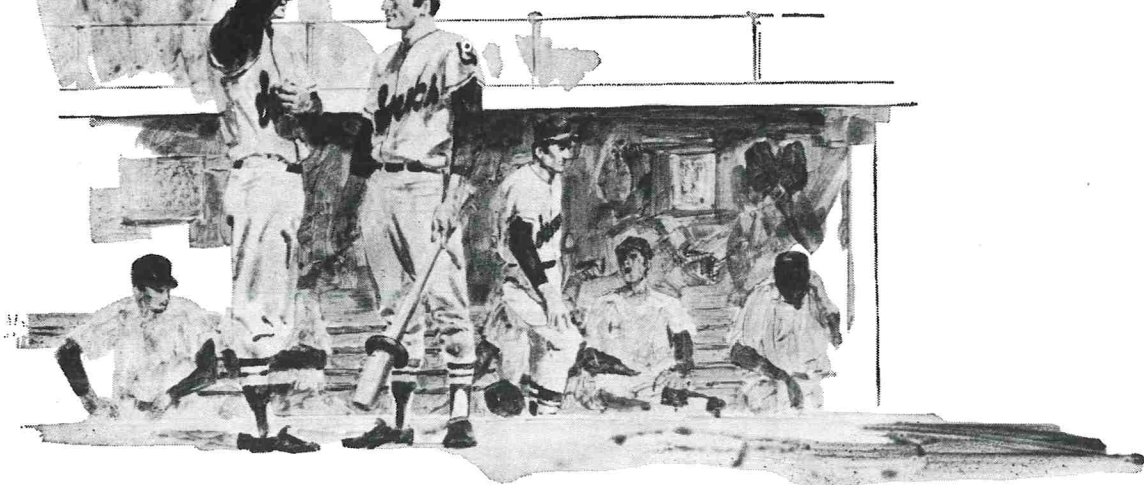# Journal of
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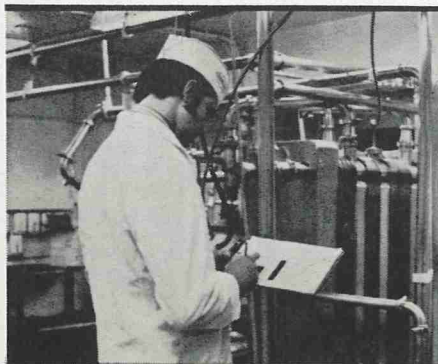
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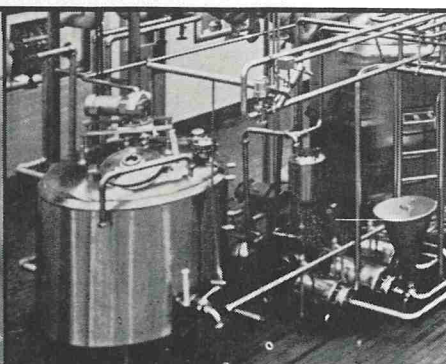
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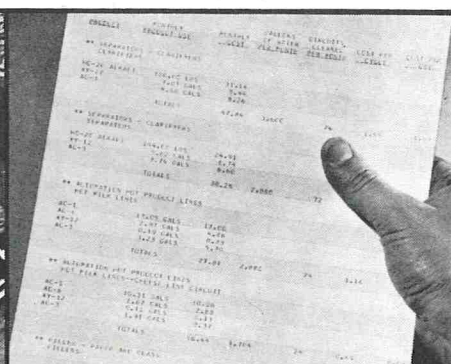
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IV

EFFECT OF TEMPERATURE AND pH ON GROWTH AND ENTEROTOXIN PRODUCTION BY *STAPHYLOCOCCUS AUREUS*[1]

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ABSTRACT

Four strains of *Staphylococcus aureus* producing enterotoxin types A, B, C, and D were inoculated into buffered Brain Heart Infusion (BHI) broth at a concentration of 10^6 cells/ml and incubated with agitation at temperatures ranging from 7 to 50 C for intervals extending to 4 weeks. At 45 C, strains 265 (type A), 493 (type C), and 315 (type D) grew and produced enterotoxin, but there was a rapid decrease in viable cells and no enterotoxin produced by strain 243 (type B). In the range of 19 to 39 C, all strains grew and produced enterotoxin. At 13 C, strains 265, 243, and 493 grew but only strain 243 produced enterotoxin. Strain 315 did not grow at 13 C and none of the strains grew at 7 or 50 C. The population of *S. aureus* varied from 1.3×10^7 to 5.5×10^8 cells/ml when enterotoxin was first detected.

S. aureus strain 243 was inoculated at a concentration of 10^6 cells/ml into BHI broth with and without 0.2 M sodium phosphate buffer and incubated at 37 C for time intervals extending to 172 hr. The initial pH of the broth was adjusted to various values from 3.62 to 9.84. Growth occurred when the initial pH of the broth was 4.96 to 9.02 and a slight increase in population was noted at pH 4.76 and 9.40. Production of detectable amounts of enterotoxin was restricted to pH 5.15 to 9.02. Detectable enterotoxin was produced in 4 to 6 hr in non-buffered broth, but a minimum of 9 hr was required in buffered broth.

Ingestion of staphylococcal enterotoxin produced by *Staphylococcus aureus* may result in bacterial food poisoning. Since *S. aureus* may occur in low numbers in many foods *(1)*, it is important that food be handled to prevent multiplication of cells and production of enterotoxin. Several environmental factors such as water activity *(17)*, NaCl concentration *(10)*, atmosphere *(16)*, growth medium *(9)*, presence of other microorganisms *(12)*, temperature *(1, 4, 18)* and pH *(5, 13)* influence growth of staphylococci and may influence production of enterotoxin. Four immunologically different enterotoxins (A-D) have been purified and made available for study *(3)*. Enterotoxin A alone or in combinations with other enterotoxins is found in about 75% of the outbreaks of staphylococcal food poisoning *(3)*. Enterotoxin B is found in relatively few outbreaks but it has been studied more extensively than the other enterotoxins because relatively high concentrations are produced in laboratory

media *(15)*. The objective of this investigation was to determine the effect of temperature on growth and enterotoxin production of four immunologically different strains of *S. aureus* and the effect of pH on production of enterotoxin B by *S. aureus* strain 243.

MATERIALS AND METHODS

Media

Mannitol Salt Agar (MSA) was used to enumerate staphylococci and Brain Heart Infusion (BHI) broth was used to grow the cultures. BHI broth containing 0.2 M sodium phosphate buffer standardized to pH 7.0 after sterilization was used in all experiments involving effect of temperature in buffered broth. In experiments involving effect of pH, 0.2 M phosphate buffered BHI broth was prepared with the pH at various values between 3.62 and 9.84. When necessary, the pH of both buffered and non-buffered broth was adjusted with 6 N HCL or NaOH.

Cultures

Four strains of *S. aureus*, 265, 243, 493, and 315, which produce enterotoxins A, B, C, and D, respectively, were obtained from the late Dr. E. P. Casman of the Food and Drug Administration Laboratory in Washington, D. C. Each strain produces only one enterotoxin.

Culture propagation

The cultures were grown in BHI broth at 37 C and transferred daily for at least 3 days before use. Cultures in the stationary phase, incubated 12-24 hr, were used for inoculation. Sufficient inoculum was added to yield a population of approximately 10^6 cells/ml in 300 ml of BHI broth in 1-liter screw-cap erlenmeyer flasks. The flasks were incubated for time intervals extending to 4 weeks at selected temperatures in a rotary shaker operated at 175 rpm. Samples used to determine the effect of temperature were thermostatically controlled at 7, 13, 19, 26, 32, 37, 39, 45, and 50 C by the heating unit on the shaker and/or placing the shaker in a refrigerated room. The samples used to determine effect of pH were incubated at 37 C under the same physical conditions indicated above. At selected intervals a portion of the broth was removed aseptically to determine population, pH, and enterotoxin.

Determination of population

The broth samples were diluted as necessary in 0.01 M phosphate buffer. The populations of *S. aureus* were determined by the spread plate technique using pre-poured MSA plates prepared no more than 24 hr before use. Plates were incubated at 37 C for 48 hr and all colonies were counted.

Determination of pH

A Corning single electrode on a Beckman pH Meter (Model 1019) was used for all pH determinations. At each sampling period, approximately 10 ml of broth was placed in a 50 ml

TABLE 1. POPULATION, pH, AND MINIMUM INCUBATION TIME ASSOCIATED WITH PRODUCTION OF MEASURABLE AMOUNTS OF ENTEROTOXIN FORMED BY FOUR STRAINS OF S. *aureus* WHEN INCUBATED WITH AGITATION IN BHI BROTH BUFFERED AT pH 7.0 WITH 0.2 M PHOSPHATE AT THE TEMPERATURES INDICATED

Incubation temperature (C)	S. *aureus* 265 (A Toxin)			S. *aureus* 243 (B Toxin)			S. *aureus* 493 (C Toxin)			S. *aureus* 315 (D Toxin)		
	Time (hr)	pH	Count* $\times 10^6$	Time (hr)	pH	Count* $\times 10^6$	Time (hr)	pH	Count* $\times 10^6$	Time (hr)	pH	Count* $\times 10^6$
7	(a)	6.92	<.0001	(a)	6.94	<.01	(a)	6.91	<.001	(a)	6.99	<.01
13	(b)	6.79	3	158	6.77	32	(b)	6.78	13	(b)	6.81	0.1
19	98	6.96	16	78	6.94	80	78	6.91	130	98	6.94	320
26	16	6.66	360	16	6.66	190	14	6.62	450	14	6.66	110
32	22	6.85	550	13	6.82	50	11	6.83	69	13	6.86	50
39	18	6.82	440	10	6.96	20	6	6.98	13	16	6.79	80
45	78	6.83	20	(c)	6.91	<.0001	38	6.78	120	38	6.82	15
50	(d)	6.90	<.0001	(d)	6.91	<.0001	(d)	6.90	<.0001	(d)	6.87	<.0001

*Counts were made on MSA. The initial inoculation was approximately 10^6/ml.
(a) No toxin detected after incubation for 673 hr.
(b) No toxin detected after incubation for 338 hr.
(c) No toxin detected after incubation for 122 hr.
(d) No toxin detected after incubation for 73 hr.

tube and centrifuged at 12,000 $\times$ g for 15 min at 2 C. The pellet was discarded. A small portion of the supernatant fluid was used for the pH determination, and the remainder of the fluid was stored at -30 C for enterotoxin assay at a later time.

Enterotoxin determination

The microslide gel-diffusion method (2) was used to determine the presence of staphylococcal enterotoxin. Purified staphylococcal enterotoxins A, B, C, and D, and corresponding antitoxins used as standards were obtained from Dr. Casman.

RESULTS AND DISCUSSION

Effect of temperature

Samples of broth taken immediately after inoculation did not contain detectable enterotoxin. All four strains of S. *aureus* grew over a temperature range of 13 to 45 C except strain 243 which failed to grow at 45 C and strain 315 which failed to grow at 13 C (Table 1). None of the strains grew at 7 or 50 C. When enterotoxin was first detected the population of S. *aureus* in the BHI broth varied from 1.3×10^7 to 5.5×10^8 cells/ml and the pH of the broth varied from 6.62 to 6.98. The temperature at which enterotoxin was produced was restricted to the range of 19 to 45 C, with the exception of S. *aureus* 243 which produced enterotoxin B over the range of 13 to 39 C. The incubation time required for enterotoxin production varied from 6 to 22 hr in the temperature range of 26 to 39 C and the time increased as the temperature varied above or below this range. Strains which produced enterotoxin at the incubation temperatures used did so within 98 hr, except strain 243 which produced enterotoxin B in 158 hr at 13 C.

Data in Table 1 show little difference among the four strains of S. *aureus* in the pH, population, or time required to produce measurable toxin during incubation at 26 C. At temperatures above and below 26 C the variation among strains in these cate-

gories was more apparent. There was a particularly abrupt increase in time required to produce toxin at 19 C. Since none of the strains grew at 50 C and only three of the four strains produced enterotoxin at 45 C, it is probable that 45 C is near the maximum temperature for toxin production. Likewise 13 C is near the minimum temperature for toxin production, since S. *aureus* 243 produced toxin at 13 C after 158 hr but the other strains did not produce enterotoxin during incubation for 2 wk at 13 C.

The incubation times and S. *aureus* populations associated with detectable amounts of enterotoxins A, B, C, and D in buffered BHI broth (Table 1) are similar to the times and populations reported by Donnelly et al. (4) for production of enterotoxin A in milk at 25-35 C. The minimum concentration of enterotoxin which can be detected by the microslide gel-diffusion technique is about 1 μg/ml (2). Since about 20 μg of enterotoxin B, and considerably less enterotoxin A, may be toxic for man (14), an enterotoxin concentration of 1 μg/g in an average serving of food is potentially hazardous. Data reported herein indicate that it is possible to predict the incubation time required at a particular temperature for cells of the four strains of S. *aureus* to produce measurable amounts of enterotoxin in buffered BHI broth, but it is not possible to predict the presence of enterotoxin in a particular sample by the population of S. *aureus* in that sample.

Genigeorgis et al. (6) used five strains of S. *aureus*, four of which were different from those used in the work reported here, and found statistically significant differences in growth rates among the strains. Under the conditions used in this investigation, no distinguishing differences were apparent among the four strains of S. *aureus*, except that each strain produces a different enterotoxin. Differences in growth rates

TABLE 2. POPULATION, pH, AND MINIMUM INCUBATION TIME ASSOCIATED WITH PRODUCTION OF DETECTABLE AMOUNTS OF ENTEROTOXIN B BY S. *aureus* STRAIN 243 GROWN AT 37 C WITH AGITATION IN BHI BROTH CONTAINING 0.2 M PHOSPHATE BUFFER AT VARIOUS pH VALUES

Initial pH	Incubation time (hr)	Terminal pH	Terminal count on MSA[1] $\times 10^6$
3.62	ND[2]	3.61	<0.1
4.48	ND	4.50	<0.1
4.76	ND	4.92	1.2
4.96	ND	5.05	4
5.15	154	5.66	350
5.38	32	5.42	57
5.48	124	6.08	220
5.56	26	5.58	90
6.44	9	6.48	32
6.45	9	6.44	30
6.53	14	6.42	720
6.79	23	6.78	1700
6.94	19	6.79	570
7.03	12	6.97	25
7.20	23	7.17	1600
7.72	52	7.71	7
7.73	48	7.61	46
7.96	47	7.80	3000
7.97	58	7.75	800
8.14	56	7.89	1500
8.40	40	7.86	1200
8.61	52	7.71	400
8.67	36	8.02	680
9.02	24	8.89	3
9.40	ND		1.5
9.84	ND		<0.1

[1]Initial counts on MSA were approximately 10^6 cells/ml.
[2]ND = No detectable toxin produced within 172 hr.

of S. *aureus* exist but these differences do not appear to be related to differences in enterotoxin type.

Effect of pH

Detectable amounts of enterotoxin B were produced in phosphate buffered BHI broth with an initial pH of 5.15 to 9.02 and incubation times ranging from 9 hr at pH 6.44 to 154 hr at pH 5.15 (Table 2). In non-buffered BHI broth the incubation times required for production of detectable amounts of enterotoxin varied from 4 to 6 hr at pH 6.14 to 7.95 (Table 3).

Growth of S. *aureus* strain 243 occurred over a greater range of pH than toxin production. Gennison and Wadsworth (8) have calculated statistically that there is a 68% probability that plate count values determined experimentally will be within 6.6% of actual values. Therefore we must recognize the possibility of experimental error in the small increases in population indicated with (a) strain 265 at 13 C (Table 1); (b) strain 243 in buffered broth at pH 4.76, 9.02, and 9.40 (Table 2); and (c) strain 243 in non-buffered broth at pH 5.02 and 9.08. In buffered BHI broth, the incubation time required for pro-

duction of a detectable quantity of enterotoxin varied considerably and was dependent upon the initial pH of the growth medium (Table 2). Less time was required for production of detectable toxin at pH 6.44 to 7.20 than at pH above and below these values.

The incubation time required to produce a detectable quantity of enterotoxin in non-buffered BHI broth varied little within the pH range in which toxin production occurred (Table 3). When detectable amounts of enterotoxin first appeared, the staphylococcal populations varied from 3.0×10^6 to 3.0×10^9 cells/ml in buffered BHI broth (Table 2) and from 7.5×10^6 to 1.2×10^9 cells/ml in non-buffered BHI broth (Table 3).

Morse et al. (11) and Peterson et al. (13) observed that the pH of the medium changes while the S. *aureus* culture is growing. If the pH of the medium is low there is a tendency for the pH to increase and if the pH is high there is a tendency for it to decrease. An increase in pH of the medium, an increase in offensive odor, and a darker colored pellet usually occurred in samples which produced toxin in the shortest incubation times.

Since the incubation time required to produce a detectable quantity of enterotoxin was less in non-buffered than in buffered BHI broth (Tables 2 and 3), it is reasonable to conclude that the phosphate buffer affected enterotoxin production. The 0.2 M sodium phosphate buffer delayed, but did not prevent, change in the pH of the growth medium. Phosphate has a high buffering capacity at pH 8 and in the range of 7.7 to 8.6 the incubation times required before enterotoxin could be detected in buffered BHI broth were considerably longer than the incubation times at higher or lower pH values. The differences in incubation times needed for measurable amounts of enterotoxin to be produced in BHI broth buffered at different pH values as well as the diff-

TABLE 3. POPULATION, pH, AND MINIMUM INCUBATION TIME ASSOCIATED WITH PRODUCTION OF DETECTABLE AMOUNTS OF ENTEROTOXIN B BY S. *aureus* STRAIN 243 GROWN AT 37 C WITH AGITATION IN NON-BUFFERED BHI BROTH AT VARIOUS INITIAL pH VALUES

Initial pH	Incubation time (hr)	Terminal pH	Terminal count on MSA[1] $\times 10^6$
5.02	ND[2]	5.00	2
6.14	6	5.34	500
6.62	6	5.70	750
7.13	4	6.78	7.5
7.55	6	6.62	1200
7.95	4	7.63	76
9.08	ND	8.90	3
9.86	ND	9.85	<0.1

[1]Initial counts on MSA were approximately 10^6 cells/ml.
[2]ND = No detectable toxin produced within 172 hr.

erence between incubation times required for entero-
toxin production in buffered and non-buffered BHI
broth may be caused by stabilization of the pH of
the medium.

In both buffered and non-buffered BHI broth,
growth of S. *aureus* occurred over a wider range of
pH than did enterotoxin production. It is theoretical-
ly possible, but frequently not feasible, to adjust the
pH of food above or below the pH range for entero-
toxin production. Few foods would be accepted
by the consumer if the pH were above 9; although
a number of acceptable foods naturally have a pH
below 5. Genigeorgis et al. (5) reported that NaCl
in the growth medium narrows the pH range in which
enterotoxin production occurs. Genigeorgis et al. (7)
also showed that growth of S. *aureus* occurred in meat
in which they were unable to detect enterotoxin pro-
duction. According to Reiser and Weiss (15), the
growth medium is known to affect the total amount
of enterotoxin produced. The nature of the growth
medium would also affect the incubation time need-
ed for a measurable amount of enterotoxin to be pro-
duced. The pH ranges for growth and enterotoxin
production by S. *aureus* are probably similar in broth
and food. Assuming this is true, the data suggest
that foods within a pH range of 5.1 to 9.0 which con-
tain staphylococci may be considered to have a po-
tential for staphylococcal food poisoning if other en-
vironmental conditions are favorable.

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MICROBIOLOGICAL, CHEMICAL, AND ORGANOLEPTIC CHARACTERISTICS OF FROZEN BREADED RAW SHRIMP[1,2]

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Abstract

Thirty percent of the samples of fresh or frozen shrimp received for processing into breaded shrimp had bacterial counts in excess of 10^6/g. The bacterial counts of 56% of plant processed samples of frozen breaded raw shrimp exceeded 10^6/g. Counts with plate incubation at 7 or 25 C were significantly higher than at 35 C. Little relationship existed between aerobic plate counts and coliform or enterococcal counts. Neither *Salmonella* nor *Vibrio parahaemolyticus* was isolated from plant processed samples. Frozen storage of breaded shrimp for 3 to 12 months caused minor reductions in aerobic plate count and coliform count and only minor changes in trimethylamine nitrogen (TMN), total volatile nitrogen (TVN), and odor scores. When frozen breaded raw shrimp were exposed to elevated temperatures (0-10 C) increases in count occurred at 0 C after 3-5 days, at 5.5 C after 2-3 days, and at 10 C after 1-2 days. Coliform bacteria increased at 5.5 C, *Escherichia coli* and enterococci at 10 C. In samples held at 0-10 C, marked increases in TMN and TVN and decreases in pH value occurred when bacterial counts had increased sharply and when off-odors became noticeable. Gram-positive forms, *Bacillus*, *Microbacterium*, *Micrococcus*, and coryneform bacteria were predominant in retail samples. Many of these samples showed evidence of repeated thawing and freezing. Of the retail samples, 52% had aerobic plate counts of 10^6/g or higher.

Production and distribution of breaded shrimp is an important part of the seafood industry. In 1971 production of breaded shrimp was 104,284,000 lb. with a value at the processors level of $121 million (18). In production of frozen breaded raw shrimp, peeled and deveined shrimp are washed, covered with batter, and coated with breading material. Breaded shrimp then are packed in cartons and frozen. Several reports are available on the conditions that influence number and types of microorganisms on Gulf Coast shrimp at time of landing (2, 5, 20, 24). Microbial counts at time of catch usually are low (10^2-10^4/g) and typical spoilage bacteria are not numerous at that time. Handling and storage procedures on board are primarily responsible for increased counts and introduction of large numbers of psychrotrophic spoilage bacteria.

In tests made on retail samples of frozen breaded raw shrimp in 1961, Silverman et al. (14) reported that the bacterial counts of about 28% of the samples exceeded 10^6/g. In a similar study published in 1972, Nickerson and Pollak (11) reported that 48% of the samples had bacterial counts in excess of 10^6/g. Surkiewicz et al. (17) reported that breaded shrimp samples collected from plants operating under good conditions of sanitation had an average (geometric) MPN of < 1000 coliform bacteria/g and that 85% had an average (geometric) aerobic plate count of < $10/^6$g. In 81% of the samples, *Escherichia coli* was absent from 80% of the units (0.1-g portions). Of the units, 57% were negative for coagulase-positive staphylococci (0.1-g portion) and 95% had < 1000 coagulase-positive staphylococci/g. They showed that production of shrimp of good bacteriological quality requires (a) raw shrimp and batter of good bacterial quality, (b) clean and sanitized equipment, and (c) sanitary handling of the product along the processing line. Little information is available about (a) the composition of the microbial flora of breaded shrimp, (b) the potential shelf life of products of varying microbial composition, and (c) the influence of storage conditions in wholesale and retail channels on the organoleptic and bacteriological quality of this product. This study provides a detailed analysis of the microbiological conditions of raw and plant processed products. Included are tests on samples subjected to warm-up treatments to mimic loss of freezer operation during transit or in freezer cabinets.

Materials and Methods

Collection of samples

Shrimp and frozen breaded raw shrimp, except for store samples, were obtained from commercial operations along the Gulf Coast. Samples were packed in dry ice at the processing plant and transported immediately to the laboratory. Samples were subjected immediately to the various analyses. If additional analyses were required at a later date, sample units were stored at −23 C. Store samples of frozen breaded raw shrimp were purchased in Fort Worth, Dallas, and Houston, and transported to the laboratory packed in dry ice. These samples were from the same commercial sources as the plant samples.

Microbiological examination

Aerobic plate counts of shrimp were determined with the
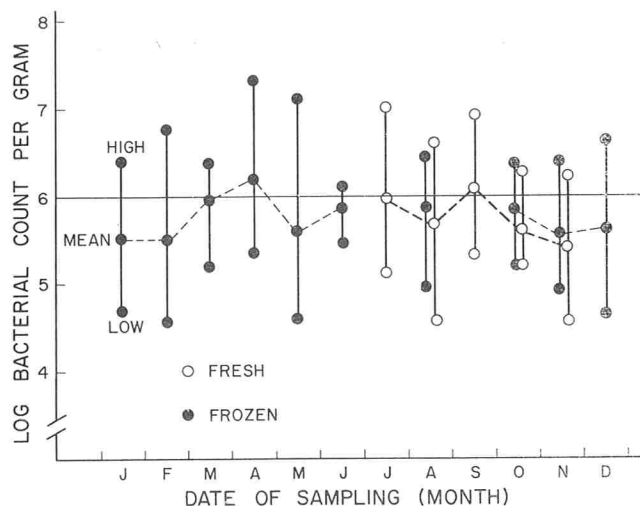
Figure 1. Aerobic plate count at 35 C of fresh and frozen shrimp used in the manufacture of breaded shrimp.

spread plate method by placing 0.1 ml of appropriate dilutions on Standard Methods agar (SMA, BBL) plates. Preparation of samples and dilutions was as described by Surkiewicz (16). Sets of triplicate plates were incubated aerobically at 7 C for 10 days, 25 C for 2 days, and 35 C for 2 days. To determine microbial types, approximately 40 colonies were picked at random from countable plates. Diagnostic procedures and schemes for identification of the microbial flora were presented previously by Vanderzant and Nickelson (21). Previous reports describe the procedures for determining the MPN of coliform bacteria and E. coli (16), coagulase-positive staphylococci (1), and enterococci (12). Established procedures were used to detect Salmonella (4) and Vibrio parahaemolyticus (23).

Chemical and organoleptic examination

Trimethylamine nitrogen (TMN) and total volatile nitrogen (TVN) were determined as described in a previous paper (3). Organoleptic evaluations were carried out by a trained three-member taste panel using a 5-point hedonic scale both for taste and odor (1 = excellent, no defect; 5 = marked defect, unacceptable). Breaded shrimp were evaluated fried with breading on and also boiled (4 min) with breading removed before boiling. Shrimp were fried in Wesson oil for 3 min at 191 C. Data were analyzed using analysis of variance, the mean separation technique of Duncan, and simple correlations (15).

RESULTS

Microbiological examination of raw and processed breaded shrimp

In Fig. 1 data are presented on the aerobic plate counts of 276 samples of fresh and frozen shrimp used to manufacture breaded shrimp. For each month, the high, low, and average (geometric) count are presented. About 30% of the samples had bacterial counts of 10^6/g or higher. An examination of 89 plant samples of frozen breaded raw shrimp showed that aerobic plate counts with plate incubation at 35 C ranged from 1.1×10^4 to 6.8×10^6, at 25 C from 6.0×10^4 to 2.7×10^7, and at 7 C from $8.0 \times$

10^3 to 3.5×10^7/g (Fig. 2, 3, 4). Geometric means of aerobic plate counts at 35, 25, and 7 C were 2.8×10^5, 1.5×10^6, and 1.2×10^6/g respectively. Analysis of variance (Table 1) indicated that highly significant differences in aerobic plate count resulted from temperature of plate incubation. By Duncan's test (15), counts with plate incubation at 35 C differed significantly from the others and ranked lowest (Table 2). Differences between counts determined at 25 and 7 C were not statistically significant. When plates were incubated at 35 C, 15 samples (17%) had counts of 10^6/g or higher. However, with plate incubation at 25 or 7 C about 50 samples or 56% showed counts of 10^6/g or higher. Although the geometric means of counts at 25 and 7 C remained high during the summer months (July through September), similar values were obtained in April and November.

