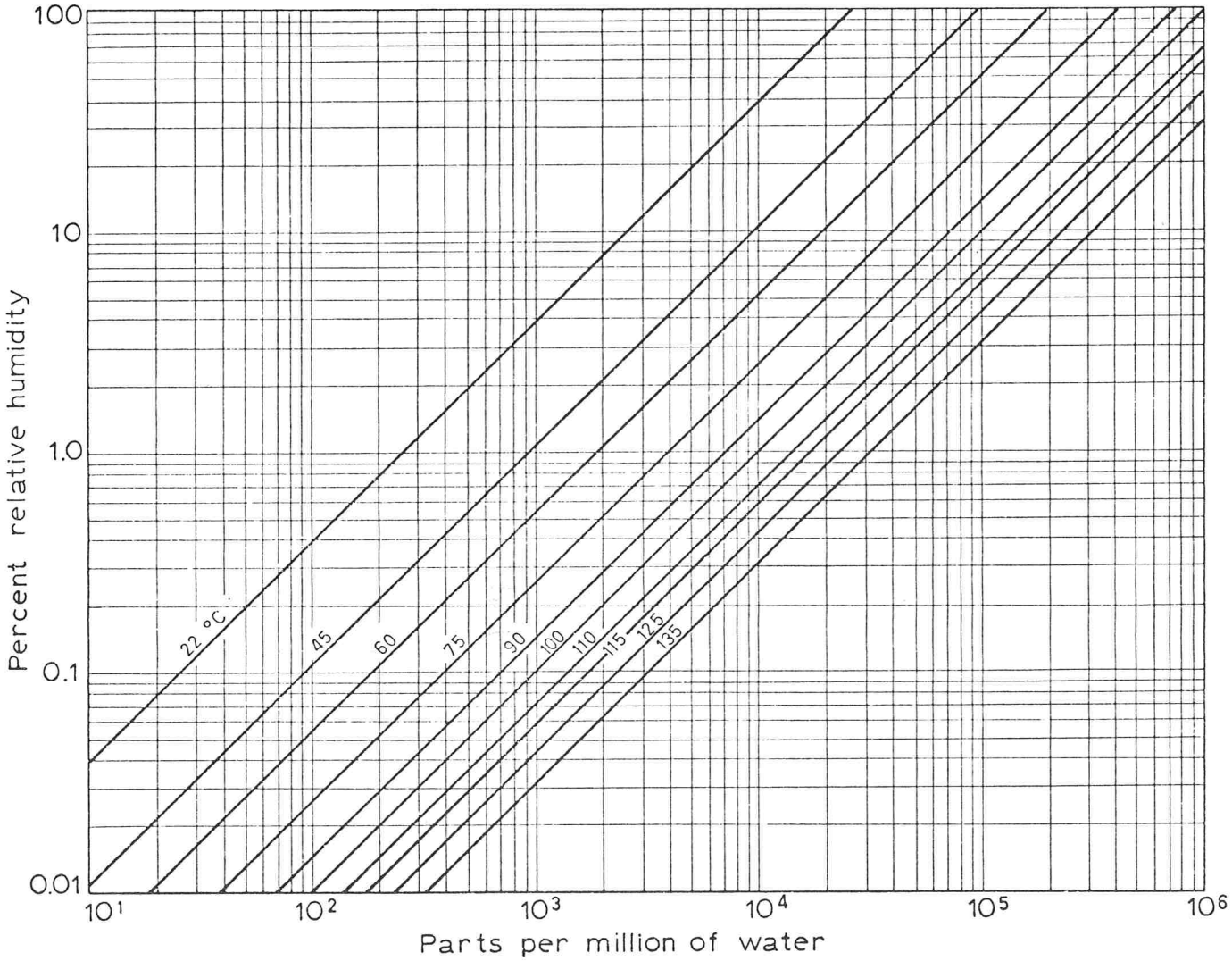

Figure 2. *Conversion graph for parts per million of water and relative humidity for an open system (P = 760 mm Hg).*

$$\%RH_{(T)} = \frac{ppm \times P}{10^4 \times p_{s(T)}} \qquad [14]$$

For an open system where the total pressure is constant at one atmosphere, this relationship is linear and equation 14 becomes

$$\%RH_{(T)} = \beta_{(T)}\ ppm \qquad [15]$$

the temperature dependency of $\beta_{(T)} = \frac{760}{10^4\ p_{s(T)}}$ can be found in Table 2.[5]

Where an atmosphere is 100% water vapor (10^6 ppm) and the temperature is above 100 C at one atmosphere of pressure (p = 760 mm Hg), a superheated water vapor condition exists. The relative humidity will be the total pressure (760 mm Hg) divided by the saturation pressure corresponding to the gas temperature. This condition is found in Figure 2 on the 10^6 ppm water content line.

TABLE 2. *Values of β(T) at Various Test Temperatures*

T C	$P_{s(T)}$ [mm Hg]	$\beta(T)$
22	19.80	3.827×10^{-3}
45	71.83	1.058×10^{-3}
60	149.35	5.088×10^{-4}
75	289.05	2.629×10^{-4}
90	525.78	1.445×10^{-4}
100	760.00	1.000×10^{-4}
110	1074.56	7.072×10^{-5}
115	1267.98	5.993×10^{-5}
125	1740.93	4.365×10^{-5}
135	2347.26	3.237×10^{-5}

For a closed system that is sealed at ambient pressure and temperature (T_a), the relationship at other temperatures (T) is more complicated since pressure will also change with temperature. The final total pressure will be the sum of the partial pressures of the component gases. For the dry gas component, the final partial

[5] *The total pressure was taken as 760 mm Hg or I standard atmosphere. This total pressure varies depending on weather and location.*

