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Journal of Milk and Food Technology

CALL FOR RESEARCH PAPERS FOR 1975 IAMFES MEETING

Contributed research papers will be an important part of the program at the 1975 Annual Meeting of IAMFES scheduled for August 10-13 at the Royal York Hotel, Toronto, Canada. Abstract forms and complete information about presenting papers can be found in this issue.

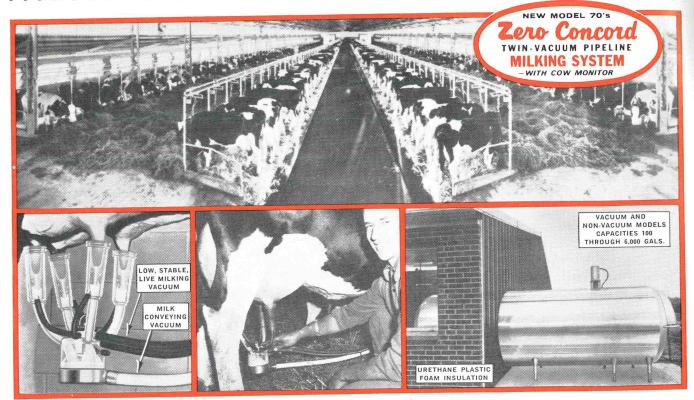
NATIONAL MASTITIS COUNCIL ANNUAL MEETING FEBRUARY 10-12, 1975 (SEE PAGE 1)

Official



Publication

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NATIONAL MASTITIS COUNCIL ANNUAL MEETING

February 10-12, 1975

RADISSON HOTEL – MINNEAPOLIS, MINNESOTA

Everyone interested in prevention and control of bovine mastitis is cordially invited to attend the 14th Annual Meeting of the National Mastitis Council.

Vice President and Program Chairman Burdet Heinemann has planned an outstanding program for this meeting. Subject matter of interest to all segments will be presented.

International authorities from England, Dr. G. C. Brander, Beecham Research Laboratories, and Dr. James M. Booth, Milk Marketing Board, have been secured for the program. Dr. Brander will relate the Somerset mastitis control scheme and report on research results concerning the problem of gram-negative organisms in mastitis control. Dr. Booth will discuss mastitis control in the field and England's mastitis awareness scheme.

Dairy farmers will have an active part in this year's program. L. F. Viney, Arlen Schwinke, Melvin Leppo, and Elbridge Sullivan will present a panel discussion with John B. Adams as moderator. James R. Lefebvre will relate personal experience. John H. Nicolai Jr. will describe what the dairyman expects.

Subjects relating to recent research include: (1) teat dips by W. Nelson Philpot; (2) dry cow therapy by W. D. Schultze; (3) coliform mastitis by Louis Newman.

Reports dealing with somatic cell counts will include: (1) estimates of cells from WMT results by R. D. Mochrie; (2) electronic counting as a screening test by W. E. Ragsdale; (3) use of a mastitis test in DHIA programs by L. H. Schultz.

Dr. Curtis C. Miller, The Upjohn Company, will present considerations in the evaluation of mastitis treatment programs.

Make your plans to attend this excellent meeting. It will start at 8:45 a.m. on February 11 and will adjourn at noon on February 12. Request advance registration form from the National Mastitis Council, 910-17th Street, N. W., Washington, D. C. 20006.

Send request for room reservation directly to the Radisson Hotel, 45 South 7th Street, Minneapolis, Minnesota 55402. Ask for special NMC rates: Single – \$19.00 per day; Twin – \$27.00 per day.

W. Nelson Philpot, President National Mastitis Council

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413 Kellogg Ave., F. O. DOX (01, Ames, A. 50010. 2nd Class postage paid at Ames, Ia. 50010 and additional entry at Shelbyville, Ind. 46176. EDITORIAL OFFICES: Dr. Elmer H. Marth Dept. of Food Science, University of Wisconsin, Madison, Wis. 53706. Earl O. Wright, P. O. Box 701, Ames, Ia. 50010.

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INHIBITION OF BACTERIA BY SOME VOLATILE AND NON-VOLATILE COMPOUNDS ASSOCIATED WITH MILK

II. SALMONELLA TYPHIMURIUM¹

D. C. KULSHRESTHA AND E. H. MARTH Department of Food Science and The Food Research Institute University of Wisconsin-Madison, Madison, Wisconsin 53706

(Received for publication April 19, 1974)

Abstract

Nutrient broth inoculated with Salmonella typhimurium was dispensed into epoxy-lined aerosol cans. Twenty-five volatile compounds were then individually added to the cans to yield or non-volatile compounds were then individually added to the cans to yield final concentrations of 1, 10, 100, and 1000 ppm of each compound. Compounds tested included fatty acids (formic, acetic, butyric, hexanoic, octanoic and decanoic), aldehydes (formaldehyde, acetaldehyde, propionaldehyde, and glyoxal), ketones (acetone, 2-butanone, and diacetyl), amines (propyl and hexylamine), alcohols (furfurol and methanol), sulfur compounds (methylsulfide, methylsulfone, methanethiol, and ethanethiol), acetonitrile, chloroform, ether, and ethylenedichloride. Bacteria were enumerated at intervals during incubation at 37 C.

Shorter chain fatty acids generally inhibited *S. typhimurium* more than did longer chain acids. At 10 ppm formic acid was most effective of those tested and at 1 ppm fatty acids were generally not inhibitory. Formaldehyde and glyoxal were more inhibitory than acetaldehyde and propionaldehyde. Diacetyl was most effective of the three ketones tested. Low concentrations of acetone or 2-butanone sometimes enhanced growth of *S. typhimurium*. Acetonitrile at all concentrations tested significantly inhibited *S. typhimurium* during the terminal stages of incubation. Ether (10 ppm), chloroform (10 ppm), ethylenedichloride (100 ppm), and methylsulfone (100 ppm) generally caused significant reduction in growth of *S. typhimurium*. Ethanethiol was more detrimental to growth of *S. typhimurium* than were methylsulfide or methanethiol; amines were more inhibitory than alcohols.

The importance of salmonellae as human pathogens has been recognized for over 100 years. These bacteria recently received attention in the dairy industry after the recovery, in 1966, of Salmonella newbrunswick from nonfat dry milk (11). If infected carriers of salmonellae handle milk or other dairy products, the bacteria can be passed on to the consumer (2, 12).

Presence of a wide variety of volatile and nonvolatile compounds in milk has been demonstrated by many workers. We listed many of these compounds in an earlier report (7). How these compounds might affect Salmonella typhimurium has received only little attention. Goepfert and Hicks (3) found that formic, acetic, propionic, and butyric acid inhibited S. typhimurium. Other investigators also noted that acetic acid (10, 15) and other fatty acids (1, 4, 6, 7,) inhibited growth of salmonellae. Kulshrestha and Marth (7) used a disc assay procedure and reported that growth of *S. typhimurium* was inhibited by a variety of compounds including aldehydes, fatty acids, amines, and diacetyl. Hedgecock and Jones (5) found that diacetyl inhibited gram-negative rods.

Experiments described in this paper were done to evaluate in some detail how certain volatile or nonvolatile compounds associated with milk affect growth of *S. typhimurium*. A preliminary report of the results has been presented (8).

MATERIALS AND METHODS

Chemicals and procedures used for this study were described earlier (9). The culture of S. *typhimurium* used in this work was obtained from the Department of Bacteriology, University of Wisconsin, Madison. The culture was maintained in brain heart infusion (BHI) agar (Difco) and was transferred twice in nutrient broth (Difco) before being used. Sufficient of a 12- to 16-h old culture was added so inoculated broth contained about 10^3 to 10^4 organisms/ml. Incubation was at 37 C.

RESULTS AND DISCUSSION

Results obtained when fatty acids were tested are given in Tables 1 and 2. At 1000 ppm all fatty acids, except acetic, were so detrimental to S. typhimurium that the organism was inactivated in less than 2 h. Acetic acid needed from 2 to 5 h to cause a similar effect. At 100 ppm all fatty acids regularly caused a significant reduction in growth of S. typhimurium. At 10 ppm acetic and butyric acid usually were significantly inhibitory to the organism, whereas the other acids always caused significant inhibition. Formic acid, at 10 ppm, was most inhibitory of the acids at that concentration and caused up to a 12% reduction in growth of S. typhimurium. When 1 ppm of fatty acids was used, generally slight inhibition was noted, which was rarely significant. In general, inhibitory activity of the fatty acids was greatest during the initial stages of incubation and then tended to decline. Short chain rather than long chain fatty acids were generally more inhibitory.

Of the compounds listed in Table 3 formaldehyde was most inhibitory to S. typhimurium and it was

¹Research supported by the College of Agricultural and Life Sciences, University of Wisconsin-Madison.

		Differenc	es (%) from contro	l in log of population	after hours of incuba	tion
Chemical	Conc. (ppm)	2	5	8	11	14 *
Formic acid ¹	1	(-) 1.5	(-) 1.1*	(-) 1.4*	2	(-) 1.1*
	10	(−) 2.3*	(−) 2.3*	(-)12.6*	(-) 5.2*	(−) 4.00*
	100	(-) 5.5*	(−) 9.1*	(-)12.1*	$(-) 6.7^*$	(-) 5.5*
	1000	NG ^{3*}	NG*	NG*	NG*	NG*
Acetic acid ¹	1	(-) 1.3	(-) 0.5	(-) 1.0	·	(-) 0.6*
	10	(−) 3.8 [*]	(−) 2.1*	(-) 2.3*	(-) 0.9	$(-) 1.2^*$
	100	$(-)11.1^{*}$	(-) 5.1*	$(-)15.7^*$	(-) 6.9*	(-) 5.8*
	1000	(-)82.4*	NG*	NG*	NG*	NG*
Butyric acid ¹	1	(-) 0.8	(-) 0.2	(-) 0.8	(-) 0.1	(-) 0.2
100 d • 0 • 96 96000	10	$(-) 1.5^*$	(-) 1.0	$(-) 1.3^*$	(-) 2.2*	(−) 1.7*
	100	(-) 3.8*	(-) 5.1*	(-)14.7*	(-) 7.5*	(-) 6.2*
	1000	NG*	NG*	NG*	NG*	NG*

TABLE 1. DIFFERENCES IN POPULATION OF Salmonella typhimurium IN NUTRIENT BROTH CAUSED BY ADDED FORMIC, ACETIC, AND BUTYRIC ACID

¹Control: Log of no./ml: 3.98, 6.13, 7.94, 8.12, and 8.25 at 2, 5, 8, 11, and 14 h, respectively.

²-: No difference.

³NG: Less than 10 organisms/ml of test liquid.

*: Population significantly different from control at 5% level.

TABLE 2.	DIFFERENCES IN POPULATION OF	Salmonella typhimurium	IN NUTRIENT BROTH	CAUSED BY ADDED	HEXANOIC, OCTANOIC,				
AND DECANOIC ACID									

		Differences (%) from control in log of population after hours of incubation						
Chemical	Conc. (ppm)	2	5	8	11	14		
Hexanoic acid ¹	1	(-) 0.7	(-) 0.5	(-) 0.5	(-) 2.1*	2		
	10	(−) 2.0*	(−) 1.4*	(-) 1.5*	(-) 3.3*	(-) 2.3*		
	100	(-) 3.5*	(-) 4.2*	(-) 3.2*	(-) 4.4*	(-) 4.6*		
	1000	NG ^{3*}	NG*	NG*	NG*	NG*		
Octanoic acid ¹	1	(-) 0.5	(-) 0.2	(-) 0.1	(-) 0.9*	(-) 0.1		
	10	(−) 3.2 *	(-) 2.6*	(-) 0.9*	(−) 3.1 *	$(-) 1.1^*$		
	100	$(-) 6.7^*$	(-) 3.7*	(-) 2.8*	(-) 3.9*	(−) 3.9 [*]		
	1000	NG*	NG*	NG*	NG*	NG*		
Decanoic acid ¹	1	(-) 0.3	(−) 1.0 *	(-) 0.8	(−) 1.8 *	(-) 0.5*		
	10	$(-) 3.7^{*}$	$(-) 2.4^{*}$	(-) 1.3*	(-) 3.8*	$(-) 1.3^*$		
	100	(-) 7.4*	(-) 4.5*	(-) 3.0*	(-) 4.9*	(-) 4.9*		
	1000	NG*	NG*	NG*	NG*	NG*		

'Control: Log of no./ml: 4.03, 6.26, 7.91, 8.16, and 8.30 at 2, 5, 8, 11, and 14 h, respectively.

²-: No difference. ³NG: Less than 10 organisms/ml of test liquid. *: Population significantly different from control at 5% level.

followed in order by acetaldehyde and propionaldehyde. Formaldehyde at 1000 ppm inactivated the organism in less than 2 h, and at 100 ppm virtually complete inactivation of the organism occurred in about 8 h. At 10 ppm inhibition by formaldehyde was always significant, but with 1 ppm of this compound inhibition was slight and was significant only in the terminal stages of incubation. When 1000 ppm of acetaldehyde or propionaldehyde were used, substantial inhibition was noted. At 100 ppm of both compounds significant inhibition occurred throughout the incubation period. Propionaldehyde at 10 ppm also was significantly inhibitory to S. typhi*murium* except early during the incubation; generally a reversed trend was noted with acetaldehyde at this concentration. Slight but generally insignificant inhibition occurred when 1 ppm of acetaldehyde or propionaldehyde were tested; and an insignificant increase in growth also was noted at 8 h with 1 or 10 ppm of acetaldehyde.

Table 4 lists results obtained when acetone, 2butanone, or diacetyl were tested. Diacetyl was most detrimental to the growth of S. typhimurium and the highest concentration (1000 ppm) of this chemical inactivated S. typhimurium in less than 2 h. Diacetyl at 100 ppm inhibited growth by more than 33%. Incubation for 11 to 14 h was accompanied by significant inhibition with 10 ppm of diacetyl. The lowest concentration (1 ppm) of this compound resulted in a slight but insignificant increase in numbers of S. typhimurium at 2 h, but during the later stages of incubation slight inhibition was noted. When 1000 ppm of acetone or 2-butanone were used, the numbers of organisms in treated samples always were significantly lower than in the control. 2-Butanone and acetone at 100 ppm caused some inhibition of S. typhimurium which was insignificant during the early stages of incubation. Acetone at 1 ppm and 2butanone at 1 and 10 ppm appeared to enhance growth initially during the incubation, but this effect was insignificant. Later in the incubation both acetone and 2-butanone at 1 or 10 ppm generally caused limited and inconsistently significant inhibition of growth. growth of S. typhimurium is shown by data in Table 5. Data for acetonitrile indicate that slight but always significant inhibition was caused by 1000 ppm. Lower concentration (1, 10, or 100 ppm) of this compound were less inhibitory. Such inhibition generally was not significant early during the incubation, but acetonitrile at all concentrations reduced growth significantly toward the end of the growth period. Chloroform and ether had nearly comparable effects. Inhibition by these compounds at 1000 ppm always was significant. At lower concentrations (10 and 100 ppm) both chemicals were substantially less effective but still significantly inhibitory, especially during the later stages of incubation. Ether at 1 ppm was without significant effect during the entire incubation

How acetonitrile, chloroform, and ether affected

TABLE 3. DIFFERENCES IN POPULATION OF Salmonella typhimurium IN NUTRIENT BROTH CAUSED BY ADDED FORMALDEHYDE, ACETALDEHYDE, AND PROPIONALDEHYDE

		Diffe	rences (%) from co	ntrol in log of populat	ion after hours of in-	cubation
Chemical	Conc. (ppm)	2	5	8	11	14
Formaldehyde ¹	1	(-) 0.5	(-) 0.9	(-) 0.5	(-) 0.2	(_) 1.1*
r ormaniceny ac	10	(-) 5.6*	(−)12.8 [*]	(_) 8.0*	(−) 3.7 *	(—) 2.3°
	100	(-)32.1*	(−)64.3 [*]	NG ^{2*}	NG*	NG*
	1000	NG*	NG*	NG*	NG*	NG*
Acetaldehyde ¹	1	3	(-) 0.2	(+) 0.1	(-) 0.1	(+) 0.1
	10	$(-) 3.2^*$	$(-) 1.0^*$	(+) 0.4	(−) 1.0 *	
	100	$(-) 6.1^*$	$(-)11.3^{*}$	(_) 9.4*	(-) 8.1*	(_) 3.4*
	1000	(−)24.7 °	(-)52.2*	(-)66.0*	(-)69.7*	(-)72.3*
Propionaldehyde ¹	1	(-) 0.3	(−) 2.6 [*]	(_) 0.1	(-) 0.1	(_) 0.5*
ropionalicon, ee	10	(-) 1.3	(-) 4.5°	(-) 1.8*	(−) 1.1 °	(_) 1.1*
	100	(-) 3.5*	$(-)12.5^{\circ}$	(_) 5.9*	(−) 3.9 *	(_) 2.1*
	1000	$(-)23.3^*$	(-)51.3*	(-)66.4*	(-)67.9*	(_)68.1*

¹Control: Log of no./ml: 3.77, 5.77, 7.67, 8.28, and 8.45 at 2, 5, 8, 11, and 14 h, respectively.

³NG: Less than 10 organisms/ml of test liquid. ³-: No difference.

*: Population significantly different from control at 5% level.

TABLE 4. DIFFERENCES IN POPULATION OF Salmonella typhimurium in nutrient broth caused by added acetone, 2-butanone, AND DIACETYL

		Diffe	erences (%) from con	ntrol in log of populat	tion after hours of inc	cubation
Chemical	Conc. (ppm)	2	5	8	11	14
Acetone ¹	1	(+) 0.3	2	(-) 0.7	(+) 0.1	(-) 0.6
	10	(-) 0.5	(-) 1.8*	(-) 0.4	(-) 0.3	(_) 1.8°
	100	(-) 0.8	(-) 4.9*	(-) 0.8	(_) 1.6*	(—) 2.3 °
	1000	(-) 1.6*	(-) 6.6*	(-) 6.4*	(-) 4.1*	(-) 5.1*
2-butanone ¹	1	(+) 0.8	(+) 0.2	(-) 0.5	(-) 0.3	(-) 0.1
	10	(+) 0.5	(_) 1.6	(-) 1.6	(_) 0.6*	$(-) 2.2^*$
	100	(-) 1.3	(-) 3.0*	(−) 5.6 *	(_) 3.3*	(-) 3.1*
	1000	(-) 1.6*	(-) 3.9*	(-) 8.3*	(-) 6.5*	(-) 8.7*
Diacetyl ¹	1	(+) 0.3	(-) 0.8*	(-) 0.4	(_) 0.7*	(-) 0.1
Diacotyr	10	(-) 1.3	$(-) 2.6^*$	(-) 0.8	(_) 1.7*	(-) 2.4*
4	100	$(-)19.7^{*}$	$(-)33.2^*$	(-)33.9*	(-)23.3 *	(-)17.3*
	1000	NG ^{3*}	NG*	NG*	NG*	NG*

¹Control: Log of no./ml: 3.76, 6.11, 7.69, 8.11, and 8.23 at 2, 5, 8, 11, and 14 h, respectively.

²—: No difference.
³NG: Less than 10 organisms/ml of test liquid.

*: Population significantly different from control at 5% level.

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		Diffe	erences (%) from co	ontrol in log of populati	ion after hours of inc	ubation
Chemical	Conc. (ppm)	2	5	8	11	14
rt.			() 07	(+) 01	()06	(-) 0.8*
Acetonitrile ¹	1	<u>_</u> ²	(_) 0.7	(+) 0.1	(-) 0.6	
	10	(_) 0.5	(-) 1.3	(_) 0.4	$(-) 1.7^*$	(-) 1.8*
	100	(-) 1.9	(−) 1.3*	(-) 1.1	(-) 2.3*	(−) 2.2*
	1000	(_) 3.5*	(-) 5.2*	(-) 2.5*	(-) 2.9*	(-) 5.1*
Chloroform ¹	1	(+) 1.1	(+) 0.7	(+) 0.3	(-) 0.5	(-) 0.4*
	10	(-) 0.8	$(-) 1.8^*$	(-) 0.5	(−) 2.1 [*]	$(-) 1.5^{\circ}$
	100	(-) 1.6	(-) 3.2*	(-) 1.6	$(-) 2.7^*$	(−) 2.5 [*]
	1000	(_) 6.4*	(_) 6.1*	(-) 3.0*	(-) 3.4*	(-) 5.6*
Ether ¹	1	(+) 2.1	(-) 0.5	(+) 0.1	_	
	10	(-) 0.3	$(-) 2.4^*$	(+) 0.3	$(-) 1.2^*$	(-) 0.7*
	100	(-) 1.1	$(-) 3.7^*$	(-) 1.4	(−) 2.4 [*]	(-) 3.1*
	1000	(-) 2.7*	(-) 7.1*	(_) 3.0*	(-) 3.6*	(-) 5.8*

TABLE 5. DIFFERENCES IN POPULATION OF Salmonella typhimrium in nutrient broth caused by added acetonitrile, chloroform, and ether

'Control: Log of no./ml: 3.76, 5.95, 7.64, 8.25, and 8.47 at 2, 5, 8, 11, and 14 h, respectively.