Coliform counts of processed plant samples (Fig. 5) ranged from 3.6 to 4,600/g with a geometric mean of 66/g. Only 7 samples (8%) had a coliform count in excess of 10^3/g. E. coli was recovered from 7 (8%) samples at a level of 3 to 3.6/g. Enterococci in plant samples (Fig. 6) ranged from 9.1 to 11,000/g, with a geometric mean of 6×10^2/g. For plant samples, the correlation between aerobic plate counts at 35 C and enterococcal counts was −0.01, whereas the correlation coefficient between aerobic plate counts and coliform counts was 0.14. Coagulase-positive staphylococci (Fig. 7) were recovered from 45 (50%) samples.

TABLE 1. ANALYSIS OF VARIANCE RESULTING FROM EFFECT OF TEMPERATURE OF PLATE INCUBATION ON THE AEROBIC PLATE COUNT OF FRESHLY PROCESSED AND STORED FROZEN BREADED RAW SHRIMP

Source of variation	d.f.	Mean squares	
		Fresh shrimp	Stored shrimp
Total	266		
Treatment	2	13.7100**	8.7690**
Error	264	0.4505	0.4337

**<0.01 level of probability

TABLE 2. MEAN LOG COUNT OF FRESHLY PROCESSED AND STORED FROZEN BREADED RAW SHRIMP WITH DIFFERENT TEMPERATURES OF PLATE INCUBATION

Plate incubation temperature	Means[1]	
	Fresh shrimp	Stored Shrimp
35 C	5.4510[b]	5.3940[b]
25 C	6.1666[a]	6.0006[a]
7 C	6.0882[a]	5.9000[a]

[1]Means in the same vertical column bearing different superscripts differ significantly (P<0.01).

Results of microbiological analyses of the same plant samples after 3 months of storage at −23 C were in many respects similar to those obtained immediately following processing. Counts at 35 C differed significantly from those determined on plates incubated at 25 or 7 C, and ranked lowest (Tables 1, 2). The range and geometric means of aerobic plate counts of the stored samples were similar to those of the freshly processed samples. At 35 C counts ranged from 1.0×10^4 to 9.3×10^6, at 25 C from 4.1×10^1 to 2.0×10^7, and at 7 C from 3.7×10^3 to 2.0×10^7/g. Average (geometric) counts at 35, 25 and 7 C were respectively 2.5×10^5, 1.0×10^6, and 8.0×10^5/g. Correlations between aerobic plate counts and coliform or enterococcal counts again were low, 0.04 and 0.16 respectively.

Coliform counts of stored samples ranged from 3 to 11,000/g with an average (geometric) count of 55/g. Enterococcal counts of stored samples ranged from 9.1 to >11,000/g with an average (geometric) count of 3.8×10^2/g. Coagulase-positive staphylococci were recovered from 48 (56%) of the stored
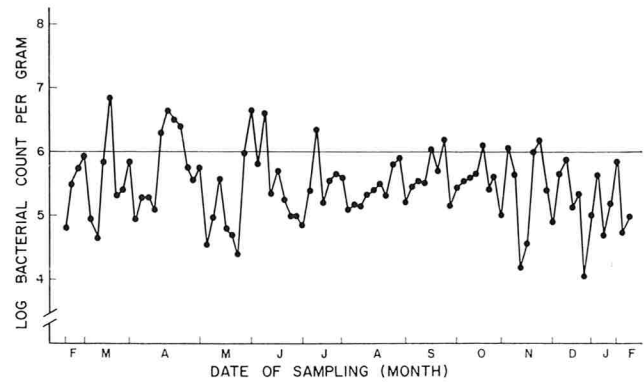
Figure 2. Aerobic plate count at 35 C of freshly processed frozen breaded raw shrimp.

samples. Storage of plant processed samples for 3 months at −23 C caused small decreases in aerobic plate counts (at 35, 25, or 7 C) and coliform counts in 56-59% of the samples. A statistical analysis indicated that differences in aerobic plate counts and coliform counts between stored and non-stored samples were not significant at the 0.05 level of probability.

TABLE 3. MICROBIOLOGICAL, CHEMICAL AND ORGANOLEPTIC EXAMINATION OF FROZEN BREADED SHRIMP SAMPLE 8 DURING FROZEN STORAGE

Age (month)	APC[a] at 25 C	Coliform MPN/g	E. coli MPN/g	Coag.-pos. staph.	pH	TMN mg/100 g	TVN mg/100 g	Organoleptic evaluation Fried Taste[b]	Odor[c]	Boiled Taste	Odor
0	2.5×10^6	43	<3	0[d]	7.0	0.3	7.3	2.2	2.2	3.5	3.3
0.5	1.6×10^6	3.6	<3	0	7.2	0.1	7.3	−	−	−	−
1	7.3×10^6	23	3.6	0	7.0	0.3	5.6	1.8	2.5	3.0	3.3
2	4.9×10^6	3.6	<3	0	7.1	0.2	7.6	2.3	2.2	3.8	3.0
3	5.0×10^6	<3	<3	0	−	−	−	2.3	2.7	2.8	3.0
4	7.6×10^6	3.6	<3	0	−	0.2	6.6	2.8	2.8	4.5	3.0
6	3.6×10^6	3.6	<3	0	7.4	−	−	−	−	−	−
8	2.1×10^6	<3	<3	0	7.2	−	−	−	−	−	−
12	1.1×10^6	3.6	<3	0	7.3	0.2	6.6	2.7	2.0	3.8	3.3

[a]APC Aerobic Plate Count
[b]Values are based on a 5-point scale [1 = excellent, no defect;]
[c]Values are based on a 5-point scale [5 = marked defect, unacceptable]
[d]None present in 10^{-1} g.

TABLE 4. MICROBIOLOGICAL, CHEMICAL AND ORGANOLEPTIC CHANGES IN SAMPLE 212 SUBJECTED TO HOLDING AT 0 C[a]

Day	Aerobic plate count/g at 35 C	25 C	7 C	Coliform MPN/g	E. coli MPN/g	Coag.-pos. staph.	Entero-cocci MPN/g	pH	TMN mg/100g	TVN mg/100g	Organoleptic evaluation Odor
0	3.9×10^5	1.2×10^6	1.7×10^6	43	7.3	0	43	7.1	0.9	9.6	2.0
0.5	5.3×10^5	2.7×10^6	3.8×10^6	150	<3	10^{-1b}	43	7.1	0.8	7.6	−
1	1.7×10^6	6.3×10^6	8.1×10^6	93	<3	10^{-1}	93	7.2	1.2	11.6	3.0
3	7.8×10^5	9.8×10^6	1.7×10^7	93	<3	0	93	7.6	1.1	9.6	2.8
5	4.5×10^6	1.1×10^8	1.7×10^8	93	3.6	0	150	7.4	3.3	15.4	3.3
7	6.8×10^7	7.2×10^8	7.6×10^8	3.6	<3	0	460	6.8	2.5	12.1	4.5
10	4.0×10^7	1.4×10^9	1.6×10^9	16	<3	10^{-1}	460	6.9	6.9	25.6	4.8
15	1.8×10^8	6.7×10^9	5.5×10^9	460	<3	0	93	6.3	7.3	37.6	4.8

[a]Footnotes in Table 3 apply.
[b]Present in 10^{-1} g.

TABLE 5. PERCENTAGE DISTRIBUTION OF MICROBIAL FLORA OF BREADED SHRIMP SAMPLE 2 A BEFORE AND AFTER STORAGE AT 0 AND 10 C WITH PLATE INCUBATION AT 25 C

Type	Initially	0 C (10 d)	10 C (5 d)
Pseudomonas	63.9	22.5	15.0
Flavobacterium	8.3	—	—
Moraxella	—	10.0	10.0
Aeromonas	5.6	—	10.0
Enterobacteriaceae	2.8	7.5	5.0
Bacillus	2.8	30.0	37.5
Coryneform	8.3	—	2.5
Microbacterium	8.3	22.5	17.5
Micrococcus	—	7.5	—
Sarcina	—	—	2.5

TABLE 6. PERCENTAGE DISTRIBUTION OF MICROBIAL FLORA OF BATTER AND BREADING

Type	Percentage distribution[a]	
	Batter	Breading
Pseudomonas	—	7.5
Moraxella	—	5.0
Bacillus	70.0	50.0
Coryneform	7.5	17.5
Microbacterium	22.5	20.0

[a]Colonies were picked from SMA plates incubated at 25 C.

Neither *Salmonella* nor *V. parahaemolyticus* was isolated from plant samples of frozen breaded raw shrimp. Only plant samples of breaded shrimp were examined for these species.

Microbiological, chemical, and organoleptic examination of stored samples

Ten samples of freshly processed plant samples ranging in aerobic plate count from about 10^4 to 10^7/g were stored in the laboratory for 12 months at -23 C and examined periodically. An example of the analyses for a representative sample (No. 8) is presented in Table 3. Initially, aerobic plate counts of the 10 samples ranged from 1.4×10^4 to 9.1×10^6 and after 12 months of frozen storage from 3.9×10^4 to 2.5×10^6/g. Bacterial counts at 35 and 7 C are not presented here because compared with those at 25 C the pattern of relationship was the same as presented in Fig. 2, 3, and 4. In general, little change in aerobic plate count occurred during frozen storage for 12 months. Initial coliform counts were low (3.6 to 460/g). In 8 of the 10 samples a decrease in coliform count occurred during frozen storage. The available data are too limited to arrive at any conclusions about the effect of the storage conditions on coagulase-positive staphylococci.

Initial TMN values of all samples ranged from 0 to 3.7 (average 0.6) mg/100 g. Initial TVN values

ranged from 3.8 to 12.3 (average 7.5) mg/100 g. Including all analyses, TMN values ranged from 0 to 3.8 mg/100 g, TVN values from 3.8 to 13.3 mg/100 g. Both minor decreases and increases in TVN and TMN occurred during frozen storage. The pH of the samples ranged from 6.8 to 7.6.

A comparison of the organoleptic evaluation of fried and boiled shrimp showed that the boiled samples usually were more severely criticized for taste and odor defects. A frequent comment of the judges on the taste of fried breaded shrimp was "taste breading material only." Of the fried samples, only two ranked in the range "slight off-flavor to unacceptable" (score 4-5) and/or "moderate to marked off-odor" (score 4-5). However, four of the boiled samples were in that range. The most common defects recorded were: bitter, stale, iodine, salty, fishy, petroleum, and amine-like.

In most samples only small changes in taste and odor scores were observed between the beginning and end of the storage period. For the fried samples, a difference of one point either in taste or odor score over this period was recorded for two samples.

A comparison of TMN, TVN, and organoleptic evaluations indicated that in some samples high TMN
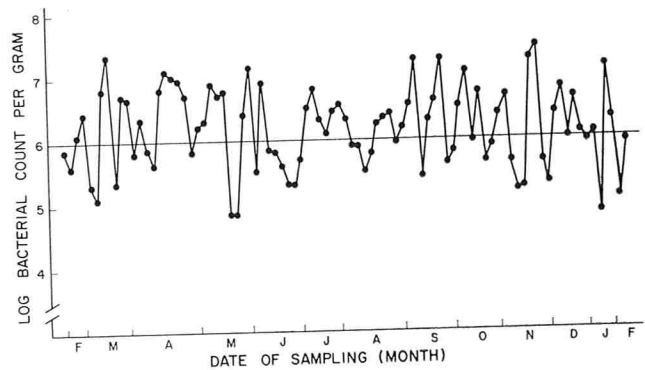
Figure 3. Aerobic plate count at 25 C of freshly processed frozen breaded raw shrimp.
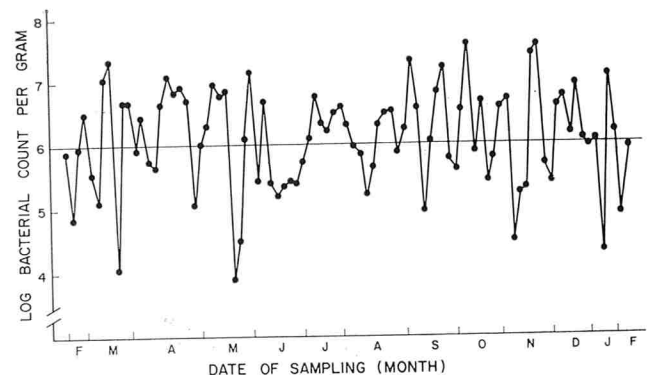
Figure 4. Aerobic plate count at 7 C of freshly processed frozen breaded raw shrimp.
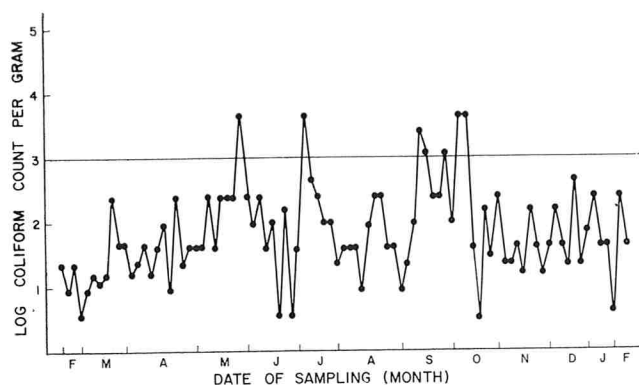
Figure 5. Coliform counts of freshly processed frozen breaded raw shrimp.
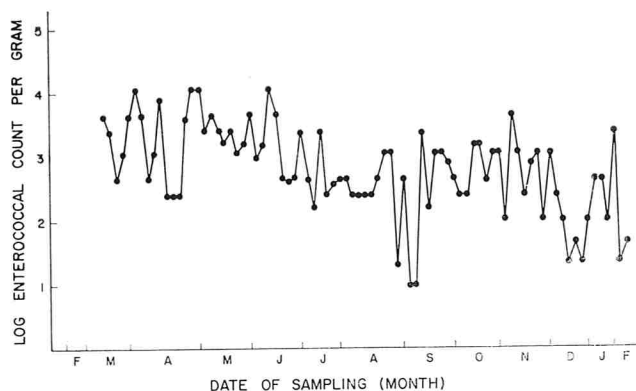
Figure 6. Enterococcal counts of freshly processed frozen breaded raw shrimp.
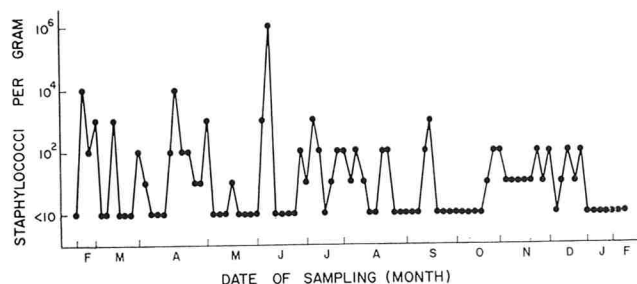
Figure 7. Staphylococci in freshly processed frozen breaded raw shrimp.

and/or TVN values were associated with moderate or marked taste and/or odor defects. In other samples, however, far less serious problems of taste and/or odor were encountered at similar or higher levels of TMN and/or TVN.

Examination of plant samples held at elevated temperatures

Three samples (1-lb. boxes) of plant processed breaded shrimp which were held at −18 to −23 C were placed at 0, 5.5, and 10 C in controlled temperature storage cabinets. Temperature changes in the products were recorded by insertion of thermocouples with a Tele-Thermometer (Yellow Springs

Instrument Company). The temperature of the shrimp reached the cabinet temperature of 0, 5.5, and 10 C after 13, 10, and 8 hr, respectively (Fig. 8). An example of the microbiological, chemical, and organoleptic changes of one of the samples (No. 212) during storage for 15 days at 0 C is presented in Table 4. In general, with storage at 0 C, marked increases in aerobic plate count (at 25 C) occurred after 3 to 5 days. Storage for 15 days at 0 C increased bacterial counts from 1.2-4 $\times$ 10^6 to 0.67-2.2 $\times$ 10^{10}/g. With plate incubation at 7 C, increases in count upon storage were recorded a few days earlier. As was observed previously, bacterial counts at 35 C were significantly lower than those with plate incubation at either 25 or 7 C. Although some fluctuations were observed in the coliform, *E. coli*, enterococcal, and staphylococcal counts during the holding period, no definite increases could be attributed to the increased storage temperature.

Holding at 5.5 C caused large increases in aerobic plate count (at 25 C) after 2 to 3 days. At 5.5 C increases also occurred in the coliform count. Usually no significant increases occurred in *E. coli*, enterococcal, or staphylococcal counts at 5.5 C. With storage at 10 C, bacterial counts increased greatly after 1 to 2 days. Within 5 days marked increases were noted in coliform count and enterococci. Smaller increases were noted in numbers of *E. coli*. Coagulase-positive staphylococci were isolated after holding samples for 5 days at 10 C but not from the original samples. Storage at 0, 5.5, and 10 C caused marked decreases in the pH of the breaded shrimp.

A comparison of the aerobic plate counts at 25 C, TMN and TVN values, and organoleptic scores indicates that large increases in TMN and TVN oc-
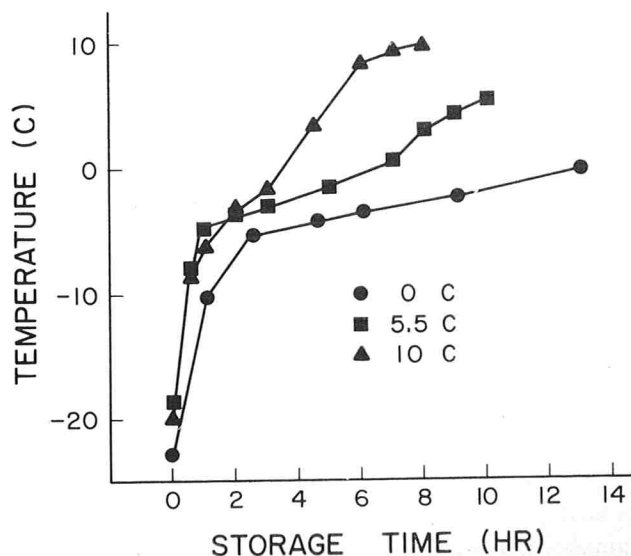
Figure 8. Temperature changes in shrimp stored at 0, 5.5 and 10 C after removal from about −20 C.

Table 7. Microbiological analyses and pH of 27 retail samples of frozen breaded raw shrimp

Age of sample (month)	Aerobic plate count per g	Coliform per g (MPN)	Escherichia coli per g (MPN)	Coagulase-pos. staphylococci per 0.1 g	Enterococci per g (MPN)	pH
1	1.1×10^7	23	<3	—	240	7.0
		79[a]	<3	+		
1	1.8×10^6	3.6	<3	—	240	7.2
		33	<3	+		
1	8.7×10^5	43	<3	+	44	6.8
1	3.2×10^6	43	3.6	+	460	6.9
2	4.9×10^6	9.1	<3	+	440	7.1
		65	<3	+		
2	8.1×10^6	<3	<3	—	240	7.2
		186	<3	—		
3	1.5×10^6	<3	<3	+	1,100	6.7
		146	<3	+		
3	1.1×10^7	<3	<3	+	11,000	7.8
		378	<3	+		
3	2.4×10^7	<3	<3	+	2,800	7.7
		378	<3	+		
3	6.9×10^4	<3	<3	—	11,000	7.0
		20	<3	+		
5	2.4×10^5	<3	<3	+	1,500	7.3
		13	<3	+		
5	3.5×10^6	<3	<3	—	21	7.1
7	8.3×10^4	<3	<3	—	93	6.9
		15	<3	+		
8	8.7×10^3	9.1	<3	—	3.6	6.7
		77	<3	+		
8	2.0×10^5	3	<3	—	7.3	7.1
		30	<3	+		
9	8.9×10^5	9.1	<3	+	150	6.9
		23	<3	+		
9	9.3×10^4	3.6	<3	+	3.6	7.0
		95	<3	—		
12	6.0×10^5	<3	<3	+	43	7.3
		68	<3	—		
12	1.0×10^6	43	<3	+	3.6	7.3
		68	<3	—		
12	2.8×10^6	<3	<3	—	3	7.2
		68	<3	—		
16	1.2×10^6	3.6	<3	+	93	6.9
		23	<3	+		
16	5.2×10^4	9.1	<3	—	240	6.9
19	8.9×10^4	<3	<3	—	23	6.8
		17	<3	—		
23	8.0×10^8	3.6	<3	+	240	6.8
		23	<3	+		
28	1.9×10^4	<3	<3	+	9.1	7.4
		$>11,000$	<3	—		
28	1.1×10^4	<3	<3	+	43	7.2
		$>11,000$	<3	—		
31	1.2×10^6	<3	<3	+	30	7.4

[a]Second line of data on coliforms, *E. coli*, and coagulase-positive staphylococci are results of analyses conducted at plant before samples were shipped out.

curred when bacterial counts had increased greatly and when organoleptic scores for taste and/or odor indicated the presence of defects. For example, with sample 212, after 10 days of storage at 0 C, the bacterial count had increased from 1.2×10^6 to 1.4×10^9/g, TMN values from 0.9 to 6.9 mg/100 g, TVN from 9.6 to 25.6 mg/100 g and marked off-odors were present. A similar pattern but occurring earlier was observed during storage at 5.5 and 10 C. When organoleptic defects first became noticeable bacterial counts ranged from 3.2×10^7 to 1.3×10^9/g, TMN from 2.2 to 9.6 (average 5.8) mg/100 g, and TVN

TABLE 8. DISTRIBUTION OF MICROBIAL FLORA OF 12 RETAIL SAMPLES OF FROZEN BREADED RAW SHRIMP

	No. of samples species were isolated from	Percentage distribution of isolates (Avg.)	Range of percentage distribution
Bacillus	12	33	9-68
Coryneform	7	14	0-42
Lactobacillus	4	4	0-20
Microbacterium	5	9	0-27
Streptococcus	3	3	0-14
Micrococcus	8	22	0-50
Staphylococcus	2	2	0-14
Acinetobacter	3	3	0-13
Flavobacterium	5	5	0-14
Moraxella	2	2	0-14
Enterobacteriaceae	2	2	0-13

from 12.1 to 38.7 (average 22.5) mg/100 g. Correlation between TMN and odor scores was 0.81 and between TVN and odor scores 0.78.

The distribution of the microbial flora of breaded shrimp before and after holding at 0, 5.5, and 10 C also was determined. Differences in the initial microflora of the samples were noted. *Pseudomonas* species predominated in one sample (No. 2A, Table 5), in the others *Achromobacter, Bacillus*, coryneform bacteria, *Microbacterium, Micrococcus*, and *Moraxella* were more prevalent. In some samples, significant differences in the distribution of the initial microbial flora also were associated with plate incubation temperature. Data on distribution of the microbial flora of sample 2A before and after holding at 0 and 10 C are presented in Table 5. With plates incubated at 25 C, holding shrimp at 0 and 10 C caused large decreases in *Pseudomonas* and increases in *Moraxella, Bacillus*, and *Microbacterium*. An analysis of the breading and batter used in commercial operations showed that *Bacillus* and *Microbacterium* species and coryneforms predominated (Table 6).

Microbiological examination of retail samples

Age of the 27 retail samples ranged from 1 to 31 months (Table 7). Aerobic plate counts at 25 C ranged from 8.7×10^3 to 8.0×10^8/g, coliform counts from <3 to 43/g. Bacterial counts of 14 (52%) samples exceeded 10^6/g. Coliform counts in general had decreased somewhat during storage in wholesale and retail channels. Coliform counts before distribution ranged from 13 to >11,000/g. All samples at time of purchase and about 90% before distribution had <1000 coliforms/g. *E. coli* was recovered from only 1 sample. Coagulase-positive staphylococci (in 0.1-g portions) were present in 16 (59%) of the retail samples. Enterococci ranged from 3 to 11,000/g. A wide range of enterococcal counts was encountered both with samples of low and high aerobic plate

counts. No relationship existed between coliform counts of products before shipment and enterococcal counts of product at time of purchase.

Bacillus, coryneform bacteria, *Microbacterium*, and *Micrococcus* species were dominant in retail samples (Table 8). Only *Bacillus* species were isolated from all retail samples. A majority of the retail samples showed evidence of thawing and refreezing.

DISCUSSION

Bacterial counts of shrimp received for processing frequently exceeded 10^6/g. The average counts of monthly supplies ranged from 2.5×10^5 to 1.6×10^6/g. Previous studies (20, 22) have shown that bacterial counts of freshly harvested Gulf Coast shrimp ranged from about 10^2 to 10^4/g. Low bacterial counts can be maintained if shrimp is handled on board under sanitary conditions, and is iced promptly. Microbial activity frequently causes extensive deterioration of quality characteristics particularly when counts reach levels of 10^6 to 10^7/g (22). Differences in degree of quality losses at similar count level can be caused by a difference in microbial species. In addition, in some instances high counts are caused by bacterial growth, whereas in others contact with heavily contaminated surfaces or equipment may lead to high counts. The present results indicate a need to improve the bacteriological condition of shrimp that is used for processing.

Although the quality of the raw shrimp undoubtedly is in part responsible for the high counts of many processed breaded products, inspection of plant facilities and processing methods showed that factors related to the processing phase can contribute to additional bacterial contamination. Thorough washing of raw shrimp can reduce the microbial load. If strict sanitary procedures were maintained in the plant and batter and breading of low bacterial count were applied, one could obtain processed products with bacterial counts lower than those of the initial fresh or frozen shrimp.

Bacterial counts with plate incubation at 25 or 7 C were significantly higher than those determined with plate incubation at 35 C. This has been reported previously (10, 19) with other foods in which psychrotrophic bacteria constitute a significant part of the microbial flora. A high plate incubation temperature probably has a limiting effect on the growth of some psychrotrophic bacteria that are part of the natural flora of the product or enter as contaminants during harvesting, handling and processing. With plate incubation at 35 C, 17% of the samples had counts in excess of 10^6/g. This figure agrees with that reported by Surkiewicz et al. (17). However, when plates in the present study were incubated at

25 or 7 C, 56% of the samples had counts in excess of 10^6/g. In this study, little relationship existed between coliform or enterococcal counts and aerobic plate count of breaded shrimp. Nickerson and Pollak *(11)* made a similar observation.

Frozen storage of breaded raw shrimp for 3 to 12 months at −23 C frequently caused minor reductions in total and coliform count. Freezing can destroy or sublethally injure bacterial cells. The latter often cannot be recovered under regular conditions of medium composition and plate incubation *(7, 9)*. This may also account for somewhat smaller colonies with plate incubation at 35 C, particularly with samples stored frozen for long periods. Poor recovery of sublethally injured cells on selective media with inhibitors may in part explain the decrease in coliform count.

In most samples of breaded shrimp stored for 12 months at −23 C changes in TMN, TVN, and odor scores were small. Careful control of the temperature probably kept chemical and microbial changes to a minimum. However, it is known that repeated changes in temperature, between the frozen state (−20 C) and the melting point can cause large decreases in bacterial counts of frozen fish *(13)*.

Laboratory conditions for holding frozen breaded shrimp at elevated temperatures were limited and do not duplicate the conditions in a truck or warehouse when freezer equipment fails. The data are useful in so far as they show the conditions of time and temperature at which changes in microbiological, chemical, and organoleptic conditions occur. Increases in coliform counts were recorded at 5.5 C, for *E. coli* and enterococci at 10 C. Storage at 0 to 10 C caused marked decreases in the pH of breaded shrimp. For iced shrimp, extensive holding usually causes an increase in pH *(22)*. In iced shrimp both the arginase-urease enzyme system and microbial activity are responsible for pH changes *(3)*. A difference in microbial activities because of differences in microbial flora of the products may be responsible for this observation. In stored iced shrimp, gram-negative species usually predominate *(20)*. Gram-positive species formed a large part of the microbial flora of breaded shrimp examined in the present study.

