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# MILK and FOOD TECHNOLOGY.

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*Official Publication*

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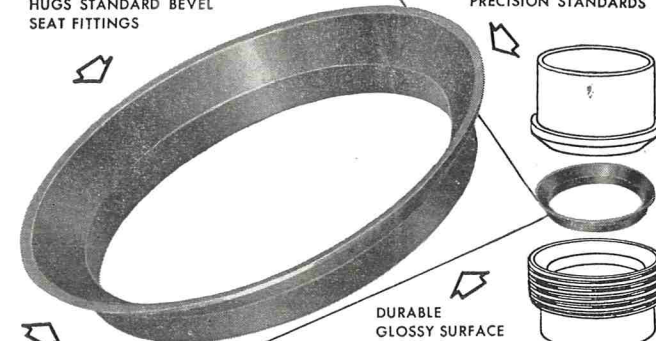
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Journal of

# MILK and FOOD TECHNOLOGY

INCLUDING MILK AND FOOD SANITATION

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# INHIBITION OF LACTIC STREPTOCOCCI AND SOME PATHOGENIC BACTERIA BY CERTAIN MILK-ASSOCIATED VOLATILE COMPOUNDS AS MEASURED BY THE DISC ASSAY<sup>1</sup>

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(Received for publication February 13, 1970)

## ABSTRACT

Twenty-seven milk-associated volatile compounds were tested with the disc assay procedure for their ability to inhibit *Streptococcus cremoris*, *Streptococcus lactis*, *Streptococcus diacetylactis*, *Leuconostoc citrovorum*, *Escherichia coli*, *Salmonella typhimurium*, and *Staphylococcus aureus*. Anisaldehyde, butyraldehyde, formaldehyde, glyoxal, diacetyl, butyric acid, decanoic acid, formic acid, hexanoic acid, octanoic acid, hexylamine, and propylamine inhibited all test cultures although frequently only at concentrations of 10,000 and 100,000 ppm. In addition, isobutyraldehyde (100,000 ppm) inhibited *L. citrovorum*, *E. coli*, *S. typhimurium*, and *S. aureus* and acetaldehyde (100,000 ppm) inhibited *S. aureus*. Compounds most active (inhibition at 1,000 ppm) against *S. lactis*, *L. citrovorum*, and *S. aureus* were formaldehyde, glyoxal, and decanoic acid; against *S. cremoris* were formaldehyde, butyric acid, decanoic acid, formic acid, and hexanoic acid; against one strain of *S. diacetylactis* were glyoxal, diacetyl, and decanoic acid; against another strain of *S. diacetylactis* were formaldehyde, decanoic acid, and formic acid; against *E. coli* were formaldehyde and formic acid; and against *S. typhimurium* were formaldehyde, diacetyl, butyric acid, formic acid, and hexanoic acid. At a concentration of 1,000 ppm formaldehyde inhibited all test cultures except the fast acid-producing *S. diacetylactis* and decanoic acid all but *E. coli* and *S. typhimurium*. The disc assay procedure was unsatisfactory for evaluating highly volatile compounds since they evaporated before adequate diffusion into the agar occurred.

The presence of volatile compounds in raw and heated skimmilk, whole milk, and cream has attracted the attention of research workers since about the mid 1950's although some isolated reports have appeared earlier. Since then many different volatile compounds have been identified as present in raw or mildly heated milks. Table 1 lists many of these compounds together with the source(s) from which the chemicals were recovered, and provides references to the literature where details about recovery procedures and identification methods can be found. Although the list in Table 1 is not intended to be exhaustive, it does indicate the great variety of volatile compounds which have been identified as present in milk.

With the exception of fatty acids, relatively little attention has been given to the possible effects of these volatile compounds on the growth of bacteria. As far back as 1940 Tarassuk and Smith (57) confirmed the inhibitory effect of rancid milk on *Streptococcus lactis*, a phenomenon first observed by Koestler in 1928 (33). Since then other investigators have reported that free fatty acids inhibited lactic acid bacteria (1, 12, 13, 20, 42, 43, 45, 46), *Escherichia coli* (13, 20), *Aerobacter* (20), *Proteus* (20), and *Staphylococcus aureus* (61).

Diacetyl, at concentrations of 0.05 to 0.25%, has been found most active against gram-negative bacteria and increasingly less inhibitory to gram-positive streptococci and lactobacilli (21). Acetaldehyde, produced by the acid forming lactic streptococci, has been found to stimulate *Leuconostoc citrovorum* (39).

The experiments described in this paper were designed to determine if a number of volatile compounds associated with raw or mildly heated milk are able to inhibit growth of certain bacteria as measured by the disc assay procedure. A preliminary report of some of the results has been presented (38).

## MATERIALS AND METHODS

### Chemicals

The 27 chemicals used in this study included some of the compounds reported as present in raw or mildly heated milk or they were commercially available representatives of a general class of compounds found to be present in such milks. From such a class of compounds only those with the lower boiling points were selected for this study. The chemicals used and their sources are: acetaldehyde, chloroform, ether, and formaldehyde from Mallinckrodt Chemical Works, St. Louis; acetone from University of Wisconsin Stores; acetonitrile, ethylene dichloride, and formic acid from J. T. Baker Chemical Co., Phillipsburg, N. J.; anisaldehyde, 2-butanone, butyric acid, decanoic acid, hexanoic acid, hexylamine, methanethiol, methyl sulfide, octanoic acid, 2-pentanone, propionaldehyde, and propylamine from Eastman Organic Chemicals, Distillation Products Industries, Rochester, N. Y.; and butyraldehyde, diacetyl, ethanethiol, glyoxal, isobutyraldehyde, 1-propanethiol, and 2-propanethiol from Aldrich Chemical Co., Inc., Milwaukee, Wisconsin.

### Cultures

The following cultures were used in these experiments: *Streptococcus lactis* (G. W. Reinbold, Iowa State Univ., Ames);

<sup>1</sup>Published with approval of the Director of the Research Division, College of Agricultural and Life Sciences, University of Wisconsin.

*Streptococcus cremoris* (J. J. Jezeski, Univ. of Minnesota, St. Paul); *Streptococcus diacetilactis*, fast (Reinhold) and slow (B. J. Liska, Purdue Univ., Lafayette, Ind.) acid-producing strains; *Leuconostoc citrovorum* (U. W. Food Science culture collection), *Staphylococcus aureus* (K. F. Weiss, U. W. Food Research Institute), *Escherichia coli* (U. W. Food Science culture collection), and *Salmonella typhimurium* (J. M. Goepfert, U. W. Food Research Institute).

#### Disc assay procedure

The inhibitory effect of the chemicals on the above listed organisms was tested using a disc assay procedure. The test was performed with over-lay agar plates as described for the antibiotic assay (14). Elliker's agar (15) was used for lactic acid bacteria and plate count agar (Difco) for the other organisms. Five milliliters of the suitable medium served as the bottom layer and 3 ml of the same seeded soft agar was added as the top layer. Soft agars were used to permit better diffusion of chemicals from discs and were prepared with one-half the amount of agar normally used in the two media. Soft agars were seeded with an active 18-24 hr old culture of the test organism. Autoclaved skim milk (for lactic acid bacteria), brain heart infusion broth-Difco (for *S. typhimurium* and *S. aureus*), and nutrient broth-Difco (for *E. coli*) were used to grow the cultures used in these tests. Soft agar was inoculated so that it contained  $10^{-2}$  and  $10^{-3}$  dilutions of test cultures. Greater sensitivity was obtained when the  $10^{-3}$  dilution was used and hence only those results are included in this paper.

Duplicate antibiotic assay discs (0.5 inch in diameter) moistened with decimal dilutions (1 to 100,000 ppm) of the test chemical were placed on the seeded soft agar. Plates were incubated for 18 hr at 30 C when inoculated with lactic acid bacteria and at 37 C when inoculated with the other organisms. A clear zone around the disc was considered as a positive indication of inhibition. The diameter of each zone was determined and recorded.

## RESULTS AND DISCUSSION

### Inhibition of *Streptococcus cremoris* and *Streptococcus lactis*

Data recorded in Table 2 indicate that of those chemicals tested, formaldehyde, butyric acid, decanoic acid, formic acid, and hexanoic acid were most active against *S. cremoris*, whereas similar activity (inhibition at the 1,000 ppm concentration) against *S. lactis* was only exhibited by formaldehyde, glyoxal, and decanoic acid. It is interesting to note that the two lactic streptococci were not affected equally by the fatty acids—compounds which have been found inhibitory to starter cultures.

It is evident from the data in Table 2, as well as from that in Tables 3 and 4, that the zone of inhibition increased in size as the concentration of chemical increased but not proportionally. According to observations recorded in Table 2, 15 of the test chemicals failed to register inhibition of these organisms by the method employed in these trials. These were the highly volatile compounds and they may have evaporated from the discs prior to diffusion into the agar. In fact, as will be discussed in another paper, when added to milk most of them

tended to retard the growth of *S. cremoris*. Consequently, the values in this and subsequent tables which clearly indicate inhibition are most meaningful.

### Inhibition of *Streptococcus diacetilactis* and *Leuconostoc citrovorum*

Two strains of *S. diacetilactis* and one of *L. citrovorum* served as test organisms and results of trials with these bacteria are recorded in Table 3. Decanoic acid was the single compound which proved to be most inhibitory to both strains of *S. diacetilactis* and *L. citrovorum*. In addition, glyoxal and diacetyl were most active against the fast acid producing strain of *S. diacetilactis*, whereas formaldehyde and formic acid proved most detrimental to the slow acid producing strain of this bacterium. In addition to decanoic acid, formaldehyde and glyoxal exhibited greatest activity against *L. citrovorum*.

Of those chemicals which were inhibitory, least activity was exhibited by anisaldehyde and butyraldehyde. A similar observation also was made when *S. cremoris* and *S. lactis* served as the test organisms. Isobutyraldehyde, which failed to inhibit the other lactic acid bacteria, exhibited some activity against *L. citrovorum* but only at a concentration of 100,000 ppm.

### Inhibition of *Escherichia coli*, *Salmonella typhimurium*, and *Staphylococcus aureus*

Data in Table 4 indicate that *E. coli*, *S. typhimurium*, and *S. aureus* were inhibited by virtually the same group of compounds which proved active against *L. citrovorum*. Acetaldehyde was the single exception and it was active only at the 100,000 ppm concentration and only against *S. aureus*. Again, anisaldehyde, butyraldehyde, and isobutyraldehyde were least active of those compounds which retarded growth of *E. coli*, *S. typhimurium*, and *S. aureus*.

It is interesting to note that one compound each, at the 100 ppm concentration, inhibited *S. typhimurium* (formic acid) and *S. aureus* (glyoxal). Other compounds with marked inhibitory activity against *S. typhimurium* include formaldehyde, diacetyl, butyric acid, and hexanoic acid and against *S. aureus* include formaldehyde and decanoic acid. Only formic acid and formaldehyde, at a concentration of 1,000 ppm, were able to inhibit *E. coli*.

It is evident from the data in this and the preceding tables that nearly always at least 1,000 ppm (0.1%) of the active chemicals was required to inhibit the test organism. This is a far higher concentration of these substances than might be expected to occur in milk. Milk, however, contains a mixture of the test chemicals as well as other compounds and additive or synergistic effects of certain chemicals, if present, may make some milks more and others less



TABLE 1. VOLATILE COMPOUNDS REPORTED AS PRESENT IN RAW OR TREATED MILK OR IN CERTAIN CONSTITUENTS OF MILK.

Chemical	Reported sources(s)	References
<i>Acids</i>		
Free fatty	In milk	8, 19, 22, 24, 25, 26, 31, 34, 35, 41, 54, 55, 59
Acetic acid	In milk	19
Butyric acid	In milk and pasteurized milk	19, 35, 59
Capric (decanoic) acid	In milk and pasteurized milk	35, 59
Caproic (hexanoic) acid	In milk	19, 35
Caprylic (octanoic) acid	In milk	19, 35
Linoleic acid	In milk	54
Linolenic acid	In milk	54
Oleic acid	In milk	19
Propionic acid	In milk	19
Valeric acid	In milk	19
4:0 to 26:0 even no.	In raw, standardized, pasteurized, and pasteurized homogenized milk	22, 31, 54
5:0 to 25:0 odd no.	In milk	22
10:1 to 24:1 even no.	In milk	22
15:1 to 23:1 odd no.	In milk	22
Formic acid	In fresh raw and heated milks	2, 3, 17, 18, 19
<i>Alcohols</i>		
Acetyl-methyl-carbinol	In fresh milk	9
Ethanol	In milk	40, 67
Furfuryl	In heated skim milk	50
2-Hydroxypropanol	In milk fat	28
Methanol	In milk	40
n-Propanol	In milk	40
<i>Aldehydes</i>		
Acetaldehyde	In normal and sunlight treated milk	7, 16, 64, 66, 67, 68, 69
Crotonaldehyde	In skim milk	16
C <sub>5</sub> -C <sub>11</sub> 2-unsaturated	In skim milk	16
Formaldehyde	In raw milk and cream and in sunlight treated milk	62, 64, 66
Propionaldehyde	In milk	7, 40
<i>Amines</i>		
Hexylamine	In heated milk with feed flavor	11
Propylamine	In heated milk with feed flavor	11
<i>Ammonia</i>	In pasteurized and boiled milk	4, 10, 11
<i>Ketones</i>		
Acetone	In normal milk and skim milk with oxidized flavor, and in milk fat	6, 7, 16, 40, 44, 64, 66, 67, 68, 69
Butanone	In milk and cream, winter milk, and sunlight treated milk	6, 7, 40, 64, 65, 66, 67, 68
Butanone-2	In heated milk fat	44
C <sub>3</sub> (acetone) through C <sub>15</sub> (pentadecanone)	In milk fat	48
Diacetyl	In fresh milk	9, 60
Heptanone-2	In heated milk fat	44
Hexanone-2	In heated milk fat, milk, cream	44, 66, 67
Nonanone-2	In heated milk fat	44
Octanone-2	In heated milk fat	44
Pentanone-2	In heated milk fat, milk, cream	40, 44, 65, 66, 67
Undecanone-2	In heated milk fat	44
<i>Lactones</i>		
Delta-decalactone	In fresh and heated milk fat, and pasteurized homogenized milk	29, 47, 58
Delta-dodecalactone	In fresh milk fat and in pasteurized homogenized milk	47, 58
Trans-4-methyl-5-hydroxy-hexanoic acid lactone	In normal milk and skim milk	23
<i>Sulfur compounds</i>		
Dimethyl sulfone	In solvent extracts of milk	63
Disulfide compounds	In raw and heated milk	52, 56
Hydrogen sulfide	In fresh, pasteurized, homogenized, and heated milks	5, 36, 37, 51, 52, 53, 51, 52, 53
Mercaptans	In raw and heated milk	27, 40, 49, 67, 69
Methyl sulfide	In raw, heated, and vacuum treated milk	27, 40, 49, 67, 69
Sulphydryl compounds	In raw and heated milk, skim milk, and heated whey	30, 32, 56, 70
Thiamine disulfide	In heated milk	37
Thionals	In heated milk	52
Thiones	In heated milk	52
<i>Other compounds</i>		
Acetonitrile	In milk	67
Chloroform	In milk	67
Ethylether	In milk	40, 67
Ethylene chloride	In milk	67

TABLE 2. INHIBITION OF *Streptococcus cremoris* AND *Streptococcus lactis* BY VARIOUS VOLATILE COMPOUNDS AS MEASURED BY DISC ASSAY PROCEDURE<sup>1</sup>

Chemical <sup>2</sup>	<i>S. cremoris</i>			<i>S. lactis</i>		
	Conc. of chemical (ppm)			Conc. of chemical (ppm)		
	1,000	10,000	100,000	1,000	10,000	100,000
	-----Diameter of zones in mm-----					
Anisaldehyde	— <sup>3</sup>	23.5	28.0	—	—	17.5
Butyraldehyde	—	—	20.0	—	—	15.0
Formaldehyde	30.0	66.0	NG <sup>4</sup>	18.5	46.0	NG
Glyoxal	—	30.0	48.0	15.5	22.0	42.5
Diacetyl	—	16.5	25.5	—	15.0	NG
Butyric acid <sup>5</sup>	19.5	56.0	NG	—	16.5	42.5
Decanoic acid <sup>5</sup>	22.5	42.5	43.0	17.0	25.5	26.0
Formic acid <sup>5</sup>	15.0	37.5	41.0	—	28.0	51.0
Hexanoic acid <sup>5</sup>	20.0	60.5	NG	—	17.0	38.0
Octanoic acid	—	29.0	45.5	—	22.0	27.5
Hexylamine	—	16.5	NG	—	17.5	66.0
Propylamine	—	25.0	NG	—	15.5	41.5

<sup>1</sup>Values are averages of two replicates obtained when agar was seeded with a 10<sup>-3</sup> dilution of test culture.

<sup>2</sup>The following chemicals were without effect when tested by the disc assay procedure: acetaldehyde, acetone, acetonitrile, 2-butanone, chloroform, ethanethiol, ether, ethylenedichloride, isobutyraldehyde, methanethiol, methylsulfide, 1-propanethiol, 2-propanethiol, 2-pentanone, and propionaldehyde.

<sup>3</sup>Dash = no inhibition.

<sup>4</sup>NG = no growth (e.g., complete inhibition).

<sup>5</sup>No inhibition detected when 100 ppm was tested.

suitable for the growth of certain bacteria, particularly starter cultures. These data suggest that removal of some volatile compounds from milk, as might occur in certain processing procedures which employ a vacuum treatment, may affect its suitability as a substrate for some bacteria.

#### REFERENCES

- Anders, R. F., and G. R. Jago. 1964. The effect of fatty-acids on the metabolism of lactic streptococci. I. Inhibition of bacterial growth and proteolysis. *J. Dairy Res.* 31:81-89.
- Auclair, J. E., and A. Portman. 1958. The effect of heat treating milk on bacterial growth. II. The stimulatory effect of autoclaved milk on the growth of *L. lactis*. Role of formic acid. *Ann. Inst. Nat. Rechn. agron., Paris Ser. E. (Ann. Tech. Agric.)*. 7:129-150. (*Dairy Sci. Abstr.* 22:88).
- Auclair, J. E., and A. Portman. 1959. Influence of heating of milk on the development of bacteria. The stimulating effect of autoclaved milk in the growth of *Lactobacillus lactis*: Role of formic acid. *Lait* 39:496-519. (*Chem. Abstr.* 54:18817).
- Bagnulo, R. 1958. Technique for the true determination of ammonia. *Latte* 32:623-624. (*Dairy Sci. Abstr.* 21:40-41).
- Bartkiewicz, S. A. 1955. Critical analytical chemical study of light activated flavor in milk. *Diss. Abstr.* 15 (1): 22.
- Bassette, R., and C. H. Whitnah. 1961. Mass spectrometry and gas chromatography applied to the study of volatiles in milk. *J. Dairy Sci.* 44:1164.
- Bassette, R., S. Ozeris, and C. H. Whitnah. 1963. Direct chromatographic analysis of milk. *J. Food Sci.* 28:84-90.
- Bills, D. D., L. L. Khatri, and E. A. Day. 1963. Methods for the determination of the free fatty acids of milk fat. *J. Dairy Sci.* 46:1342-1347.
- Catalano, M. 1956. Contents of citric acid, diacetyl and acetylmethylcarbinol in fresh cow's milk. *Ann. Fac. Agr. Bari.* 10:155-162. (*Dairy Sci. Abstr.* 21:169).
- Choi, R. P., C. M. O'Malley, and B. W. Fairbanks. 1946. Colorimetric determination of ammonia in milk and dry products of milk. *J. Dairy Sci.* 29:645-649.
- Cole, D. D., W. J. Harper, and C. L. Hankinson. 1961. Observations on ammonia and volatile amines in milk. *J. Dairy Sci.* 44:171-173.
- Costilow, R. N., and M. L. Speck. 1951. Inhibition of *Streptococcus lactis* in milk by fatty acids. *J. Dairy Sci.* 34:1104-1110.
- Costilow, R. N., and M. L. Speck. 1951. Inhibitory effect of rancid milk on certain bacteria. *J. Dairy Sci.* 34:1119-1127.
- Difco Manual. 9th ed. 1966. Difco Laboratories, Inc. Detroit, Michigan.
- Elliker, P. R., A. W. Anderson, and G. Hannesson. 1956. An agar culture medium for lactic acid streptococci and lactobacilli. *J. Dairy Sci.* 39:1611-1612.
- Forss, D. A., E. G. Pont, and W. Stark. 1955. The volatile compounds associated with oxidized flavor in skim milk. *J. Dairy Res.* 22:91-102.
- Gould, I. A., 1945. The formation of volatile acids in milk by high temperature heat treatment. *J. Dairy Sci.* 28:379-386.
- Gould, I. A., and R. S. Frantz. 1946. Formic acid content of milk heated to high temperatures as determined by the distillation procedure. *J. Dairy Sci.* 29:27-31.
- Hankinson, C. L., W. J. Harper, and E. Mikolajcik. 1958. A gas liquid chromatographic method for volatile fatty-acids in milk. *J. Dairy Sci.* 41:1502-1509.
- Hassinen, J. B., G. T. Durbin, and F. W. Bernhart. 1951. The bacteriostatic effects of saturated fatty acids. *Arch. Biochem. Biophys.* 31:183-189.
- Hedgecock, L. W., and L. R. Jones. 1950. Antibacterial activity of diacetyl. *Bact. Proc.* 1950:127-128.
- Herb, S. F., P. Magidman, F. E. Luddy, and R. W. Riemenschneider. 1962. Fatty acids of cows' milk. B. Composition by gas liquid chromatography aided by other methods of fractionation. *J. Amer. Oil Chem. Soc.* 39:142-146.
- Henkanen, E., T. Moisio, P. Karvonen, A. I. Virtanen, and J. Paasivirta. 1968. On the occurrence of a new lactone compound trans-4-methyl-5-hydroxy-hexanoic acid lactone, in milk. *Acta Chem. Scand.* 22:2041-2043. (*Dairy Sci. Abstr.* 31:98).
- Jenness, R., and S. Patton. 1959. Principals of dairy chemistry. John Wiley and Sons, Inc. New York.
- Jensen, R. G., G. W. Gander, and J. Sampugna. 1962. Fatty-acid composition of the lipids from pooled raw milk. *J. Dairy Sci.* 45:329-331.
- Jensen, R. G., J. G. Quinn, D. L. Carpenter, and J. Sampugna. 1967. Gas liquid chromatographic analysis of

TABLE 3. INHIBITION OF *Streptococcus diacetylactis* AND *Leuconostoc citrovorum* BY VARIOUS VOLATILE COMPOUNDS AS MEASURED BY THE DISC ASSAY PROCEDURE<sup>1</sup>

Chemical <sup>4</sup>	<i>S. diacetylactis</i> <sup>2</sup>			<i>S. diacetylactis</i> <sup>3</sup>			<i>L. citrovorum</i>		
	Conc. of chemical (ppm)			Conc. of chemical (ppm)			Conc. of chemical (ppm)		
	1,000	10,000	100,000	1,000	10,000	100,000	1,000	10,000	100,000
	Diameter of zones in mm								
Anisaldehyde	— <sup>5</sup>	—	16.0	—	—	20.0	—	—	20.5
Butyraldehyde	—	—	15.5	—	—	15.0	—	—	15.5
Isobutyraldehyde	—	—	—	—	—	—	—	—	17.5
Formaldehyde <sup>7</sup>	—	58.0	NG <sup>6</sup>	20.5	55.0	NG	21.5	55.5	NG
Glyoxal <sup>7</sup>	14.5	30.5	43.0	—	31.0	44.5	17.0	35.0	53.5
Diacetyl <sup>7</sup>	23.5	20.5	NG	—	17.5	NG	—	—	NG
Butyric acid	—	22.0	46.0	—	27.0	54.0	—	16.0	41.5
Decanoic acid <sup>7</sup>	22.0	36.0	40.5	20.5	34.0	35.0	18.0	31.5	35.0
Formic acid <sup>7</sup>	—	29.0	56.5	15.5	42.0	63.5	—	28.5	58.0
Hexanoic acid	—	24.0	44.0	—	25.5	51.5	—	19.5	46.5
Octanoic acid	—	34.5	40.5	—	29.5	39.5	—	30.5	35.0
Hexylamine	—	20.5	52.0	—	20.5	51.0	—	16.0	57.5
Propylamine	—	16.5	46.0	—	17.0	41.0	—	17.5	60.0

<sup>1</sup>Values are averages of two replicates obtained when agar was seeded with 10<sup>-3</sup> dilution of test culture.

<sup>2</sup>Fast acid producing strain.

<sup>3</sup>Slow acid producing strain.

<sup>4</sup>The following chemicals were without effect when tested by the disc assay procedure: acetaldehyde, acetone, acetonitrile, 2-butanone, chloroform, ethanethiol, ether, ethylene dichloride, methanethiol, methylsulfide, 1-propanethiol, 2-propanethiol, 2-pentanone, and propionaldehyde.

<sup>5</sup>Dash = no inhibition.

<sup>6</sup>NG = no growth (e.g., complete inhibition).

<sup>7</sup>No inhibition detected when 100 ppm was tested.

TABLE 4. INHIBITION OF *Escherichia coli*, *Salmonella typhimurium*, AND *Staphylococcus aureus* BY VARIOUS COMPOUNDS AS MEASURED BY THE DISC ASSAY TEST PROCEDURE<sup>1</sup>

Chemical <sup>2</sup>	<i>E. coli</i>			<i>S. typhimurium</i>				<i>S. aureus</i>			
	Conc. of chemical (ppm)			Conc. of chemical (ppm)				Conc. of chemical (ppm)			
	1,000	10,000	100,000	100	1,000	10,000	100,000	100	1,000	10,000	100,000
	Diameter of zones in mm										
Acetaldehyde	— <sup>3</sup>	—	—	—	—	—	—	—	—	—	18.0
Anisaldehyde	—	—	20.5	—	—	—	37.5	—	—	22.0	36.5
Butyraldehyde	—	—	16.5	—	—	—	15.0	—	—	—	17.5
Isobutyraldehyde	—	—	23.0	—	—	—	20.0	—	—	—	19.5
Formaldehyde <sup>5</sup>	21.0	42.5	NG <sup>4</sup>	—	22.0	54.5	NG	—	33.5	68.0	NG
Glyoxal <sup>6</sup>	—	26.0	44.0	—	—	22.5	44.0	15.5	33.5	55.0	67.0
Diacetyl	—	NG	NG	—	17.0	NG	NG	—	—	NG	NG
Butyric acid	—	24.0	60.5	—	16.0	16.0	61.5	—	—	28.5	68.5
Decanoic acid	—	—	16.5	—	—	15.0	16.0	—	18.5	30.5	38.0
Formic acid <sup>7</sup>	15.0	34.0	57.0	15.0	15.0	31.0	35.0	—	—	41.0	NG
Hexanoic acid	—	17.5	59.0	—	15.0	17.5	52.5	—	—	31.5	61.5
Octanoic acid	—	16.5	30.0	—	—	24.5	34.0	—	—	36.0	42.5
Hexylamine	—	17.0	NG	—	—	37.0	NG	—	—	20.5	NG
Propylamine	—	16.5	NG	—	—	20.0	NG	—	—	—	NG

<sup>1</sup>Values are averages of two replicates obtained when agar was seeded with 10<sup>-3</sup> dilution of test culture.

<sup>2</sup>The following chemicals were without effect when tested by the disc assay procedure: acetone, acetonitrile, 2-butanone, chloroform, ethanethiol, ether, ethylenedichloride, methanethiol, methylsulfide, 1-propanethiol, 2-propanethiol, 2-pentanone, and propionaldehyde.

<sup>3</sup>Dash = no inhibition.

<sup>4</sup>NG = No growth (e.g., complete inhibition).

<sup>5</sup>No inhibition when 100 ppm was tested.

<sup>6</sup>No inhibition of *S. aureus* when 10 ppm was tested.

<sup>7</sup>No inhibition of *E. coli* when 100 ppm was tested or of *S. typhimurium* when 10 ppm was tested.

- milk fatty acids. A review. *J. Dairy Sci.* 50:119-126.
27. Keenan, T. W., and R. C. Lindsay. 1967. Dimethyl sulfide and its precursor in milk. *J. Dairy Sci.* 50:950.
  28. Keeney, M., and F. J. Doan. 1951. Studies on oxidized milk fat. II. Preparation of 2, 4-dinitrophenylhydrazones from the volatile material from oxidized milk fat. *J. Dairy Sci.* 34:719-727.
  29. Keeney, P. G. 1955. Identification of the coconut-like flavor defect of milk fat. *Abstr. Doct. Diss. Pa. St. Coll.* 18:172-173.
  30. Kiermeier, F., and M. G. E. Hamed. 1961. Sulfhydryl content of milk and milk products as influenced by technical processes. I. Influence of temperature and heating time. *Z. Lebensm. Untersuch. Forsch.* 115:322-330. (*Chem. Abstr.* 56:743.)
  31. Kinter, J. A., and E. A. Day. 1965. Major free fatty acids in milk. *J. Dairy Sci.* 48:1575-1581.
  32. Kleyn, D. H., and W. F. Shipe, Jr. 1961. Effects of direct steam heating and vacuum treatments on the chemical composition of milk with especial reference to substances involved in oxidized flavor development. *J. Dairy Sci.* 44:1603-1620.
  33. Koestler, G. 1928. Ranzige Milch und ihr entwicklungshemmender Einfluss auf Bakterien. *Schweiz. Milchztg*, No. 103, December 1928. (Cited in reference 57).
  34. Kosikowski, F. V. 1955. Analysis of milk for free volatile fatty acids. *J. Dairy Sci.* 38:592.
  35. Kosikowski, F. V. 1959. Free volatile fatty acids in homogenized raw milk. 15th Intern. Dairy Cong. 3:1545-1551.
  36. Kristofferson, T. 1958. Effect of treatment of raw milk on heat liberated hydrogen sulfide. *J. Dairy Sci.* 41:702.
  37. Kristofferson, T., I. A. Gould, and P. S. Rao. 1962. Certain factors affecting the heat production of sulfhydryl groups in milk. 16th Intern. Dairy Cong., Sect. A:793-798.
  38. Kulshrestha, D. C., and E. H. Marth. 1969. Effect of volatile compounds associated with milk on lactic acid bacteria. *J. Dairy Sci.* 52:894-895.
  39. Lindsay, R. C., T. W. Keenan, and E. A. Day. 1965. Acetaldehyde production and utilization by lactic starter organisms. *J. Dairy Sci.* 48:783-784.
  40. Loney, B. E., R. Bassette, and G. M. Ward. 1963. Some volatile components in milk, blood and urine from cows fed silage bromegrass and hay and grain. *J. Dairy Sci.* 46:922-926.
  41. Magidman, P., S. F. Herb, R. A. Barford, and R. W. Riemenschneider. 1962. Fatty acids of cows milk. A. Techniques employed in supplementing gas liquid chromatography for identification of fatty acids. *J. Amer. Oil Chem. Soc.* 39:137-142.
  42. Maxcy, R. B. 1964. Influence of surface active agents on some lactic streptococci. *J. Dairy Sci.* 47:1285-1290.
  43. Maxcy, R. B., and R. C. Chandan. 1962. Inhibitory effects of fatty acids and other surface active compounds on *Streptococcus lactis*. *J. Dairy Sci.* 45:654.
  44. Nawar, W. W., and I. S. Fagerson. 1961. Heat induced changes in milk fat. *J. Dairy Sci.* 44:1171.
  45. Nieman, C. 1954. Influence of trace amounts of fatty acids on the growth of microorganisms. *Bact. Rev.* 18:147-163.
  46. Nilsson, R., and S. Willart. 1966. Influence of fatty acids on activity of starter culture. *Sevenska Mejeritidn* 58:253-256. (*Dairy Sci. Abstr.* 28:427-428).
  47. Patton, S. 1961. Gas chromatographic analysis of flavor in processed milks. *J. Dairy Sci.* 44:207-214.
  48. Patton, S., and B. W. Tharp. 1959. Formation of methyl ketones from milk fat during steam distillation for saponification. *J. Dairy Sci.* 42:49-55.
  49. Patton, S., D. A. Forss, and E. A. Day. 1956. Methyl sulfide and the flavor of milk. *J. Dairy Sci.* 39:1469-1470.
  50. Patton, S., and D. V. Josephson. 1949. The isolation of furfuryl alcohol from heated skim milk. *J. Dairy Sci.* 32:222-227.
  51. Pereira, R. R. 1966. Volatile sulfur compounds from high heat treatment of milk. *Diss. Abstr.* 26:6639-6640.
  52. Pereira, R. R., W. J. Harper, and I. A. Gould. 1964. Volatile sulfur compounds in heated milk. *J. Dairy Sci.* 47:662.
  53. Pereira, R. R., W. J. Harper, and I. A. Gould. 1966. Volatile sulfur compounds in milk. I. Effect of chemical form of <sup>35</sup>S on selective labelling of milk constituents and free sulfur compounds. *J. Dairy Sci.* 49:1325-1330.
  54. Ryoki, T., and T. Kudo. 1964. Study on the fatty acid composition of cow's and human milk fat by gas chromatography. *Japan J. Dairy Sci.* 13(5)A:143-156. (*Dairy Sci. Abstr.* 29:225).
  55. Sara, C., A. Canale, and E. Carmello. 1966. The variations of free fatty acids in the lipid fraction of crude milk in comparison with pasteurized milk. *Atti. Soc. Ital. Sci. Vet.* 20:621-624 (*Chem. Abstr.* 66:10639).
  56. Sasago, K., H. K. Wilson, and E. O. Herried. 1963. Determination of sulfhydryl and disulfide groups in milk by the p-chloremercuibenzoate-dithiozone method. *J. Dairy Sci.* 46:1348-1351.
  57. Tarassuk, N. P., and F. R. Smith. 1940. Relation of surface tension of rancid milk to its inhibitory effect on the growth and acid fermentation of *Streptococcus lactis*. *J. Dairy Sci.* 23:1163-1170.
  58. Tharp, B. W., and S. Patton. 1960. Coconut-like flavor defect of milk fat. IV. Determination of Δ-dodecylactone in the steam distillate from milk fat. *J. Dairy Sci.* 43:475-479.
  59. Trammel, J. A., and J. J. Janzen. 1961. A method for determining the free fatty acids in milk with gas chromatography. *J. Dairy Sci.* 44:1170-1171.
  60. Trout, G. M. 1968. Heat induced volatile compounds in milk. *Dairy Ice Cream Field.* 151(2):28-30.
  61. Vadhera, D. V., and L. G. Harmon. 1964. Lipolytic activity of *Staphylococcus aureus* and the effect of free fatty acids on growth. *Bact. Proc.* 1964:2.
  62. Vas, K. 1947. The decrease of formaldehyde content in preserved milk. *Rec. Hung. Agric. Exp. Sta.* 47-49:93-102. (*Dairy Sci. Abstr.* 12:199).
  63. Williams, K. I. H., S. H. Burstein, and D. S. Layne. 1966. Dimethylsulfone: Isolation from cow's milk. *Proc. Soc. Exp. Biol. Med.* 122:865-866.
  64. Wishner, L. A., and M. Keeney. 1961. Some carbonyl compounds formed by the action of sunlight on milk. *J. Dairy Sci.* 44:1164.
  65. Wishner, L. A., and M. Keeney. 1963. Carbonyl pattern of sunlight exposed milk. *J. Dairy Sci.* 46:785-788.
  66. Wong, N. P. 1962. Investigations on the flavor of normal milk and cream. *Diss. Abstr.* 22:2356.
  67. Wong, N. P., and S. Patton. 1962. Identification of some volatile compounds related to the flavor of milk and cream. *J. Dairy Sci.* 45:724-728.
  68. Woods, A. E. 1963. Studies on the volatile carbonyl compounds of Ladino clover and their influence on the flavor of milk. *Diss. Abstr.* 23:3858-3859.
  69. Wynn, J. D., J. R. Brunner, and G. M. Trout. 1960. Gas chromatography as a means of detecting odors in milk. *Food Technol.* 14:248-250.
  70. Zweig, G., and R. J. Block. 1953. The effect of heat treatment on the sulfhydryl groups in skim-milk and nonfat dry milk. *J. Dairy Sci.* 36:427-436.