²—: No difference.

*: Population significantly different from control at 5% level.

TABLE 6. DIFFERENCES IN POPULATION OF Salmonella typhimurium in nutrient broth caused by added glyoxal, ethyl-Lenedichloride, and methylsulfone

		Diffe	erences (%) from co	ntrol in log of populat	tion after hours of in	cubation
Chemical	Conc. (ppm)	2	5	8	11	14
Glyoxal ¹	1	(-) 0.3	(-) 0.5	(-) 1.2*	(_) 0.1	(-) 0.2
	10	$(-) 5.7^{*}$	(_) 4.5*	(-) 2.3*	(_) 1.7*	(_) 0.8*
	100	(-) 9.1*	(-) 5.5*	(-) 7.3*	(_) 4.7*	(_) 2.2♥
	1000	NG ^{2*}	NG*	NG*	NG*	NG*
Ethylenedichloride ¹	1	(+) 0.5	(+) 0.2	(-) 0.6	(+) 0.1	(+) 0.1
	10	(-) 0.3	(-) 0.7	(-) 1.9	(_) 0.5*	_3
	100	(-) 3.7*	(-) 4.2*	(-) 2.5*	(-) 1.0*	(_) 0.7*
	1000	(-)17.8*	(_) 9.9*	(-) 3.7*	(-) 1.7*	(-) 1.6*
Methylsulfone ¹	1	_	(-) 0.3	(-) 0.3	_	(+) 0.2
inotity is direction	10	(-) 1.8	$(-) 2.2^*$	(-) 0.6	(-) 0.7*	(-) 0.2
	100	(-) 6.0*	(-) 4.7*	$(-) 1.8^*$	$(-) 1.7^{*}$	$(-) 1.6^*$
	1000	(_) 7.8*	(-) 6.7*	(-) 3.6*	(-) 2.3*	(-) 2.8*

¹Control: Log of no./ml: 3.83, 5.99, 7.77, 8.22, and 8.36 at 2, 5, 8, 11, and 14 h, respectively.

²NG: Less than 10 organisms/ml of test liquid.

³-: No difference.

*: Population significantly different from control at 5% level.

period. Insignificant stimulation was noted for up to 8 h when 1 ppm of chloroform was used. Later this turned to slight but significant inhibition.

Glyoxal was quite detrimental to S. *typhimurium* as is evident from data in Table 6. At 1000 ppm the organism was inactivated in less than 2 h. At 10 and 100 ppm inhibition was usually greatest early during the incubation, but was significant during the entire observation period. Only marginal and generally insignificant reduction in growth was caused by 1 ppm of glyoxal. Ethylenedichloride at 100 and 1000 ppm caused a gradually declining (during incubation) but always significant inhibition. Consistently insignificant effects were obtained with 1 and 10 ppm of this compound. Methylsulfone at 1000 ppm tended to be less effective than ethylenedichloride. Inhibition by 100 or 1000 ppm of methylsulfone was minimal but always significant. Effects were generally insignificant when 1 or 10 ppm of this compound were present.

Results obtained when methylsulfide, methanethiol, and ethanethiol were tested against *S. typhimurium* are given in Table 7. Ethanethiol was the most inhibitory of the three compounds. With 1000 ppm of ethanethiol, reduction in number of viable cells exceeded 57% at 8 h, and then declined. Less inhibition was noted with 10 or 100 ppm of this compound. Greatest inhibition at several concentrations occurred at 5 h. The inhibitory effect by ethanethiol was always significant when 10, 100, or 1000 ppm were present. At 1 ppm marginal and generally insignificant inhibition was noted. Methanethiol was slightly less effective than ethanethiol but was more inhibitory than methylsulfide. With 1000 ppm of methanethiol, inhibition became more pronounced as the incubation progressed, whereas with 10 or 100 ppm of this compound, inhibition was greatest at 5 h. As with ethanethiol, methanethiol always was significantly inhibitory at the higher concentration (10, 100, and 1000 ppm). Methylsulfide at 1000 ppm was significantly inhibitory only after 5 h and was inconsistently detri-

mental at 100 ppm. At lower concentrations (1 and 10 ppm) methylsulfide never had any significant effect on growth of S. typhimurium. Moderate but insignificant stimulation was noted when 1 ppm of this compound was tested.

Results obtained with alcohols and amines are listed in Table 8. Amines were more inhibitory to S. typhimurium than were alcohols. Hexylamine at 1000 ppm inactivated the organism in less than 2 h, whereas 5 h were needed for propylamine to do the same. Inhibition by both amines, at all concentrations tested was greatest early during the incubation

TABLE 7. DIFFERENCE IN POPULATION OF Salmonella typhimurium in nutrient broth caused by added methylsulfide, METHANETHIOL, AND ETHANETHIOL

		Diffe	erences (%) from co	ontrol in log of populat	ion after hours of ine	cubation
Chemical	Conc. (ppm)	2	5	8	11	14
Methylsulfide ¹	1 10 100 1000	$(-) 0.4 \\ -^2 \\ (-) 2.1 \\ (-) 4.6$	(+) 0.6 (-) 0.4 $(-) 3.5^{\circ}$ $(-)10.8^{\circ}$	(+) 1.2 (+) 0.2 (-) 1.2 (-) 5.0*	(+) 0.8 (-) 0.1 (-) 0.6° (-) 2.7°	(+) 0.1 (-) 0.4 (-) 1.5° (-) 1.8°
Methanethiol ¹	1 10 100 1000	(-) 2.1 (-) 5.7* (-) 8.5° (-)14.5°	(-) 2.7* (-) 6.8* (-)18.2* (-)26.3*	(-) 1.3 (-) 4.0° (-)18.1° (-)44.9°	(-) 0.1 (-) 2.6° (-) 7.8° (-)52.4°	(-) 0.2 (-) 1.1* (-) 2.3* (-)54.9*
Ethanethiol ¹	1 10 100 1000	(-) 1.1 (-) 7.1° (-) 9.9° (-)17.7°	(-) 1.9° (-) 6.6° (-)18.2° (-)56.9°	(-) 0.4 (-) 3.9° (-)12.3° (-)57.8°	(-) 0.3 (-) 4.7° (-) 9.7° (-)48.8°	(-) 0.4 $(-) 0.6^{\circ}$ $(-) 1.0^{\circ}$ $(-)35.2^{\circ}$

¹Control: Log of no./ml: 2.82, 4.83, 6.75, 7.81, and 8.23 at 2, 5, 8, 11, and 14 h, respectively.

²-: No difference.

*: Population significantly different from control at 5% level.

TABLE 8.	DIFFERENCES IN POPULATION OF Salmonella typhimurium IN NUTRIENT BROTH	CAUSED	BY	ADDED	FURFURYL	AND
4	METHYL ALCOHOL AND PROPYL- AND HEXYLAMINE					

		Differences (%) from control in log of population after hours of incubation					
Chemical	Conc. (ppm)	2	5	8	11	14	
Furfuryl alcohol ¹	1	(-) 0.7	(-) 1.0*	(-) 0.4	(-) 0.6	(+) 0.3	
	10	(-) 4.4*	(−) 3.1 [*]	(-) 0.5	(-) 1.8*	_2	
	100	(-) 5.4*	(−) 7.8 [*]	(-) 1.3*	(−) 3.4 *	(_) 1.9*	
	1000	(-) 8.0*	(-) 9.5*	(-) 1.8*	(-) 4.4°	(_) 3.4*	
Methyl alcohol ¹	1	(-) 0.5	(-) 2.3*	(-) 0.1	(-) 0.5	(+) 0.1	
	10	(-) 1.5	(-) 3.9*	-	$(-) 1.1^*$	(_) 0.1	
	100	(-) 4.1*	(-) 6.8*	(-) 2.0*	(−) 3.0 *	(_) 1.6°	
	1000	(-) 7.5*	(-) 8.5*	(-) 1.8*	(-) 3.6*	(_) 2.8*	
Propylamine ¹	1	(-) 8.0*	(−) 5.1 °	(-) 1.6	(-) 0.6	() 0.4	
17	10	(-)10.7*	(-) 6.5*	(-) 1.3	(-) 2.1*	(-) 1.1°	
	100	(-)13.1*	(-) 7.3*	(-) 5.2*	(-) 3.8*	(_) 1.9*	
	1000	(-)55.0*	NG ³ *	NG*	NG*	NG*	
Hexylamine ¹	1	(−) 3.2 *	(-) 3.3*	_	(-) 0.5	÷	
	10	(-) 7.1*	(-) 4.4°	(-) 0.5	(-) 2.0*	(_) 0.6	
<u>)</u>	100	(-) 7.8*	(-) 7.7*	(-) 4.6*	(_) 3.3*	(-) 1.5°	
	1000	NG*	NG*	NG*	NG*	NG*	

¹Control: Log of no./ml: 4.11, 6.14, 7.63, 7.97, and 8.05 at 2, 5, 8, 11, and 14 h, respectively.

²-: No difference. ³NG: Less than 10 organisms/ml of test liquid.

*: Population significantly different from control at 5% level.

and gradually declined as time progressed. At 10, 100, and 1000 ppm amines almost always were significantly inhibitory, but at 1 ppm significant inhibition was noted only up to 5 h of incubation. Alcohols (furfurol and methanol) at 100 and 1000 ppm also always caused significant inhibition. Only minimal and inconsistently significant inhibition was caused by lower concentrations (1 and 10 ppm) of alcohols. A small but insignificant increase in numbers of the organism was noted at 14 h when 1 ppm of either alcohol was used.

All chemicals tested were most detrimental to S. *typhimurium* at the higher concentrations. At lower concentrations only some compounds were significantly inhibitory. How these compounds might effect survival and/or growth of S. *typhimurium* in milk or milk products is more difficult to predict from these findings, because milk is a very complex system in which inhibition by some compounds may be balanced by stimulation from others. This could be why Park et al. (13, 14) observed that S. *typhimurium* survived in low-acid Cheddar cheese and in cold pack cheese food for long periods even though these foods could be expected to contain some of these compounds in sufficient amounts to be inhibitory to S. *typhimurium.*

Inhibition of *S. typhimurium* by some of these compounds, especially fatty acids and amines, suggests they may be of benefit to control this organism. Many foods contain fatty acids and amines at levels found to be inhibitory to *S. typhimurium*. Whether these compounds affect growth of this organism in such foods needs to be investigated.

Most of the compounds tested, other than fatty acids and amines, are present in milk in such small quantities that they probably would not be inhibitory individually, but the combined effect of the compounds, although unknown, might influence the growth of some bacteria.

Our findings confirm earlier reports (1, 3, 6) that an increase in chain length resulted in a decrease in the inhibitory activity of fatty acids. Only minor differences were noted when these results were compared with those obtained when we tested *Escherichia coli* (9). Of six fatty acids tested at 1000 ppm, acetic acid was least effective against S. *typhimurium*, but not against *E. coli*; in this instance, it was hexanoic acid. Acetaldehyde, propionaldehyde, ethanethiol, and alcohols were generally less inhibitory to *S. typhimurium* than to *E. coli*, whereas glyoxal was more detrimental to *S. typhimurium* than to *E. coli* (9).

Addendum

The reader is referred to the Addendum to the first paper in this series (*Escherichia coli*) (9) for information on the boiling point and solubility in water of all test chemicals. "The initial pH of nutrient broth that contained the various amounts of the chemicals also is given. The low (3.6-5.5) pH values caused by 1000 ppm of formic, acetic, butyric, hexanoic, octanoic, and decanoic acid, and the high (8.9-9.3) pH values obtained with 1000 ppm of propylamine and hexylamine undoubtedly contributed to the antibacterial property of these chemicals at the highest concentration that was tested.

When the reduction in population of *S. typhimurium* was 22% or greater, a given chemical at the appropriate concentration was bactericidal. A reduction in population of 0.6-15.7% represents bacteriostatic action by the chemical.

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INHIBITION OF BACTERIA BY SOME VOLATILE AND NON-VOLATILE COMPOUNDS ASSOCIATED WITH MILK

III. STAPHYLOCOCCUS AUREUS¹

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Abstract

Nutrient broth inoculated with Staphylococcus aureus was dispensed into aerosol cans with an epoxy lining. Twentyfive volatile or non-volatile compounds were individually added to these cans to yield final concentrations of 1, 10, 100, and 1000 ppm of each compound. The compounds used included fatty acids (formic, acetic, butyric, hexanoic, octanoic, and decanoic), aldehydes (formaldehyde, acetaldehyde, propionaldehyde, and glyoxal), ketones (acetone, 2-butanone, and diacetyl, amines (propyl- and hexylamine), alcohols (furfurol and methanol), sulfur compounds (methylsulfide, methylsulfone, methanethiol, and ethanethiol), acetonitrile, chloroform, ether, and ethylenedichloride. Bacteria were enumerated at intervals during incubation at 37 C.

At 1000 ppm butyric acid was least inhibitory of the fatty acids, but at 10 ppm, butyric, octanoic, and decanoic acid were more effective against *S. aureus* than were the other fatty acids. Formaldehyde was most inhibitory of the aldehydes tested, whereas propionaldehyde was least effective. Diacetyl was most detrimental of the ketones tested. Chloroform at 1000 ppm inactivated *S. aureus*. Acetonitrile, ether, ethylenedichloride, and methylsulfone were significantly inhibitory to *S. aureus* only at higher concentrations. Lower concentrations of methylsulfide, methanethiol, and ethanethiol sometimes significantly enhanced growth of *S. aureus*. Amines were more inhibitory to *S. aureus* than were the alcohols.

Staphylococcus aureus is widely recognized as a food poisoning agent. Outbreaks of foodborne illness caused by this organism have been associated with milk and some milk products (14) and other foods (15). Raw and heated milks contain many volatile and other compounds (7); but how these compounds affect growth of S. aureus has received only little attention. Kodicek and Worden (6) reported that 8 µg of fatty acids/ml, particularly of linolenic and linoleic acid, were inhibitory to S. aureus. Acetic acid (11, 12, 13) and other fatty acids (4, 7, 16, 17, 18) also have been reported to retard growth of this bacterium. Kulshrestha and Marth (7) used the disc assay procedure to test 27 volatile compounds and found that fatty acids, aldehydes, amines, and diacetyl were inhibitory to S. aureus. Hedgecock and Jones (3) also noted that diacetyl inhibited gram-positive cocci. It is well recognized (1, 2, 5) that S. aureus is inhibited

by some actively growing food starter cultures.

This paper describes a detailed evaluation of the effect of some volatile or non-volatile compounds associated with milk on growth of *S. aureus*. Results were obtained by confining the bacterium and the chemicals in an air tight vessel. A preliminary report of some of these results has been presented (8).

MATERIALS AND METHODS

Chemicals and procedures used for this study were the same as described earlier (9). The culture of S. aureus 100 used in this work was obtained from K. F. Weiss, formerly of the Food Research Institute, University of Wisconsin, Madison. The culture was maintained on brain heart infusion (BHI) agar (Difco), and was transferred twice in nutrient broth (Difco) before it was used in experiments. A 12- to 16-h old culture was used to inoculate nutrient broth (Difco) before it was dispensed into cans. All incubations were at 37 C.

RESULTS AND DISCUSSION

Tables 1 and 2 summarize results obtained when six fatty acids were tested. At 1000 ppm formic, hexanoic, and decanoic acid virtually inactivated S. aureus in less than 2 h. When octanoic or acetic acid was used a similar effect occurred at 5 and 8 h, respectively. Butyric acid at this concentration caused a marked increase in inhibition of S. aureus as the incubation progressed but the organism was not inactivated after 14-h. Substantial inhibition of S. aureus was noted when 100 ppm of the different fatty acids were used. Generally more inhibition appeared in the later stages of incubation. Acetic acid (10 ppm) and hexanoic acid (10 ppm) always caused a significant reduction in growth of S. aureus as compared to the control. At the same concentration other fatty acids also were inhibitory but not always significantly. Inhibition was generally not significant when 1 ppm of fatty acid was used. Butyric, octanoic, and decanoic acid were more inhibitory than were the other fatty acids when they were tested at the 10 ppm concentration. At this concentration hexanoic acid was least inhibitory.

Results obtained when different concentrations of formaldehyde, acetaldehyde, or propionaldehyde were

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		Difference	s (%) from control i	fter hours of incubat	tion	
Chemical	Conc. (ppm)	2	5	8	11	14 ,
Formic acid ¹	1	(-) 3.8*	4	(-) 0.8	(−) 1.2 *	(-) 1.1
Forme actu	10	$(-) 6.2^*$	(-) 0.6	(-) 1.0	$(-) 2.5^*$	(-) 3.7*
	100	$(-) 5.6^{*}$	$(-) 3.4^*$	(-) 6.3*	(-) 4.4*	(-) 5.8*
	1000	NG5*	NG*	NG*	NG*	NG*
Acetic acid ²	1000	(+) 0.6	(-) 0.7	(-) 0.2	(-) 0.4	(-) 0.4
Acetic acid	10	(-) 5.3*	$(-) 6.4^*$	(-) 5.3*	(-) 5.3*	(-) 4.1*
	100	$(-)13.7^*$	(-)14.2*	$(-)17.4^{*}$	(-)23.3*	(-)12.8*
	1000	$(-)65.5^*$	$(-)63.5^{*}$	NG*	NG*	NG*
Butyric acid³	1	(-) 0.3	(-) 0.8		(-) 0.2	(-) 0.2
Dutylic dela	10	(-) 1.2*	(-) 3.1*	(-) 0.2	(-) 2.4*	$(-)12.2^{*}$
	100	(-) 3.0*	(-) 9.7*	(-) 5.2*	(-)20.6*	$(-)16.4^*$
	1000	$(-)14.6^{*}$	(-)28.0*	(-)36.2*	$(-)55.1^*$	(-)62.6*

TABLE 1.	DIFFERENCES IN POPULATION OF Staphylococcus aur	CUS IN NUTRIENT BROTH
	CAUSED BY ADDED FORMIC, ACETIC, AND BUTYR	IC ACID

¹Control: Log of no./ml: 3.40, 3.53, 3.99, 5.18, and 5.66 at 2, 5, 8, 11, and 14 h, respectively. ²Control: Log of no./ml: 3.42, 4.52, 5.63, 6.75, and 7.13 at 2, 5, 8, 11, and 14 h, respectively. ³Control: Log of no./ml: 3.30, 3.92, 4.22, 5.43, and 6.09 at 2, 5, 8, 11, and 14 h, respectively. ⁴-: No difference. ⁵NG: Less than 10 organisms/ml of test liquid. ^{*}: Population significantly different from control at 5% level.

TABLE 2.	DIFFERENCES IN POPULATION OF Staphylococcus aureus in nutrient broth
	CAUSED BY ADDED HEXANOIC, OCTANOIC, AND DECANOIC ACID

0

		Difference	es (%) from control	in log of population a	after hours of incuba	ition
Chemical	Conc. (ppm)	2	5	8	11	14
Hexanoic acid ¹	1	(+) 0.3	(-) 0.5	(-) 0.2	3	
Tionumore word	10	(-) 1.8*	$(-) 2.1^*$	(-) 2.7*	$(-) 1.7^*$	(_) 3.3*
	100	$(-)11.1^*$	$(-) 6.6^*$	(−) 4.4*	$(-)$ 6.1^*	(_) 7.2*
	1000	NG ^{4*}	NG*	NG*	NG*	NG*
Octanoic acid ²	1	(+) 0.3	(-) 1.0	(+) 0.2	(-) 0.2	-
octanoio acia	10	(-) 0.3	(-) 2.8*	(_) 0.7	(-) 1.5*	(_)10.3*
	100	(-) 5.5*	(-) 5.9*	(-) 6.2*	(-)21.4*	(_)18.7*
	1000	(-)69.7*	NG*	NG*	NG*	NG*
Decanoic acid ²	1	· · ·	(-) 2.3	(+) 0.2	(-) 0.2	(+) 0.3
Decanore usia	10	$(-) 1.8^*$	(-) 5.1*	_	(-) 1.8*	(_)13.1*
	100	() 8.5*	(-) 8.9*	(-) 6.9*	(-)22.7*	(_)24.1*
	1000	NG*	NG*	NG*	NG*	NG*

¹Control: Log of no./ml: 3.32, 3.77, 4.11, 5.43, and 6.08 at 2, 5, 8, 11, and 14 h, respectively. ²Control: Log of no./ml: 3.30, 3.92, 4.22, 5.43, and 6.09 at 2, 5, 8, 11, and 14 h, respectively. ³—: No difference. ⁴NG: Less than 10 organisms/ml of test liquid. ^{*}: Population significantly different from control at 5% level.