Large increases in TMN and TVN of breaded shrimp occurred when bacterial counts had increased significantly and taste and/or odor defects became noticeable. The lower TMN and TVN values of samples with moderate to severe organoleptic defects were respectively 4.4 mg and 24.2 mg/100 g shrimp excluding breading material. These values compare favorably with limits of acceptability reported by Montgomery et al. (5 mg TMN, 30 mg TVN) *(8)* and Iyengar et al. (15 mg TVN) *(6)*. Correlation between TMN, TVN values and odor scores were high.

The initial microbial flora of frozen breaded raw shrimp consisted primarily of *Pseudomonas, Achromobacter, Bacillus, Moraxella, Microbacterium, Micrococcus,* or coryneform bacteria. Holding samples at 0 to 10 C caused increases in *Microbacterium, Micrococcus, Bacillus,* or coryneform bacteria. Gram-positive species contributed 50 to 90% of the microbial flora of these samples. The microbial flora of batter and breading probably contributed to the large number of *Bacillus* and *Microbacterium* species. In retail samples of frozen breaded shrimp gram-positive forms also were predominant. Many of these samples had been held in storage for up to 31 months and showed evidence of repeated thawing and refreezing. This condition may have contributed to a predominance of gram-positive species and apparent lack of typical gram-negative spoilage bacteria. The number of retail samples with counts in excess of 10^6/g was similar to that reported by Nickerson and Pollak *(11)*.

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REPORT OF THE COMMITTEE ON FOOD EQUIPMENT SANITARY STANDARDS: 1971-1972

The IAMFES Committee on Food Equipment Sanitary Standards, known hereafter as the Committee, is charged with the responsibility of cooperating with other interested health organizations and related industries in the formulation of sanitary standards and educational materials for fabrication, installation, and operation of food equipment and to present to the membership those standards and educational materials which the Committee recommends be endorsed by the Association.

The purpose of this cooperative program is to aid industry in improving the design, construction, and installation of equipment so that it will lead to easy cleaning and proper functioning when placed into service in food establishments. It is the Committee's further purpose to cooperate with industry in the preparation of standards or guidelines which public health agencies will accept, thereby securing uniformity in the manufacture and nationwide acceptance of such equipment.

The following report outlines the Committee's activities during the past year in working with two health and industry organizations (National Sanitation Foundation's Joint Committee on Food Equipment Standards and the National Automatic Merchandising Association's Automatic Merchandising Health-Industry Council) and progress in meeting its purposes and objectives. It is expected these organizations will be the two groups that the Committee will work with during the coming year.

NATIONAL SANITATION FOUNDATION (NSF)

The Committee was represented at the 1972 meeting of the National Sanitation Foundation's Joint Committee on Food Equipment Standards, where action was taken on several proposals; and before the meeting, the Committee reviewed and submitted comments on each draft of these proposals. Since the meeting, the Committee has also reviewed and submitted comments on proposed changes to standards.

Basic criteria C-2 for special devices and equipment

Item 4.01 of Basic Criteria C-2 on the cleanability of food contact surfaces was revised as follows:

4.01 CLEANABILITY: All food contact surfaces shall be readily accessible for cleaning and inspection, either in an assembled position or when removed. *4.011:* In equipment where components are of such weight or design that food contact surfaces are not readily removable and/or cleaning without disassembling is intended, the component parts of the equipment shall be so arranged that cleaning and sanitizing solutions can be effectively applied to all food contact surfaces. The equipment shall be self-draining or otherwise completely evacuated, and the manufacturer's recommended cleaning procedures shall result in thorough cleaning and sanitizing of the equipment. *4.012:* In equipment of such design that heavy duty high speed operation is intended and/or

BACTERIOPHAGE TYPING OF STAPHYLOCOCCI ISOLATED FROM FOODSTUFFS AND FOOD HANDLERS IN TEHRAN

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Abstract

Studies were done to assess contamination of 3247 samples of foods and food handlers with coagulase-positive staphylococci. Food specimens were collected from retail shops and a Tehran milk pasteurizing plant; in addition 510 food handlers of different professional groups were examined. Three swabs for bacteriological examination were randomly collected from nose, hands, and throat. Of samples tested for coagulase-positive staphylococci, 26.02% were positive and almost half of them were obtained from foodstuffs. Of 546 isolates that were phage typed, we found that group III predominated in the nose and cream cake, while group II was more prevalent in raw cream, throat, and hands. Although group IV was not prominent, nose and hands yielded 3 and 1 positives, respectively. Untypable isolates comprised about 47.2% with most from hands and nose, 61.7 and 60.5%, respectively, and the least from raw cream, 33.3%.

Wide-spread adoption of phage typing for grouping coagulase-positive staphylococci makes this technique very useful for comparing the results reported by different laboratories (2). Phage typing of staphylococci isolated from milk and cheese (24); raw milk (8); certified milk (16); Cheddar cheese (4, 5); skin of pig and chickens (22); whipping cream, half cream, and butter milk (10); fish (21); market meats and non-frozen meats (14, 15); minced kebab (15); and some other foods and food handlers (4, 7, 19) has been reported from different countries in recent years, but in Iran no report is available concerning the phage typing of staphylococci of food origin.

Food poisoning, although common in Tehran, particularly during the warmer months, is not reportable, therefore there are no data available regarding the extent of food poisoning and the role that different organisms play in these episodes (6, 11). It is hoped that further studies towards isolation and phage typing of staphylococci of food origin and comparison of the results with similar studies elsewhere may help towards the understanding the epidemiology of these bacteria in food poisoning.

Materials and Methods

Food specimens collected from 717 retail shops and a Tehran milk pasteurizing plant were prepared and cultured on plates containing Baird-Parker medium (1) for isolation of coagulase-positive staphylococci.

Sampling food handlers

Five hundred and ten food handlers from different professional groups were examined. Three swabs for bacteriological examination were randomly collected from nose, hands, and throat of each person.

Before collection swabs were soaked in trypticase soy broth (TSB) and the specimens were then kept in TSB medium for 24 hr at 37 C. A loopful of each incubated sample was inoculated on Baird-Parker medium, and plates were incubated at 37 C for 48 hr. Typical colonies were transfered to 10 ml of broth and after sufficient growth, the coagulase test was done using 1:3 dilution of fresh rabbit plasma.

Phage typing of staphylococci

Bacteriophage typing was done according to the standard method (4, 27), using the following phages: Group I: 29, 52, 52A, 79, 80; Group II: 3A, 3B, 3C, 55, 71; Group III: 67, 42E, 47, 53, 54, 75, 77; Group IV: 42D; and Miscellaneous Group: 81, 187, 83A, 77ad, D, B5.

Results

Table 1 demonstrates that from 3247 samples of foods and food handlers that were tested for coagulase-positive staphylococci, 846 were positive and of these 367 were collected from food handlers and 479 were obtained from foodstuffs. In this table, we also have shown the percentage of coagulase-positive staphylococci.

The phage type strains from each source are presented separately in Tables 2-8. Group III staphylococci prevailed in the nose and cream cake, while Group II was more prevalent in raw cream, throat and hands. There were 3 and 1 group IV staphylococci recovered from nose and hands, respectively. About 47.2% of all samples were untypable, with most from hands and nose, 61.7 and 60.5%, respectively, and the lowest from raw cream, 33.3%.

Discussion

It was found that contamination of minced kebab in Tehran with coagulase-positive staphylococci was much greater than that of other foodstuffs tested in

TABLE 1. NUMBER AND PERCENTAGE OF COAGULASE-POSITIVE STAPHYLOCOCCI ISOLATED
FROM FOODSTUFFS AND FOOD HANDLERS

| Source | Cream cake | Raw milk | Minced kebab | Raw cream | Food handlers | | | Total |
					Nose	Hands	Throat	
No. samples	250	1000	350	117	510	510	510	47
No. of staph. coa-pos.	19	147	307	6	190	141	36	846
% Pos.	7.6	14.7	87.7	5.1	37.2	27.6	7.1	26.0
No. of strains typed	19	95	63	6	190	141	36	550

our study. More than 87% of the samples contained the bacteria. This high percentage can be attributed to the nature and composition of the minced kebab which contains about 90% meat.

It has been reported that meat marketed in developing countries is so contaminated that one has to consider it as poor quality meat (25). Kebab is excessively manipulated during processing and before roasting, and this can also account for such a high level of staphylococcal contamination. Although the percentage of staphylococcal contamination is too high, there is no report of a high incidence of food poisoning produced by kebab in Tehran areas. The number of staphylococci in the kebab specimens

TABLE 2. TYPE DISTRIBUTION OF STAPHYLOCOCCI FROM CREAM CAKE

Group	No. of strains	Percentage
Group I:		
80	1	5.3
Group II:		
3A	1	5.3
71	2	10.5
Total Group II	3	15.8
Group III:		
6/42E	1	5.3
6	1	5.3
Total Group III	2	10.5
Group IV	0	0
Mixed Group		
80/71	1	5.3
42E/53/81	1	5.3
52/79/80/6/47/54/83A/81	2	10.5
Total Group	4	21.0
Non typable	9	47.3
Total Strains	19	

examined were < 400,000/g, and only four specimens exceeded such a value (15). Hobbs (12) has shown that the enterotoxin produced by < 500,000 staphylococci/g of food is not sufficient to induce poisoning. It is necessary to mention that all kebab, milk, and cream specimens examined were in the raw condition.

Since staphylococci are usually killed in roasted kebab and pasteurized milk and cream, the enterotoxin produced by these microorganisms persist and may cause food poisoning which can not be traced to staphylococci (12).

Table 1 indicates that the number of coagulase-positive staphylococci isolated from raw milk, raw cream, and cream cake is very low in comparison with kebab specimens. It is suggested that such a discrepancy in milk and cream is due to a high level of contamination of these materials with other microorganisms, particularly lactic bacteria. The latter act to inhibit staphylococcal growth (6). It was observed that, in typable strains, the nose of food handlers examined was more afflicted with strains of group III-IV, and hands and throat with group II staphylococci.

Strains of group I were most frequently observed in minced kebab and raw milk. Petersen (18) reported that in Great Britain the yearly incidence of food-borne intoxication, from 1950 to 1962, was due to strains of staphylococci in phage group III, ranging from 64.5 to 96.7%. Another investigation has also revealed that strains more commonly implicated in food poisoning are members of phage groups III and IV (20). In our experiments a high proportion (about 47.2%) of the strains isolated from food handlers and foodstuffs were untypable, but these also may produce enterotoxins (3, 24). Hence, staphylococcal food poisoning in Tehran is associated with group III and untypable strains of staphylococci.

Tables 6 and 7 show that the percentages of untypable strains of staphylococci isolated from the nose

Table 3. Type distribution of staphylococci from raw milk

Group	No. of strains	Percentage
Group I:		
79/52A	1	1.06
29/52/52A/79/80	5	5.31
52/52A/79	1	1.06
Total Group I	7	7.43
Group II:		
3C/55/71	4	4.2
3A/3C/55/71	1	1.06
3A	1	1.06
Total Group II	6	6.32
Group III:		
75	2	2.1
53/54	2	2.1
42E/53	2	2.1
Total Group III	6	6.3
Group IV	0	0
Mixed group		
81/6/7/42E/54/75/83A	2	2.1
52/80/6/42E/81	1	1.06
52A/80/81	1	1.06
52/79/80/3A/3C/47	9	9.5
83A	5	5.3
80/6/42E/75/81	6	6.3
52A/42E/75	1	1.06
53/81/80/3A/3C/6	7	7.4
Total Group	32	33.78
Non typable	44	46.7
Total Strains	95	

Table 4. Type distribution of staphylococci from minced kebab

Group	No. of strains	Percentage
Group I:		
79/80	5	7.9
52/79	2	3.1
52A/79/80	2	3.1
Total Group I:	9	14.1
Group II:		
3A	2	3.1
55	1	1.5
71	1	1.5
Total Group II:	4	6.1
Group III:		
54	1	1.5
53/6/7/42E/47	2	3.1
6/75/54	3	4.7
6/7/47/53/54/75	1	1.5
Total Group III	7	10.8
Group IV	0	0
Mixed Group		
42E/81/52/7/54	1	1.5
54/53/79/6/7/42E/83A	10	15.8
42E/81/7/52	5	7.9
52/53	1	1.5
79/7/53	1	1.5
Total Group	18	28.2
Non typable	25	39.6
Total Strains	63	

Table 5. Type distribution of staphylococci from raw cream

Group	No. of strains	Percentage
Group I:		
79	1	16.6
Group II:		
3A/3C	2	33.3
Group III:		
54	1	16.6
Group IV	0	0
Non typable	2	33.3
Total Strains	6	

and hands of food handlers are very similar, namely 60.4 and 61.7% respectively.

This is compatible with findings of other investigators who reported that 15 to 20% of the people who carry staphylococci in their nose, also carry the organisms on their hands (7, 13). In addition, phage type 42D, which is the only group IV staphylococcus, was isolated from the nose and hands at a frequency of 1.5 and 0.7%, respectively. Tables 6 and 7 also reveal that the incidence of the following phage types was similar in both nose and hands: 29, 52, 52A, 80, 3A, 3C, 55, 71, 7, 42E, 187, 83A. It is therefore suggested that a significant relationship exists between contamination of nose and hands with coagulase-positive staphylococci.

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TABLE 6. TYPE DISTRIBUTION OF STAPHYLOCOCCI
FROM THE NOSE

Group	No. of strains	Percentage
Group I:		
29/52/52A/79	10	5.2
80	6	3.1
22A/79	2	1.05
Total Group I	18	9.35
Group II:		
55/71	14	7.3
3A	1	0.5
3C	3	1.5
Total Group II	18	9.3
Group III:		
7/42E	7	3.6
77	7	3.6
53	3	1.5
7/54	3	1.5
6	2	1.05
75	1	0.5
Total Group III	23	11.75
Group IV	3	1.5
Mixed Group		
83A/53/77	3	1.5
3A/7	1	0.5
3C/55/71/75	2	1.05
57/52A/79/80/7	1	0.5
Total Group	7	3.55

Group	No. of strains	Percentage
Type 81	1	0.5
Type 187	2	1.05
Type 83A	3	1.5
Non typable	115	60.5
Total strains	190	

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TABLE 7. TYPE DISTRIBUTION OF STAPHYLOCOCCI FROM HANDS

Group	No. of strains	Percentage
Group I:		
52/52A/79/80	5	3.5
80/29	3	2.1
52	2	1.4
52A	1	0.7
Total Group I	11	7.7
Group II:		
71/3C	12	8.5
3A/3C	1	0.7
55/3A/71	5	3.5
Total Group II	18	12.7
Group III:		
77	3	2.1
6/7/42E/47/53	10	7.0
47/53	1	0.7
7/75	1	0.7
Total Group III	15	10.5
Group IV:	1	0.7
Mixed Group		
29/80/53	1	0.7
6/54/75/83A	1	0.7
55/71/7	1	0.7
6/7/42E/47/53	1	0.7
54/75/187	1	0.7
52/6	1	0.7
52/83A/75/53	2	1.4
42E/6	1	0.7
Total Group	9	6.3
Non Typable	87	61.7
Total Strains	141	

TABLE 8. TYPE DISTRIBUTION OF STAPHYLOCOCCI
FROM THE THROAT

Group	No. of strains	Percentage
Group I:		
80	1	2.7
Group II:		
3A	2	5.5
71	3	8.3
3A/3C	2	5.5
3C/55/71	3	8.3
Total Group II	10	27.6
Group III:		
7	2	5.5
47/54/75/77	1	2.7
77	1	2.7
Total Group III	4	10.9
Group IV	0	0
Mixed Group		
52/53	1	2.7
75/81	1	2.7
Total Group	2	5.4
83 A	3	8.3
Non typable	16	44.4
Total strains	36	

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REPORT OF COMMITTEE ON
FOOD EQUIPMENT

where close tolerances are required on assembled parts and where secure fastening is necessary for safety or proper continuing function, simple tools for disassembly are acceptable provided that all parts which require cleaning shall be accessible by use of the simple tools and the manufacturer's recommended cleaning procedures shall result in thorough cleaning and sanitizing of the equipment.

In addition to this amendment, a few minor changes incorporating, for the most part, previously approved wording or concepts in other Standards/Criteria were also recommended for approval by the Joint Committee.

Standard No. 2—Food service equipment

A review of the requirements of Item 4.10, General Design and Construction, of NSF Standard No. 2, as it related to corrugated transport carts was carried out. After discussion with the manufacturer's representatives and much deliberation, the public health representatives requested that an NSF Standards Task Committee be formed to further study this problem and recommend appropriate revisions in the Standard to delineate the design and construction requirements for this type of equipment.

According to the Foundation staff, the provision of Item 4.312 of NSF Standard No. 2 as it pertained to drain size of small bain maries and steam tables has created unnecessary problems for manufacturers. After a brief discussion, the public health representatives concurred in the concept of permitting drains as small as 1/2 inch for containers holding one gallon or less of liquid.

Standard No. 4—Commercial cooking and warming equipment

Item 4.01 of Standard No. 4 on cleanability of food contact surfaces was amended to read the same as stated under Criteria C-2.

The Foundation staff reviewed the need for clarification of the intent in the coverage of Standard No. 4 as it related to mobile barbecue equipment. After considerable discussion, it was agreed that Standard No. 4 was the appropriate document under which to evaluate such equipment, provided adequate attention was given to the need for protection of the equipment against dust during transportation via streets and highways from the basic commissary to the point of use.

The report of the Standards Task Committee for Fat Filters recommending deletion of requirements for removing particles

USE OF THIOLATED AMINOETHYL CELLULOSE TO REMOVE MERCURY BOUND TO SOLUBILIZED FISH PROTEIN

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Abstract

Aminoethyl cellulose (AEC) was thiolated with S-acetylmercaptosuccinic anhydride. The S-acetyl protective groups were removed at pH 11.5 to yield thiolated aminoethyl cellulose (TAEC) preparations with 0.077 to 0.109 $\times$ 10^{-3} mole SH per g TAEC. TAEC bound approximately 0.1 $\times$ 10^{-3} mole of p-mercuribenzoate/g.

Mercury was removed from fish protein by stirring solubilized tuna fish protein concentrate with TAEC at pH values from 6 to 11. The fish protein was solubilized by either a high temperature, high pH process, or by succinylation. The amount of mercury removed was pH dependent, being maximal at pH values 6.4 and 9. Under reducing conditions, that is, TAEC treated with dithiothreitol, and the mercury removed under nitrogen, 80% removal from a 2% protein solution at pH 7 was achieved. Srafion NMRR, a commercial chelator for dissolved organic and inorganic mercuric salts, was ineffective in removing mercury from soluble fish protein concentrate.

Of all forms of mercury, methylmercury is best absorbed and most slowly excreted by man and animal (3). Swedish investigators (26) reported that mercury in fish tissue from lakes in Sweden and Japan exists almost entirely as methylmercury. Smith et al. (18) reported the same to be true in North American fish. The hazards of mercury have been reviewed extensively by Miller, Berg and co-authors (15) and by Nelson et al. (16). However, Ganther et el. (6) have indicated that the danger of mercury in tuna for humans and other animals may be less than anticipated, since selenium in tuna and, possibly other modifying factors, may reduce methylmercury toxicity.

High levels of mercury in tuna have been reported in museum specimens caught 62 to 93 years ago, and there is little difference in mercury concentration between the museum samples and samples caught recently (14). Thus, the high mercury levels now being found in ocean fish are not the consequence of man-made pollution, but apparently are of natural origin.

Attempts to accelerate excretion of mercury by animals after ingestion or injection have been reported by Takahashi and Hirayama (23), and by Trojanowska et al. (25); however, ideally, the best approach is to prevent ingestion of mercury initially. Recently, Regier (18) used acidified isopropanol to extract mercury from fish protein concentrate. Four extractions with 2.6% concentrated HCl in 99% isopropanol were necessary to remove 93.1% of the mercury. Methylmercury in fish is protein bound, distributed in muscle evenly, and is excreted very slowly (2, 13). A material, such as a sulfhydryl-containing resin, that has a capacity for binding methylmercury could be used to remove mercury from fish protein.

In this paper, we report the capacity of thiolated aminoethyl cellulose to remove mercury from a solution of tuna protein at pH values ranging from 6 to 11. In addition, a resin reported by Law (11) to have a high affinity for both methylmercury and inorganic mercury was tested.

Materials and Methods

Materials

Freeze-dried tuna, which contained levels of mercury > 0.5 ppm, was obtained from Dr. M. L. Sunde of the Department of Poultry Science, University of Wisconsin. Aminoethyl cellulose was purchased from Bio-Rad, whereas S-actylmercaptosuccinic anhydride, 5,5'-dithiobis (2-nitrobenzoic acid), and dithiothreitol were purchased from Calbiochem. Srafion NMRR resin was a product of Ayalon Water Conditioning Company of Haifa, Israel, 2,4,6-trinitrobenzenesulfonic acid was from Eastman Chemical, and p-chloromercuribenzoic acid (Na) was from Nutritional Biochemicals. All other chemicals were reagent grade.

Methods

Thiolation of aminoethyl cellulose. Aminoethyl cellulose (AEC) was thiosuccinylated by the method of Klotz and Heiney (9), using S-acetylmercaptosuccinic anhydride at pH 7.0 in 0.1 M phosphate buffer. A 20-fold molar excess of anhydride over exchange groups on the AEC was employed. Acetyl protective groups were removed by base hydrolysis at pH 11.5 for 45 min. The thiolated aminoethyl cellulose (TAEC) was washed and freeze dried. The sulfhydryl content of the TAEC was determined by the method of Ellman (5).

Mercury binding capacities of AEC and TAEC were determined at pH 7 by stirring a known weight of dry cellulose with a solution of p-chloromercuribenzoic acid, sodium salt (pCMB), in 0.1 M phosphate buffer under nitrogen. At various intervals a 1-ml aliquot was read at 231 nm in a Beckman DU-2 spectrophotometer. The amount of pCMB remaining in solution was determined from a standard curve.

Solubilization of tuna protein. Fish protein concentrate (FPC) was prepared from tuna according to the isopropanol extraction method of Power (17). The FPC was solubilized by either of the two following procedures: I. The high temperature, high pH method described by Tannenbaum et al. (22). II. Succinylation of the FPC with succinic anhydride at

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pH 7.0 (Chen and Richardson, *unpublished*).

In both instances, protein was recovered by precipitation at pH 4.8 (preparation I) and 4.0 (preparation II), respectively, where maximum precipitation occurred. The protein precipitates were washed several times with deionized water and freeze dried. Preparation I was readily soluble at pH 8 or above, and preparation II at pH 6 or above. The approximate degree of succinylation of preparation II was determined by labeling the epsilon amino groups of lysine and N-terminal amino groups with 2,4,6-trinitrobenzenesulfonic acid (TNBS) according to the method of Habeeb (7). The degree of succinylation was approximated from the number of free amino groups in preparation II compared with its total lysine content, determined by amino acid analysis with a Phoenix model M-7800 amino acid analyzer, according to the method of Spackman et al. (20).

Removal of mercury from fish protein. A 1-g sample of preparation I or II was dissolved in 50 ml of either 0.1 M phosphate buffer (pH 6 and 7) or 0.1 M Tris buffer (pH 8, 9, and 11). Then 30 ml of the solution was stirred with 0.5 g TAEC for 2 hr. The level of sulfhydryl groups in the TAEC was in 3,000-fold molar excess over the mercury in the sample. The mixture was centrifuged to remove the TAEC, the protein was precipitated at the appropriate pH, then taken up in deionized water and titrated with 1 N NaOH until completely dissolved. The solution was made up to 25 ml with deionized water, and aliquots were taken for mercury and protein analyses. The remaining 20 ml of the protein solution was used as a control and was treated exactly as the sample, except that it was not stirred with TAEC.

Srafion NMRR resin was also stirred with fish protein solution. Amount of resin used was 2 g/g protein, and the subsequent procedures were the same as those for TAEC.

Determination of mercury. A 5-ml aliquot of fish protein solution was digested as described by the AOAC (1). The digested mixture was diluted to 50 ml with deionized water, and mercury was measured by the method of Uthe et al. (26).

Determination of protein. Freeze dried preparation II, its nitrogen content determined by semi-micro Kjeldahl, was used as a standard to determine the protein content of various fish protein solutions by the method of Lowry et al. (12).

RESULTS AND DISCUSSION

Thiolation of aminoethyl cellulose

Optimum conditions for removal of acetyl protecting groups after thiosuccinylation, and for analysis of sulfhydryl groups on cellulose are given in Fig. 1. It is evident that about 45 min at pH 11.5 is sufficient to yield maximum sulfhydryl content. From curves B and C, the optimum conditions for sulfhydryl analysis were established at 0.08 ml of standard Ellman's solution (5) with a reaction time of 2 hr.

Sulfhydryl contents and mercury binding capacities of TAEC and AEC are reported in Table 1 and Fig. 2. The exchange capacity of AEC was 0.27×10^{-3} mole/g cellulose, and by using a 20-fold molar excess of S-acetylmercaptosuccinic anhydride, we were able to thiolate 30 to 40% of the amino groups. Furthermore, when a 60-fold molar excess of anhydride was used, the degree of thiolation was the same. It is evident from Table 1 and Fig. 2 that TAEC binds pCMB very effectively whereas AEC has a negligible
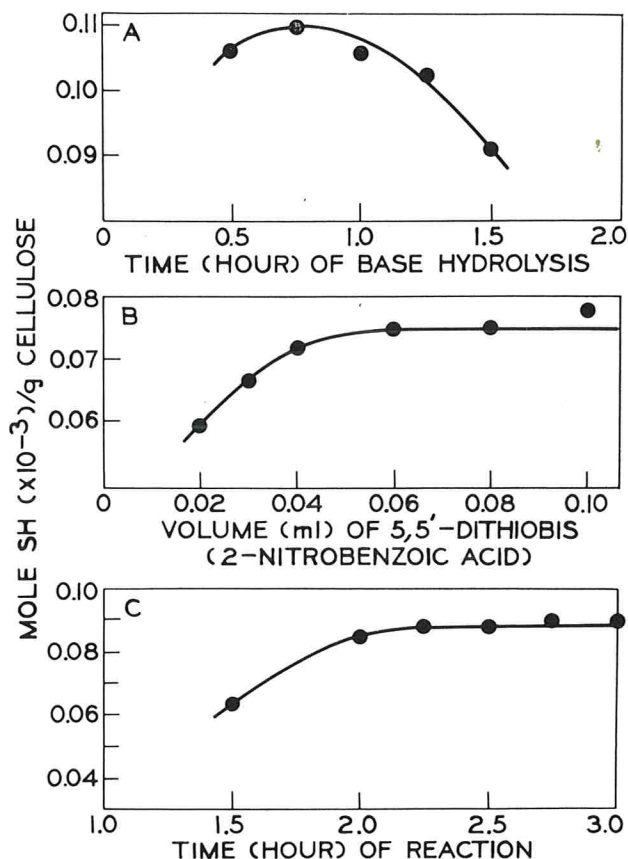
Figure 1. Optimum conditions for preparation and analysis of thiolated aminoethyl cellulose. A. Time of base hydrolysis (pH 11.5) of the acetyl thiosuccinylated aminoethyl cellulose to remove acetyl groups. B. Amount of standard 5,5'-dithiobis (2-nitrobenzoic acid) solution used to determine sulfhydryl groups. C. Time of reaction of TAEC with 5,5'-dithiobis-(2-nitrobenzoic acid) to determine the sulfhydryl contents.