# EFFECT OF DILUENTS ON THE RECOVERY OF MICROORGANISMS FROM FOODS

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## ABSTRACT

Two aspects of the recovery of bacteria from foods were examined: a comparison of the effects of two diluents, phosphate-buffered dilution water and 0.1% peptone on the recovery of eight bacterial strains and the total counts; and the effect of these diluents on the recovery during a period of 1 hr. No marked superiority of either diluent in terms of initial recoveries or of protection over a 1-hr time period was noted. On the average, however, recovery was as good or better in 0.1% peptone as in phosphate-buffered dilution water; the choice of diluent, therefore, is slightly in favor of 0.1% peptone, which seemed to yield higher recovery on more samples.

The bacteriological examination of foods involved in outbreaks of foodborne illness requires the preparation of a homogenate and subsequent dilution of this for plating in differential media. In order for the results to depict accurately the microbiological condition of the food at the time of testing there should be no die-off or build-up of the bacteria during this process. Lewis and Angelotti (3) recommended the use of phosphate-buffered dilution water (1) for the preparation of the homogenate and subsequent dilutions. This selection was based on their experience and the results obtained by Hartman and Huntsberger (2). Straka and Stokes (4) showed that 0.1% peptone solution was superior to both distilled water and phosphate-buffered water for preserving bacterial numbers without allowing bacterial growth.

The findings and recommendations of these and other workers have created two schools of thought concerning the proper diluent to be used in the bacterial examination of foods. In the interest of uniformity of approach, however, it is desirable that one or the other of these diluents be established as the better one so that methods may be standardized in this respect. Toward this end a study was carried out in which a food item, commercial beef pot pies, was examined for total counts and for the recovery of added pathogenic and indicator organisms, using the two diluents.

## MATERIALS AND METHODS

### Food

A single brand of commercially prepared beef pot pie was

purchased from local stores and held in frozen storage until used.

### Bacterial cultures

All cultures were from the stock culture collection of the Food Microbiology Unit and included: *Escherichia coli* B-15, *Citrobacter freundii* B-5, *Streptococcus faecalis*, *Streptococcus zymogenes*, *Clostridium perfringens* strains A86 and A91, and *Staphylococcus aureus* strains 230 and 243.

### Media

Except for the *C. perfringens* strains, all cultures were maintained by weekly transfer on trypticase soy agar (BBL) slants from which trypticase soy broth (BBL) was inoculated to produce the inocula. The *C. perfringens* strains were maintained in cooked meat broth and subcultured to fluid thioglycollate medium (BBL) to produce the inocula. Total counts were determined in Standard Methods agar, and various lots were used for this purpose. *Escherichia coli* and *C. freundii* were plated in violet red bile agar (Difco Lot No. 4970C3) with an overlay. *Streptococcus faecalis* and *S. zymogenes* were plated in K. F. agar (Difco Lot No. 489714) to which 6.4 ml of 10% Na<sub>2</sub>CO<sub>3</sub> per liter and 10 ml of 1% triphenyl tetrazolium chloride (TTC) per liter had been added. An overlay also was used with this medium. The *C. perfringens* strains were plated in SPS agar (BBL Lot No. 512615). The *S. aureus* strains were surface plated on staphylococcus medium 110 (Difco Lot No. 468481).

### Preparation of the food homogenate

The 1:10 dilution was prepared by homogenizing 50 g of beef pot pie in 450 ml APHA (1) phosphate-buffered dilution water (buffer) or in 0.1% peptone (Difco Lot No. 468789) for 2 min at high speed in a Waring Blendor jar of 1-liter capacity. For total counts the beef pot pies had been incubated at 35 C for 24 hr before being examined. For recovery studies, they were examined frozen, and 1 to 2 ml of an 18- to 24-hr broth culture of the organisms being tested was added before homogenization. Preliminary work had shown that the frozen pies did not add sufficient numbers of organisms to affect the final counts on the selective media.

### Preparation of dilutions

The 1:100 dilution was prepared from the food homogenate by transferring 11 ml to dilution blanks containing either buffer or peptone. Each dilution blank was shaken 25 times through a distance of 1 ft in 7 sec before transferring material to the next dilution blank. Subsequent dilutions were made by transferring either 11 ml or 1 ml to similar blanks in the same manner.

### Plating procedure

Appropriately marked plastic petri dishes (15 x 100 mm) were inoculated in duplicate with 1 ml or 0.1 ml of suitable

<sup>1</sup>Deceased May 28, 1970.

<sup>1</sup>Mention of commercial products does not imply endorsement by the Public Health Service.

TABLE 1. SUMMARY OF ANALYSIS OF VARIANCE RESULTS

Source	Recovery medium	Time A	Buffer B	Samples C	AB	AC	BC	ABC	Variation of replicate log counts
<i>S. aureus</i> 243	110	NS <sup>a</sup>	** <sup>b</sup>	**	**	NS	**	NS	0.01013 (80) <sup>c</sup>
<i>S. aureus</i> 230	110	NS	**	**	NS	**	NS	NS	0.00981 (72)
<i>E. coli</i> B15	VRB Agar	NS	**	**	NS	NS	**	NS	0.00402 (80)
<i>C. freundii</i> B5	VRB Agar	**	**	**	NS	NS	**	NS	0.00380 (80)
<i>C. perfringens</i> A86	SPS Agar	**	NS	**	NS	**	**	NS	0.00324 (80)
<i>C. perfringens</i> A91	SPS Agar	**	NS	**	NS	**	**	NS	0.00202 (72)
<i>S. faecalis</i>	KF Agar	NS	NS	**	NS	NS	NS	NS	0.00287 (80)
<i>S. zymogenes</i>	KF Agar	NS	NS	**	NS	NS	NS	NS	0.00322 (80)
Total Count 35 C	PCA	NS	**	**	NS	**	**	NS	0.00373 (80)

<sup>a</sup>NS not significant.

<sup>b</sup>\*\* significant at  $\alpha = 0.01$  level.

<sup>c</sup>degrees of freedom.

TABLE 2. SUMMARY OF DUNCAN'S TEST FOR FACTORS. [A (TIME) AND B (BUFFERS)—GEOMETRIC MEANS  $\times 10^7$ ]

Source	Plating time in minutes				Buffers	
	0	15	30	60	Buffer	Peptone
<i>S. aureus</i> 243	25	27	28	27 <sub>a</sub>	25	29
<i>S. aureus</i> 230	25	27	27	26	25	28
<i>E. coli</i> B15	47	51	51	49	47	53
<i>C. freundii</i> B5	63	59	56	52	51	65
<i>C. perfringens</i> A86	105	92	88	84	92	92
<i>C. perfringens</i> A91	101	91	83	79	88	88
<i>S. faecalis</i>	53	53	55	53	53	54
<i>S. zymogenes</i>	43	42	43	43	43	43
Total Count 35 C	93	94	95	91	98	88

<sup>a</sup>Indicates no difference was detected

dilutions, usually  $10^{-5}$ ,  $10^{-6}$ , or  $10^{-7}$ . Approximately 15 ml of the recovery medium previously cooled to 46 C was poured into each petri dish, and mixing was accomplished by rotating the dishes 10 times clockwise and 10 times counter-clockwise. The medium was allowed to solidify, and, when appropriate, a cover layer of 5 ml of the same medium was poured over the surface. The plates were then inverted and incubated at 35 C for 24 or 48 hr as appropriate. In the recovery of *S. aureus*, 0.1 ml of inoculum was spread over the surface of the medium with a bent glass rod. The *C. perfringens* recovery plates were incubated anaerobically in glass jars with an atmosphere of 90% N<sub>2</sub> and 10% CO<sub>2</sub>. Platings were made at 0 time (as soon as dilutions were completed) and at 15, 30, and 60 min after the 0-time platings. Dilution blanks were shaken vigorously 15 times before each plating.

#### RESULTS AND DISCUSSION

Two aspects of the recovery of bacteria from foods were examined in this study: a comparison of the effects of the two diluents, phosphate-buffered dilution water (1) and 0.1% peptone solution, on the recovery of eight bacterial strains and the total counts, and the effect of these diluents on the recovery during a period of 1 hr. The results obtained were analyzed statistically and Table 1 lists the summary of results and F ratio tests for significance where each factor and interaction is noted. The variance is also given for each analysis in terms of log<sub>10</sub> units. Log<sub>10</sub> counts were computed and used in the statistical analysis in

TABLE 3. GEOMETRIC MEANS  $\times 10^7$  FOR RECOVERY OF *S. aureus* 243 IN TWO DILUENTS

Sample	Recovery in buffer	Recovery in peptone	% difference
C <sub>1</sub>	15	17	13.3
C <sub>2</sub>	27	31	14.7
C <sub>3</sub>	21	28	33.3
C <sub>4</sub>	10	18	80.0
C <sub>5</sub>	12	17	41.6
C <sub>6</sub>	37	41	10.7
C <sub>7</sub>	28	43	53.5
C <sub>8</sub>	26	26	0
C <sub>9</sub>	56	58	3.6
C <sub>10</sub>	51	27	47.0

order to satisfy the analysis of variance assumptions. Significant differences as related to plating time were noted with *C. freundii* and the two *C. perfringens* strains. A significant effect related to the buffer was noted with the two *S. aureus* strains, *E. coli*, *C. freundii*, and the total count. Inasmuch as no attempt was made to use a standardized inoculum, all samples showed significant variations. Significant interactions were noted between buffer and time with *S. aureus* 243 only; and between time and sample with *S. aureus* 230, the two *C. perfringens* strains, and the total counts. Similar variations occurred with *S. aureus* 243, *E. coli*, *C. freundii*, the two *C. perfringens* strains, and the total count in relation to buffer and sample interactions. The tests were not significant for the three factor interaction time, buffer, and sample. Only the two strains of *S. aureus* showed a high variance (0.005 or less is considered normal in our laboratories) in the replicate  $\log_{10}$  counts. Whether this is related to the fact that these counts were obtained by a spread plate method or to other factors cannot be determined from the results. However, all counts observed on pour plates yielded variances of 0.00402 or less.

Table 2 summarizes results of Duncan's test for the two factors, time and buffer, expressed as the geometric means times  $10^7$ . The significant differences indicate a decrease in count over time. It can be seen that only with *C. freundii* and the two strains of *C. perfringens* did the plating time have a significant effect. With *C. freundii* the 0- and 15-min platings were statistically similar as were the 15- and 30- and 60-min platings. There was, however, a

significant difference between the 0 plating and the 30- and 60-min recoveries. With the *C. perfringens* strains, a significant reduction in recovery was noted after 15 min and another at the 60-min plating time. In regard to the *C. perfringens* strain, we might assume that the reduction in counts was related to oxygen exposure caused by aeration as the dilution blanks were shaken before each plating. This would not, however, explain the similar, if less marked reduction in recovery of *C. freundii*. In this instance, the results indicate a failure of both buffer and peptone solution to provide conditions favorable to the sustained viability of the strain.

Table 2 also shows the effect of the diluents on recovery of the organisms. With four strains and the total count there was a significant difference in recovery, although these differences were not great. *Staphylococcus aureus* strain 243 showed a 14% better recovery in peptone than in buffer, and *S. aureus* strain 230 11%. *Escherichia coli* B15 also showed an 11% better recovery in peptone and *C. freundii* a 22% better recovery in peptone. On the other hand, the total count recovery was 10% better in buffer than in peptone. Although none of these differences are very great, it is obvious that with the individual strains the recovery is as good, or better, in 0.1% peptone solution as in phosphate buffered dilution water. These differences, however, were not consistent over all samples, which resulted in the interaction between buffer and samples noted in Table 1. Although on the average, peptone gave better recovery, there were individual instances when this was not true. An example of this is seen in Table 3, which shows the results of the 10 tests using *S. aureus* 243. In eight of the 10 tests recovery was better in peptone (3.6-80%), but with specimen C<sub>8</sub> recoveries were identical, and with specimen C<sub>10</sub> recovery was better in buffer than in peptone (47%).

## REFERENCES

1. American Public Health Association. 1967. Standard methods for the examination of dairy products. Twelfth edition. William G. Walter, (ed.). Amer. Public Health Ass. Inc., New York, N. Y. 10019, pp. 254-255.
2. Hartman, P. A., and D. V. Huntsberger. 1961. Influence of subtle differences in plating procedure on bacterial counts of prepared frozen foods. *Appl. Microbiol.* 9:32-38.
3. Lewis, K. H., and R. Angelotti. 1964. Examination of foods for enteropathogenic and indicator bacteria. Public Health Service Publication No. 1142. U.S. Government Printing Office, Washington, D.C. 20402.
4. Straka, R. P., and J. L. Stokes. 1957. Rapid destruction of bacteria in commonly used diluents and its elimination. *Appl. Microbiol.* 5:21-25.

## THE REFUSE DISPOSAL PROBLEM: PREVENTION COULD BE THE CURE

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### ABSTRACT

Production of solid waste in the United States is beginning to outrun disposal capacity. Solid waste production must be sharply curtailed; eventually there will be no adequate alternative. Means to achieve reduction include: reinstating returnable bottles and creating returnable cans, recycling metals, paper, plastics and cans, banning heavy newspaper advertising and advertising by mail, giving up large paper grocery bags, regulating packaging to reduce waste, developing biodegradable containers for frozen and convenience foods, and returning from "planned obsolescence" to easily available parts and repairs. Financial incentives built into the reduction measures and sophisticated advertising techniques will be needed to gain the cooperation of the public.

In these days of "the Affluent Society" the garbage-man comes and too often finds too much refuse waiting to be hauled away. As many as seven trash cans per household are allowed: that fact alone indicates that America is in trouble in the refuse disposal field. *The garbage man is finding it more and more difficult to dispose of the collected effluent from the affluent.* "The affluent society" is one and the same as "the Affluent Society." We find (or, more correctly, we are allowed to continue under the delusion) that we can afford to discard tons of paper and metal every day; large quantities of glass and plastics are similarly discarded. Society cannot afford this because there is a limit on the means with which to dispose of refuse. Land fill is definitely limited. Burning is a bad idea because it uses up vast quantities of oxygen. We cannot afford to consider the oxygen supply limitless. Green plants are the sole providers of oxygen on this earth; water pollution, which continues and which is beginning to seriously affect even the ocean, means that algal life is seriously impaired. Every oil slick from every dirty or leaking freighter whittles away at the little plants that make breathing possible. One must eventually come to the inescapable conclusion that "the only way to stop pollution is to stop polluting"; a truism which sounds both glib and impossible to carry out, but for which there is ultimately no alternative.

Remedies to the unfortunate state of affairs in which we find ourselves will be very unpopular at first and almost impossible to implement since they will run afoul of so many people with deeply vested interests. The only way in which these difficulties can be overcome is to create new vested interests

running parallel to the remedies. Non-pollution must be made to pay and also must be made into a *sine qua non* of social respectability. The government may well be forced to enlist the services of Madison Avenue and to employ a system of financial incentives and tax benefits. A few of the measures that immediately come to mind are the following:

#### 1. Reinstating returnable bottles and creating returnable cans

Everyone knows what a problem no-return bottles and cans are; both when they are scattered over the countryside and when stuffed into trash cans. Enterprising children and Scouts, charitable organizations, and churches could both benefit from and aid the clean-up effort. Ideally, the government should outlaw no-return bottles and cans; but failing that, a stiff tax, to be paid by the consumer on no-return bottles and cans would help a great deal. Cans used for ordinary grocery items should be included in the plan. Anti-litter can't be legislated directly, but appeals to the pocketbook work. Housewives would save cans if they knew they could thereby get a penny or two off on the next can of food (besides saving the tax on no-return cans). Deposits on containers are incentives.

#### 2. Recycling of metals, paper, plastics, and glass

If suitable processes do not exist to accomplish recycling, research to find them should be a top priority item in the engineering laboratories in this country. Scrap dealers are needed again. Inflation and the population explosion will aid in the conditioning of people to this program. Most of the contents of trash cans today are solid refuse rather than garbage. People do not like to throw away food, as a general rule, because it is seen as expensive and valuable. As the population increases and costs of living rise, the same point will be able to be made about recyclable materials. Our raw materials supply is not inexhaustible.

#### 3. Banning heavy newspaper advertising

Sizes of ads, and therefore amounts of newspaper, should be strictly limited—by stiff taxes, if a law is found to be unenforceable. What is



the proportion of ads to news in the average daily paper (let alone Sunday?) And how many people read the giant ads and take them seriously? If advertising efficacy depended on ad size, the classified ad section would be a complete failure instead of the successful medium it is. Good products sell themselves, as do good stores. The very best products do not need to be advertized at all, and aren't.

4. *Banning advertising by mail*

There is a paper blizzard coming into nearly everyone's mailbox and drifting into the wastebasket. The postal service can't afford to carry it anyway.

5. *Giving up the use of large paper grocery bags*

Each person should furnish his own containers and use them over and over. This is the procedure in Britain and one soon becomes accustomed to it. A stiff luxury tax should be put on the bags and stores should be forced to charge for them instead of giving them away free.

6. *Closely regulating packaging so that unnecessarily elaborate and paper wasting packages would be eliminated*

Packets inside boxes and boxes far bigger than needed to hold contents are the worst offenders. Snack, cereal, and children's toy and game articles are particularly bad.

7. *Developing simpler, and if possible biodegradable, containers for "convenience foods" such as TV dinners*

If these convenience foods are good, people will continue to buy them without a cardboard "production in cinemascope and living color" on top showing what people fondly hope will be within.

8. *Discouraging such schemes as making "disposable messes"*

Ads for making charcoal fires on aluminum foil for "outdoor cooking" and then wrapping up the whole mess and throwing it away (widely seen on TV) are deplorable.

9. *Reinstating, through tax incentives to big business, the idea of interchangeable parts and easily available, more reliable, and cheaper repairs.*

The "planned obsolescence" of the 1950's and 1960's must stop.

10. *Using Madison Avenue techniques to sell anti-litter and anti-pollution actions*

Compost heaps should be made respectable

and conforming instead of eccentric. Using sewage sludge instead of chemical fertilizers for lawns and gardens should be made to sound clever and thrifty. Simply inventing a new name for it would help greatly. Waste must be made to sound sinful and shameful.

People must be educated to the fact that the tables are turned for the moment: we, "the most advanced culture" are the greatest sinners and most backward when it comes to environmental despoiling. An Asian peasant or a New Guinea headhunter does not pollute one-tenth as much as the average U. S. citizen. "Civilizing" the "uncivilized" should cease until we have solved our pollution problem.

Women who "demand" convenience in the form of elaborate packaging and disposable items should be made to realize how they are made slaves to the trash can and incinerator and how much they pay for fancy, colorful containers and the luxury of throwing away; not only in the immediate cash outlay at the store but also in the environmental sense.

Human beings are amazingly capable of adjusting: *Man gewöhnt sich an alles* is very nearly true. The cost and labor aspects of the objections which are sure to be raised to anti-pollution remedies such as those suggested above are certainly not insurmountable. Any environmental improvements are going to cost money. We will have to pay for them in the same way a sick person has to pay for medicines. There is no alternative in the end, except to perish in our own mess. As surely as some jobs will be eliminated and unemployment created, new jobs and new employment will develop. A great deal of fiction surrounds cost and labor questions. For example, one is told that "labor costs and machinery costs make returnable bottles uneconomic and no-return bottles cheaper;" cheaper to whom? uneconomic to whom? Who is now saving the money that was formerly spent when all bottles were returnable and that is now not spent? Not the consumer; that much can be verified by a visit to the store. Milk (so-called "jug milk") in returnable glass bottles and soft drinks in returnable bottles are still cheaper than the same beverages in no-return containers. And even if they *were* more expensive, it would be preferable, in the long run, to return to them as an anti-pollution measure rather than pay the environmental as well as monetary price of coping with the no-return container problem.

If people find that they must follow a new set-up in their daily living patterns, they may grumble but they will do so. People generally are rather sheep-like, and also endlessly acquisitive and full of cupidity. If momentum can once be established in a new direction, people will follow and a profit somewhere will be made as usual.

## QUALITY CONTROL COMMITTEE LABORATORY OF THE MINNEAPOLIS-ST. PAUL MARKET<sup>1</sup>

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### ABSTRACT

The Quality Control Committee laboratory is a unique organization which was started approximately 32 years ago by Dr. Harold Macy of the University of Minnesota. The dairy industry operates a laboratory which does most of the official testing for the health agencies in the Minneapolis-St. Paul market. With higher costs of operations many health agencies are trying to find ways of saving money, and still have a satisfactory laboratory program to protect the public's health. Some health agencies are using industry laboratories, and the cost is passed on to the customer rather than the taxpayer.

The laboratory functions are to evaluate the quality of the raw milk supply from 4238 Grade A producers, and the finished products from 17 processing plants. The laboratory also does the official butterfat testing for the Federal Milk Market Administrator for Order 68.

This organization is supervised by a Steering Committee of nine individuals who represent the University of Minnesota; the producer cooperatives, who supply the raw milk; and the Grade A fluid milk processors from the Minneapolis-St. Paul market.

All of the routine results from the laboratory are provided to the health agencies. The health agencies and laboratory manager have a close working relationship to coordinate the program. In order for an organization like this to work, it takes cooperation from all parties involved.

There are two philosophies in operating a control laboratory to insure the quality of milk and milk products in a market. A control laboratory can be operated either by regulatory agencies or by industry. Of course, in most instances the taxpayer pays the bill if a regulatory agency operates the laboratory; and the consumer pays the bill when industry operates the laboratory. Like all public agencies, the cost of operation has been increasing; and if there is duplication of laboratory testing, a direct way to save money is to stop needless duplication.

### DEVELOPMENT OF LABORATORY

The Minneapolis-St. Paul market chose to have an industry operated official testing laboratory. This came about 32 years ago when fluid milk processors were charged with a series of violations by the health agency for high bacteria counts on finished products. The processors approached Dr. Harold Macy, Professor of Bacteriology, University of Minnesota, to help them solve their problems. An industry meeting was set up in 1936 to discuss the quality problems of

of this meeting, a laboratory was set up in Dairy Bacteriology at the University of Minnesota, under Dr. Macy's guidance, to evaluate both the raw milk supply and finished products. By the fall of 1937, it was decided that the results were such that steps should be taken to establish a permanent organization. Thus, the Quality Control Committee came into being and held its first meeting January 3, 1938 (2).

### PRESENT ORGANIZATION

The organization as it stands today has representatives of the University of Minnesota Food Science Department to give guidance when needed. This concept has continued from the very beginning of the organization and is probably a key factor for its success over these many years of operation. The manager of the laboratory may encounter a technical problem which he cannot handle, and can usually get expert advice from one or more staff members at the University. Each year a grant of \$3,000 is made to the Food Science Department as a token of appreciation for the support the University gives the organization. Dr. S. T. Coulter of the University of Minnesota is the Chairman of the Steering Committee, and Dr. J. J. Jezeski, also of the University of Minnesota, is the technical advisor. Dr. J. C. Olson, Jr. acted as technical advisor to the organization for many years during his time at the University of Minnesota. The Steering Committee is the governing board of this organization. This board sets policy and approves the budget. The only officer of the organization is the Executive Secretary who also serves as laboratory manager. The producer cooperatives have representatives on the Steering Committee and there are also representatives from both the Minneapolis and St. Paul fluid milk processors to give a total of 11 members including the Executive Secretary. Each member on the Steering Committee has one vote regardless of the size of the organization he represents. In addition to the Steering Committee Meetings which are called periodically by the Executive Secretary, there is an annual business meeting of the entire membership. The Quality Control Committee has been an informal organization from the very beginning but has survived because a needed service was performed at a reasonable cost. The cost factor and the willingness of the various dairy organizations of the market to cooperate are the key factors of this organization. There is no

<sup>1</sup>Presented at the Fifty-sixth Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, Inc., Louisville, Kentucky, August 17-21, 1969. the Minneapolis-St. Paul milk market. As a result

question in my mind that a parent organization of this kind with strategically located divisions could cover a complete state with an adequate quality control program for either Grade A or manufacture-grade milk.

#### *Financing the laboratory*

The program is financed equally by both the producer cooperatives and the fluid milk processors. The producers are assessed approximately 1/3 of a cent for each hundredweight of Grade A milk produced and the processors are assessed approximately 3/4 of a cent for each hundredweight of Class I milk processed. Last year it cost approximately \$57,000 each for our producer cooperatives and our fluid milk processors to operate the laboratory. The income from the Federal Milk Market Administrator for butterfat testing was approximately \$21,000. In 1968, there were 191,069 various tests conducted for approximately \$135,000.

#### *Laboratory staff*

The laboratory and technicians are certified under the Interstate Milk Shippers Agreement. This certification is done by agents of the Minnesota Department of Agriculture.

### LABORATORY FUNCTIONS

The laboratory function could be divided into four categories: (a) to evaluate the quality of the raw milk supply; (b) to evaluate the quality of the finished products of the market; (c) to study applied laboratory methodology for the market; and (d) to perform the official butterfat testing for the Federal Milk Market Administrator on a contractual basis. The manager of the laboratory is available to the various members of the organization to consult on quality control problems.

#### *Producer samples*

The individual producer's milk is check-tested 4 times in a 6-month period. The various tests conducted are the Standard Plate Count, disc assay for bacterial growth inhibitors, and a catalase test for abnormal milk. A freezing point determination is done during each 6-month period on individual producer milk.

In 1968, we had an average of 4,238 Grade A producers in our market which we check tested. Eight samples of each producer's milk were tested and 43.6% had a Standard Plate Count of <11,000 per ml; 85.5% had a standard Plate Count of <51,000 per ml; and, 7.5% had a Standard Plate Count of >100,000 per ml. We conducted 34,465 disc assays for bacterial growth inhibitors on producer milk samples in 1968, and detected 46 positive samples or 0.13%. We are using the tube catalase test procedure as our

screening test for the abnormal milk program. A total of 34,182 samples were tested in 1968, and 3.2% had 30% gas or greater.

#### *Commingled milk*

Another program to further insure the quality of the commingled raw milk was put into operation over a year ago. Twice each week a Standard Plate Count and a disc assay for bacterial growth inhibitors are conducted on all milk delivered to the 17 Grade A fluid processing plants. This milk is delivered directly from the farm in bulk tank trucks or from receiving stations in over-the-road tank trucks. This program was instituted when we found raw milk to be pasteurized in the fluid milk processing plants with high total bacteria counts. This program revealed that some of the over-the-road tankers were not being properly washed; also, some improper sampling and sample handling was detected. By investigation, a high bacteria count on a farm bulk load could be traced to an individual problem farm. If a bacterial growth inhibitor, such as penicillin, is detected in a farm bulk load, we will check all of the producers' milk on that load for bacterial growth inhibitors. By checking farm bulk loads, in several instances we have caught a producer that did not hold out milk from a treated cow. This program gives us constant control on the commingled raw milk supply. In most markets, quality evaluation of raw milk has been concentrated at the farm level, and the commingled milk at receiving stations and in transportation vessels has been somewhat neglected.

#### *Finished products.*

The second function of the laboratory is to evaluate the finished products which are sold in our market. We obtain a complete set of finished product samples from the coolers of 17 plants *twice* each month. On the one set of samples the standard routine tests, such as butterfat content, total bacteria count, coliform count, phosphatase, growth inhibitors, and freezing point are made. On the other set of samples a "shelf-life" determination is made. The samples are held for 5 days at 45 F and then a Standard Plate Count is made. This program pointed out that even though our fluid milk processing plants were meeting the regulatory standards, many needed to be upgraded from the "shelf life" standpoint. After 18 months of concentrated effort in this area, the improvement has been very gratifying. This again points out that all of us should evaluate our routine laboratory tests and programs and see if they are really giving us the correct information.

The health agencies of Minneapolis and St. Paul receive all routine testing results on both the raw milk and finished products. Results from raw milk

testing are used by the health agencies as official tests. All results from tests on raw milk are punched on computer cards, and there is no delay in these results reaching the farmer or the sanitarian. Results from tests on finished products are used by both health agencies to evaluate the processors' performance. In addition to this, the city of St. Paul uses the results for official evaluation.

We also do diagnostic testing, such as line trials, when requested by the fluid milk processing plants. Some of the plants have their own laboratories to do this kind of work, but most of them rely on the Committee Laboratory. In many instances, the processor will request the laboratory manager to come into his plant and make recommendations pertaining to quality problems.

#### *Applied research*

Over many years this laboratory has been involved in applied research of new laboratory methodology. An example of this is the study which was recently published in the *Journal of Milk and Food Technology* on the Milko Tester (1). A laboratory of this type can be of value in collaborative studies in new methodology.

#### *Butterfat testing*

Another unique function of this laboratory is the butterfat testing which we do for the Federal Milk Market Administrator of Order 68. A butterfat test is made on all Grade A tank truck milk received at all of the Grade A fluid milk processing plants in the market. Each day we will have approximately 100 tank samples for butterfat analysis. These samples are taken in sterile containers and are also used for our bacteriological testing program which was mentioned earlier. We also have a butterfat check-testing program which the Market Administrator uses to verify producer butterfat payment. Four fresh samples of all individual producer milk are tested and then the Administrator's office conducts an audit against the composite butterfat tests which are done by the cooperatives. This is a self-imposed program by industry, and each receiving station will be check-tested once each year. This again points out that industry in our market wants the job done right and can do a satisfactory job of self-policing. This work is done on a contractual cost basis. This Federal Milk Market Administrator's butterfat testing program makes up about 25% of our work load, since our market expanded May 1, 1969.

#### *Sampling program*

We also have a unique sampling program in this market which is worthy of mention. In order to reflect a true picture of a quality testing program, proper sampling as well as the unannounced sam-

pling time is essential. Each time milk is picked up on the farm a sample is taken by the bulk milk hauler in a sterile container. This sample is used to build the composite butterfat sample, or it is used once approximately every 6 weeks for bacteriological testing purposes; it is also used for the fresh milk butterfat check-testing program mentioned above. Most samples are picked up early the following morning so that they can be plated before they are 24 hr old. We drive 9,000 to 10,000 miles each month to secure these samples from the various receiving stations in Minnesota and Wisconsin and from the fluid milk processing plants in the market which receive direct shipped milk from the farm.

The question will come to mind as to how many miles can be covered economically from a centralized laboratory. Most of our plants are within a 100 mile radius. The highways are good and we can easily get around from one plant to another. It would seem that if plants were beyond 100 miles, it would be difficult to get samples to the laboratory within the 24 hr period. The 8 hr working day could also be a factor.

#### *Laboratory reports*

Bimonthly, a complete laboratory report is compiled and a noon luncheon meeting is held to discuss the test results and mutual problems. There are usually 35 to 40 interested individuals who attend. These are people from the University of Minnesota, the various health agencies, the State Department of Agriculture, fluid milk processors, and milk distributors. Each year an annual laboratory business meeting is held which most of the membership attends.