TABLE	3.	Diff	FERI	ENCES	IN	POPULATION	OF	Staphylococcu	is at	ireus	IN	NUTRIENT	Г BR	OTH	C
								CETALDEHYDE,							
												100 m 100	200	1.000	

		Differenc	es (%) from control	in log of population af	ter hours of incubat	tion
Chemical	Conc. (ppm)	2	5	8	11	14
Formaldehyde ¹	1	(-) 0.9	(-) 2.7	(-) 0.7*	(-) 1.4*	(-) 0.9
1 officiate for the second s	10	(-) 7.3*	(-) 9.6*	$(-)17.5^*$	$(-)14.5^*$	(_) 3.8*
	100	$(-) 9.8^*$	$(-)25.4^*$	$(-)42.6^*$	NG ^{*3}	NG*
	1000	NG*	NG*	NG*	NG*	NG*
Acetaldehyde ¹	1	(-) 0.3	(-) 1.9	(-) 0.2	(-) 0.5	4
neetuiden) de	10	(-) 1.3*	(-) 2.4	(-) 4.0*	(-) 0.7*	(_) 1.3*
	100	$(-) 2.8^*$	(-)13.6*	(-)26.0*	(-)25.3*	(-)33.9*
	1000	$(-)12.0^{*}$	(-)30.2*	(-)50.0*	NG*	NG*
Propionaldehyde ²	-1	(+) 0.6	(+) 1.4	(-) 0.5	-	(_) 0.2
110pionalaoni, ao	10	· · · _ ·	(+) 0.3	(-) 6.3*	(-)21.0*	(_) 2.8*
	100	$(-) 2.7^*$	(-) 2.0*	(-)10.0*	(-)18.0*	(_) 5.8*
	1000	(-)13.5*	(-) 9.4*	(-)26.3*	(-)62.2*	NG*

¹Control: Log of no./ml: 3.16, 3.74, 4.46, 5.88, and 7.04 at 2, 5, 8, 11, and 14 h, respectively. ²Control: Log of no./ml: 3.40, 3.53, 3.99, 5.18, and 5.66 at 2, 5, 8, 11, and 14 h, respectively. ³NG: Less than 10 organisms/ml of test liquid. ⁴-: No difference.

*: Population significantly different from control at 5% level.

used are given in Table 3. Of the three compounds, formaldehyde was most detrimental to S. aureus, and at 100 ppm virtually inactivated the organism in less than 2 h. Acetaldehyde (1000 ppm), propionaldehyde (1000 ppm), and formaldehyde (100 ppm) caused a very conspicuous and ever increasing inhibitory effect that terminated in inactivation of S. aureus. At 10 ppm formaldehyde always was significantly inhibitory to S. aureus. The degree of inhibition caused by this concentration of formaldehyde generally increased for 8 to 11 h of incubation and then declined. When 1 ppm of this aldehyde was used, only marginal and often insignificant inhibition was noted. With 100 ppm of acetaldehyde regularly significant inhibition was observed and it increased in magnitude with time. Propionaldehyde at a similar concentration exerted maximum inhibition at 11 h. Acetaldehyde (10 ppm) was generally significantly inhibitory to S. aureus but at 1 ppm the compound had no significant effect. Significant inhibition, especially in the later stages of incubation, was noted when 10 ppm of propionaldehyde were used although 1 ppm of this chemical had a minimal effect on S. aureus.

Of the ketones tested diacetyl was most detrimental to growth of S. aureus, as is evident from data in Table 4. At 1000 ppm diacetyl caused more than 50% reduction in the number of S. aureus in 2 h, and at 5 h the organism was inactivated. A generally significant inhibition that increased with time was noted when 100 ppm of diacetyl were tested. Lower concentrations (1 and 10 ppm) of diacetyl were generally without effect on S. aureus. At 1000 ppm both acetone and 2-butanone were almost equally inhibitory to S. aureus. At 100 ppm acetone probably was somewhat more inhibitory early in the incubation than was 2-butanone. When 10 ppm of 2-butanone were present, the chemical caused slightly more inhibition than the same amount of acetone; at 1 ppm neither compound had any significant effect on the growth of S. aureus.

Table 5 lists results obtained when different concentrations of acetonitrile, chloroform, or ether were tested. Chloroform was so detrimental to S. aureus that 1000 ppm of this compound inactivated the organism in less than 2 h. As the incubation progressed, 100 ppm chloroform caused a continuously declining but regularly significant inhibition of S. aureus. Lower concentrations (1 and 10 ppm) were only marginally or not at all inhibitory. Data obtained with acetonitrile (1000 ppm) indicate that except at 2 h of incubation, the chemical always was significantly inhibitory to S. aureus. At lower concentration (1 and 10 ppm) acetonitrile was without

significant effect against S. aureus. During the later stages of incubation when 100 ppm of acetonitrile were used, significant inhibition was noted. The effects caused by 1 or 10 ppm of acetonitrile were never significant. Ether at 100 and 1000 ppm was less inhibitory to S. aureus than was acetonitrile. In spite of this, inhibition was always significant; inhibition was maximal at 5 h and then steadily declined during the incubation. At lower concentrations (1 and 10 ppm) ether was essentially ineffective against S. aureus.

Glyoxal was very effective against S. aureus as is evident from data in Table 6. With 1000 ppm of the chemical the organism was inactivated in less than 2 h and with 100 ppm after 11 h. When 10 ppm of glyoxal were tested, regularly significant inhibition occurred and it was greatest in the early stages of incubation. Glyoxal at a concentration of 1 ppm was essentially without any effect on S. aureus. Ethylenedichloride (1000 ppm) was markedly inhibitory to S. aureus after 5 h of incubation. The magnitude of inhibition increased as the incubation progressed. Less but generally significant inhibition which was greatest at 8 h of incubation was caused by 100 ppm of this compound. Lower (1 to 10 ppm) concentrations of ethylenedichloride were essentially without effect on S. aureus. Methylsulfone was least effective of the three compounds listed in this table. Neither 1000 nor 100 ppm of the chemical were always significantly inhibitory to S. aureus. Inhibition by 10 or 1 ppm was even less and generally was not significant.

Table 7 summarizes results obtained with methylsulfide, methanethiol, and ethanethiol. At 1000 ppm both of the thiols were nearly equally inhibitory and were far more detrimental to growth of S. aureus than was methylsulfide. When 100 ppm of the mercaptans were used, methanethiol was somewhat more inhibitory than ethanethiol. Inhibition of S. aureus caused by 100 or 1000 ppm of either thiol was always statistically significant. With 10 ppm of either mercaptan, minimal and usually insignificant inhibition was noted. Both thiols, at 1 ppm, stimulated growth of S. aureus and the extent of the stimulation was often significant. Methylsulfide was only minimally inhibitory even at the 1000 ppm concentration, although the inhibition was statistically significant. Lower concentrations (1, 10, and 100 ppm) of methylsulfide generally favored growth of S. aureus; this stimulation was sometimes statistically significant.

Results obtained when alcohols and amines were tested are listed in Table 8. Amines were more unfavorable than alcohols for growth of *S. aureus*. At 1000 ppm propylamine was somewhat more active

		Difference	es (%) from control i	n log of population a	fter hours of incuba	tion
Chemical	Conc. (ppm)	2	5	8	11	14
Acetone ¹	1	4	(+) 0.2	(-) 0.2	(-) 0.3	
rectone	10	(-) 0.9	(-) 1.1	(-) 0.4	(-) 1.0	(_) 0.7
	100	(-) 5.6*	(-) 3.3*	(-) 1.1	(_) 3.0*	(-) 1.7*
	1000	$(-) 8.5^*$	(-) 4.4*	(-) 3.6*	(-) 4.4*	(_) 3.0*
-Butanone ²	1000	(-) 0.9	_	_	(-) 0.2	(_) 0.3
-Dutanone	10	$(-) 2.5^*$	(_) 1.3	(-) 0.9*	— .	$(-) 1.6^*$
	100	(-) 3.2*	$(-) 2.4^{\circ}$	(-) 2.0*	(-) 1.5*	(_) 3.3*
	1000	(-) 4.4*	$(-) 5.6^*$	(−) 3.1 *	(-) 7.8*	(_) 6.7*
Diacetyl ³	1	(-) 0.3	(+) 0.2	(+) 0.2	(-) 0.5	(+) 0.1
Jiacetyi	10	(-) 0.6	(-) 1.0	$(-) 2.1^*$	(-) 1.2	(_) 0.9
	100	(-) 3.9	(-) 7.5°	$(-)17.5^{*}$	$(-)18.9^*$	(_)18.4*
	1000	$(-)54.6^*$	NG ⁵ *	NG*	NG*	NG*

TABLE 4.	DIFFERENCES IN POPULATION OF	Staphylococcus aureus IN NUTRIENT BROTH
	CAUSED BY ADDED ACETONE,	2-BUTANONE, AND DIACETYL

¹Control: Log of no./ml: 3.42, 4.52, 5.63, 6.75, and 7.13 at 2, 5, 8, 11, and 14 h, respectively. ²Control: Log of no./ml: 3.16, 3.74, 4.46, 5.88, and 7.04 at 2, 5, 8, 11, and 14 h, respectively. ³Control: Log of no./ml: 3.62, 4.13, 5.21, 6.08, and 6.91 at 2, 5, 8, 11, and 14 h, respectively.

TABLE 5.	DIFFERENCES IN POPULATION OF Staphylococcus aureus IN NUTRIENT BR	OTH
1110000 01	CAUSED BY ADDED ACETONITRILE, CHLOROFORM, AND ETHER	

		Difference	es (%) from control in	n log of population a	after hours of incuba	ation
Chemical	Conc. (ppm)	2	5	8	11	14
Acetonitrile ¹	1	(+) 1.6	(-) 0.2	2	(+) 0.1	(+) 0.3
leetomtino	10	(+) 1.1		(-) 0.2	(+) 0.1	(_) 0.3
	100	(+) 0.8	(−) 2.4 [*]	(-) 2.3*	(_) 0.9*	(_) 1.2*
	1000	(-) 3.0	$(-)14.5^{*}$	(-) 5.8*	(-) 2.7*	(_) 1.5*
Chloroform ¹	1000	(-) 1.1	(-) 1.6	(-) 0.3	_	—
	10	(-) 1.6	(-) 2.2*	(-) 0.2	(_) 0.1	(_) 0.3
	100	$(-) 6.0^*$	(_) 3.8*	(−) 2.6 [*]	(_) 1.3*	(_) 1.2*
	1000	NG ³ *	NG*	NG*	NG*	NG*
Ether ¹	1	_	(-) 1.2	-	(+) 0.1	(_) 0.1
Liller	10	(-) 0.5	(-) 1.0	_	(+) 0.1	(_) 0.1
	100	$(-) 2.2^*$	(-) 3.2*	$(-) 1.7^*$	(_) 0.7*	(_) 0.7*
	1000	(-) 2.2	(−) 4.2 *	(_) 3.8*	(-) 2.3*	(_) 1.2*

¹Control: Log of no./ml: 3.64, 4.95, 6.04, 7.00, and 7.50 at 2, 5, 8, 11, and 14 h, respectively. ²-: No difference. ³NG: Less than 10 organisms/ml of test liquid. ^{*}: Population significantly different from control at 5% level.

TABLE 6. DIFFERENCES IN POPULATION OF Staphylococcus aureus in nutrient broth CAUSED BY ADDED GLYOXAL, ETHYLENEDICHLORIDE, AND METHYLSULFONE

		fter hours of incuba	urs of incubation			
Chemical	Conc. (ppm)	2	5	8	11	14
Glyoxal ¹	1	(_) 0.3	(-) 0.3	(_) 0.7	(-) 0.2	(_) 1.3*
Giyoxai	10	$(-)16.3^*$	$(-)12.7^{*}$	(_) 7.8*	$(-)12.7^{\circ}$	(_) 9.5*
	100	$(-)23.8^*$	$(-)34.5^{\circ}$	$(-)48.9^{*}$	NG*	NG*
	1000	NG ⁴ *	NG*	NG*	NG*	NG*
Ethylenedichloride ²	1	(+) 0.6	(-) 0.7	(_) 0.4	_5	(+) 0.4
Emplehenemoniae	10	(-) 0.3	(-) 1.0	(_) 0.6	(-) 1.2	(+) 0.3
	100	(-) 0.8	(-) 1.5*	(_) 3.7*	(-) 3.0*	(_) 2.5*
	1000	(-) 1.4	$(-)14.5^{\circ}$	(-)42.8*	(-)63.8*	(_)60.9*
Methylsulfone ³	1	(-) 0.6	(-) 0.3	(+) 1.1	(-) 0.2	(+) 0.1
wiethyisunone	10	(-) 2.8*	(-) 1.1	(+) 0.9	(-) 0.2	(_) 0.1
	100	$(-) 5.4^{*}$	(-) 0.8	(_) 0.9*	(-) 1.0	(_) 0.6
	1000	(_) 9.1*	(-) 2.6	(-) 2.4*	(−) 1.9 *	(_) 1.0

¹Control: Log of no./ml: 3.32, 3.77, 4.11, 5.43, and 6.08 at 2, 5, 8, 11, and 14 h, respectively. ²Control: Log of no./ml: 3.62, 4.13, 5.21, 6.08, and 6.91 at 2, 5, 8, 11, and 14 h, respectively. ³Control: Log of no./ml: 3.17, 3.79, 4.49, 5.88, and 6.87 at 2, 5, 8, 11, and 14 h, respectively. ⁴NG: Less than 10 organisms/ml of test liquid. ⁵-: No difference.

°: Population significantly different from control at 5% level.

		Difference	es (%) from control	in log of population	after hours of incub	ation
Chemical	Conc. (ppm)	2	5	8	11	14
Methylsulfide ¹	1	(+) 0.4	(+) 1.9*	(+) 0.6	(+) 0.4	(+) 0.8*
	10	2	(+) 1.0	(+) 0.7*	(+) 0.6	$(+) 0.6^*$
	100	(-) 1.8	(+) 1.2*	(+) 0.2	(+) 0.3	(+) 0.2
	1000	(-) 4.3*	(-) 3.7*	(-) 4.7*	$(-) 2.7^*$	$(-) 2.8^*$
Methanethiol ¹	1	-	(+) 3.9*	(+) 3.1*	(+) 1.4	$(+) 0.6^*$
	10	(-) 1.4	(-) 9.7*	(-) 7.6*	(-) 1.2	-
	100	(−) 7.5 [*]	(-)19.1*	$(-)17.7^{*}$	$(-)10.3^*$	(-) 2.8*
	1000	(-)10.7*	(-)51.4*	$(-)52.3^*$	$(-)41.8^{*}$	$(-)34.6^*$
Ethanethiol ¹	1	(+) 1.4	(+) 3.9*	(+) 2.9*	(+) 1.2	(+) 0.6*
	10	(-) 3.2*	(+) 0.4	(-) 0.2	(-) 1.4	_
	100	(-) 8.5*	(-)11.1*	(-) 9.2*	(-) 4.2*	(-) 1.9*
	1000	(_)15.3*	(-)54.1*	(-)51.6*	(-)40.0*	$(-)23.0^{*}$

TABLE	7.	Diffe	REN	ICES	IN	POPULATION	OF	Staphylococcus	aurei	S IN	NUTRIENT	BROTH	
		CAUSED	BY	ADD	ED	METHYLSULF	IDE,	METHANETHIOL,	AND	ETHA	NETHIOL		

¹Control: Log of no./ml: 2.81, 4.86, 6.84, 7.80, and 8.29 at 2, 5, 8, 11, and 14 h, respectively.

-: No difference.

*: Population significantly different from control at 5% level.

	CAUSED BY ADI	DED FURFURYL, M	ETHYL ALCOHOL,	PROPYLAMINE, AND	HEXYLAMINE	
		Differenc	es (%) from control	in log of population a	fter hours of incub	ation
Chemical	Conc. (ppm)	2	5	8	11	14
Furfuryl	1	_	(-) 0.3	(+) 0.4		(+) 0.1
alcohol ¹	10	(-) 1.3*	(-) 0.8	(+) 0.7	(-) 0.2	(-) 0.1
	100	(-) 2.8*	(-) 1.1	(-) 0.2	(-) 0.5	(-) 0.4
	1000	(_)11.0*	(-) 2.6	(-) 0.7*	$(-) 2.2^*$	(-) 0.9
Methyl alcohol ¹	1	(_) 0.3		(+) 0.4	(-) 0.3	(-) 0.4
	10	() 2.8*	(-) 0.5	(_) 0.2	(-) 0.7	(-) 0.6
	100	(_) 3.8*	(-) 1.1	(-) 0.4*	(-) 0.8	(-) 1.3
an an an an	1000	(-) 5.4*	(-) 3.2	(-) 2.2*	$(-) 2.5^*$	(-) 1.9*
Propylamine ²	1	() 0.3	(-) 0.5	(_) 0.2	(-) 0.2	(-) 0.3*
	10	(-) 0.9*	(-) 1.6	() 1.5	$(-) 1.1^*$	$(-) 2.8^*$
	100	() 1.5*	(-) 5.3*	(-) 3.4*	$(-) 6.1^*$	(-) 6.3*
	1000	(-)67.5*	NG ⁵ *	NG*	NG*	NG*
Hexylamine ³	1	(+) 0.6	(-) 0.7	(-) 0.2	(-) 0.4	(-) 0.4
	10	(—) 5.3	(-) 6.4	(-) 5.3*	(-) 5.3*	(-) 4.1
	100	(-)13.7*	(-)14.2*	(-)17.4*	(-)23.3*	(-)12.8*
	1000	(-)65.5*	$(-)63.5^{*}$	NG*	NG*	NG*

TABLE 8. DIFF	ERENCES IN POPULA	TION OF	Staphyloco	CCUS aureus IN	NUTRI	ENT BROTH
CAUSED BY	ADDED FURFURYL,	METHYL	ALCOHOL,	PROPYLAMINE,	AND	HEXYLAMINI

¹Control: Log of no./ml: 3.17, 3.79, 4.49, 5.90, and 6.87 at 2, 5, 8, 11, and 14 h, respectively. ²Control: Log of no./ml: 3.32, 3.78, 4.11, 5.43, and 6.08 at 2, 5, 8, 11, and 14 h, respectively. ³Control: Log of no./ml: 3.42, 4.52, 5.63, 6.75, and 7.13 at 2, 5, 8, 11, and 14 h, respectively.

⁴-: No difference. ⁵NG: Less than 10 organisms/ml of test liquid.

*: Population significantly different from control at 5% level.

than was hexylamine; the former inactivated the organism in 2 to 5 h, whereas 5 to 8 h were required by the latter compound. At 100 ppm hexylamine was more inhibitory than propylamine, but both compounds caused significant inhibition of S. aureus. The lower (10 and 1 ppm) concentrations were rarely significantly inhibitory to S. aureus. Alcohols were not regularly significantly inhibitory even at 1000 ppm. Furfuryl alcohol at this concentration sometimes was more inhibitory than was methyl alcohol. At lower concentrations (100, 10, and 1 ppm) inhibition was less pronounced and only sometimes significant.

Higher concentrations of all compounds tested were inhibitory to S. aureus, but only some compounds

were significantly inhibitory when low concentrations were used. Concentrations of test compounds, other than fatty acids and amines required for significant inhibition generally were greater than the amounts of these compounds likely to be present in milk. Podesta and Bertoldini (16) found that butyric acid was less inhibitory to S. aureus than the C6 to C_{12} fatty acids. This was confirmed by our results especially those obtained when we used higher concentrations of the chemicals. They also found that fatty acids did not greatly alter growth conditions for S. aureus in milk cultures. This is contrary to the findings of Vadhera and Harmon (17, 18) who noted that 500 to 1000 ppm of decanoic acid and 1000 ppm of octanoic acid caused complete inhibition of

S. aureus. Findings of Daly et al. (2) and Minor and Marth (11, 12, 13) that acetic and formic acid inhibited S. aureus also were confirmed by these studies.

Our results obtained with S. aureus were somewhat different from those obtained with Escherichia coli (9) and Salmonella typhimurium (10). At 1000 ppm acetic and hexanoic acid were least inhibitory to S. typhimurium and E. coli, respectively; but butyric acid was least effective against S. aureus at this concentration. Acetaldehyde and propionaldehyde were more inhibitory to S. aureus than to S. typhimurium, and chloroform, glyoxal, and ethylenedichloride were more detrimental to S. aureus than to E. coli and S. typhimurium. Diacetyl was less effective against S. aureus than against E. coli or S. typhimurium, whereas E. coli was more susceptible than S. aureus to ethanethiol and hexylamine.