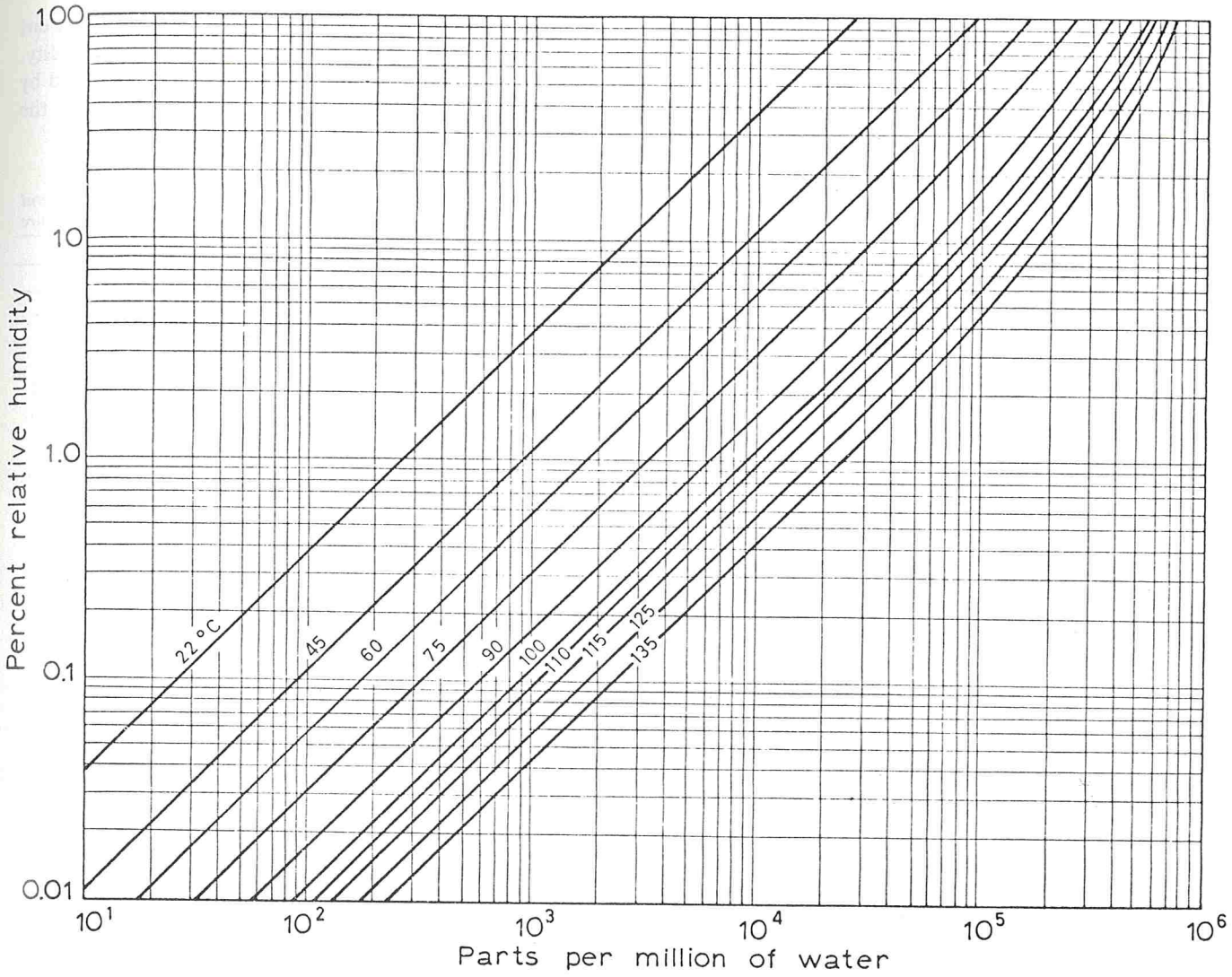

Figure 3. *Conversion graph for parts per million of water and relative humidity for a closed system* (p_{dg} = *760 mm Hg*).

pressure, $p_{dg(T)}$, upon being heated, increases in accordance with

$$p_{dg(T)} = p_{dg(a)} \frac{T}{T_{(a)}} \qquad [16]$$

$p_{dg(a)}$ = the partial pressure of the dry gas at the time of sealing

This equation is only valid for closed systems where the gaseous volumes remain constant. By definition of relative humidity, the partial pressure of water at test temperature is

$$p_w = \frac{\%RH_{(T)}}{100} \; p_{s(T)} \qquad [17]$$

Presumably, the relative humidity at test temperature ($RH\%_{(T)}$) is calculatable from the amount of liquid water added (see equation 11), and/or the relative humidity of the ambient gas (see equation 8) which is sealed in the closed system. At any rate, the total pressure will be

$$P = p_{dg(a)} \frac{T}{T_{(a)}} + \frac{\%RH_{(T)}}{100} \; p_{s(T)} \qquad [18]$$

Example. Thus, for a closed system having been sealed at 22 C with the dry air having a partial pressure of 760 mm Hg, the total pressure at test temperature is

$$P = 760 \frac{T}{295} + \frac{\%RH_{(T)}}{100} \; p_{s(T)} \qquad [19]$$

By combining equations 14, 17, and 19 the relationship necessary to convert from ppm to %RH is

$$\%RH_{(T)} = \frac{\frac{T}{295} \beta(T) ppm}{1 - \frac{ppm}{10^6}} \qquad [20]$$

Figures 2 and 3, respectively, show the relationship of water in ppm to %RH for an open and for a closed system. The results are linear for the open system and nonlinear for a closed system, especially at the larger values of ppm.

Dew point to relative humidity

Some researchers report water content in terms of dew point or the temperature at which condensation starts upon an isobaric cooling (cooling at constant total pressure) of the gas mixture. Since the pressure at dew point equals the partial pressure of water vapor, relative humidity can be calculated as

$$RH_{(T)} = \frac{p_{s\ (DPT)}}{p_{s(T)}} \qquad [21]$$

$p_{s(DPT)}$ = saturation vapor pressure at the dew point temperature

Figure 1 shows the dew point temperature for gas mixtures of varying temperatures and relative humidities.

Psychrometric forms to relative humidity

Wet and dry bulb psychrometry forms are basic to most common humidity measurements. Conversion of wet and dry bulb temperatures to relative humidity relies heavily on use of psychrometric charts (*2, 9*). Such charts generally describe, for the user's information, the proper technique to determine relative humidity for any psychrometric determination.

SUMMARY

The common methods of measuring water in water-air systems are described. Equations are developed that make possible the conversion of data from the different type of measuring unit into percent relative humidity. The location of the relevant conversions can be found by consulting Table 3. Equations to be used to carry out the measuring unit conversions are listed in Table 3.

TABLE 3. *Equations to be used to convert from the several measuring units to percent relative humidity at treatment temperature*

Measurement unit	Equation number	
	In open system	In closed system
Ambient RH	7	8
Dew point	21	21
Parts per million	15	20
Specific humidity	13	13
Vapor density	11	11

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Energy Conservation in the Food Processing Industry

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ABSTRACT

The food system utilizes 12.8% of the total energy consumed in the United States. Higher fuel costs increase the need for operating only efficient steam systems in food plants. Checking combustion efficiency by monitoring flue gases and maintaining an exhaust gas temperature no higher than 150 F above that of the saturated steam helps save fuel. The importance of insulating pipelines, buildings, and some equipment is increased as available energy sources become limited. Enlarging a regenerator of a milk pasteurizer from 80 to 90% reduces steam requirements by one-half. Inserting a heat exchanger to capture heat lost through liquid or air discharges saves energy. Infra-red systems can reduce fuel consumption 50% in heating poorly insulated or partially exposed shipping, receiving, or similar areas.

The energy efficiency ratio is an important consideration in selection of a refrigeration unit. When operating at low temperatures such as −28 F, a two stage ammonia system can save about 25% of the electricity used by a single stage installation. Modern light sources use electric energy more efficiently than some older systems. Employee involvement and cooperation is necessary for an energy conservation program to reach its potential.

Growth in energy consumption without adequate expansion of supply suggests the need for conservation. Rising costs of the energy sources now available have greatly increased the economic impetus for initiating conservation measures for both the private and industrial sectors. In energy consumption the food processing industry ranks fourth behind metals, chemical and petroleum refining in the Standard Industrial Classification grouping (*15*).

Estimating the energy consumption by various segments of our food system is complex, therefore, some inconsistencies in the estimated values reported may be expected (*6*). Steinhart (*18*) reported that all of the functions involved in the food system from production through consumption accounted for about 12.8% of the total energy used in the United States in 1970. Hirst (*8*) reports that on a per capita basis we are approximately 32.4×10^6 BTU (British thermal unit) annually in the food system. The ratio of energy input into the system to food energy consumed has increased from approximately 3:1 to 7:1 between 1940 and 1970.

FOOD INDUSTRY ENERGY REQUIREMENTS

A 1972 survey of the California food industry (*2*) indicates that for some agricultural products the combined production and transportation energy requirements exceeded the amount needed for processing one ton of product. The relative energy requirements for production, transportation and processing to a stable form before distribution is presented in Fig. 1. For many fruits and vegetables canning plants use about 3.5×10^6 BTU per ton of finished product. Milk processing operations were found to consume about 1.3×10^6 BTU per ton of raw product. Electrical energy requirements, however, are higher for processing one ton of milk than to can or freeze either peaches or green beans, as indicated in Fig. 2.

A wide variation may exist in the energy required to convert raw products into different processed forms. Nielsen (*14*) compared the heat needed to pasteurize and package fluid milk with the amount necessary to manufacture cheese and dry whey. To convert 100 lb of milk to cheese and spray dried whey requires 81,620 BTU whereas 15,600 BTU are used to process the bottled product.

Food plants performing similar processing functions vary in their energy requirements as influenced by location, physical arrangement, and overall efficiency. Data obtained from one relatively large dairy plant provide some information on usage. This dairy processes over 500,000 lb of milk per day into fluid products, ice cream, and cottage cheese and utilized 29.5 Kwh and 215.3 ft^3 of natural gas per 1,000 lb of milk during the first 6 months of 1974.