#### THE FUTURE

In my opinion, the future of this organization looks good. Today we are all cost conscious and this certainly is a very economical way to do routine laboratory testing; but there is much more to a program like this than just being economical. The trust and cooperation between industry and regulatory agencies is a must to make an organization like this work. This concept has worked in Minneapolis-St. Paul for 32 years. The factual information which we can accumulate is another asset of this organization. Programs, like our "shelf life" program, can be done on a market wide basis, thus benefiting the whole market.

#### REFERENCES

1. Ginn, R. E., and V. S. Packard, Jr. 1969. Comparisons of milkfat tests on commercial milk samples determined by the Milko-Tester and Babcock methods. *J. Milk Food Technol.* 32:203-205.
2. Lawton, W. C. 1957. A quality control committee and a quality milk supply. *Amer. Milk Review.* 19(12):28-32.

# EFFECT OF THE NITROFURAN-FURYLURAMIDE- ON CRAB MEAT QUALITY

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## ABSTRACT

The effect of furylfuramide [2-(2-furyl)-3-(5-nitro-2 furyl) acrylamide] on extending the shelf life of fresh crab meat was investigated. Results show that furylfuramide inhibited growth of the natural flora of crab meat (*in vitro* study). Very little growth occurred in a medium containing 1 ppm of furylfuramide. Even less growth occurred in one containing 5 ppm. Aerobic plate counts of treated crab meat stored for 12 days indicated that treatment in a 5-ppm solution was as effective as treatment in a 20-ppm solution. Exposure of crab meat for 5 min to a 5 ppm solution gave optimum inhibition, all factors considered.

The pH and ammonia content of samples correlated well with results of organoleptic tests. Organoleptic analysis show that furylfuramide extended the shelf life of crab meat by 2 to 4 days. Treatment of fresh picked crab meat in a 5-ppm solution of furylfuramide does not affect its appearance.

Withdrawal of the tetracycline antibiotics from the list of approved food additives has left the fishing industry without an effective preservative for iced seafoods. Although many other preservatives are available to the industry, the tetracycline antibiotics are by far the most effective. However, resistant strains of bacteria develop when exposed to the tetracyclines for long periods of time. Compounds such as sodium benzoate, sodium nitrate, carbon dioxide, and calcium propionate have been suggested as bacteriostats, but they have proved ineffective against the vast number of the more hardy microorganisms associated with fresh seafoods.

Levin (7) demonstrated the beneficial effects of EDTA (ethylenediaminetetraacetic acid) against several species of bacteria of the genus *Pseudomonas* commonly found on fish and shellfish. Although EDTA extended the organoleptic quality of fish fillets, the total bacterial count was only slightly suppressed. Cox and Eagon (2) and Eagon and Carson (4) reported favorably on the use of EDTA and lysozyme to inhibit the growth of bacteria belonging to the genus *Pseudomonas*. Hugo and Frier (6) stated that dequalinium acetate has a detrimental effect on bacterial cells and suggest that nucleic acid containing components of the cell may be the prime target of this compound.

The Japanese have successfully used nitrofurans to combat spoilage bacteria indigenous to fish and

shellfish. Several of these compounds were approved by the Minister of Welfare of Japan; however, only one nitrofuran, furylfuramide, is currently being used. Although not approved for use in foods in the United States, furylfuramide has proven successful in suppressing the bacterial flora in Japanese-produced fish sausage and whole dressed fish (8). The level of residue of furylfuramide allowed by Japanese law varies. For example, 0.02 g of furylfuramide/kg of fish sausage is authorized while as little as 0.0025 g/kg of other fish paste products is allowed. The potential value of two nitrofurans in the preservation of raw iced shrimp was previously reported (10). Of the two compounds investigated, furylfuramide demonstrated the greater degree of inhibition; nitrofuryl acrylamide was less effective. Because furylfuramide has exhibited good antibacterial properties, studies were extended to include its use with crab meat.

Fresh crab meat is one of the most perishable seafood items. Because of the processing method and the necessity of much handling, crab meat has a relatively short shelf life—6 to 8 days when stored in ice. The fresh meat requires 2 days to reach distant markets, as in New York, Philadelphia, and Boston. Hence, only 4 to 6 days of shelf life remain after the product reaches the market.

Current processing methods do not include the addition of preservatives to fresh crab meat (pasteurization is used during the peak season of production). A 4-day extension of shelf life could result in considerable economic gain to the producer and give him more latitude in the distribution of his product. The purpose of this paper is to report the results of a study on the use of furylfuramide in extending the shelf life of fresh crab meat (blue crab, *Callinectes sapidus*).

## METHODS

### Indole

Indole was determined by the method of Duggan and Strasburger (3) as modified by Turner (9). The breakdown of tryptophan to indole is a measure of spoilage.

### Ammonia

Ammonia was determined by a method suggested recently

by chemists of the Food and Drug Administration (5). This method utilized thymol and bromine which are reacted with the liberated ammonia. It is said to be very sensitive.

#### pH

The pH was determined using the Beckman Zeromatic pH-Meter<sup>1</sup>. An increase in pH almost always accompanies spoilage of crab meat.

#### Color

Color-reflectance analysis was made using an Agron color reflectance reflectometer.

#### Total aerobic plate count

Aerobic counts were made using standard pour plate techniques and nutrient agar incubated at 35 C for 48 hr.

#### Organoleptic analysis

Odor was the criterion used in organoleptic analysis. Odor was judged by an expert 5-member panel on a scale of 1-5; 5 being excellent quality, 3 being borderline, and 1 being spoiled.

#### Moisture

Moisture was determined according to methods described by the A.O.A.C. (1).

#### Protein

Protein was determined by the microKjeldahl method of the A.O.A.C. (1).

#### Turbidity

Turbidity of cultures was determined using a colorimeter set at a wavelength of 600m $\mu$ .

## RESULTS

### Effect of furylfuramide on natural flora

Before a detailed study was undertaken, it was necessary to determine if furylfuramide was effective in retarding the growth of bacteria native to picked crab meat. Type(s) of bacteria present were not determined since this was beyond the scope of this study. Effectiveness was determined by an *in vitro* study using three flasks of nutrient broth containing 1, 3, and 5 ppm of furylfuramide, and a control flask without the chemical added. Each flask was inoculated with 1 ml of a dilution of crab meat containing 9,700 microorganisms and incubated at 35 C for 56 hr.

Turbidity of the cultures was measured at regular intervals using a Bausch and Lomb Spectronic 20 colorimeter set at a wavelength of 600 m $\mu$ . The work was carried out in triplicate. Optical density of the control increased very rapidly, reaching a value of 0.575 at the end of incubation. The 1 ppm furylfuramide culture reached an optical density of 0.026, and the 5 ppm culture increased to 0.019. Results of the 3 ppm culture were almost identical to the 5 ppm curve. These results agree with those of Waters and Hamdy (10) using the natural flora of shrimp as an inoculum and/or a pure culture of *Achromobacter aquamarinus*. Furylfuramide had a significant effect in inhibiting the growth of the natural flora of picked crab meat assuming the growth pattern of

microorganisms in the broth was the same as that in fresh crab meat.

### Effect of concentration of furylfuramide

The next step was to ascertain what concentration of furylfuramide in a dipping solution was most effective in preventing a bacterial buildup during storage of crab meat. Twelve pounds of fresh crab meat were obtained from a local processor. The crab meat was thoroughly mixed to provide a homogenous sample, and the sample was equally divided into four lots. One lot was dipped for 5 min in a 5-ppm solution of furylfuramide, a second lot in a 10-ppm solution, and a third in a 20-ppm solution. The fourth lot, which served as the control, was dipped in distilled water. The treated crab meat was drained for 2 min, packed in 1-lb. cans, iced down, and held in a cooler at 5 C. The treatments and analyses were carried out in triplicate. Portions were removed from each treatment at regular intervals. They were assessed organoleptically and for total aerobic plate counts.

The results (Fig. 1) indicate that inhibition depends on concentration; the greater the concentration of furylfuramide, the greater the inhibition. In general, the 5-ppm solution suppressed bacterial growth 2 days as compared with the control; 10 ppm

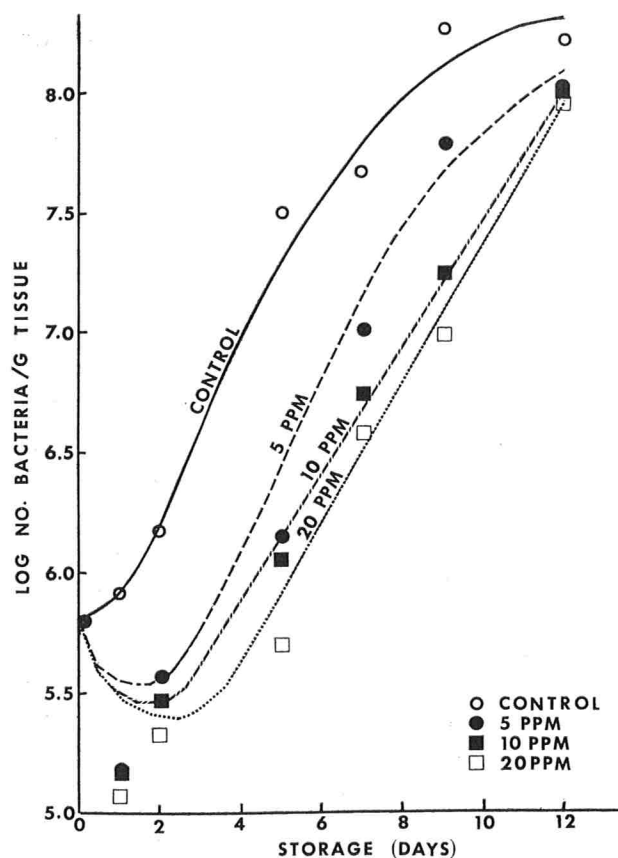


Figure 1. Effect of furylfuramide on growth of aerobic bacteria.

<sup>1</sup>Use of trade name is merely to make descriptions more precise; no endorsement is implied.

suppressed growth 3 days; and 20 ppm suppressed it about 4 days. Organoleptic analysis showed that the control reached borderline quality after 7 days of storage and had spoiled completely after 9 days. All furylfuramide-treated samples were of borderline quality after 9 days and were spoiled after 12 to 13 days. Treatment with furylfuramide thus extended the shelf life of crab meat 2 to 4 days.

Although the 10 and 20 ppm treatments increased the shelf life 1 and 2 days more than the 5 ppm treatment, respectively, concentrations of more than 5 ppm were not suitable. The 10- and 20-ppm solutions caused a yellowish-red color in the crab meat; the color contributed by the 20-ppm solution being more noticeable. The 5-ppm solution did not change the color of the meat appreciably, yet it demonstrated good bacterial inhibitory effects. Consequently, the 5-ppm concentration was selected for further work.

#### Effect of immersion time in a furylfuramide solution

A third factor had to be resolved: How long should the crab meat be immersed in a 5-ppm solution of furylfuramide to be effective yet not cause the color of the meat to change? As before, crab meat was obtained and thoroughly mixed. The sample was divided into four lots to provide samples for dipping times of 1, 5, 10, and 20 min. After dipping, each lot was drained 2 min, packed in 1-lb. cans, and held as was previously outlined. Bacterial and organoleptic analysis were conducted as previously stated.

The bacterial growth curve of all treatments followed the typical growth curve in that there was a lag period of 3 days followed by the logarithmic phase to the end of storage (12 days). As the exposure time increased, the inhibition increased, i.e., there was an inverse relationship between the exposure time and the number of organisms surviving. The lot receiving the 20-min treatment exhibited the lowest bacterial count. The appearance of the crab meat did not change when the 1- and 5-min dips were used. However, when the immersion time was increased to 10 and/or 20 min, the meat appeared to turn a very pale yellow (the color of the dipping solution). Therefore, future immersion times were limited to 5 min.

#### Correlation of chemical, bacteriological, and organoleptic results

The last experiment was conducted to correlate bacteriological, chemical, and organoleptic results of treated and nontreated crab meat. Twenty pounds of freshly picked crab meat were obtained and divided in half. One-half of the crab meat was treated 5 min in a 5-ppm solution of furylfuramide. The second half was treated similarly by dipping the meat in water without the preservative. After each treatment, the meat was drained 2 min and packed in 1-

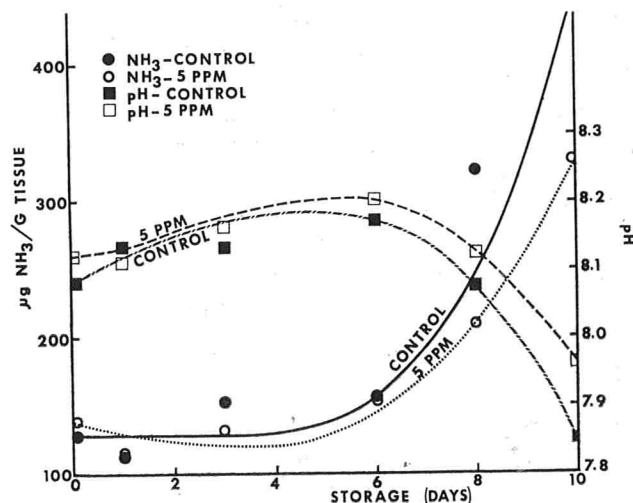


Figure 2. NH<sub>3</sub> content and pH of crab meat during storage.

lb. cans, and the cans were held in crushed ice.

Representative samples were taken during storage for the analyses previously described. Chemical analyses included indole, ammonia, and pH. Total plate counts, organoleptic ratings, and color-reflectance values were also determined.

Results of the bacterial analysis were very similar to those already shown in Fig. 1, the treated sample showing significant inhibition of microbial growth over the control (about 1 log cycle) through the 10th day of storage. However, during the last 2 days of storage, the bacterial count of the furylfuramide-treated sample almost equaled that of the control. Organoleptic analysis also conformed to those reported earlier; the furylfuramide-treated sample showed a 2-day extension of shelf life over the control.

Figure 2 shows the pH of the control and the treated sample. The pH of both samples paralleled each other, the furylfuramide-treated sample exhibiting a higher pH than the control. It is not clear why the chemically-treated sample exhibited a higher pH. Possibly this higher pH resulted from some reaction of the nitrofurans with compounds present in the crab meat or to suppression of acid-producing bacteria. The pH reached maximum on the 6th day of storage, the same time that the control sample was judged to be borderline by organoleptic analysis. The treated sample, however, was rated as "good". After the 6th day the pH began to decrease, indicating the production of acids (such as formic and acetic), canceling out the production of amines and ammonia. In this instance pH did not appear to verify the beneficial effects of furylfuramide.

Figure 2 also shows the results of the ammonia analysis. Interestingly, ammonia increased in the

control over that of the furyluramide sample after 6 days. At the time the control was judged borderline organoleptically, the treated samples rated "good." The pH at this point also changed. After 6 days storage, ammonia in the control increased at a faster rate than in the chemically treated sample. These results verify that furyluramide is effective in reducing the ammonia content and hence in retarding spoilage as determined by odor. Fernandez-Flores and Salwin (5) claim that this method is very sensitive and can detect the onset of spoilage.

Indole was not detected in any of the experimental samples. Apparently, indole was not a byproduct of spoilage in this study.

#### *Color change due to treatment with furyluramide*

In previous experiments it became apparent that the highly colored furyluramide solution was contributing somewhat to discoloration of the crab meat, particularly in the higher concentrations. Consequently, it was desirable to know if treating crab meat with furyluramide would appreciably affect the color, even though a change in color was not apparent to the eye. A color analysis was made before and after treating the crab meat and throughout storage. Values obtained using the blue filter showed a significant color change in the chemically treated sample. The color change, however, was not obvious visually. The treatment can, therefore, be employed commercially without seriously affecting the appearance. Interestingly, the control sample appeared whiter in color after being dipped in distilled water than before being dipped.

#### *Effects of dipping on nutrient value*

It was necessary to determine if crab meat picked up moisture during the treating process and if an appreciable amount of protein was lost due to the treatment. A portion of the meat was taken prior to and after treatment and analyzed for protein and moisture.

Following treatment, samples of the treating solutions (distilled water and 5 ppm furyluramide) were analyzed for protein. Results of the analysis of the meat indicated that 0.32 to 0.48% nitrogen was lost due to the treatments. Analysis of the solutions verified this fact. Moisture analysis showed an increase of 2.5% in the treated samples over the non-treated samples. A longer draining time after treatments, however, could eliminate much of this increase in moisture.

#### *Approval for use as a food preservative*

The Food and Drug Administration has not ap-

proved nitrofurans as food additives. However, after the inhibitory effects of these compounds have been clearly established (assuming, of course, that these effects can be established clearly and lack of toxicity established), an effort should be made by appropriate parties to get these compounds approved for food use.

#### CONCLUSIONS

Furyluramide is effective in inhibiting the activity of aerobic bacteria native to fresh picked crab meat and extends its shelf life by 2 to 4 days as judged organoleptically. A 5-min dip in a 5-ppm solution of furyluramide proved to be the most effective treatment, all factors considered. Although instrumental analysis showed a significant change in the color of treated crab meat over the control, this change does not seriously affect the appearance. Indole was not produced due to bacterial degradation; consequently, the test for indole could not be used to verify spoilage. A new method for determining ammonia in spoiled crab meat showed promise as an indicator of spoilage but did not detect the onset of spoilage. Organoleptic analysis is still the most reliable method for judging quality of crab meat.

#### REFERENCES

1. Association of Official Analytical Chemists (A. O. A. C.). 1965. Official methods of analysis. The Association of Analytical Chemists, Washington, D. C. 20044.
2. Cox, S. T., and R. G. Eagon. 1968. Action of ethylenediaminetetraacetic acid, tris(hydroxymethyl)-aminomethane, and lysozyme on cell walls of *Pseudomonas aeruginosa*. *Can. J. Microbiol.* 14:913-922.
3. Duggan, R. E., and L. W. Strasburger. 1946. Indole in shrimp. *J. Ass. Off. Agr. Chem.* 29:177-188.
4. Eagon, R. G., and K. J. Carson. 1965. Lysis of cell walls and intact cells of *Pseudomonas aeruginosa* by ethylenediaminetetraacetic acid and by lysozyme. *Can. J. Microbiol.* 11:193-201.
5. Fernandez-Flores, E., and H. Salwin. 1968. Determination of ammonia in seafoods. *J. Ass. Off. Anal. Chem.* 51:1109-1110.
6. Hugo, W. B., and M. Frier. 1969. Mode of action of the antibacterial compound dequalinium acetate. *Appl. Microbiol.* 17:118-129.
7. Levin, R. E. 1967. The effectiveness of EDTA as a fish preservative. *J. Milk Food Technol.* 30:277-283.
8. Obatake, A., and T. Matsuda. 1965. Studies on new nitrofurans derivatives as food preservatives. I. On the preservative effect of AF-2 and AF-5. *Bull. Jap. Soc. Sci. Fish.* 31:138-145.
9. Turner, J. M. 1961. A new reagent for the assay of indole in the tryptophanase reaction. *Biochem. J.* 78:790-792.
10. Waters, M. E., and M. K. Hamdy. 1969. Effect of nitrofurans and chlortetracycline on microorganisms associated with shrimp. *Appl. Microbiol.* 17:21-25.



# INCIDENCE AND IDENTIFICATION OF SOME BETA-HEMOLYTIC STREPTOCOCCI IN FOODS<sup>1</sup>

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## ABSTRACT

A total of 109 food products were surveyed for the incidence of *beta*-hemolytic streptococci. The organisms were isolated and enumerated using a pre-enrichment-Most Probable Number technique. Eighty seven isolates were obtained from 18 of 53 meat and fish products. No *beta*-hemolytic streptococci were isolated from the remainder of the samples which included vegetables, dairy products, dehydrated foods, and miscellaneous food items. Serological and biochemical procedures indicated 73 isolates belonged to groups C, D, F, B, L, A, and G, in order of frequency. Fourteen isolates remained ungrouped. The quantity of these organisms, as determined by the Most Probable Number (MPN) technique, ranged from 91 to >24,000 per 100 g of sample.

A number of the *beta*-hemolytic streptococci are frequently associated with diseases of man and animals. Although there are numerous reports on milk-borne (4, 17) and food-borne (2, 5, 6) outbreaks of scarlet fever or septic sore throat caused by group A streptococci, only limited work has been conducted on determining the incidence and types of other *beta*-hemolytic streptococci occurring in retail food products. Since these organisms are generally present in small numbers in comparison with the remaining flora, a selective medium must be used for their isolation. Traditionally sodium azide has been used in media for the selection of streptococci (3, 8, 13, 14). However, azide enrichment broth has been reported to have limited application in the recovery of group A streptococci (8). Nevertheless, its use was continued in this study because of the relatively greater inhibitory effect on gram-negative organisms than on the streptococci.

The Most Probable Number (MPN) technique was employed to estimate the number of these organisms, when present. Following isolation, the precipitin test of Lancefield and biochemical tests were used in classification of the isolates.

## MATERIALS AND METHODS

### Food samples

One hundred and nine food samples, purchased from various food stores in Morgantown, West Virginia, were surveyed for the presence of *beta*-hemolytic streptococci. The samples included meat, fish, vegetables, dehydrated, and miscellaneous products (i.e., pie, tea, cake roll) (Table 1). The food samples were prepared by blending 20 g of the food sample in 180 ml of sterile distilled water for 2 min.

### Isolation procedures

Tryptic Soy Agar (Difco) containing 5% sheep blood, was used for isolating and studying the isolates. Portions of 1 g, 0.1 g, and 0.01 g of the samples (10 ml, 1 ml, and 0.1 ml portions of the 1/10 dilution) were used to inoculate three replicate tubes containing Streptosel Broth (BBL). Following incubation at 32 C for 24 and 48 hr, 0.01 ml from each tube was streaked onto a blood agar plate and incubated at 32 C. Gram stains of hemolytic colonies were made from each plate. Those showing typical streptococcal morphology were transferred to Tryptic Soy Agar slants and stored at 4 C for further study. The MPN of *beta*-hemolytic streptococci present in the sample was then computed (1).

### Identification of isolates

The isolates were streaked on blood agar plates and re-isolated to ensure purity and to verify hemolytic properties before identifications were made. The inocula in the following tests consisted of 0.1 ml of a 24-hr culture in Tryptic Soy Broth.

Two strains of *Streptococcus pyogenes*, which were isolated from infected sore throats, and one strain of *Streptococcus faecalis* var. *zymogenes* obtained from the West Virginia University Medical Center, were used as controls in the biochemical and serological tests.

**Fermentation reactions.** Ten per cent aqueous solutions of sugars and sugar alcohols, arabinose, lactose, sucrose, raffinose, trehalose, mannitol, glycerol, sorbitol, and salicin, were filter sterilized.<sup>1</sup> Prior to inoculation, 1.0 ml of the 10% carbohydrate solution was aseptically added to 9 ml sterile Purple Broth Base (Difco), yielding a 1% concentration of carbohydrate in each tube. Tubes were inoculated and observed at daily intervals, and final readings recorded at the end of 7 days.

**Final pH in dextrose broth.** The 1% dextrose broth was prepared in the same manner as the broth used in the fermentation tests. Following 4 days incubation, pH was obtained using a Beckman Zeromatic pH meter.

**Growth in methylene blue milk.** Tubes (10 ml) of 0.1%

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<sup>1</sup>MS Type HA, pore size 0.45  $\mu$ , Millipore Corporation, Bedford, Massachusetts.

methylene blue milk were inoculated. Readings were made after 24 hr incubation at 32 C and at daily intervals for a week.

*The pH 9.6 tolerance test.* Sterile Tryptic Soy Broth was adjusted to pH 9.6 with sterilized 1N NaOH solution and dispensed aseptically into tubes. The tubes were inoculated and observed for growth after 24 hr incubation.

*Growth at 10 C and 45 C.* Two sets of previously tempered Tryptic Soy Broth tubes were inoculated, with one set of cultures incubated at 10 C and the other at 45 C. Observations for growth were made at 24-hr intervals for a week.

*Production of ammonia from arginine.* The medium of Niven et al. (12) was used. The presence of ammonia was detected on a spot plate with Nessler's reagent after growth for two days at 32 C.

*Precipitin test.* Streptococcal extracts for the precipitin test were prepared by the autoclave method. The antisera for groups A, B, C, D, E, F, G, H, K, L, M, and O were purchased from Difco. The precipitin tests were performed in capillary tubes (16).

## RESULTS

In 109 food products surveyed the occurrence of *beta*-hemolytic streptococci was restricted to meat and fish products (Table 1). They were isolated from ground beef, chicken, lamb stew, sausage, chicken liver, lamb patties, smelt, whiting, and boneless beef stew. Their numbers ranged from 91 to greater than 24,000 per 100 g of food sample. No *beta*-hemolytic streptococci were isolated from any dairy products, fresh and frozen vegetables, dehydrated food, or miscellaneous products tested.

The classification of the isolates depended mainly on the precipitin tests. In cases of weak precipitation or cross reactions, biochemical reactions were then relied upon for grouping. The unclassified isolates were those for which both the serological and biochemical reactions were ambiguous. Among the 87 isolates studied, 73 were classified into groups and 14 isolates remained ungrouped. The distribution of the groups is shown in Table 2.

Three isolates (3.4%), classified as group A, formed precipitates only with group A antiserum; they also exhibited biochemical reactions similar to those of group A streptococci. The five group B isolates (5.7%) also showed a weak precipitate with group F antiserum and they fermented glycerol aerobically.

The largest percentage (41.4%) of the 87 isolates studied were classified as members of Group C, forming heavy precipitates with group C antiserum. The biochemical tests of the majority of the strains were in close agreement with the serological tests, except for one isolate which grew in the presence of 6.5% NaCl, three isolates which grew at pH 9.6, and ten isolates which grew slightly in 0.1% methylene blue milk after a week's incubation.

Sixteen isolates (18.5%) were classified as members of group D. Some isolates did not show the

TABLE 1. DISTRIBUTION OF *beta*-HEMOLYTIC STREPTOCOCCI IN FOOD PRODUCTS

Food products	No. of samples	No. of positive samples	Positive (%)
Meat and fish	53	18	34
Dairy	24	0	0
Vegetables	14	0	0
Dehydrated	14	0	0
Miscellaneous	4	0	0
Total	109	18	16.5

TABLE 2. SEROLOGICAL GROUPING OF THE *beta*-HEMOLYTIC STREPTOCOCCI ISOLATES OBTAINED FROM FOOD SAMPLES

Group	No. of isolates	Per cent
A	3	3.4
B	5	5.7
C	36	41.4
D	16	18.5
E	0	0
F	7	8.0
G	2	2.3
H	0	0
K	0	0
L	4	4.6
M	0	0
O	0	0
Ungrouped	14	16.1
Total	87	100.0

typical Sherman's tolerance reactions but did form strong precipitates with group D antiserum. The reason for the variable biochemical reactions is not known.

Seven isolates (8.0%) were classified as members of group F and two of group G. Strong precipitates were observed. Four isolates gave weak precipitates with group L antiserum.

Among the 14 unclassified isolates, antigen extracts of two unusual organisms gave strong cross reactions immediately with all the antisera tested except group K antiserum. The extract of another isolate precipitated with antisera A, B, C, and F. These three isolates showed no growth at pH 9.6, 10 C or 45 C, in medium containing 6.5% NaCl, and did not reduce 0.1% methylene blue milk. No further studies were made to identify them.

## DISCUSSION

Among the *beta*-hemolytic streptococci, only those of group A have been reported to cause food-borne human diseases, whereas group D are suspect (11). Foods generally become contaminated with group A streptococci from the active infections of the hand-

lers of milk and food. The products involved are usually cold foods such as salads, sandwiches, etc., because these organisms cannot survive pasteurization or the time-temperature conditions of normal cooking. In addition, there is always a time interval between food preparation and food consumption that allows for multiplication of the microorganisms.

Results of this study showed that the largest number of *beta*-hemolytic streptococci isolated were members of group C (36 of 87 isolates). One possible explanation for this high frequency of isolation is that sodium azide is not overly toxic to these organisms. Since these organisms can be infectious to man as can other *beta*-streptococci, adequate heat treatment of these foods is desirable (7, 15).

Milk and dairy products usually are excellent sources of group D and E *beta*-hemolytic streptococci, but surprisingly in this study none were isolated from 24 pasteurized or non-pasteurized milk and dairy products tested. Also no isolates were recovered from beef, turkey, or tuna pot pie. Enterococci have been found in these products (10), however the non-*beta*-hemolytic streptococci of group D were the predominant forms present.

One interesting finding was that ground meats and sausages were always positive for *beta*-hemolytic streptococci. The sausages examined were "home-made," and "country style," both link and roll types which are classified as fresh sausages, as they are neither smoked nor cooked. Since the meats are ground during preparation, it is likely that this step contributes to contamination as well as a more uniform dispersal of the flora.

From a total of 109 samples examined, 16.5% were positive for the presence of *beta*-hemolytic streptococci. Fresh meat and fish were the only sources of these organisms and only 3.4% of the isolates were classified as group A streptococci. All foods involved were of the type requiring further cooking. These organisms therefore would only present a hazard if the food were not thoroughly cooked or if they were to contaminate foods which had already received a terminal heat treatment.

## REFERENCES

1. American Public Health Association. 1965. Standard methods for the examination of water and wastewater. 12th ed. American Public Health Ass., New York.
2. Barron, R. D. 1960. Report of an outbreak of food-borne streptococcal throat infection in the Canadian Army. *Med. Services J. Canada* 16:35-38.
3. Bryan, C. S., E. D. Devereux, W. C. Hirschey, and A. C. Corbett. 1939. The use of brilliant green, sodium azide, and dextrose in the microscopic and Hotis tests for streptococcal mastitis. *North Amer. Veterinarian* 20:4-46.
4. Camps, F. E., A. T. Miller, and J. L. Wood. 1936. An outbreak of epidemic sore throats of milk-borne origin. *Lancet* 231:756-760.
5. Commission on acute respiratory diseases. 1945. A study of a food-borne epidemic of tonsillitis and pharyngitis due to *beta*-hemolytic streptococcus type 5. *Bull. Johns Hopkins Hosp.* 77:143-210.
6. Dudding, B. A., H. C. Dillon, L. W. Wannamaker, R. M. Kilton, S. S. Chapman, and B. F. Anthony. 1969. Post-epidemic surveillance studies of a food-borne epidemic of streptococcal pharyngitis at the United States Air Force Academy. *J. Infect. Dis.* 120:225-236.
7. Evans, A. C. 1944. Studies on hemolytic streptococci VIII. *S. equisimilis*. *J. Bacteriol.* 48:267-284.
8. Hunter, D. H., E. B. Blair, and J. H. Rust, Jr. 1962. A comparison of cultural techniques for the isolation of *beta*-hemolytic streptococci from acute respiratory infections. *Bacteriol. Proc.* 1962:81.
9. Lichstein, H. C., and M. H. Soule. 1944. Studies of the effect of sodium azide on microbial growth and respiration. *J. Bacteriol.* 47:221-257.
10. Litsky, W., I. S. Fagerson, and C. R. Fellers. 1957. A bacteriological survey of commercially frozen beef, poultry and tuna pies. *J. Milk Food Technol.* 20:216-219.
11. Moore, B. 1955. Streptococci and food poisoning. *J. Appl. Bacteriol.* 18:606-618.
12. Niven, C. F. Jr., K. L. Smiley, and J. M. Sherman. 1942. The hydrolysis of arginine by streptococci. *J. Bacteriol.* 43:651-660.
13. Packer, R. A. 1943. The use of sodium azide and crystal violet in a selective medium for streptococci and *Erysipelothrix rhusiopathiae*. *J. Bacteriol.* 46:343-349.
14. Pike, R. M. 1945. The isolation of hemolytic streptococci from throat swabs. Experiments with sodium azide and crystal violet in enrichment broth. *Amer. J. Hyg.* 41:211-220.
15. Sanders, V. 1963. Bacterial endocarditis due to a group C *beta*-hemolytic streptococcus. *Ann. Internal. Med.* 58:858-861.
16. Swift, H. F., A. T. Wilson, and R. C. Lancefield. 1943. Typing group A hemolytic streptococci by M precipitin reaction in capillary pipettes. *J. Exptl. Med.* 78:127-133.
17. Taylor, P. J., and M. A. MacDonald. 1959. Milkborne streptococcal sore throat. *Lancet* 1:330-333.

## SANITATION STUDIES OF A REVERSE OSMOSIS UNIT USED FOR CONCENTRATION OF MAPLE SAP

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### ABSTRACT

Sanitation studies of an industrial-scale reverse osmosis unit used for the concentration of maple sap are discussed. Bacterial plate counts of the concentrate and permeate effluent streams declined steadily during the initial 10-12 hr of operation, followed by respective increases in count from  $2.8 \times 10^4$  per ml (12 hr) to  $2.0 \times 10^5$  per ml (36 hr) and from  $3.3 \times 10^3$  per ml (12 hr) to  $2.4 \times 10^4$  per ml (36 hr). Membrane modules were maintained in good sanitary condition in the reverse osmosis unit pressure vessels for as long as one month when the modules were kept in contact with an acidified chlorine dioxide sanitizer.

Production of 1 gal of maple sirup requires the concentration of 35-40 gal of maple sap (2-3° Brix). In modern maple evaporator plants, this is usually done by boiling the sap in oil-heated atmospheric evaporator pans. Since the characteristic color and flavor of maple sirup are developed during boiling, a modicum of heat must be used in sirup production. But a typical maple evaporator plant consumes 3 gal of fuel oil for every gallon of sirup produced, and the prolonged exposure of sap to heat during the concentration process favors the production of darker colored sirup which has a lower market value.