TABLE 1. SULFHYDRYL CONTENT AND MERCURY BINDING CAPACITY OF THIOLATED AMINOETHYL CELLULOSE (TAEC) AND AMINOETHYL CELLULOSE (AEC)

	Mole SH/g cellulose	Mole Hg[1]/g cellulose[2]
TAEC	0.109×10^{-3}	—
	0.077×10^{-3}	0.115×10^{-3}
AEC	0.09×10^{-5}	0.175×10^{-5}

[1] As p-chloromercuribenzoate
[2] Calculated from Fig. 2

affinity for the mercurial. In each instance, the cellulose derivative binds more pCMB than anticipated from its "sulfhydryl" content. Presumably this results from nonspecific binding of pCMB by the cellulose derivatives.

Mercury content of tuna protein

Canned tuna was processed through several steps to yield a soluble protein, and, as shown in Table 2, the protein concentration increased at each stage due to elimination of water, lipid, and other nonprotein
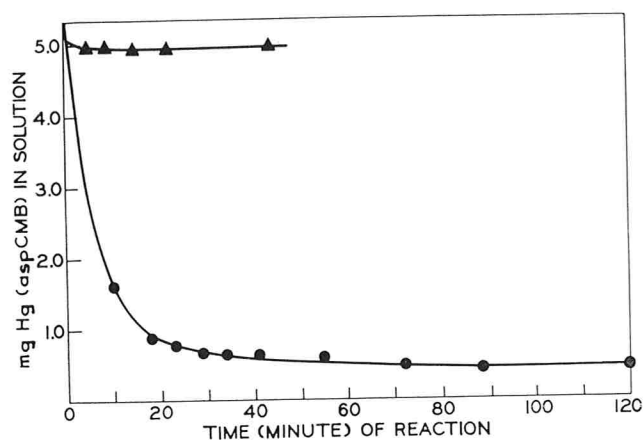
Figure 2. Binding of mercury (as p-chloromercuribenzoate) by thiolated aminoethyl cellulose (closed circles) and aminoethyl cellulose (closed triangles) as a function of time. The amount of cellulose used in each instance was 0.2 g.

substances. The level of mercury remains essentially constant at about 5 ppm based on protein indicating the mercury is associated with the protein. Data in Table 2 indicate there is no significant change in mercury levels due to heating and freeze drying. Westoo (28) reported that frying or boiling of fish did not remove methylmercury.

Removal of mercury from tuna protein

Preparations I and II treated with TAEC revealed a decrease in mercury level, as shown in Table 3. The mean removal varied from 49% to 70% with a high of 78%. It is evident that elimination of mercury from fish was pH dependent, having maxima at about

TABLE 2. PROTEIN AND MERCURY LEVELS OF TUNA AT VARIOUS STAGES OF PROCESSING

	% Protein	ug Hg/g protein
Lyophilized canned tuna	57.12	5.10
Tuna FPC	83.46	5.32
Preparation I[1]	99.30	5.05
Preparation II[2]	98.90	5.05

[1]Protein solubilized by the method of Tannenbaum et al. (22)
[2]Protein solubilized by succinylation

6.4 and 9. In studies using cysteine, penicillamine, glutathione, mercaptoacetic acid and related compounds to remove mercury from hemoglobin, Sugiura et al. (21) reported that there was a linear correlation between percent ethylmercuric chloride removed and dissociation constants of chelator sulfhydryl groups. The dissociation constants of sulfhydryl groups on TAEC and the protein-methyl-mercury complex are not known, but they could be responsible for the slightly increased removal at these two pH values. Further studies on this matter are needed.

With the TNBS reagent, the number of amino groups in preparation II was calculated to be 0.28×10^{-3} mole/g protein, using 1.09×10^4 as the molar extinction coefficient of one trinitrophenylamino group (8). From amino acid analysis, the total lysine content for this preparation was 0.60×10^{-3} mole/g protein. The difference between the above two values should reflect the amount of succinylated lysine. The degree of succinylation thus calculated was 53%. Presumably, most of the N-terminal amino groups were succinylated because of the lower pK of the alpha amino group; however, the uncertainty as to the extent of this reaction renders the above value only an approximation.

Because succinylated preparation II would possess a large net negative charge compared to preparation I, it was thought that the protein molecules might be more "open" as a result of charge repulsion, and protein bound mercury would be removed more easily. But Table 3 shows that mercury removal was similar, indicating that succinylation probably did not have any effect on the removal process.

Sulfhydryl groups on TAEC were quite stable to oxidation when the cellulose was stored in the dry state. In one preparation, the amount of these groups was 0.109×10^{-3} mole/g TAEC immediately after thiolation, and 0.102×10^{-3} mole/g after two months' storage at 4 C as a powder. However, during stirring with the protein solutions, air was incorporated into the solution, and sulfhydryl groups might be oxidized

TABLE 3. REMOVAL OF MERCURY FROM FISH PROTEIN WITH THIOLATED AMINOETHYL CELLULOSE (TAEC) AS A FUNCTION OF pH

	Preparation I		Preparation II	
pH	Untreated	Treated	Untreated	Treated
	(ug Hg/g protein)[1]			
6.0	5.1 ± 0.1	2.6 ± 0.1	---	---
6.4	5.2 ± 0.1	1.8 ± 0.1	---	---
7.4	5.1 ± 0.2	2.3 ± 0.2	5.17 ± 0.05	2.1 ± 0.1
8.0	---	---	---	---
8.4	5.1 ± 0.1	2.1 ± 0.2	5.1 ± 0.2	1.54 ± 0.08
9.0	---	---	5.2 ± 0.1	1.66 ± 0.06
11.2	---	---		

[1]Mean ± S.D. of mean (n = 4)

to perhaps lose mercury binding capacity. This possibility was studied by first treating 0.5 g TAEC (0.077 $\times 10^{-3}$ mole SH/g) with a dithiothreitol solution under nitrogen for 1 hr to maximize the sulfhydryl content of TAEC. Ratio of sulfhydryl groups on TAEC and dithiothreitol was 1:10. The mixture was then centrifuged, and the TAEC was washed several times with deionized water (under nitrogen). The reduced TAEC was stirred with a 2% solution of preparation II at pH 7 for 2 hr, with nitrogen bubbling through the solution during the course of reaction. Mercury removal was 74%, compared with 65% obtained by using 0.5 g untreated TAEC (0.109 $\times 10^{-3}$ mole SH/g) in air.

The effect of protein concentration (preparation II) on mercury removal was also studied. All samples were stirred with reduced TAEC under nitrogen at pH 7. Low protein concentrations showed a slight but insignificant tendency toward higher mercury removal. Protein solutions greater than 4% were difficult to prepare because of solubility limits, and the higher concentrations were quite viscous to work with. Recovery of protein after treatment with TAEC was between 80 and 100%.

Since AEC did not bind mercury in the form of pCMB (Table 1), it was assumed that it also would not remove mercury from fish. This was further demonstrated by the failure of 0.5 g AEC when stirred with 30 ml of 2% preparation I at pH 9 to remove any mercury.

The ability of Srafion NMRR resin to remove mercury from fish was also tested. This product is a chelating resin for noble metals, and, as shown by Law (11), has a high affinity for methylmercury and inorganic mercuric salts. The resin contains positively charged amidine groups on a styrene-divinylbenzene copolymer matrix, and its properties and selectivities were reported by Koster and Schmuckler (10), and Law (11). However, this resin was totally ineffective in removing mercury from preparation I at pH 9, and from preparation II at pH 6. Possibly, the strong positive charges on the resin bound the protein which masked the chelating groups.

Westoo (28) postulated that methylmercury was attached to protein probably in the form of R-S-Hg-CH₃. Sugiura et al. (21) identified a ternary Hb-Hg-cysteine complex by gel filtration as an intermediate in removing mercury from hemoglobin, using sulfhydryl containing reagents, but the mechanism of TAEC in removing mercury from fish protein needs further investigation.

There are several binding sites for methylmercury in fish organs and muscles (16), but these sites have not been identified. It is possible that some sites bind mercury more strongly than others, and mercury will probably bind to those strong sites first, and consequently be removed last. Clarkson and Magos(4) reported that in rat liver and kidney homogenates, two classes of mercury-binding sites were observed, one class having a chemical affinity for mercury 100-fold greater than the other. So the higher the mercury level in fish, the easier it probably will be to remove a large percent of it, only the amount that binds to the stronger sites will be difficult to eliminate. In the present studies, failure of TAEC to remove all mercury from tuna protein may reflect a stronger affinity of mercury for protein, or it may result from steric factors preventing the interaction of bound mercury with TAEC.

The necessity for treating soluble proteins with insoluble TAEC tends to limit the practical application of this system in processing relatively insoluble fish proteins. Consequently, thiolated polymers of this type may be more useful when fed with contaminated fish to prevent absorption of mercury, by poultry for example. Presumably, mercury would be bound to the undigestible thiolated cellulose in the gastrointestinal tract and be excreted with the thiolated cellulose.

Digestion of fish proteins in the intestinal tract tends to obviate any steric factors preventing interaction of bound mercury with TAEC. Thus, TAEC might be more effective in binding mercury in fish protein as part of the feeding regimen. Although the insoluble nature and undigestibility of cellulose derivatives by monogastric animals seems to render TAEC innocuous, the safety of feeding such compounds remains to be determined.

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REPORT OF COMMITTEE ON FOOD EQUIPMENT

of a specified size was presented by the Foundation staff. After considerable discussion, the recommendation of the Task Committee to delete the provisions of Item 4.404 of NSF Standard No. 4, was not approved; thus, the requirements of Item 4.404 will remain as issued. The public health representatives were not satisfied with either the Committee's recommendation or with the specifications to remove paricles 10μ or larger, and they indicated more stringent requirements should be adopted as soon as technology would permit the manufacturing and evaluating of such equipment.

Standard No. 5—Hot water generating equipment

The Foundation staff next presented the problems encountered in evaluating equipment and the reaction of industry relating to elimination of the 40° temperature rise in the listing of hot water generating equipment under the provisions of NSF Standard No. 5. They also noted that a decision was necessary as regards the efficiency ratings to be utilized in determining recovery capabilities of hot water generating equipment. After a brief discussion, the Joint Committee agreed that the 40° temperature rise would be acceptable and that the following efficiency should be utilized in determining recovery rates of such equipment: electric, 100%; and gas, 70%.

Standard No. 7—Food service refrigerators and freezers

The Foundation staff reviewed the activities of the Standards Task Committee for Alternate Methods of Effecting Coves in Walk-ins. According to one member of the Joint Committee, there was no possible method by which coves could be applied in the field and comply with provisions of the Standard requiring smooth continuous radii. The Foundation staff then outlined the numerous existing requirements relative to radii in 2 and 3 plane intersections of interior liners of refrigerators under various NSF Standards No. 1, 2, and 7 which indicated a lack of uniformity of specifications.

After a brief discussion, the Joint Committee recommended that the current Task Committee or another NSF Standards Task Committee be established to review this issue and recommend appropriate revisions in applicable NSF Standards to provide for uniformity; and it was requested that the Task Committee also be instructed to review the feasibility of drains in both walk-in and reach-in refrigerators and to recommend appropriate revisions in Items 4.154 and 5.01.

Standard No. 8—Commercial powered food preparation equipment

Item 4.01 of Standard No. 8 on cleanability of food contact surfaces, which was used as a guide in amending the same item in Basic Criteria C-2 and Standard No. 4, was amended as noted above under C-2.

The proposed extensive revisions in NSF Standard No. 8,

EFFECT OF SELECTED COATING MATERIALS ON THE BACTERIAL PENETRATION OF THE AVIAN EGG SHELL[1]

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Abstract

Whole shell eggs were coated with the following materials: Zein (corn prolamine), Polidene 930-H (polyvinylidene chloride), Epolene Wax E-45 (epolene wax emulsion), and 974-1 (hydrolyzed sugar derivative plus shellac). Surfaces of coated and uncoated eggs were inoculated with *Salmonella typhimurium* and *Pseudomonas fluorescens*. Contents of each egg were then replaced aseptically with a sterile agar medium containing triphenyl tetrazolium chloride. Eggs were sealed, incubated, and examined for shell penetration and growth of *P. fluorescens* and *S. typhimurium*. The ability of the two microorganisms to decompose films of the dry coatings was also tested. All coatings greatly retarded penetration by both microorganisms, although *P. fluorescens* was retarded more than *S. typhimurium*. When incubated for 7 days, heavy suspensions of *P. fluorescens* and *S. typhimurium* did not decompose films of dried coatings. After 48 hr incubation, growth of either organism was not obtained in media containing only dried coating films as added substrates. The dry coatings did not inhibit growth of either organism.

Application of various coatings to whole shell eggs for such purposes as increasing the shell strength and preserving the internal quality has been studied (4, 9). Recently, Meyer and Spencer (8) coated eggs with acrylic resin, casein, polyvinyl acetate, polyvinylidene chloride, prolamine, and epolene wax emulsion. They found that these coatings strengthened the shell, reduced moisture loss, and retarded loss of internal quality and increase in albumen pH.

The rate of bacterial contamination of eggs has been shown to be related to the porosity of the shell (3). The blunt end of the egg, where the shell porosity is maximal, has been shown to be the most vulnerable to infection by *Pseudomonas aeruginosa* (11). Correspondingly, regions of lesser porosity, i.e. the equatorial region and the narrow end of the egg, were found to be less vulnerable to infection. Lifshitz et al. (7) demonstrated that common egg-invading organisms, *Pseudomonas fluorescens*, *Salmonella paratyphi*, and *Alcaligenes bookeri* could pass through the barrier of a clean egg shell with no additional source of nutrients except those found naturally in the cuticle and shell. Reproduction of the microorganisms was demonstrated to be essential for the penetration process. The shell of an egg has been shown to be a less effective barrier to bacterial penetration than the inner shell membrane (6).

Penetration through the shell is the most common way by which salmonella organisms enter and infect the egg, with the exception of *Salmonella pullorum* and *Salmonella gallinarum* which usually enter through ovule infection (1). Solowey et al. (10) in an investigation of the source and mode of entry of salmonella in spray-dried whole egg powder indicated that 16% of dirty eggs and at least 2% of clean eggs contained the organism on the shell and in the pores of the shell.

A method to prevent multiplication on egg shell surfaces and penetration of egg shells by such organisms could be of public health importance. For this reason, this study was undertaken to determine the effect of selected coating materials on the microbial penetration of egg shells.

Methods and Materials

Nest clean, nonfertile eggs were obtained from one strain of White Leghorn hens one day post oviposition. The eggs were candled and those having cracked or checked shells were discarded. The remaining eggs were wiped with a wet cloth and then sanitized by dipping in 70% ethanol and flaming.

Three sets of ten sanitized eggs each were used for the uncoated, uninoculated controls and the uncoated inoculated controls.

Coating of eggs

Triplicate sets of eggs were also coated with each of the following materials: Zein[3] (a prolamine from corn gluten), Polidene 930-H[4] (polyvinylidene chloride), Epolene Wax E-45[5] (a wax emulsion), and 974-1[6] (hydrolyzed sugar derivative plus shellac). Characteristics of the coatings used, with the exception of the 974-1 were described by Meyer and Spencer (8). The material 974-1 consisted of approximately 10% hydrolyzed sugar derivative, 12 to 15% shellac, a small amount of ammonium oleate, and alcohol. This coating was used undiluted as supplied by the manufacturer. Sanitized

Table 1. Percentage of eggs[1] penetrated by *S. typhimurium* and by *P. fluorescens*

Penetration score[2]	Inoculated, uncoated controls				Uninoculated, uncoated controls				Polidene 930 H			
	+++	++	+	0	+++	++	+	0	+++	++	+	0
S. typhimurium	86.7	6.7	6.7	0.0	0.0	0.0	3.3	96.7	7.1	14.2	57.1	21.4
P. fluorescens	46.7	26.7	26.7	0.0	0.0	0.0	3.3	96.7	0.0	13.3	23.3	63.3

Penetration score	Zein				974-1				Epolene wax E-45			
	+++	++	+	0	+++	++	+	0	+++	++	+	0
S. typhimurium	0.0	0.0	16.7	83.3	0.0	3.3	33.3	63.3	6.9	3.4	34.5	55.2
P. fluorescens	0.0	0.0	3.3	96.7	0.0	0.0	0.0	100	0.0	0.0	13.3	86.7

[1]Based on 30 eggs per treatment (3 replicates, 10 eggs each).
[2]+ indicates less than 20 bacterial penetration sites.
++ 20 to 40 bacterial penetration sites.
+++ greater than 40 bacterial penetration sites.

eggs were coated by immersing in the appropriate coating solution, allowing the egg to drain briefly, and then drying by use of forced air. Details of the coating procedure were described by Meyer and Spencer (8).

Inoculation of eggs with test microorganisms

After each coating had thoroughly dried, three separate sets each were inoculated with suspensions of *Salmonella typhimurium* and *Pseudomonas fluorescens*. Organisms were grown 18 to 24 hr on Standard Methods Agar (BBL) slants and harvested by washing the slants with 2-ml quantities of sterile 0.25% saline. Suspensions of each organism were diluted to 100 ml with 0.25% sterile saline and the turbidity determined at 625 mμ using a Bausch and Lomb Spectronic 20 spectrophotometer. The turbidity of each suspension was then adjusted to give approximately 1 to 5 × 10⁷ organisms/ml according to a predetermined relationship between turbidity and resultant number of viable cells. One hundred milliliters of each diluted suspension were added to 900 ml of sterile 0.25% saline in a stainless steel beaker in which a teflon-coated stirring bar was placed underneath a wire gauze egg support. The inoculum was kept at a temperature of 5 to 8 C and continuously stirred by placing on a magnetic stirrer.

Eggs that had been held at room temperature were inoculated for 15 min. The eggs were then removed to a sterile, specially constructed drying board consisting of points of nails driven through plywood spaced 2.54 cm apart.

After eggs were completely dried, five uncoated, inoculated eggs were used to determine the resultant surface population using the blending method described by Gunaratne and Spencer (5). The contents of each egg were discarded and the egg shell, membranes and adhering albumen were blended in 100 ml of sterile 0.1% peptone using a Sorvall Omni-mixer. Serial dilutions were made and plated on Standard Methods Agar (BBL).

Detection of bacterial penetration of coated and uncoated eggs

The remaining coated and uncoated eggs were used to determine the extent of bacterial penetration. A method described by Board and Board (2) was used with the following modifications. Approximately 1 to 2 cm² of the shell at the pointed end of completely dry eggs was removed by first gently cracking the shell with the edge of a sterile knife, then removing shell segments with sterile rat-tooth forceps. The contents of eggs were then aseptically removed using suction. Sterile distilled water was used to rinse the inside shell and membranes and similarly removed. The emptied shells were then filled with a sterile medium similar to that used by Board and Board (2) and of the following composition: gly-

cerol, 0.5% (w/v); yeast extract (Difco), 0.05% (w/v); agar (Difco), 2%; triphenyl tetrazolium chloride (Difco), 0.02%; tap water, pH 7.2, 97.43%.

After the agar had solidified, eggs were inverted and the open end sealed with sterile paraffin wax contained in the depressions of aluminum palette trays. Eggs in trays were then incubated at 25 C for growth of *P. fluorescens* and at 37 C for growth of *S. typhimurium*.

After 24 and 48 hr of incubation, eggs were examined by placing the sealed end of the egg into the aperture of an egg candling lamp. The dark red spots of triphenyl formazan were qualitatively estimated according to the following scale: (+) — indicated the appearance of < 20 spots, (++) — indicated 20 to 40 spots, and (+++) — indicated > 40 spots of triphenyl formazan.

Tests for the microbial degradation of coatings

The ability of the test microorganisms to attack or utilize the coatings as growth substrates was examined by the following two methods. (a) Sterile glass slides were coated with the materials to be tested and allowed to dry thoroughly for 24 hr at 25 C in sterile petri dishes. Sterile filter paper discs (1 cm diameter) were dipped in suspensions of *S. typhimurium* (2.7 × 10⁸ cells/ml) and *P. fluorescens* (1.6 × 10⁷ cells/ml) and placed on each coating material. Coated slides with inoculated discs and the coated slides with non-inoculated discs were placed in desiccators containing 400 ml of 4% H_2SO_4 to give a relative humidity of about 97% (12). The desiccators were sealed and incubated at 25 and 37 C for growth of *P. fluorescens* and *S. typhimurium*, respectively. After 7 days, coated slides were removed and examined for evidence of microbial degradation and growth. (b) Segments of aseptically prepared dried films of coating materials and dry 1 × 10 cm strips of sterile Whatman No. 4 filter paper impregnated with coating materials were added to separate tubes of sterile saline media (0.5% NaCl in triple distilled water) and sterile saline-peptone media [0.5% NaCl, 0.5% peptone (Difco) in triple distilled water]. One-tenth milliliter of each bacterial suspension used in the first method was added to a set of tubes of both types of media plus coatings and incubated at the approximate temperatures. After 48 hr, the tubes were examined for presence or absence of microbial growth and/or inhibition.

RESULTS AND DISCUSSION

Penetration of coated egg shells

The method described by Board and Board (2)

was effective in demonstrating penetration of the egg shell by the microorganisms *P. fluorescens* and *S. typhimurium*. Uncoated inoculated eggs were frequently penetrated, as evidenced by the numerous spots of triphenyl formazan, which resulted from the reduction of triphenyl tetrazolium chloride by the actively growing microorganisms. This penetration was prevented or reduced to a great extent by the application of the various coatings studied.

Microbial growth within the agar was not observed in any of the eggs. Deposition of triphenyl formazan was limited to the inner surface of the shell and the shell membranes, indicating that the actively growing organisms had progressed only this far within the 48 hr incubation period.

The percentage of eggs which were penetrated by *S. typhimurium* and *P. fluorescens* is presented in Table 1. All of the inoculated uncoated controls were penetrated by both organisms. Only 3.3% of the uninoculated uncoated control eggs were penetrated by organisms present on the egg shell.

The average shell population of the uncoated eggs was found to be 1.1×10^7 microorganisms per egg when inoculated with *S. typhimurium* and 2.6×10^7 when inoculated with *P. fluorescens*.

Zein was the most effective coating against the penetration of *S. typhimurium*, preventing penetration in 83.3% of the eggs. Coatings 974-1 and Epolene wax prevented penetration in 63.3% and 55.2% of the eggs, respectively, while Polidene 930-H prevented penetration of *S. typhimurium* in only 21.4% of the eggs inoculated. When viewed with transmitted light, the shell of eggs coated with Polidene 930-H had a mottled appearance, indicating the coating may not have covered the shell surface evenly.

All coatings were more effective in preventing penetration of *P. fluorescens* than *S. typhimurium*, even though the inoculum level was of approximately the same order. Coating material 974-1 prevented penetration by *P. fluorescens* in 100% of the eggs. The success of this coating was unexpected because during the inoculation process the inoculum acquired a slight oily appearance which seemed to indicate that part of the coating had been removed. It is possible that the shellac present in the coating remained in the pores of the egg, preventing the bacterial penetration.

Zein prevented penetration in 96.7% of the eggs inoculated with *P. fluorescens* while Epolene wax and Polidene 930-H prevented penetration in 86.7% and 63.3%, respectively.

Microbial degradation of coating materials

Sterile filter paper discs dipped in the two test microorganisms did not result in any growth of the microbes when placed on the slides coated with the various coating materials, nor was any decomposition of the coating materials detected either microscopically or macroscopically after incubation for 7 days at 97% relative humidity.

Further evidence for the resistance of the coating materials to microbial decomposition was obtained in the tests where the dried coating films or coatings applied to filter paper were incubated in liquid media. No growth of either *P. fluorescens* or *S. typhimurium* was obtained in saline containing only the coating materials as the substrates. Heavy growth of both organisms was obtained in the saline + peptone media; however, no decomposition of the coatings was apparent. Strips of the dried coating 974-1 dissolved in the liquid media, causing it to be slightly milky in appearance. Additional turbidity from microbial growth in the saline was not evident when comparisons were made with the uninoculated control tubes of saline and the coating material.

Some inhibitory effect on the growth of *P. fluorescens*, *Salmonella derby*, and *Aerobacter aerogenes* has been shown by the application of wet filter paper discs soaked in the coating solutions to inoculated agar plates *(8)*. In their tests, Meyer and Spencer showed that Epolene wax inhibited growth of all three microorganisms, Polidene 930-H inhibited the growth of *P. fluorescens*, and Zein had no inhibitory effect. It is possible, therefore, that application of certain coating materials would have a sanitizing effect or at least prevent further growth of the microbes present. Inhibitory properties of the coatings tested in this study were less than those observed by Meyer and Spencer *(8)*.

In the event that any one of these coatings might be used commercially to increase egg shell strength, they would have the added advantage of reducing bacterial penetration through the egg shell. For maximum resistance to bacterial penetration, it appears that the coatings Zein and 974-1 would be the best choices. Although shelf-life studies of coated inoculated intact eggs were not done, from data obtained in these experiments, it seems reasonable to hypothesize that coating of shell eggs would markedly increase their shelf-life.

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REPORT OF COMMITTEE ON FOOD EQUIPMENT
(Continued from Page 271)

Commercial Powered Food Preparation Equipment, were reviewed by the Joint Committee. Following completion of the review, the proposed amendments, as revised, were recommended for adoption. Copies of this and all other Standards or Criteria are available from the National Sanitation Foundation, NSF Building, Ann Arbor, Michigan, 48105.

Standard No. 12—Automatic ice making equipment

Following the 3-year review of the proposed revisions in NSF Standard No. 12, Automatic Ice-Making Equipment, the amendments, as revised, were recommended for adoption. During the course of reviewing the Standard, the need for further study of Item 4.302 relating to the cleanability of cold plates including their appurtenances and the containers for the storage of edible ice where such plates are installed in the edible ice compartment.

Standard No. 29—Detergent and chemical feeders for commercial spray-type dishwashing machines

The current provisions of NSF Standard No. 29, Detergent and Chemical Feeders for Commercial Spray-Type Dishwashing Machines, were discussed as they related to other than detergent feeders. The Joint Committee agreed that it was its intent to include performance requirements for all types of chemical feeders. To this end, the Foundation staff was requested to prepare a proposed revision in the Standard to provide such requirements.

Covers and doors over the food zone (Applicable to almost all standards)

The following specifications for covers and doors over the food zone have been approved by the Joint Committee following a 2-year study to provide a feasible plan and uniformity between the applicable Standards:

(a) Where under anticipated use condition, surfaces adjacent to top openings to food zone may permit contaminated drainage to enter the food zone, such openings shall be protected by a raised rim which shall extend at least 3/16 inch above the level to which liquids may accumulate.

(b) Where under anticipated use conditions, top openings to food zones are required to be covered to protect against the entrance of contaminants. Such openings shall be covered to effectively preclude the entrance of contamination. Covers shall meet the following requirements: *(i)* The cover or door shall be provided with a flange which overlaps the opening and shall be designed to prevent spillage or other foreign materials from entering the food zone in the closed position or when being opened. *(ii)* The cover or door shall be sloped to provide drainage from the door or cover surface. *(iii)* Doors and covers shall be designed with sufficient clearance to avoid contact with the foods which they cover. *(iv)* Hinges or pivots shall be designed to be easily cleanable and of simple, take-apart design and construction. Piano hinges are not acceptable in the food zone. Covers shall be readily removable or easily cleanable in place.