Since the impact of the energy situation in 1973 many food plants have made considerable effort to reduce utility consumption. For example, one dairy achieved the following reductions during Jan., Feb., and March, 1974 compared with the same period of 1973:

Kwh	9.5% reduction
Natural gas	4.5% reduction
Water	15.0% reduction

The reduction in water usage was achieved mainly by recycling the discharge water from compressed air after-coolers and ammonia compressor heads over a cooling tower permitting its reuse as a coolant. Careful use of power and steam also reduced electricity and fuel consumption in this plant.

Another medium size dairy operation significantly decreased the natural gas burned through conservation measures beginning in 1972. Steps taken included installation of a water softener for boiler feedwater, thorough boiler descaling, discontinuance of direct steam injection processing of fluid milk, lowering steam

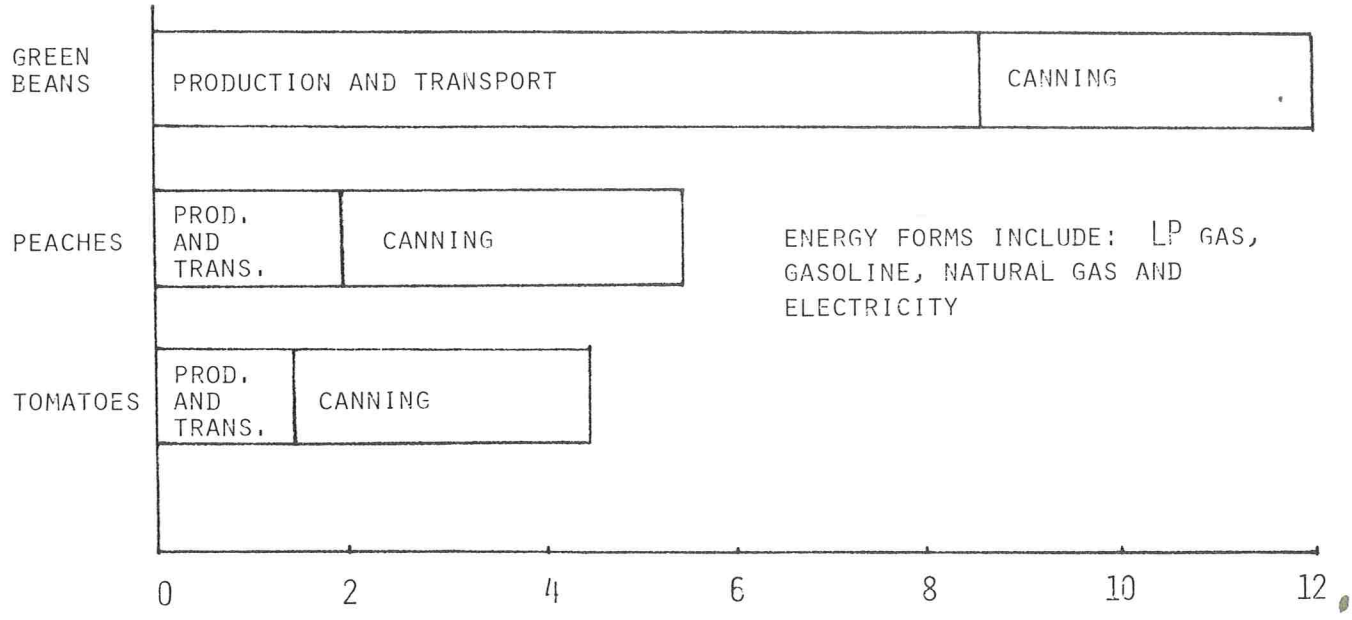

Figure 1. *Energy required to produce, transport and can one ton of three products in California in 1972.*

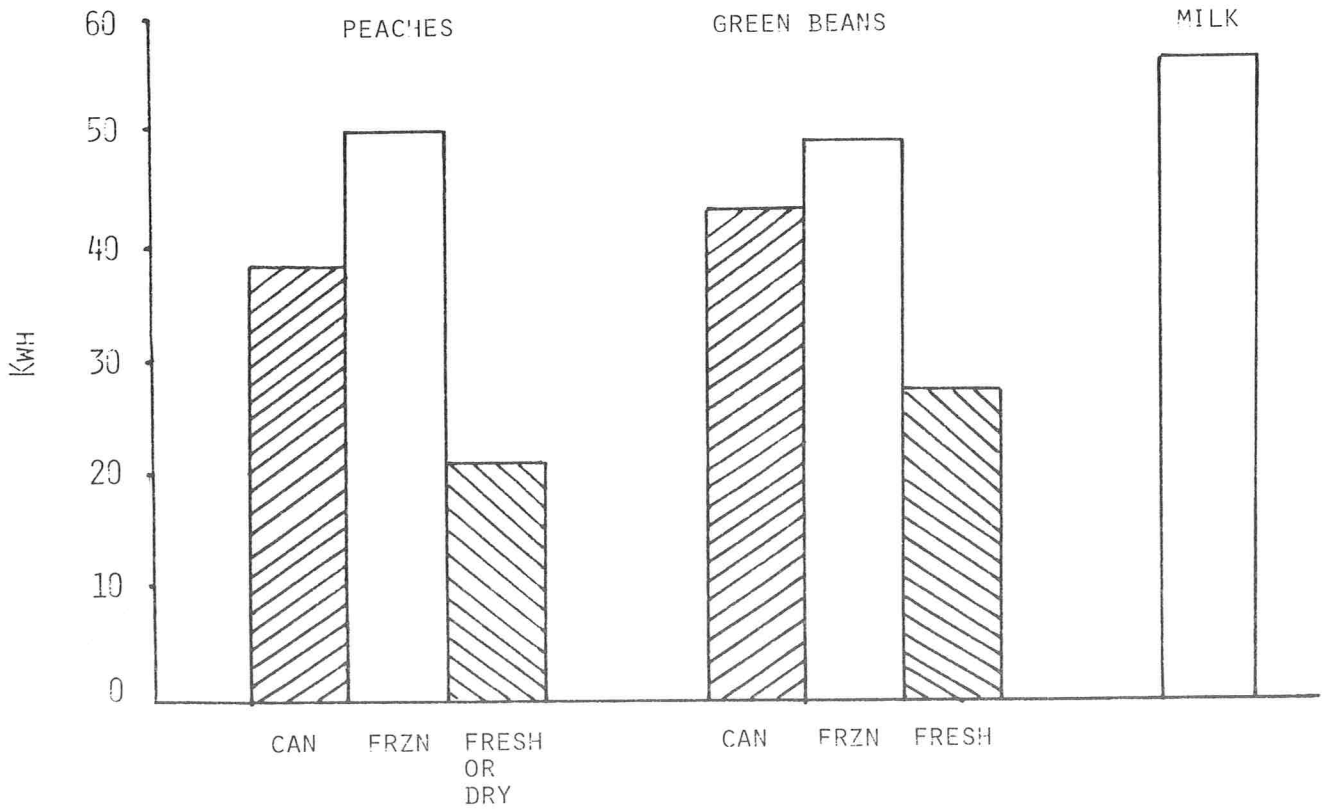

Figure 2. *Electrical energy required to process one ton of three different products in California in 1972.*

pressures, and boiler shut-down on weekends. The effect on gas purchases is given in Fig. 3. An indication of the monetary savings which was a direct benefit to this plant is evident from the data in Table 1.