Partial concentration of maple sap by reverse osmosis offers an attractive alternative to the conventional boiling process. Up to 75% of the water can be removed from sap by this process at about 1/25 the energy cost required for evaporation by conventional methods (5). The concentrated sap can then be rapidly boiled to standard sirup density in a small oil or steam heated finishing pan evaporator which eliminates much of the danger of scorching encountered when large evaporator pans are used. Thus, the sirup producer could cut processing costs and run less risk of product damage from scorching by utilizing reverse osmosis in his plant. An additional advantage lies in the fact that reverse osmosis equipment is compact, requiring little floor space in comparison to that needed for the evaporator pans in current use. However, these advantages must be weighed against the current high cost of reverse osmosis equipment.

The use of reverse osmosis as an intermediate step in the production of maple sirup poses several prob-

lems to the sanitarian. Raw maple sap is readily contaminated by the microflora of the farm-forest environment in which it is harvested and can contain bacterial counts well above  $1.0 \times 10^8$  per ml when it is delivered to the evaporator plant. Once at the plant, bacterial growth in the sap can be controlled by ultraviolet irradiation (2) until the sap is sterilized by the boiling process. However, passage of raw sap through a reverse osmosis (R.O.) unit exposes the sap to further contamination. The concentration of sap by R. O. implies an increase of bacterial count in the concentrate commensurate with the volume of water removed by permeation through the membranes. Bacterial slimes also may develop on the membrane surfaces and impair permeation of water through the membranes thereby cutting operating efficiency. These latter factors are further complicated by the fact that the R.O. units operate as a closed system which cannot be readily disassembled for cleaning. Hence, C.I.P. cleaning techniques must be used in the course of normal plant operation with only occasional shut-downs for more rigorous clean-up.

Initial small scale studies conducted at this laboratory indicated the feasibility of partial concentration of maple sap by reverse osmosis (6). Based on these findings, a large pilot scale reverse osmosis concentrator (EUROC) was designed and built (4). The operational characteristics of the EUROC were tested and optimum operating conditions established in a series of short, 2-6 hr, runs using a 2.5° Brix sucrose solution. Bacterial counts were made on samples taken from the EUROC effluent streams, and a C.I.P. system for sanitizing the unit was developed (3). These studies showed that bacterial growth took place in the unit during short periods of idleness, and that bacterial populations in the effluent streams decreased from beginning to end of a short period of operation. They gave no indication of the sanitation problems which might arise in the course of prolonged operation and concentration of raw maple sap under typical field conditions.

The EUROC was installed in a central evaporator plant and was used for partial concentration of maple

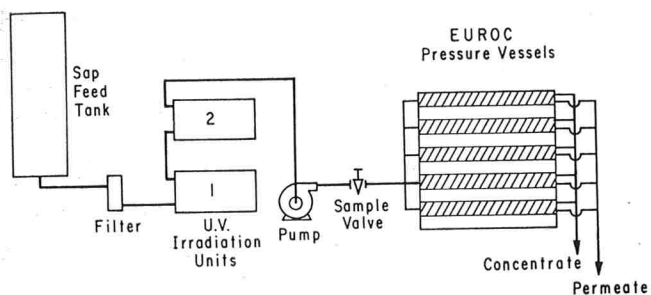


Figure 1. Flow diagram of EUROC maple sap concentration system.

sap during two sap runs late in the sap flow season of 1968. This paper reports the results of sanitation surveys conducted while the EUROC was used to concentrate raw sap delivered to the evaporator plant by independent sap producers or collected by plant employees from plant-owned sugar bushes.

#### MATERIALS AND METHODS

##### Reverse osmosis unit

(a) A reverse osmosis unit (EUROC), capable of processing sap at feed rates up to 12 gpm and pressures up to 700 psig was designed and constructed at this laboratory (4).

(b) The modified cellulose acetate reverse osmosis membranes were spirally wound modules (ROGA<sup>1</sup>) 4 inches in diameter x 12 inches long containing approximately 10 ft<sup>2</sup> of membrane and were obtained from Gulf General Atomic<sup>1</sup>.

##### Operation of EUROC unit

A flow diagram of the EUROC maple sap concentration process is shown in Fig. 1. As raw sap was received at the plant, it was pumped into the sap feed tank from which it was passed first through a Cuno P 110 cartridge filter and then through the UV irradiation units. The filtered, irradiated sap was pumped into the pressure vessels of the EUROC. The concentrated sap effluent from the pressure vessels was fed to a conventional finishing pan evaporator, where it was evaporated to standard density (65.5° Brix) sirup. The permeate effluent was discarded.

##### Sampling and plating

Samples for bacterial counts were taken aseptically from the sap feed tank; the sample valve located in the pump discharge line and from both concentrate and permeate effluent lines (see Fig. 1). Dilution and plating procedures were carried out by A.P.H.A. standards methods (1). Standard tryptone glucose extract agar (Difco) was used for all bacterial counts, with incubation at 30 C for 48 hr. Counts were made using a Quebec colony counter.

#### RESULTS AND DISCUSSION

After the EUROC was emplaced at the central evaporator plant, it was sanitized with a 100-gal rinse of an acidified sodium hypochlorite solution containing 50 ppm available Cl<sub>2</sub> which had been adjusted to pH 4.5 with glacial acetic acid (3). The pH adjustment was made on the advice of the manufacturer of the modules to avoid possible adverse effects caus-

ed by alkaline pH on the membranes. The fresh sap was pumped through the EUROC at 6 gpm and 600 psig. Sap feed temperatures ranged from 52-57 F during the period of operation. Samples for bacterial counts were taken as previously described at the start of operation and at 6-hr intervals, during 36 hr of continuous operation.

The data from this sampling program are shown in Fig. 2. The bacterial count of the raw sap held in the feed tank was low, never exceeding  $1.0 \times 10^5$  per ml during the 36 hr of operation, and the in-line irradiation units effectively reduced this population so that the sap entering the pressure vessels of the EUROC contained less than  $5.0 \times 10^3$  per ml. The zero hour samples of the concentrate and permeate had bacterial counts of  $9.5 \times 10^5$  and  $9.0 \times 10^5$  per ml, respectively, indicating that a considerable buildup of bacteria had taken place in the EUROC pressure tubes during the period of idleness. The bacterial counts in the effluent streams decreased rapidly to  $2.8 \times 10^4$  per ml in the concentrate and  $3.3 \times 10^3$  per ml in the permeate after 12 hr of operation. Then the counts of both discharge streams increased until at the end of the 36-hr operating period the concentrate contained  $2.0 \times 10^5$  per ml and the permeate  $2.4 \times 10^4$  per ml. This increase indicated a progressive build-up of bacterial growth in the pressure vessels which could ultimately produce sliming of the membranes with a resulting loss of operating efficiency.

During the 36 hr of continuous operation, 13,000 gal of maple sap were concentrated; and a top grade, light amber, delicately flavored sirup was made from the concentrated sap.

At the end of the maple season, the EUROC was stored in an air-conditioned room (65 F). The main-

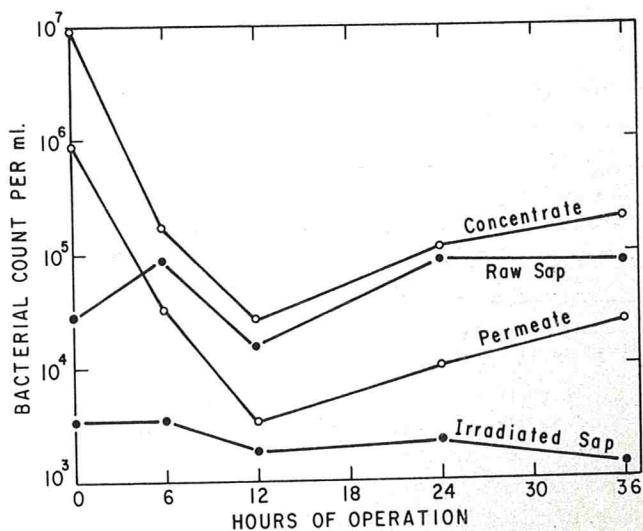


Figure 2. Numbers of bacteria in raw sap, irradiated sap, and effluent stream during 36 hr of continuous operation of EUROC.

<sup>1</sup>Mention of company or trade names does not imply endorsement by the Department over others not named.

TABLE I. BACTERIAL COUNT OF SAMPLES TAKEN FROM REVERSE OSMOSIS UNIT PRESSURE VESSELS AFTER SANITATION AND DURING STORAGE

Sanitizer	Storage time (weeks)	Bacterial count per ml	
		Concentrate	Permeate
Acidified Hypochlorite with H <sub>2</sub> O rinse	0	<10.0	4 x 10 <sup>1</sup>
	1	2.6 x 10 <sup>5</sup>	2.7 x 10 <sup>4</sup>
	2	4.6 x 10 <sup>6</sup>	2.3 x 10 <sup>6</sup>
Acidified ClO <sub>2</sub> with H <sub>2</sub> O rinse	0	<1.0	<1.0
	1	1.1 x 10 <sup>3</sup>	1.1 x 10 <sup>2</sup>
	2	1.5 x 10 <sup>5</sup>	4.4 x 10 <sup>4</sup>
Acidified ClO <sub>2</sub> held in R.O. tubes	0	<1.0	<1.0
	1	<1.0	<1.0
	2	<1.0	<1.0
	3	<1.0	3.0 x 10 <sup>1</sup>
	4	7.0 x 10 <sup>2</sup>	1.2 x 10 <sup>3</sup>

tenance of good sanitary conditions in the EUROCC during long-term storage (8 month) was difficult because it was designed for experimental purposes and therefore was fitted with a more intricate system of valves, controls, flow meters, and piping than would be used in commercial practice. Moreover, the configuration of the spiralwound membrane modules supported by latex-glass bead backing materials further complicated C.I.P. sanitation procedures by impeding turbulent flow across membrane surfaces, and it was also possible that the high pressures exerted in the tubes during operation had compacted the modules creating small "dead" areas which could not be readily sanitized by these procedures. These problems suggested the need for rigorous sanitation techniques.

The following sanitation procedures were studied during the 8-month storage period:

(a) One-hundred gallons of a sodium hypochlorite sanitizer (50 ppm available Cl<sub>2</sub>) acidified to pH 4.5 with glacial acetic acid were pumped through the EUROCC. The final portion of the sanitizer was allowed to stand in contact with the membranes for 30 min. Then, the sanitizer was rinsed from the unit with 200 gal of ultraviolet irradiated tap water.

(b) The above procedure was repeated using a chlorine dioxide sanitizer (50 ppm available Cl<sub>2</sub>) acidified to pH 4.5 with glacial acetic acid.

(c) One-hundred gallons of the acidified chlorine dioxide sanitizer were pumped through the unit. The final portion of the sanitizer was allowed to remain in the unit in contact with the membranes for the duration of the storage period.

Bacterial counts were made on samples taken from both the concentrate and permeate sectors of the

EUROCC at the beginning of each storage period and at weekly intervals thereafter, until the rise in bacterial counts indicated the need for further sanitization.

The bacterial counts made during the final tests of each of the three sanitization techniques are shown in Table I. The hypochlorite sanitizer did not maintain the unit in good sanitary condition. After 2-weeks of storage, bacterial counts of samples taken from both sectors of the pressure tubes exceeded 2.0 x 10<sup>6</sup> per ml and a foul odor was associated with the samples. Because of these results and a persistent hypochlorite flavor residue problem, the use of this sanitizer was discontinued.

A more rigorous sanitizer which could be readily rinsed from the unit was required, and the use of a chlorine dioxide sanitizer (FDA approval for food sanitation pending) was investigated. When this sanitizer was rinsed from the unit with 200 gal of irradiated tap water, as described in procedure (b), a slower build-up of bacterial growth took place, but after two weeks of storage, samples taken from the concentrate and permeate sectors had counts of 1.5 x 10<sup>5</sup> and 4.4 x 10<sup>4</sup> per ml, respectively. The strong odor noted in the previous study was not present in the samples taken from either sector of the unit. However, the growth of bacteria in the pressure tubes indicated that this system would not permit long-term storage of the modules in the pressure tubes.

The final sanitization procedure in which the sanitizer was permitted to remain in contact with the membrane modules was more effective in controlling bacterial growth, but at the end of four weeks of storage bacterial counts of 7.0 x 10<sup>2</sup> per ml in the concentrate sector sample and 1.2 x 10<sup>3</sup> per ml in the permeate showed that the sanitary condition of the EUROCC was deteriorating. Tests for the presence of Cl<sub>2</sub> in the samples were negative. More study is required to develop better cleaning procedures for the EUROCC and methods for maintaining spiralwound membranes in good sanitary condition during prolonged storage.

#### CONCLUSIONS

(a) During 36 hr of continuous operation, the bacterial counts of the concentrated sap and permeate effluents decreased steadily for the first 12 hr, but then, though the bacterial count of the feed declined, the count of the concentrate increased from 2.8 x 10<sup>4</sup> per ml (12 hr) to 2.0 x 10<sup>5</sup> per ml (36 hr) and that of the permeate increased from 3.3 x 10<sup>3</sup> per ml (12 hr) to 2.4 x 10<sup>4</sup> per ml (36 hr). This indicated that bacteria could survive and multiply in the EUROCC pressure vessels while the unit was in operation concentrating maple sap.

(b) C.I.P. cleaning of the EUROCC with 200 gal of

an acidified chlorine dioxide sanitizer (pH 4.5-4.7, 50 ppm available  $\text{Cl}_2$ ) effectively reduced bacterial populations in the effluent streams to  $<1.0$  per ml.

(c) When the sanitizer was flushed from the EU-ROC tubes with irradiated tap water, a slow build-up of bacterial contamination took place during 2 weeks of storage.

(d) An acidified chlorine dioxide sanitizer (pH 4.5-4.7, 50 ppm available  $\text{Cl}_2$ ), when left in the EUROCC pressure vessels in contact with the membrane modules, maintained the pressure vessels in good sanitary condition for a storage period of one month.

#### ACKNOWLEDGMENTS

The author acknowledges the assistance of Mr. L. H. Sipple, Bainbridge, New York whose central evaporator plant facilities were used in the course of this work, and of Mr. R. A. Bell of this laboratory who assisted in the sampling and plating program.

#### AUTHORS' NOTE

Shortly after the completion of this work, a new spiral

wound membrane module became available. This module has new backing material, improved separators, and its rigid construction resists compaction. The new modules should eliminate much of the sanitation problem posed by the now obsolete modules used in the work reported here.

#### REFERENCES

1. American Public Health Association. 1960. Standard methods for the examination of dairy products. 11th ed. Amer. Public Health Ass., Inc., New York, N. Y.
2. Kissinger, J. C., C. O. Willits, and R. A. Bell. 1969. Control of bacterial growth in stored maple sap by irradiation of the sap surface with germicidal lamps. *Dev. Ind. Microbiol.* 10:140-149.
3. Kissinger, J. C., and C. O. Willits. Preservation of reverse osmosis membranes from microbial attack. *Food Technol. In press.*
4. Moore, W. H. Jr., and C. O. Willits. A reverse osmosis plant for maple sap concentration. *Food Technol. In press.*
5. Underwood, J. C., and C. O. Willits. 1969. Operation of a reverse osmosis plant for the partial concentration of maple sap. *Food Technol.* 23:787-790.
6. Willits, C. O., J. C. Underwood, and U. Merten. 1967. Concentration by reverse osmosis of maple sap. *Food Technol.* 21:24-26.

## NUTRITIONAL ASPECTS OF DAIRY PRODUCTS<sup>1</sup>

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#### ABSTRACT

The materials used in building the human body must be chosen with care so that good health and long life are possible. Research in the study of nutrition has shown that certain food nutrients are essential in promoting physical fitness. When taken into the body in adequate amounts, these nutrients, together with good habits of health and hygiene, promote growth and sound physical development.

As the study of nutrition continues, the value of milk in the diet becomes more apparent. Everyone needs milk every day in order to receive the essential food nutrients their bodies require daily.

Millions of Americans are crumbling on the inside while showing no outward sign. The fault lies with a "hidden hunger" for calcium—a hunger that could easily be met and overcome by proper diet. Milk is the best source of calcium in our food supply. It is almost impossible to supply the amounts of calcium recommended unless milk in some form is used daily and cheese and other milk containing products are eaten frequently.

Calcium is just one of many nutrients found in milk.

Altogether, at least 100 chemical components have been identified. Milk's value as a whole is greater than just the sum of its known components.

Milk gives more food for the money than any other food material available. A quart of milk weighs 2.15 pounds. It is a package of nutrients, safety, convenience, flavor, and economy. A true daily investment in health.

In order to fully appreciate the nutritional aspects of dairy products, I think it good to take a moment and think about the meaning of the word nutrition. Nutrition does begin with food, but it is more than food. It is the food itself plus all the things that happen to it from the time it is eaten until it actually nourishes the body. Nutrition is really a process in which food is digested and its nutrients are absorbed and finally distributed to the parts of the body where they are utilized in all metabolic activities. This process may be entirely successful, or it may be faulty in varying degrees at different points. The faults may consist of too little, too much, or the wrong kinds of food; or there may be functional failure in any of the steps through which food passes

(Continued on Page 334.)

<sup>1</sup>Presented at the Fifty-Sixth Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, Inc., Louisville, Kentucky, August 17-21, 1969.

# IRRADIATION OF PACIFIC COAST FISH AT SEA<sup>1</sup>

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## ABSTRACT

An experimental shipboard irradiator was installed aboard the Bureau Of Commercial Fisheries research vessel *Miller Freeman* and utilized to irradiate West Coast fish at sea. The optimum dose range for irradiating whole English sole (*Parophrys vetulus*), Dover sole (*Microstomus pacificus*), Pacific cod (*Gadus macrocephalus*), Pacific ocean perch (*Sebastes alutus*), and black rockfish (*Sebastes melanops*) is in the range of 50 to 100 kilorads. The shelf life (at a high level of quality) of the irradiated products was extended by one and one-half to two times that of the nonirradiated samples. Among the five species irradiated at various states of rigor, prerigor-rigor fish generally had better storage characteristics than postrigor fish.

The Bureau of Commercial Fisheries Technology Laboratory at Seattle, Washington has completed a program to determine the feasibility of irradiating West Coast fish at sea. The program was sponsored by the United States Atomic Energy Commission.

The rationale for irradiating whole fish at sea is to apply the radiation treatment at a time when it is most effective: (a) while the bacterial population is still at relatively low numbers in the lag phase of growth, and (b) before penetration of surface bacteria into tissues has occurred and produced metabolic end products which, together with the endogenous biochemical and physical changes in fish tissue, lead to spoilage.

By irradiating fish at sea, we hope to be able to: (a) land fish of very high quality, (b) widen the areas of sale of the product by increasing its shelf life at a high level of quality, (c) utilize a smaller radiation dose than that required for irradiating fish after landing ashore to attain an appreciable shelf-life extension, and (d) improve the economics of operating the fishing vessels by permitting them to remain at sea longer and come in with a larger catch.

Slavin and Ronsivalli (5) demonstrated that the better the quality of fish at the time of irradiation, the better the quality and the longer the refrigerated storage life of treated fish. Liston et al. (2) found that the effectiveness of pasteurizing radiation in increasing the shelf life of the fish held under refrigeration is greatest for fresh fish with a low bacterial count. It is evident then that the best time to irra-

diate fish for extending the shelf life at a high level of quality would be immediately after capture.

Irradiation of East Coast fish and shellfish at sea was studied by the Bureau of Commercial Fisheries at Gloucester, Massachusetts (1). A shipboard irradiator installed aboard the *MV Delaware* was utilized to irradiate surf clams (*Spisula solidissima*), herring smelt (*Argentina silus*), and headed and gutted haddock (*Melanogrammus aeglefinus*) and codfish (*Gadus morhua*). The shelf life of the irradiated products was double or triple that of the nonirradiated products. The prerigor fish appeared to be more suitable for irradiation than postrigor fish (1).

In contrast to the Atlantic Coast where trawl-caught fish are headed and gutted aboard the vessel, on the Pacific Coast the trawl-caught fish are iced in the round aboard the vessel. In the study reported here, we simulated the current custom of the West Coast fishermen and used fish in the round for irradiation studies. The purpose of this study was to determine the benefits of irradiating West Coast fish at sea immediately after catching.

This paper is divided into three sections: (a) the shipboard irradiator installation aboard the *MV Miller Freeman* and its calibration, (b) optimum dose range for irradiating fish at sea, and (c) effect of pre-irradiation storage time on the post-irradiation storage characteristics of Pacific Coast fish.

## THE SHIPBOARD IRRADIATOR

### General description and installation

Physically and operationally, the shipboard irradiator is similar to that used by the Bureau of Commercial Fisheries, Gloucester, Massachusetts (1).

The irradiator (Fig. 1) was mounted on the platform deck at midship on the port side of the *Miller Freeman* (Fig. 2) in accordance with a design engineered by the naval architect.

### Dosimetry of the shipboard irradiator

In the majority of the radiation experiments, maximum capacity of stainless steel product containers (16 inches long, 6 inches wide, and 9 inches high) would be utilized. With that in mind, the minimum and maximum radiation doses that a sample would receive per hour of dwell time was determined using the general procedures outlined in the *U.S. Atomic Energy Commission Food Irradiator Calibration*

### Manual (4).

When the stainless steel containers were filled with rockfish fillets and a 20 min dwell time was used, the maximum

<sup>1</sup>This work was done under a Division of Isotopes Development Contract from U.S. Atomic Energy Commission.



TABLE 1. SENSORY CHANGES OBSERVED IN THE VARIOUS SPECIES OF FISH IRRADIATED IN THE SHIPBOARD IRRADIATOR AT MEAN DOSES OF 50, 100, AND 200 KILORADS UP TO 24 HOURS AFTER CATCH AND THEN STORED AT 33 TO 34 F<sup>1</sup>

Species	Sensory changes observed	Type of fish sample	Time changes first observed days
English sole	Free drip readily apparent	Whole	14
	Rancidity	Fillets	14
	Slight discoloration of scales	Whole	14
	Slight erosion of scales in stomach region	Whole	14
	Red discoloration of flesh	Fillets	14 to 21
	Greenish discoloration of flesh	Fillets	14 to 21
	"Barnyard" odors	Whole	14
Dover sole	Slight reddening	Fillets	11
	Slight greenish discoloration	Fillets	13
	Slight autolysis	Whole	11
Pacific cod	Autolysis in gut cavity	Whole	14
Black rockfish	Slight discoloration of flesh	Fillets	15
	Slight autolysis in region of stomach	Whole	21
Pacific ocean perch	Slight discoloration	Fillets	20

<sup>1</sup>The samples were irradiated at mean doses of 50, 100, and 200 kilorads.

dose found was  $41.5 \times 10^4$  rads and the minimum dose  $21 \times 10^4$  rads. The maximum and minimum dose rates were 1245 kilorads and 630 kilorads for each hour of dwell time, respectively. The uniformity ratio is  $U = \frac{1245}{630} = 1.98$ .

#### OPTIMUM DOSE RANGE FOR IRRADIATING FISH AT SEA

When considering irradiation of fish at sea, we need to know what would be an optimum dose to use. The optimum dose is the dose range that would least alter the sensory characteristics of the irradiated products as compared to the nonirradiated counterparts and still significantly extend the storage life of the samples at above freezing refrigerated temperatures.

In determining the optimum dose for irradiating whole fish, we assumed that the density of the whole fish is the same as that of the fillets and that the product containers are always filled to capacity for irradiation.

#### Procedure

Five species of commercially important trawl fish—namely, English sole (*Parophrys vetulus*), Dover sole (*Microstomus pacificus*), Pacific cod (*Gadus macrocephalus*), black rockfish (*Sebastes melanops*), and Pacific ocean perch (*Sebastes alutus*) were caught off the West Coast of the United States during a cruise by the *Miller Freeman*. The fish from each species were divided into four groups, three of which were irradiated within 24 hr after catch in the shipboard irradiator and the fourth was retained as a nonirradiated control. Group 1 received a mean dose of 50<sup>1</sup> kilorads, Group 2 a mean dose of 100<sup>1</sup> kilorads, and Group 3 a mean dose of 200<sup>1</sup> kilorads.

Throughout the tests, fish were stored at 33 to 34 F.

<sup>1</sup>Groups 1, 2, and 3 received maximum doses of 70, 140, and 270 kilorads, respectively, and minimum doses of 35, 70, and 140 kilorads, respectively.

Periodic sensory evaluations were made on two to five whole fish from each treatment and on the raw and cooked fillets cut from these fish at the time of evaluation. An experienced panel of 5 to 10 judges evaluated the cooked samples using a 10-point scale (3). The limit of the storage life for any sample was reached when the average quality rating was below 5.

#### RESULTS AND DISCUSSION

The various sensory changes and overall scores for the five species of fish are presented in Tables 1 and 2.

For the five species studied, the optimum dose appeared to be 50 to 100 kilorads.

For *English sole*, the shelf life was limited by development of rancidity and reddish and greenish discolorations. These changes became readily apparent after storage of 14 to 21 days and point to the necessity of filleting the fish as soon after landing ashore as possible.

For *Dover sole*, a reddish discoloration in the flesh first observed at 11 days of storage and a greenish discoloration first observed at 13 days steadily increased with storage time. These fish should also be filleted soon after landing ashore.

For *Pacific cod*, the 200-kilorad samples had off-flavors that appeared to be related to the higher radiation dose. Pacific cod also should not be stored whole for more than 2 weeks owing to autolysis that occurs in the gut cavity region.

For *black rockfish*, the 200-kilorad samples had off-

TABLE 2. SENSORY EVALUATION OF VARIOUS SPECIES OF FISH IRRADIATED IN THE SHIPBOARD IRRADIATOR WITHIN 24 HOURS OF CATCH AND THEN STORED AT 33 TO 34 F

Species	Storage time after irradiation days	Overall sensory scores of fish samples <sup>1</sup> irradiated at:			
		0 kilorad	50 kilorads	100 kilorads	200 kilorads
English sole ( <i>Parophrys vetulus</i> )	0	9.5	9.2	9.2	9.2
	6	7.8	7.5	8.0	8.0
	10	5.2	7.4	—	—
	13	4.7	5.2	—	—
	15	—	4.6	7.0	—
	20	—	—	6.6	—
	24	—	—	5.0	6.0
27	—	—	—	3.8	
Dover sole ( <i>Microstomus pacificus</i> )	6	7.9	8.2	8.0	—
	11	3.2	7.2	5.0	—
	13	—	5.2	5.8	—
	18	—	2.0	2.0	—
True cod ( <i>Gadus macrocephalus</i> )	1	9.4	9.0	9.0	9.4
	7	6.7	7.4	8.0	8.1
	14	4.3	8.0	8.0	5.8
Black rockfish ( <i>Sebastes melanops</i> )	9	8.2	8.6	8.2	7.2
	15	2.0	6.8	6.8	4.4
	21	—	5.2	5.7	—
	24	—	2.0	3.5	—
Pacific ocean perch ( <i>Sebastes alutus</i> )	5	7.8	8.1	7.9	7.4
	11	7.0	7.0	7.0	6.3
	15	4.3	6.5	6.0	5.7
	20	—	4.5	1.8	3.7

<sup>1</sup>Two to five fish from each irradiation treatment were examined at each sampling interval.

flavors that appeared to be related to the higher radiation dose rather than to bacterial spoilage. Throughout the tests, the appearance of the whole fish remained good, but the flesh gradually darkened with increasing time of storage of the whole fish. Slight autolysis in the region of the stomach was observed at 3 weeks of storage.

For *Pacific ocean perch*, the appearance of the whole fish and of the fillets cut from these fish at the time of evaluation remained good throughout the tests. However, the odor gradually deteriorated with time of storage.

### Conclusions

With English and Dover sole, black rockfish, Pacific cod, and Pacific ocean perch, the optimum dose for irradiating whole Pacific fish at sea is in the range of 50 to 100 kilorads.

### EFFECT OF PRE-IRRADIATION STORAGE TIME ON THE POST-IRRADIATION STORAGE CHARACTERISTICS OF PACIFIC COAST FISH

Having found that the optimum dose range for irradiating English and Dover sole, Pacific cod, black rockfish, and Pacific ocean perch soon after catch to be in the range of 50 to 100 kilorads, next we studied the storage characteristics of the several species of fish when irradiated at various states of rigor.

### Procedure

Fish of medium size were divided into three groups: Group 1 was irradiated at a mean dose of 50 kilorads, Group 2 at a mean dose of 100 kilorads, and Group 3 was retained as a nonirradiated control. At the time of irradiation, the fish were either in the prerigor (0 to 3 hr after catch), rigor (6 to 24 hr after catch), or postrigor (2 to 3 days after catch) state. The irradiated and nonirradiated samples were stored at 33 to 34 F throughout the tests. Periodically, several fish from each treatment were evaluated by sensory means for

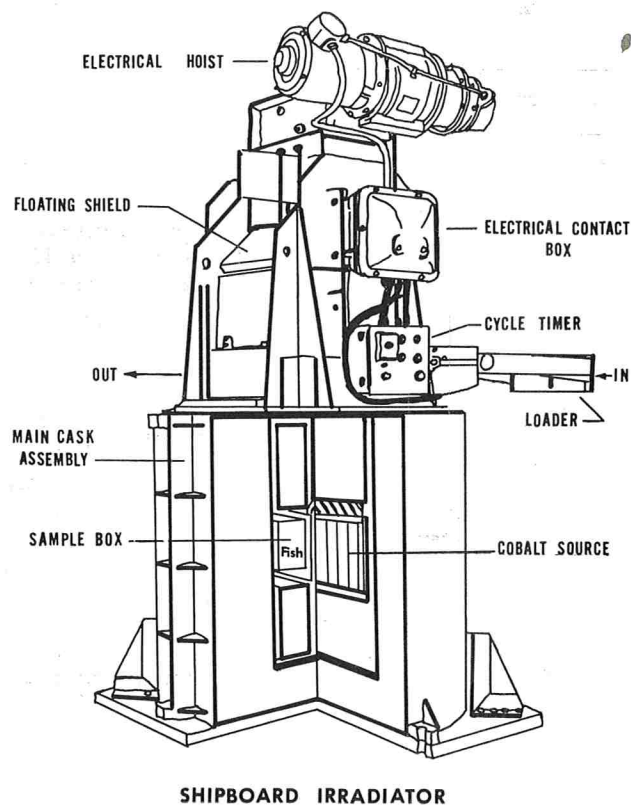


Figure 1. The shipboard irradiator and a cut-away view of the irradiation chamber.

changes in the quality of the raw and cooked samples. The differences in the quality attributes between the test samples were subjected to statistical analysis by Student's "t" tests (6). The limit of the storage life for any sample was reached when the average quality rating was below 5.

English sole and cod were irradiated at a mean dose of 100 kilorads only, as the amount of fish available for irradiation was limited.

The planned bacteriological tests on the various fish samples were not made owing to the extremely rough seas during the cruise.

TABLE 3. SENSORY EVALUATIONS OF FISH FILLETS CUT AT EACH EXAMINATION PERIOD FROM SEVERAL SPECIES OF FISH WHICH HAD BEEN IRRADIATED DURING THE VARIOUS STATES OF RIGOR AT MEAN DOSES OF 50 KILOBRADS AND 100 KILOBRADS AND THEN STORED AT 33 TO 34 F

Species	Storage time days	Overall sensory scores of fish <sup>1</sup> irradiated at										
		50 kilorads							100 kilorads			
		0 kilorad	Pre-rigor	Rigor	Postrigor			Pre-rigor	Rigor	Postrigor		
					2 days	5 days	8 days			2 days	5 days	8 days
Black rockfish ( <i>Sebastes melanops</i> )	9	8.2	8.8	8.6	7.8	—	7.5	8.2	8.3	8.3	—	8.0
	15	2.0	6.8	7.0	7.0	—	3.2	6.8	6.0	6.0	—	4.5
	21	—	5.2	3.8	5.0	—	—	5.2	5.0	3.2	—	2.0
	24	—	2.0	—	—	—	—	3.5	2.0	—	—	—
Pacific ocean perch ( <i>Sebastes alutus</i> )	5	7.2	8.1	7.7	—	7.2	—	7.9	8.2	—	7.4	—
	11	7.0	7.0	7.0	—	6.0	6.0	7.0	7.0	—	6.2	5.5
	15	4.3	6.5	6.0	—	6.3	2.0	6.0	5.7	—	5.2	5.3
	20	—	4.5	4.5	—	2.0	—	1.8	4.8	—	3.7	3.7
Dover sole ( <i>Microstomus pacificus</i> )	6	7.9	8.2	—	—	7.3	—	8.0	—	—	7.0	—
	11	3.2	7.2	—	—	5.2	—	5.2	—	—	5.0	4.0
	13	—	5.0	—	—	3.7	—	5.7	—	—	3.5	—
	18	—	2.0	—	—	—	—	2.0	—	—	—	—
English sole ( <i>Parophrys vetulus</i> )	8	8.0	—	—	—	—	—	7.1	7.3	7.6	—	7.7
	14	2.0	—	—	—	—	—	6.0	4.3	2.0	—	2.0
Lingcod ( <i>Ophiodon elongatus</i> )	7	8.0	—	—	—	—	—	7.6	8.1	—	—	8.2
	14	4.8	—	—	—	—	—	7.3	7.2	—	—	6.7
	18	—	—	—	—	—	—	5.0	5.3	—	—	—

<sup>1</sup>Two to five fish from each irradiation treatment were examined at each sampling interval.

## RESULTS AND DISCUSSION

The results of the sensory evaluations of the cooked fillets cut at each examination from fish irradiated at the various states of rigor are presented in Table 3.