When under anticipated use condition, top openings to food zones do not require protective covers or where the cover is provided as a functional part of the equipment such as covers for pressurized vessels, steam retaining enclosures, as exampled by bun warmers, or in units where a cover is provided but not required for protection against contamination, the cover or door need not comply with the requirements of Item 2(a) through 2(c). The covers, however, should comply with Item 2(d). Covers designed to permit stacking of units need not comply with Item 2(b).

Future plans

The scheduled 3-year review of Standards Nos. 1, 2, and 20 should be ready for study and comment by this Committee before 1973. In addition, plans for Standards for cart washers, retail food store refrigeration equipment, high pressure spray cleaning equipment, carpeting for food service areas, compactors, and beverage cases are being considered.

Furthermore, the Foundation staff reported that a number of comments had been received suggesting that the Foundation include in its Standards appropriate references to the metric system. Following a brief discussion, the Joint Committee agreed that the Foundation Standards should include dual references to the English and metric systems.

THE WATER RELATIONS OF FOOD-BORNE BACTERIAL PATHOGENS. A REVIEW

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ABSTRACT

Water limitation techniques can be useful tools to preserve food materials not only from autodecomposition, but also from microbial decomposition. Besides those organisms that might render a food unfit to eat because they can produce adverse compositional and textural changes, food-borne bacterial pathogens are also influenced by the amount of available water. This review considers several major food-borne bacterial pathogens and the effect of water limitation on various aspects of their growth. Wherever possible, references have been included which pertain to the influence of water limitation on these organisms in foods. The data reviewed indicate that food-borne bacterial pathogens in general can grow at water activity levels of 0.83 to 0.999. The implications of this wide a_w range for prevention of growth of food pathogens and thus, the safety of foods, are discussed.

Water content and condition affect foods in many ways. Enzymatic breakdown, textural and flavor anomalies, and decomposition as a result of the metabolic activities of microorganisms can result when the moisture content of a given food is not properly controlled. Besides spoilage, water influences growth, survival, and/or toxin production by food-borne pathogens which find their way into many foods. Thus, enlightened knowledge pertaining to methods which result in the limitation of moisture and the effect that this limitation has on food-borne pathogens are powerful techniques in the food scientist's armamentarium for controlling these pathogens.

Although the early studies of DeFreytag (19), Petterson (63), and Tanner and Evans (80) laid much of the groundwork for ensuing studies on how water affects microorganisms, it was not until Scott and his co-workers (71) at the C.I.R.S.O. Laboratories in Australia began their investigations on the moisture requirements of microorganisms that much of our present knowledge began to accumulate. This work clearly established the usefulness of the concept of water activity as a means of quantifying moisture content and availability. For the reader who wishes to provide himself with basic information on the study of the water requirements of microorganisms, Scott's excellent review (73) is recommended. An additional series of papers by Wodzinski and Frazier (85-87), while not dealing directly with food-borne pathogens, further extended the concept of water activity, or a_w, and its relationship to microbial growth.

The present review has by choice been limited to the moisture requirements of food-borne, bacterial pathogens. In many instances the authors of published articles have utilized the term "percent" to quantify a particular solute employed to limit water. The reader should be cautioned against referring to published tables relating solute concentrations to water activity to obtain the more broadly applicable a_w term. Almost invariably the particular solute is in a medium or food, the components of which may independently contribute to the amount of water available to microorganisms.

It is hoped that the present review, while focusing on previous work will emphasize, to at least some degree, the necessity for additional studies on this important subject.

CLOSTRIDIUM BOTULINUM

The effect of water activity on spore germination, growth, and toxin formation by *Clostridium botulinum* is of critical importance in many food systems. In many instances, water relations have been studied in conjunction with other growth conditions such as pH or temperature. Because this organism causes one of the most serious of the food-borne illnesses, at least from the standpoint of its high mortality, it is extremely important that food microbiologists consider all factors which impinge on its growth and toxin formation not the least of which is water activity.

Spore germination

Baird-Parker and Freame (2) found that the minimal a_w for spore germination varied with the solute chosen for a_w adjustment. Types A, B, and E spores germinated at a minimal a_w of 0.93 if NaCl was the adjusting solute, however, if glycerol was used, the minimal a_w for germination was 0.89. Halvorson (30) tested the spores of several *C. botulinum* strains to determine the effect of various salts on their germination. Concentrations of 9% NaCl, KCl, or $MgCl_2$ were required to prevent germination, whereas as much as 15% Na_2SO_4 and $MgSO_4$ had

no effect on germination. These results are probably more consistent than they appear because, extrapolating from the data of Ingram (36), a 15% solution of Na_2SO_4 possesses a higher a_w than 9% NaCl or KCl solutions. Others (54) have observed spore germination at 8% NaCl without further growth. Pedersen (62) tested the effect of salt-containing media and curing brines on spore germination and survival of Type A, B, and E strains. Spore germination of the Type A strain was prevented at 7.4% NaCl in a broth medium, the Type B strain was inhibited at a salt concentration of 6.4%, and the two Type E strains were inhibited at only 4.1%. Spores of all strains tested survived storage for at least 21 days in curing brines containing from 14.8 to 22.4% NaCl.

Yesair and Cameron (88) examined the effect of various salts, alone and in combination, on germination of C. botulinum 62-A. When only NaCl was tested, 5.0% of this salt produced 100% inhibition of germination, however, complete inhibition was also obtained with 3.5% NaCl plus 0.188% $NaNO_3$. A similar amount of NaCl plus only 0.0156% $NaNO_2$ produced 99.6% inhibition of germination.

Spore outgrowth

Glycerol, when used to adjust the a_w of experimental media, appears to be less inhibitory than NaCl to spore outgrowth as well as to germination (2). This effect was particularly apparent with the Type E strain which formed vegetative cells at a minimal a_w of 0.94 in a glycerol-adjusted medium and at a minimal a_w of 0.97 in a medium adjusted with NaCl. Both germination and vegetative cell outgrowth occurred at lower a_w levels with a 30 C rather than 20 C incubation temperature. Schmidt and Segner (70) reported 3- to 4-fold extensions of outgrowth time of four C. botulinum Type E strains as the salt concentration of a trypticase, peptone, glucose medium was increased to 4.0% at temperatures of 46 and 50 F. Because no outgrowth time extension was noted at 85 F, these authors suggested that normal salt concentrations of refrigerated, cured meats might provide some protection against outgrowth of Type E spores. Observing spore outgrowth of several Type E strains over extended incubation times, Segner et al. (74) found that 5% NaCl was required to inhibit outgrowth at 16, 21, and 30 C, whereas significant inhibition at lower temperatures occurred in the presence of 4.5% NaCl. These results confirm those reported earlier by Schmidt and Segner (70) that salt concentrations that do not appreciably inhibit outgrowth at optimal temperatures can extend outgrowth at lower temperatures. These results further suggest that Type E strains are significantly more sensitive to

NaCl than Type A or B strains. Pedersen (62) noted that spore outgrowth was more sensitive to elevated salt concentrations than vegetative cell growth at optimal temperatures. This author also reported some differences in response to NaCl between various C. botulinum types.

Growth

Five solutes, sodium formate, sodium chloride, potassium chloride, sucrose, and glucose, were used by Emodi and Lechowich (21) to study the effect of water limitation on the growth of C. botulinum. Minimal a_w levels supporting growth of the four Type E test strains were 0.980 to 0.977 with sucrose, 0.972 to 0.970 with glucose, 0.976 to 0.971 with sodium formate, and 0.978 to 0,976 with KCl and NaCl. The inhibitory a_w for growth appeared to be in good agreement with the 0.97 minimal a_w for growth reported by Baird-Parker and Freame (2) using NaCl as a solute. Emodi and Lechowich also examined the possibility that ionic and osmotic effects could be responsible for the observed inhibition at high solute concentration. They concluded that ionic effects were probably not important in inhibiting growth although inhibition as a function of osmotic phenomena could not be discounted. Marshall et al. (48) also supported the finding that growth of C. botulinum occurred at a slightly lower a_w level (0.93) when glycerol was the solute as compared to NaCl (0.94). Unfortunately, the report did not mention the type strain used in these studies.

Growth rates of four Type E strains at various a_w levels were determined by Ohye et al. (60) who showed that maximal rates occurred at a_w 0.995. No significant differences in growth response were reported between the two solute systems used to reduce a_w, KCl and a mixture of NaCl:KCl:Na_2SO_4. It was further reported that a 15 C reduction in temperature greatly increased the water requirements for both growth and toxin formation. Minimal a_w levels reported by Ohye et al. (59) as supporting growth at optimal temperature and pH were 0.95 (5.1% NaCl) for the two Type E strains tested. Sensitivity to a_w was increased as measured by increase in lag time when pH levels were reduced from pH 7.0 to 6.0. These authors concluded that 10% NaCl (a_w = 0.938) which is reported to inhibit all three types of C. botulinum, may produce marginal inhibition at optimal pH and temperature levels, however, in foods in which some acidification is present, an ample safety margin would exist.

Baird-Parker and Freame (2) found significant solute differences when the minimum a_w permitting growth of Types A, B, and E C. botulinum were determined. Glycerol allowed growth at much lower

a_w levels than did NaCl. Like Segner et al. (74) these workers noted that the Type E strain appeared to be much more sensitive to water limitation than either the Type A or B strains.

Heat resistance

The heat resistance of spores of several bacterial species in heating menstruums with various water activity values was determined by Murrell and Scott (55). Spores of *C. botulinum* Type E were equilibrated with atmospheres of specific relative humidities at 25 C and heated. Spores equilibrated to an a_w of 0.8 to 0.9 before heating had the greatest degree of heat resistance. This work suffered from an absence of adequate water activity control during heating, a deficiency which was rectified to some extent in a second report by Murrell and Scott (56) by heating equilibrated spores in solutions of controlled water activity and by calculating the actual a_w in the heating ampules at various temperatures from existing tables. The heat resistance of the spores of six bacterial species were tested, including *C. botulinum* Types B and E. A linear relationship was observed between the log of the number of viable spores and the time of heating, with greatest heat resistance occurring at a_w levels in the range of 0.2-0.4. When spore suspensions were heated (110 C) at a_w levels in excess of 0.4, heat resistance decreased as a direct function of a_w increase. The Type E strain tested produced a Q_{10} value of 10 at high a_w values decreasing to 2 or less at a_w levels below 0.3. Of the six organisms tested, *C. botulinum* Type E spores appeared to be the least heat resistant whereas Type B spores were among the most heat resistant. Other species studied were *Bacillus subtilis* var. *niger*, *Bacillus coagulans*, *Bacillus megaterium*, and *Bacillus stearothermophilus*.

The effect of salts (3.5% NaCl and/or 0.2% NaNO₃) on the thermal resistance of *C. botulinum* No. 62-A was reported by Yesair and Cameron (88). No appreciable effect could be produced by the added salts within a heating range of 230-235 F, however, at temperatures < 230 F, NaCl alone, NaNO₃ alone, and NaCl plus NaNO₃ decreased heat resistance.

Most of the studies concerning heat resistance of microorganisms in liquid systems suffer from difficulty in maintaining water vapor equilibrium during rapid heating and cooling. In addition, as pointed out by Murrell and Scott (56), the a_w of the heating menstruums and of the spores themselves will be altered as the temperature is changed and thus erroneous results can be obtained unless accurate compensations are made.

Toxin formation

Tanner and Evans (80) investigated the effect of meat curing solutions on growth and toxin formation by *C. botulinum* Types A and B. All of the *C. botulinum* strains tested failed to produce toxin in a glucose broth containing 8.720% NaCl, however, 10.446% NaCl prevented toxin formation in pork infusion medium, whereas lower salt levels did not inhibit toxin formation. These authors also found that the test strains varied somewhat in their reaction to NaCl. Surprisingly, they also found that toxin formation occurred at NaCl concentrations in excess of those permitting growth. This was especially true with one Type B strain in which growth was inhibited at 8.619% NaCl, whereas 10.446% NaCl inhibited toxin production. Riemann (66) found that Type E toxin formation did not occur at NaCl concentrations in excess of 2% whereas spore outgrowth occurred at a NaCl concentration of 4.5%.

Ohye and Christian (59) found that minimal a_w levels for growth (0.95 Type A, 0.94 Type B, and .97 Type E) were identical with minimal a_w levels for toxin formation. Toxin production was not observed to occur in the absence of growth. It appears from these studies (59, 66) that growth and toxin formation by Type E strains are more sensitive to a_w reductions than are Type A and B strains.

C. botulinum in foods

All studies relating to water requirements of *C. botulinum* in foods have pertained to meats of various types. Scott (72) investigated the effect of curing ingredients on the growth of *C. botulinum* and concluded that none of the curing factors; pH, nitrate, nitrite, and NaCl, when used at levels normally found in foods, alone would prevent growth, however, it was possible that combinations of factors could produce control. In an investigation on the possibility that *C. botulinum* Types A, B, and E could produce toxins in cured meats which remained organoleptically acceptable, Pivnick and Barnett (64) noted that increasing concentrations of salt and nitrate inhibited toxin production by the Type A and Type B strains. Type A and B toxin production occurred in inoculated, cooked hams over an NaCl range of 2.1 to 3.4%; however, it was somewhat delayed at the highest NaCl concentration tested, 3.4%, especially if the ham was incubated at 20 C rather than 25 or 30 C. Type E toxin production in a different meat preparation did not occur at a salt concentration of 3.3% and was almost completely inhibited at 2.7%. It would thus appear that salt plays a part in protecting cured meats from toxin production by *C. botulinum* and that, as noted previously, Type E toxin formation is somewhat more sensitive to this solute than the formation of either Type A or B toxin. Greenberg et al. (29) investigated the amount of NaCl required to

TABLE 1. SUMMARY OF MAXIMAL NaCl CONCENTRATIONS AND MINIMAL A_w LEVELS PREVENTING SPORE GERMINATION, OUT-GROWTH, AND TOXIN FORMATION BY *C. botulinum* IN LABORATORY MEDIA

| | *C. botulinum* type | | | | | |
| | A | | B | | E | |
	a_w	% NaCl	a_w	% NaCl	a_w	% NaCl
Germination	.93[a] (2) .89[b] (2)	7.4 (62)	.93[a] (2) .89[b] (2)	6.4 (62)	.93[a] (2) .89[b] (2)	4.1 (62)
Spore outgrowth	—	6.5 (2)	.91[b] (2)	6.5 (2)	.97[a] (2) .94[b] (2)	5.1 (59)
Growth	.95[a] (59) .93[b] (2)	8.0 (59)	.94[a] (59) .93[b] (2)	9.4 (59)	.97[a] (59) .95[b] (2)	5.1 (59)
Toxin formation	.95[a] (59)	10.5 (80)	.94[a] (59)	10.5 (80)	.97[a] (59)	4.4 (7)

— No information.
Superscript letters refer to specific solute used to adjust a_w; a = NaCl, b = glycerol.
Numbers in parentheses refer to listed references.

prevent toxin formation in cured meat inoculated with mixed (Types A and B), heat-shocked spore suspensions of *C. botulinum*. These workers showed that significant numbers of samples (14 of 104 studied) were toxic when the meat was cured with 7.12% brine whereas none of 103 samples prepared with 8.95% brine were toxic. These authors attributed the absence of a history of botulism in canned hams to the very low levels of anaerobes in this product and to the fact that product mishandling results in obvious organoleptic unacceptability. They did not believe that the 5-6% brine level, normally used to cure hams, was sufficient to prevent the growth of *C. botulinum;* however, no mention was made of the effect of other curing brine components, such as nitrates and nitrites, acting in combination with NaCl, on toxin production. Williams and Purnell *(84)* investigated the relationship between moisture and spore germination, sporulation, and growth of a Type A toxin-producing strain of *C. botulinum* in a liver paste medium. These authors showed that at an equilibrium relative humidity (E.R.H.) > 96%, rapid growth and sporulation occurred. Although growth occurred within an E.R.H. range of 96 to 98%, sporulation appeared to be diminished and at 94 to 96% the rates of growth and sporulation were sharply reduced. Germination reportedly occurred at < 90%, however, the basis for determining germination, a decrease in spore counts, was somewhat uncertain.

Despite the intense interest during the period 1965 to 1970 in the factors affecting growth of *C. botulinum* Type E in fish and fish products, relatively little published information exists on the effect of water activity on growth and toxin production of Type E strains in foods of this type. Angelotti *(1)* suggested that processing of smoked fish could produce a reduction in a_w with the result that increased heat resistance of the spores could occur. If this were true, it was reasoned that the a_w of the fish could be the principal factor influencing spore survival. Another report by Boyd and Southcott *(7)*

was concerned with the effect of NaCl concentration on growth and Type E toxin production by three strains of *C. botulinum*. Toxin production, but not growth, of one strain was inhibited at 3.76% NaCl and of a second strain at 3.84%. The third strain studied produced toxin at 4.40% NaCl. A salt concentration of 5.05% appeared to be the maximal level at which spore germination and outgrowth would occur. These data agree with those of Sheneman *(76)* who found that smoked whitefish chunks supported Type E toxin production at a brine content of 3% but not at 5.5%.

In summary, the a_w levels required to inhibit spore germination and outgrowth of a given *C. botulinum* type are similar and lower than those required for growth and toxin formation. The Type E strains appear to be significantly more sensitive to water limitation than either Type A or Type B strains. These relationships are illustrated in Table 1. Lechowich *(45)* has briefly reviewed many of these effects. It should be emphasized that strong differences between various solutes and their effect on growth parameters appear to exist and, in addition, interactions between temperature, pH, and type of medium or food systems can also greatly affect the response to water limitation.

STAPHYLOCOCCUS AUREUS

The staphylococci probably represent the most salt-tolerant group of the food-borne bacterial pathogens. Microbiologists have recognized and exploited this property to develop media selective for staphylococci. Hill and White *(32)* in 1929 noted that gram-positive cocci could grow in the presence of high NaCl concentrations and suggested that media containing NaCl be utilized to select for these organisms. Koch *(42)* later incorporated 7.5% NaCl in an agar medium for the purpose of isolating staphylococci. Chapman *(9)* also utilized 7.5% NaCl, adding this solute to agar containing phenol red and mannitol, a medium which continues in use today.

Growth

Scott *(71)* determined the optimal a_w level for growth rate and maximal cell yield in a medium containing various nitrogen sources plus yeast extract. Adjustment of this medium to various a_w levels was achieved with a mixture of NaCl, KCl, and Na_2SO_4. The fourteen *S. aureus* strains tested appeared to produce maximal growth at $a_w = 0.995$ to 0.99. Both rate and cell yield growth declined as a function of a_w and the minimal a_w at which growth occurred was found to be 0.86. Scott further concluded that the range of water activities permitting *S. aureus* growth was relatively independent of the type of solutes in the medium. These findings were in disagreement with those of Marshall et al. *(48)* who compared two solutes, glycerol and NaCl, for their ability to inhibit various species of bacteria. They found that inhibition of staphylococcal growth rates at comparable a_w levels was 10% greater in glycerol than in NaCl.

Iandolo et al. *(35)* studied the effect of salt on lag time and growth rate of *S. aureus* MF-31 and found that lag times were significantly extended as the NaCl concentration was increased from 0.5% to 4% and 8%. A similar response in growth rates, however, could not be demonstrated and in fact, growth rates were more rapid at 4.0% than either 0.5 or 8.0%. Maximal populations also were somewhat reduced as the NaCl concentration of the medium was increased; these effects were amplified by incubation temperatures and pH levels above and below optimal levels.

Parventjev and Catelli *(61)* found that introduction of 10% NaCl in Tryptose Phosphate broth failed to halt growth of either pathogenic or nonpathogenic strains of *S. aureus*. In contrast, solutions containing only NaCl were very injurious to the staphylococci; however, this inhibition could be reversed by addition of .03 $\mu g/ml$ of the above medium. Growth rates of *S. aureus* C-243 at various water activities were measured by Troller *(82)* who found that reductions in a_w from 0.99 to 0.89 or 0.90 increased generation times from approximately 30 min to > 300 min. Maximal counts of staphylococci were decreased and lag times were extended as the a_w levels of the media were reduced.

Enterotoxin production

Much of the work on the influence of water activity on enterotoxin production is relatively current, reflecting the recent development of sensitive immunological techniques for quantitatively estimating enterotoxins. Genigeorgis and Sadler *(22)* found that enterotoxin B could be detected in Brain Heart Infusion (BHI) broth at pH 6.9 containing as high as 10% NaCl or at pH 5.1 in a medium containing 4% NaCl.

These authors concluded that enterotoxin production was dependent on the interaction of pH and salt.

Hojvat and Jackson *(33)* found that enterotoxin B production and staphylococcal growth were inhibited as the NaCl concentration in BHI broth was increased from 0 to 12%. They further concluded that the effect of NaCl was more marked on enterotoxin production than on staphylococcal growth. The effect of curing solutions on enterotoxin B production was also studied by McLean et al. *(50)* who similarly noted that increases in NaCl concentration from 0 to 10% reduced toxin levels more rapidly than growth as measured by culture turbidity. The concentrations producing most rapid enterotoxin diminution were between 2 and 4% NaCl. Troller *(82)* related growth and enterotoxin B production to a_w and found that very slight reductions in water activity extensively reduced the amount of toxin produced. Similar studies by Troller *(83)* utilizing an enterotoxin A-producing strain of *S. aureus*, indicated that this effect was not as pronounced with this organism. Markus and Silverman *(47)* similarly noted a reduction in enterotoxin A production with an increase in NaCl content from 0 to 10% in a basal medium containing N-Z Amine A, a casein hydrolysate, and yeast extract. However, when toxin production was related to growth (toxin/Klett units), this parameter remained constant with increasing salt concentration.

The influence of solutes in skim milk on the thermal resistance of *S. aureus* was studied by Kadan et al. *(37)* who demonstrated that sucrose concentrations to 14% decreased killing time at 60 C. However, if a greater amount of sugar was employed (27 to 57%), a protective effect was observed. Calhoun and Frazier *(8)* studied the thermal resistance of *S. aureus* 196E previously cultured in media adjusted to a_w 0.95 with glucose or NaCl. Previous growth at the reduced a_w level had a minimal effect on heat resistance. However, if the a_w of the heating menstruum was lowered to 0.95, a solute specific effect was noted with NaCl producing a protective effect. The heating menstruum adjusted to a_w 0.95 with glucose did not demonstrate this effect and in fact was somewhat more sensitive to thermal destruction than the phosphate-buffered control (a_w 0.994).

Influence of pH and other factors on a_w effects

In addition to the effects of water limitation on *S. aureus*, a number of additional reports exist pertaining to a_w reduction in combination with pH reduction to inhibit growth and toxin production of staphylococci. Much of this work has been motivated by the desire to understand the combined effect of NaCl, pH, and/or nitrates in meat curing brines.

Hucker and Haynes (34) speculated that a reduction in viable counts of toxin-producing "micrococci" following prolonged incubation was the result of combined action of high sucrose concentrations added to the medium to limit water availability and to the development by the organism of an acid from the added sucrose. Nunheimer and Fabian (58) investigated the effect of mixtures of acids with sucrose or NaCl solutions on six S. aureus strains. Only 5% NaCl plus 0.15 millequivalents of HCl was required to achieve a 50% reduction in growth, however, 11.5% NaCl was required to produce a similar growth reduction with 0.4 millequivalents of citric acid. Based on these results, these workers concluded that organic acids were less effective when combined with NaCl than inorganic acids.

Genigeorgis et al. (24) studied production of enterotoxin C in laboratory media containing from 0 to 12% NaCl and adjusted to various initial pH levels. They found that the initial pH of the medium that allowed toxin production narrowed as the NaCl concentration was increased. In a medium containing 10% NaCl, enterotoxin C was produced in a pH range of 5.45 to 7.30 with a large inoculum. However, a smaller inoculum in a similar medium produced enterotoxin in a pH range of 6.38 to 7.30. No enterotoxin C could be detected in media containing 12% NaCl in the pH ranges 4.50 to 8.55 despite active growth as demonstrated by optical density increases in inoculated media.

McLean et al. (50) investigated the effects of combinations of low levels of NaNO₃ plus 2% NaCl and noted a reduction in the amount of enterotoxin B produced by S. aureus 243, however, it was doubtful if the effect was more than additive. It should also be noted that the low concentration of NaCl used in this portion of the study probably had little effect on the amount of available water in the medium.

Water activity effects in foods

Investigations on the limitation of staphylococcal growth and enterotoxin production in foods are, with few exceptions, limited to studies in which meats or meat curing solutions were studied. This is primarily caused by the importance of curing brines as a means to adjust the water activity levels of hams and other preserved meats, and to the high incidence of meats implicated in food poisoning outbreaks. Kelly and Dack (39) studied the influence of salted meats, i.e. ham, beef, tongue, and chicken, on the growth of a *Staphylococcus* isolated from sandwiches involved in a food poisoning outbreak. They found that the test strain multiplied at NaCl concentrations as high as 10% and that salt was highly selective for the staphylococci in the test foods.

Lechowich et al. (44) investigated the effect of curing salts on the growth of four enterotoxin-producing strains of S. aureus. Curing solutions containing 5% NaCl, 1.5% glucose, 0.25% NaNO₃ and varying, but low levels of NaNO₂ were inoculated and pumped into hams. Incubation was at 40 F. The counts of inoculated staphylococci declined rapidly in these hams and were undetectable after 72 hr of incubation. Supplementation of curing pickles with meat juices, however, was protective and appreciable counts of staphylococci were detected after 7 days of incubation under these conditions. Genigeorgis et al. (22) showed that enterotoxin production could occur in hams cured in brines containing as much as 9.2% NaCl, a value 2 to 5 times higher than found in commercially cured meats. Thatcher (81) noted enterotoxin production in inoculated Canadian bacon containing from 0.3 to 3.2% NaCl. Eddy and Ingram (20) have reported the presence of S. aureus in bacon and its curing brines and there is a recent report (18) of a staphylococcal food poisoning outbreak resulting from ingestion of fried bacon. Working with two intermediate moisture foods, one meat-based and the other fruit-based, Labuza et al. (43) found that the number of inoculated staphylococci increased in the meat-based food ($a_w = 0.84$) from initial counts of 8.6×10^3 per gram to 5.2×10^9/g after 1 month of storage at 25 C. This a_w level is somewhat lower than the minimal a_w for staphylococcal growth, 0.86, reported by others (70).

Minimal water activities for growth of S. aureus 49/174 in dried milk, dried mutton, and a dried soup mixture were examined by Scott (71). In each instance, the food was rehydrated to specific a_w levels and incubated at 30 C for 30 days. In all of the foods at a_w 0.88, slow growth occurred, however, at a_w 0.86 only the soup mix and milk supported staphylococcal growth. In foods adjusted to a_w 0.84, growth could not be detected. Growth of an S. aureus strain on the top and cut surfaces of custard pies was studied by Preonis et al. (65). These workers noted that initiation of growth on the top surfaces of the pie was prolonged as compared to the cut surfaces and postulated that this was caused by a moisture gradient which resulted in less available moisture on the top surface. Lag times were further extended when the a_w was lowered by means of additional glucose or when propionic or sorbic acid was present as a preservative in the pies.