THE STEAM SYSTEM

A food processor in Michigan paid \$0.82 per 1000 ft^3 of natural gas in 1973 and \$1.55 in 1975 indicating the sharp increase in the cost of fuel. Higher fuel costs virtually mandates the operation of only efficient combustion and heat transfer systems. A comparison of the cost of steam produced by two hypothetical boilers with fuel to steam efficiencies of 70 and 80% serves to illustrate the need to operate efficient units. Table 2

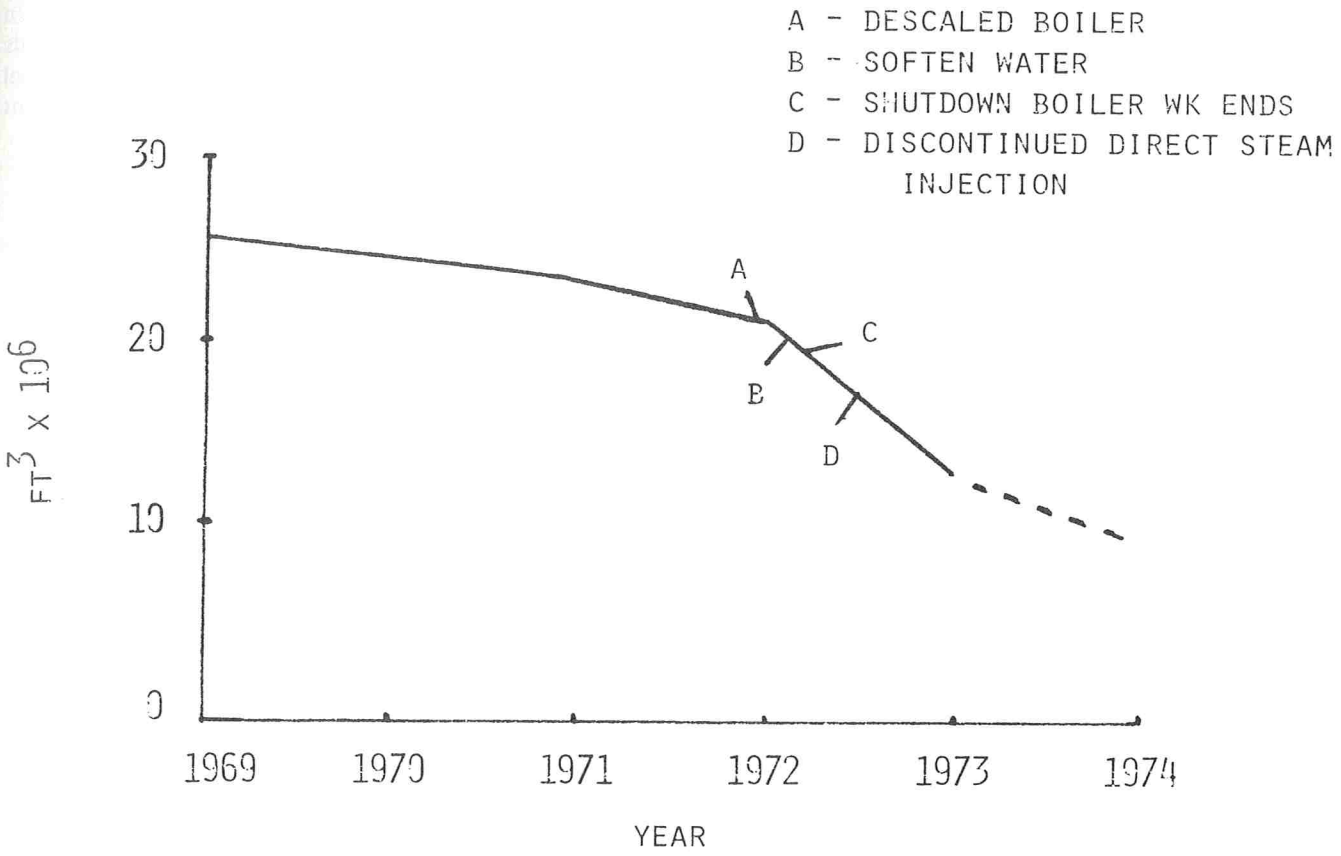

Figure 3. *Reduction in natural gas consumption in a Michigan dairy plant.*

TABLE 1. *Amount paid for natural gas by a Michigan dairy plant*

Year	Rate per 1000 ft^3	6 mo. period Jan. through June
1971	$0.82	$9,740
1972	0.82	9,115
1973	0.82	5,691
1974	0.92	5,243
1975 est.	1.55	8,400

TABLE 2. *Cost of producing 1,000 lb of steam with boilers of 70 and 80% efficiency and two fuel prices*

Natural gas cost per 1000 ft^3	Boiler efficiency		Cost advantage of 80% boiler
	70%	80%	
$0.82	$1.13	$1.00	$0.13
1.55	2.15	1.89	0.26

shows that the boiler with an 80% fuel to steam conversion efficiency has a cost advantage of $0.13 per 1000 lb of steam when gas costs $0.82 per 1000 ft^3. However, the cost advantage increases to $0.26 per 1000 lb of steam when fuel costs $1.55 per 1000 ft^3. Careful evaluation of the steam system is recommended with special consideration of the effect of higher fuel prices. Some plant operators have discovered that the time for recovering the cost of a new boiler to replace an older, inefficient unit has been shortened substantially.

The burner on any boiler must be adjusted to obtain complete combustion and maximum heat from the fuel. An analysis of the flue gas for CO_2 (carbon dioxide) content is important. Table 3 gives the combustion efficiency rating for three different fuels as determined by the amount of CO_2 in the flue gas (*13*). Efficiency declines with a decrease in CO_2. Carbon monoxide is zero with complete combustion. Exhaust gases should contain 1 to 2% oxygen to assure an adequate amount to completely burn the fuel. Manufacturers of boilers can assist in obtaining testing equipment for regularly checking flue gases.

TABLE 3. *Effect of carbon dioxide level in flue gas on combustion efficiency rating for three fuels*

Rating	Gas	Percent CO_2 in flue gas	
		No. 2 oil	No. 6 oil
Excellent	10.0	12.8	13.8
Good	9.0	11.5	13.0
Fair	8.5	10.0	12.5
Poor	8.0	9.0	12.0

Boilers should obviously be kept clean and free of scale (*7*). An increase in the stack temperature is an indication of scale buildup. A general guide used by some food plant operators is to avoid having exhaust gas temperatures exceed the saturated steam temperature by more than 150 F (*16*). This figure can generally be applied in the operation of most common fire tube boilers. A heat exchanger installed to recover some of the heat from exhaust gases for preheating incoming air before combustion also saves fuel. Each 100 F rise in the temperature of the air entering the burner results in a fuel saving of approximately 2 to 2.5% (*10*).

Steam condensate returned to the boiler saves approximately 1% in fuel cost for every 11 F increase in feedwater temperature. Condensate also will usually reduce the amount of chemicals needed for feedwater treatment.

REDUCING HEAT LOSSES

Heat passing from buildings to the outside atmosphere or that radiate from pipe lines or equipment from which no benefit is gained represents a loss. Installing insulation at the interface between where heat is desired and where it yields no benefit is one of our principal approaches to reducing heat losses. Most food processing plants can probably benefit from a study of the various areas of the building to determine insulation recommendations for each area. Because of variations in processes, climate, location, and many other factors, a general recommendation will not be attempted. In colder climates where space heating is done for comfort or to facilitate operations, some insulation will save fuel. Storm windows cut heat losses through a single pane of glass in half. For a climate with 7,000 winter degree days storm windows save about $17.00 annually per 100 ft² of glass area assuming steam costs $1.90 per 1000 lb. Heat transfer through a single glass window is given as 1.13 BTU/h/ft²/°F temperature differential (*1*).

Uninsulated steam pipes radiate more heat than may be realized. For example, a bare 2-inch pipe 100 ft long carrying steam at 1000 psig (lb per in² gage) will lose 1.25×10^6 BTU in 24 h (*13*). Recovering the cost of steam pipe insulation through fuel savings may require no more than 1 or 2 years in industrial plants.

Insulating refrigeration lines saves energy and is economically feasible in most food processing plants. Approximately 43 BTU/F/ft² of bare pipe is added to the refrigeration load in 24 h (*7*). This amounts to 3.8 tons of refrigeration per year if the pipe is maintained at 0 F in an atmosphere of 70 F.