For black rockfish, the samples irradiated prerigor, rigor, and 2 days after catch had significantly better storage characteristics than their corresponding nonirradiated counterparts. No improvement in quality nor extension in shelf life was realized by irradiating 8-day-old fish. Only small differences in quality were found among the fish irradiated prerigor, rigor, or 2-day-old fish. The prerigor fish had the best storage characteristics and were followed by the rigor and 2-day-old fish.

For cod, irradiation of samples up to 8 days after catch improved the storage characteristics of the fish. The shelf life of the irradiated fish was up to 18 days compared to 7 to 14 days for the nonirradiated control samples. No significant differences in quality and storage-life extension were found between fish irradiated prerigor or while in rigor; however, both were slightly better in quality than the fish irradiated after 8 days of storage.

For Pacific ocean perch, slight differences existed among the fish irradiated prerigor, in rigor, and 5 days after catch. The prerigor and rigor fish received the highest sensory scores. Only slight improvement in storage characteristics was realized by irradiating 8-day-old fish.

The Dover sole irradiated prerigor and 5 days after catch had significantly better storage characteristics than the corresponding nonirradiated fish or fish irradiated 8 days after catch. Among the prerigor and the 5-day-old fish, the prerigor fish had

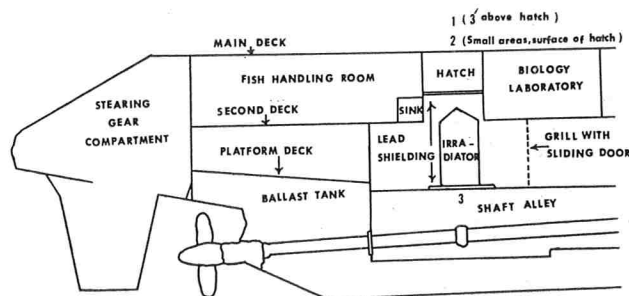


Figure 2. Location of irradiator on the vessel Miller Freeman and areas tested in the radiation survey.

the better storage characteristics. Irradiation of 8-day-old fish produced no improvement in quality or shelf-life extension.

For *English sole*, the prerigor-rigor irradiated fish had significantly better storage characteristics than the postrigor fish. However, when *English sole* was stored in ice, no significant differences were found in the quality of the fish irradiated at rigor or 8 days after catch.

For the five species studied, the inferior storage characteristics of the samples irradiated postrigor in comparison to those irradiated prerigor may be caused by the presence in the postrigor fish of more bacterial enzymes and end products, which may have already diffused into the tissues, than there were in the prerigor fish. Both the prerigor and postrigor irradiated fish, however, when held for extended periods of time at above-freezing refrigerated temperatures, suffer from autolysis in the digestive system. In our studies, this was readily apparent in cod at 2 weeks and in rockfish at 3 weeks. Autolysis of the food causes off-odors and a degradation in the quality of the flesh, especially in the proximity of the gut cavity. These quality defects resulting from enzymatic action certainly point to the necessity of gutting the fish as soon as possible. The combined activities of the enzymes and end products produced by the inactivated microorganisms and by those organisms that survived the irradiation treatment lead

to eventual spoilage. Based on the findings of this study, it appears that greater benefits from the irradiation process would be realized if the fish are headed and gutted prior to irradiation.

#### Conclusion

Among the five species of Pacific Coast fish irradiated at different states of rigor, the prerigor-rigor fish generally had better storage characteristics than the postrigor fish.

#### REFERENCES

1. Carver, J. H., T. J. Conners, and J. W. Slavin. 1969. Irradiation of fish at sea. pp. 509-513. In Rudolph Kreuzer (ed.) Freezing and irradiation of fish. Fishing News (Books) Limited, London (FAO).
2. Liston, J., A. M. Dollar, and J. R. Matches. 1969. Irradiation of fish at sea. pp. 463-471. In Rudolph Kreuzer (ed.) Freezing and irradiation of fish. Fishing News (Books) Limited, London (FAO).
3. Miyauchi, D., M. Eklund, J. Spinelli, and N. Stoll. 1968. Irradiation preservation of Pacific coast shellfish. I. Storage life of king crab meats at 33 and 42 F. Food Technol. 18:138.
4. Rizzo, F. X. 1968. Atomic Energy Commission food irradiation calibration manual. Brookhaven National Laboratory, New York.
5. Slavin, J. W., and L. J. Ronsivalli. 1963. Study of irradiated-pasteurized fishery products. In Annual report for the period October 1962 to September 1963. USAEC TID-19969.
6. Snedecor, G. W. 1956. Statistical methods, 5th ed., The Iowa State College Press, Ames, Iowa.

#### NUTRITIONAL ASPECTS OF DAIRY PRODUCTS (Continued from Page 329)

before it is ready for body use.

Food goes beyond its purely physical functions. It is said to be essential for the "spirit" as well as the body. Food is associated with every human emotion. For the individual it may symbolize joy or sorrow, comfort or fear, security or conflict. As these psychological factors operate, they may affect the amounts and kinds of food one eats and how well the body utilizes food for its own nourishment.

The outcome of such physical and emotional processes—the product of them—is the individual himself. How well he is nourished depends on how well these physical and emotional factors function. His level of nourishment is referred to as his nutritional status. His nutritional status is an essential aspect of his total health, which implies not only freedom from disease, but physical, mental, and emotional fitness as well.

Two chief factors determine the rate at which a

person grows and the size which he attains: his in-born capacity to grow, and various environmental conditions, very important among which is nutrition. The two are interdependent in the sense that heredity limits the final size a person may become; nutrition largely determines whether an individual achieves this limit. This concept has been aptly paraphrased: *The blue print is in the chromosomes, but the bricks and the mortar for the building of a strong healthy body are found in the food market basket.*

So it is with pride that I discuss a food that is so important in the nutritional status of man—milk.

Milk—one of man's oldest foods—is still one of his favorites. He's been quenching his thirst with it since 9000 B.C., according to historic drawings found in the Sahara Desert.

Many important early civilizations—Egyptian, Greek, and Roman—left evidence in writings and drawings of the importance of milk. And that inveterate

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# REMOVAL OF BACTERIA FROM LIQUID EGG WHITE BY CENTRIFUGATION

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## ABSTRACT

Continuous bacterial centrifugation, or bactofugation, of clarified, liquid egg white reduced total bacterial count 50 to 99.7%, depending on processing temperatures.

Coliforms and enteric bacteria were found in liquid egg white in small numbers and their reduction by bactofugation was generally less than obtained for total bacteria. Effectiveness of removal of bacteria from liquid egg white by bactofugation was related to temperature, initial bacterial numbers, and prior mechanical clarification.

The application of bactofugation to liquid egg white as a supplement to pasteurization or hydrogen peroxide-catalase treatment requires more intensive study for proper assessment of value.

Liquid egg products are now pasteurized to obtain increased safety and keeping quality. As a rule, liquid egg does not withstand high temperatures (7, 8, 13, 14). Therefore, the heat margins are not wide because of the usually adverse effects upon the physical and functional (foamability) properties of liquid egg. Specifically, untreated liquid egg white, or albumen, is particularly labile and heating to 59.4 C for 3.5 min is considered maximum. Cunningham and Lineweaver (4) have extended this heat level (59.4 C) about 1 C by lowering the pH from 9.0 to 7.0 and introducing aluminum salts as stabilizers. This heat maximum is lower than that for liquid whole egg but bacterial reduction efficiency is higher for liquid egg white than for liquid egg. Another new process is designed to keep the heat treatment of liquid egg white low and bacterial destruction high by applying hydrogen peroxide at 54 C. Then catalase is added to remove excess peroxide (11).

Lineweaver (10) reported that pasteurization does not always adversely affect whipping, although the whipping quality of liquid egg white may deteriorate when exposed to temperatures below pasteurization. When adverse effects do occur, the performance can be improved materially by adding a triethylcitrate type whipping aid.

Effectiveness of bacterial reduction in liquid egg white under any treatment depends on many factors, including initial load and type of microbial species.

Angelotti (3) stated that liquid eggs containing 5,000-10,000 *Salmonella* organisms per ml and processed at 60 C for 3.5-4 min expectantly can have these counts reduced four log cycle concentration to non-detectable levels but that liquid eggs containing a larger number of these organisms would probably contain *Salmonella* cells after pasteurization.

In 1953 Simonart and Debeer (15) introduced a centrifugal process, called bactofugation, for removing bacteria from milk heated to 70-72 C in order to improve keeping quality. Later Kosikowski and O'Sullivan (9) showed that coliforms were effectively reduced 99.9% in cheese milk heated to only 54 C.

Since any significant bacterial reduction prior to standard processing of liquid egg is obviously advantageous, particularly at a relatively low temperature, the effectiveness of bactofugation to reduce bacteria in liquid egg white at 54 C, or lower, was studied. The results are given in this report.

## MATERIALS AND METHODS

### Eggs

Fresh, liquid egg white in 30-lb cans was obtained in a chilled state from a New York State commercial egg-breaking concern. The liquid egg white was mixed together in stainless steel vats prior to processing.

### Treatment

Approximately 2,000 lb of liquid egg white were processed in the first trial; thereafter each pilot scale trial was conducted with about 1,000 lb. In experiments involving different heat treatments prior to bactofugating, the liquid egg white was divided into three equal portions and heated to the stipulated temperature in separate stainless steel vats, using jacketed steam, except that for liquid egg white heating to 54 C occurred in a DeLaval, type P5-VEB, plate heat exchanger. Samples of the raw, heated, and bactofugated liquid egg white were collected in sterile flasks and analyzed.

### Mechanical apparatus and treatment

Except for the first trial where no clarification was carried out, liquid egg white was first mechanically clarified in an air-tight DeLaval Model 266 Milk Clarifier. The clarified liquid egg white was pumped through two Alfa Laval Bactofuges, type D3187M, at 13,000 lb per hr and chilled thereafter to 5 C in the cooling section of a DeLaval plate heat exchanger.

### Bacterial determination

Bacterial counts selected from three dilutions of the liquid egg white were made in duplicate, using serial dilution and

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TABLE 1. EFFECT OF LABORATORY CENTRIFUGATION OF LIQUID EGG WHITE, OR ALBUMEN, AT DIFFERENT pHs ON THE STANDARD PLATE COUNT

Centrifuge treatment of raw, liquid egg white	pH of liquid egg white <sup>a</sup>			
	9.0		6.6	
	(bact/ml)	(% reduction)	(bact/ml)	(% reduction)
None	2,100,000	—	930,000	—
Laboratory centrifuged at 9,000 G	2,650	99.87	1,900	99.73

<sup>a</sup>Liquid egg white adjusted with lactic acid to obtain pH 6.6.

pour plate techniques recommended in *Standard Methods for the Examination of Milk and Milk Products (1)*. The following media were used: standard plate count agar for total count; violet red bile agar for coliforms; and MacConkey, Salmonella-Shigella, Bismuth Sulfite, and Desoxycholate Citrate agar for enteric bacterial types.

Total protein was determined by the Kjeldahl method and pH by a Beckman glass electrode potentiometer.

## RESULTS

### *Bactofugation of unclarified egg white*

In the first trial, 2,000 lb of liquid egg white were bactofugated at 43 to 54 C without prior mechanical clarification. Extremely variable bacterial counts were obtained which raised a question as to whether liquid egg white was properly adaptable to bactofugation. A laboratory trial on two small lots of liquid egg white at 15 C, utilizing an International Model B20 Centrifuge, was conducted to determine whether numbers of bacteria could be reduced effectively by centrifugation. One lot of liquid egg white was held at its natural pH of 9.0; the other lot was adjusted downward with lactic acid to pH 6.6. Each was centrifuged in 50-ml glass bottles for 5 min at 9,000 G. Bacterial reduction in liquid egg white ranged from 1-2 million per ml and was obtained irrespective of the pH of the medium (Table 1).

### *Effect of mechanical clarification alone*

Fresh, raw, liquid egg white was heated to 21 C and mechanically clarified without bactofugation at 13,000 lb/hr, then pumped into a clean stainless steel vat. Bacterial counts of clarified and non-clarified samples showed mechanical clarification did not significantly reduce microbial populations of liquid egg white (Table 2). In fact, probably because of cluster break-up, the total bacterial count increased from 250,000 to 380,000 per ml.

### *Bactofugation of clarified liquid egg white*

Three lots of mechanically clarified egg white were bactofugated at different low temperatures. A consistent total count reduction related to temperatures was observed. Total bacteria levels were reduced 50.0, 75.5, and 92.7%, respectively, at 21, 32, and 43 C (Table 3). In another trial several weeks later on low-count mechanically clarified liquid egg white, bactofugation was undertaken at 49 and 54 C (Table 4). Reductions of total bacteria by the bactofugation process were greater in mechanically clarified liquid egg white at these higher temperatures. For example, a reduction of 96.5% occurred at 49 C, and 99.7% at 54 C (Table 4).

Coliform and enteric bacteria in liquid egg white were reduced by bactofugation at low temperatures (Table 5), but not with the same degree of efficiency as total bacteria numbers. For example, at low bactofugation temperatures between 21 and 43 C, coliforms were reduced about 90% and enteric bacteria growing on Salmonella-Shigella agar were reduced only 40-60%, and in some instances not at all (Table 5). When the same raw, liquid egg white was bactofugated at 49 and 54 C, no bacteria growing on Salmonella-Shigella agar were detectable in the treated egg white.

The pH of the egg white ranged from 8.35-8.85 and the total protein content was 10.6% before bactofugation and 10.5% after.

TABLE 2. EFFECT OF MECHANICAL CLARIFICATION ON BACTERIAL POPULATIONS OF LIQUID EGG WHITE, OR ALBUMEN, AT ROOM TEMPERATURE

Treatment of raw egg white	Populations in liquid egg white using following media					
	Total count <sup>a</sup>	Coliform <sup>b</sup>	MacConkey	Bismuth sulfite	Salmonella-Shigella	Desoxycholate citrate
	(bact/ml)					
None	250,000	30,000	18,000	6,700	550	4,900
Clarified at 21 C	380,000	27,000	15,000	6,800	490	4,500

<sup>a</sup>SPC or standard plate count agar.

<sup>b</sup>Violet red bile agar.

TABLE 3. EFFECT OF BACTOFUGATION ON TOTAL BACTERIAL NUMBERS OF POOR QUALITY LIQUID EGG WHITE, OR ALBUMEN, AT LOW TEMPERATURES

Treatment of clarified, raw, liquid egg white <sup>a</sup>	Total bacterial count <sup>b</sup>	
	(per ml)	(reduction - %)
Heated to 21 C only	380,000	
Bactofugated at 21 C	190,000	50.0
Heated to 32 C only	590,000	
Bactofugated at 32 C	145,000	75.5
Heated to 43 C only	530,000	
Bactofugated at 43 C	46,000	92.7

<sup>a</sup>Prior mechanical clarification occurred at 21 C. All bactofugated egg whites were heated before bactofugation similarly to their respective controls.

<sup>b</sup>Using standard plate count agar.

TABLE 4. EFFECT OF BACTOFUGATION ON TOTAL BACTERIAL NUMBERS OF HIGH QUALITY LIQUID EGG WHITE, OR ALBUMEN, AT MEDIUM TEMPERATURES

Treatment of clarified, raw, liquid egg white <sup>a</sup>	Total bacterial count <sup>b</sup>	
	(per ml)	(reduction - %)
Heated to 49 C only	2,300	
Bactofugated at 49 C	80	96.5
Heated to 54 C only	9,500	
Bactofugated at 54 C	30	99.7

<sup>a</sup>Prior mechanical clarification occurred at 21 C. All bactofugated egg whites were heated before bactofugation similarly to their respective controls.

<sup>b</sup>On standard plate agar.

## DISCUSSION

The principle and the operational characteristics of the continuous bactofugation apparatus have been recently reviewed by Fjaervoll (6). Though a high degree of efficiency in removing bacteria from milk (9, 15) has been demonstrated, each liquid food, because of viscosity, specific gravity, protein heat lability differences, and other characteristics, requires independent testing to properly assess bactofugation efficiency.

With liquid egg white, prior mechanical clarification, though by itself incapable of removing bacteria, appears necessary prior to bactofugation for high reduction of total bacterial count. Improvement in efficiency results apparently because clarification removed extraneous material such as shell fragments, which otherwise may clog the bactofuge exit nozzles. According to Anderson (2) clarifiers are not generally used for processing egg products, although some yolk product processes require clarification.

Reduction of bacterial numbers in preclarified, bactofugated, liquid egg white at low temperatures, 21-43 C, reflects mostly the effect of G force. With an increase in temperature to 49 and 54 C, the destructive influence of heating in a high pH medium, 9.0, highly endowed with the bacterial-inhibiting enzyme, lysozyme, combines with G force to sharply reduce bacteria. Under influences peculiar to egg white, total bacterial reduction efficiency at the higher temperatures can be very high in liquid egg white with a low count. On the other hand, coliform and enteric bacteria removal from liquid egg white bactofugated at low temperatures, 21-49 C, was relatively ineffective, probably as a result of initial low counts and the high viscosities of egg whites at these low temperatures.

The present study was directed more to measuring

TABLE 5. THE EFFECT OF BACTOFUGATION ON ENTERIC BACTERIAL NUMBERS IN POOR QUALITY LIQUID EGG WHITE, OR ALBUMEN, AT LOW TEMPERATURES

Treatment of clarified, raw liquid egg white <sup>a</sup>	Populations in liquid egg white on following agars				
	Coliform	MacConkey	Bismuth sulfite	Salmonella-Shigella	Desoxycholate citrate
	(bact/ml)				
Heated to 21 C only	27,000	15,000	6,800	490	4,500
Bactofugated at 21 C	3,900	3,100	4,000	300	950
Heated to 32 C only	31,000	8,900	3,700	280	1,200
Bactofugated at 32 C	3,300	3,500	600	330	800
Heated to 43 C only	42,000	3,700	1,030	250	1,800
Bactofugated at 43 C	550	2,000	870	100	480

<sup>a</sup>All bactofugated egg whites were heated before bactofugation similarly to their respective controls.

bacterial reduction than to ascertaining the physical properties of the clarified, bacto-fugated, liquid egg white. Within the past decade the introduction of pasteurization to liquid egg white has led to some physical changes in the product, but with certain adjustments heated egg products apparently have become acceptable. Commercial assessment of a lot of these bacto-fugated egg whites indicated good food-processing qualities (5). Furthermore, in laboratory measurements of specific gravity conducted by Naworski (12), the mean values of foam volume and foam stability showed the untreated liquid egg white to be only slightly superior to that of the bacto-fugated product and no significant differences were observed between nonbacto-fugated-heated and bacto-fugated-heated egg whites.

Bacto-fugation of liquid egg white is not a substitute for proper pasteurization, but insofar as it reduces the load of high bacterial count products prior to pasteurization or to hydrogen peroxide-catalase treatment, this may be a reason to give such supplemental control measure more extensive and critical study.

#### ACKNOWLEDGEMENT

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#### REFERENCES

1. American Public Health Association. 1967. Standard methods for the examination of dairy products. 12th ed. American Public Health Association, New York.
2. Anderson, O. R. 1969. Personal communication. Seymour Foods, Inc., Topeka, Kansas.
3. Angelotti, R. 1966. Quality control of liquid egg products. Proc. Rutgers Liquid Egg Processing Course, Rutgers University, New Brunswick, New Jersey.
4. Cunningham, F. E., and H. Lineweaver. 1965. Stabilization of egg white proteins to pasteurization temperatures at 60 C. Food Technol. 19:1442-1447.
5. Dodge, J. W. 1966. Personal communication, March 24. Agway, Inc., Ithaca, New York.
6. Fjaervoll, A. 1968. The theory and use of centrifugal machines in the dairy industry. J. Soc. Dairy Technol. 21:180-189.
7. Goresline, H. E., K. M. Hayes, R. E. Moser, Jr., M. A. Howe, and E. E. Drewniak. 1951. Pasteurization of liquid whole egg under commercial conditions to eliminate *Salmonella*. USDA Circular No. 897:1-15.
8. Kline, L. T. F., M. L. Sugihara, M. L. Bean, and K. Ljichi. 1965. Heat pasteurization of raw liquid egg white. Food Technol. 19:1709-1718.
9. Kosikowski, F. V., and A. C. O'Sullivan. 1966. Bacterial centrifugation of low grade milk for Cheddar cheese. Proc. 17th Intern. Dairy Congr. D1:25-32.
10. Lineweaver, H. 1966. Pasteurization and the functional properties of egg products. Proc. Rutgers Liquid Egg Processing Course, Rutgers University, New Brunswick, New Jersey.
11. Lloyd, W. E., and L. A. Harriman. 1957. Method of treating egg whites. U. S. Patent No. 2,776,214.
12. Naworski, Priscilla. 1966. Personal report, March 19. Dept. Food and Nutrition, Cornell University, Ithaca, New York.
13. Romanoff, A. L., and A. L. Romanoff. 1949. The avian egg. John Wiley and Son, New York. 766 pp.
14. Sebring, M., A. B. Rogers, J. G. Heck, and G. Pankey. 1965. Low temperature pasteurization of egg products. Poultry Sci. J. 44:1414.
15. Simonart, P., and G. Debeer. 1953. Recherches en vue d'ameliorer la qualite microbiologique des laits par ultracentrifugation. Neth. Milk Dairy J. 7:117.

#### NUTRITIONAL ASPECTS OF DAIRY PRODUCTS (Continued from Page 334)

traveler, Marco Polo, attributed much of the Tartars' endurance and strength to their milk drinking habits.

In this country, the introduction of milk predates the formation of the Republic. In 1611, the first dairy cows helped alleviate starvation and death in the Jamestown Colony. Early Spanish explorers brought cattle into this country on their first venture in the new world.

When the American populace started its massive westward shift, the covered wagons were shadowed by one or two cows, which were considered vital members of the family. Early settlers thought of their bovine friends as the family's food factory on the move.

Milk is relied upon as the principle source of many of the nutritional elements necessary for proper growth of our bodies and for the maintenance of good health. Millions of gallons of milk are sent

from farm to city every day where it is pasteurized, delivered to our homes, and consumed with confidence. This is possible because it is safeguarded by sanitary measures at every step of production, processing, and distribution.

In the natural state, milk is consumed directly at its source by the young animal. Problems arise when milk is stored and transported after it is produced. Milk is such a good food that microorganisms compete with man for it. Consequently, milk must have constant protection from the healthy cow to the glass on the dining table.

Research in the study of nutrition has shown that certain food nutrients are essential in promoting physical fitness. These materials are classified into five general groups, namely—vitamins, minerals, proteins, fats, and carbohydrates. When taken into the body in adequate amounts, these nutrients together with good habits of health and hygiene, promote

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# EFFECT OF THE COW'S BODY CONDITION AND STAGE OF LACTATION ON DEVELOPMENT OF MILK RANCIDITY<sup>1</sup>

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## ABSTRACT

An objective of this study was to investigate the relationship between body condition of the dairy cow and susceptibility of milk to rancidity. Initial milk samples were obtained from 166 cows in Spring, 1969, and all animals were retested after an interval of two months. Additional observations included the effects of stage of lactation, level of milk production, and herd effects. Each cow was evaluated for body condition, at the times when milk samples were obtained, by a method based on measures of weight and skeletal size and by a subjective scoring system. Spontaneous rancidity of the milk fat was allowed to develop upon storage for 48 hr; and induced rancidity was brought about by controlled agitation. As measured by acid degree values (ADV) the treatments imposed on the milk samples were successful in simulating development of spontaneous and induced lipolysis of the milk fat. However, the magnitude of the ADV was not related to body condition score. It is concluded that in well-fed herds, such as those used in this experiment, body condition of the cow does not influence the susceptibility of her milk to rancidity. These data may not apply under conditions of poor feeding and management. Advancing stage of lactation was associated with increases in both spontaneous and induced rancidity. This was indicated by positive correlations between days in milk and ADV, and by significantly higher values in the second sampling period than in the first. There was a negative correlation between the amount of milk produced and ADV. This may have been related to the decline in milk flow which accompanied advancing lactation. An unexplained herd difference existed with respect to the susceptibility of milk to induced rancidity.

Much effort has been expended on the causes of hydrolytic rancidity and on methods of reduction. Although many suggestions have been made to minimize the problem, rancid flavor continues to be a common defect. It is generally agreed that lipase enzymes are responsible for hydrolysis of milk fat. Apparently these enzymes are inactive until milk is drawn, after which they may become activated.

Factors other than lipase concentration and activity may affect the amount of fat hydrolysis and the incidence of rancid flavor. Individual cow differences appear to be important (4, 7, 8, 11). Diet of the

animal has been suggested as a contributing cause. It appears that the metabolic state of the cow may be an important consideration. An earlier field trial at this station appeared to indicate a relationship between body condition and incidence of milk rancidity. There was a significant positive correlation between the percentage of thin cows in a given herd and the average ADV of the milk (1).

A major objective of this investigation was to study the possible relationship between body condition of the cow and the development of hydrolytic rancidity in her milk. It was postulated that the mammary gland of the thin cow may have to depend more heavily upon the products of rumen fermentation for a supply of milk fat precursors than would be true if there was more depot fat. Jensen (12) and Al-Shabibi et al. (2) have noted that the short-chain free fatty acids mainly are responsible for the flavor normally associated with rancidity. An additional objective was to observe the effect of advancing lactation on susceptibility of the milk from individual cows to spontaneous and induced rancidity. It has been reported that advancing lactation is associated with an increase in rancidity (6, 8, 13) although other studies have indicated that there is little relationship (5, 9, 10). Most of these have been based on mixed herd milk.

## EXPERIMENTAL PROCEDURES

From April through September, 1969, 166 cows were used in a study of the possible relationship between body condition and the development of hydrolytic rancidity in the milk produced by each individual. Each animal was evaluated for body condition, and spontaneous and induced acid degree values (ADV) were determined on the milk produced. Re-evaluations were made after two months. The University research and instructional Holstein herds were used, as were the University instructional Ayrshires and Guernseys. Also included was one Holstein herd at the Pennsylvania Correctional Institution at Huntingdon. Animals selected for use included all of those in the herd which appeared healthy and which had been in lactation at least 10 but not over 215 days at the start of the experiment.

All herds received limited pasture plus hay and corn silage or green chop. Supplemental concentrate was fed according to production. There were no great differences in the nutrition of the various herds.

<sup>1</sup>Authorized for publication on January 20, 1970, as paper No. 3730 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

<sup>2</sup>Present address: Cooperative Extension Service, University of Puerto Rico, P. O. Box AR, Rio Piedras.

Two methods were selected to evaluate the fleshing condition of each cow. In one, a score was assigned based on a modification of a formula used by Brody (3), as follows:

$$\frac{\text{Body Weight}}{2 (\text{Height at withers} + \text{Length from shoulders to pinbones})} \times 100$$

Weight was expressed in kilograms and the linear measures, in centimeters. A second method depended on subjective evaluations of each animal. Numerical observation scores were assigned, based on a scale ranging from one, extremely thin, to eight, a very fat cow. Scores were assigned by three experienced persons, and averaged for each cow.

Milk samples were collected at one farm at a time by groups that varied from 20 to 33 cows. Milk samples and weights were taken at the evening milking, and the samples were placed in an ice chest. They were taken to the laboratory immediately after milking and the milk from each cow was divided into three portions of about 150 ml each. The three portions were labeled with a code number to identify the cow and with the letters A, B, or C to describe the type of treatment to which the portion was to be subjected.

Each sample under Treatment A was heated immediately upon arrival at the laboratory to 71 C for 5 min to inactivate lipase enzymes. It then was cooled quickly in an ice water bath and subsequently analyzed for the ADV of the milk fat.

Treatment B samples were held at 5 C for 48 hr. They then were heated to 71 C for 5 min, cooled, and analyzed for ADV. This treatment was designated as a measure of the development of spontaneous hydrolytic rancidity.

Milk samples constituting Treatment C were warmed to 38 C in a water bath and immediately agitated for a period of 10 sec in a Waring Blendor controlled by an autotransformer set at 80 volts. The agitated samples then were cooled and stored at 5 C for 48 hr. They then were heated to 71 C and analyzed for ADV. The values thus derived were considered to be measures of induced rancidity.

For purposes of data analysis, two other values were calculated. The ADVs of Treatment A were subtracted from the corresponding values of Treatments B and C for each cow. Designated B-A and C-A, respectively, they were viewed as measures of actual fat hydrolysis caused by the corresponding treatments.

Analysis for ADV was by the method of Thomas et al. (15) as modified by Speer et al. (13).

Analysis of data was by use of linear correlations and analysis of variance according to Steele (14).

## RESULTS AND DISCUSSION

Milk samples were obtained between April 22 and June 26 during the first experimental period. The time lapse between Periods 1 and 2 varied by herds from 59 to 63 days. The mean stage of lactation was 137 days and the average age of the animals was 59 months at the beginning of the experiment. Milk production at the evening milking when sampling occurred averaged 10.5 kg in Period 1 and 8.2 kg in Period 2.

Fleshing condition scores based on measurements (3) indicated that the Holsteins in Herd B were in higher condition than were the Guernseys, Table 1. Holstein Herds A and C and Ayrshires scored similarly. Observation scores, based on the subjective eval-

TABLE 1. AVERAGE FLESHING CONDITION SCORES AND ACID DEGREE VALUES BY HERD AND SAMPLING PERIOD

Herd	Number of cows	Condition Scores		ADV, <sup>1</sup> treatment		
		Measurement	Observation	A	B	C
<i>Period 1</i>						
Holstein, herd A	33	95.7	5.1	0.32	0.60	1.05
Holstein, herd B	63	111.5	5.4	0.32	0.61	1.03
Holstein, herd C	28	95.8	5.3	0.43	0.85	2.98
Ayrshire	29	96.5	5.4	0.32	0.65	1.37
Guernsey	13	91.0	5.0	0.33	0.57	1.02
<i>Period 2</i>						
Holstein, herd A	33	99.6	5.1	0.40	1.02	2.44
Holstein, herd B	63	107.1	5.1	0.36	1.00	1.60
Holstein, herd C	28	94.9	5.2	0.39	0.97	2.87
Ayrshire	29	95.2	5.5	0.30	0.69	1.26
Guernsey	13	89.3	5.4	0.31	0.62	1.18

<sup>1</sup>ADV is defined as the number of milliliters of KOH required to neutralize the free fatty acids in 100 g of milk fat.

uations of three judges, varied around a mean of 5.2 and did not differ greatly between herds. A score of 5.0 was considered to indicate average condition. Average acid degree values also are summarized in Table 1. The averaged values for Treatment A were in the accepted range for normal milk. Under Treatment B, intended to simulate development of spontaneous rancidity, it was evident that hydrolysis of the milk fat did occur; and the agitation of Treatment C was effective in inducing rancidity. Treatment effects were more pronounced during Period 2 than in Period 1.

Simple linear correlation analyses were performed, using the data from individual cows. The two measures of fleshing condition were shown to be positively related ( $P < .01$ ).

In general, none of the measures of ADV were related to condition scores, Table 2. Although it is true that the ADV of Treatments C and C-A were negatively correlated with measurement scores in Period 1, the "r" value was low, and the trend was not repeated in Period 2. Similarly, the ADV of Treatment A was positively correlated with measurement scores of fleshing condition in Period 2; but this was not true in Period 1. Observation scores of fleshing condition were not correlated with any measure of ADV. When the data of both periods were combined there were no significant relationships.

As an additional determinant of the possibility that body condition of the cow is related to rancidity,

TABLE 2. LINEAR CORRELATIONS BETWEEN FLESHING CONDITION AND ACID DEGREE VALUES

Dependent variables, ADV of:	Independent variables					
	Period 1		Period 2		Both periods	
	Measurement score	Observation score	Measurement score	Observation score	Measurement score	Observation score
Treatment A	0.03	0.11	0.21 <sup>2</sup>	0.05	0.09	0.08
Treatment B	0.02	0.10	0.14	0.12	0.07	0.09
Treatment C	-0.17 <sup>1</sup>	0.01	0.08	0.06	-0.07	0.03
B - A	0.01	0.08	0.12	0.12	0.06	0.08
C - A	-0.18 <sup>1</sup>	0.00	0.07	0.06	0.08	0.02

<sup>1</sup>Significant at  $p < .05$ .<sup>2</sup>Significant at  $p < .01$ .

analyses of variance were applied. The data were arranged into classes based on condition measurement scores and also on observation scores. Analyses were performed on the individual ADV values from each treatment and by periods. None of the F-ratios were significant, thus strengthening the conclusion that body condition was not related to the development of milk fat rancidity.

Milk production at the evening milking when samples were obtained appeared to be negatively correlated with the development of spontaneous rancidity as measured by Treatment B, Table 3. The "r" value was not high, however, and was significant only in Period 2 and when the data were combined for both periods. For the combined periods, there also was a significant negative relationship between milk production and induced rancidity as measured by Treatment C. Since production by individual cows varies considerably between milkings, it is possible that the observed correlation may have been different if a more reliable measure of production level had been used. A positive and highly significant relationship was observed between the stage of lactation (days in milk) and ADV of both Treatment B and C.

An additional analysis of variance was conducted to determine the possible effects of herd and time of sampling (period) on the ADV of the milk, Table 4. The herds were in three locations and each was subjected to somewhat different management procedures. The sampling periods represented two stages in the lactation of each cow, Period 2 being approximately 61 days after Period 1. A two-factor analysis was used. The B-A and C-A treatment data are presented in Table 4. The results would not have been different if the uncorrected values of Treatments A and B had been used.