Effect of a_w on intracellular constituents of staphylococci

Intracellular contents of K⁺ and Na⁺ of a number of halotolerant (including staphylococci) and non-halotolerant bacterial species were determined by

Christian and Waltho (14). These workers reported that halotolerance was closely correlated with increased intracellular levels of ions; especially K^+. Additional work by Christian and Ingram (13) and Christian and Waltho (15) demonstrated that intracellular a_w levels never exceeded the a_w of the medium in which the cells were grown and that the intracellular water content of S. aureus cells grown at decreasing a_w levels also decreased. The amino acid content of cells grown at decreasing a_w levels also increased gradually with decreasing a_w levels, however, from a_w 0.92 to 0.90 a very sharp increase in intracellular amino acid levels was noted.

These data suggest that the ability of staphylococci to accumulate low molecular weight solutes may strongly influence growth of this organism under reduced water conditions. These results also indicate that the staphylococcal cell membrane does not possess an inherent ability to maintain an intracellular a_w level above that of the medium when these cells are grown at low a_w. The increase in intracellular amino acid content in staphylococci grown at low a_w levels noted above could be caused by direct inhibition of amino acid incorporation or by reduced protein synthesis. Additional research by Christian and Waltho (16) in which an 80% inhibition of respiration was observed at reduced a_w levels could result in a shortage of energy for protein synthesis and thus favors the latter hypothesis. The final resolution of the mechanism by which a_w inhibits staphylococcal growth awaits additional work on the metabolism of this organism. The work of Christian and his colleagues has provided an excellent basis for subsequent efforts hopefully directed at the oxidative machinery of the cell at low a_w levels.

Much additional work is required to obtain a more complete understanding of the effect of water limitation on growth and toxin formation by S. aureus. Effects of a_w on the heat resistance of various strains, better definition of minimal and optimal water activities, and further elucidation of solute effects under a variety of conditions are all worthy of further research.

CLOSTRIDIUM PERFRINGENS

Reports describing the effect of water activity on growth, sporulation, and spore germination of C. perfringens, while few in number, are relatively recent.

Germination and growth
Gough and Alford (27) investigated the effects of curing salts on growth and heat resistance of this organism. These authors found that 10,000 ppm $NaNO_3$ or 400 ppm $NaNO_2$ or 6.0% NaCl were required to inhibit growth in a laboratory medium. Unfortunately, these findings were not related to a_w levels of the curing brines and thus it is difficult to correlate these data with those of succeeding workers. Kim (40) and Kang et al. (38) reported that the minimal a_w supporting growth of several C. perfringens strains was between 0.97 and 0.95 when either NaCl or sucrose were used to adjust the medium, and between 0.95 and 0.93 if glycerol was added. It was further found that the minimal a_w levels for growth were similar to those for spore germination.

Several C. perfringens strains were used by Mead (51) to demonstrate that this organism grows in a medium containing 6% NaCl. Growth was also observed in media containing 0.5% NaCl at Eh values of +194 to +238 mv; however, if the NaCl concentration was increased ten-fold, lower Eh values more closely approximating anaerobic conditions were required for growth to occur. Strong et al. (79) reported minimal a_w levels for growth similar to those observed by Kim (40) and Kang et al. (38) and like these authors, also noted significant solute effects. Addition of glucose to adjust a_w permitted growth at a_w 0.960 and when this compound was used to adjust to higher a_w levels, it uniformly supported more growth than other solutes. Media adjusted with KCl appeared to be the most inhibitory at comparable a_w levels.

Spore formation and survival
Spores of 50% of the C. perfringens strains tested by Gough and Alford (27) remained viable for at least 35 days in a brine containing 21.5% NaCl, 1,800 ppm $NaNO_3$, and 1,200 ppm $NaNO_2$. Spores, when inoculated into hams with pumped curing solutions containing 17.9% NaCl, survived both curing and smoking processes. Sporulation rates decreased dramatically as the a_w of Ellner's medium was reduced from 0.993 to 0.98 or 0.97 (38). These effects could be alleviated to some extent by supplementing this medium with ground veal. In addition, the observed sporulation rates were dependent on the solute and the particular strain used.

Like C. botulinum, solute effects appear to influence the growth of C. perfringens at comparable a_w levels. The minimal reported a_w which allows growth of this organism is 0.93 (38) adjusted with glycerol; however, NaCl and KCl are somewhat more inhibitory and limit growth at a_w 0.95 to 0.97, respectively. Spore germination reacts to water limitation in a manner similar to growth whereas sporulation requires a_w levels in the optimal growth range (a_w = 0.99).

Probably because of its relatively recent discovery, there currently exist no published data on the effect

of water activity on *C. perfringens* enterotoxin production, however, such information would be useful. In addition, much additional work is required to extend the data of Kang *(38)* and particularly the influence of proteinaceous materials on a_w effects.

SALMONELLA

Effect of a_w on growth

The effect of water activity on growth of *Salmonella* has been studied for almost 20 years, and like much of the early and excellent work on water activity, this research was mostly done in the laboratory of W. J. Scott in Australia. Christian and Scott *(10)* grew 16 strains of *Salmonella* in casamino acids—yeast extract—casitone medium adjusted to various a_w levels by a mixture of NaCl, KCl, and Na_2SO_4. Growth occurred at a_w levels between 0.999 and 0.945 with an average optimum of 0.995. Reductions of a_w below 0.99 produced an increase in lag period as well as in total cell yield, regardless of whether growth was aerobic or anaerobic. Severens and Tanner *(75)* reported that *Salmonella pullorum* and *Salmonella schottmuelleri* could be adapted to grow in a medium containing 8% NaCl after growth for 10 weeks in 3% NaCl. No attempt was made in these studies to relate growth to water activity levels. Using sucrose, glucose, NaCl, or KCl to control a_w, Christian *(12)* found that *Salmonella oranienburg* grew at a_w 0.97, regardless of which solute was used to adjust the a_w. Only glycerol, however, allowed growth at 0.96 but not at 0.95. The rate of oxygen uptake by this organism decreased as the a_w was reduced to 0.97 and 0.96 with NaCl, KCl, sucrose, or glucose; however, addition of glycerol to adjust a_w produced little or no reduction in oxygen uptake. In studies on the influence of nutritional factors on the limiting water for *S. oranienburg*, Christian *(11)* noted that the lower a_w limit for growth in three complex media was between 0.94 and 0.95 but was slightly higher (0.96 to 0.97) in a defined medium. However, if proline and methionine were added to this medium, growth was obtained at 0.96. Similarly, addition of eight water-soluble vitamins plus proline and methionine further extended the minimal a_w range to 0.95, however, addition of this vitamin mixture alone did not extend the minimal a_w for growth below 0.97.

Water activity effects in foods

The effect of water limitation on growth and/or survival of salmonellae in foods has received only limited investigation. Christian and Scott *(10)* studied growth of *S. oranienburg* in dehydrated milk, meat, and soups adjusted to various water contents. Growth occurred at a_w levels of 0.95 or 0.94 in all three foods, but at a_w 0.93, only the rehydrated soup supported growth. Increases in numbers could not be detected in foods adjusted to a_w 0.92 and incubated for 21 days. These authors concluded that growth in foods paralleled growth in laboratory media but occurred at slightly lower a_w levels in foods. In a study on the factors affecting microbial stability of cream fillings, Silliker and McHugh *(77)* briefly considered growth of salmonellae. They found that an inoculum mixture of two species, *Salmonella montevideo* and *Salmonella paratyphi* B, did not grow within 12 days at room temperature in fillings adjusted with sucrose to a_w levels in the range 0.795 to 0.830. These results were not too surprising, however, considering the low a_w of the fillings. Hargrove et al. *(31)* observed no differences in inhibition of four inoculated *Salmonella* species in cheese containing from 1.4 to 2.7% NaCl. These authors concluded that salt plays little or no part in the observed decline in numbers of *Salmonella* during cheese making, however, the salt concentrations used in these studies probably were not within the inhibitory range for *Salmonella*. Clayson and Blood *(17)* studied the survival and growth of salmonellae in thin films in an attempt to determine the fate of these organisms during surface drying or during baking. *Salmonella typhimurium* was prevented from growing in films at or below 92% equilibrium relative humidity. It is noteworthy that these authors attempted to adapt cultures of the test organism to increasing NaCl concentrations but unlike Severens and Tanner *(75)*, achieved variable results.

Influence of a_w on heat resistance

Because *Salmonella* species are considered potentially infectious agents, theoretically, even very low numbers of this genus are undesirable in foods. For this reason, much effort has been devoted to thermal destruction of salmonellae in foods and to the combined action of thermal kill and water activity. In this regard, the literature pertaining to the influence of a_w on the heat resistance of salmonellae can be divided into earlier work which describes an intimate relationship between these two factors and more recent research which indicates that a number of factors are probably at least as critical as a_w in their influence on heat resistance.

A_w has a pronounced effect on the thermal lability of all microorganisms; generally, the lower the a_w, the greater the heat resistance of the organism. Banwart and Ayres *(4)* investigated the combined effect of these two factors on the survival of salmonellae in albumen. They found that thermal kill increased rapidly at 50 to 70 C as the water content of the albumen was increased from 3-5% to 6-12%. Mossel and Koopman *(53)* inoculated *Salmonella senften-*

berg into fish and meat meals and casein previously adjusted to a_w 0.46. Based on the rate of diminution in *Salmonella* counts over a 5-day period, these authors stated that while the chemical composition of the heating menstruum was important, the observed lethal effect seemed to be mainly due to exposing the organism to an environment of different a_w. The heat resistance of salmonellae in dried milk was investigated by McDonough and Hargrove (49) who found that storage at 37 C of nonfat dry milk previously equilibrated to various moisture levels resulted in a reduction in viable count corresponding with a rise in moisture to 15 to 20%. Beyond 20%, no further reduction in counts occurred and growth of salmonellae was observed at moisture levels in excess of 40%. These results were confirmed in moistened milk powders at 85 and 115.5 C. At both temperatures the destructive effect of heating was enhanced with increasing levels of moisture. With milk samples held at 85 C, 2 hr were insufficient to obtain negative counts at 4 and 7% moisture, whereas only 30 min were required to reach a negative count at the 25% moisture level. Riemann (67) noted similar results in bone meals inoculated with S. *senftenberg* 775W, a heat resistant strain, and S. *typhimurium*.

More recently, work at the Food Research Institute and in England has shown that factors other than a_w may be more important in influencing the heat sensitivity of salmonellae. Goepfert et al. (25) compared the heat resistance of five serotypes of *Salmonella* and could not find a direct correlation between a_w and heat resistance. These authors found that S. *senftenberg* 775W, previously reported as highly heat resistant, showed the least heat resistance as the a_w was lowered. When glycerol rather than sucrose was used to control a_w, increased heat resistance could be observed in organisms previously grown at reduced a_w levels. Similarly, Baird-Parker et al. (3) found sucrose to be more protective than either NaCl or glycerol, and like Goepfert et al., these workers were unable to predict the heat resistance of salmonellae at low a_w levels. It is also evident from these studies that there is a large strain-to-strain variability in heat resistance among the salmonellae at a variety of water activities.

From these data, it appears that the salmonellae are not particularly tolerant of low a_w levels. The lowest a_w reported which supports growth is 0.945, with an optimum reported a_w of approximately 0.99. However, in view of the large solute and strain differences in response to limiting water activities, it is difficult to draw generalized conclusions concerning the effects of these factors until additional work is done to expand the scope of many of the existing studies.

VIBRIO PARAHAEMOLYTICUS

Vibrio parahaemolyticus has received increasing attention in the U. S. as a causative agent of foodborne infections usually resulting from the ingestion of raw or partially cooked sea food. Because of the high rate of consumption in Japan of these types of foods, the incidence of infections caused by this organism far outnumber all other types of food-borne infections in that country. As a result, it is not surprising that the preponderance of research on this organism has been done by the Japanese workers and the reviews by Sakazaki (68) and Nickelson and Vanderzant (57) are suggested as excellent background reading on this organism and the disease which it causes.

One of the most obvious growth characteristics of *V. parahaemolyticus* is its halophilic, or salt-requiring nature. Sakazaki (69) has reported that growth is easily obtained in culture media to which 1 to 3% NaCl has been added, with optimal growth occurring in the 2 to 4% range. Maximal salt concentrations at which growth will occur are in the 8 to 9% range. According to LeClair et al. (46), growth will not occur in 1% peptone water in the absence of NaCl; however, growth could be obtained in this medium plus either 3 or 7% NaCl. Like Sakazaki, these workers reported no growth of this organism in a medium containing approximately 10% NaCl. Baross and Liston (5), in a comparison of Japanese strains to isolates obtained from Puget Sound, reported that all of the test strains from both sources grew in the presence of 7.0% NaCl, however, none of the 40 Japanese strains grew in media containing 10% NaCl and only 3 of 200 of the Puget Sound strains grew at this salt concentration. Based on these and other biochemical characteristics, these workers concluded that the two groups of *V. parahaemolyticus* strains were identical.

The halophilic nature of *V. parahaemolyticus* presumes that this organism has a specific requirement for NaCl, however, the lack of definitive data makes such a judgment doubtful. Additional research on the solute requirements of this organism and the relationship of these requirements to a_w is warranted.

BACILLUS CEREUS

Bacillus cereus has been incriminated as the causative agent of numerous outbreaks of food poisoning, especially in European countries. A recent article by Goepfert et al. (26) reviews many of these outbreaks and summarizes much of the work which

has been done on this organism and its relationship to foodborne disease.

Smith et al. *(78)* in their classical, taxonomic study of the aerobic, sporeforming bacilli listed one of the characteristics of *B. cereus* as its ability to grow in 5 and 7% but not in 10% NaCl. Kim and Goepfert *(41)* also noted that 48 of 51 egg yolk-positive strains and 52 of 52 "narrow zone" strains could grow in 7% NaCl. The use of NaCl as a selective agent for *B. cereus* was investigated by Mossel et al. *(52)* who concluded that 5% NaCl was more useful than 10% when incorporated into a medium containing meat extract, peptone, mannitol, and phenol red. Addition of 10% NaCl to this medium produced highly variable results in respect to recovery of test strains.

There appear to be no reports specifically related to the effect of a_w on germination and outgrowth of *B. cereus* spores. Gould *(28)*, however, did investigate the effect of NaCl on spore germination and outgrowth and determined that 15% NaCl was required to inhibit germination of *B. cereus* spores, however, only 4 to 5% was required to prevent spore outgrowth.

It is apparent that much work remains to be done to determine the factors, including water relations, influencing the growth, sporulation, and spore germination of *B. cereus*, both in laboratory media and in foods. The possibility that this organism may produce disease symptoms by elaboration of a toxin [Goepfert *(26)*] further complicates the issue.

SUMMARY

Although excellent work has already been done to define the water requirements of food-borne bacterial pathogens, there is need for additional information. Probably one of the most pressing needs is for the general recognition among workers in this area of the usefulness of the water activity term. Although most of the research currently being reported utilizes this term, occasional articles continue to appear referring to solute concentrations on a weight per cent basis. As a result, it is often difficult to relate this work to that of others who have used the more broadly applicable a_w term. It is recognized that many researchers, for example those investigating curing brines, will find percent NaCl a more appropriate term, however, this writer advocates the use of both percent and a_w terms in such instances to describe the system under investigation. Along with the more general utilization of the a_w term in food research, there continues to be a need to evaluate the methods for measurement of water activity in terms of the food system or bacteriological medium employed. Frequently, these methods are complex and therefore difficult to use or are highly inaccurate, insensitive, or subject to contamination by the system being measured. An objective comparison of currently used methodology is urgently needed to enable researchers to select appropriate instrumentation for the system to be measured.

Basic information on the mechanisms by which water limitation exerts its effects on microorganisms is vitally needed. Only relatively recently have data [Christian and Waltho *(14, 16)*] pertaining to the intracellular responses of microorganisms to a_w adjustment begun to appear. We know virtually nothing of the response of cell-free extracts, electron transport systems, and genetic transfer to limited water. Similarly, we have little knowledge of nutritional transport across bacterial membranes although, surely, osmotic conditions must affect the ability of nutrients to cross these membranes. Some food-borne pathogens produce toxins in foods before these foods are consumed, yet relatively little is known about the effect of water limitation on production and biological activity of these toxins. The interactive effects of a_w and other factors in foods, such as pH and nutrients, on toxins also require thorough investigation.

Solute specific effects are frequently cited in this review. One of the most notable of these is the tendency for glycerol to allow growth at lower a_w levels than other solutes utilized for a_w adjustment. In at least some instances, it is possible that the organisms under investigation could have utilized a portion of the glycerol as a carbon source and thus lowered the concentration of this compound with a concomitant increase in a_w. Because a_w levels following growth and/or analyses of final concentrations of the solute were not done, or at least were not reported, solute specific effects of this type must be regarded with some suspicion. Recognition of possible utilization of solutes by the test organism should be considered, especially when utilizable or potentially utilizable carbohydrates, such as glycerol, sucrose, or glucose are involved. On the other hand, the greater tolerance of glycerol shown by many food-borne pathogens could be attributed to the fact that glycerol solutions tend to show a lower a_w level than salt solutions, especially when measured by electric hygrometers or dew point devices. These data are discussed in greater detail by Black et al. *(6)*.

The limitation of water as a means of preserving foods and preventing the growth of food-borne pathogens continues to be of great significance to food scientists. The growing interest in intermediate moisture foods is an excellent example of the future applicability of such information, however, existing foods and food processing techniques must also be interpreted in terms of water availability. As addi-

tional data become available, the utilization and exploitation of water limitation as a useful means of preserving food materials will assume ever increasing status.

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REPORT OF COMMITTEE ON
FOOD EQUIPMENT
(Continued from Page 275)

NATIONAL AUTOMATIC MERCHANDISING ASSOCIATION (NAMA)

The National Automatic Merchandising Association's Automatic Merchandising Health-Industry Council (AMHIC) held its 16th annual meeting during October 1971, and this Association and other public health organizations and the affected industries were represented and participated in AMHIC's discussions.

The afternoon of the first day was reserved solely for a meeting of the public health representatives and was used by them to discuss and clarify their view on public health objectives and policies to be followed in their work with the entire membership of the AMHIC. The Chairman of the IAMFES Food Equipment Committee was re-elected Chairman of the Public Health Group and also served as Co-Chairman of AMHIC during 1971-1972.

Evaluation manual for food and beverage vending machines
Carbonation backflow prevention. At the 1970 meeting the members of AMHIC revised the NAMA Vending Machine Evaluation Manual to require the use of a vented check valve or other positive venting device, in addition to the normal check valves, in all vending machines with carbonated water. This more stringent amendment to Item 603.1 still failed, in the opinion of several members of AMHIC, to offer adequate protection to the consumer; and the following proposal was discussed: in air-gapped machines, no toxic materials should be permitted downstream (from the air gap) in the water supply contact zones. However, in lieu of immediate application of this proposal, the following additional charge was given to the Committee on Carbonation Back-flow Prevention: to survey industry and operator effects of its implementation, the operational history of vented valves and industry trends, and to report the Committtee's findings and recommendations on or before the next meeting.

Cut-off controls. At the request of the Committee on Cut-off Controls, two different field tests were developed by members of AMHIC to determine the cut-off temperature which would de-activate the potentially hazardous food vending machine and cause it to indicate "sold out." Both of these tests using a thermometer equipped with a remote sensing device were successfully demonstrated at the 1971 meeting of AMHIC and were proven effective and applicable for use by sanitarians in the field.

In addition, there was a general reporting of changes made by manufacturers to improve accessibility to controls in the field and to phase out limited vapor filled controls which have proven difficult to field test with body heat.

In view of progress made by the Committee on Cut-off Controls, there was agreement that the NAMA staff and the AMHIC Committee on Education and Training are with sufficient data to develop a *Handbook on Location and Field Testing of Health Code Cut-off Controls*. Such a *Handbook* is urgently needed by industry and public health to aid in safe guarding the health of the consumer and not only to make the applicable provisions of the *Evaluation Manual* and the *PHS Recommended Vending Code* meaningful but also to promote their compliance.

Coin-operated special devices or dispensers. The Committee on Coin-operated Special Devices or Dispensers presented a report which indicated a growing demand for this "office" type of equipment and a need to develop adequate sanitary specifications which would aid in fabricating acceptable equipment to satisfy this demand.

The NAMA Staff reviewed the two distinct problems associated with small coin-operated dispensers: (a) the present definition of "vending machine" which has forced states and municipalities to license and inspect this equipment whether they deemed it necessary or not; and (b) inability of NAMA to accept most such dispensers for evaluation because of the lack of a reasonable standard.

The PHS-FDA representative on AMHIC, in response to

SOLIDS–LIQUID SEPARATION: AN IMPORTANT STEP IN THE RECYCLING OF DAIRY COW WASTES[1,2]

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ABSTRACT

Use of a solids–liquid separator in the management of dairy cattle wastes may (a) reduce labor requirements, (b) make mechanical handling more feasible, (c) improve automation, (d) produce solids with economic value, and (e) produce a liquid that may be handled by ordinary equipment and treated to produce potable water and fertilizer. In solids-liquid separation of dairy cattle wastes, macro-colloidal ($>5\mu$) and larger solid particles are partially removed from the liquid portion by screens, sieves, and compressors. The two products produced are (a) wet solids and (b) a dilute liquid. The solids contain about 45 to 80% water depending on the systems used and are stable in nature. The solids have little or no odor and may be dried and used for bedding, refeeding, or mulch, thus having economic value. The liquid is dark brown in color and contains about 85-90% of the 5-day BOD and only about 1-3% suspended and dissolved solids. It may be handled by ordinary equipment and does not require special liquid pumps and high-powered tractors for mixing, pumping, and conveying. The liquid may be irrigated directly onto crops and soils or it may be treated to make potable water and a more concentrated fertilizer.

For some 5 to 7 years several researchers have been diligently seeking a "break through" in farm animal waste handling and management. They have been looking for a system that has or is: (a) low labor requirements, (b) mechanical handling and treatment, (c) automatic or nearly automatic controls, (d) non-pollutional (low odor, BOD, and nutrient discharge), and (e) economical. Furthermore, it seems that the farm animal producer expects such a development. Solids-liquid separation appears to be a step in this direction.

The dairy farm situation

In general the dairy farm has not needed a new system for large quantities of wastes as much as the beef producer, swine grower, or poultry raiser. Dairy sizes in terms of numbers of animals per acre generally have not been as great as these other enterprises. For example, it is difficult to name a 10,000 head dairy operation. Yet there are many such beef cattle operations and some equivalent swine and poultry operations. Although there are rumors that the dairy herd size is going to increase to 80 to 85 within the near future, the average dairy herd is still less than 50 cows (2).

Dairy operations have also generally been connected directly to or with a farming operation of some 160 or more acres of land. Thus, the ratio of animals per acre has been generally quite low, and it has been a problem of simply (if it may be put in such terms) returning the wastes (manure, waste water, etc.) to the land by some means. With the relatively small quantity of wastes, it was almost impossible to pollute land, water, or air if normal discretion was used. Nevertheless, many dairy farmers would like to have a "better system" for waste management in their present operation. Increases in the size of operations in the future may require such improvements.

Waste disposal is a very important aspect of commercial dairy farming. A cow produces several pounds of manure/pound of milk and this waste must be disposed of in a safe, inoffensive manner. Tables 1 and 2 give the properties of fresh dairy

TABLE 1. SOME PROPERTIES OF DAIRY CATTLE MANURE

Average daily manure production	107.8 lb.
Portion of manure as feces	68.8%
Portion of manure as urine	31.2%
Percent body weight	7.22%
Moisture content of feces	82.7%
Moisture content of urine	95.1%
Average moisture content of manure	87.5%
Fixed solids in feces	13.8%
Fixed solids in urine	37.6%
BOD of straight manure (urine plus feces)	25,600 ppm
BOD per day	2.76 lb.

TABLE 2. CHEMICAL CONTENTS OF DAIRY CATTLE MANURE

Moisture content	Lb/ton manure								
	N	P	K	S	Ca	Fe	Mg	US	Fat
79%	11.2	2.0	10.2	1.0	5.6	0.08	2.2	322	7

cow manure (3). At present there are numerous disposal or treatment methods being used, each with its own advantages and disadvantages. One modification that may improve these methods in solids-liquid separation.

Some general waste handling terminology

Several waste handling terms are being used which may not be entirely clear. Some of them are discussed below:

Recycling. This term usually means one or two things. It can mean simply returning the waste materials to the soil where they are degraded by the natural organisms for reuse by plants along with the sun's energy to produce more feed. Others have used the term to mean the direct return of the wastes to the animal as a feed or in combination with other feeds for direct consumption by the animal. Both are correct terms and are used in this paper, however, they are differentiated between as used. The use of a portion of the solids for bedding may also be referred to as recycling.

Solids-liquid separation. Generally solids-liquid separation means the removal of the macro-colloidal and larger solid particles from its liquid support media. The division is not "clear cut" but generally any particle less than about 5 μ is considered to act about the same as if it were dissolved and generally remains in suspension with only slight settling if any. Some engineers prefer to use the terms suspended and settleable solids but again they do not apply to all conditions as some suspended material in animal wastes may be quite large. Therefore, it is assumed that solids-liquid separation is the removal of most of the matter above 5 μ or slightly larger from the liquid.

Solids-liquid separation is a fairly new technique in livestock waste management. There are presently about 30 solids-liquid separators installed in dairies, mostly in California, to screen out fiber, undigested feed, and other large particles contained in liquid manure *(6)*. There is also one in Ohio *(12)*.

Biodegradability and non-biodegradability. Most or maybe all organic materials are theoretically biodegradable if given sufficient time with the right bacteria at the correct temperature. Nevertheless the terms biodegradable and non-biodegradable have come to mean something slightly different to many sanitary engineers and perhaps to others. Generally the term "biodegradable" has come to mean something that can be readily hydrolyzed and broken down into more basic forms in a few days.

If the above definition holds for ordinary treatment (anaerobic or aerobic treatment for 4 or 5 days), animal wastes could be said to contain much non-biodegradable material. This "non-biodegradable" quantity may be as high as 60 or 70% of the total solids in the wastes (manure). The author once conducted an aerobic digestion experiment with dairy cow manure for over 180 days only to find that over one-half of the organic solids still remained in the unreduced form. The remaining organic matter consisted generally of fibrous material and undigested feed particles which would have eventually broken down but which would have required more time.

The separated solids and liquid

Products from the solids-liquid separator still contain the original materials found in the wastes but they are in a different form, and are more manageable and usable.

The solids contain about 40 to 60% of the suspended and settleable solid of the raw wastes. These solids are made up mainly of undigested feed particles such as stems of hay, particles of corn, silage cuts, and other large pieces that have defied digestion within the ruminant. Of course some bacterial cells and other stable organics and inorganics are also in the mixture. These materials are often referred to as non-biodegradable even though they could possibly be decomposed over a long period (6 months to 2 years).

Because of the non-biodegradability of the solids they are referred to as stable and contain only about 5 to 15% of the 5-day BOD of the raw wastes. The moisture content of the solids depends on the efficiency of the separator and seems to vary between about 45 and 85%. No doubt further development of the separators will provide a lower moisture content in the future.

The dried solids are light and fluffy. They are unobnoxious and may be stored or used almost any place desired. Odor is nil.