Heat flow from higher to lower temperature can be reduced if the temperature difference is decreased. Plant operators can take advantage of this pinciple by reducing the system steam pressure to the minimum necessary for processing. The extent to which lower pressures reduce heat losses is shown for 80 and 120 psig steam pressures in Table 4. Gradually lowering the steam pressure to the point where processing functions begin to be affected helps to determine the minimum pressure required to operate the plant. Dialing down room thermostats in winter and up a few degrees in air conditioned rooms in summer takes advantage of the temperature differential principle in energy conservation. For example, over an 8-h period at night, a fuel saving of 1% or more results for each degree F the temperature is lowered (*12*). Fuel savings diminish if the thermostat is lower than about 60 F at night.

TABLE 4. *Effect of steam pressure on heat losses from 100 ft of two size pipes with 1½ in of insulation*

Pipe size	BTU per hour* 80 psig 324F	120 psig 350F	Difference
2-inch	7,400	8,200	800
3-inch	9,700	10,700	1,000

* In ambient 70 F.

PROCESSING OPERATIONS

Food processing usually requires heating or cooling and often both in many plants. Because heating and cooling are frequently used in an operation, opportunities are provided to employ the regenerative heat exchange principle. The dairy industry has successfully used milk-to-milk regeneration in the pasteurization process for about 25 years. During this time the industry has saved a huge amount of steam with the milk-to-milk regenerator. All processes where heating or cooling is involved should be analyzed for the possibility of utilizing regeneration (*17*). In the analysis of an operation the discharges of hot or cold wastes should be considered as a possible heating or cooling medium. Using a suitable heat exchanger the heat may be transferred directly to a product or to another medium useful to the process. Passing warm water discharges from a blancher through a heat exchanger to preheat incoming fresh water or boiler feedwater is an example of recovering heat which would probably be lost.

Increasing the amount of regenerative heating and cooling of an existing regenerator can result in additional fuel savings. More heat exchange surface must be added to increase the amount of regeneration. Perhaps additional controls will be necessary for satisfactory performance. There is a practical limit of around 87 to 90% regeneration for present milk pasteurizers to avoid operational difficulties. However, it should be noted that increasing the regeneration of a HTST (high temperature short time) pasteurizer from 80 to 90% reduces the steam consumed by one-half.

Processing temperatures must obviously be adequate to achieve food safety and preservation. But there may be occasions where some temperatures could be adjusted slightly without sacrificing quality or safety. An example is the possibility of lowering the pasteurization temperature of milk a few degrees in those instances where the actual processing temperature greatly exceeds the legal minimum requirement. With a typical HTST unit, 8 to 9% saving in steam results by lowering the process temperature from 176 to 165 F. The refrigeration requirement also is reduced 7% while processing at the lower temperature. If a program of lowering the temperature is undertaken, it should be done gradually and with alertness to detect any adverse changes which might develop affecting product quality, particularly shelf life.

SPACE HEATING

Space heating requirements differ between areas of a food processing plant depending on the design and the kind of operation conducted in each area. But at times

during cold weather, most areas need some heat. Infra-red heating systems offer economy and comfort with less fuel in rooms with high, poorly-insulated ceilings or where there is considerable exposure to outside weather conditions. Loading or receiving docks which are not tightly enclosed, warehouses and supply storage rooms and, in some instances, processing areas lend themselves to infra-red heating systems. In some instances fuel consumption by gas type infra-red heaters was observed to be about 50% less than that for direct gas fired space heaters for heating comparable industrial buildings (*11*).

Certain processing operations can be a heat source for space heating by directing warm exhaust air through a heat exchanger. If conditions warrant, it may be satisfactory to discharge high quality warm air directly into an adjacent room. Humidity and condensation control become important considerations, however, when utilizing warm moisture-laden air for space heating.

ELECTRICITY

Sharp increases in the fuel cost adjustment portion of electric bills during 1974 reflects directly the fuel situation and probably portends things to come. Projections of electric power demand in the United States indicate the need to increase generating capacity from the present 450,000 to 650,000 megawatts by 1985 (*19*). The steady rise in construction costs of generating plants leaves little doubt that further price increases for electrical energy lies ahead.

The refrigeration system is the biggest electrical load in many food plants. It is, therefore, necessary to keep the system performing efficiently. Good overall maintenance is required with particular emphasis on eliminating any obstructions to heat flow at all heat transfer surfaces.

Ammonia systems used for cooling low temperature storages or for rapid freezing of food should be the two stage type. Single stage type ammonia systems using reciprocating compressors are not recommended for suction pressures of 0 psig (–28 F) or below. Even at 0 psig the two stage system has about 25% lower brake horsepower requirement than a single stage installation (*5*). The difference increases to approximately 40% in favor of the two stage system when operated at 5.4 inches Hg vacuum (–35 F). In this comparison condensing pressures to 185 psig were considered.

The energy efficiency ratio (electrical energy required per unit of capacity) should be considered when selecting a freon condensing unit for a specific refrigeration job. Most manufacturers can provide data on power consumption (BTU/kwh) under given operating conditions. A slight change in the bore size, for example, can make a difference in excess of 15% in the energy used when performing under certain conditions (*4*).

Electric power requirements can generally be lowered by carefully scheduling operations so that equipment is doing productive work when running. Frequent production interruptions are inefficient and also tend to increase power consumption.

Utility companies may insist on adjustments in equipment if the power factor becomes too low. A power factor below 0.85 causes concern for utility firms.

Artificial lighting represents about 5% of the nation's total energy consumption. However, lighting accounts for approximately 40% of the electrical energy used in the commercial and institutional sector of our economy (*3*). For this group effective application of lighting systems is an important consideration. Some general areas which have been suggested for conserving energy in commercial lighting systems are as follows: (*a*) Survey present lighting levels by area or operation and establish minimum requirements consistent with good lighting practices. The survey should also note location and type of light source including switches and other controls. (*b*) Develop recommended changes in fixture locations, type, controls, light intensity, and others as appropriate. (*c*) Follow a maintenance program for regular luminaire cleaning, lamp replacement, and fixture ventilation. (*e*) Develop a program to obtain employee cooperation in turning off lights when not in use.

Plant operators may discover economic advantages by replacing a portion or all light sources with newer, more efficient systems. In any event, the potential for energy saving warrants investigation. Comparative efficiencies of some commonly used light sources are given in Table 5.

TABLE 5. *Efficiency of various light sources*

Type	Lumens per watt
Incandescent	10-20
Mercury	40-60
Fluorescent	50-70
Metal halide	70-90
High pressure sodium	90-120

EMPLOYEE INVOLVEMENT

Energy conservation will usually require some engineering, scheduling, or operational changes; but unless employees are motivated in an energy saving effort, the goals may not be reached. Employee suggestions should be solicited. Their voice in planning and goal setting and their sincerity in executing a conservation program may well determine the success or failure of the project. Some firms have effectively utilized a committee of employees to develop and conduct a conservation program (*9*). Prominently displaying a sizeable chart showing the amount of the various utilities used each month per unit of production helps maintain the interest and cooperation of everyone.

CONCLUSION

Food processing is a necessary function in our food system. Those performing this important function must assume the responsibility of using the available energy wisely. Although progress appears to have been made,

additional efforts and changes are needed to reduce energy requirements.

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International Institute of Refrigeration Meeting

The Australian National Committee for the I.I.R. is organizing a Joint Meeting of Commissions in Melbourne in September 1976. This will be the first occasion on which the Institute has held a meeting in that country, and it is being arranged by the Committee in collaboration with local professional and technological Societies.

The aim of the meeting is to bring Australian research and development in the relevant fields to the notice of an international audience, and to acquaint Australian science and industry with current work being carried out in other countries.

A Call for Papers for the meeting will be issued shortly, setting out the selected theme and a list of preferred topics. Papers will however also be invited in the general areas of interest of the five Commissions. Limited funds will be available to assist selected authors with travel costs.