With respect to Treatment B-A values, the differences between herds were not significant, with values

ranging from 0.287 to 0.523. Period 2 mean values were significantly higher ( $P < .01$ ) than those of Period 1. It appears that as cows advanced in lac-

TABLE 3. LINEAR CORRELATIONS BETWEEN MILK PRODUCTION AT TIMES OF SAMPLING, STAGE OF LACTATION, AND ACID DEGREE VALUES OF TREATMENTS B AND C

Dependent variables, ADV of:	Independent variables					
	Period 1		Period 2		Both periods	
	P.M. milk	Days in milk	P.M. milk	Days in milk	P.M. milk	Days in milk
Treatment B	-0.13	0.25 <sup>1</sup>	-0.21 <sup>1</sup>	0.33 <sup>1</sup>	-0.22 <sup>1</sup>	0.35 <sup>1</sup>
Treatment C	-0.14	0.17 <sup>1</sup>	-0.10	0.31 <sup>1</sup>	-0.16 <sup>1</sup>	0.27 <sup>1</sup>

<sup>1</sup>Significant at  $p < .01$ .

TABLE 4. EFFECTS OF HERD AND SAMPLING PERIOD ON TREATMENTS B-A AND C-A ACID DEGREE VALUES OF MILK

Criteria	Cows per herd	Observations	Mean ADV	
			B-A	C-A
<i>Herd</i>				
Holstein, herd A	33	66	0.45	1.38 <sup>1</sup>
Holstein, herd B	63	126	0.47	0.98
Holstein, herd C	28	56	0.52	2.50 <sup>2</sup>
Ayrshire	29	58	0.36	1.01
Guernsey	13	26	0.29	0.83
<i>Period</i>				
1	—	166	0.32	1.10
2	—	166	0.56 <sup>3</sup>	1.52 <sup>3</sup>

<sup>1</sup>Significantly greater than Guernsey herd ( $p < .01$ ).<sup>2</sup>Significantly greater than all other herds ( $p < .01$ ).<sup>3</sup>Significantly greater than Period 1 ( $p < .01$ ).

tation their milk tended to become more susceptible to spontaneous rancidity.

The Treatment C-A difference between periods again was highly significant, Table 4. Advancing lactation apparently caused the milk to be more susceptible to induced rancidity. For this treatment, herd differences also were significantly different, as was the interaction between herd and period ( $P < .01$ ). Application of Duncan's multiple range test (14) to the herd means revealed that the mean ADV value of Holstein Herd C was significantly greater than that of any other herd. The mean value for Holstein Herd A was greater than that of the Guernseys but not different from Holstein Herd B or Ayrshires. Since the numbers of Guernseys was small, this latter difference may be questioned. However, it is obvious that for some reason Holstein Herd C, and perhaps Holstein Herd A, produced milk that was more susceptible to induced rancidity than was true of the other herds. In light of the significant interaction between herd and period, it is probable that the susceptibility became more pronounced with advancing lactation. Stage of lactation, as such, most likely was not responsible for the observed herd difference, because the average days in milk of the Holstein Herds B and C were within six days.

#### REFERENCES

1. Adams, R. S. 1969. Personal communication. The Pennsylvania State University.
2. Al-Shabibi, M. M. A., E. H. Lagner, J. Tobias, and S. L. Tuckey. 1964. Effect of added fatty acids on the flavor of milk. *J. Dairy Sci.* 47:295-296.
3. Brody, S. 1927. Growth and development. VII. The relation between weight growth and linear growth with special reference to dairy cattle. *Mo. Exp. Sta. Res. Bull.* 103.
4. Cannon, R. Y., and G. H. Rollins. 1964. Effect of source of nutrients and nutritional level of the cow on development of hydrolytic rancidity in the milk. *J. Dairy Sci.* 47:41-45.
5. Chen, H. S., and C. R. Bates. 1962. Observations on the pipeline milker operation and its effect on rancidity. *J. Milk Food Technol.* 25:176-182.
6. Chimanbhai, V. P. 1967. Relationship of phospholipids to enzymatic hydrolysis of milk fat. *Diss. Abstracts, Sect. B.* 28:227.
7. Demott, B. J., and L. R. Brandt. 1956. A study of some factors related to rancidity in pipeline milkers. *J. Dairy Sci.* 39:1481.
8. Fredeen, H., J. E. Browstead, W. L. Dunkley, and L. M. Smith. 1951. Hydrolytic rancidity in milk: II. Some management and environmental factors influencing lipolysis. *J. Dairy Sci.* 34:521-528.
9. Guthrie, E. S., and B. L. Herrington. 1960. Further studies of lipase activity in the milk of individual cows. *J. Dairy Sci.* 43:843.
10. Henningson, R. W. 1964. Influence of late lactation on the acid value of herd milk fat. *J. Dairy Sci.* 47:337.
11. Jensen, R. G. 1959. Spontaneous lipolysis in milk throughout the stages of lactation of individual cows. *J. Dairy Sci.* 42:1619-1621.
12. Jensen, R. G. 1964. Lipolysis. *J. Dairy Sci.* 47:210-215.
13. Speer, J. F., G. H. Watrous, Jr., and E. M. Kesler. 1958. The relationship of certain factors effecting hydrolytic rancidity in milk. *J. Milk Food Technol.* 21:33-37.
14. Steele, R. G. D., and J. H. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill Book Company, Inc., New York, N.Y.
15. Thomas, E. L., A. J. Nielson, and J. C. Olson, Jr. 1955. Hydrolytic rancidity in milk: A simplified method for estimating the extent of its development. *Am. Milk Review*, 17:50-52, 85.

## NUTRITIONAL ASPECTS OF DAIRY PRODUCTS

(Continued from Page 338)

growth and sound physical development.

As the study of nutrition continues in research laboratories throughout the country, the value of milk and dairy products in the diet becomes more apparent. While it has been known for many years that milk is the only substance in nature designed to serve solely as food, it was not until the results of research during the past half century, that its importance in the human diet was proved. Today nutrition authorities tell us that everyone needs milk each and every day in order to supply many of the essential food nutrients we require daily.

Although milk is a liquid food, it contains an average of 12 to 13% total solids, an amount comparable to the solids content of many other foods. Altogether at least 100 chemical components have been identified in milk.

There is a geometric law which states that the whole is equal to the sum of its parts. I would like to disprove this law. The nutritional value of milk as a whole is certainly greater than the total value of its individual nutrients. The key here is interaction. The chemical components, or nutrients in milk, each possess a biological value. We know the nutritional attributes of the major milk components, but have we ever put the nutrients together and assessed the chemical profile of the product as a complete entity? No, we have not realized the unsurpassed nutritional balance of the whole milk. Today, science is still trying to uncover the complex chemical profile of milk.

It was impressive to note in a study made several years ago that dairy products contributed only 12% of the calories used in the United States, yet they

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# ELECTROMETRIC pH DETERMINATIONS OF DIFFERENT AGAR MEDIA AT 25 AND 45 C

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## ABSTRACT

In all tests performed using antibiotic test medium No. 1, APHA standard reference, BBL standard methods, violet red bile, and desoxycholate lactose agars, the overall mean values of pH readings were significantly higher ( $p < 0.01$ ) when determined at 25 C than at 45 C. This difference varied between 0.14 and 0.38 pH unit. Whether this difference has practical significance in routine laboratory use of these media was not determined.

In determining the pH of Standard Methods Agar, the 11th edition of *Standard Methods for the Examination of Dairy Products* (SMEDP) stipulates: "Make determinations at 45 C without diluting medium or, providing laboratory can demonstrate that results are equivalent, at lower temperatures (20 C to 35 C) if medium is diluted 1:1 with freshly distilled water or 1:2 with freshly boiled distilled water". The 12th edition of SMEDP specifies: "Determine hydrogen-ion concentration of culture media at 25 C either electrometrically or colorimetrically and record reaction in terms of pH".

No published reports have been found indicating the advantages of one temperature over the other for making pH determinations. However, correspondence from representatives of Difco and Bioquest (BBL) indicates that they adjust their media at 25 C and that when the pH of Standard Methods Agar was determined at 45 C low and erratic values 0.1 to 0.3 of a pH unit below the pH specified on the bottles of the media were obtained.

The present study was conducted to obtain specific information regarding pH values of Standard Methods Agar as well as of several other media at 25 C and 45 C.

## MATERIALS AND METHODS

### pH meter

A Beckman (Zeromatic II, Model 96 A) was employed.

### Media

The following media were employed: APHA Reference Standard as used in the Productivity Test for Standard Methods Agar; Standard Methods Agar, BBL Lot. No. 610639; Violet Red Bile Agar BBL Lot No. 903607; Desoxycholate

TABLE 1. COMPARISON OF pH DETERMINATIONS ON APHA STANDARD REFERENCE AGAR MEDIUM<sup>1, 2</sup>

Trial	Before autoclaving		After autoclaving	
	25 C	45 C	25 C	45 C
1	7.02	6.79	7.05	6.73
	7.00	6.78	7.00	6.75
	7.01	6.73	7.07	6.78
	7.02	6.70	7.03	6.70
2	7.09	6.85	7.09	6.83
	7.08	6.85	7.08	6.83
	7.09	6.87	7.10	6.83
	7.09	6.86	7.09	6.82
3	7.00	6.87	7.02	6.82
	6.98	6.89	7.01	6.82
	7.00	6.90	6.99	6.82
	6.99	6.89	7.01	6.82
4	7.05	6.79	7.08	6.79
	7.05	6.80	7.03	6.80
	7.00	6.75	7.00	6.78
	7.01	6.83	7.04	6.79

<sup>1</sup>At 25 C: overall mean 7.04 with standard deviation of 0.04

<sup>2</sup>At 45 C: overall mean 6.80 with standard deviation of 0.05

Lactose Agar BBL Lot No. 708642; and Antibiotic Medium No. 1, Difco Lot No. 485630.

### Water baths

A National Appliance Company (NAPCO) Model 8725, water bath was maintained at 45 C  $\pm$  2 C and another NAPCO bath Model 8735-4 was maintained at 25 C  $\pm$  1 C.

### Procedure

Six hundred milliliters of each medium were prepared. The Standard Methods agars and the antibiotic medium were each divided into two 300 ml samples. One portion was then autoclaved. Neither coliform medium was autoclaved. All samples were prepared and divided randomly by one technician into four 75 ml portions contained in 250 ml beakers. The other technician determined the pH values of the media, not knowing the identity of any sample.

The water bath contained the numbered beakers of media, distilled water, certified Coleman buffer (pH 7.00  $\pm$  0.01 at 30 C), and certified Coleman buffer (pH 9.02  $\pm$  0.01 at 30 C).

The pH meter electrodes were washed with distilled water and allowed to equilibrate at 45 C in the distilled water. The

TABLE 2. COMPARISON OF pH DETERMINATIONS ON BBL STANDARD METHODS AGAR AT TWO TEMPERATURES BEFORE AND AFTER AUTOCLAVING<sup>1, 2</sup>

Trial	Before autoclaving		After autoclaving	
	25 C	45 C	25 C	45 C
1	7.00	6.70	6.91	6.72
	7.02	6.72	7.01	6.72
	7.00	6.72	7.01	6.70
	7.02	6.69	7.02	6.69
2	7.08	6.82	7.05	6.83
	7.00	6.83	7.08	6.87
	7.09	6.83	7.09	6.83
	7.08	6.83	7.10	6.86
3	7.00	6.82	6.98	6.80
	7.00	6.82	6.98	6.80
	7.04	6.87	6.99	6.80
	7.00	6.87	6.97	6.80
4	7.02	6.73	7.04	6.77
	7.05	6.77	7.02	6.79
	7.05	6.78	7.01	6.80
	7.02	6.80	7.00	6.75

<sup>1</sup>At 25 C: overall mean 7.02 with standard deviation of 0.04

<sup>2</sup>At 45 C: overall mean 6.78 with standard deviation of 0.06

TABLE 3. COMPARISON OF pH DETERMINATIONS OF ANTIBIOTIC MEDIUM NO. 1 AT TWO TEMPERATURES BEFORE AND AFTER AUTOCLAVING<sup>1, 2</sup>

Trial	Before autoclaving		After autoclaving	
	25 C	45 C	25 C	45 C
1	6.47	6.22	6.48	6.22
	6.48	6.22	6.47	6.21
	6.46	6.22	6.47	6.22
	6.43	6.22	6.47	6.22
2	6.47	6.22	6.48	6.21
	6.42	6.21	6.46	6.22
	6.45	6.21	6.48	6.22
	6.48	6.19	6.45	6.22
3	6.43	6.25	6.43	6.25
	6.43	6.22	6.43	6.22
	6.43	6.23	6.43	6.23
	6.43	6.21	6.45	6.23
4	6.43	6.23	6.45	6.23
	6.45	6.22	6.43	6.23
	6.45	6.22	6.45	6.23
	6.43	6.22	6.43	6.23

<sup>1</sup>At 25 C: overall mean 6.45 with standard deviation of 0.02

<sup>2</sup>At 45 C: overall mean 6.22 with standard deviation of 0.01

temperature compensator was set at 45 C and the pH meter was then standardized at 45 C with the 7.00 buffer and checked for accuracy with the 9.02 buffer. The electrodes were washed with distilled water between measurements and carefully wiped with Kimwipes. The pH of the medium in each beaker was then determined.

All of the beakers of media and buffers were then transferred to a 25 C water bath. Fresh distilled water was used.

The solidified agar was macerated with a glass rod while the pH meter was equilibrating at 25 C. The temperature compensator was set at 25 C and the pH meter was restandardized. The pH of the media at 25 C was determined following the same procedure as given for the measurement at 45 C. Electrodes were then inserted into boiling distilled water to insure removal of agar. When not in use, electrodes were placed in distilled water.

Similar pH determinations were made on split samples of the plate count agars after media had been autoclaved at 121 C for 15 min. All data were analyzed statistically by analysis of variance.

## RESULTS

A comparison of pH findings at 25 C and 45 C before and after autoclaving APHA standard reference agar media is presented in Table 1. No significant difference was found between the readings obtained before and after autoclaving and therefore the data at the respective temperatures were pooled. The mean pH value of 7.04 at 25 C was significantly greater ( $p < 0.01$ ) than the mean pH value of 6.80 at 45 C. Similar results were found when BBL standard methods agar was tested (Table 2), viz. at 25 C the mean pH was 7.02 and at 45 C it was 6.78

TABLE 4. COMPARISON OF pH VALUES OBTAINED ON VIOLET RED BILE AGAR AND DESOXYCHOLATE LACTOSE AGAR AT TWO TEMPERATURES

Trial	Violet red bile		Desoxycholate lactose		
	25 C	45 C	25 C	45 C	
1	7.30	7.00	7.19	6.87	
	7.29	6.95	7.19	6.85	
	7.29	6.97	7.19	6.90	
	7.31	6.93	7.20	6.90	
	7.32	6.98	7.19	6.90	
	7.30	6.97	7.20	6.90	
	7.32	6.90	7.19	6.90	
	7.35	6.98	7.19	6.89	
	2	7.27	6.92	7.18	6.85
		7.30	6.93	7.13	6.87
7.30		6.93	7.18	6.83	
7.30		6.92	7.18	6.85	
7.30		6.91	7.18	6.82	
7.31		6.92	7.18	6.83	
7.30		6.92	7.19	6.83	
7.30		6.91	7.19	6.82	
3		7.42	7.00	7.25	6.83
		7.40	7.02	7.25	6.84
	7.38	7.01	7.23	6.86	
	7.43	7.01	7.24	6.85	
	7.45	7.02	7.25	6.84	
	7.50	7.01	7.22	6.83	
	7.39	7.02	7.24	6.84	
	7.40	7.00	7.24	6.85	
Overall means	7.34	6.96	7.20	6.86	
Standard deviation	0.06	0.04	0.03	0.03	

—a difference of 0.24 unit. Likewise, antibiotic test medium No. 1 gave significantly higher readings at 25 C—a mean pH value of 6.45 as compared to one of 6.22 at 45 C—a difference of 0.23 unit (Table 3).

Violet red bile and desoxycholate lactose agars were investigated and pH readings obtained at 25 C were significantly higher than those at 45 C (Table 4). With violet red bile this difference was 0.38 pH

unit, whereas, 0.14 unit was the difference with the desoxycholate lactose agar.

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## NUTRITIONAL ASPECTS OF DAIRY PRODUCTS

(Continued from Page 342)

provided 76% of the calcium, 44% of the riboflavin, 38% of the phosphorus, 23% of the protein, 12% of the vitamin A, 10% of the thiamine, as well as significant amounts of other nutrients, such as vitamin D, magnesium, and niacin. In no other food can we obtain the quality and quantity of nutrients per calorie as in milk, all very vital to functions of body processes.

In nutrition, a variety of foods consumed as part of a meal, more completely and efficiently meet nutrient needs than single foods taken separately, no matter how necessary each one alone may be. Recent studies also show that the body will make better use of those necessary nutrients when they are obtained along with a variety of other nutrients. In thinking of the nutrient interactions that exist in whole milk, let us first evaluate the protein in milk. Remember there are five general groups of essential nutrients.

### PROTEIN IN MILK

The excellent biological value of milk has been attributed to the amount and manner in which the amino acids are arranged in its proteins. Milk is a combination of several proteins. An important one is casein which is found only in milk and comprises around 80% of the total milk proteins.

The proteins of milk are of high quality. They contain, in varying amounts, all of the amino acids commonly found in protein, essential and otherwise. Moreover, the pattern of the distribution of amino acids in milk protein is excellent. A pint of milk can provide more than a woman's or man's minimal daily requirements for all the essential amino acids except the sulfur containing amino acid, methionine.

Milk proteins are only slightly affected by the processing temperatures used in pasteurization. Even the sterilization of evaporated milk and the modern technological processes used in the manufacture of

dry milk yield products of high protein quality.

### FAT IN MILK

Another important nutrient to be discussed is fat. (In recent years fat has almost become a bad word.) The fat I'm going to mention is not that 50 million pounds of excess that Americans are accused of lugging around, but the butterfat of milk.

Butterfat has withstood the brunt of criticism for many years. Whole milk is believed to be fattening. We all know the fallacy of this argument. Although the diet-heart controversy has exploited the saturation of butterfat, it has stimulated a multitude of very basic studies into butterfat chemistry. At this time there is no scientific evidence to substantiate the claim that animal fats in a balanced diet can cause heart attacks. Here are a few facts about milk fat. Butterfat is 60% saturated. However, only certain saturated fatty acids are believed to raise blood cholesterol levels. These fatty acids collectively comprise only 36% of butterfat, of which milk in turn is only 4% fat; they do not enter the diet in significant amounts from dairy foods. In a recent report, butterfat has been shown superior to vegetable fats in increasing calcium absorption; particularly at low levels of calcium intake.

Both vitamin A and carotene are present in high concentration in the fat portion of milk. The carotene, which forms vitamin A in the body, gives milk its characteristic creamy color. Although the natural vitamin D content of milk is low, about 85% of all fluid whole milk in the U.S.A. is fortified with vitamin D. Traces of other vitamins also occur in the fat of milk.

### CARBOHYDRATES OF MILK

Lactose, the major carbohydrate of milk, is synthesized by the mammary gland, in a manner that is not yet completely understood. It accounts for about

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# MICROBIAL FLORA OF GULF OF MEXICO AND POND SHRIMP<sup>1, 2</sup>

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## ABSTRACT

Bacterial counts of shrimp delivered by fishing vessels to processing plants varied greatly. Aerobic plate counts at 28 C ranged from 870-1,300,000 per gram. Either natural seawater or distilled water could be used in media preparation. The use of artificial seawater usually resulted in lower counts. The microbial flora of Gulf shrimp was dominated by coryneforms and species of *Pseudomonas*, *Moraxella*, and *Micrococcus*. Refrigerated storage usually caused an increase in *Pseudomonas* species. Bacterial counts of pond shrimp were much lower than those of Gulf shrimp. In some samples of pond shrimp *Bacillus* and *Lactobacillus* species were predominant.

The number and types of microorganisms on fish at time of landing depends on many factors such as species, season, fishing grounds, methods of catching, handling on board, and time and temperature of storage. Microbial activity is one of the main causes of quality deterioration of shrimp. To determine the significance and role of individual microbial species in this process, a detailed analysis of the microbial flora is useful. Information on this subject is limited. Green (5, 6) reported on the quantitative changes in bacterial populations of shrimp from catching to landing. She determined the influence of various handling procedures such as washing, heading, icing, and storage on the bacterial count. In general, if shrimp was handled under sanitary conditions and iced promptly, low bacterial counts could be maintained for several days. Campbell and Williams (3) and Williams et al. (10) showed that species of *Achromobacter*, *Bacillus*, *Micrococcus*, *Flavobacterium*, and *Pseudomonas* predominated in Gulf Coast shrimp. In Pacific shrimp, *Acinetobacter-Moraxella* species were predominant (7).

Raising shrimp in artificial ponds has become increasingly important in recent years. Texas has about 200,000 acres of coastal marshland, part of which could be used for this purpose. The objective of this study was to determine the level and type of microbial population of shrimp from the Gulf of Mexico and from marshland ponds.

## MATERIALS AND METHODS

Samples of shrimp were collected monthly during 1969 and were taken directly from commercial boats at Galveston just before they were unloaded. The boats had been in the Gulf of Mexico for periods ranging from 1-8 days. A representative sample of the middle layer of iced shrimp in a bin was obtained, avoiding the top and bottom layers. The exact age of the sample was not established. Shrimp were either brown (*Penaeus aztecus*) or white (*Penaeus setiferus*) or a mixture of the two types. All shrimp were headed and iced after being caught. The samples were brought to the laboratory in sterile jars packed in ice and were plated within 4-5 hr.

Five samples of white shrimp were obtained from artificial ponds located in a marshland area on the West Galveston bay shore in Brazoria County. Ponds were stocked with postlarvae shrimp (about 3/4-inch long) at a rate of 20,000 per acre. They were harvested 80-120 days later. These shrimp were headed immediately and placed in sterile containers packed in ice. The time interval between obtaining the samples and plating was 4 to 5 hr.

Bacterial counts were determined by plating appropriate dilutions of blended shrimp on Standard Methods agar (SMA, Difco) by the spreadplate method. Approximately 50 g of shrimp (3 shrimp) were placed in 450 ml of sterile phosphate buffer (1) and blended. Further dilutions were made with sterile phosphate buffer. Plating media were prepared with three different types of water, artificial seawater, natural seawater, and distilled water. The artificial seawater was prepared from synthetic sea salts (Aquarium Systems, Inc., Wickliffe, Ohio) according to the directions supplied by the manufacturer. The natural seawater was obtained from the Gulf of Mexico at Galveston Bay. Duplicate sets of plates were incubated aerobically and anaerobically at 5 C for 7 days and at 28 C for 2 days. To obtain anaerobic conditions, jars with GasPak Lids (BBL) were used. The GasPak envelope (BBL) was used to produce hydrogen and carbon dioxide gases. The same procedure was followed for stored shrimp. Stored shrimp consisted of fresh shrimp (shrimp from boats at time of landing) stored for 7 days at 1 C. Bacterial counts on shrimp from artificial ponds were obtained in the same way.

Approximately 40 representative colonies were picked from countable plates to determine microbial types isolated from shrimp. Colony characteristics were recorded before transfer to slants of the same medium. Smears for microscopic examination were prepared using the Gram stain (Hucker's modification). Diagnostic procedures and schemes for identification were the same as presented by Vanderzant and Nickelson (9). Analysis of variance, assuming a fixed model, was employed in the statistical treatment of the data. The individual plate counts were transformed to logarithms before analysis of variance was applied.

<sup>1</sup>Technical paper No. 8313 of the Texas Agricultural Experiment Station.

<sup>2</sup>This work was funded in part by the NSF Sea Grant Program Institutional Grant GH-59 made to Texas A&M University.



TABLE 1. AEROBIC PLATE COUNTS (APC) OF FRESH AND STORED GULF SHRIMP WITH PLATE INCUBATION AT 28 C FOR 2 DAYS.

Sample	APC/g of fresh shrimp <sup>a</sup>	APC/g of stored shrimp <sup>b</sup>	Period at sea (days)
1	670,000	13,000,000	2
2	150,000	7,900,000	3
3	870	34,000	1
4	8,100	8,500,000	7
5	51,000	480,000	2
6	65,000	2,500,000	5
7	12,000	4,000,000	5
8	3,800	2,100,000	7
9	1,300,000	15,000,000	6
10	63,000	8,800,000	1
11	130,000	70,000,000	8
12	23,000	2,000,000	2

<sup>a</sup>Fresh shrimp: shrimp as sampled from the boat at time of landing.

<sup>b</sup>Stored shrimp: fresh shrimp stored at 1 C for 7 days.

TABLE 2. ANALYSIS OF VARIANCE RESULTING FROM EFFECTS OF CONDITIONS OF PLATE INCUBATION AND COMPOSITION OF PLATING MEDIA ON THE BACTERIAL COUNT OF FRESH AND STORED GULF SHRIMP.

Source of variation	d.f.	Mean squares	
		Fresh shrimp	Stored shrimp
Plate incubation (P)	3	3.76368**	2.88180**
Plating media (M)	2	0.33031	1.44117*
P x M	6	0.07802	0.05722
Residual	132	0.78792	0.36564
Total	143		

\* < 0.05 level of probability

\*\* < 0.01 level of probability

### RESULTS AND DISCUSSION

The bacterial counts of fresh Gulf shrimp varied greatly. They ranged from 870-1,300,000 per gram on SMA plates prepared with distilled water and incubated at 28 C for 2 days (Table 1). In addition to factors such as season, area of catch, and length of trawling, these differences in count most likely reflect differences in the handling of shrimp on the boat, sanitary conditions of hold and ice, and time and temperature of storage. For example, an inspection of the boats revealed that sample 1 was poor-

ly iced and that samples 9 and 11 were from dirty bins in need of repair. These conditions probably contributed to the relatively high bacterial counts of these samples (Table 1).

Aerobic plate counts of fresh shrimp were generally somewhat lower at 5 C than at 28 C and ranged from 400-1,100,000 bacteria per gram on SMA plates prepared with distilled water. However, 5 of the 12 samples gave similar counts at both incubation temperatures. The same was true but to a lesser extent on SMA plates prepared with either regular seawater or artificial seawater. When fresh shrimp was stored on ice for 7 days, the bacterial counts increased greatly. Aerobic plate counts on stored shrimp ranged from 34,000 to 70,000,000 per gram on SMA agar prepared with distilled water with plate incubation at 28 C for 2 days. With stored shrimp, a majority of the samples showed similar aerobic plate counts at 28 and 5 C. The bacterial counts at both plate incubation temperatures usually were highest on media with distilled water or natural seawater and lowest on those with artificial seawater. Counts on plates incubated under anaerobic conditions were usually lower than comparable plates incubated under aerobic conditions.

Based on the data on fresh shrimp, the analysis of variance (Table 2) indicated that highly significant differences in viable count resulted from conditions of plate incubation. By Duncan's test (8), aerobic plate counts with plate incubation at 28 C differed significantly from the others and ranked highest (Table 3). No statistically significant differences were observed among media.

With respect to the data on stored shrimp, signifi-

TABLE 3. MEAN LOG COUNT OF FRESH AND STORED GULF SHRIMP WITH DIFFERENT CONDITIONS OF PLATE INCUBATION AND MEDIA

Effect	Means <sup>1</sup>	
	Fresh shrimp	Stored shrimp
<i>Plate incubation at:</i>		
28 C - 2 days - aerobic	4.55622 <sup>a</sup>	6.58151 <sup>a</sup>
5 C - 7 days - aerobic	4.14004 <sup>b</sup>	6.54894 <sup>a</sup>
28 C - 2 days - anaerobic	3.98131 <sup>b</sup>	6.12481 <sup>b</sup>
5 C - 7 days - anaerobic	3.79794 <sup>b</sup>	6.03491 <sup>b</sup>
<i>Media (SMA) with:</i>		
Seawater	4.17344 <sup>a</sup>	6.43274 <sup>a</sup>
Artificial seawater	4.02341 <sup>a</sup>	6.12282 <sup>b</sup>
Distilled water	4.15978 <sup>a</sup>	6.41207 <sup>a</sup>

<sup>1</sup>Means with different superscript are significantly different (P < 0.05).

TABLE 4. DISTRIBUTION OF MICROORGANISMS FROM FRESH SHRIMP SAMPLES ISOLATED FROM MEDIA WITH REGULAR SEAWATER.

Type	Percentage distribution during months of 1969											
	1	2	3	4	5	6	7	8	9	10	11	12
<i>Achromobacter</i>	0	0	0	0	0	0	2.5	0	0	0	0	0
<i>Alcaligenes</i>	5.7	0	2.5	0	0	0	0	0	0	0	0	0
<i>Bacillus</i>	2.9	0	0	0	0	0	0	0	0	0	0	0
Coryneform	22.9	37.5	65.0	45.0	75.7	35.0	25.0	35.0	15.0	42.5	37.5	47.5
<i>Flavobacterium</i>	11.4	5.0	0	2.5	2.7	7.5	7.5	7.5	2.5	45.0	7.5	2.5
<i>Lactobacillus</i>	0	0	0	0	2.7	2.5	7.5	2.5	0	0	0	0
<i>Microbacterium</i>	0	0	0	0	0	0	2.5	0	0	0	0	0
<i>Micrococcus</i>	14.3	10.0	17.5	10.0	18.9	5.0	10.0	2.5	0	2.5	7.5	7.5
<i>Moraxella</i>	8.6	25.0	7.5	12.5	0	12.5	30.0	5.0	2.5	10.0	22.5	32.5
<i>Pseudomonas</i>	31.4	17.5	0	27.5	0	30.0	15.0	47.5	65.0	0	25.0	2.5
<i>Staphylococcus</i>	2.9	5.0	7.5	2.5	0	0	0	0	0	0	0	7.5
<i>Vibrio</i>	0	0	0	0	0	7.5	0	0	15.0	0	0	0

TABLE 5. DISTRIBUTION OF MICROORGANISMS FROM STORED SHRIMP SAMPLES ISOLATED FROM MEDIA WITH REGULAR SEAWATER.

Type	Percentage distribution during months of 1969											
	1	2	3	4	5	6	7	8	9	10	11	12
<i>Achromobacter</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Alcaligenes</i>	12.8	2.6	0	2.5	0	0	0	0	0	0	0	0
<i>Bacillus</i>	10.3	2.6	2.5	0	0	0	0	0	0	0	5.0	0
Coryneform	17.9	7.7	52.5	32.5	25.0	10.0	20.0	30.0	7.5	17.5	25.0	12.5
<i>Flavobacterium</i>	0	5.1	0	0	0	2.5	0	0	0	0	0	2.5
<i>Lactobacillus</i>	0	23.0	2.5	0	0	0	20.0	0	0	0	0	0
<i>Microbacterium</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Micrococcus</i>	12.8	2.6	12.5	0	2.5	5.0	0	7.5	0	10.0	0	17.5
Mold	0	0	2.5	0	0	0	0	0	0	0	0	0
<i>Moraxella</i>	2.6	41.0	27.5	17.5	37.5	12.5	7.5	22.5	2.5	65.0	10.0	20.0
<i>Pseudomonas</i>	35.9	15.4	0	47.5	35.0	62.5	52.5	37.5	90.0	0	60.0	47.5
<i>Staphylococcus</i>	2.6	0	0	0	0	2.5	0	0	0	0	0	0
<i>Vibrio</i>	5.1	0	0	0	0	0	0	2.5	0	0	0	0

cant differences in bacterial counts resulted from conditions of plate incubation and composition of plating media. Counts on media with artificial seawater differed significantly from the others and ranked lowest. Aerobic plate counts differed significantly from anaerobic counts. Aerobic plate counts at 28 and 5 C did not differ significantly. This could be expected because during refrigerated storage of shrimp only the psychrotrophic bacteria will increase in number. These bacteria can be expected to show up on plating media at both 28 and 5 C.

The composition of the microbial flora of fresh and stored shrimp is presented in Tables 4 and 5. Although variations in microbial types were noted between samples, coryneforms and *Pseudomonas*, *Moraxella*, and *Micrococcus* species predominated in fresh and stored shrimp. The microbial flora of fresh shrimp was usually dominated by coryneforms; that of stored shrimp by *Pseudomonas* species. No consistent differences in type of bacterial flora were observed between the media prepared with seawater or distilled water. The present data do not allow any conclusions about the influence of season on the

distribution of the microbial flora in shrimp. The experimental samples came from different boats and the trawling locations and duration of trawling were different. The composition of the microbial flora shown in this study is in some respect different from that of Gulf Coast shrimp reported in 1952 (3, 10) and that of Pacific shrimp (7). According to Campbell and Williams, and Williams et al. (3, 10) species of *Achromobacter*, *Micrococcus*, *Pseudomonas*, *Flavobacterium*, and *Bacillus* predominated in Gulf coast shrimp. In Pacific shrimp (7) the initial flora in order of predominance was *Acinetobacter*, *Moraxella*, *Flavobacterium*, *Pseudomonas*, gram-positive cocci, and *Bacillus* species. Some of the differences in microflora of shrimp can probably be attributed to differences in shrimp species, marine environment, shrimp handling on board, and time and temperature of storage. The small number of *Achromobacter* species reported in Gulf shrimp in this study and also in Pacific shrimp (7) most likely reflects a change in taxonomic status. Some of the biochemically inert, gram-negative, short stout rods, formerly classified as *Achromobacter* species are now frequently reclas-

TABLE 6. AEROBIC AND ANAEROBIC PLATE COUNTS OF "POND SHRIMP" ON SMA PREPARED WITH DIFFERENT WATERS.