These results and our earlier findings with E. coli (9) and S. typhimurium (10) suggest that fatty acids, amines, and many other volatile compounds, which are responsible for imparting the typical flavor to many dairy and non-dairy foods, might influence growth and/or survival of these organisms. Whether the amounts of such compounds naturally present affect the growth and/or survival of these potentially hazardous organisms in different foods is still not understood. Use of these flavor compounds to retard growth of such bacteria needs further study.

Addendum

The reader is referred to the Addendum to the first paper in this series (*Escherichia coli*) (9) for information on the boiling point and solubility in water of all test chemicals. The initial pH of nutrient broth that contained the various amounts of chemicals also is given. The low (3.6-5.5) pH values caused by 1000 ppm of formic, acetic, butyric, hexanoic, octanoic, and decanoic acid, and the high (8.9-9.3) pH values obtained with 1000 ppm of propylamine and hexylamine undoubtedly contributed to the antibacterial property of these chemicals at the highest concentration that was tested.

When the reduction in population of *S. aureus* was 24% or greater, a given chemical at the appropriate concentration was bactericidal. A reduction in population of approximately 1-24% represents bacteriostatic action by the chemical.

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LOSS OF ASCORBIC ACID FROM GRAPEFRUIT JUICE WHEN CULTURED WITH ASPERGILLUS PARASITICUS

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Abstract

Ascorbic acid was added to single-strength grapefruit juice that was then inoculated with *Aspergillus parasiticus*. Up to approximately 9.8 mg ascorbic acid/ml juice failed to inhibit either growth or aflatoxin synthesis by the mold. Loss of ascorbic acid during 18 days at 28 C was greater from inoculated than from uninoculated grapefruit juice that initially contained about 20 times the normal amount of the vitamin.

Single-strength and concentrated grapefruit juice (1, 3, 4) as well as intact grapefruit (2) can support growth of and aflatoxin production by toxigenic aspergilli. Citrus fruits, including grapefruit, contain appreciable amounts of ascorbic acid. Since ascorbic acid is an important constituent of citrus products, a study was done to learn if the vitamin could affect aflatoxin production and also if mold growth caused a decrease in the amount of ascorbic acid in grapefruit juice. Results are reported in this paper.

MATERIALS AND METHODS

Organism A. parasiticus NRRL 2999, a known toxigenic strain, was obtained from the Northern Regional Research Laboratory, Peoria, Illinois. Stock cultures were maintained at 5 C on slants of mycological agar (Difco).

Preparation of spore suspension

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

Preparation of grapefruit juice with added ascorbic acid

Grapefruit juice contains about 0.4 mg ascorbic acid/ml of juice (8). Appropriate amounts of L-ascorbic acid (J. T. Baker Chemical Co., Phillipsburg, N. J.) were added to steamed and cooled grapefruit juice (single-strength juice prepared from thawed frozen concentrated juice) to provide approximately 3 and 20 times the amount found naturally in the juice.

Sampling

Contents of all flasks were sampled after 2, 8, 12, and 18 days of incubation at 28 C.

Determination of ascorbic acid

Amounts of ascorbic acid initially present and remaining after incubation were measured by the iodine titration method (6)that is especially applicable to fruit juices.

Aflatoxin analysis

Aflatoxin was determined according to procedures that were described earlier (4).

RESULTS AND DISCUSSION

Although the acidity of citrus juices is primarily caused by citric acid, the pH value can also be influenced somewhat by the amount of ascorbic acid that is present (5). This also is evident from our data in Table 1 since the pH dropped by 0.1 unit when the content of ascorbic acid was increased from 3- to 20fold the normal concentration. Furthermore, the data indicate that the pH of inoculated juice increased appreciably during the incubation of 18 days. The increase occurred toward the end of the incubation and was more pronounced in grapefruit juice with three rather than 20 times the amount of ascorbic acid found naturally. These data on the change in pH of grapefruit juice when it is cultured with A. parasiticus are in accord with our earlier observations (1, 3).

The added ascorbic acid (up to 1 g/100 ml juice) in grapefruit juice caused no appreciable change in mold growth or aflatoxin production from those observed in regular grapefruit juice (data not shown). This suggests that *A. parasiticus* is not sensitive to high concentrations of ascorbic acid.

Loss of ascorbic acid from inoculated juice with the highest concentration of the vitamin was appreciably greater than from the uninoculated control (Table 2). Ascorbic acid was rapidly depleted from inoculated juice during the first 2 days of incubation and again during the interval between the 12th and 18th day of incubation. The rapid loss of vitamin toward the end of the incubation coincided with an

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		Inocula	ted juice			Uninoc	ulated juice	
	Amo	unt of add	led ascorbic acid		Amo	ount of add	led ascorbic acid	
	3 ×3		20×4		3 ×		$20 \times$	
Days	Residual ascorbic acid	рН	Residual ascorbic acid	pH	Residual ascorbic acid	pH	Residual ascorbic acid	pH
	(mg/ml)		(mg/ml)		(mg/ml)		(mg/ml)	
0 2 8 12 18	1.28 1.06 0.66 0.43 ND ⁵	3.30 3.30 3.55 4.12 7.08	9.76 8.88 8.08 7.15 5.22	3.20 3.20 3.40 3.87 5.67	1.55 1.48 0.80 0.23 0.15	3.35 3.35 3.35 3.40 3.53	$11.93 \\ 11.75 \\ 10.60 \\ 9.18 \\ 8.95$	3.25 3.25 3.30 3.30 3.30

TABLE 1. Residual ascorbic acid in and final pH values of grapefruit juice¹ fortified with ascorbic acid, inoculated with A. parasiticus, and incubated quiescently at 28 C²

'Thawed frozen grapefruit juice diluted with three equal volumes of water.

²Each value is the average of three trials.

³Three times the normal concentration.

⁴Twenty times the normal concentration.

⁵Not determined.

TABLE 2. LOSS OF ASCORBIC ACID FROM UNINOCULATED AND INOCULATED (WITH A. parasiticus) GRAPEFRUIT JUICE¹ CONTAINING APPROXIMATELY 20 TIMES THE NORMAL CONCENTRATION OF ASCORBIC ACID^{2, 3}

	Loss of asc	orbic acid (%)
Days	Inoculated	Uninoculated
0-2	9.0	1.6
2-8	8.3	9.6
8-12	9.5	11.9
12-18	19.8	1.1
8-18	29.3	13.0
0-18	46.6	24.2

¹Thawed frozen concentrated grapefruit juice diluted with three equal volumes of water.

²Quiescent incubation at 28 C.

³Each value is the average of three trials.

increase in the pH of the juice (Table 1); this suggests that ascorbic acid may have been utilized by the mold and/or that the vitamin became less stable when the pH increased as a consequence or fungal activity. Molds have been reported as able to oxidize ascorbic acid (7).

Ascorbic acid also disappeared from the uninoculated grapefruit juice, probably because the juice was exposed to the air during the incubation period. However, the amount of vitamin lost from the inoculated juice (46.6%) was almost again as much as from the uninoculated juice (24.2%). This difference is attributable to fungal activity. When grapefruit juice contained three times the normal concentration of ascorbic acid, loss through fungal activity was not nearly as apparent as when the juice contained the high concentration of vitamin. This was true because juice only exposed to air (uninoculated) lost the vitamin as rapidly as did the inoculated product. Hence, the amount of ascorbic acid, in this instance, was inadequate to demonstrate its use by the mold.

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INFLUENCES OF RECOVERY MEDIA AND INCUBATION TEMPERATURES ON THE TYPES OF MICROORGANISMS ISOLATED FROM SEAFOODS

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Abstract

Four hundred fifty-five microbial isolates from seafoods were replicated on tryptone-peptone-yeast extract agar with 0.5% NaCl (TPE), trypticase soy agar with 3.0% NaCl (TSA), and plate count agar with no added NaCl (PCA). Daughter plates were incubated at 5, 25, 35, and 37 C, and colonies that developed on the plates were identified. At 25 C, 7% of TPE isolates failed to grow on PCA and 4% on TSA. The difference was mainly caused by Pseudomonas type III species, 24% of which failed to grow on PCA; Pseudomonas type II species, 13% of which failed on PCA; and Flavobacterium-Cytophaga species, 18% of which failed on TSA. Except for the reference mesophilic, cultures of Staphylococcus, Micrococcus, Escherichia coli, and Vibrio parahaemolyticus-which grew equally well at 25 and 35 C but failed to grow at 5 C-49% of colonies growing at 25 C failed to grow at 35 C, and 17% at 5 C. Microbial groups in order of sensitivity to 35 C were Arthrobacter, Pseudomonas, Moraxella, Micrococcus, Flavobacterium-Cutophaga, and Acinetobacter, with the respective growth failures at 35 C of 68, 51, 46, 42, 33, and 29%. Microbial groups that failed to grow at 5 C in the order of sensitivity were, Flavobacterium-Cytophaga, Acinetobacter, Arthrobacter, and Moraxella with respective growth failures of 40, 26, 12, and 9%.

The 35 C incubation temperature commonly employed for the aerobic plate count (APC) (1, 7, 9, 11) is detrimental to recovery of psychrotrophic microorganisms (8, 10, 11). Although the difference between plate counts of seafoods at 25 and 35 C could range from 1 to 4 logs, the identities of the psychrotrophs responsible for this difference are not clearly known. Information on the identities of such microorganisms will be needed for optimum modification of the APC suitable for seafoods and will also provide insight into types of microorganisms that would be missed by the 35 C APC procedure.

Three commonly employed media for seafoods that represented the NaCl concentrations of 0, 0.5, and 3.0% were examined with microorganisms isolated from seafoods. The identities of microorganisms that grew at 25 C but failed to grow at 5, 35, or 37 C on any of the media were then determined.

MATERIALS AND METHODS

Microorganisms

Most of the microbial cultures tested had been isolated from two Dungeness crab samples, a frozen shrimp sample, and an iced fillet of sole. One crab sample was obtained fresh from a commercial processing plant and the remaining seafoods were purchased from retail stores. The length of refrigerated storage for these samples, therefore, was unknown. Except for the mesophilic reference cultures, microorganisms from seafoods were isolated by spread plating the diluted homogenates on one of the test media, TPE, that contained 0.5% Bacto-tryptone, 0.5% Bacto-peptone, 0.25% Bacto-yeast extract, 0.1% glucose, 0.5% NaCl, and 1.5% Bacto-agar. Plates were incubated at 25 C for 48 h.

After incubation, all microbial colonies that developed on countable plates were transferred onto TPE master plates with sterile toothpicks. Each master plate contained 30 colonies on the marked spots that corresponded to the positions of the 30, 22 gauge nickel-chrome wires of the stab replicator. Plates were incubated at 25 C for 48 h. Master plates of the mesophilic reference cultures were prepared similarly. Mesophile cultures included in this study were five *Staphylococcus*, four *Micrococcus*, and three *Escherichia coli* from the stock culture collections of this laboratory and four *Vibrio parahaemolyticus* strains obtained from M. Fishbein, U.S. Food and Drug Administration, Washington, D. C.

Test procedures

Colonies that developed on master plates were replicated onto three test agar plates in duplicate and incubated at 5, 25, 35, and 37 C in a Hotpack Refrigerated Incubator (Hotpack Corp., Philadelphia, PA) and three Naplco Incubators (National Appliance Co., Portland, OR). Incubator temperature variation was less than ± 1 C.

One of the test media was standard plate count agar (PCA) that contained 0.5% Bacto-peptone, 0.25% Bacto-yeast extract, 0.1% glucose, and 1.5% Bacto-agar. This medium did not contain NaCl. The second medium was trypticase soy agar plus 3% NaCl (TSA). It contained 1.7% BBL- trypticase, 0.3% BBL-phytone, 0.25% K_2 HPO₄, 0.25% glucose, 1.5% Bacto-agar, and 3.6% NaCl. This medium has been used by Vanderzant et al. (15) for microbiological investigation of Gulf coast seafoods.

The composition of TPE, or the reference medium, was listed earlier. This medium has been employed by us for a number of years to enumerate microorganisms from seafoods and it represented media that contained 0.5% NaCl. The 0.5% NaCl level had been found optimum for microorganisms from seafoods by Pelroy and Eklund (12) and Silverio and Levin (14).

After incubation of 48 h for 25-, 35-, and 37-C plates and at 78 h for 5-C plates growth on daughter plates was recorded.

¹Technical Paper No. 3798, Oregon Agricultural Experiment Station.

TABLE 1. EFFECTS INCUBATION TEMPERATURES ON THE NUMBER OF COLONIES RECOVERED ON THREE MEDIA

		Media ¹		
Temp. — (C)	PCA	TPE	TSA	Total
5	360	399	285	1044
25	424	455^{2}	437	1316
35	221	286	276	783
37	223	293	262	778

¹PCA = plate count agar (0% NaCl), TPE = tryptone-peptone-extract agar (0.5% NaCl), and TSA = trypticase-soy agar (3.0% NaCl).

²Identical to the conditions of initial isolation of test microorganisms from seafoods.

TABLE 2. EFFECT OF MEDIA¹ ON THE 25-C GROWTH OF MICROORGANISMS ISOLATED FROM SEAFOODS

Microorganisms	PCA	TPE	TSA
Pseudomonas I	3	3	2
Pseudomonas II	33	38	38
Pseudomonas III	57	75	74
Moraxella	155	161	150
Acinetobacter	120	120	119
Flavobacterium-			
Cytophaga	26	28	23
Arthrobacter	30	30	30
TOTAL	424	455	437

¹See Table 1.

TABLE 3. EFFECT OF MEDIA AND NACL ON THE 5-C GROWTH OF SEAFOOD ISOLATES

	N	fedia (% Na	21)
Microorganisms	PCA (0)	TPE (0.5)	TSA (3.0)
Pseudomonas I	3	3	3
Pseudomonas II	32	38	34
Pseudomonas III	43	64	60
Moraxella	141	146	135
Acinetobacter	101	102	61
Flavobacterium-			
Cytophaga	16	20	10
Arthrobacter	24	27	28
TOTAL	360	400	331

In addition, plates were kept at each test temperature for a week to 10 days to detect any delayed growth.

Microbial identification was made according to the general schemes and procedures reported earlier from this laboratory (5). Additional details of the modification and the improvement of this technique will be reported elsewhere.

RESULTS

Effects of incubation temperature and growth medium

Test microorganisms were initially isolated from TPE plates and incubated at 25 C. Counts on PCA or TSA at 25 C therefore, could not exceed the count on TPE at this temperature. Despite this built in bias, the total number of colonies that developed on PCA or TSA was comparable to that of TPE at all four incubation temperatures (Table 1). Maximum num-

ber of colonies developed on all three media at 25 C and the influence of NaCl concentration was also apparently the lowest at this temperature.

Growth at 25 C

Listed in Table 2 are the microorganisms that grew on three test media at 25 C. At this temperature the lack of NaCl in PCA was especially detrimental to *Pseudomonas* type III species. Eighteen of 75 or 25% of TPE colonies failed to grow on PCA. The 3% NaCl in TSA also appeared somewhat detrimental to *Flavobacterium-Cytophaga* species and 5 of 28, or 18%, did not develop on TSA. Not listed in Table 2 are the mesophilic reference cultures. As expected, all four *V. parahaemolyticus* failed to grow on PCA, but stock cultures of five *Staphylococcus*, four *Micrococcus*, three *E. coli*, and 54 *Proteus* species isolated from a seafood processing plant, grew equally well in all 3 media.

Growth at 5 C.

The number of colonies that developed at 5 C were 1,044, compared to 1,316 at 25 C or 83% of the maximum (Table 1). Data in Table 1 also show that the colony recovery on TSA was the least of three media at 5 C.

Identities of microorganisms that grew at 5 C are presented in Table 3. The data suggest that the poor recovery on TSA at 5 C was almost exclusively due to the inability of nearly 50% of *Acintobacter* and *Flavobacterium-Cytophaga* species to grow on this medium. The PCA, on the other hand, did not support growth of nearly 33% of *Pseudomonas* III species.

Growth at 35 C.

The effect of 35 or 37 C on recovery of the 25-C growth was far more pronounced than that of 5 C. From the total of 1,316 colonies recoverable at 25 C, only 783 or 51% of the colonies developed at 35 C on three media. The levels of recovery at 35 and 37 C were similar (Table 1). In contrast to what was observed at 5 C, PCA yielded the least number of colonies at 35, 37, and 25 C. *Pseudomonas, Moraxella*, and *Flavobacterium-Cytophaga* species grew poorly on PCA when compared to growth of other species on TPE and TSA at 35 C (Table 4).

Salt appears to either stimulate or inhibit colony development. Each microbial group responded differently to the combined effects of NaCl and incubation temperature.

DISCUSSION

The effects of NaCl and incubation temperature on growth of microorganisms isolated from seafoods apparently are interrelated. Tolerance to inhibitory concentrations of NaCl is known to depend on the physiological conditions of the microorganisms. Salt tolerance of *Streptococcus faecalis*, for example, was reduced when the cells had been injured by the sub-lethal heat treatment (4).

Microorganisms from seafoods had been grown under apparently optimum conditions before they were replicated onto the test media. Replication was made from the actively growing microbial colony with a 22 gauge nickel-chrome wire, to insure a heavy inoculum. This was done to solicit the maximum response from each test culture. Despite this, responses of microorganisms to NaCl concentrations in three test media at 5, 35, and 37 C incubation temperatures were more pronounced than that at 25 C (Table 1). This seems to indicate that the microorganisms had responded to the combination of NaCl and suboptimum growth conditions, in a manner similar to that observed for sublethally injured cells.

The temperature-dependent nature of NaCl effect may also explain some of the conflicting reports on the NaCl requirements of microorganisms. Castell et al. (2) indicated that some *Pseudomonas putrefaciens* strains isolated from cod fillets required NaCl for growth. Chai et al. (3) examined *P. putrefaciens* strains isolated from haddock and concluded that NaCl was not required for growth but that it enhanced development of the characteristic reddishpink pigmentation. The incubation temperature employed by the former investigators was not reported while the later work was done at 20 C.

We identified 14 colonies of Pseudomonas II species as P. putrefaciens, based on the H₂S production and the salmon pink pigmentation (3). They were replicated on TPE agar containing from 0 to 5% NaCl in 0.5% increments. At 5 and 25 C, colonies developed on all media. At 35 and 37 C, however, no growth was observed at 0% NaCl, although some of them could grow at 35 and 37 C on agar containing NaCl (unpublished data). The relationship between NaCl and incubation temperature for optimum recovery of microorganisms from seafoods would undoubtedly include additional factors which are beyond the scope of this investigation. A whole host of factors both intrinsic to the microorganisms and extrinsic relating to conditions of recovery need to be examined before this phenomenon can be fully explained.

An additional effect of NaCl observed was that 3% NaCl in TSA reduced the swarming of *Proteus* species and also prevented the spreading of many mucoid colonies. This could be viewed as a desirable attribute of this medium, except for the fact that whenever a large proportion of microorganisms failed to grow on TSA, the growth of the remaining micro-

TABLE 4. EFFECT OF MEDIA AND NACL ON THE 35-C GROWTH OF SEAFOOD ISOLATES

	N	Iedia (% NaC	21)
Micromganisms	PCA (0)	TPE (0.5)	TSA (3.0)
Pseudomonas I	1	2	1
Pseudomonas II	14	25	20
Pseudomonas III	23	33	41
Moraxella	68	95	105
Acinetobacter	90	98	83
Flavobacterium-			
Cytophaga	15	22	18
Arthrobacter	10	11	8
TOTAL	221	286	276

TABLE 5. COMPARISON OF 25- AND 35-C GROWTH OF MICRO-ORGANISMS ISOLATED FROM SEAFOOD¹

Microorganisms ²	No. colonies 35 C/25 C	% of 25-C growth
Arthrobacter	29/90	32.2
Pseudomonas I	4/9	44.4
Pseudomonas III	97/206	47.1
Pseudomonas II	59/109	54.1
Moraxella	268/466	57.5
Flavobacterium-		
Cytophaga	55/77	71.4
Acinetobacter	271/359	75.5

¹Combination of data from Tables 2 and 4.

²Proteus, Staphylococcus, Micrococcus, V. parahaemolyticus and E. coli tested grew equally well at 25 C and 35 C.