The organizer of the meeting is Mr. F. G. Hogg, I.I.R. Liaison Officer in Australia, from whom further details can be obtained. His address is P.O. Box 26, Highett, Victoria, 3190, Australia.

Erratum

Comparative Validity of Members of the Total Coliform and Fecal Coliform Groups for Indicating the Presence of *Salmonella* in the Eastern Oyster, *Crassostrea virginica*

W. H. ANDREWS, C. D. DIGGS, M. W. PRESNELL, J. J. MIESCIER, C. R. WILSON, C. P. GOODWIN, W. N. ADAMS, S. A. FURFARI, and J. F. MUSSELMANN

Division of Microbiology, Washington, D.C. 20204;
Shellfish Sanitation Branch, Dauphin Island, Alabama 36528 and
Davisville, Rhode Island 02854
Food and Drug Administration, Public Health Service,
Department of Health, Education, and Welfare

This paper appeared on pages 453-456 of the August (No. 8) issue of Volume 38 (1975) of the *Journal of Milk and Food Technology*. On page 454, beginning at the fifth sentence of the fourth paragraph it should read: Tubes of LST broth, inoculated with dilutions of the water or shellfish samples, were incubated at 35 ± 0.5 C, and at 24- and 48-h intervals, sterile applicator sticks were used to make transfers from gassing tubes of LST broth to tubes containing brilliant green lactose bile (BGLB) broth (Difco). Cultures producing gas in BGLB broth after 48 h of incubation at 35 C were classified as total coliforms. Fecal coliform confirmation was made by transferring all tubes of LST broth positive for gas after 24 to 48 h of incubation at 35 ± 0.5 C to EC medium.

Erratum

Behavior of Selected Food-Borne Pathogens in Raw Ground Beef

J. M. GOEPFERT and H. U. KIM

Food Research Institute, University of Wisconsin
1925 Willow Drive, Madison, Wisconsin 53706

This paper appeared on pages 449-452 of the August, 1975 (No. 8, Vol. 38) issue of the *Journal of Milk and Food Technology*. Table 2E. on page 450 should be entitled S. AUREUS rather than ENTEROCOCCI.

News and Events

Ice Cream Short Course

The annual Ice Cream Short Course of The Pennsylvania State University will be held January 12-22, 1976. Lectures, demonstrations, laboratory practice and visits to large and small ice cream plants are integrated to provide information about the formulation, manufacture, and distribution of both hard and soft ice cream and ice milk, sherbets, ices, vegetable fat products, and other types of frozen desserts. Because of the diverse interests and backgrounds of the students who attend, course content is broad in scope.

The registration fee, which includes field trip transportation costs, is $50 for Pennsylvanians and $60 for non-Pennsylvanians. Enrollment is limited to 45 students.

Application blanks and further information can be secured from the Director of Short Courses, 306 Ag. Admin. Bldg., University Park, PA 16802.

Ohio Evaluates their Food Service Manager Certification Course

The Ohio Department of Health recently completed an evaluation of Ohio's Food Service Manager Certification Course. This evaluation was sponsored in part by a grant from the U.S. Food and Drug Administration.

The results of this evaluation indicated that the level of sanitation significantly increased after a owner/operator/manager completed the certification course and was certified. The sanitation level increased from a pre-course inspection average of an 84 percent compliance level to a 6 month post-course score of 89 percent. This 89 percent was also significantly higher than non-certified managers which were used as a control group.

This study also revealed that there was a lower turnover of management in food service operations operated by certified managers than by non-certified managers. During a six month period the Certified Manager group had a 20 percent turnover rate as compared to 30 percent turnover in the noncertified manager sample.

The Ohio Department of Health, a pioneer in the development of the food service manager certification concept, is again expanding into a new area. The demand for the certification courses by the food service industry has far surpassed the capabilities of Ohio Department of Health. As a result various teaching bases are being implemented throughout the state to conduct the certification courses. These teaching bases will involve colleges, universities, and some local health departments. Furthermore, the possibility of approving specific companies and foodservice industry organizations to act as instructional bases is also being considered.

The Ohio Department of Health has produced a mechanism which will raise the level of food protection by training the responsible food service management personnel and incorporating them in a continuous self-improvement process. This mechanism was developed, implemented and evaluated and has been found to be a successful tool for increasing the level of sanitation and food protection in food service operations in Ohio.

New Kit for Rapid Determination of Antibiotics in Milk

Delvotest P, a new compact economical kit for the determination of inhibitory antibiotics in milk, is now available in the United States through Enzyme Development Corporation, N.Y.C., 10001. The kit, manufactured by Gist Brocades, N.V., Holland, has gained widespread acceptance in Europe as a standard procedure for antibiotic monitoring.

The advantages of the Delvotest P kit over existing methods include: 1) SHORTER INCUBATION TIME—Precise and accurate results are obtained within 2½ hours; 2) GREATER SENSITIVITY—Accurate determination is achieved for as low as 0.003 I.U. Penicillin G per ml.; 3) The kit is SELF-CONTAINED consisting of 100 ampules with agar and spores, nutrient tablets, dosing syringe and disposable syringe tips and requires no preparation of culture, reagents, etc.; 4) The testing procedure is simple, practical and low cost whether used for 1 or 100 determinations.

For further information, contact "DELVOTEST DEPT.," Enzyme Development Corp., 2 Penn Plaza, New York, N.Y. 10001. (212) 736-1580.

Literature on New Standardizing Package Available

Foss America Inc. has just released a new four page brochure on their new milk standardizing package.

The new package is described and explained by means of drawings which show how the system is installed to operate from storage tanks or separators.

A number of features of the package are also pointed out, such as it having the ability to save $67. on every ten thousand gallons of milk that is processed; the standardizing package is simple to understand and operate without having to deal with any computers or keyboards; the package comes complete, requiring a minimum of installation work required of the milk plant.

A list of the components included in the package is also shown.

For copies of the literature contact: Foss America Inc. Route 82, Fishkill, N.Y. 12524.

News and Events

NSF Announces Seminars

Again during 1976 the National Sanitation Foundation will conduct a series of one and two-day seminars relating to the "SANITATION REQUIREMENTS OF FOODSERVICE EQUIPMENT." This program is tailored to assist:

FOODSERVICE MANAGEMENT in the selection, installation, and use of equipment.

HEALTH REGULATORY OFFICIALS in the evaluation of equipment in the field.

EQUIPMENT MANUFACTURES in sound design and construction practices.

FOODSERVICE CONSULTANTS, DEALERS AND ARCHITECTS in the planning and layout of operations.

CONTENT:

Discussion to focus on "Sanitation Requirements for Food Service Equipment" based on NSF Standards #1 and #2. Included will be a coverage of:

(1) materials; (2) design features; and (3) construction considerations.

Review of "Commercial Spray-Type Dishwashing Machines" including:

(1) design features; (2) installation; and (3) operation.

Outline of "Sanitation Aspects of Installation of Food Service Equipment."

LOCATIONS:

To date one-day programs have been arranged for:

Pittsburgh, Pennsylvania	January 6, 1976
Washington, D.C.	February 10, 1976
Kansas City, Kansas	March 2, 1976
Denver, Colorado	March 25, 1976
San Francisco, California	March 27, 1976
Louisville, Kentucky	April 6, 1976
Newark, New Jersey	May 11, 1976
Albany, New York	June 22, 1976

Two-day programs have been arranged for:

Ann Arbor, Michigan	February 25-26, 1976
Atlanta, Georgia	April 21-22, 1976
Dallas, Texas	June 9-10, 1976

NOTE: The two-day program also covers foodservice refrigeration, dispensing equipment, and ice machines.

Additional locations will be announced as arrangements are made.

REGISTRATION:

All participants must be pre-registered with no registrations being accepted less than *10 days* prior to the seminar.

Registration fee includes—Reference Materials, Lunch, and Coffee Break.