Conditions of plate incubation	Agar plate count per g on medium prepared with		
	Seawater	Artif. seawater	Dist. water
2d at 28 C - aerobic	1400-6800	180-2000	72-2000
7d at 5 C - aerobic	<10-47	<10	<10-42
2d at 28 C - anaerobic	200-1900	130-1100	29-500
7d at 5 C - anaerobic	<10-67	<10	<10-21

TABLE 7. DISTRIBUTION OF MICROBIAL FLORA OF POND SHRIMP PLATED ON MEDIA WITH DIFFERENT WATERS.

Type	Percentage distribution of isolates from plates prepared with	
	Regular seawater	Dist. water
<i>Achromobacter</i>	0 (0) <sup>a</sup>	0-2.5 (0.5)
<i>Alcaligenes</i>	0-40.0 <sup>b</sup> (11.5)	0-5.0 (1)
<i>Bacillus</i>	0-2.5 (0.5)	0-87.5 (23.4)
Coryneform	5-60.0 (43.0)	0-39.1 (19.5)
<i>Flavobacterium</i>	0-47.5 (24.5)	0-17.9 (5.6)
<i>Lactobacillus</i>	0-5.0 (1.0)	0-80.0 (32.2)
<i>Microbacterium</i>	0 (0)	0-2.6 (0.5)
<i>Micrococcus</i>	0-2.5 (1.5)	0-17.4 (5.0)
<i>Moraxella</i>	0-42.5 (15.0)	0-8.7 (2.8)
<i>Pseudomonas</i>	0-5.0 (2.0)	0-12.8 (2.6)
<i>Staphylococcus</i>	0-2.5 (1.0)	0-28.2 (5.8)
<i>Vibrio</i>	0 (0)	0-2.5 (0.5)

<sup>a</sup>Mean percentage

<sup>b</sup>Range of percentage distribution

sified as *Acinetobacter* or *Moraxella* species (2, 9). In the other studies, *Achromobacter* or *Acinetobacter-Moraxella* species predominated. In contrast, coryneforms usually were predominant in this study. Although some of the factors mentioned above may have been responsible for this difference in microflora, it is possible that recovery conditions (composition of plating medium and conditions of plate incubation) are involved. Some coryneforms isolated from shrimp were difficult to maintain on ordinary laboratory media because of their nutritional requirements. This may be another reason for the low numbers reported in other studies.

The data obtained on shrimp raised in ponds (Table 6) indicate that the bacterial counts of these

samples were lower than those of most commercial samples from the Gulf of Mexico. Counts on media prepared with distilled water and incubated at 28 C ranged from 72 to 2,000 bacteria per gram. Psychrotrophic bacterial counts (5 C for 7 days) were very low. The low microbial load of pond shrimp compared to Gulf shrimp may be attributed in part to differences in environment. In addition, elimination of extensive handling and storage on board probably are responsible for the differences in counts. The microflora of pond shrimp on seawater medium (Table 7) was dominated primarily by coryneforms and *Flavobacterium* species and to a lesser extent by *Alcaligenes* and *Moraxella* species. On media prepared with distilled water, coryneforms and species of *Lactobacillus* and *Bacillus* predominated. This observation again emphasizes that the microbial flora recovered from foods might depend on many factors including the composition of the plating medium. A difference in microflora distribution between media prepared with different waters was not observed with Gulf shrimp. *Bacillus* and *Lactobacillus* species on both media constituted less than 2% of the microflora of Gulf shrimp. Hence, *Bacillus* and *Lactobacillus* probably were of greater significance on pond than on Gulf shrimp. Pond shrimp also contained fewer *Pseudomonas* species (mean % 2-2.6) as compared with Gulf shrimp (mean % 18-22). Differences in microbial flora of pond and Gulf shrimp might be attributed to differences in environmental conditions, amount of handling, and age of shrimp. In general, the artificial ponds were quite different from the marine conditions in the Gulf of Mexico. The ponds were shallow and muddy. The temperature of the water which is pumped into the ponds through filters is higher than that of the water in the Gulf of Mexico. Shrimp in the ponds are fed artificially with either fish meal or poultry by-products. Little is known about the effect of these feedstuffs on the microbial flora of pond shrimp. In view of the uncertain microbiological quality of some of these feedstuffs (4) it would be advisable to ascertain that no human pathogens are present in these materials.

The present study indicates that shrimp from ponds contained few psychrotrophic bacteria. With improved methods of harvesting shrimp from ponds, the level of psychrotrophic bacteria may be controlled which would benefit the shelf life of the product. Further studies on the effect of the microbial flora of pond shrimp on shrimp quality and shelf life as warranted.

## REFERENCES

1. American Public Health Association. 1967. Standard methods for the examination of dairy products (12th ed.). American Public Health Association, Inc., New York.

2. Baumann, P., M. Doudoroff, and R. Y. Stanier. 1968. Study of the *Moraxella* group. II. Oxidase-negative species (genus *Acinetobacter*). *J. Bacteriol.* 95:1520-1541.
3. Campbell, L. L., Jr., and O. B. Williams. 1952. The bacteriology of Gulf Coast shrimp. IV. Bacteriological, chemical, and organoleptic changes with iced storage. *J. Milk Food Technol.* 6:125-126.
4. Chou, C. C., and E. H. Marth. 1969. Microbiology of some frozen and dried feedstuffs. *J. Milk Food Technol.* 32:372-378.
5. Green, M. 1949. Bacteriology of shrimp. I. Introduction and development of experimental procedures. *Food Res.* 14:365-371.
6. Green, M. 1949. Bacteriology of shrimp. II. Quantitative studies on freshly caught and iced shrimp. *Food Res.* 14:372-383.
7. Harrison, J. M., and J. S. Lee. 1969. Microbial evaluation of Pacific shrimp processing. *Appl. Microbiol.* 18:188-192.
8. Snedecor, G. W. 1956. *Statistical methods.* 5th ed. Iowa State College Press, Ames.
9. Vanderzant, C., and R. Nickelson. 1969. A microbiological examination of muscle tissue of beef, pork and lamb carcasses. *J. Milk Food Technol.* 32:357-361.
10. Williams, O. B., L. L. Campbell, Jr., and H. B. Reese, Jr. 1952. The bacteriology of Gulf coast shrimp. II. Qualitative observations on the external flora. *Texas J. Sci.* 4:53-54.

## NUTRITIONAL ASPECTS OF DAIRY PRODUCTS (Continued from Page 342)

one-half of the total solids-not-fat in milk, and contributes about 30% of the calories of whole milk.

Lactose is only about one-sixth as sweet as sucrose. Dr. John Yudkin, Professor of Nutrition, Queen Elizabeth College, University of London, England recently stated: "There is now quite strong evidence that dietary sugar—sucrose—is an important factor in the etiology of coronary heart disease" Again, as in so many things this is a theory. An important characteristic of lactose is its ability to promote the growth of certain beneficial lactic acid producing bacteria, with their possible displacement of undesirable forms in the intestinal tract.

That the milk sugar lactose stimulates absorption of calcium is well established although the exact mechanism is not understood. It is also known that this effect of stimulating absorption includes magnesium, barium, and phosphorus.

### VITAMINS IN MILK

All the vitamins known to be required by man are found in milk, the water-soluble vitamins being in the nonfat portion of milk and others in the butterfat of milk; many in significant amounts and others at a lower level.

All the B vitamins occur in significant amounts in milk. Milk is an important source of riboflavin, and this vitamin is only slightly affected by the heat of pasteurization, and the riboflavin content of milk is only slightly reduced by exposure to light. Riboflavin, sometimes called vitamin B-2, helps cells use oxygen, helps the body use protein, and helps keep tissues healthy. Although niacin occurs in milk at a low level, it is considerably higher than indicated by a measure of its niacin content. This is evident from milk's effectiveness in curing pellagra, a disease that

can be cured by administering niacin. Pasteurization does not reduce the niacin content of milk.

A significant amount of thiamine (B-1) occurs in milk, and in general about 90% of this thiamine is retained when milk is pasteurized.

### MINERALS IN MILK

Milk contains seven minerals as major constituents and many more in minor or trace amounts. Calcium and phosphorus are considered dietary essentials.

*Hoard's Dairyman* in the May 25, 1969 issue, had a very interesting article in regard to calcium. Women, of course, received the bold black print—"WOMEN ARE IN BAD SHAPE—NUTRITIONALLY" Small print below—"And Adult Men Are Not Much Better Off."

Highlights in this article: (a) Millions of Americans are crumbling on the inside while showing no outward sign. The fault lies with a hidden hunger for calcium. (b) Seven million women are afflicted with osteoporosis, a bone deterioration disease. (c) It is estimated that more than one-half million women have fractures of their vertebrae without knowing it. (d) Hip and spine fractures in older people may be the result of bone failure, causing the fall. (e) Research with experimental animals suggests human reproduction may be impaired. (f) Life span may be shortened. (g) Height and strength may be reduced.

All of this and more may be the result of lower than recommended milk consumption. They are not drinking enough milk or eating enough cheese and ice cream. Neither are they getting enough whole grain or enriched breads and cereals, nor dark, green, leafy vegetables.

Two eminent nutritionists, Ruth M. Leverton and Millicent L. Hathaway, have written: "A body well

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# A VISCOMETRIC METHOD FOR THE ESTIMATION OF MILK CELL COUNT

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## ABSTRACT

The use of a rolling ball viscometer to estimate the cell count of milk treated with California Mastitis Reagent is described. The method compares favorably with the direct microscopic count and requires relatively simple apparatus and unskilled operators. A correlation coefficient for mean count and log viscometer time of 0.93 with a coefficient of variation of 8.1% were obtained. The repeatability of viscometer readings was 0.97.

Increasing emphasis on the definition of the normality of milk in terms of its cell content (1, 3, 5) has resulted in a need for a method of assessing the cell count of milk at the factory or treatment station which is rapid, simple, and reliable. The California Mastitis Test (CMT) (8) and its modifications have an important place as a cow-side test for mastitis, but because of their subjectivity, they are not suitable for testing at the factory. The introduction of the Brabant Test (4) and the Wisconsin Test (9) provided the foundation for a factory-type test. Both are viscometric tests based on the reaction of certain surface-active agents with cells in milk in which the flow rate of the mixture through an orifice is used to determine the relative viscosity. In the Wisconsin Test the viscosity is estimated by measuring the liquid remaining in a tube 12.5 by 125 mm with an orifice 1.15 mm in diameter drilled in a cap at the top end. A fixed volume of milk-reagent mixture is added, and the tube inverted for a period of 15 sec. It is allowed to stand upright for 1 min and the liquid level then measured.

A possible criticism of this and similar tests is the fact that the index of viscosity used (the volume or height of remaining liquid) is not directly related to viscosity. The rate of flow through the orifice is not a linear function of the head of the liquid above it. The resultant reading of liquid level after inversion is therefore not directly related to viscosity. If there is to be any standardization of milk tests of this type there is merit in their being defined in terms of true viscosity changes regardless of the form of apparatus.

A second objection to all "small orifice" tests is the occasional plugging of the orifice by small clots in the milk. Postle and Smith (6) mention this problem and recommend the use of duplicates in applications

of the Wisconsin Test. Rotary viscometers are unsuitable for highly thixotropic gels such as that formed by the CMT reaction. Preliminary studies showed that such viscometers produce large errors.

If leucocyte or somatic cell assessment is to become a routine factory determination, it is desirable that it be made as nearly automatic as possible, finally perhaps being totally automated. "Multiple tube" tests are more difficult to automate than tests involving sequential operations in an apparatus which runs continuously. An example of a satisfactory test which is readily adapted to automation is the application of the Coulter Particle Counter to milk cell counting (2, 10). This is, however, expensive.

Viscometric methods for the estimation of cell counts have been shown to be more effective than other rapid methods for both farm and tanker samples (1, 3, 6). The latter has shown that in a classification system based on the direct microscopic cell count (DMCC) on both producer and tanker milk, the correlation coefficients between the DMCC and the Wisconsin Mastitis Test were 0.97 and 0.99, respectively.

The viscometric technique to be described is an attempt to develop a low-cost method suitable for automation which gives a result which is directly proportional to viscosity. Further, the method avoids the difficulty caused by "small orifice" techniques and gives a result which is a true average for a non-homogeneous thixotropic gel. The gel resulting from the CMT reaction is frequently non-homogeneous. Portions of high viscosity may cause a partial obstruction of a small orifice because of their "structural viscosity" component. In the rolling ball method the ball "squeezes past" a clot which would block a small orifice.

## METHODS

### *Viscometer*

A preliminary report on the use of the rolling-ball viscometer has been published (11). The instrument was described and the operating sequence used in this study explained by Whittlestone and Allan (12).

The milk sample (10 ml) and reagent<sup>1</sup> (10 ml) were mixed in a 25 ml test tube for 45 sec using a roll-tube mixer which is illustrated in Fig. 1. The holding period prior to testing was a further 45 sec. The operations were performed at room

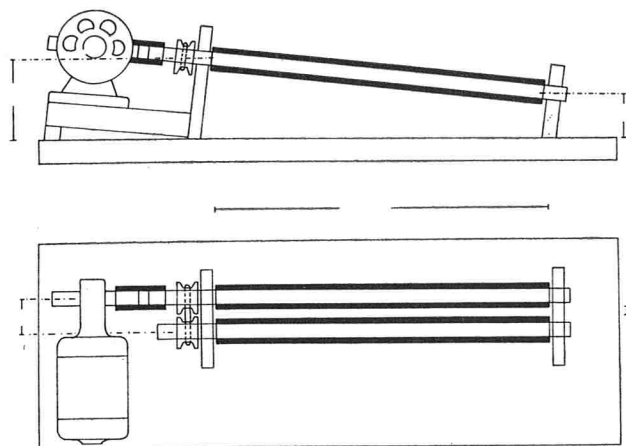


Figure 1. Diagram of roll-tube mixer. The milk/reagent mixture is placed in a stoppered tube which is permitted to rotate slowly on the sloping rollers of the mixer which runs for a fixed period of 45 sec then stops automatically.

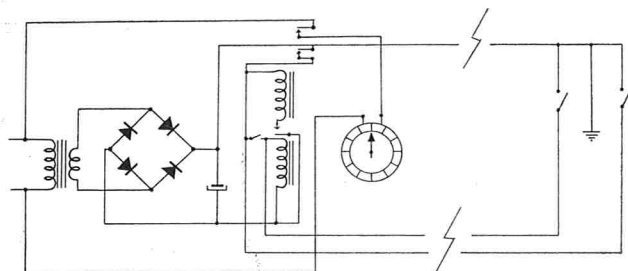


Figure 2. Circuit diagram of timing mechanism of rolling-ball viscometer. The automatic timer is driven by a synchronous clock motor controlled by two relays. The contacts on the right are fitted to the viscometer. The left hand contact closes when the viscometer tube is tipped to the running position. This closes the top relay and starts the clock. On arrival at the bottom of the viscometer tube the stainless steel ball causes the right hand contact to close. This energizes the lower relay whose contacts open so releasing the upper relay and stopping the clock. The contacts to the left of the lower relay are manually operated to stop the clock in the event of encountering a sample with a running time beyond the capacity of the timer which is 60 sec.

temperature which causes a small error but makes the method simple for factory use. The viscometer was operated at an angle of 26° to the horizontal. A circuit diagram of the timing mechanism is given in Fig. 2.

#### Direct microscopic count

A modification of the method of Prescott and Breed (7) was used for the microscopic counting of somatic cells in milk. With a platinum loop calibrated to deliver 0.01 ml, the milk sample was spread over a circular area (diameter 1.5 cm) marked on a microscope slide. The milk film was dried, defatted in xylol, fixed in 95% ethanol, and stained in a methylene blue solution. Cells were counted in 100 random fields and the count expressed as cells per milliliter of milk.

Bulk samples of milk of high count (5,000,000 cells per ml) and low count (300,000 cells per ml) were collected. By serial dilution (high: low; 4:1) ten sub-samples were obtained. These were submitted to viscometric estimation and microscopic count within 4 hr of collection.

<sup>1</sup>CMT reagent, National Dairy Association of N.Z.

One operator performed five separate viscosity determinations on each sample. Microscopic counts were carried out by three operators using three different microscopes having a working factor of 3,250 (operator 1); 8,150 (operator 2); and 4,975 (operator 3). For each sample three loops were taken and 100 fields counted per loop.

## RESULTS

Microscopic cell counts (the mean of all operators) and mean viscometer test results are given in Table 1. Cell numbers of the 10 samples range from 280,000 to 5,530,000 cells per milliliter. The time taken for the ball to roll through the milk-reagent mixture, which was taken as an estimate of the viscosity, ranged from 2.72 to 14.08 sec. The transformation ( $\frac{1}{\text{viscometer time}}$ ) is given because it was found to have an error variance which does not depend on the level of cell count.

There was a highly significant relationship between the estimates of viscosity and cell count. The correlation coefficient for mean count and viscometer time was 0.91, for mean count and log viscometer time 0.93, and for log count and viscometer rate ( $\frac{1}{\text{time}}$ ) 0.84. There were differences between "operators" (including the difference between microscopes) the correlation coefficients for log count and viscometer rate being 0.81 (operator 1), 0.92 (operator 2), and 0.90 (operator 3). The regression relationship between log cell count and viscometer rate is summarized in Table 2 and a graph of the relationship given in Figure 3.

An analysis of variance of viscometer test results is given in Table 3. The variation of replicates within samples was a very small proportion of the variance, having a standard deviation of 0.018. The

TABLE 1. MICROSCOPIC CELL COUNTS AND VISCOMETER TEST RESULTS ON TEN MILK SAMPLES

	Cell count		Viscosity	
	Number <sup>1</sup> (x10 <sup>6</sup> )	Log <sup>2</sup>	Time <sup>3</sup>	Rate ( $\frac{1}{T}$ ) <sup>4</sup>
1.	5.53	2.73	14.08	0.074
2.	4.33	2.62	8.16	0.126
3.	4.93	2.69	7.86	0.129
4.	3.67	2.56	9.24	0.117
5.	2.39	2.35	5.42	0.186
6.	2.50	2.39	3.90	0.256
7.	1.94	2.27	3.30	0.303
8.	1.68	2.22	3.10	0.323
9.	1.27	2.17	2.98	0.336
10.	0.28	1.41	2.72	0.367

<sup>1</sup>Mean of 3 operators.

<sup>2</sup>Mean of 9 loops.

<sup>3</sup>Time of travel for ball, sec.

<sup>4</sup>Rate of travel for ball ( $\frac{1}{\text{time}}$ ).

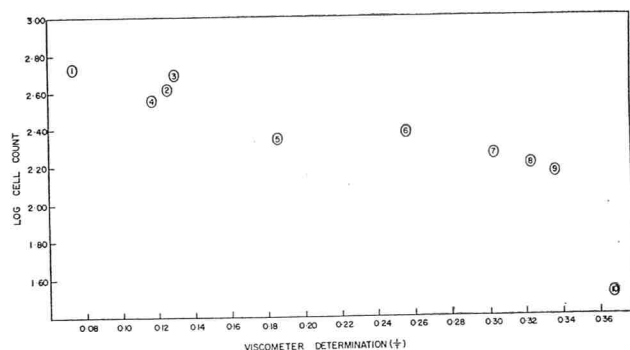


Figure 3. Relationship between log cell count and viscometer rate results.

coefficient of variation for the method was therefore 8.1%.

An analysis of variance of the cell count data (log cell count plus one) is given in Table 4. The difference between samples was the major component of variance, but variation between "operators" which includes the difference in working factor between microscopes, was also large. The standard deviation for loops was 0.051.

Repeatabilities of the cell count and viscometer methods were high, being 0.95 (operator 1), 0.98 (operator 2), 0.96 (operator 3), and 0.97 (viscometer).

#### DISCUSSION

There is real difficulty in making a comparison between a viscosity determination which is inherently repeatable and reasonably accurate on the one hand and a cell count which is beset with many errors on the other. The standard deviation of a single loop used in cell counting is a much larger proportion of the mean than that for a viscometer determination. In addition there are such sources of error in cell counting as operator performance and microscope factor which add to make the method highly error prone.

Figure 3 shows a "ceiling effect" at the lower end of the scale which contributes to the residual variance. The transformation  $(1/T + 2.5)$  would have improved the linearity but this is purely a matter of convenience. The point at which the "ceiling effect" commences, and the sensitivity of the method may be adjusted by altering the rolling angle. This feature makes it possible to use the instrument at different levels of sensitivity for quarter and bulk milk samples, a distinct advantage.

If the present test were applied to the grading of milk samples as was done by Janzen (1) using the Wisconsin test the same satisfactory result would have been achieved with the additional advantages of obtaining results directly related to viscosity and

TABLE 2. REGRESSION OF LOG CELL COUNT AND VISCOMETER

Rate $\left(\frac{1}{\text{Time}}\right)$				
Regression sum of squares	Residual sum of squares	Degrees of freedom	Mean square	Regression coefficient
0.92932	0.38777	8	0.04847	-2.988878

$^{\circ}$  ( $p=0.01$ )

TABLE 3. ANALYSIS OF VARIANCE VISCOSITY

MEAN = 0.222

	df	Mean square	Variance component
Samples	9	0.05786	0.01151
Runs within samples	40	0.00032	0.00032

TABLE 4. ANALYSIS OF VARIANCE CELL COUNT LOG (C+1)  
MEAN = 0.372

	df	Mean square	Variance component
Operators	2	48.1186	0.0156
Samples	9	55.7859	0.0605
Operators x Samples	18	1.3674	0.0037
Loops	60	0.2561	0.0017
Counts within loops	8910	0.0842	0.0842

using apparatus which may be made automatic for large scale testing using unskilled staff.

#### ACKNOWLEDGMENTS

The authors are grateful to Mr. D. Duganzich of this Centre for carrying out the statistical work involved in this study and for his suggestions in planning the work.

#### REFERENCES

1. Janzen, J. J. 1969. Evaluation of five screening tests for estimating leucocyte counts in bulk milk. *J. Dairy Sci.* 52:329-334.
2. Kleinschroth, E. Von, O. Richter, and H. Schumann. 1968. Die Anwendung der elektronischen Bestimmung des Milchzellgehaltes in Eutergesundheitsdienst und bei der Qualitätsüberwachung der Milch. *Milchwissenschaft* 23:147-153.
3. Kroger, D., and D. E. Jasper. 1967. Relationships between Wisconsin mastitis test scores and cell counts in milk. *J. Dairy Sci.* 50:1226-1233.
4. Matschullat, G. 1963. Die Brabanter Mastitis-Reaktion. *Molkerei- und Kaserei-Zeitung* 14:1550-1552.
5. Miller, D. D., and J. V. Kearns. 1966. Effectiveness of the California mastitis test as a measurement of the leucocyte content of quarter samples of milk. *J. Dairy Sci.* 50:683-686.

6. Postle, D. S., and A. R. Smith. 1965. Applications of the Wisconsin mastitis test under field conditions. *J. Milk Food Technol.* 28:310-313.
7. Prescott, S. C., and R. S. Breed. 1910. The determination of the number of body cells in milk by a direct method. *J. Infect. Dis.* 7:632-640.
8. Schalm, O. W., and D. O. Noorlander. 1957. Experiments and observations leading to the development of the California Mastitis Test. *J. Amer. Veterinary Med. Ass.* 130:199-204.
9. Thompson D. I., and D. S. Postle. 1964. The Wisconsin mastitis test—An indirect estimation of the leucocyte count in milk. *J. Milk Food Technol.* 27:271-275.
10. Tolle, A. Von, H. Zeidler, and W. Heesch. 1966. Ein Verfahren zur elektronischen Zählung von Milchzellen. *Milchwissenschaft* 21:93-98.
11. Whittlestone, W. G., and L. R. Fell. 1965. A viscometer for routine mastitis tests. *Proc. 36th Ann. Conf., N.Z. Dairy Sci. Ass.*:21-22.
12. Whittlestone, W. G., and D. J. Allen. 1966. An automatic viscometer for the measurement of the California mastitis test reaction. *Aust. J. Dairy Technol.* 12:139.

## NUTRITIONAL ASPECTS OF DAIRY PRODUCTS

(Continued from Page 350)

nourished with calcium and other nutrients can be expected to have good bone growth and development, a well functioning nervous system, a high level of vigor and positive health at every age, and a longer period of the prime of life." Sherman and Lanford say: "Calcium salts are absolutely necessary to the normal action of the heart muscle."

In addition calcium helps the blood to clot and regulates the use of other minerals in the body. Calcium is essential in the action of certain enzymes and the control or passage of fluids through cell walls. In combination with phosphorus, calcium gives rigidity and hardness to teeth and bones. So you can see why some are crumbling on the inside—hunger for calcium.

Following the Daily Recommended Dietary Allowances revised in 1968 by the Food and Nutrition Board of the National Academy of Sciences—National Research Council, nutritionists say it is almost impossible to supply the amount of calcium that is recommended unless milk in some form is used daily, and cheese and other milk-containing products are eaten frequently. To match the calcium in one quart of milk, you would have to eat: 28 oranges, or 39 eggs, or 27 lb potatoes, or 6.75 lb cabbage, or 7 lb of carrots. Some of the foods we need every day, but not for that all important calcium. All nutrients work in harmony as a team.

Some very interesting things were found out in the National Nutrition Survey. The survey is still going on and hopefully someday will include every state in the Union. Now it has covered Texas, Louisiana, California, Washington, Kentucky, West Virginia, Massachusetts, New York, South Carolina, and Michigan.

Incomes ranged from \$180 to \$42,000 a year—not just the low income group.

Every kind of malnutrition that one usually connects with Central American, Africa, and Asia was found to exist. It is alarming to discover such malnourishment in our "Land of Plenty."

About 56 years ago, it was not unusual to find cases of pellagra and rickets, and other deficiency diseases. As years have gone by we have become complacent about many such diseases. Thinking "Oh, not in the good old U.S.A." Maybe in some of the underdeveloped countries. This survey has made us sit up and take notice. In 10 states: (a) 18 cases of rickets—lack of vitamin D, (b) 1/3 pre-school children have iron deficiency anemia, (c) endemic goiter—5% of ones examined had enlarged thyroid gland—lack of iodine, (d) 8 cases of bitot spots—often causes blindness—vitamin A deficiency, (e) 7 cases of Kwaskiorkor—African word coined by a gold coast tribe—protein deficiency, and (f) bone deficiencies—calcium. A contributing factor, in several instances, was the mother's ignorance of health care and even lack of concern.

We know where some of the problems are—we must do something about it. Maybe the ball will get rolling in the right direction at the White House Conference on Food, Nutrition and Health, December 2-4, 1969 in Washington, D.C.

In summing up these 5 general groups of nutrients found in milk and dairy products, we need to think again as to how they help build a healthy body:

### VITAMINS

- Regulate body processes
- Needed for clear skin, good eyesight
- Helps cells use oxygen
- Make walls of blood vessels firm

### MINERALS

- Helps build bones and teeth
- Proper function of nerves and muscles
- Proper clotting of blood
- Helps delay fatigue

### PROTEINS

- Builds and repairs all body tissues
- Helps fight infection
- Helps supply energy

### FATS AND CARBOHYDRATES

- Helps in digestion and absorption
- Supplies energy
- Added flavor

The best is being done through intensive research to find out more about these components of milk and why they work together so well for good health.

(Continued on Page 360)



# POLYMYXIN-COAGULASE-MANNITOL-AGAR

## II. ISOLATION AND IDENTIFICATION OF COAGULASE-POSITIVE STAPHYLOCOCCI FROM FROZEN SHRIMP<sup>1</sup>

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(Received for publication February 25, 1970)

### ABSTRACT

Polymyxin-Coagulase-Mannitol-Agar (PCMA) was compared with three other selective media for the ability to rapidly isolate coagulase-positive staphylococci from frozen shrimp. When spread plating was used, PCMA recovered higher numbers of coagulase-positive staphylococci than did Vogel and Johnson Agar, Staphylococcus Medium No. 110 with 0.75 mM sodium azide, and Trypticase Soy Agar with 10% NaCl. PCMA allowed identification of coagulase-positive staphylococci within 24 hr; whereas, the other selective media required 48 hr for growth plus 24 hr for confirmation of identity of the coagulase-positive staphylococci.

Cross-replica-plating was used to compare the effectiveness of the four selective media studied. In general, PCMA allowed fewer total number of colonies to develop than did the other selective media, but a greater percentage of the colonies that developed on PCMA were coagulase-positive staphylococci.

Coagulase production is generally accepted as an indicator of the pathogenicity of a *Staphylococcus* (1, 2, 5). The recommended procedure for the isolation and identification of *Staphylococcus aureus* from foods (1) requires a minimum of 3 days to isolate and identify coagulase-positive staphylococci. The Polymyxin-Coagulase-Mannitol-Agar (PCMA) developed by Orth and Anderson (12) offers a more rapid means for quantitating the coagulase-positive and mannitol-fermenting staphylococci in food samples.

The purpose of this study was to compare the ability of PCMA, Vogel and Johnson Agar (V-J), Staphylococcus Medium No. 110 with azide (S-110-Az), and Trypticase Soy Agar containing 10% NaCl (TRY-SOY) to isolate *S. aureus* from frozen foods on the market. The samples were selected at random in order to obtain foods contaminated with *S. aureus* from "natural" sources. The data reported here deal with frozen Pacific shrimp which yielded coagulase-positive staphylococci under the prescribed test conditions.

### MATERIALS AND METHODS

#### *Foods examined*

Several market products were screened for the presence

of *S. aureus*. These products included frozen meat, chicken pie, frozen mixed vegetables, and frozen Pacific shrimp (*Pandalus jordani*). Only the shrimp samples yielded staphylococci, therefore, this comparative study of selective media was made with samples from 4 lots of frozen shrimp. The shrimp samples were obtained from a local seafood processor and had been stored frozen at -15 C for one month at the time of this investigation.

#### *Media<sup>1</sup>*

The basic non-selective medium (TPN) was prepared by the formula of Corlett et al. (4). The TRY-SOY was prepared from Trypticase Soy Agar (Baltimore Biological Laboratory, Baltimore, Md.) plus 9.5% NaCl. The S-110-Az contained 0.75 mM sodium azide and was prepared by the method of Smuckler and Appleman (14). The V-J agar was made in accordance with the manufacturer's directions, and PCMA was prepared by the method of Orth and Anderson (12). Before replica plating, the agar surfaces were dried by incubating the plates at 37 C for approximately 24 hr.

#### *Sampling and direct plating*

Ten grams of each shrimp sample was added aseptically to 90 ml of 0.2% peptone water with glass beads (4). After shaking, serial 10-fold dilutions were made in this diluent. Six 0.1 ml aliquots of each dilution were then spread-plated on TPN, TRY-SOY, S-110-Az, V-J, and PCMA. Additional spread plates of TPN were prepared in triplicate and incubated at 27 C to determine the mesophilic and psychrotropic load of the sample. All other plates were incubated at 37 C for 24 to 48 hr. After 24 hr incubation, the coagulase-positive colonies on the PCMA plates were enumerated. After 48 hr incubation, representative colonies on all the selective media were selected for coagulase testing by the recommended method (1). At the same time, three plates of each of the selective media and the TPN plates were used in the cross-replica-plating experiments. A microscopic examination after gram staining also was made with 10% of the colonies selected at random from each selective medium.

#### *Replica plating*

After growth, three plates of each type of medium were designated "mother plates" and were used for replica plating (9) onto TPN, PCMA, V-J, S-110-Az, and TRY-SOY. The last plate in the replication series was the same medium as the mother plate to confirm the successful inoculation to all preceding daughter plates. The daughter plates were incubated at 37 C for 24 to 48 hr.

#### *Confirmation of coagulase activity by the tube test*

All PCMA-positive colonies and at least one of each of the colony types on all the selective media were picked and streaked on Brain Heart Infusion Agar. The pure cultures

<sup>1</sup>Technical Paper No. 2787, Oregon Agricultural Experiment Station.

<sup>1</sup>All media used were obtained from Difco, Detroit, Mich. unless otherwise stated.

TABLE 1. COMPARISON OF THE TOTAL COUNTS ON THE TEST MEDIA USING THE TWO FROZEN SHRIMP SAMPLES WHICH YIELDED NO COAGULASE-POSITIVE STAPHYLOCOCCI.

Medium	Count/100 g <sup>1</sup>	% Recovery <sup>2</sup>
TPN (27 C)	130,000	—
TPN (37 C)	30,000	100
TRY-SOY	6,700	22
S-110-Az	4,100	14
V-J	3,300	11
PCMA	370	1

<sup>1</sup>Average of three tests per sample

<sup>2</sup>Based on TPN incubated at 37 C as 100% recovery

thus isolated were tested for coagulase production by the tube method using Bacto-coagulase plasma with EDTA (10).

## RESULTS AND DISCUSSION

### PCMA as a direct isolation medium

When dilutions of the shrimp samples were plated on PCMA, 230 and 1,160 coagulase-positive staphylococci per 100 g were detected from two samples after incubation for 24 hr at 37 C. The two additional shrimp samples tested were PCMA-negative. When tested with V-J, the same four samples yielded one coagulase-positive sample with a count of 30 coagulase-positive staphylococci per 100 g.

### Comparison of selective media

The selectivity of the four media were compared by plating the corresponding serial dilutions of shrimp on each medium in triplicate. The average counts of the two negative samples are presented in Table 1. Since these samples did not yield coagulase-positive staphylococci, the degree of inhibition was taken as the ability of each medium to inhibit interfering microorganisms. Incubation at 37 C alone inhibited 77% of the growth at 27 C. Pacific shrimp has been shown to contain up to 84.5% *Acinetobacter-Moraxella* species (8), and 93% of these organisms could not grow at 35 C (*unpublished observation*).