TABLE 6. COMPARISON OF 25- AND 5-C GROWTH OF MICRO-ORGANISMS ISOLATED FROM SEAFOODS¹

Microorganisms ²	No. colonies 5 C/25 C	% of 25-C growth
Flavobacterium-		
Cytophaga	46/77	59.7
Acinetobacter	264/359	73.5
Pseudomonas III	167/206	81.1
Arthrobacter	79/90	87.8
Moraxella	422/466	90.6
Pseudomonas II	105/109	96.3
Pseudomonas I	9/9	100.0

¹Combination of data from Tables 2 and 3.

²Proteus, Staphylococcus, Micrococcus, V. parahaemolyticus and E. coli tested did not grow at 5 C.

organisms also had been delayed (Acinetobacter on TSA at 5 C shown in Table 3).

In an attempt to neutralize the effect of NaCl and to examine the effects of incubation temperature alone, data from Tables 2, 3, and 4 were pooled and recovery of each microbial group at 35 and 5 C compared with that of 25 C (Tables 5 and 6). Microbial groups were listed in the order of sensitivity to the incubation temperatures. Therefore, an estimate of the detrimental effect of 35 C employed for APC could be made from data shown in Table 5. Similarly Table 6 lists the microorganisms in the order of sensitivity to 5 C.

The percentage of growth shown for each microbial group at 5 and 35 C in Tables 5 and 6 would have undoubtedly represented the maximum figures. The inoculum was heavy, the cultures were actively growing at the time of inoculation, and the colonies, well isolated from each other, were given ample time to develop. Such favorable conditions would be absent during initial isolation and this may account for the greater differences between reported counts and our data (Table 1). Vanderzant et al. (15) reported 1.6 to 3.7 logs difference between 25- and 37-C counts and a log difference was reported by Walker et al. (16) between 20 and 37 C counts of iced scampi. Our earlier study of TPE and PCA, also showed that TPE, as the initial isolation medium, yielded from 2 to 3 times more colonies than did PCA (10).

It is reasonable to assume that the order of sensitivity of each microbial group to 35 and 5 C, shown in Tables 5 and 6, would be proportionally reflected in the microbial flora of seafoods subjected to these temperatures. Indirect evidence in support of this has been provided by Farber and Lerke (6) who observed that pigmented microorganisms from fish fillet disappeared during refrigerated storage. Our data showed that pigmented *Flavobacterium-Cytophaga* species were most sensitive to 5 C (Table 6).

The microbial flora of four seafoods combined for this study were comparable to those shown by Shewan (13) for Northsea fish. The microbial flora of gulf coast oysters enumerated with TSA by Vanderzant et al. (15), however, contained higher percentages of Vibrio (22%) and Aeromonas (21%) than the Northsea fish, which contained less than 1% of each. We failed to recover psychrotrophic Vibrio or Aeromonas from the seafood samples tested for this study. We do not know, therefore, how Vibrio (except V. parahaemolyticus) and Aeromonas would have fared under our test conditions.

The microorganisms employed in this study were the primary isolates. Although it was intended that the experimental conditions duplicate those in nature as closely as possible, control was not possible over the types and the numbers of microorganisms to be tested. They were solely decided by the types and the numbers of microorganisms recoverable from the samples. Some of the microorganisms tested could therefore have been the progenies of a single clone. Efforts made to compensate for these limitations were: selection of different seafoods from different sources, inclusion in the test of all microorganisms that formed isolated colonies on initial isolation plates, and the large sample size. Nevertheless, the extent of redundancy is unknown and the data reported here should not be construed in a statistical sense.

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3-A SANITARY STANDARDS FOR FILLERS AND SEALERS OF SINGLE SERVICE CONTAINERS FOR MILK AND FLUID MILK PRODUCTS

Serial #17-04

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

It is the purpose of the IAMFES, USPHS, and DIC in connection with the development of the 3-A Sanitary Standards program to allow and encourage full freedom for inventive genius or new development. Specifications for Fillers and Sealers of Single Service Containers for Milk and Fluid Milk Products heretofore and hereafter developed which so differ in design, material, fabrication, or otherwise as not to conform with the following standards, but which, in the fabricator's opinion, are equivalent or better, may be submitted for the joint consideration of the IAMFES, USPHS, and DIC at any time.

Α.

SCOPE

A.1

These standards cover the sanitary aspects of equipment for performing all or a part of the following functions: mechanically opening, filling, and sealing single service containers and all parts which are essential to these functions that are furnished by the filler and sealer manufacturer. It does not pertain to other integral equipment embodied on certain machines which perform such functions as container fabricating; nor to the single service container.

A.2

In order to conform with these 3-A Sanitary Standards, fillers and sealers of single service containers shall comply with the following design, material, and fabrication criteria that are applicable.

B.

DEFINITIONS

B.1

PRODUCT: Shall mean the milk or fluid milk product which is filled into the container.

B.2

CONTAINER: Shall mean a single service package which is to be filled with the product.

B.3

MECHANICAL OPENING EQUIPMENT: Shall mean the equipment for opening a container without manual contact with any product contact surface of the container.

B.4

MECHANICAL FILLING EQUIPMENT: Shall mean the equipment for mechanically filling the container with the product.

B.5

MECHANICAL SEALING EQUIPMENT: Shall mean the equipment for mechanically closing and/ or sealing the filled container.

B.6

SURFACES

B.6.1

PRODUCT CONTACT SURFACES: Shall mean all surfaces which are exposed to the product, surfaces from which liquids may drain, drop or be drawn into the product or into the container, and surfaces that touch product contact surfaces of the container.

B.6.2

NON-PRODUCT CONTACT SURFACES: Shall mean all other exposed surfaces.

B.7

MECHANICAL CLEANING OR MECHANICAL-LY CLEANING: Shall denote cleaning solely by circulation and/or flowing chemical detergent solutions and water rinses onto and over the surfaces to be cleaned, by mechanical means.

B.8

ENGINEERING PLATING: Shall mean plated to specific dimensions or processed to specified dimensions after plating.¹

¹QQ-C-320a Federal Specification for Chromium Plating (Electrodeposited). July 26, 1954. Available from: General Services Administration, Seventh and D Streets, NW, Room 1643, Washington, D.C.

QQ-N-290 Federal Specification for Nickel Plating (Electrodeposited), April 5, 1954, and Amendment 1, December 13, 1961. Available from: General Services Administration, Seventh and D Streets, NW, Room 1643, Washington, D.C.

C.

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MATERIALS

C.1

All product contact surfaces shall be of stainless steel of the AISI 300 series² or corresponding ACI³ types (See Appendix, Section E.), or metal which under conditions of intended use is at least as corrosion resistant as stainless steel of the foregoing types and is non-toxic and non-absorbent, except that:

C.1.1

Optional metal alloy may be used but only in applications requiring disassembly and manual cleaning. (See Appendix, Section F. for the composition of an acceptable optional metal alloy.)

C.1.2

Those surfaces of container opening, closing and sealing devices which touch the product contact surfaces of the container or from which liquids may drain or drop into the container may be made of a non-toxic, non-absorbent metal that is corrosion resistant under conditions of intended use or may be made of metal made corrosion-resistant and wear-resistant by a covering of an engineering plating of chromium or nickel or an equally corrosion and wear-resistant non-toxic metal.

C.1.3

The valve plugs of compression-type valves may be covered with rubber or rubber-like materials or plastic materials. Rubber or rubber-like materials and plastic materials used as a coating shall be of such composition as to retain their surface and conformation characteristics under conditions encountered in the environment of intended use and in cleaning and bactericidal treatment.

C.1.4

Rubber or rubber-like materials or plastic materials may be used for filling nozzles, plungers, bonded or removable gaskets, diaphragms, sealing rings, rollers, belts, drip shields, protective caps for sanitary connections, container opening and closing parts, filling valve members, seals and parts used in similar applications. Plastic materials may be used for short flexible transparent connectors.

C.1.5

Rubber and rubber-like materials when used for

specified applications shall comply with the applicable provisions of the "3-A Sanitary Standards for Multiple-Use Rubber and Rubber-Like Materials Used as Product Contact Surfaces in Dairy Equipment, Serial #18-00."

C.1.6

Plastic materials when used for specified applications shall comply with the applicable provisions of the "3-A Sanitary Standards for Multiple-Use Plastic Materials Used as Product Contact Surfaces for Dairy Equipment, Serial #20-00," as amended.

C.1.7

Silver solder material shall be non-toxic and corrosion resistant.

C.1.8

Single service gaskets of a sanitary type may be used.

C.2

All non-product contact surfaces shall be of corrosion-resistant material or material that is rendered corrosion-resistant. If coated, the coating used shall adhere. All non-product contact surfaces shall be relatively non-absorbent, durable and cleanable. Parts removable for cleaning having both product contact and non-product contact surfaces shall not be painted.

D.

FABRICATION

D.1

All product contact surfaces shall be at least as smooth as a No. 4 finish on stainless steel sheets. (See Appendix, Section G.)

D.2

All permanent joints in product contact surfaces shall be welded or may be silver soldered if welding is not feasible. An exception is made to the foregoing for product connections which may have rolled-on sanitary pipeline ferrules or flanges. All welded or silver soldered areas of product contact surfaces shall be at least as smooth as the adjoining surfaces.

D.3

The minimum thickness of engineering plating shall be 0.0002-inch for all product contact surfaces except that when the parts listed in C.1.2 that are to be plated are other than stainless steel, the minimum thickness of the engineering plating shall be 0.002-inch.

D.4

All product contact surfaces shall be easily accessible, visible, and readily cleanable, either when in an assembled position or when removed. Re-

²The data for this series are contained in the following reference: AISI Steel Products Manual, Stainless & Heat Resisting Steels, April 1963, Table 2-1, pp. 16-17. Available from American Iron and Steel Institute, 150 East 42nd Street, New York, N.Y. 10017.

³Alloy Casting Institute Division, Steel Founders' Society of America, 21010 Center Ridge Road, Rocky River, OH 44116.

movable parts shall be readily demountable. Fillers designed to be mechanically cleaned shall be accessible for manual cleaning and inspection.

D.5

All product contact surfaces shall be self-draining or self-purging except for normal clingage. The bottom of the filler bowl shall have a minimum pitch of 1/8 inch per foot toward the plane of the outlets.

D.6

The filler bowl shall be equipped with a cover having a drop-flange which overlaps the rim of the bowl by at least 3/8 inch. The edges of all openings in the bowl cover shall extend upward at least 3/8 inch or be fitted with a permanently attached sanitary pipeline connection conforming to D.13. Openings in the bowl cover, except those fitted with a permanently installed sanitary pipeline connection, shall be provided with covers having a downward flange of not less than 1/4 inch so designed as to prevent liquid from entering the filler bowl. Covers shall be self-draining.

D.7

The filling equipment shall be so designed that adjustments necessary during the operation may be made without raising or removing the filler bowl cover(s).

D.8

Rubber or rubber-like materials and plastic materials having product contact surfaces that are a covering or a gasket to be bonded shall be bonded in such a manner that the bond is continuous and mechanically sound, and so that when exposed to the conditions encountered in the environment of intended use and in cleaning and bactericidal treatment, the rubber and rubber-like material or the plastic material does not separate from the base material. The final bond shall conform to the criteria in C.1.5 or C.1.6.

D.9

Any gasket groove or gasket retaining groove shall not exceed 1/4 inch in depth or be less than 1/4 inch wide except those for standard O-Rings smaller than 1/4 inch.

D.10

All internal angles of 135° or less on product contact surfaces shall have minimum radii of 1/4inch, except that:

D.10.1

Where smaller radii are required for essential functional reasons, such as those in filler nozzles. In no case shall such radii be less than 1/32 inch.

D.10.2

The minimum radii in gasket grooves or gasket retaining grooves other than those for standard 1/4inch and smaller O-Rings shall be not less than 1/8 inch.

D.10.3

The minimum radii in grooves for standard 1/4 inch O-Rings shall be not less than 3/32 inch and for standard 1/8 inch O-Rings shall be not less than 1/32 inch.

D.11

Shields or guards shall be provided and shall be so designed and located to prevent liquid or other contaminants from draining or dropping into the container or product, or onto product contact surfaces.

D.12

There shall be no threads on product contact surfaces.

D.13

All sanitary fittings and connections shall conform with the applicable provisions of the "3-A Sanitary Standards for Fittings Used on Milk and Milk Products Equipment and Used on Sanitary Lines Conducting Milk and Milk Products, Serial #08-09", as amended and supplements thereto except that sanitary fittings made of optional metal alloy shall not be used if the filler is designed for mechanical cleaning.

D.14

Coil springs having product contact surfaces shall have at least 3/32 inch openings between coils including the ends when the spring is in a free position. Coil springs shall be readily accessible for cleaning and inspection.

D.15

The filler shall be mounted on legs or casters that will provide a clearance between the lowest fixed point on the filler and the floor of at least 4 inches when the base outlines an area in which no point is more than $12 \ 1/2$ inches from the nearest edge, or a clearance of at least 6 inches when any point is more than $12 \ 1/2$ inches from the nearest edge.

D.15.1

Legs, if provided, shall be smooth with rounded ends and have no exposed threads. Legs made of hollow stock shall be sealed.

D.15.2

Casters, if provided, shall be durable and of a size that will permit easy movement of the filler.

D.16

Any guard(s) required by a safety standard that

F.

will not permit accessibility for cleaning and inspection, shall be designed so it (they) can be removed without tools.

D.17

Non-product contact surfaces shall be smooth, free of pockets and crevices and be readily cleanable and those to be coated shall be effectively prepared for coating.

D.18

A defoamer system, if provided, shall comply with the applicable parts of the following:

D.18.1

Steam defoamer systems shall be provided with a suitable self-draining water condensation trap and strainer on the steam supply line just prior to the defoamer head. The defoamer head shall be constructed in conformance with D.4. (See Appendix, Section H. for suggested design of water condensation trap and strainer and recommendations.)

D.18.2

A vacuum system designed to return foam continuously to the filler bowl. In this type all surfaces from which foam may drain, drop or be drawn into the product shall be constructed in conformance with D.4. All surfaces of blower or vacuum lines subject to contact with foam shall be constructed in such a manner as to be readily accessible for cleaning. (See Appendix, Section I. for suggested design.)

D.18.3

A vacuum system designed not to return foam to the filler bowl. In this type all surfaces from which foam may drain, drop or be drawn into the product or the sanitary container shall conform with D.4. All surfaces of blower or vacuum lines subject to contact with foam shall be constructed in such a manner as to be readily accessible for cleaning. (See Appendix, Section J. for suggested design and recommended operation.)

APPENDIX

E.

STAINLESS STEEL MATERIALS

Stainless steel conforming to the applicable composition ranges established by $AISI^2$ for wrought products, or by ACI^3 for cast products, should be considered in compliance with the requirements of Section C.1 herein. Where welding is involved the carbon content of the stainless steel should not exceed 0.08%. The first reference cited in C.1 sets forth the chemical ranges and limits of acceptable stainless steels of the 300 series. Cast grades of stainless steel corresponding to types 303, 304, and 316 are designated CF-16F, CF-8 and CF-8M, respectively. These cast grades are covered by $ASTM^4$ specifications A 296-68 and A 351-70.

OPTIONAL METAL ALLOY

An optional metal alloy having the following minimum and maximum composition is deemed to be in compliance with C.1.1 herein.

Zinc	— 8% maximum
Nickel	- 19 1/2% minimum
Tin	- 3 1/2% minimum
Lead	— 5% maximum
Iron	-1 1/2% maximum
Copper	- the balance

An alloy of the composition given above is properly designated "nickel silver," or according to ASTM⁴ Specification B 149-70, may be entitled, "leaded nickel bronze."

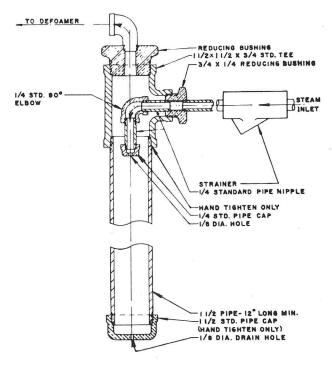
G.

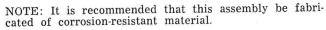
PRODUCT CONTACT SURFACE FINISH

Surface finish equivalent to 150 grit or better as obtained with silicon carbide, is considered in compliance with the requirements of Section D.1 herein.

APPENDIX, SECTION H.

SELF DRAINING WATER CONDENSATION TRAP

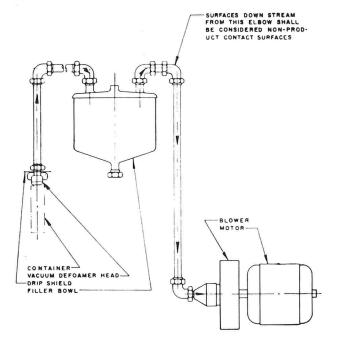




⁴Available from American Society for Testing and Materials, 1916 Race Street, Philadelphia, PA 19103.

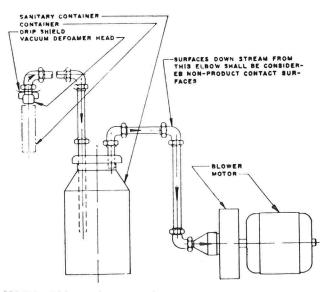
APPENDIX, SECTION I. VACUUM DEFOAMER SYSTEM

CONTINUOUS RETURN TYPE



NOTE: This entire assembly, including blower, should be cleaned after each day's operation.

APPENDIX, SECTION J. VACUUM DEFOAMER SYSTEM NON-RETURN TYPE



NOTE: This entire assembly, including blower, should be cleaned after each day's operation.

i.

К.

HANDLING OF COLLECTED MILK

If the milk or milk product collected in the defoamer system is intended to be used for human consumption, the following procedures are recommended:

к.1

It should be protected from contamination during collection and in subsequent handling.

K.2

It should be maintained at or below the legal temperature requirement for milk for pasteurization.

К.3

It should be repasteurized.

These Standards are effective Feb. 12, 1975, at which time the "3-A Sanitary Standards for Fillers and Sealers of Single Service Containers for Milk and Fluid Milk Products, Serial #17-00" and the amendments to it are rescinded and become null and void.

3-A SANITARY STANDARDS FOR UNINSULATED TANKS FOR MILK AND MILK PRODUCTS

Serial #32-00

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

It is the purpose of the IAMFES, USPHS, and DIC in connection with the development of the 3-A Sanitary Standards program to allow and encourage full freedom for inventive genius of new developments. Specifications for uninsulated tanks heretofore and hereafter developed which so differ in design, material, fabrication, or otherwise as not to conform with the following standards, but which, in the fabricator's opinion, are equivalent or better, may be submitted for the joint consideration of the IAMFES, USPHS, and DIC at any time.

Α.

SCOPE

A.1

These standards cover the sanitary aspects of uninsulated tanks, both open top and closed types and both single and multiple compartment types, that are intended to be used for one of the following purposes:

A.1.1

Mixing of milk and milk products and ingredients.

A.1.2

Storage of milk and milk products in tanks intended to be located in a room to which the air surrounding the tank will maintain the product temperature.

A.1.3

Storage of milk and milk products in tanks having heat exchange surface to maintain the product temperature.

A.1.4

As a raw product constant level tank in a pasteurizing. system.

A.2

In order to conform with these 3-A Sanitary Standards, uninsulated tanks shall comply with the following design, material, fabrication, and cleaning criteria.

B.

DEFINITIONS

B.1

B.2

Product: Shall mean milk, milk products and ingredients.

Uninsulated Tank: Shall mean a cylindrical, rec-

tangular, oval or other equally satisfactory shape tank that is not insulated, used for the storage or mixing and storage of product or used as a raw product constant level tank in a pasteurizing system.

B.3

Open Top Type Tank: Shall mean a tank that (1) can only be operated at atmospheric pressure and (2) the opening(s) for inspection and/or access for manual cleaning have removable or hinged covers other than pressure type cover(s) such as a manhole cover(s).

B.4

Closed Type Tank: Shall mean a tank that (1) can be operated at atmospheric pressure or at a pressure above or below that of the atmosphere and (2)the opening(s) for inspection and/or access for manual cleaning is a manhole(s) with a pressure type cover(s).

B.5

SURFACES

B.5.1

Product Contact Surface: Shall mean all surfaces which are exposed to the product and surfaces from which liquids may drain, drop, or be drawn into the product.

B.5.2

Non-Product Contact Surfaces: Shall mean all other exposed surfaces.

B.6

Mechanical Cleaning or Mechanically Cleaning: Shall denote cleaning, solely by circulation and/or flowing chemical detergent solutions and water rinses onto and over the surfaces to be cleaned, by mechanical means. C. MATERIALS

C.1

All product contact surfaces shall be of stainless steel of the AISI 300 series¹ or corresponding ACI² types (See Appendix, Section E.), or metal which under conditions of intended use is at least as corrosion resistant as stainless steel of the foregoing types and is non-toxic and non-absorbent, except that:

C.1.1

Rubber and rubber-like materials may be used for umbrellas for vertical agitator assemblies, slingers, drip shields, agitator seals, bearings, O-Rings, seals, gaskets, and parts used in similar applications. These materials shall comply with the applicable provisions of the "3-A Sanitary Standards for Multiple-Use Rubber and Rubber-Like Materials Used as Product Contact Surfaces in Dairy Equipment, Serial #18-00."