Each participant completing a seminar will receive a certificate.

For registration information contact Education Services, National Sanitation Foundation, NSF Building, Ann Arbor, Michigan 48105.

"Dr. H. C. Olson, Dairy Consultant, Stillwater, Oklahoma (L) and Erik Lundstedt, International Dairy Consultant, Boca Raton, Florida (R) proudly display awards for meritorious service received at the 1975 American Cultured Dairy Products Institute Annual Meeting in Louisville. Pictured with the award recipients is the Institute's immediate past-president, George Grant, Dairylea Cooperative, Vernon, New York.

The September 17-18 cultured products conclave at Stouffer's Inn drew 180 delegates from thirty states."

Announcement

PROCEEDINGS, MASTITIS CONTROL SEMINAR, Sponsored by the International Dairy Federation. The proceedings represent the most important collection of papers ever assembled on mastitis control. More than 60 papers by authorities from 20 countries are included on such timely subjects as detection, prevention, and therapy. Excellent reference. Copies available postage paid by sea mail for $13.00 from Secretary General, International Dairy Federation, Square Vergote, 41, B-1040 Brussels, Belgium.

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Letter to the Editor

Suggestions for testing hamburger

DEAR SIR:

The difficulties reported by Dr. R. L. Winslow (*9*) in the laudable and impressive attempts made by his Company to meet the bacteriological standards for, amongst other items, fresh meats, set by the State of Oregon, prompt me to suggest two points of advice. These are based on experience with the bacteriological monitoring of ground fresh meats which are one of the main red meat staple foods in The Netherlands.

1. It is indeed most frustrating when microbiological monitoring requires a week in instances where the food under investigation is as perishable as comminuted fresh meat. However, there is no need at all to use such a tardy method for enumeration of *Escherichia coli*. Based on Clegg and Sherwood's classical work, carried out in Britain in the thirties (*1*) we have elaborated a technique that gives fully reliable results within 20 h (*4*). Such a period will certainly not lead to any significant proliferation of *E. coli* in consignments of minced meats kept at temperatures not exceeding 7 C (*2*). When using this procedure compliance with the limit considered violative by a U.S. Authority, viz. 50 cfu/g (*9*) can be assessed simply, rapidly, and with much greater accuracy than by a MPN procedure which Dr. Winslow rightly criticizes for its almost unacceptable variability amounting to almost a factor of 30 for lower and higher limit of confidence.

2. Dr. Winslow states that he fails to see how a microbiological standard for comminuted fresh meats may help to protect the health of the consumer. Were this indeed the case, his assignment would be even more frustrating. May I offer some counseling from the field of microbial ecology?

As our colleagues at the University of Wisconsin have rightly stressed (*2*) the direct risk of higher numbers of enteric pathogens in minced meats is remote, because such meats are generally—although not always (*7*)—sufficiently heated before consumption. As we have shown, confirming the experience of many investigators, the real hazard stems from recontamination of well cooked meats from infectious foci in the food environment (*3*). This risk obviously increases with what is called 'epidemiological pressure.' In this instance this amounts to the median of the cfu/g of enteric pathogens, particularly *Salmonella* species. Hence it is imperative to reduce such median values by measures such as: (*a*) adequate animal husbandry; (*b*) excellent sanitary care all along the slaughter and meat processing lines; and (*c*) uninterrupted, adequate chilling practices. When GMPs satisfying these requirements are followed, the product as it reaches the consumer can indeed meet strict bacteriological standards—provided the latter have been carefully assessed as a result of surveys of the type introduced many years ago on the U.S. continent (*8*). We have found (*5*) that, e.g., limits for *E. coli,* or the entire group of mesophilic *Enterobacteriaceae*—both criteria being available within 20 to 24 h—are fully attainable. Obviously, tolerances have to be built into such limits; but this hardly anybody will discuss anymore these days (*6*).

It is my true hope that these two brief reports on research carried out on the European Continent may be of some assistance to the food industry and the authorities in the U.S. alike.

D. A. A. MOSSEL
Chair of Food Microbiology
Faculty of Veterinary Medicine
The University of Utrecht
Utrecht, The Netherlands

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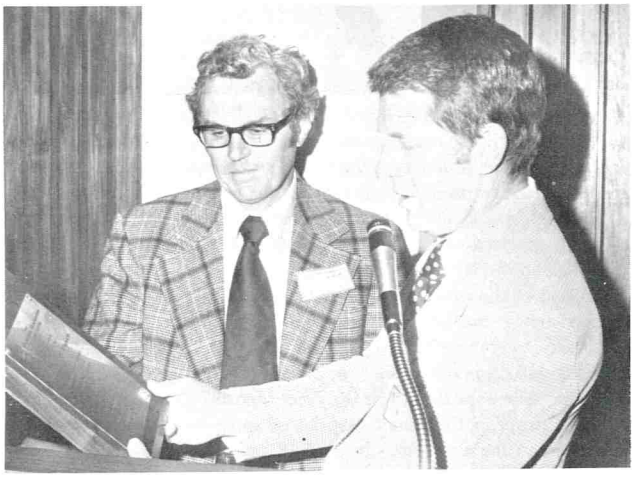
Minnesota Sanitarians Association

Dr. H. Macy, Alfred Ratzlaff, Leonard Sinton, Elwin Omundson.

The annual conference of the Minnesota Sanitarians Association was held September 11-12, 1975, at the University of Minnesota, St. Paul Campus.

The two day program had many interesting subjects from "Dry Cow Therapy" to "What the Sanitarian Should Know about Home-Canning."



Orlowe Osten of the Minn. Dept. of Agriculture presenting the Achievement Award to Donald Pusch of the Pillsbury Company.

At the business meeting Secretary-Treasurer, Roy Ginn, reported that all the legal work had been completed for the incorporation of the association, which is now named "The Minnesota Sanitarians Association, Incorporated."

Honorary Life Memberships were given to Leonard Sinton, retired, Minneaspolis Health Department; Dr. H. Macy, Dean Emeritus, University of Minnesota; Alfred Ratzlaff, Marigold Foods, Rochester, Minnesota; and Elwin C. Omundson of the Albert Lea Health Department.

The Achievement Award was presented to Donald Pusch of the Pillsbury Company who was a past president of MSA for exceptional service to the association.

President Zottola of the University of Minnesota passing the presidency on to Edward Kaeder of Mid-America Dairymen, Inc.

New York Affiliate Holds Annual Meeting

Maurice A. Guerrette became the 49th president of the New York State Association of Milk and Food Sanitarians at its annual meeting held in conjunction with the Cornell University Food Science Department, September 17-19 at the Granit Hotel in Kerhonkson, N.Y. Mr. Guerrette is the Assistant Director of Food Control of the New York State Department of Agriculture and Markets.

Other new officers and board members are Dr. William K. Jordan of the Cornell Food Science Department who was elected junior member of the executive board and Donald A. Brownell of Cooperative Marketing Agency in Oneida who was named president-elect. Retiring president is John W. Burke of Borden, Inc. in Watertown. The conference was attended by 425 people.

At the annual awards banquet engraved plaques were presented to four members of the Association. Dr. Robert F. Holland, retired head of the Cornell Food Science Department and 39th president of the Association was the recipient of the Emmet R. Gauhn award given in memory of the Association's first president. This award recognizes outstanding service and leadership in behalf of the Association. DeForest May, a retired New York State Department of Agriculture and Markets senior milk inspector, was presented with the Brooks award in recognition of his contribution to the welfare and progress of the affiliates of the Association. The Marlatt Memorial Award went to Douglas W. Friend, owner of a regional testing laboratory, for his outstanding service in the field of laboratory technology. The annual award for the outstanding fieldman, given in memory of Dr. T. H. Reich, was presented to Joseph A. Leo who is currently employed by Kraft Foods. Five Honorary Life Memberships were presented to Alfred H.