The microscopic examination of the colonies picked from each selective medium revealed that no gram-negative bacteria were present in the dilutions examined. The TPN growth at 37 C was 60% gram-positive cocci and 40% gram-positive rods. PCMA and S-110-Az yielded 80% gram-positive cocci and 20% gram-positive rods; whereas, TRY-SOY and V-J allowed the growth of only gram-positive cocci.

### Replica plating

The initial isolation plates from a shrimp sample which contained coagulase-positive staphylococci were cross-replicated (i.e. plated onto fresh plates of all other media used in this experiment). The resulting replication series with the TPN mother plate

showed that the degree of inhibition increased in the same order as that observed with direct plating: TPN < TRY-SOY < S-110-Az < V-J < PCMA (Table 2). Data in Table 2 also show that the recovery of coagulase-positive staphylococci from the shrimp sample improved when the initial isolation plate was less selective. It is recognized that the physiological state of a microorganism could influence its ability to grow on selective media (3, 7, 13). Freeze-injury could be responsible for the improved recovery of coagulase-positive staphylococci following growth on the less selective media. Finegold and Sweeney (6) indicated that 75 µg polymyxin B per milliliter was the optimal amount for selective media for *S. aureus*. In general, PCMA allowed fewer colonies to develop than did the other selective media; however, a greater percentage of the colonies that developed on PCMA were coagulase-positive staphylococci.

### False reactions on PCMA

Neither false-positive nor false-negative reactions on PCMA was observed in this study. All coagulase-positive staphylococci isolated produced fibrin halos when streaked onto PCMA and gave a positive coagu-

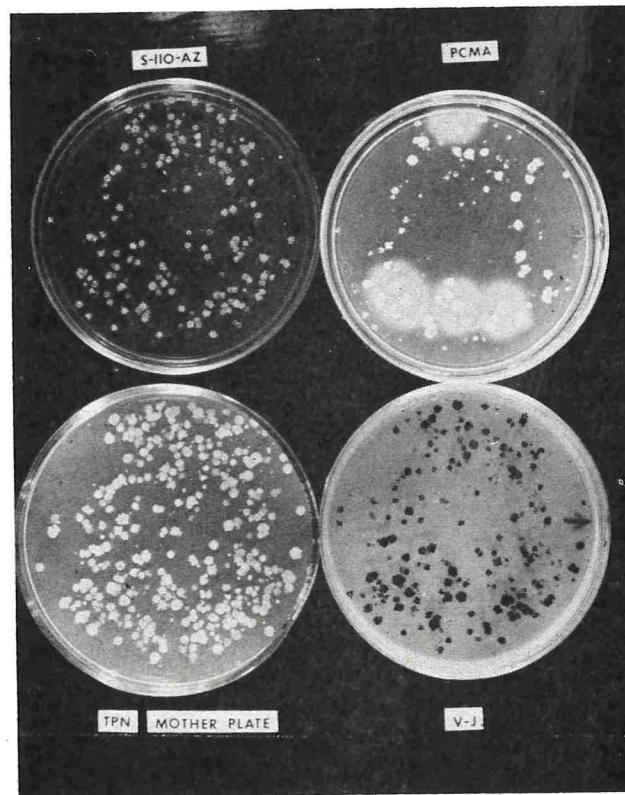


Figure 1. Replica-plating of TPN (mother plate) onto S-110-Az, PCMA and V-J. This photograph was taken after 48 hr incubation at 37 C. The 6 fibrin halos around the *S. aureus* colonies on the PCMA plate were discrete at 24 hr; however, continued incubation resulted in overlapping of the fibrin zones. The corresponding PCMA-positive colonies on V-J and S-110-Az are difficult to distinguish from the non-*S. aureus* colonies.

lase reaction in the tube test. The *S. aureus* strains isolated from the shrimp samples gave a distinct fibrin halo, 2 to 6 mm in diameter, within 24 hr. The replication series in which the TPN plate was used as the mother plate is shown in Fig. 1.

False-negative reactions may occur on PCMA if the *S. aureus* strain is strongly proteolytic (12). Some proteolytic strains isolated from shrimp began to hydrolyze the center of their respective fibrin halos, but the degree of proteolysis was not sufficient to interfere with the coagulase reading after five days of incubation. This finding conflicts with the observation of McDivitt and Jerome (11). They showed that more coagulase-positive strains were detected by the tube method than by the reaction on fibrinogen-polymyxin medium. PCMA was prepared with plasminogen-free coagulase-reacting factor. The elimination of fibrinolysis due to the Müller phenomenon and staphylokinase action is believed to be responsible for the stability of the fibrin halos on PCMA.

Mannitol fermentation was indicated on PCMA by the yellow acid reaction of the brom cresol purple indicator in and around the colony. Although all coagulase-positive staphylococci isolated were mannitol-positive, the usefulness of this test was limited by the presence of coagulase-negative strains which were mannitol-positive.

PCMA was successfully used to demonstrate coagulase-positive staphylococci from nasal swabs and purulent exudates in 12 to 24 hr. PCMA allowed faster identification of the coagulase-positive staphylococci in frozen shrimp than did V-J, TRY-SOY, and S-110-Az. A study is in progress to adapt PCMA for the detection of *S. aureus* in other food commodities.

#### ACKNOWLEDGMENTS

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#### REFERENCES

1. Anon. 1969. Bacteriological analytical manual. U.S. Department of Health, Education and Welfare, Public Health Service, Consumer Protection and Environmental Health Service, F.D.A. Division of Microbiology, Washington, D. C. 20204.
2. Chapman, G. H., C. Berens, E. L. Nilson, and L. G. Curcio. 1938. The differentiation of pathogenic staphylococci from non-pathogenic types. *J. Bacteriol.* 35:311-334.
3. Clark, C. W., and Z. J. Ordal. 1969. Thermal injury and recovery of *Salmonella typhimurium* and its effect on enumeration procedures. *Appl. Microbiol.* 18:332-336.
4. Corlett, D. A., Jr., J. S. Lee, and R. O. Sinnhuber. 1965. Application of replica plating and computer analysis for rapid identification of bacteria in some foods. I. Identification scheme. *Appl. Microbiol.* 13:808-817.
5. Evans, J. B., and C. F. Niven, Jr. 1950. A comparative study of known food poisoning staphylococci and related varieties. *J. Bacteriol.* 59:545-550.
6. Finegold, S. M., and E. E. Sweeney. 1961. New selective and differential medium for coagulase-positive staphylococci allowing rapid growth and strain differentiation. *J. Bacteriol.* 81:636-641.
7. Harris, N. D. 1963. The influence of the recovery medium and the incubation temperature on the survival of damaged bacteria. *J. Appl. Bacteriol.* 26:387-397.
8. Harrison, J. M., and J. S. Lee. 1969. Microbiological evaluation of Pacific shrimp processing. *Appl. Microbiol.* 18:188-192.
9. Lederberg, J., and E. M. Lederberg. 1952. Replica plating and the indirect selection of bacterial mutants. *J. Bacteriol.* 63:399-406.
10. Litkenhous, C. J., E. F. Baer, M. M. Gilden, and M. B. Bromer. 1968. False-positive coagulase reactions in the characterization of microorganisms isolated from foods. *J. Ass. Off. Anal. Chem.* 51:1-3.
11. McDivitt, M. E., and N. W. Jerome. 1965. Limitation of fibrinogen-polymyxin medium in detecting coagulase-positive staphylococci in raw milk. *Appl. Microbiol.* 13:157-159.
12. Orth, D. S., and A. W. Anderson. 1970. Polymyxin-coagulase-mannitol-agar. I. A selective isolation medium for coagulase-positive staphylococci. *Appl. Microbiol.* 19:73-75.
13. Postgate, R. J., and J. R. Hunter. 1963. Metabolic injury in frozen bacteria. *J. Appl. Bacteriol.* 26:405-414.
14. Smuckler, S. A., and M. D. Appleman. 1964. Improved *Staphylococcus* medium No. 110. *Appl. Microbiol.* 12:355-359.

# FOOD AND DRUG ADMINISTRATION VIEW OF STAPHYLOCOCCAL CONTAMINATION OF FOOD<sup>1</sup>

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All strains of coagulase positive staphylococci are potentially pathogenic. These organisms are ubiquitous in man's environment and may be isolated with ease from air, water, food, milk, dust, feces, and sewerage. Man is colonized extensively with staphylococci and the carrier rate approximates 30 to 50% of the normal population. The organisms typically are carried in and on the skin and the mucous membranes of the nasopharynx. Since they are prevalent in the environment and exist as "normal flora" of the skin and nasopharynx, it is no wonder that staphylococci are present in foods, particularly those that come in intimate contact with food handlers during processing and preparation. Except for milk and dairy products, in which staphylococci may be present as a result of shedding from the bovine udder, the single most important source of *Staphylococcus aureus* in foods is man, and until he is no longer directly involved in the preparation and service of food, staphylococci will remain a foodborne health hazard.

## SURVIVAL AND GROWTH

The staphylococci are capable of surviving many food processing treatments. They withstand desiccation well and drying on glass beads is commonly employed to preserve cultures in the laboratory. Freezing causes an immediate but slight reduction in numbers and once frozen the organisms last indefinitely. Growth in foods can occur at 44 F but not at 42 F, and toxin may be produced at temperatures of 64.4 F and above. Though cellular multiplication occurs at temperatures up to 114 F, higher temperatures result in death. In a study reported by this author, 59 min exposure to 140 F was required to reduce  $1 \times 10^7$  cells of a food-poisoning strain of *S. aureus* to a non-detectable level in food. Salt concentrations usually sufficient to inhibit many other bacterial species, up to 10% sodium chloride, have little or no effect on growth of *S. aureus*.

## INCIDENCE

The incidence of staphylococcal food poisoning has increased over the years to the point that today it is the most frequently reported foodborne illness. The incidence remains high in spite of continued efforts of Federal, State, and municipal health agencies to improve environmental sanitation in food processing, preparation, and service establishments and to educate food handlers to the dangers of subjecting potentially hazardous foods to conditions that permit staphylococcal growth and toxin production. Better control of staphylococcal food poisoning is possible through application of current knowledge related to food sanitation and proper heating and cooling practices, but it is doubtful that full control can ever be achieved.

## VIEWS ON STAPHYLOCOCCI

Because we recognize that much of the staphylococcal contamination in foods is contributed by people involved in the processing and preparation of foods and because we further recognize that the presence of toxin in food is the result of staphylococcal proliferation, we view staphylococcal contamination in two ways. Firstly, staphylococcal numbers serve as an index of the care exerted during processing in the maintenance of time-temperature control and environmental sanitation, as well as for the minimization of unnecessary human contact. Secondly, when present in large numbers they signal the potential danger of illness from preformed toxin and indicate further that poor environmental control and personal hygienic practices probably prevailed during processing.

Though staphylococcal contamination in many foods is unavoidable within the present technical capabilities of the processing industries, numbers can be kept to a minimum and growth and toxin production is controllable. Extensive outgrowth of staphylococci in food from an initially small number introduced by people or from the processing environment is to be prevented. Our experience indicates that some manufacturers are able to produce many foods with low levels of staphylococcal contamination. Our

<sup>1</sup>Presented at the annual meeting of the Food Research Institute, Madison, Wisconsin, March 31 to April 1, 1970.

intention is for all processors of potentially hazardous foods to do the same.

#### MICROBIOLOGICAL CRITERIA

There are, of course, a wide variety of prepared convenience foods in which staphylococci may be a potential hazard. The ideal approach would be to establish microbiological criteria within attainable and reasonable limits which would serve as guidelines or standards for industry and regulatory officials. Unfortunately, the development of such standards is not a simple matter. Before microbiological standards can be set, industries must be surveyed, with samples taken throughout the manufacturing process, and at the same time, correlated with the sanitary conditions in the plants. Consideration must be given to the normal flora of each product and ingredient. Seasonal, regional, and technological factors must also be weighed. Sufficient samples from plants operating under good manufacturing practices must be examined and correlated to establish the capability of each industry. Thus it becomes apparent that development of valid attainable microbial standards is not a simple process accomplished without sound scientific data.

#### *Administrative guidelines*

Within FDA, for internal use of our District administrative officers, we have established some "Administrative Guidelines" with a staphylococcus specification for nine categories of foods encompassing 19 separate products. Seventeen of the 19 products are frozen foods and all are considered convenience items. Integrated into these guidelines are requirements for correlated insanitary factory conditions and practices. The quantitative limits, standing alone, are of limited value and even when evaluated with correlated factory evidence, they serve only to identify those situations and cases which may be submitted with recommendation for regulatory action. We have not published or disseminated these internal guides, for unless their limitations are fully understood, the guidelines could be misleading and amount to a disservice to food processors.

#### *Good manufacturing practices guidelines*

In addition to Administrative Guidelines, the Administration is working toward the development and promulgation of Good Manufacturing Practices (GMP) Guidelines. The "umbrella" or general GMP for food processing has been issued and other GMP's for specific foods are being developed. In some instances an industry may approach the FDA with a request to develop cooperatively with FDA a GMP for the industry, whereas, in other instances, the FDA may initiate such an action independently. An exam-

ple of the former is the GMP for frozen, breaded shrimp and an example of the latter is the GMP for hot, smoked, fish.

Through the mechanisms of plant inspection for adherence to good manufacturing practices and compliance of products with certain microbiological criteria it is intended that improved sanitation practices will become commonplace in food processing and that microbial contamination, including staphylococcal contamination, will be kept to a minimum.

#### PREFORMED TOXIN: HEALTH HAZARD

The second view of staphylococcal contamination has already been mentioned, namely, the threat to health of many persons because of the possible presence of preformed toxin. In the event that preformed toxin is detected in a food, regardless of the number of staphylococci present, the only sensible course is to prevent such a food from reaching the consumer. In those instances in which large numbers of coagulase positive staphylococci are present, but toxin is not detected, we would again take a position that the food should be kept from the consumer because: (a) it may contain one or more, as yet, unidentified enterotoxins and (b) it has been produced under insanitary conditions that are not consistent with good manufacturing practices for the production of human foods.

#### LIMITS FOR STAPHYLOCOCCI

Excessive numbers of staphylococci in foods are inexcusable and enlightened processors are striving to prevent both the entry and multiplication of staphylococci in their products. The initial level of staphylococci in a product is affected by a number of factors. For example, a product that requires hand-picking such as crabmeat or deboned chicken would be expected to contain more staphylococci than one not involving intimate human contact. Raw milk often times contains staphylococci and may serve as a source of these organisms in a food. For these reasons one cannot, in the name of consumer protection, arbitrarily set a general limit for staphylococcal contamination that is applicable to all foods.

Limits when set, must take into account the factor of technical achievability under good manufacturing practices. With this in mind, we recently made operational within FDA the National Center for Microbiological Analysis which is located in Minneapolis. One of the functions of this Center is to obtain base line data on the microbiological quality of categories of foods for which no data or incomplete data exist. Not only are the types and numbers of organisms in a finished product determined, but also, those critical

points in a processing line are identified that contribute significantly to the microbial load. Microbiological data are correlated to manufacturing practices and plants are evaluated in terms of adherence to good manufacturing practices. Through such a process it is anticipated that microbiological guidelines can be established which are scientifically

sound, afford improved consumer protection, are achievable within the technical capabilities of the affected industries, and are consistent with good manufacturing practices. Staphylococci figure prominently in this program and we expect that in the near future limits for these organisms will be established for several additional food categories.

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## NUTRITIONAL ASPECTS OF DAIRY PRODUCTS (Continued from Page 354)

But as prominent research workers have repeatedly emphasized—the exact formula of milk is not known and the nutritional equality cannot therefore be duplicated.

### ACKNOWLEDGMENT

Appreciation is expressed to Dr. Marion F. Brink and Dr.

Elwood W. Speckman—Nutrition Research, National Dairy Council.

### REFERENCES

1. Newer Knowledge of Milk—Third Revised Edition. 1968. National Dairy Council, 111 North Canal St., Chicago, Illinois 60606.
2. Recommended Dietary Allowances, Seventh Revised Edition. 1968. Food and Nutrition Board, National Academy of Sciences, National Research Council.
3. Hoard's Dairyman, May 25, 1969 Issue.

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# NEWS AND EVENTS

## UNIVERSITY OF GEORGIA INSTITUTES DEGREE PROGRAM IN ENVIRONMENTAL HEALTH SCIENCE

Announcement was made recently by Dr. George Parthemos, University of Georgia Vice President for Instruction that a program leading to the B. S. degree in Environmental Health Science will be offered at the University of Georgia beginning in the Fall of 1970. The program was approved by the University Council at the Fall meeting, and by the Board of Regents in January.

The program is inter-departmental and will be in the College of Agriculture. Dr. Henry Garren, Dean and Coordinator of the College announced that Professor H. B. Henderson, Head of the Dairy Science Department will advise students in the new program. Courses in the basic sciences will constitute a considerable portion of the program, but numerous courses will be included in engineering, dairy and food sciences, political science, and veterinary medicine.

In citing the need for this program Professor Henderson said, "The demands of an ever changing environment present the State and the Southeast with a constant need for young people who are academically qualified in the field of environmental science. Local public health departments, the Georgia De-

partment of Public Health, Georgia Department of Agriculture, other governmental agencies, and institutions as well as many private industries are seeking personnel with the type of training this program will afford."

Workers in the field of environmental science were given legal recognition in 1957 by the passage of legislation providing for the examination and licensing of "Registered Professional Sanitarians" by the State Examining Board for Registered Professional Sanitarians. Professor Henderson has served on this Board for a number of years. It is anticipated that the program will involve at least 50 students within three years.

In commenting on the importance of this new program in view of the increasing interest in environmental control, Dr. John H. Venable, Director of the Georgia Department of Public Health, said "In the present emphasis on improving and maintaining a healthy environment, it is extremely gratifying that the State University System has recognized the need for more well trained environmentalists. This is a real service to Georgia."

Mr. Milton Trippe, Environmental Health Training Officer, Health Education and Training Service for the Georgia Department of Public Health was equally emphatic in endorsing the program. "Academic

preparation specifically in the area of the environmental sciences should go a long way toward minimizing the man-power problem that has plagued environmental health programs for years," said Mr. Trippe. He added that the new program in environmental health science at the University will contribute to the duration and effectiveness of orientation training required for new employees in specific program areas. "We are delighted," he said.

**REQUIREMENTS FOR DEGREE**  
**BACHELOR OF SCIENCE IN ENVIRONMENTAL HEALTH**  
**UNIVERSITY OF GEORGIA**  
**FRESHMAN-SOPHOMORE**

HUMANITIES -----TOTAL 20 HOURS  
English Composition (10); Speech (5); Report Writing (Journalism) (5)

SOCIAL SCIENCES -----TOTAL 20 HOURS  
American and Georgia Government (5); Survey of American and Georgia History (5); Principles of Economics (5); Elective (5)

MATHEMATICS AND SCIENCE -----TOTAL 20 HOURS  
College Algebra (5); Trigonometry (5); General Chemistry (10)

COURSES RELATED TO THE MAJOR -----TOTAL 30 HOURS  
Principles of Biology (10); General Physics or Physical Science (5); Zoology or Botany (5); Elementary Statistics (5); Graphics (Drafting) (5)

*Total Academic Hours For Freshman and*

*Sophomore Years -----90 hours*

**JUNIOR AND SENIOR**

Economic Entomology (5); Medical Entomology (5)	10 hours
Introductory Microbiology (5)	5
Dairy Microbiology (5); Microbiology for Sanitarians (5)	10
Food Industries Survey (5); Food Microbiology (5)	15
Environmental Microbiology (5)	15
Principles of Public Administration (5) Public Personnel Administration (5); Government Organization (5)	15
Public Health (Microbiology (5); Epidemiology (5)	10
Organic Chemistry (5)	5
Drainage & Irrigation (3); Soil & Water Technology (5); Buildings & Equipment (3); Functional Planning of Structures (5)	16
Electives from the following	20
Ecology; Educational Psychology; Veterinary Hygiene; Organic Chemistry; Public Speaking; Man and Physical Environment; World Human Geography; Earth Science Survey; English Derivatives; Dairy Chemistry; Introduction to Food Science; Meat Processing; Poultry Processing; Physics; Wood and Masonry Technology; Other science courses with approval of adviser.	

*Total Academic Hours For Junior & Senior Years ----- 106 Hours*

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With a larger bulk tank the dairyman will have the milk cooling and storing capacity to go ahead and enlarge their herd for greater milk production and increased profit. Also benefit from the hauling savings is made possible by every-other-day pick-up. ZERO THRU-THE-WALL bulk milk tanks are available in standard capacities from 600 gallons to 5,000 gallons. Bulk tanks are manufactured in accordance with 3-A Sanitary Standards.

**ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS ANNUAL MEETING**  
**OCTOBER 12-15, 1970**

The Association of Official Analytical Chemists will hold its 84th annual meeting October 12-15, 1970, at the Marriott Motor Hotel, Twin Bridges, Washington, D. C. The latest developments in analytical methodology for many commodities and materials important to agriculture and public health areas will be presented and discussed.

About 1,500 chemists, microbiologists, physicists, and their administrators are expected to attend, representing Federal, State, Provincial, and local government agencies, universities, and industries throughout North America. Over 230 papers will be given on new techniques, methods, and instrumentation for analysis of drugs, feeds, fertilizers, foods, food additives, pesticides, flavors, beverages, contamination of foods, aflatoxins, and related subjects.

A special feature of the meeting will be a symposium on analytical methods for forensic science areas to be held all day Thursday, October 15. Another symposium will also be held on international cooperation in study and standardization of analytical methods. A roundtable discussion of pesticide formulations problems will be held in conjunction with

a scientific session on the subject.

Dr. L. S. Stuart of the U. S. Department of Agriculture will present his AOAC Presidential address on October 12 at the opening session. Other notable speakers will be on the daily program.

A highlight of the meeting will be a banquet on Monday evening, October 12. A special event of the evening will be the presentation of the Harvey W. Wiley Award to a winner for his outstanding contributions to analytical chemistry.

About 35 firms will exhibit the latest laboratory equipment and supplies. Registration will continue from Sunday afternoon, October 11, through Thursday morning, October 15. The registration fee will be \$3.00. Anyone interested is invited to attend. Contact Luther G. Ensminger, Executive Secretary, AOAC, P. O. Box 540, Benjamin Franklin Station, Washington, D. C. 20044.

### NEW SURGE MILK STAMPS KEEP ON PROMOTING MILK

"An easy, attractive way for dairymen to actively promote milk." Those words probably best explain the tremendous response and interest generated by Surge Milk Stamps. Babson Bros. Co. estimates that more than 2,000,000 letters carried the words "Milk . . . Good with Anything," in the last 12 months.

Following-up on this acceptance, Babson Bros. Co. and their network of Surge Dealers have distributed more than 100,000 new 1970 Surge Milk Stamp Folders. The folder promises "Home Run Power" in every glass of milk and the 21 colorful stamps advise "Add Milk Power to Every Meal." The stamps point out that milk is an important part of every meal and a source of energy for today's active sports-minded generation.

In keeping with Babson Bros. Co.'s efforts to help promote milk through advertisements, free posters and milk stamps, the 1970 Surge Milk Stamp Folder is available free from Surge Dealers or by writing to Babson Bros. Co., 2100 So. York Road, Oak Brook, Illinois 60521.

### MULTIPLE FARM INSPECTIONS KEY TOPIC AT PENNSYLVANIA FIELDMEN'S CONFERENCE

SIDNEY E. BARNARD  
*Extension Dairy Specialist*  
*Pennsylvania State University*  
*University Park, Pennsylvania*

Fieldmen are being called upon more and more to do things that make milk more acceptable to the

consumer, James R. Donnan, Board Secretary of Dairylea Cooperative, Inc., told the more than 350 industry representatives attending the 1970 Pennsylvania Dairy Fieldmen's Conference at the Pennsylvania State University in June.

The conference was sponsored by the Pennsylvania State University, Pennsylvania Association of Milk Dealers, Pennsylvania Dairy Sanitarians Association, and the Pennsylvania Department of Agriculture.

Donnan said that regulatory agency inspection through routine farm and plant checks is necessary, but coordination and not duplication is suggested. He recommended that fewer than the seven agencies now inspecting Pennsylvania supplies would reduce costs and make it possible for field personnel to work on the things consumers want outside public health safety. He added that flavor and shelf life are of prime importance.

A three-member panel reviewed "The Fieldman's Position in a Changing Market." Dr. Earl Cook, QC Inc., outlined the commercial laboratory viewpoint. He indicated changes in production, marketing, quality control testing, and inspection have made the fieldman's responsibilities much more complex and diversified.

Dr. Roy F. Davenport, retired Krafcó Director of Field Services, suggested the Interstate Milk Shipper's Program as a solution to the many inspections now required by regulatory and industry representatives. States could then confine their activity within their boundaries and fieldmen would be able to explain the objectives of a uniform inspection program. This should improve the prestige of inspectors, he emphasized.

Mr. Boyd M. Cook, Maryland Cooperative Milk Producers' Director of Field Services, outlined the awards and penalties included in their quality control program. "A quality premium with their monthly check and annual plaques encourage producers to maintain excellent quality records," the Co-op Director said. Producers and haulers must pay for rejected milk—receiving Class II price or less. Quality penalties and inspection fees are assessed to those with continuing poor quality records. He also stressed the increased effort on consumer acceptance, namely flavor and shelf life of dairy products.

Mr. Bernhard Larsen, Chief, Bureau of Foods and Chemistry, Pennsylvania Department of Agriculture, reviewed the revised Pennsylvania milk sanitation regulations and definitions and standards of identity for frozen desserts.

The revised Pennsylvania Abnormal Milk Program was discussed by Mr. George Fouse, Chief, Division of Milk Sanitation of the Pennsylvania Department of Agriculture.



Solutions to water and air pollution problems were suggested by three speakers on the Penn State Co-operative Extension service staff. Larry Click, Extension agricultural engineer, emphasized that septic tanks are not satisfactory for handling dairy farm wastes. Acceptable systems include: liquid manure; tank-storage and spread; and sprinkler-irrigation lagoons.

Construction and operation of lagoons for parlor and milkhouse wastes were discussed by N. Henry Woodling of the Penn State Extension agricultural engineering section. Specifications and operational procedures were provided to all participants.

Dr. Samuel B. Guss, Penn State Extension veterinarian, reviewed dairy cattle health problems caused by air pollution of feeds. Among these are lead and lime dust which gather on forage crops harvested for dairy cattle feed.

The central laboratory at Penn State for testing DHIA samples now has three automatic Milk-O-Testers. Professor Herbert Gilmore of the Extension dairy science section, described procedures for sampling, handling, and testing.

More than 60,000 samples in two-ounce plastic bags are tested each month. Testing and transportation costs are roughly 11 cents per sample.

Donald H. Race, Director of Quality Control, Dairy Lea, reported on problems in collecting quality samples from farm bulk tanks and transporting them to laboratories. "Establishing sampling procedures and training milk haulers reduced unsatisfactory Standard Plate Counts by 50%" he said. Race also stressed the importance of training drivers to evaluate milk for flavor prior to pickup as the key to consumer acceptance.

Milking machine inspection methods were outlined by Stephen Spencer, Penn State Extension dairy specialist. He pointed out that dairy fieldmen should determine the mechanical adequacy of the milking system and whether it is clean. He emphasized advising dairymen on the installation and cleaning of milking systems.

Mr. James Welch, Klenzade Co., stressed the problems of CIP of farm bulk tanks. These problems are operator caused and include not measuring the volume of wash solution and cutting back on the recommended amount of cleaner. He suggested wash water temperatures of 160 F, 10 min circulation time, and proper rinsing to satisfactorily CIP farm tanks with adequate strength solutions.

Objectives of quality control were discussed by Dr. George H. Watrous, Jr., Professor of Food Science and Industry at Penn State. Dr. Watrous said the two reasons for performing quality tests are public health safety and assuring consumers that products

have acceptable flavor and shelf life. Quality tests should be performed only to insure a safe product and stimulate products sales, the dairy specialist noted.

Mr. Richard Weaver, Milk Control District No. 1 sanitarian, received the Outstanding Sanitarian Award for 1970. Banquet speaker James T. De Voss, president of the U. S. Philatelic Society, related stories of counterfeiting stamps.

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### NATIONAL AUTOMATIC MERCHANDISING ASSOCIATION PUBLISHES NEW SAFETY BULLETINS

Copper Poisoning Prevention (a Guide for Post-Mix Soft Drink Equipment Operators) and Microwave Oven Safety are the titles of two recent publications which may be obtained from National Automatic Merchandising Association, 7 South Dearborn St., Chicago, Illinois 60603.

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### SCHEDULES OF 1970-71 CONFERENCES UNIVERSITY OF MARYLAND

26th Annual Dairy Technology Conference—Thursday, November 12, 1970, University of Maryland.

Ice Cream Short Course—January 25 through February 4, 1971, Department of Dairy Science, University of Maryland.

Ice Cream Conference—February 4, 1971, University of Maryland.

Cottage Cheese & Cultured Milk Products Symposium—March 10, 1971, University of Maryland.

For further information contact Wendell S. Arbuckle, Dept. of Dairy Science, U. of Maryland, College Park, Maryland 20742.

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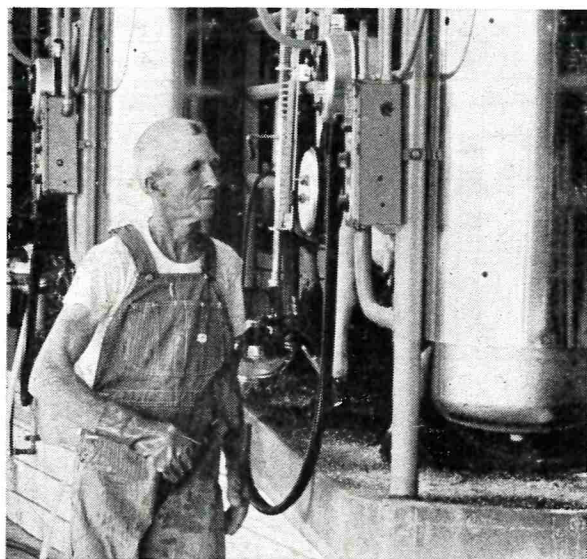
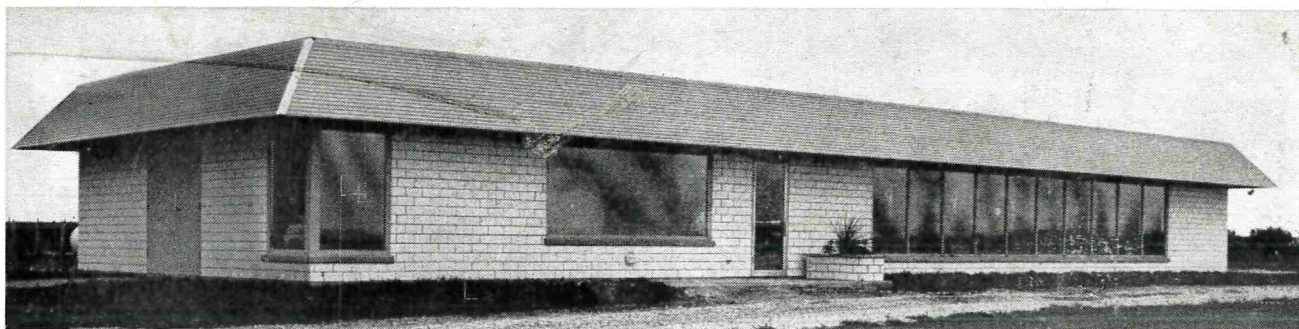
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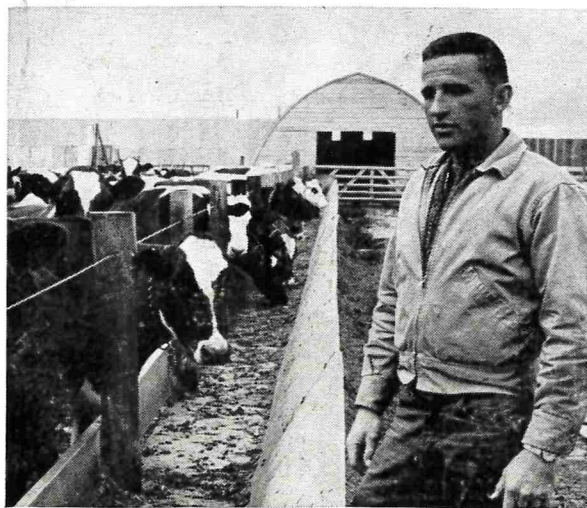
“My son Randi initiated the interest in dairying while he was still in high school,” Walter Vining continues.

“Our goal is to have 200 cows in milk at a 15,000-pound herd average within five years.”

The Vinings’ milking parlor testifies to the determination and optimism with which they have approached dairying. Constructed of tile both inside and out, the building contains almost every available feature . . . plus a few the Vinings developed themselves.

A six-unit picture window design, the parlor features forced air heating from two gas furnaces . . . with air conditioning to be added soon. The emphasis is on labor and time saving equipment such as power operated milking stall gates, Surge Automatic Feed Controls and a 2-inch Tonganoxie Milking System that is automatically cleaned-in-place.

“I think we will be able to handle 200 cows in the new system as easily as we do 85 now,” states Randi Vining. Besides the 15,000-pound average milk production goal, Randi noted another objective: “We hope to have the cleanest and best kept dairy in the state of Iowa.”



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