C.1.2

Plastic materials may be used for umbrellas for vertical agitator assemblies, slingers, drip shields, agitator seals, bearings, O-Rings, seals, gaskets, direct reading gauge tubes, in sight and/or light openings and parts used in similar applications. These materials shall comply with the applicable provisions of the "3-A Sanitary Standards for Multiple-Use Plastic Materials Used as Product Contact Surfaces for Dairy Equipment, Serial #20-00," as amended.

C.1.3

Where materials having certain inherent functional properties are required for specific applications, such as bearing surfaces and rotary seals, carbon, and/or ceramic materials may be used. Ceramic materials shall be inert, non-porous, non-toxic, non-absorbent, insoluble, resistant to scratching, scoring, and distortion when exposed to the conditions encountered in the environment of intended use and in cleaning and bactericidal treatment.

C.1.4

Glass may be used in sight and/or light openings and for direct reading gauge tubes and when used shall be of a clear heat resistant type.

C.1.5

Sanitary fittings shall be made of materials pro-

vided for in the "3-A Sanitary Standards for Fittings Used on Milk and Milk Products Equipment and Used on Sanitary Lines Conducting Milk and Milk Products, Revised, Serial #08-09," as amended and supplements thereto.

C.1.6

Single-service sanitary type gaskets may be used.

C.2

All non-product contact surfaces shall be of corrosion-resistant material or material that is rendered corrosion-resistant. If coated, the coating used shall adhere. All non-product contact surfaces shall be relatively non-absorbent, durable and cleanable. Parts removable for cleaning having both product contact and non-product contact surfaces shall not be painted.

FABRICATION

D.1

D.

All product contact surfaces shall be at least as smooth as a No. 4 finish on stainless steel sheets. (See Appendix, Section F.)

D.2

All permanent joints in product contact surfaces shall be welded except that rolled on sanitary pipeline ferrules or flanges may be used on connections. All welded areas of product contact surfaces shall be at least as smooth as the adjoining surfaces.

D.3

All product contact surfaces shall be easily accessible for cleaning, either when in an assembled position or when removed. Removable parts shall be readily demountable.

D.4

All product contact surfaces shall be self draining except for normal clingage.

D.4.1

The bottom of a vertical tank designed for mechanical cleaning, if flat, shall have a minimum slope of 3/4 inch per foot toward the outlet, or if the bottom of the tank is of the reverse dish-type, the portion of the bottom adjacent to the sidewall shall have a minimum slope of 3/4 inch per foot toward the outlet.

D.4.2

Horizontal rectangular tanks designed for mechanical cleaning which have built-in bottom pitch, shall have a pitch of at least 1/4 inch per foot toward the outlet.

D.4.3

Horizontal tanks shall be so constructed that they will not sag, buckle, or prevent complete drainage

¹The data for this series are contained in the following reference: AISI Steel Products Manual, Stainless & Heat Resisting Steels, April 1963, Table 2-1, pp. 16-17. Available from American Iron and Steel Institute, 150 East 42nd Street, New York, N.Y. 10017.

²Alloy Casting Institute Division, Steel Founders' Society of America, 21010 Center Ridge Road, Rocky River, OH 44116.

of water when the tank has a pitch of not more than 1 inch in 100 inches.

D.4.4

The bottom of a raw product constant level tank to be used in a pasteurizing system shall have a built in pitch of at least 1/4 inch per foot toward the outlet.

D.5

The top head of a vertical tank designed for mechanical cleaning shall be dished or otherwise shaped so that it readily facilitates mechanical cleaning.

D.6

Gaskets shall be removable. Any gasket groove or gasket retaining groove shall not exceed 1/4 inch in depth or be less than 1/4 inch wide except those for standard O-Rings smaller than 1/4 inch.

D.7

All internal angles of 135° or less on product contact surfaces shall have minimum radii of 1/4 inch, except that:

D.7.1

Radii in agitator shaft bottom guide bearings and in gasket grooves or gasket retaining grooves other than those for standard 1/4 inch and smaller O-Rings shall be not less than 1/8 inch.

D.7.2

Radii in grooves for standard 1/4 inch O-Rings shall be not less than 3/32 inch and for standard 1/8 inch O-Rings shall be not less than 1/32 inch.

D.7.3

The radius at the juncture of the end(s), side wall(s), top and bottom shall not be less than 3/4 inch.

D.8

There shall be no threads on product contact surfaces.

D.9

All sanitary fittings and connections shall conform with the applicable provisions of the "3-A Sanitary Standards for Fittings Used on Milk and Milk Products Equipment and Used on Sanitary Lines Conducting Milk and Milk Products, Serial #08-09," as amended and supplements thereto.

D.10

Covers Covers shall be furnished for all openings in the tank except those fitted with permanently attached sanitary fittings.

D.10.1

Covers and Bridges for Open Top Type Tanks.

D.10.1.1

Covers (1) shall be sufficiently rigid to prevent buckling, (2) shall be self draining, (3) shall be provided with an adequate, conveniently located and durable handle(s) of sanitary design, which is welded in place or formed into the cover material, (4) unless gasketed, shall have downward flanges not less than 3/8 inch along all edges, except that covers of raw product constant level tanks to be used in pasteurizing systems shall have downward flanges not less than 1/2 inch, and (5) shall be close fitting. If the cover is not gasketed, the clearance between the surface of the cover and the surface of the tank it is designed to contact shall not exceed 3/32 inch.

D.10.1.1.1

Non-removable covers (1) shall be of a type that can be opened and maintained in an open position, (2) shall be designed so that when the covers are in any open position liquid from the upper surface will not drain into the tank and (3) shall be designed so that when the covers are in their fully opened position, drops of condensation on the underside will not drain into the tank.

Covers of openings that will be held in place by gravity or vacuum may be of the lift-off type and may be provided with a clamp(s) or other device to maintain them in position.

D.10.1.1.2

Bridges and fixed covers shall pitch to the outside edge(s) of the tank for complete drainage, and shall have a raised flange not less than 3/8inch in height where the edge(s) meets the main cover(s). Bridges and fixed covers shall be integral or welded to the tank lining, and shall be installed so the underside is accessible for cleaning and inspection without completely entering the tank.

D.10.2

Covers for Closed Type Tanks.

Covers for manholes in side walls and/or ends shall be of the inside or outside swing type. If the cover swings inside, it shall also swing outside, away from the opening. Threads or ball joints employed to attach the manhole cover(s) and its appendages shall not be located within the tank. Covers for manholes in the top of tanks shall be of the outside swing type or be of a removable type.

D.10.3

All openings in the tank or in covers or in bridges shall be provided with overlapping removable covers, which are designed to make close contact with the upper edges of the opening or cover Sixty-Second Annual Meeting of 9AMJES

ROYAL YORK HOTEL, TORONTO, ONTARIO, CANADA AUGUST 10-13, 1975

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- 2. Type in the title, capitalize the first letter of the first word and of any proper nouns.
- 3. List authors and institution(s). Capitalize first letters and initials. Indicate with an asterisk the author who will present the paper. Give complete mailing address of the author who will present the paper.
- 4. Type the Abstract *double-spaced*, in the space provided on the Abstract form.
- 5. Mail two copies of the Abstract before February 15, 1975 to:

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6. Enclose *two* self-addressed standard post cards. One will be used to acknowledge receipt of the Abstract and the other to notify the speaker about the scheduling of the paper. Two cards must be included with *each* Abstract that is submitted.

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Annual Meeting

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

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surface, and when in a non-removable main cover the removable cover(s) shall remain in position when the main cover is in an open position. An exception to the foregoing requirement is made for openings fitted with permanently attached sanitary fittings.

D.10.4

An umbrella or drip shield of sanitary design that can be raised or dismantled, to permit cleaning of all of its surfaces, shall be provided to protect against the entrance of dust, oil, insects and other contaminants into the tank through the space around the agitator shaft.

D.11

Openings

All openings in the tank shall be within a processing area. The edges of all openings in the tank or in a cover or in a bridge that are upward or horizontal, shall extend upward or outward at least 3/8 inch beyond the tank or be fitted with a permanently installed sanitary pipeline fitting. In the cover for a raw product constant level tank to be used in a pasteurizing system, the opening(s) for an appurtenance(s) or a sanitary pipe(s) shall be not more than 1/4 inch larger in diameter than the entering sanitary pipe or appurtenance.

D.11.1

The main opening(s) of tanks shall be of sufficient number, adequate in size, and so located that all product contact surfaces are easily accessible and, except for the product contact surfaces of parts removable for cleaning, can be inspected visually without completely entering the tank.

D.11.2

An exception to the requirements of D.11.1 is made for closed type tanks having product contact surfaces that cannot be manually cleaned and inspected without entering the tank. The minimum inside height of this type of tank shall be 42 inches and if the inside height exceeds 96 inches, means shall be provided for mechanically cleaning the product contact surfaces of the tank and all appurtenances thereto (See Appendix, Section I). This type of tank shall have a manhole opening(s) complying with the provisions of D.11.4.

D.11.3

Agitator openings: The opening for a vertical agitator shall have a minimum diameter of 1 inch on tanks which require removal of the agitator shaft for cleaning or be of a diameter that will

provide a 1 inch minimum annular space between the agitator shaft and the inside surface of the opening on a tank which does not require the removal of the agitator for cleaning.

D.11.4

A manhole(s) shall be provided in closed type tanks. The inside dimensions of the manhole opening shall not be less than 15 inches by 20 inches oval, or 18 inches diameter. The sleeve or collar of a manhole opening for an inside swing type manhole cover shall be pitched so that liquids cannot accumulate.

D.11.5

Sight and Light Openings: When provided, shall be of such design and construction that the inner surfaces drain inwardly; and if the tank is designed for mechanical cleaning, the inner surface of the glass (or plastic) shall be relatively flush with the inner surface of the tank. The inside diameter of the opening shall be at least 3 3/4 inches. The external flare of the opening shall be pitched so that liquid cannot accumulate.

D.11.6

Thermometer Openings or Connections.

D.11.6.1

Tanks designed to be used in applications found in A.1.2. and A.1.3 shall be provided with one or more fittings to accommodate indicating and/or recording thermometer temperature sensing devices.

D.11.6.2

They shall conform to one of the following types:

D.11.6.2.1

Fittings conforming to thermometer well supplements to "3-A Sanitary Standards for Instrument Fittings and Connections Used on Milk and Milk Products Equipment, Serial #09-00", as amended.

D.11.6.2.2

Fittings for temperature sensing devices which do not pierce the tank lining, either temperature sensing element receptacles securely attached to the exterior of the lining or means to attach temperature sensing elements securely to the exterior of the lining.

D.11.6.3

The fittings for temperature sensing devices shall be located to permit the registering of the temperature of the product when the tank contains no more than 20 percent of its capacity; and if the tank has heat exchange surface, they shall be located so that the sensing element is not influenced by the heating or cooling medium.

D.11.7

A fitting for a pressure sensor, if provided, shall be installed so that the sensor will be relatively flush with the inner surface of the tank.

D.11.8

Covers of raw product constant level tanks to be used in pasteurizing systems that are 18 inches or more in diameter, or more than 2 square feet in horizontal cross section area shall be provided with an inspection port or opening at least 4 inches in diameter.

D.12

Inlets and Outlets

D.12.1

The inlet shall be an opening(s) to accommodate at least 1 1/2 inch 3-A sanitary tubing or be a sanitary pipeline connection at least as large as a 1 1/2inch 3-A sanitary fitting.

D.12.2

The outlet shall provide complete drainage of the tank. If the tank is designed for mechanical cleaning or if it is designed to be used as a raw product constant level tank in a pasteurizing system, the top of the terminal end of the outlet passage shall be lower than the low point of the inner surface of the tank bottom at the outlet. The outside diameter of the outlet opening shall be at least as large as that of 1 1/2 inch 3-A sanitary tubing.

D.12.3

Outlet and permanently installed inlet connections in the tank shall be provided with welding stub ends, bolted or clamp type flanges or 3-A sanitary threaded connections. The face of a bolted or clamp type flange or a 3-A sanitary threaded connection below the maximum normal product level shall be as close as practical to the outer shell of the tank. (See Appendix, Section G and Section H.)

D.13

Agitation

Unless otherwise specified, means for mechanical and/or air agitation of product shall be provided that when operated intermittently or continuously shall be sufficient to maintain the butterfat content of whóle milk throughout the tank within a variation of plus or minus 0.1 percent as determined by an official AOAC Milk Fat Test.³ The agitator, if not designed for mechanical cleaning, shall be located in such a manner that it shall be readily accessible and removable for manual cleaning.

Mechanical agitators may be of a vertical or horizontal type. They shall comply with the applicable provisions of D.13.1, D.13.2, and D.13.3.

D.13.1

Vertical mechanical agitators. The agitator, if removable, shall be provided with an easily accessible, readily demountable coupling of either a sanitary type located within the tank or a coupling located outside the tank provided that it is above the umbrellas provided to protect the annular space around the shaft. A bottom support or guide, if used, shall be welded to the tank and shall not interfere with drainage of the tank and the inside angles shall have minimum radii of 1/8 inch. When the agitator shaft has a bearing cavity, the diameter of the cavity shall be greater than the depth.

D.13.2

Horizontal mechanical agitators, if not designed for mechanical cleaning, shall be readily accessible and removable for manual cleaning. 0

D.13.3

A seal for the agitator shaft, if provided, shall be of a packless type and sanitary in design. A seal for the agitator shaft shall be provided for (1)a horizontal agitator and (2) an agitator in a tank having means for mechanically cleaning the tank.

D.13.4

The means for air agitation or movement of product, which is supplied as an integral part of the tank shall comply with D.14.

D.14

Air for Agitation or Movement of Product

Means for applying air under pressure shall conform to the applicable provisions of the "3-A Accepted Practices for Supplying Air Under Pressure in Contact with Milk, Milk Products and Product Contact Surfaces, Serial #604-03", and the following:

D.14.1

Clamp type fittings shall not be used within the tank.

D.14.2

Tubing and related fittings within the tank shall be readily and easily removable for cleaning outside the tank or be designed for mechanical cleaning. If designed for mechanical cleaning, the tubing and all related fittings shall be self-draining.

³The method of making these tests will be found in the following reference: Official Methods of Analysis: Available from the Association of Official Analytical Chemists, P.O. Box 540, Benjamin Franklin Station, Washington, D.C. 20044.

D.14.3

Permanently mounted air tubing shall be constructed and installed so that it will not sag, buckle, vibrate or prevent complete drainage of the tank or tubing and shall be located so that the distance from the outside of the tubing to the inside of the tank shall be at least two inches, except at point of entrance.

D.15

Mechanical Agitator Driving Mechanism Mounting: The driving mechanism when above the tank shall be securely mounted in a position that will provide a minimum distance of 4 inches measured vertically downward from the bottom of the driving mechanism housing, excluding bearing bosses and mounting bosses to the nearest surface of the tank; and in such a manner that all surfaces of the tank under or adjacent to the driving mechanism shall be readily accessible for cleaning and inspection.

D.16

Vents Closed type tanks shall be provided with a hooded sanitary vent of sufficient diameter to prevent back pressure during filling and to prevent vacuum during emptying of the tank. It shall be in the front head near the top of the tank or in the top of the tank or in the sidewall of a vertical tank near the top of the tank. It shall be provided with a perforated cover having openings not greater than 1/16 inch diameter, or slots not more than 1/32 inch wide. Woven wire mesh shall not be used for this purpose. It shall be so designed that parts are readily accessible and readily removable for cleaning. (See Appendix, Section J.)

D.16.1

In a vertical tank designed for mechanical cleaning, when the re-vent method or overflow line method is used to prevent siphonage, the terminal ends of the cleaning and/or vent line(s) and/or overflow line(s) shall be arranged or means provided to prevent liquids or objects being drawn up in the re-vent line.

D.17

Raw product constant level tanks to be used in a pasteurizing system shall be equipped with an automatic device of sanitary construction to control the raw product level.

D.18

Cleaning: Tanks having an inside height of more than 96 inches shall be provided with means for mechanically cleaning the product contact surfaces of the tank and all non-removable appurtenances thereto. (See Appendix, Section I.)

D.19

Sample Cock: A sample cock, if provided, shall be of a type that has its sealing surface relatively flush with the product contact surface of the tank and have an inside diameter no less than that of 1 inch 3-A sanitary tubing.

D.20

A direct reading gauge of the glass tube or plastic tube type, if provided, shall be sanitary in design and construction and shall be readily accessible for cleaning or shall be designed for mechanical cleaning. If designed for mechanical cleaning, the inside diameter of the gauge parts shall be sufficiently uniform that all product contact surfaces will be cleaned. It shall be designed and constructed so that all product in the gauge may be discarded. Means to accomplish this shall be provided at the lowest point and in such a manner that product in the gauge will not enter the tank outlet line nor re-enter the tank.

The valve shall be close coupled. The distance, measured along the passage for the product in the tank to the gauge valve, from the nearest point on the tank to the ferrule or flange for the valve shall not be more than the smaller of (1) twice the nominal diameter of the passage of (2) five inches.

D.21

Tank Supports

The means of supporting a tank shall be one of the following:

D.21.1

With legs: Adjustable legs shall be provided of sufficient number and strength and so spaced that the filled tank will be adequately supported. Legs shall be smooth with rounded ends and have no exposed threads. Legs made of hollow stock shall be sealed. Legs for tanks, except those for raw product constant level tanks to be used in a pasteurizing system, shall be such that the product outlet is sufficiently high to allow for adequate cleaning and will provide an 8 inch minimum clearance between the floor and the tank outlet valve or bracing whichever is lower. The legs of cylindrical horizontal tanks shall be installed so that the leg will be vertical when the tank lining is pitched 1/4 inch per foot toward the outlet.

D.21.2

Mounted on a slab or island: The base of the tank shall be such that it may be sealed to the mounting surface. (See Appendix, Section K.)

D.22

Any guard(s) required by a safety standard that will not permit accessibility for cleaning and inspection shall be designed so that it (they) can be removed without the use of tools.

D.23

Non-product contact surfaces shall be smooth, free of pockets and crevices and be readily cleanable and those to be coated shall be effectively prepared for coating. Outside welds need not be ground.

D.24

Tanks shall have an information plate in juxtaposition to the name plate giving the following information or the information shall appear on the name plate:

If the tank is or is not designed for mechanical cleaning.

APPENDIX

E.

STAINLESS STEEL MATERIALS

Stainless steel conforming to the applicable composition ranges established by AISI for wrought products, or by ACI for cast products, should be considered in compliance with the requirements of Section C.1 herein. Where welding is involved the carbon content of the stainless steel should not exceed 0.08%. The first reference cited in C.1 sets forth the chemical ranges and limits of acceptable stainless steels of the 300 series.

Cast grades of stainless steel corresponding to types 303, 304, and 316, are designated CF-16F, CF-8, and CF-8M, respectively. These cast grades are covered by $ASTM^4$ specifications A296-68 and A351-70.

F.

PRODUCT CONTACT SURFACE FINISH

Surface finish equivalent to 150 grit or better as obtained with silicon carbide, is considered in compliance with the requirements of Section D.1 herein.

G.

INLET AND OUTLET CONNECTIONS

The distance between the nearest point on the outside of the tank to (1) the face of a bolted or clamp type flange or (2) the face of a 3-A sanitary threaded connection on an inlet or outlet connection below the normal product level should not exceed the smaller of (1) twice the nominal diameter of the connection or (2) five inches.

н.

VALVES

Valves on inlet and outlet connections in the tank below the maximum normal product level should be of the close coupled plug-type or of the close coupled compression-type.

I.

MECHANICAL CLEANING

One cleaning method found to be satisfactory is to pump the cleaning solution to the dome of the tank or the upper portion of the tank surface, as the case may be, through stainless steel lines with C-I-P fittings or welded joints and distribute it in such a manner as to provide flooding over all interior surfaces. The tank should be installed with sufficient pitch to accomplish draining and to have a fast flushing action across the bottom (See D.4.1 and D.4.2) Means should be provided for manual cleaning of all surfaces not cleaned satisfactorily by mechanical cleaning procedures. NOTE: Cleaning and/or sanitizing solutions should be made up in a separate tank—not the storage tank.

J.