Ahrens, Charles G. Ashe, Worthington P. Howe, Richard M. Parry, Joseph F. Tiernan, and John Vorperian.

Twenty-six papers were given during the two-day conference by Association members, Cornell faculty, and guest speakers from industry and regulatory agencies. There was one woman speaker—Ms. Rosemary Pooler, head of the New York State Consumer Protection Board. Separate sessions for fieldmen and for people whose interests are in the areas of laboratory, plant, and food were held as well as one General Session.

Index To Advertisers

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Notice of Vacancy

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

Affiliate Annual Meetings

California—October 20-22, 1975, Queensway Hilton, Long Beach.
Connecticut—January 1976.
Indiana—October 7-9, 1975, Holiday Inn, Merrillville,
Iowa—March 22, 1976, Ames.
Kansas—October 1-3, 1975, Holiday Inn, Manhattan.
Kentucky—February 24-25, 1976, Stouffer's Inn, Louisville.
Michigan—March 1976.
New York—September 17-19, 1975, Granit Hotel, Kerhonkson.
Ontario—Eastern, November 1975, Kemptville.
Oregon—November 17, 1975, Oregon Department of Agriculture & Kings Table of International Restaurant, Salem.
South Dakota—May 11-14, 1976, Holiday Inn, Aberdeen.
Washington—September 9, 1975, Sheraton-Renton Inn, Renton.
Wisconsin—September 25-26, 1975, Holiday Inn, Tomah, Wisconsin.

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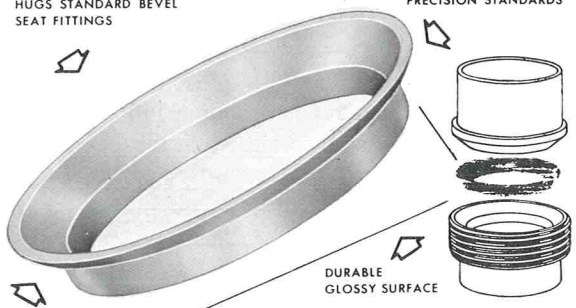

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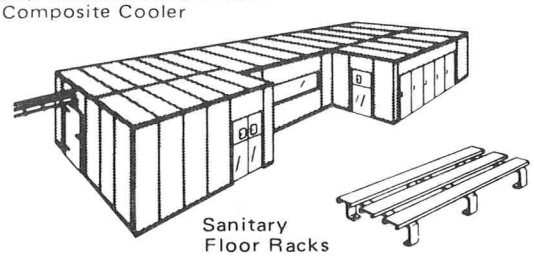

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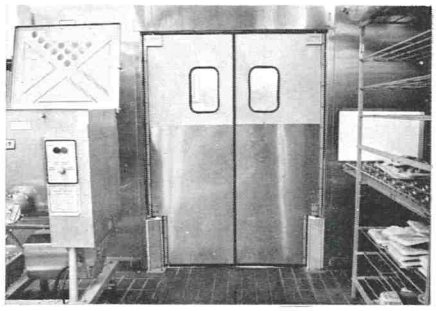

with DOOR JAMB GUARDS in nickel stainless steel meat processing or storage

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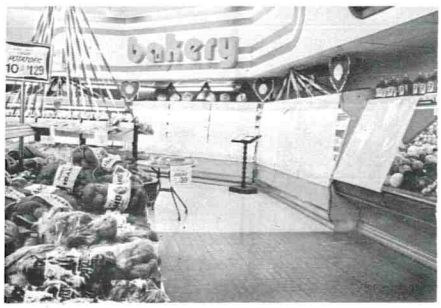

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Dairy authorities speak out on better cow milking

Stephen B. Spencer
Extension Dairy Specialist,
Pennsylvania State University

What's your score on vacuum?

An inadequate vacuum pump can affect the operation of the milking units drastically. Excessive "drop-off," slower milking, incomplete milking and an increase in the incidence of mastitis are likely results.

As a dairyman, your net dollar income depends on the vacuum pump and it probably is used more hours each year than most farm tractors.

Few people would hitch six plows behind a four plow tractor and head for the field to do a day's work. Yet many of these same people milk cows with a short vacuum supply and never question whether the pump is adequate.

Vacuum pumps used to be rated according to the number of units that could be operated. Today we measure the pump capacity in terms of Cubic Feet of air per Minute (CFM). Just as "horsepower" is more reliable than "plow rating" as an indication of tractor size, "CFM" is more reliable than "unit rating" when sizing a vacuum pump.

CFM output depends upon vacuum level. As vacuum level increases, the CFM output decreases. It's important to operate the system at the vacuum level specified by the manufacturer or the output of the pump will be altered.

The important consideration of any vacuum pump is the CFM output at the milking vacuum level. The pump must have adequate CFM output to meet the vacuum requirements of the system and provide sufficient reserve to maintain a constant vacuum level.

Vacuum Requirement
The milking unit is the most important of the machine components which admit air into the system. The air consumption of milking units varies depending upon shell and inflation size, pulsation rate and length and size of pulsated air tubes. Typically, the air requirement of a milking unit while it is not milking is three to four CFM. The pulsator consumes 50 to 70% of this volume. Considerably larger air pumping capacity (a reserve) must be provided to make a milking system operational. Other components which consume air are such things as vacuum operated door openers, milk metering devices, and the vacuum regulator. The requirements of each component must be added together to determine the system requirements.

Vacuum Reserve
The vacuum reserve is the air pumping capacity which remains after the vacuum requirement of all components has been satisfied. That's the problem. We've thought in terms of the vacuum reserve as the amount of CFM capacity that's *left over*. We really should be thinking in terms of a *base reserve* for the operator(s) before we begin to compute the system requirements.

The reserve is all-important in order to maintain vacuum stability. The reserve is necessary in order to make allowances for operator usage and possible leaks in the system or other contingencies.

The most important reason for an adequate vacuum reserve is to provide for the amount of air that the operator will use. The operator is the largest user of the vacuum reserve. Some operators are very wasteful of the available reserve. This occurs as units are being attached and removed. Improper unit adjustment is also a significant factor. When teat cups start to leak and "squeal" during milking, the vacuum reserve is depleted rapidly.

Some operators may deplete vacuum reserve as much as 30 or more CFM for short periods of time. The careful operator will use but half that amount during the milking process. The real test of any milking system is when a milking unit falls off. It takes huge reserves of air just to keep the remaining units on the cows. Reserve tanks aid a little during these occurrences but basically the vacuum pump must be relied upon to maintain vacuum level. It all adds up to the fact that an adequate pump is a *must* for every dairyman.

Research in Ireland, Wisconsin, Pennsylvania and California indicates that inadequate vacuum reserve is associated with higher leucocyte counts. In plain language it means that mastitis can result if your vacuum pump isn't large enough.

What's your Vacuum Score?
Don't make a mistake and just assume that your pump is putting out enough air. Have it checked with an air flow meter once a year. Many dealers are equipped to do this for you.

And how will you know for sure that they're not just trying to sell you a pump? Frankly, I've found most dealers to be very reliable in this respect.

There have been many different recommendations about pump sizes. It's hard to give one that's exactly right for each system. Here's a guide for you to check your vacuum needs. It's based upon the New Zealand Standard. The American Standard would give values equal to one-half of the New Zealand Standard.

For bucket users:
Allow 4 CFM per unit + 20 CFM base reserve.

For pipeline users:
Allow 5 CFM per unit + 40 CFM base reserve for the first operator and 20 CFM for each additional operator.

The resulting CFM values would give you the minimum size vacuum pump capacity. If your system has more than this, fine. If you have less vacuum capacity than this you should carefully investigate your vacuum needs.

This method of determining vacuum capacity is different from what you may have seen before. A 50 percent reserve is commonly used. While a 50 percent reserve may be satisfactory on a system of six or more units, our field studies indicate that using a 50 percent reserve is not adequate for the smaller system.

Remember this: There is no substitute for an adequate vacuum system. Make sure you know your score on your vacuum needs.