The liquid effluent from the separators contains more pollutional materials than the solids but it is in a form that may be readily handled (pumped, conveyed, etc.). Depending on the separator used and the dilution water added, the liquid contains from 0.5% to about 4% finely suspended and dissolved solids. It may be irrigated directly onto crops and soils or it may be treated further.

The liquid portion contains approximately 85 to 95% of the 5-day BOD. This means it contains by far the greatest percent of the biodegradable portion of the raw wastes. However, since it does not contain the large quantity of suspended and settleable solids that the raw wastes contain, it may be more readily aerated in an oxidation ditch or lagoon. Also the alpha factor (transfer of oxygen from the air to the mixture) would probably be considerably higher, thus lowering power requirements for providing oxygen and reducing the cost of treatment.

A solids—liquid waste management system

Solids-liquid separation should be thought of as an integral part in a new system such as the following. *(a)* Solids and liquids are flushed or scraped to a 2- or 3-day capacity holding tank. *(b)* The waste mixture is mixed and pumped by a submersible pump

(about 5 horsepower) to the solids-liquid separator. (c) The solids are separated from the liquids by the solids-liquid separator using screens, sieves, presses, etc. (d) Solids are discharged to an open storage for drying before reuse as (i) bedding, (ii) feed, (iii) mulch, or (iv) returned to land. (e) Liquids are discharged to a holding lagoon or tank prior to (i) irrigation onto soil and crops or (ii) further treatment. A 2 to 3-horsepower, electrically driven pump will move the liquids for either purpose. (f) A simple, low cost irrigation system can be permanently installed to sprinkle or flood irrigate some given crop and land. One and one-half-inch plastic pipe can be placed underground to convey the liquids to desired locations in the field. If an irrigation system is used, it should be designed properly taking into account the permeability of the soil, sealing characteristics of the liquid, and the nutrients therein.

Winter operation requires some special consideration. For example, the holding tank or lagoon must be large enough to hold the liquids through the season of frozen soil if irrigation is not permitted during such a period. This is a time when maximum recirculation of the wash water should be used to avoid any excess water use which would require a larger storage capacity.

The storage provided should be given some special attention and consideration. An outdoor lagoon type storage provides the most economical unit. However such a storage should be located where no runoff will enter it. The cost of such a lagoon is relatively low regardless of location as the excavated soil may be used to elevate the sides thereby increasing the volume. A capacity of about 2 ft³/cow/day for about 180 days should be the minimum lagoon storage.

Odor control may be an important item for consideration in the near future in all systems. With the liquid stored in the lagoon this may be somewhat of a problem but it can be easily controlled by aeration if necessary. It appears that aeration equivalent to about the 5-day BOD would do a satisfactory job of controlling odor. This past year at the Purdue Dairy Center, the odor of whole wastes accumulating in a storage lagoon from about 130 dairy cows during November to April, was controlled by one 5-horsepower floating aerator. The aerator was not turned on until the ice melted in March. So odors can be controlled if necessary but it costs extra. According to Koelliker and Miner (8) effluent irrigated from an anaerobic lagoon loses its odor producing capabilities within hours after being exposed to the open air.

The *solids* produced by the above waste management systems with a solids-liquid separator may have many useful or even profitable uses. As mentioned before, the separated solids are large, relatively inert particles and may be dried, incinerated, composted, or used directly as soil conditioners. Dried or composted solids have been used for livestock bedding, refeeding, or mulch or organic matter for soil conditioning. When properly dried, the waste solids form a light fluffy litter that has been found satisfactory for bedding in free stalls with no staining of the cows (4). It may be an even better source of calf bedding than wood shavings because the calves do not eat the manure solids and because the dry solids absorbs about four times their own weight in liquids. With money saved on litter the dairyman may be able to cover much of the expense of separation as well as making the remaining liquid manure considerably easier to dispose of. Dairy manure solids have also been tested in poultry houses and has been reported to be an excellent floor litter for growing chicks (14).

Dried or composted solids may have a potential as a mulch or soil conditioner. In California, a golf course has used the dried manure as a mulch over grass seed placed on bald spots caused by excess salinity. When spread over the seed at 3/8 to 3/4-inch thick, vigorous green grass sprang up within several days. The mulch apparently worked to overcome the salinity problem by holding moisture near the soil surface and preventing the grass seed from drying out. In Washington state, a county purchased manure solids from a local dairy at $3/yard for use in flower and shrub beds. They reported excellent results (4).

Research in refeeding of livestock solids has been reported for swine, poultry, and sheep wastes but it is unlikely that government health agencies will permit refeeding for dairy cows for some time to come. The Federal Food, Drug, and Cosmetic Act of 1938 prohibits interstate commerce in adulterated or misbranded foods and drugs. "The Act" does not distinguish between food used solely for animals as opposed to food used solely for man. It deems a food adulterated if that food contains any poisonous or deleterious substance which may render it injurious to health, if it contains any food additive which is unsafe, or if it consists in whole or in part of any filthy, putrid or decomposed substance (14).

At present, while not sanctioning the reuse of manure as an animal feed, the FDA does not discourage research in this area. It will continue to review information on waste products as research makes the facts available. When sufficient information is available on whether a specific waste is safe for consumption by the target species and that the meat and by-products from the animals are safe for consumption, the FDA will review their objections to the use of animal wastes in livestock diet (14). At present, refeeding is still in the research stage and

should not be attempted without government permission and strict controls (15).

The liquid portion has a potential use in the production of protein by yeast or bacteria (9, 10). Although the mechanics of such a system need to be simplified somewhat, this type of conversion may have much potential, not only in refeeding, but in the manufacture of commercial products. It seems that refeeding, either directly or through protein production, would yield significant improvements in feed conversion by making several passes through the animal.

Solids-liquid separators

Currently there are at least three solids-liquid separators and separation systems in limited use for dairy cow wastes. There are other separators being developed but the status of their adaptation to dairy cow wastes is unknown.

Basically all the solids-liquid separators require that dairy cow wastes be collected, diluted (amount of dilution varies with separators), and mixed in a sump prior to discharge or pumping to the separator mechanism. From this point on the separation is performed by the equipment. Three such separators are discussed briefly below:

The SWECO Vibro-Energy Separator is a screening device that vibrates about its center of mass. Vibration is accomplished by eccentric weights on the upper and lower ends of the motor shaft. Rotation of the top weight creates vibration in the horizontal plane, which causes materials to move across the screen to the periphery. The lower weight acts to tilt the machine, causing vibration in the vertical and tangential planes. The screen (available in various meshes) which varies in diameter from 18 to 60 inches is dosed in the center with the diluted wastes. The liquid portion falls on through. Solids are caught on the screen which vibrates (moves) them to the exterior from whence they are discharged at the periphery next to the shroud into a stack. According to Fairbank (5), the separator works quite well as long as the manure is diluted with 10 to 12 volumes of water. This might be called a high dilution rate and could lead to problems of water supply.

The solids as they come from the above separator contain approximately 80% moisture. However, they are relative "clean" and stable as they have been washed with the highly diluted water. After the solids have been spread in the sun to dry for a few days they become an ordorless, inert material which may be used for bedding, chicken litter, or a mushroom growing medium.

The liquid portion contains less than 0.5% solids and is ready for irrigating onto crops and soils. It is usually not acceptable for discharge into ditches without further treatment. Treatment which would

remove more solids may be desirable as it could then be used to dilute the raw wastes.

The Bauer Hydrasieve for solids-liquid separation has no moving parts. It was developed primarily as a thickener for the pulp and paper industry. However, it has been found to be adaptable to other industries such as sewage treatment, food processing, chemical processing, etc. The heart of the Hydrasieve is a screen assembly of specially designed wires with unique singular curves. The physical structure of the device resembles the traditional flat side-hill screen, but the technique of separation of the solids from fluids is basically different from other static screens. The Hydrasieve achieves fluid removal by a hydraulic surface attachment utilizing the Coanda effect on a multiplicity of essentially V-section screen bars which between the supporting members are formed in a single arc, the apex of which is directed in the sense of the slurry flow (7).

Solids of animal wastes coming from the Hydrasieve contain approximately 80% water and are ready for further processing (drying, composting, etc.).

The liquid contains around 1 to 2% solids and is ready for further treatment or irrigation onto crops and soils. It is not of a quality that is acceptable to be discharged into a stream.

Babson Bros. Company, recognizing the need for a farm animal waste treatment system has also developed a solids-liquid separator for specific use with dairy cow wastes. This separator goes beyond the normal solids-liquid separation in that plans are to treat the liquids and to produce potable water and fertilizer. The unit is referred to as Babson Bros. Co. TRU (total recycle unit). Although some successful prototypes have been fabricated and are in use it is still in the developmental stage.

Briefly the Babson Bros. solids-liquid separator consists of a loading hopper with a rewash mechanism, a sloping screen onto which the wastes are directed from the hopper, and a porous belt to accept the solids as they roll off the screen. The solids are then conveyed through a "roller-squeezer" to remove excess moisture before final discharge. A dilution of about one part water to one part manure is needed to make the separator function at its optimum. Final plans include adding a second conveyor and roller-squeezer to further reduce the moisture content to produce a more readily manageable product.

Solids produced by the separator contain about 45 to 50% moisture and give a "clean appearance." The solids dry rapidly when spread out in a thin layer and have been used successfully for bedding.

The liquid discharged from the separator contains 3% to 4% dissolved and finely suspended solids. Plans are to treat this material in such a way as to separate much of the remaining solids and nutrients thereby

producing a potable water and a concentrated fertilizer. However, it may be irrigated directly onto crops and soil without additional treatment but it contains matter that may close the pores of some soils.

Other solids-liquid separators are available but only a few are being adapted for use with animal wastes.

Solids-liquid separation and present waste management

To see how solids-liquid separation may fit into dairy wastes management systems, it may be helpful to first review dairy waste treatment systems now in operation.

Scraping and water flushing-holding tank-land disposal. This is perhaps the most commonly used method of dairy waste management. Pens, gutters, and driveways are washed down with water under pressure and the washwater drains by gravity into a manure storage tank. The waste is then stored until the dairyman's work schedule and the weather permit field disposal. One of the primary difficulties of this system is that the waste in the holding tank is under anaerobic conditions and noxious odors produced can cause difficulty unless proper ventilation is provided.

Solids-liquid separation could be provided by using two holding tanks in series with a shaker screen for solids-liquid separation in between. This would allow a small separator to work continuously at a low rate of flow even though water flushing is done periodically. Here the first tank would be merely a sump to hold the waste until it can be processed. Solids-liquid separation would not have a major beneficial effect for odor control in this system since the high pollution strength liquid would still be stored under septic conditions. The major advantage of using a separator in this system would be in transporting the separated liquid to the land disposal site. With most of the suspended solids removed, the liquid could be easily pumped to the field without fouling transport pipes or irrigation nozzles. The need of an extra tractor is eliminated and not "tied up" in pumping liquid manure since a small sewage pump can easily do the job. Also the power and time required to "homogenize" (mix) the solids and liquid is eliminated.

The effluent liquid from the separator is still very unstable and would probably not be suitable for reuse in flushing. This system would therefore require a relatively large volume of fresh water for flushing and dilution.

Water flushing-aerobic unit-land disposal. This is identical to system just described except that an aerobic unit is substituted for the holding tank. This system would not have problems with odors but does have the disadvantage of a higher cost because of a large power demand for a mechanically aerated oxidation ditch or aerated lagoon or because of a large area and construction cost for an oxidation pond.

This system could be improved by adding a solids-liquid separator before aerobic treatment. This would decrease the cost of aerobic treatment with an oxidation pond because it would decrease sedimentation in the pond and therefore increase the useful life of the pond. Separation would also decrease cost with the mechanically aerated systems since much of the power normally provided is to keep solids in the unit in suspension. Also the rate of transfer of oxygen from the air to the wastes (alpha factor) is improved with a decrease in solids concentration.

It may be advantageous to use the effluent from the aerobic unit to supplement the flushing water supply. This would tend to dilute the waste, aid the separation process, and decrease the amount of fresh water needed. Also, excess water would not accrue in the lagoon.

Water flushing-anaerobic unit-aerobic unit-land disposal. Basically, an anaerobic unit has the function of converting suspended solids in a waste to dissolved organic compounds and gases. In other words, a liquid waste is produced from a solid waste with a net loss in pollution potential.

Since the solids produced by solids-liquid separation are large relatively inert particles, they are not modified very much by either anaerobic or aerobic treatment. They may, however, cause sedimentation problems in both units and therefore a separator in the waste flow line may help to remove this material before it reaches the treatment unit. It may be feasible to use the effluent from the aerobic treatment unit for flushing here also.

In-house oxidation unit-holding unit-land disposal. If a dairy is set up with an in-house oxidation ditch, the solids-liquid separator has no place before treatment. The solids and liquids fall into the ditch through the slotted floor directly into the ditch. However, immediately after treatment in the oxidation ditch, the solids-liquid separator would be advantageous since the solids could be removed for other uses and the liquids irrigated onto land or some returned to the ditch for dilution purposes. One of the difficulties with oxidation ditches is their tendency to become "over loaded" with solids that either settle out or result in poor oxygen transfer or poor mixing in the ditch. The solids-liquid separator used in this way would eliminate this difficulty.

In-housing holding unit-land disposal. This system involves use of a holding tank under either a slotted floor or one into which wastes may be scraped. The wastes can be held in this unit for whatever storage period it was designed. The entire wastes

are then usually mixed by a tractor-driven pump and recirculator before spreading on the soil. A second tractor is used to convey the "liquid" wastes to the field for spreading onto the land. If this system is working entirely satisfactory for the dairy producer and tractor power and odors are not problems, perhaps the solids-liquid separator has no place. However if there are problems with the system perhaps the installation of a slightly deeper sump in one or two ends of the storage could serve as a collection pit for pumping to a solids-liquid separator. Then by use of such a separator solids could be removed with the liquid going (a) onto land by irrigation, (b) into an outside storage, or (c) returned to the underfloor pit for dilution of incoming wastes. Some dilution is necessary to properly operate a solids-liquid separator anyway and liquid flushing to the sump in the pit is also required.

Other waste management systems without solids-liquid separation

Drying entire wastes. If the entire waste of dairy cows is dried, solids-liquid separation has no place in the system. *Incineration* with the ash being returned to the land also has no need of a solids-liquid separator. However most animal wastes will not furnish sufficient heat to incinerate themselves. Gases and particulate matter discharged to the air are also pollutional and costly to control. Therefore this system has not received wide acceptance.

Composting. Most wastes contain too much water to compost properly without mechanical aids and addition of dry organic solids. A solids-liquid separator that does not require much dilution or one in which the separated liquids are returned for dilution could possibly improve composting of animal wastes.

Other waste management systems improved by solids-liquid separation

Refeeding. A solids-liquid separation may be a big assist for refeeding either as a dry feed mix or silage.

Protein production. The liquid portion with the nutrients is the part of the wastes that are needed for this according to Nye (9). Other fermentation processes also require the liquid without the inert solids.

Building blocks. The liquid portion must be removed in this process, thus solids-liquid separation is desirable.

Raw material for oil. Generally a solids-liquid separation would help this process. The solids are needed without much of the liquid although a quantity of the organics left in the liquid are also useful in this process.

Methane production from dairy waste could become a reality with the liquid portion of the wastes which contains much readily usable materials for the methane formers.

SUMMARY

Farm animal wastes are a mixture of water (about 87.5%) and solids (12.5%). The total solids are composed of a mixture of large particles of non-biodegradable organic solids, dissolved or finely suspended biodegradable organic solids, dissolved inorganic solids, and bacterial cells. Separation of the large non-biodegradable portion of organic solids from the mixture is a desirable step in that it provides the following: (a) Solids that may be used for bedding, refeeding, mulch and other by-products which have economic value to the dairyman. (b) A liquid that is easy to pump, contains most of the 5-day BOD but which is readily treatable by aeration to control odor, is irrigable, contains most of the nutrients, and is desirable as a fertilizer. (c) A liquid that can be readily treated to produce potable water and concentrated fertilizer. (d) An important step in mechanizing and automating waste management. (e) A step that improves present waste management systems by reducing power requirements of two or more tractors for mixing, pumping, and spreading and making possible the management of the wastes with ordinary equipment.

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REPORT OF COMMITTEE ON FOOD EQUIPMENT
(Continued from Page 288)

questions, pointed out that revision of the PHS Vending Code could require several years and that, in the meantime, his agency, in his opinion, would not disapprove of: (a) reasonable standards for specialized types of equipment; and (b) state/local amendment of definitions to specify the types of equipment not subject to licensing and routine inspection.

At the conclusion of this discussion, members of AMHIC further charged the Committee on Coin-operated Special Devices or Dispensers to appropriately consider the foregoing matters and that a proposed Standard for Special Devices or Dispensers be developed by AMHIC. The Committee on Special Devices has met, and a report should be available in the near future to this Committee and other interested persons for review and comment.

The *Evaluation Manual,* policies and procedures, and other educational materials developed by AMHIC and/or NAMA may be obtained from the National Automatic Merchandising Association, 7 South Dearborn Street, Chicago, Illinois, 60603.

Seal of approval

The advisability and feasibility of a NAMA Seal of Approval program and its application to NAMA evaluated vending machines was thoroughly discussed, and the members of AMHIC indicated their favor of an in-depth exploration of a seal program by instructing the Committee on Education and Training to explore all aspects of a seal of approval program and to submit its recommendations to AMHIC.

The Committee on Education and Training developed a survey procedure concerning essential aspects of a seal of approval program; and using this procedure, it has surveyed certain public health and industry personnel as an initial step in implementing its new charge to explore a NAMA Seal of Approval program for Vending Machines. The Committee has started tabulating initial survey findings and should have a very interesting report including recommendations for review at the next meeting of AMHIC.

Other activities

The NAMA staff reported that the relationship of the NAMA *Vending Machine Evaluation Manual* as an official standard to the application of the Occupational Safety and Health Act of 1970 is being explored. Further, the NAMA *Labeling Guide* including a statement on proposals to require ingredient listing of mandatory ingredients of standardized food has been submitted to the Federal Food and Drug Administration for approval; and the pending proposals to the U. S. Department of Agriculture to determine the application of the Federal Meat and Poultry Inspection Programs to

Commissaries has delayed completion of the AMHIC Commissary Guide. In addition, a new bulletin on Heart Pacemakers and Microwave Ovens which has been published recently by NAMA should prove beneficial to all sanitarians in answering questions concerning the hazard potential to pacemaker users from association with microwave ovens.

RECOMMENDATIONS

(a) The Association reaffirm its support of the National Sanitation Foundation and the National Automatic Merchandising Association and continue to work with these two organizations in developing acceptable standards and educational materials for the food industry and public health.

(b) The Association urges all sanitarians to obtain a complete set of the National Sanitation Foundation's Food Equipment Standards and Criteria and a copy of the National Automatic Merchandising Association—Automatic Merchandising Health-Industry Council's Vending Machine Evaluation Manual and related materials; to evaluate each piece of food equipment and vending machine in the field to determine compliance with the applicable sanitation guidelines; and to let this Committee and the appropriate evaluation agency know of any manufacturer, installer, or operator failing to comply with these guidelines.

(c) The Association urges all sanitarians and regulatory agencies to support the work of the Association's Committee, submit suggestions for developing new guidelines and for amending same, and subscribe, by law or administrative policy, to the principles represented by the *Standards, Criteria, and Evaluation Manual for Food Equipment and Vending Machines.*

This report of the Committee on Food Equipment Sanitary Standards respectfully submitted by:

Karl K. Jones, *Chairman,* Purdue University, West Lafayette, Indiana.

Irving L. Bell, State Department of Health, Frankfort, Kentucky.

Glenn Brauner, National Canners Association, Washington, D.C.

Robert R. Dalton, State Department of Public Health, Lansing, Michigan.

Carl Henderson, State Department of Health & Social Services, Santa Fe, New Mexico.

Howard Hutchings, State Department of Health, Pierre, South Dakota.

O. Donald Moore, Food & Drug Administration, Atlanta, Georgia.

W. Joel Simpson, State Department of Environmental Resources, Harrisburg, Pennsylvania.

Harold Wainess, Harold Wainess and Associates, Northfield, Illinois

PROGRAM

SIXTIETH ANNUAL MEETING

INTERNATIONAL ASSOCIATION OF MILK, FOOD AND ENVIRONMENTAL SANITARIANS, INC.

In cooperation with

NEW YORK STATE ASSOCIATION OF MILK AND FOOD SANITARIANS

and

CORNELL UNIVERSITY, FOOD SCIENCE DEPARTMENT

AUGUST 13-16, 1973

Flagship-Rochester Hotel   Rochester, New York

REGISTRATION

Sunday, August 12-3:00 P.M.-8:00 P.M.
Monday, August 13-8:30 A.M.-5:30 P.M.
Tuesday, August 14-8:30 A.M.-5:00 P.M.
Wednesday, August 15-8:30 A.M.-5:00 P.M.

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Reception: William H. DuMond, Queensboro Farms; John J. Campana, Monroe Co. Health Dept.

Milk Breaks: Lynn A. Smith, Upstate Milk; John G. Burke, Borden's; John C. Woods, Queensboro Farms, Carl F. Waterstram

Special Events: William K. Jordan, Cornell; John J. Campana

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H. E. Hansen, Iowa Assn. Milk, Food and Environmental Sanitarians

Vern Packard, Minnesota Sanitarians Assn.

Harry Furgeson, Idaho Environmental Health Ass'n.

TOPICS FOR AFFILIATE AGENDA AT ANNUAL MEETING

(To be announced later)

SUNDAY, AUGUST 12, 1973

3:00-8:00 P.M.—Registration—North Corridor, 2nd Floor

1:30-5:30 P.M.—Executive Board—Board Room

8:00-11:00 P.M.—Executive Board—Board Room

MONDAY, AUGUST 13, 1973

8:30 A.M.-5:30 P.M.—Registration—North Corridor, 2nd Floor

NATIONAL MASTITIS COUNCIL REGIONAL MEETING

9:00 A.M.-5:00 P.M.—(See separate program) Tudor Room

COMMITTEE MEETINGS

Check Bulletin Board—North Corridor, 2nd Floor

SPECIAL MEETINGS

9:00 A.M.-12:00 noon—Executive Board — Board Room
1. Report on Local Arrangements
2. Report of Executive Secretary
3. Report of Sanitarians Joint Council

1:30-5:00 P.M.—Executive Board—Board Room
1. Report of Journal Management Committee
2. Regular Agenda
3. Committee Chairman
4. Meet with Past Presidents

1:30-5:00 P.M.—Individual Committee Meetings
(See Bulletin Board) North Corridor, 2nd Floor

5:30-6:30 P.M.—Reception—Stuart Room

6:30 P.M.—New York State Association of Milk and Food Sanitarians
50th Anniversary Celebration Dinner
Windsor Room
(All are invited to attend)

7:30-9:00 P.M.—Affiliate Council—Grenadier Room

9:00 P.M.—Farm Methods Committee Meeting
Genesee and Crossroads Room

TUESDAY, AUGUST 14, 1973

8:30 A.M.-5:00 P.M.—REGISTRATION—North Corridor, 2nd Floor Lobby

8:00 A.M.-9:00 A.M.—Executive Board—Board Room
Report of Affiliate Council

MORNING—GENERAL SESSION
WINDSOR ROOM

EARL O. WRIGHT, *President-Elect, Presiding*
9:30—INVOCATION

9:35—ADDRESS OF WELCOME
CHARLES G. ASHE, President, NYSAMFS

9:50—PRESIDENTIAL ADDRESS
WALTER F. WILSON

10:15—OPPORTUNITIES UNLIMITED
WILLIAM B. LANE

11:00—CAREER OPPORTUNITIES
HAROLD E. CALBERT

11:45—NOMINATIONS, 1973

AFTERNOON—MILK SANITATION SECTION
WINDSOR ROOM

HAROLD E. THOMPSON, JR., *Presiding*

1:30—DOOR PRIZE DRAWING

1:35—FLAVOR AND SHELF LIFE
SIDNEY E. BARNARD

2:15—PASTEURIZATION OF GRADE A MILK
AND MILK PRODUCTS BY STEAM IN-
JECTION
ROGER W. DICKERSON, JR.

3:00—MILK BREAK
Regimental Corridor

3:15—LICONE SYSTEM FOR ANIMAL WASTE
MANAGEMENT
BERNARD HOFFMAN

4:00—IMPACT OF FEDERAL AND STATE POL-
LUTION CONTROL LAWS AND REGULA-
TIONS ON DAIRY PRODUCTS
JOHN B. ADAMS

AFTERNOON—FOOD AND ENVIRONMENTAL
SANITATION SECTION
TUDOR ROOM

P. J. SKULBORSTAD, *Presiding*

1:30—DOOR PRIZE DRAWING

1:35—NUTRITIONAL LABELING
IRA I. SOMERS

2:15—MICROBIOLOGICAL MONITORING OF
THE FOOD PROCESSING PLANT
DAVID BALDOCK

3:00—MILK BREAK
Regimental Corridor

3:15—MICROBIOLOGY OF EGGS
ROBERT C. BAKER

4:00—NEW YORK STATE WINE RESEARCH
WILLARD B. ROBINSON

TUESDAY EVENING, AUGUST 14, 1973

7:30-9:30—EVENING DISCUSSION GROUPS

7:30—FOOD SANITATION
State Room
JOHN BRUHN, *Moderator*
VERNON E. CORDELL
WILLIAM S. LAGRANGE
EMMANUEL SOMERS

7:30—MILK:
Discussion of the 13th edition of Standard
Methods for the Examination of Dairy Prod-
ucts and needs for changes.
Genesee and Crossroads Room
ROBERT T. MARSHALL, *Moderators*
ELMER H. MARTH
SIDNEY E. BARNARD
JAMES H. MARTIN
ROBERT WEIK

7:30—ENVIRONMENTAL SANITATION
Corinthian Room
SHELBY JOHNSON, *Moderator*
J. DAVID BALDOCK
JOHN TESKE
DICK B. WHITEHEAD

WEDNESDAY, AUGUST 15, 1973

8:30 A.M.-5:00 P.M.—Registration—North Corridor,
2nd Floor

MORNING—GENERAL SESSION
WINDSOR ROOM

WALTER F. WILSON, *President, Presiding*

8:30—DOOR PRIZE DRAWING

8:35—FILM, CHOLESTEROL STORY

9:00—ENVIRONMENTAL HEALTH PROGRAM
PLANNING AND IMPLEMENTATION
BAILUS WALKER, JR.