AIR VENTING

To insure adequate venting of the tank which will protect it from internal pressure or vacuum damage during normal operation, the critical relationship between minimum vent-size and maximum filling or emptying rates should be observed. The size of the free vent opening of a tank should be at least as large as those shown in the table below:

Minimum Free Vent	Maximum Filling or
Opening Size	Emptying Rate
(inches, I.D.)	(gallons per minute)
1-3/4	175
2-1/4	300
2-3/4	400

The above sizes are based on normal operation and are sized to accommodate air only and not liquid. A perforated vent cover, if used, should have a free opening area equal to at least 1-1/2 times the area of the vent opening in the tank. The venting system covered in the preceding paragraphs is intended to provide for venting during filling and emptying; however, it is not adequate during cleaning. During the cleaning cycle, tanks when cleaned mechanically should be vented adequately by opening the manhole door to prevent vacuum or pressure build up due to sudden changes in tem-

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⁴Available from American Society for Testing and Materials, 1916 Race Street, Philadelphia, Pa. 19103.

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perature of very large volumes of air⁵. Means should be provided to prevent excess loss of cleaning solution through the manhole opening.

The use of tempered water of about 95°F for both pre-rinsing and post-rinsing is recommended to reduce the effect of flash heating and cooling. Pro-

⁵For example, when a 6,000 gallon tank (with 800 cu. ft. of 135° F hot air after cleaning) is suddenly flash cooled by 50°F water sprayed at 100 gpm the following takes place: Within one second, the 800 cu. ft. of hot air shrinks approximately 51 cu. ft. in volume. This is the equivalent in occupied space of approximately 382 gallons of product. This shrinkage creates a vacuum sufficient to collapse the tank unless the vent, manhole, or other openings allow the air to enter the tank at approximately the same rate as it shrinks. It is obvious, therefore, that a very large air vent such as the manhole opening is required to accommodate this air flow. visions should be made to prevent overfilling with resultant vacuum or pressure damage to the tank.

к.

SLABS OR ISLANDS

When a tank is designed to be installed on a slab or an island, the dimensions of the slab or island should be such that the tank will extend beyond the slab or island at least one inch in all horizontal directions. The slab or island should be of sufficient height so that the bottom of the outlet connection is not less than 8 inches above the floor. The surface of the slab or island should be coated with a thick layer of waterproof mastic material, which will harden without cracking. The junction of the tank and the slab or island should be sealed.

These Standards are effective Feb. 12, 1975.

ATTENTION STUDENTS

You can receive the Journal of Milk and Food Technology for \$5.00 per year. To qualify you must be a full-time undergraduate or graduate student in Food Science, Dairy Science, Microbiology, Environmental Health, Public Health, or a related field. Send \$5.00 and a note from your advisor or major professor certifying your status as a student to: Mr. E. O. Wright, Executive-Secretary of IAMFES, Box 701, Ames, Iowa 50010. Your check should be sent by January 1, 1975.

EFFECT OF FERMENTED MEAT PH ON SUMMER SAUSAGE PROPERTIES

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Abstract

Beef summer sausages were fermented to pH endpoints ranging from 5.5 to 4.6. A nonfermented sausage of pH 5.9 served as control. Fermentation time requirements using a frozen concentrate of Pediococus cerevisiae ranged from 7-8 h (pH 5.5) to 19-21 h (pH 4.6). Water holding capacities (WHC) of sausage mixes during the fermentation phase rapidly decreased as sausage pH decreased, reaching a minimum at pH 5.2. An increase in WHC from pH 5.2 to pH 4.6 was attributed to the combined effect of pH reduction and remaining functional protein. Bacterial counts (total viable and lactic bacteria) showed a stepwise increase from 2.4 \times 10^6 cells/g (lactics) to 6.6×10^8 cells/g (lactics) during fermentation. Reductions of bacterial counts during heat processing appeared dependent on the sausage pH and phase of bacterial growth. Compositional changes (fat, protein, ash, salt, lactic acid) during the drying phase were significantly correlated to moisture loss of the sausages. Within drying intervals (days), sausages of pH groups 5.9 and 5.5 had significantly less weight loss and required lower shearing force when compared to pH groups 4.8 and 4.6. For summer sausages examined at 20 days of drying, panelists rated higher preference and "tanginess" scores as sausage pH decreased.

Fermented sausages possess good keeping qualities because of low pH and high salt content, and, in dry varieties, a low moisture content. Traditional production processes require 3 to 5 days for fermentation and processing before drying. Use of commercial starter cultures of *Pediococcus cerevisiae* and *Lactobacillus plantarum* in frozen concentrate form has significantly reduced fermentation time requirements to 15 to 24 h (2, 8). The controlled inoculation of lactic bacteria aids processors in maintaining uniform product characteristics from batch to batch (8, 19, 21). The fermentation phase is sometimes referred to as the "ripening" period (16).

The pH generally attained during fermentation is near 5.1 although a lower pH is desired for some products (16, 19). Final retail products range in pH from 4.8 to 5.4 (24). The isoelectric point of meat proteins (near 5.0) is approached in fermentation which aids. in moisture removal during the drying

phase. The water holding capacity of muscle proteins is at a minimum at the isoelectric point (10).

Acton et al. (1) reported that fermentation of summer sausage at either 22, 30, or 37 C did not significantly affect product flavor although less lactic acid was produced at 22 C than at 30 or 37 C. Lactic acid imparts the characteristic "tangy" flavor of fermented sausages (6, 19, 24). An acid content of 0.5 to 1.5% is generally reported for summer sausage, cervelat, and thuringer (1, 19, 23).

The solubility of meat proteins (15, 23), inhibition of pathogenic microorganisms (7), use of freeze-dried meat (18), and compositional changes during the fermentation and processing of fermented-dried sausages have been investigated.

This study was done to evaluate the effect of the pH attained during summer sausage preparation on (a) water holding capacity of the sausage mix, (b) bacterial counts on heat processing, (c) sausage weight loss ("shrinkage"), and (d) textural development during drying. Compositional changes during drying and taste panel evaluation of product flavor were also determined.

MATERIALS AND METHODS

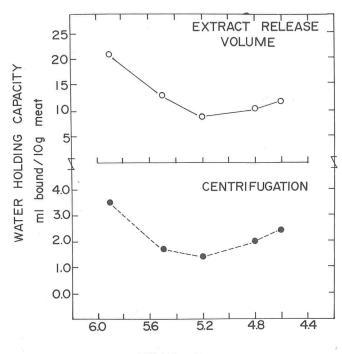
Sausage preparation and processing

A summer sausage formulation (Table 1) was used in this study. Fresh boneless beef (chucks) was obtained from the state-inspected Meats Laboratory of the Animal Science Department at Clemson University. The boneless beef was coarsely ground once through an 8-mm plate, mixed, and reground through a 6-mm plate. Packages of approximately 4.54 kg of meat were frozen at -20 C for 2 to 3 months. Thawing was done at room temperature (21 C) for 10 to 12 h followed by 6 to 8 h storage at 0 C. Proximate composition of the ground meat was 63.6% moisture, 16.9% protein, 17.3% fat, and 0.9% ash.

Sausage mixes were prepared by blending in a Hobart 4346 Mixer-Grinder equipped with two arm paddles. The curing agents, seasonings, and dextrose were blended into the meat for 4 min before addition of the starter culture. A suspension of *Pediococus cerevisiae* (LACTACEL, Merck & Co.) was added at a level of 2.4×10^6 cells/g meat and the meat mixture blended for an additional 6 min. The initial mix temperature was approximately 2 C and increased to approximately 10 C during the 10 min of blending at 29 rpm. Two replicate sausage batches were prepared in different weeks using the same lot of boneless beef,

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MEAT pH

Figure 1. Rate of pH reduction during fermentation of summer sausage.

Each sausage preparation was stuffed into 52 mm diameter D.S. fibrous casings (Union Carbide). The sausage chubs, each weighing approximately 540 g, were hung in a fermentation chamber maintained at 38 C and 95% relative humidity. One group of 12 chubs was not fermented but carried immediately through heat processing as described below. During the time course of fermentation, the meat pH was periodically monitored by examining randomly selected sausage chubs from the chamber. As the pH decreased by 0.2 to 0.4 pH unit, a set of 12 chubs were removed for immediate heat processing. Fermentation was terminated as close as possible at the following pH values: 5.5, 5.2, 4.8, and 4.6. The initial sausage group of the nonfermented mix had a pH of 5.9. It should be noted that approximately the same pH values were used for fermentation termination in the second replicate batch of sausages (Fig. 1). Approximate time requirements to reach these pH endpoints are also given in Table 2.

For heat processing, the sausage chubs were initially placed at 82 C for 2 h and then at 88 C until an internal temperature of 60 C was attained. Total heating time ranged from 3.0 to 3.3 h. The chubs were cooled to 16 C with a cold water spray and placed in a 7.5 ± 2 C drying room having 15 to 20 air changes/h. The air relative humidity ranged from 80 to 84%. Sausage chubs of each pH group were removed for analysis at 0, 5, 10, 15, and 20 days of drying.

pH and lactic acid determination

Duplicate 10-g samples of meat were blended for 60 sec with 100-ml quantities of distilled water in an Osterizer. The pH values of homogenates were recorded. Meat slurries were then titrated with 0.1 \times NaOH to an endpoint of pH 8.30. The mEq of NaOH required for titration of the initial nonfermented meat samples were subtracted from the total mEq required in titrating fermented samples. The developed acidity was assumed to be due to lactic acid production. The mEq of NaOH were converted to and expressed as percent lactic acid.

Water holding capacity

Sausage samples were evaluated during fermentation for water holding capacity (WHC). Two methods were utilized, an extract release volume (ERV) method and a centrifugation procedure.

The ERV method of Acton et al. (1) as modified from the initial procedure of Jay (12) was used for WHC determinations. ERV results were expressed as ml bound/10 g meat.

A centrifugation technique modified from Hamm (10) as reported by Wardlaw et al. (22) was also used to determine WHC. The WHC with this method was also expressed as ml bound/10 g meat.

Plate counts

Counts of total viable bacteria and of lactic acid bacteria were made on sausage samples at the following intervals of processing: (a) after initial blending of ingredients but before starter culture addition; (b) after inoculation and final blending of the sausage mix (pH of 5.9); (c) on attaining pH values of 5.5, 5.2, 4.8, and 4.6; (d) after heat processing each pH group to 60 C internal; and (e) after 20 days of drying.

Duplicate samples of 20 g of meat were blended with 180-ml quantities of 0.9% saline and subsequent decimal dilutions were prepared with the same diluent. Duplicate 1-ml samples of the appropriate dilutions were mixed with standard plate count agar (3) for total viable bacteria and the V-8 agar of Fabian et al. (9) for lactic acid bacteria. Plates were incubated at 30 C for 48 to 72 h before counting.

Sausage composition

Percentages of moisture, fat, protein, ash, and salt were determined for the initial sausage mixes and the products during drying. Moisture, fat, and ash were determined by AOAC (4) methods. The Kjeldahl nitrogen method following AOAC (4) was used for protein analysis. Total nitrogen obtained by Kjeldahl analysis was corrected for nonprotein nitrogen using 5% trichloroacetic acid filtrates. The salt content, expressed as NaCl, was measured with QUANTAB Chloride Titrators following the procedure of the AOAC (5) and Vander Werf and Free (25).

Weight loss and shear measurements

The percent weight loss or "shrink" of summer sausage chubs was determined after 3, 5, 8, 10, 15, and 20 days of drying. Five sausage chubs from each pH group were selected after heat processing for weight recording on drying.

Shear forces for slices of the sausage samples were measured with an Allo-Kramer Shear Press equipped with a 3000-lb ring. The press was operated at a downstroke of 30 sec and range 300. Meat slices were 4 mm in thickness. The shearing force was calculated as kg force/g sample/cm² surface area exposed to the shear blades. A surface area expression was included since the meat slices were of variable diameter during the drying phase.

Taste panel evaluation

Sausage slices of each of the five pH groups were evaluated for preference rating and "tanginess" flavor scores at the end of 20 days of drying. The slices were served in randomized order and at room temperature (21 C) to 8 panelists familiar with the flavor of fermented meat products. Panelists scored their preference ratings on a nine-point hedonic scale (1 = dislike extremely; 9 = like extremely). Tanginess, a descriptive flavor term commonly used with fermented meats (1, 6, 19), was also rated on a nine-point hedonic scale (1 = no tanginess of flavor; 9 = extremely tangy flavor). Panelists were informed before each session that acidity or sharpness of flavor could be described as tanginess. Two panel sessions were conducted on successive days using meat samples from

TABLE 1. SUMMER SAUSAGE INGREDIENTS

Ingredient	Quantity
Meat:	
boneless beef (chucks)	27.24 kg
Cure:	
sodium nitrite	2.10 g
sodium nitrate	4.20 g
sodium erythorbate	12.72 g
salt	680.40 g
Seasonings:	
ground black pepper	51.0 g
ground white pepper	51.0 g
mustard powder	51.0 g
sucrose	135.6 g
Starter materials:	
LACTACEL (diluted) ^a	138.0 m
dextrose	272.4 g

^aA frozen concentrate of *Pediococcus cerevisiae* prepared by Merck & Co., Rahway, N. J. A culture suspension is prepared by diluting 6 oz concentrate with 18 oz distilled water.

TABLE 2. SUMMER SAUSAGE PH AFTER FERMENTATION AND AFTER HEAT PROCESSING

Fermented		Heat pr	ocessed
Sausage pH group	Fermentation time, h	Sausage pH	Lactic acid (%)
5.9	0	5.95	0.04
5.5	7-8	5.4	0.50
5.2	10-11	5.1	0.67
4.8	13-14	4.75	0.76
4.6	19-21	4.55	1.03

different sausages of the same pH grouping. Statistical analyses

Results were analyzed using analysis of variance and the significance of means was tested by Duncan's method (20). Correlation analysis for the relationship between variables was included as part of the analyses.

RESULTS AND DISCUSSION

Fermentation phase: pH reduction and WHC response

Kramlich (16) has stated that the ". . .sausage pH, not time, is the chief factor determining the length of the ripening [fermentation] period." In this study, reference to sausage of a particular pH group refers to the pH endpoint selected to terminate fermentation. As shown in Table 2, the pH of the heat processed sausage differed from the pH of the fermented sausage by 0.05 to 0.10 pH unit. All results were analyzed and discussed using the fermented pH value for sausage grouping.

The rates of pH reduction for the replicate sausage fermentations are shown in Fig. 1. Fermentation with the frozen concentrate form of *Pediococcus cerevisiae* as used in this study proceeded in significantly less time than has been reported for the lyophilized culture form (1, 8). Sausage mixes are generally fermented to a pH near 5.1 (16) although a lower range of pH 4.5 to 4.8 may be desirable for some products

(19). Following heat processing and drying, the final retail product ranges in pH from 4.8 to 5.4 (24).

The internal temperature of the sausage chubs had reached 38 C within 6 h after placing in the fermentation chamber (Fig. 2). A 52-mm diameter casing was used for stuffing the sausages. Use of larger diameter casings would change the internal temperature pattern toward the center and possibly alter the rate of pH reduction.

The ERV and centrifugation methods for determining WHC during fermentation yielded similar responses (Fig. 3). Actual values obtained with each procedure were different although the principle of the methods was the same. The pressure exerted to free "loose" or "mobile" water was: (a) for ERV, normal gravity, and (b) for centrifugation, centrifugal force.

The WHC in either instance rapidly decreased as the sausage pH decreased, reaching a minimum at pH 5.2 (Fig. 3). Hamm (10) reported that the WHC minimum in fresh beef occurs near pH 5.0, corresponding to the approximate isoelectric point of actomyosin. The increase of WHC after pH reduction below 5.2 suggests that some of the meat protein remained functional to bind moisture and was not completely denatured by the 38 C temperature of fermentation. Hamm and Deatherage (11) reported that mild denaturation of protein occurs between 30 and 40 C, followed by substantial denaturation between 50 and 60 C. In the study of a similar summer sausage mix, Wardlaw et al. (23) found approximately 52% of the myofibrillar protein fraction still extractable after 36 h of meat fermentation at

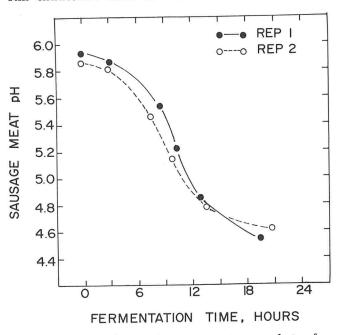


Figure 2. Rate of internal temperature increase during fermentation of summer sausage in a 52-mm diameter casing.

TABLE S	3. Тот.	AL VIABLE	AND	LACT	IC ACI	D BACTERIA	COUNTS ^a
	AFTER	FERMENTA	TION	AND	HEAT	PROCESSING	

	End of fer	mentation	End of heat	processing ^b	
Meat sample	Total viable count	Lactic bacteria count	Total viable count	Lactic bacteria count	
Boneless					
beef	1.4×10^5	$< 10^{1}$		-	
Summer				2	
sausage:					
pH 5.9°	8.6×10^{6}	2.4×10^6	2.2×10^{6}	1.8×10^{6}	
pH 5.5	5.3×10^7	3.5×10^7	3.7×10^4	5.4×10^4	
pH 5.2	1.9×10^{8}	6.9×10^7	1.9×10^6	1.2×10^{6}	
pH 4.8	6.0×10^{8}	5.8×10^8	1.2×10^{6}	8.1×10^{5}	
pH 4.6	6.3×10^{8}	6.6×10^{8}	1.9×10^{6}	1.1×10^{6}	

^aBacterial counts are cell numbers/g sample.

^bInternal temperature of 60 C.

^eNot fermented; immediately heat processed after starter culture inoculation.

38 C. Thus the initial decline in WHC (Fig. 3) was likely partly due to the combined effect of pH reduction and partial denaturation of protein whereas the increase observed at pH values below 5.2 may have been due to increased effectiveness of remaining functional protein. As pH values decrease below the isoelectric pH, increased WHC may occur (10). Further study is needed on the relationship between extent of protein denaturation and the protein's functional properties.

Bacterial changes during processing

The counts of total viable bacteria and lactic acid bacteria at intervals during summer sausage processing are given in Table 3. The boneless beef contained 10⁵ cells/g total viable bacteria of which less than 10¹ cells/g were lactic acid bacteria. The initial sausage mix (pH 5.9) for each replicate was inoculated at an average level of 2.4×10^{6} cells/g in lactic acid bacteria. After inoculation the total viable counts for the samples were essentially the same as the lactic counts. During fermentation to sausage pH values of 5.5, 5.2, 4.8, and 4.6, there was a stepwise increase in lactic bacteria counts to the highest level of 6.6×10^{8} cells/g mix.

Heat processing the sausages to an internal temperature of 60 C resulted in varied responses in the total viable and lactic bacteria counts. Each response appeared dependent on the pH value of the sausage meat. In addition, the phase of bacterial growth was considered in the following discussion.

The initial sausage mix with pH 5.9 was immediately heat processed after inoculation and, as shown in Table 3, no significant reduction in total viable or lactic bacteria counts occurred. Since the inoculum was not in a growth phase, having been freshly introduced to the sausage mix, it is possible to conclude

that the meat environment afforded protection against a significant reduction in cell numbers.

The sausage mix of pH 5.5 (fermented for 7 to 8 hr) had increased in lactic bacteria cell numbers by approximately 1-log cycle when removed for heat processing. Counts of both total viable and lactic acid bacteria in the heat processed sausage showed a 3.2 to 3.4-log reduction in cell numbers for each type of count. In the early period of exponential growth of the lactics, the organisms are most sensitive to sudden environmental changes (17) and are easily killed by heat, thus accounting for the significant reductions in bacterial counts.

Heat processing of sausage mixes fermented to pH 5.2, 4.8, and 4.6 generally showed reductions of 2.0 to 2.7-log cycles in total viable counts and 1.7 to 3.2log cycle reductions in lactic bacteria counts. These final counts were not significantly different from each other or from the final counts of the sausage of pH 5.9. Wardlaw et al. (23) reported a 5-log reduction in lactic acid bacteria counts in summer sausage processed to 71 C internal. Keller and Acton (13) also reported a 5-log reduction in counts of lactic bacteria for a turkey sausage mix heated to 71 C. Their results showed that the greatest destruction of bacteria occurred between 49 C and 60 C during heat processing. Although not reported in Table 3, sausage chubs examined at 20 days of drying had no significant differences in total viable or lactic bacteria counts when compared to those counts found immediately after heat processing.