9:45—MILK BREAK
Regimental Corridor

10:00—DOOR PRIZE DRAWING

10:05—ANNUAL BUSINESS MEETING
1. Report of Executive Secretary
2. Report of Secretary-Treasurer

3. Committee Reports
4. 3-A Symbol Council Reports
5. Report of Resolutions Committee
6. Report of Affiliate Council
7. Old Business
8. New Business
9. Election of Officers

12:00-1:30 P.M.—New York State Council of Affiliates Luncheon—Grenadier Room

WEDNESDAY, AUGUST 15, 1973
AFTERNOON—MILK SANITATION SECTION
WINDSOR ROOM

CHARLES G. ASHE, *Presiding*

1:30—DOOR PRIZE DRAWING

1:35—STANDARDS OF IDENTITY FOR FLUID MILK AND MILK PRODUCTS
ROBERT WEIK

2:15—INHIBITORS IN RAW AND FINISHED MILK PRODUCTS
JAMES W. MESSER

3:00—MILK BREAK
Regimental Corridor

3:15—SIGNIFICANCE OF SPORES IN MILK
JAMES H. MARTIN

4:00—REPORT FROM NATIONAL CONFERENCE ON INTERSTATE MILK SHIPMENTS
J. C. SCHILLING

AFTERNOON—ENVIRONMENTAL SANITATION SECTION
STUART ROOM

ORLOWE M. OSTEN, *Presiding*

1:30—DOOR PRIZE DRAWING

1:35—TRAINING FOR RECOGNITION AND EVALUATION OF TARGET HEALTH HAZARDS
JOHN TESKE

2:15—A STATE PLAN FOR OSHA
DICK B. WHITEHEAD

3:00—MILK BREAK
Regimental Corridor

3:15—INSECT CONTROL AND CHEMICAL TOXICITY
JOHN G. MATTHYSSE

4:00—AGRICULTURAL WASTE MANAGEMENT—THE BIG PICTURE
RAYMOND C. LOEHR

WEDNESDAY, AUGUST 15, 1973
AFTERNOON—FOOD INDUSTRY SANITATION SECTION
TUDOR ROOM

RICHARD A. LEDFORD, *Presiding*

1:30—DOOR PRIZE DRAWING

1:35—ENVIRONMENTAL CONTAMINANTS IN FOODS
EMMANUEL SOMERS

2:15—NEW YORK STATE SHELLFISH SANITATION PROGRAM
ROBERT B. MACMILLAN

3:00—MILK BREAK
Regimental Corridor

3:15—SOME THINGS YOU'VE WANTED TO KNOW ABOUT FOODBORNE ILLNESS BUT HESITATED TO ASK
W. S. LAGRANGE

4:00—CHARTING OF A SAFENESS COURSE OF THE FOOD INDUSTRY
VERNON CORDELL

WEDNESDAY EVENING, AUGUST 15, 1973

6:00-7:00 P.M.—COCKTAIL HOUR
Stuart Room

7:00 P.M.—ANNUAL AWARDS BANQUET
Windsor Room
WALTER F. WILSON, *President, Presiding*
INVOCATION
IVAN E. PARKIN
MASTER OF CEREMONIES
JAMES C. WHITE
INTRODUCTIONS
PRESENTATION OF AWARDS
DICK B. WHITEHEAD, *Chairman*

1. Past President's Award
2. Citation Award
3. Honorary Life Membership
4. Sanitarians Award
Sponsored by: Klenzade Products, Inc., Division Economics Laboratories, Pennwalt Chemical, Inc., Diversey Corporation, Inc.
5. Industry and/or Educator Sanitarian's Award
Sponsored by: Farm Equipment Institute

INSTALLATION OF OFFICERS
ENTERTAINMENT

THURSDAY, AUGUST 16, 1973

MORNING—GENERAL SESSION

WINDSOR ROOM

PAT J. DOLAN, *Presiding*

9:00—DOOR PRIZE DRAWING

9:15—A QUALITY ASSURANCE PROGRAM FOR SUPERMARKETS
MARY ELLEN BURRIS

10:00—THE PHOSPHATE DILEMMA
PAUL F. DERR

10:45—PARALYTIC SHELLFISH POISON: A REPORT OF AN OUTBREAK
DANIEL A. HUNT

12:30—EXECUTIVE BOARD MEETING
Board Room

AFTERNOON

1:30 P.M.—VISIT TO THE WINE COUNTRY AND TOUR OF WIDMER'S WINE CELLARS, INC.

(Limited to first 250 people who sign up for Tour)

ENTERTAINMENT MEN AND WOMEN

MONDAY, AUGUST 13, 1973

5:30-6:30 P.M.—RECEPTION
Stuart Room

6:30 P.M.—50TH ANNIVERSARY CELEBRATION DINNER
New York State Association of Milk and Food Sanitarians
Windsor Room

(All are invited to attend)

WEDNESDAY, AUGUST 15, 1973

LUNCHEON

12:00-1:30 P.M.—NYSAMFS COUNCIL OF AFFILIATES
Grenadier Room

6:00-7:00 P.M.—COCKTAIL HOUR
Stuart Room

7:00 P.M.—BANQUET AND ENTERTAINMENT
Windsor Room

THURSDAY, AUGUST 16, 1973

1:30 P.M.—VISIT TO THE WINE COUNTRY AND TOUR OF WIDMER'S WINE CELLARS, INC.

(Limited to first 250 people who sign up for Tour)

ENTERTAINMENT FOR THE LADIES

(Ladies are invited to attend any of the meeting sessions)

MONDAY, AUGUST 13, 1973
Mini-tour of Rochester
Cheese Demonstration and Tasting Session at Hickory Farms Store

TUESDAY, AUGUST 14, 1973
Tour of Xerox Building
Luncheon and Style Show at the Top of the Plaza Restaurant

WEDNESDAY, AUGUST 15, 1973
Continental Breakfast with Speaker from the Rochester Chapter of National Organization of Women (NOW)
Executive Room

LADIES HOSPITALITY ROOM

MONDAY-THURSDAY, AUGUST 13-16, 1973

9:00 A.M.-5:00 P.M.—Executive Room—Main Floor

NATIONAL MASTITIS COUNCIL

1973 REGIONAL MEETING

MONDAY, AUGUST 13, 1973

FLAGSHIP-ROCHESTER, ROCHESTER, NEW YORK

TUDOR ROOM

PROGRAM

Monday morning

9:00—GREETINGS FROM NMC PRESIDENT
HARVEY J. WILHELM

9:10—REPORT ON DAIRYMAN'S BULLETIN — NMC
JOHN H. NICOLAI, JR.

9:30—ECONOMICS OF MASTITIS CONTROL
GLEN H. SCHMIDT, PH.D.

10:15—BREAK

10:30—EVALUATING A NEW MASTITIS TREATMENT
GEORGE J. CHRISTIE, DVM

11:15—WHAT RESEARCH TELLS THE DAIRY-MAN ABOUT MASTITIS
W. Nelson Philpot, Ph.D.

12:00—BREAK

1:30—IMMUNIZATION AS A CONTROL PROCE-DURE FOR MASTITIS
Neil L. Norcross, Ph.D.

2:15—WHAT MILKING MACHINE RESEARCH TELLS US ABOUT THE FUTURE
R. L. Appleby

3:00—BREAK

3:15—COLIFORM MASTITIS — FROM A RE-SEARCH POINT OF VIEW
R. J. Eberhart, DVM, Ph.D.

3:45—COLIFORM MASTITIS — FROM A CLINI-CIAN'S POINT OF VIEW
R. B. Hillman, DVM

4:15—THE DAIRYMAN'S VIEWPOINT
to be announced

PROGRAM PARTICIPANTS

Adams, John B.—Director, Environmental and Consumer Affairs, National Milk Producers Federation, Washington, D. C.

Ashe, Charles G.—District Sales Manager, The Kendall Co., Fayetteville, New York

Baker, Robert C.—Director, Institute of Food Science and Marketing, Cornell University, Ithaca, New York

Baldock, J. David—Department of Food Science and Technology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia

Barnard, Sidney E.—Extension Dairy Specialist, Pennsylvania State University, University Park, Pennsylvania

Bruhn, John C.—Extension Food Technologist, University of California, Davis, California

Burris, Mary Ellen—Director of Consumers Affairs, Wegman Food Markets, Rochester, New York

Calbert, Harold E.—Chairman, Food Science Department, University of Wisconsin, Madison, Wisconsin

Cordell, Vernon E.—Director, Public Health, Safety, and Research Division, National Restaurant Association, Chicago, Illinois

Derr, Paul F.—Research and Development, F.M.C. Corporation, Princeton, New Jersey

Dickerson, Roger W., Jr.—Chief, Food Engineering Branch, Food and Drug Administration, Cincinnati, Ohio

Dolan, Pat J.—Regional Administrator, Bureau of Dairy Service, California Department of Agriculture, Sacramento, California

Hoffman, Bernard—Director of Research, DeLaval Separator Co., Poughkeepsie, New York

Hunt, Daniel A.—Director, Shellfish Sanitation, Food and Drug Administration, Washington, D. C.

Johnson, Shelby—Director, Environmental Services Program, Kentucky State Department of Health, Frankfort, Kentucky

LaGrange, William S.—Extension Food Technologist, Iowa State University, Ames, Iowa

Lane, William B.—Plant Manager, Crowley Foods, Inc., Arkport, New York

Ledford, Richard A.—Chairman, Department of Food Science, Cornell University, Ithaca, New York

Loehr, Raymond C.—Director of Environmental Studies, New York State College of Agriculture and Life Sciences, Cornell University, Ithaca, New York

MacMillan, Robert B.—New York State Environmental Conservation Department, Stony Brook, New York

Marshall, Robert T.—Department of Food Science and Nutrition, University of Missouri, Columbia, Missouri

Marth, Elmer H.—Department of Food Science, University of Wisconsin, Madison, Wisconsin

Martin, James H.—Professor and Head, Dairy Science Department, South Dakota State University, Brookings, South Dakota

Matthysse, John G.—Department of Entomology, Cornell University, Ithaca, New York

Messer, James W.—Microbiologist, Laboratory Branch, Food and Drug Administration, Cincinnati, Ohio

Osten, Orlowe M.—Director, Dairy Industries Division, Minnesota Department of Agriculture, St. Paul, Minnesota

Parkin, Ivan E.—Retired, Westbrook, Connecticut

Robinson, Willard B.—Chairman, Department of Food Science and Technology, New York State Agricultural Experiment Station, Cornell University, Geneva, New York

Schilling, John C.—St. Louis Health Division, St. Louis, Missouri

SKULBORSTAD, P. J.—Vice President, Sales and Marketing, Babson Brothers Co., Oak Brook, Illinois

SOMERS, EMMANUEL—Director, Food Research Laboratories, Dept. of National Health & Welfare, Tunney's Pasture, Ottawa, Ontario, Canada

SOMERS, IRA I.—Executive Vice President, National Canners Association, Washington, D. C.

TESKE, JOHN—OSHA Training Institute, U. S. Department of Labor, Rosemont, Illinois

THOMPSON, HAROLD E., JR.—U. S. Public Health Service, Food and Drug Administration, Washington, D. C.

WALKER, BAILUS, JR.—Director, District of Columbia

Environmental Health Administration, Washington, D. C.

WEIK, ROBERT—Dairy, Fats and Oil Branch, Food and Drug Administration, Washington, D. C.

WHITE, JAMES C.—School of Hotel Administration, Cornell University, Ithaca, New York

WHITEHEAD, DICK B.—Director, Occupational Safety and Health, Mississippi State Board of Health, Jackson, Mississippi

WILSON, WALTER F.—Environmental Management Deputy, Los Angeles County Department of Health, Los Angeles, California

WRIGHT, EARL O.—Extension Food Technologist, Iowa State University, Ames, Iowa

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of regional associations.

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VIRGINIA ASSOCIATION OF SANITARIANS AND DAIRY FIELDMAN OFFICERS ELECTED AT MARCH, 6, 7, 8 ANNUAL MEETING

Pres., J. O. Gunter; 2nd Vice-Pres., M. R. Cooper; 1st Vice-Pres., J. C. Bussey; International Representative, J. G. Hampton; Sec'y.-Treas., W. H. Gill.

ROCKY MOUNTAIN CONFERENCE ON FOOD SAFETY

The reorganization of the Rocky Mountain Association of Milk, Food and Environmental Sanitarians Association got off to an excellent beginning in co-operating with Rocky Mountain Chapter, Institute of Food Technology; Colorado Dairy Technology Society; The University of Wyoming, College of Agriculture, Division of Animal Sciences; Colorado State University, College of Agriculture, Department of Animal Sciences and Program in Food Technology in the Rocky Mountain Conference on Food Safety April 19 and 20 at Colorado State University, Ft. Collins.

The attendance was good in spite of a bad snow storm which prevented attendance by many from Wyoming. The tone of an excellent meeting was set by the key note address of Norman Myrick, Director of Public Relations, Single Service Division, International Paper Company, New York. His subject was, "Facts of Life."

Other excellent topics presented were: The Microbial Facts of Life, Oliver W. Kaufmann, Food and Drug Administration, Cincinnati, Ohio; Milk Section: Safety and Quality of Milk from Cow to the Consumer—A panel discussion; Coordination of personnel efforts in Milk Sanitation and Safety to Consumer—A panel discussion; Food Section: Safety and Quality Control of Meat for the Consumer—A panel discus-

sion; General Session April 20; Cooperation Between Regulatory Agencies and Food Processors: Walter F. Wilson, (President of International Association of Milk, Food and Environmental Sanitarians, Inc.) Environmental Management Deputy, Dept. of Health Service, Los Angeles, Calif.; Panel Discussion: Interfaces within the Food Industry; Douglas McCluskie, Director Environmental Health, Denver Dept. of Health; H. L. Thomasson, Exec. Sec'y., Mg. Ed., IAMFES, Inc.; Hyman S. Fischbach, Consultant, Wyoming-Colorado Restaurant Assoc.

Additional topics at general session were: Overview of Food Product Hazards: Pesticides, Additives, Toxins, Pathogens; K. G. Weckel, Professor, U. of Wis., Dept. of Food Science and Thaddeus F. Midura, Research Microbiologist, State of Calif., Dept. of Public Health, Berkeley, Calif. A special luncheon was presided over by John M. Nussbaumer, City-County of Denver Dept. of Health and Frank Yatchoske, Dairy Section, Colorado State Dept. of Agriculture. John, President and Frank, Sec'y.-Treas. of the Rocky Mountain Association presented Walter Wilson, H. L. Thomasson and K. G. Weckel who discussed history, services and objectives of IAMFES, Inc.

The final session was: Milk Sanitation Surveys: Why? Where? How? participated in by Don K. Summers, Regional Milk & Food Consultant, FDA, PHS, Denver; John Bagholt, Div. of Food and Drug, Colorado State Dept. of Health, Denver; Laurence Hertske, Hertske Brothers Dairy, Greeley; Francis Gregarson, Longmont; and Arden Wolfe, Sinton Dairy, Colorado Springs.

NEW 3-A STANDARD IS BREAK-THROUGH

Approval of a new 3-A standard with particular application to the processing of viscous products was among the major actions taken by the 3-A Sanitary Standards Committees' spring meeting at St. Paul April 10-12.

The new standard is for scraped surface heat exchangers, and is the third document written for the heating process. Earlier standards had dealt with the traditional plate heat and tubular exchangers.

Adoption of the standard "offers a substantial break-through for thermally processing of foods subject to great pressure drops during processing," said Don Williams, secretary of the 3-A committees.

In other actions an amendment to the 3-A storage tank standard, which adds new criteria for insulation

and agitation, was also adopted. "These criteria provide new flexibility for bulkheaded installation," added Williams.

Both the storage tank amendment and heat exchanger standard will be prepared for signing and publication following editorial polishing of the final drafts. Publication is scheduled for late 1973.

In addition, the first complete overhaul in more than 10 years of the farm milk cooling tank standard and first in 23 years for the instrument fittings standard were reviewed by the committees. The revisions, when finally adopted, would update the standards with respect to format, materials definitions and new fabricating technology, as well as making documents more compact, convenient and sophisticated.

Considerable discussion was given to the inclusion in 3-A standards of criteria necessary for equipment to be used in aseptic processing systems sterilized by heat and operated at temperature of 250° F or higher. The committees explored the intricacies of inclusion of such criteria in the standards program.

The committees also reviewed an amendment to the standards for silo tanks, revision of published 3-A standards for pumps, fillers and sealers of single-service containers, and a new standard for uninsulated tanks.

The 3-A program for dairy equipment is the result of cooperation among dairy processors, the users of equipment; dairy industrial suppliers and equippers, the manufacturers and sellers of dairy equipment; and public health officials and sanitarians, the regulatory officials under whose jurisdiction the equipment is installed and used.

Voluntarily supported by the national trade associations in the dairy processing industry, the program has resulted in the adoption of a total of 32 Standards and Practices for dairy equipment. Equipment complying with the standards may carry the 3-A Symbol, provided its manufacturer received authorization to do so from the 3-A Symbol Council.

Generally speaking, the 3-A Standards and Practices are acceptable in public health jurisdictions in nearly every town, city and state in the U. S. The 3-A Sanitary Standards and Practices are cited in the recommended Grade "A" Pasteurized Milk Ordinance of the U. S. Public Health Service.

INTERSOCIETY COUNCIL TO DEVELOP NEW EDITION OF STANDARD METHODS

An Intersociety Council has been organized to develop the 14th edition of *Stardard Methods for the Examination of Dairy Products.* This widely distributed and well recognized book on laboratory methods serves industrial, regulatory, and research workers concerned with milk and milk products. Many methods detailed in the book also have been used to test numerous other food products.

The Intersociety Council is headed by Dr. Elmer H. Marth, Professor of Food Science and Bacteriology, Department of Food Science, University of Wisconsin, Madison. Other members of the Council and the organizations they represent are as follows: Dr. Warren S. Clark, Jr., American Dry Milk Institute, Chicago (representative of Industry); Dr. Jim L. Dizikes, Chemist-In-Charge, Dairy Division Laboratory, U. S. Department of Agriculture, Chicago (representative of U. S. D. A.); Dr. William J. Hausler, Jr., Director, State Laboratory of Hygiene, University of Iowa, Iowa City (representative of the American Public Health Association and editor of the 13th edition of *Standard Methods*); Dr. Robert T. Marshall, Professor of Food Science and Nutrition, University of Missouri, Columbia (representative of the International Association of Milk, Food, and Environmental Sanitarians); Dr. Don W. Mather, Manager, Dairy Products Laboratory, Research and Development Division, Kraftco Corporation, Glenview, Illinois (representative of Industry); Dr. George W. Reinbold, Professor of Dairy Microbiology, Iowa State University, Ames (representative of Academia); Dr. Gary H. Richardson, Professor of Nutrition and Food Sciences, Utah State University, Logan (representative of the American Dairy Science Association); and Dr. William W. Ullmann, Director of the Laboratory Division, Connecticut Department of Health, Hartford (representative of the Association of State and Territorial Public Health Laboratory Directors).

Activities of the Council, which include sponsorship of some studies needed to improve methods described in *Standard Methods,* are funded through a contract between the American Public Health Association and the Food and Drug Administration. The Project Director is Dr. Howard L. Bodily, Staff Associate for Laboratory Programs, American Public Health Association, Midway, Utah, and the Project Officer is Dr. Ralston B. Read, Jr., Deputy Director, Division of Microbiology, Food and Drug Administration, Washington, D. C.

The Council is now identiflying changes needed in the book to update the 14th edition and to make it more useful to laboratory workers. Committees to revise the chapters and appendices are also being developed.

Anyone who is familiar with the 13th edition of *Standard Methods* and who has suggestions for improvement of the book is invited to communicate with the Chairman or any member of the Council.

TRI-CLOVER GOLD VALVE PRESENTED TO THE KROGER CO.

Accepting the gold plated valve from R. L. Nissen (right) General Sales Manager, Tri-Clover Division, are Ronald Rice (middle) Manager of the new Kroger dairy products plant in Indianapolis and William Siffermiller, Manager of production and plant engineering for the Kroger Co.

Ladish Co., Tri-Clover Division marked its 50,000th Air-Actuated Valve with an unique piece of industrial sculpture—a 24 kt. gold valve now on display at the Kroger Co.'s new Indianapolis, Indiana dairy products processing plant.

According to R. L. Nissen, Ladish Co., Tri-Clover Division's General Sales Manager, the gold valve serves ". . . to emphasize the increased use of automated process flow control and cleaning systems by processors in order to meet the almost impossible demands being placed on them by an expanding economy and population."

The first sanitary air-actuated valve was installed by Tri-Clover in 1958, the 15,000th in 1965, the 50,000th last October with more than 6,700 being prduced since then. The gold valve, though operable, will not be put on stream at Kroger where it was one of 345 Tri-Flo Valves specified for use in the Indianapolis plant.

Economics Laboratory, Inc., Equipment-Engineering Division, Beloit, Wisconsin, had the responsibility for the design and installation of the process flow control and cleaning system.

REPORT OF THE BAKING INDUSTRY EQUIPMENT COMMITTEE, 1971-1972

This committee had two meetings with the Baking Industry Sanitation Standard Committee (BISSC) since our report last year. The Fall meeting was held in Miami Beach, Florida. At this meeting Paul Laughlin, Director of Sanitation for the National Biscuit Company was elected Chairman of BISSC. Mr. Laughlin succeeds Phillip Winters of the Kroger Company who was Chairman of BISSC for the

last 10 years. It was decided to revise and update all published bakery standards including BISSC Basic Criteria. This revision is actively being pursued and will continue until all standards have been revised. This continuous revision is necessary because of the rapid strides being made in improved bakery equipment.

The Spring meeting was held in Chicago, Illinois and three standards, Conveyors, Donut Equipment, and Electric Motors and Accessory Equipment were approved as revised. Revisions of the Basic Criteria, Casters and Mechanical Ovens Standard were preliminary accepted,

To date 34 bakery standards have been approved and published. These bakery standards are available without charge to members of this Association by writing to the Executive Secretary of BISSC, 521-5th Ave., N.Y., N.Y. 10017.

This Committee believes that conversion to the metric system is making rapid strides. This committee is therefore recommending to BISSC that all standards published in the future show the metric equivalent of any dimensions given in inches or feet. The Committee also requests that sanitarians everywhere acquaint themselves with BISSC Standards and the availability of bakery equipment manufactured to BISSC Standards.

Vincent T. Foley, *Chairman*, City Health Department, 21st Floor City Hall, Kansas City, Missouri 64106.

Louis A. King, Jr., American Institute of Baking, 400 East Ontario, Chicago, Illinois 60611.

Harold Wainess, Wainess and Associates, 510 North Dearborn Street, Chicago, Illinois 60610.

A. E. Abrahamson, City Health Department, 125 Worth Street, New York, New York.

Fred Vitale, Continental Baking Company, Inc., P. O. Box 731, Rye, New York 10580.

REPORT OF THE COMMITTEE ON SANITARY PROCEDURES, 1971-1972

The Committee on Sanitary Procedures of the International Association of Milk, Food, and Environmental Sanitarians is one of the outstanding examples of work which IAMFES has done throughout the years together with the regulatory agencies, namely the Public Health Service (now PHS/FDA), and the trade associations on the 3-A Standards Committees to develop equipment standards for fabrication of dairy equipment. So much progress has been made in this field that other food industries are now looking to the 3-A Standards Committees for guidance and also, in some instances, full participation in promulgating standards for that particular industry.

Throughout the years many outstanding members of IAMFES have served on the Committee on Sanitary Procedures as working members and also as chairmen. We have been particularly fortunate in having outstanding men who have given willingly of their time to participate in development of fabrication standards for dairy equipment. These men have contributed knowledge which they gained through many years of experience in the dairy field.

During the past 2 years the Committee on Sanitary Procedures has undergone some changes in membership and also in chairmanship. We have had such outstanding chairmen as C. A. Abele who probably was one of the most outstanding sanitarians insofar as years of service and who gave many years of his life to the work of CSP. Mr. Abele was then followed by an outstanding chairman, D. B. Whitehead, who contributed tremendously in a short time. It was rather unfortunate that Mr. Whitehead went into another area of work which certainly is a challenge to him and one in which

he will gain tremendous stature but, unfortunately for CSP, he could not continue as chairman of the committee. Mr. Whitehead and the president of IAMFES, Mr. Orlowe Osten, asked me to take over the chairmanship of the committee for a period so we might reorganize and make some changes in membership of the committee. I have agreed to do this and with the cooperation of the president and Mr. Whitehead, who at this point has agreed to serve as vice-chairman of the committee, we will proceed in that direction.

At a meeting of CSP at Atlanta, Georgia a year ago, the committee made certain recommendations which we have passed on to the president of IAMFES. One of them was that the CSP be broadened to bring in new individuals who have a broad background not only in milk sanitation but in the entire food field. As you know, other food processing industries have approached 3-A for standards for their equipment.

In the coming year as chairman of CSP I intend to follow through on this recommendation. To do so I will need the help of the secretary of your affiliate to establish a reservoir of manpower that we can draw from in making our recommendations to the president of IAMFES in increasing the membership of the Committee. This will also be helpful when vacancies occur so that we can immediately make recommendations for replacements.

We would like your wholehearted cooperation in submitting to us names of outstanding members in your affiliate who have a broad background in the area of milk and food and we would like to have it evenly divided between representatives of official agencies such as health departments, state or local, and state departments of agriculture. We were wondering if you could give us a short resume on the background of each individual. We will then contact them with an invitation to serve on the Committee on Sanitary Procedures.

Would it be asking too much if you would give us your recommendations by October 1 so that we then can make our recommendations to the new president of IAMFES who has the authority to make the appointment. As chairman of CSP I am only in a position of making recommendations to the president.

When you are sending the suggested names, please remember that all members whom you recommend may not be selected because we will have to keep in mind that we have geographical areas that should be represented. We believe that any member whom you recommend should feel honored to be recommended to serve on this very important committee. I will be eagerly awaiting your reply.

C. K. LUCHTERHAND, *Chairman*
P. O. Box 309, Madison, Wis. 53701

THE PENNSYLVANIA STATE UNIVERSITY COLLEGE OF AGRICULTURE
31st Annual Dairy Fieldmen's Conference
June 12 and 13, 1973

The annual Dairy Fieldmen's Conference is going to be held at The Pennsylvania State University on June 12 and 13, 1973. All meetings will be held in the auditorium of the J. Orvis Keller Building, and the banquet will be held on Tuesday evening at the Nittany Lion Inn. Registrants should make reservations directly with the hotel or motel of their choice at their earliest opportunity, as several other large conferences will be meeting concurrently.

The fee is $15.00 per person and includes: Registration,

conference proceedings, banquet, and Dairy Fieldmen's Scholarship. For further information write: Agricultural Conference Coordinator, Room 410, J. Orvis Keller Building, The Pennsylvania State University, University Park, Pennsylvania 16802.

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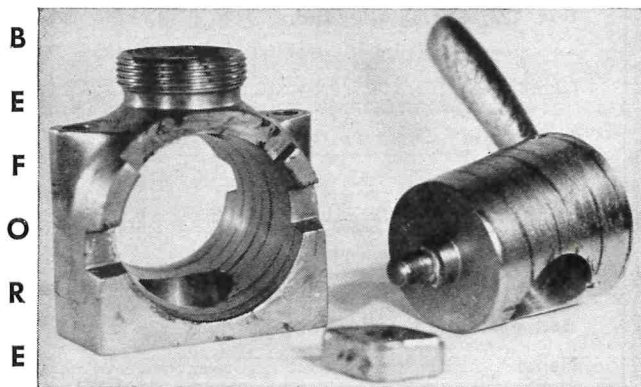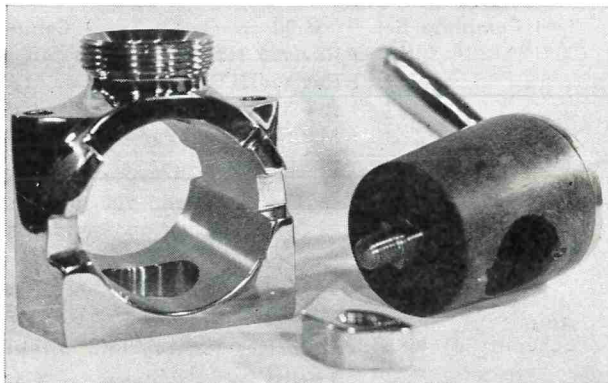
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