Sausage properties on drying

The average composition of the summer sausages during processing is given in Table 4. It should be

TABLE 4. AVERAGE COMPOSITION OF SUMMER SAUSAGES DURING PROCESSING TO DRY PRODUCT STAGE

Product stage	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	NaCl (%)
Boneless beef	63.61	16.95	17.32	0.91	Trace
Fermented sausage	64.50	13.43	15.55	3.22	2.43
0 days drying	62.24	16.79	16.47	3.39	2.37
20 days drying	40.59	24.35	27.26	5.96	4.20

Table 5. Lactic acid content of summer sausages in each pH group during drying^a

	5	Perc	cent lactic a	acid	
Saugago		Da	ys of dryi	ng	
Sausage	0	5	10	15	20
5.9	0.04ª	0.08ª	0.11ª	0.16ª	0.23ª
5.5	0.50 ^b	0.83 ^b	1.06 ^b	1.30^{b}	1.50 ^b
5.2	0.67 ^{bc}	0.98 ^b	1.24 ^b	1.64°	1.85°
4.8	0.76°	1.22°	1.53°	1.82^{d}	1.95°
4.6	1.03 ^a	1.50^{a}	1.77°	2.04^{d}	2.21^{d}

*Any two means within a column having one of the same letters are not significantly different at p < 0.05.

~			Percent	weight loss (shri	ink)		
Sausage - pH group	0	3	5	8	10	15	20
5.9	0.00	9.61 ^{ab}	16.76 ^{ab}	24.14 ^a	27.50ª	35.81ª	39.71ª
5.5	0.00	8.60ª	16.48 ^a	24.31ª	27.93 ^{ab}	36.60 ^{ab}	41.48 ^b
5.2	0.00	9.00ª	17.62 ^{bc}	25.52 ^b	29.13°	37.99°	42.70 ^{bc}
4.8	0.00	10.65 ^b	17.76 ^{bc}	25.58^{b}	28.96 ^{bc}	37.56 ^{bc}	42.20 ^{bc}
4.6	0.00	10.36 ^b	17.82°	25.68 ^b	29.86°	38.54°	43.06°

TABLE 6. WEIGHT LOSS (SHRINK) OF SUMMER SAUSAGES IN EACH PH GROUP DURING DRYING^a

*Any two means within the same column having one of the same letters are not significantly different at P < 0.05.

TABLE 7. Shearing force for sausage slices in each pH group during drying^a

	Shear force, kg/g-cm ²						
Sausage			Days of drying				
pH group	0	5	10	15	20		
5.9	0.273ª	0.523ª	0.867ª	1.268ª	1.730ª		
5.5	0.339 ^{ab}	0.533ª	0.926 ^{ab}	1.314ª	2.065^{b}		
5.2	0.356 ^b	0.556ª	0.949 ^b	1.446 ^b	2.166°		
4.8	0.405 ^{bc}	0.591 ^{ab}	0.960 ^b	1.409 ^b	2.153°		
4.6	0.429°	0.634 ^b	0.951 ^b	1.474^{b}	2.265^{d}		

^aAny two means within the same column having one of the same letters are not significantly different at P < 0.05.

noted that the same lot of boneless beef and other ingredients was used for all sausage preparations. The fermented sausages had a loss of approximately 2.3% moisture content during the heat process (0 days drying). The moisture loss in turn increased the content of the other chemical constituents. After 20 days of drying, the moisture levels was reduced by approximately 35% from the 0-day level. The increases in protein, fat, ash, and salt contents were significantly (P < 0.05) correlated with the decrease in moisture during dehydration (lowest r = 0.94). This is in agreement with the findings of Lu and Townsend (18) and Wardlaw et al. (23). The final moisture content of 40.6% at 20 days of drying is between the 50% moisture level expected for semidry sausage and the 35% moisture content of dry sausages (16). While Kramlich (16) based dryness classifications on approximate moisture content of sausages, Wilson (24) used a range of weight loss or "shrinkage" values for dryness classification. Since sausage preparations may vary in initial moisture content due to type and quantity of meat tissues and trimmings used, the latter method provides more flexibility for dry sausage classification.

The increase in lactic acid content during drying is given in Table 5 for sausages of each pH group. The level of acid in the initial (nonfermented) pH group of 5.9 represented the slight amount of acid probably present in the inoculum and its concentrating on drying. It is possible that some lactic acid production occurred during the initial drying period since a low heat process was utilized. Lu and Townsend (18) reported a substantial drop in pH during 35 days of drying a fermented salami. In their study, no heat process was used that allowed for continued fermentation in the early phase of drying. Lactic acid levels in sausages of each pH group (Table 5) were concentrated by increased drying as indicated by a significant correlation (r=0.96) between lactic acid level and moisture content.

The rates of summer sausage weight loss of each pH group are given in Table 6. Weight losses at each interval of drying were significantly (P < 0.05) different. Within drying intervals, the sausages of pH groups 5.9 and 5.5 had significantly less shrinkage as compared to the sausages of the 4.8 and 4.6 pH groups. Using Wilson's (24) classification of dryness, the fermented sausages reached the semidry stage (20 to 25% shrink) between 5 to 8 days of drying. The medium dry stage (30 to 35% shrink) was attained between 10 and 15 days. The dry stage (40% shrink) was reached in 20 days. For retail purposes, the drying period can be terminated when the desired weight loss is attained.

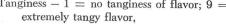
Shear values determined during drying (Table 7) showed a significant increase as drying time increased.

TABLE 8. PANEL RATINGS FOR PREFERENCE AND FLAVOR SCORES OF DRY SUMMER SAUSAGE⁸

Sausage pH group	Preference rating ^b	Tanginess rating ^b	
5.9	7.08ª	4.41ª	
5.5	7.30 ^{ab}	5.59°	
5.2	7.51 ^{ab}	6.05 ^{bc}	
4.8	7.85 ^{ab}	6.62 ^{cd}	
4.6	7.90 ^ь	6.98 ^d	

*Means within each column having one of the same letters are not significantly different at P < 0.05.

^b Hedonic	scales:	Preferen	ce –	1	=	dislike	extremely;	9	-
		like extre	mely						
		Tangines	1		no	tonging	of flower	0	



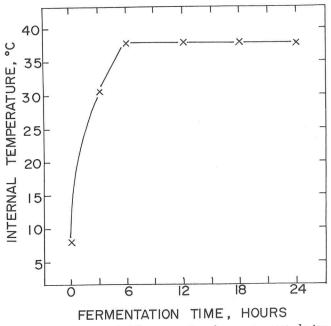


Figure 3. Water holding capacity of sausage meat during fermentation.

At each drying interval, sausages of pH groups 5.9 and 5.5 required significantly (P < 0.05) less shearing force than sausages of the pH 4.6 group. Shear values have previously been reported to increase during drying (14, 23) being correlated with drying time and dependent on the grinding procedure used in sausage preparation. Although Keller et al. (14) suggested that dry sausage having shear values above 1.2 kg/g-cm² were undesirable in eating quality due to the dried fibrous condition of the meat, no undesirable quality in hardness was noted in the present study. Lu and Townsend (18) stated that the type of microflora and sausage pH were involved in development of proper consistency of dry sausage.

Preference and "tanginess" flavor scores of the sausages at 20 days of drying are given in Table 8. Panelists rated higher preference scores for the sausages as the pH decreased although only the extreme pH groups of 5.9 and 4.6 were significantly different in ratings. Tanginess scores, reflecting the acidity or sharpness of flavor, showed a similar rating increase as the sausage pH decreased. Except for sausages of pH group 5.9 which were not fermented, approximately a 0.6 to 0.7 pH unit difference or a difference of 0.3 to 0.5 percent in lactic acid concentration was required for significant differences in tanginess scores among the fermented sausages. The effect of interactions of moisture content with lactic acid concentration on flavor development during drying are largely unknown and require further investigation.

Acknowledgments

The authors express their appreciation to R. L. Dick and L. H. McCaskill for technical assistance; to the Merck Chemical Division of Merck & Co., Inc., Rahway, N. J. for samples of their starter culture, LACTACEL; to the Films-Packaging Division of Union Carbide Corp., Chicago, Ill. for D.S. Fibrous Casings; and to Griffith Laboratories, Lithonia, Ga. for spice and seasoning ingredients.

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PRESIDENTIAL ADDRESS

Earl O. Wright

International Association of Milk, Food, and Environmental Sanitarians P. O. Box 701, Ames, Iowa 50010



E. O. Wright gives the presidential address.

This year will be recorded as one of big change for IAMFES. The office of the Executive-Secretary was relocated on January 1, 1974. At that time the headquarters and office functions were moved from Shelbyville, Indiana to Ames, Iowa. This will certainly also be a year that I will long remember. It has been gratifying that the membership has been very patient and helpful through the transition. Publication of the *Journal* will be moved to Ames where a new printer will do the work after January 1, 1975. We hope that you will again have patience with us when we make this move. The wearing of two hats since last January has been an intriguing experience for me. I am referring to serving as both President of the organization and taking on my new duties as Executive-Secretary.

AFFILIATES AND MEMBERSHIP

This year considerable effort has been directed toward strengthening the affiliate organizations and reviving affiliates that had not been active in recent years. Affiliate organizations are the local arm of IAMFES, and it is very essential that this representation be a strong one if our Association is to continue its outstanding work. There are several states or areas that do not have organized affiliates even though they do have a large number of direct members. There is a challenge for us to help organizations function in these areas. To accomplish this it will take the effort of many active affiliates. I know that the affiliates will respond when called upon to help in this effort.

It has been very gratifying to know that the Association has had 350 to 400 new direct or affiliate members join this year. This is largely due to the fine efforts of our Committee on Membership that is led by Harold Heiskell. Increasing membership should be a goal for everyone to work on. The strength of an organization is in its membership. I wonder how many prospective members each of you have talked to this year? A good active, membership is the *first*

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ingredient to a successful organization. The activity of that membership is the *second important ingredient*. Put these two together with proper leadership and a successful organization will emerge. With the successful organization that we enjoy in IAMFES, it is necessary to constantly keep reviewing these two ingredients if we are to continue to be one of the leading organizations in this field.

There is a good possibility that we will have new affiliates organized in two of our neighboring countries in the near future. With the number of our subscribers growing abroad this could well be our golden opportunity to begin to establish affiliate activity in other countries. Your Executive Board is taking a serious look at such possible developments. One new affiliate organization has become a reality at this meeting. We now have two affiliate associations with our neighbor, Canada.

COOPERATION WITH NEHA

The Association has continued its investigation of a possible future merger with the National Environmental Health Association (NEHA). This year the Executive Secretaries of the two organizations made a study of the organizations-comparing their composition, functions, and financial status. This report is now in the hand of the two Executive Boards who will be giving it further consideration. It is not an easy matter to combine two strong organizations into an amalgamated organization. It first must be determined if this type of procedure would benefit the membership. If this is a possibility then what type of an organization should be the outcome? Joining two strong organizations together is no assurance that the new or reorganized group will be successful. I have seen both successes and failures in the past decade among dairy and food plants attempting to combine into another organization. Because of the similarities of our memberships, before steps toward merger can be taken a thorough investigation should be made with both organizations participating.

COMMITTEES

Committee work is the backbone of our organization. Much effort has been put forth to strengthen committees. We have 28 committees functioning within our organization. Keeping up to-date with each of these committees is no easy task. The success of this organization is very dependent on hard working committees. The IAMFES is very fortunate in having not only hard working membership but also top notch leadership which makes things happen and gets things done. We are asking our affiliate asso-

ciations to annually make suggestions and recommendations to the President-Elect who is in charge of committee assignments, so that new leadership becomes involved in committee work. I want to thank the chairman of each committee for the help he has given me this past year. If our organization is to serve our membership properly, the committees must be in tune with the needs of the membership. The affiliate organizations can play a major role in this area.

THE JOURNAL

The Journal of Milk and Food Technology is our mouthpiece. It serves as a strong public relations medium for our organization. Subscriptions for this Journal from foreign countries are growing; we are now mailing the Journal to most foreign countries. This Journal is recognized as being outstanding in the area of milk and food safety and sanitation. Because of the reputation of the Journal and our strong organization we have clientele covering a wide spectrum of interests. Our clientele vary from the research scientist in the laboratory doing research on quality and other problems, to students, and to sanitarians who are doing quality control work in the field. The food industry has become an important section of our clientele. We need to increase and promote the interest of this group in our Journal and organization. With this wide range of clientele it is evident that our Journal can not be all things to all people. Our Journal enjoys the recognition of researchers and the academic community. Therefore, we have the good fortune of receiving outstanding research articles for publication. In addition to research papers the Journal endeavors to have an equal amount of space devoted to technical papers (sometimes in form of review articles), non-technical papers, and affiliate news published in each issue, in that order, if all these materials are available. The most difficult papers to obtain for publication are those of a non-technical nature. We ask your help in strengthening this area of the Journal.

The *Journal* is nearly a self-supporting vehicle. Printing of the *Journal* will be moved to Ames, Iowa on January 1, 1975 so that this operation is located in close proximity to the main office.

IN CONCLUSION

The IAMFES is an active and strong organization because of the input from its individual members. The IAMFES will continue to represent the members as their professional organization in a manner that is desired by the members. It is up to you as members to communicate with leaders of your organization to let your wishes be known. This can be done by working through your affiliate and also by directly contacting the Executive-Secretary's office. This office is waiting for suggestions about needed improvements. Many have already been made and changes are being planned because of your suggestions. More communication always results in better understanding.

THE SIXTY-FIRST ANNUAL MEETING OF IAMFES ST PETERSBURG, FLORIDA—AUGUST 11-14, 1974

"Change" is the word that best describes the 61st Annual Meeting of IAMFES. The meeting was hosted by the Florida Association of Milk and Food Sanitarians and was held at the Hilton Hotel in St. Petersburg. More than 600 persons attended one or several of the three meetings that were held in succession. They were the Annual Meeting of the Florida Association of Milk and Food Sanitarians on Monday, August 12th; the Annual Meeting of IAMFES on August 13th and 14th; and the Regional Meeting of the National Mastitis Meeting on August 15th.

Numerous changes were evident at this year's annual meeting. They included: a new Executive-Secretary of IAMFES (see separate story elsewhere in this issue), contributed research papers as part of the program, scheduling the National Mastitis Council meeting after rather than before the IAMFES meeting, and eliminating the traditional Thursday morning general session from the IAMFES meeting. One thing, however, did not change—the Executive Board held numerous meetings to transact the Association's business.

MEETINGS OF THE EXECUTIVE BOARD

Numerous reports were given at various sessions of the Executive Board. They included:

(a) Membership committee (given by Harold Heiskell; 384 new members were added in 1974; 47 of these were from foreign countries and New York was the state with the most new members, 44; the Arizona affiliate has been reactivated; a new affiliate, the Ontario Milk and Food Sanitarians-Eastern Division will be completely organized before the end of the year; another new affiliate in Alberta is expected to be organized early in 1975; a contest in each affiliate to increase membership by 25 members or 25% was proposed and subsequently approved by the Executive Board),

(b) Editor of the Journal of Milk and Food Technology (presented by Dr. E. H. Marth; volume 36 published in 1973 is the first volume to contain more



An attentive audience at one of the technical sessions.



Some of the speakers at the Annual Meeting included Dr. J. H. von Elbe (top, left), R. W. Bennett (top, right), Dr. R. J. Hasiak (bottom, left), and Dr. E. H. Marth (bottom, right).

papers that dealt with nondairy foods than with dairy foods; effective January, 1975 the *Journal* will be printed by Heuss Printing in Ames, Iowa rather than the Franklin Printing Co. in Franklin, Indiana; composition of the *Journal* will be by computer and this will permit a variety of improvement to make the *Journal* more attractive to readers and authors; Dr. W. G. Walter of Montana State University resigned from the Editorial Board and he has been replaced by Dr. H. W. Walker of Iowa State University),

(c) Financial report (given by E. O. Wright; there was a net loss of approximately \$4,700 during 1973-1974; this resulted because two offices (Ames and Shelbyville) were maintained for 6 months of the year, there was a slight decrease in income from dues, the cost of moving materials from Shelbyville to Ames, and the increased cost of postage for mailing the Journal),

(d) Cooperation with NEHA (presented by E. O. Wright and W. F. Wilson; the Executive-Secretary of IAMFES met with the same officer of the National Environmental Health Association, NEHA, reviewed the nature of the two organizations, and prepared a report for the officers of both groups; the Executive Board of IAMFES will attempt to meet with a similar group from NEHA before further steps toward cooperation are taken),

(e) Sanitarians' Joint Council (presented by Ray Belknap; the Council met in Cincinnati during the 1974 annual meeting of NEHA; proposed model act for registration of sanitarians will soon be available in printed form; bylaws of the Council will be updated and a history will be prepared; future objectives include developing a code of ethics, emphasizing the need for reciprocity in registration of sanitarians, and striving to further improve the national image of sanitarians),

(f) Awards committee (presented by O. M. Osten; the new forms used in 1974 yielded names of numerous candidates for the Sanitarian's and Education/ Industry Award; suggestions also were received for the Citation Award and Honorary Life Memberships; upon recommendation of the Awards Committee, the Executive Board agreed that John Schilling would receive the Citation Award and that Honorary Life Membership Awards would go to H. L. Thomasson and K. G. Weckel-details about all awards can be found in a separate article elsewhere in this issue of the Journal),

(g) Journal Management Committee (presented by Dr. W. C. Lawton; responsibilities of the committee have been defined; notice of IAMFES Annual Meeting should appear in more trade publications; sustaining memberships should be considered as a source

of support for the Journal),

(h) Committee on applied laboratory methods (presented by C. N. Huhtanen; results of two studies done last year are being prepared for publication; two additional studies dealing with the temperature of dilution blanks and the temperature of incubation for the Standard Plate Count have been completed and results are being analyzed; some members of the committee are involved in revising the 13th edition of Standard Methods for the Examination of Dairy Products),

(i) Representative to National Mastitis Council (given by A. E. Parker; Parker will continue to serve for another term; the Council will meet again in February, 1975 in Minneapolis, Minnesota),

(*j*) Observer at the International Dairy Federation (presented by Harold Wainess; Wainess is listed as an official observer at IDF since the U. S. is not a member of the federation; IDF will meet in Toronto in 1976 and interested persons are invited to attend the session; IDF is active in developing standards for products and equipment),

(k) Farm methods committee (presented by M. W. Jefferson; the report represents an interim report and was printed through the courtesy of Babson Bros.; the report honors A. K. Sauders, long-time chairman of the committee, who died early in 1974; 11 sub-committees contributed to the report).

In addition to action taken in response to some reports, the Executive Board agreed to the following: (a) ballots for the forthcoming election will again be sent by first class mail; (b) cost of reprints will be increased 20% to cover the higher cost of paper and advertising rates will be raised by 10%; (c) the fee for student members will be raised from \$4.00 to 5.00 for 1975; (d) declined participation in a proposed National Council on Environmental Health; (e) accepted the invitation of the Illinois affiliate to host the Annual Meeting of IAMFES in 1976; (f) accepted a proposed amendment to the constitution whereby the Secretary-Treasurer serves one year and then advances to the position of second vice-president -this amendment must be approved at the 1975 annual business meeting before it is effective; and (g) agreed to meet November 14 and 15, 1974 at the Royal York Hotel in Toronto to plan the program for the 1975 Annual Meeting.

The schedule for future annual meetings of IAMFES is as follows:

- 1975, August 10-13, Royal York Hotel, Toronto
- 1976, near Chicago, Illinois
- 1977, uncertain at this time but probably on the West Coast
- 1978, Kansas City

AFFILIATE COUNCIL

Another change made this year was the time of the Affiliate Council meeting. It was held during the afternoon of Monday, August 12th. Representatives of the following affiliates were present: California, Illinois, Indiana, Iowa, Kentucky, Minnesota, Mississippi, Missouri, New York, Ontario, Oregon, Pennsylvania, South Dakota, Washington, and Wisconsin.

Much time was spent to determine how membership should be reported to the Executive-Secretary and when new members should begin to receive the *Journal*. The discussion was prompted because increased costs prohibit supplying extra issues of the *Journal* to either new members or to members who fail to pay their dues.

The Affiliate Council heard a report on cooperation between IAMFES and NEHA and then voted to encourage the IAMFES Executive Board to meet with a similar group from NEHA so that detailed information about possible future cooperation can be obtained. New officers of the Council are: Chairman, Ervin Gadd of Missouri, and Secretary, Howard Hutchings of South Dakota.

TECHNICAL SESSIONS

This year 14 contributed research papers were presented at the technical sessions. Topics covered by the papers included: iron content of separator and clarifier sediment, size of disks for the sediment test, *Escherichia coli* in foods, aflatoxigenic fungi in foods in the home, betalaines as colorants in dairy products, shelf-life of pasteurized milk, milk quality in retail markets, method to isolate salmonellae from yeast, control of aflatoxin production in wild rice, patulin production in cherries, occurrence of *Geotrichum candidum* in baker's yeast, factors that affect rubratoxin production, recovery of staphylococcal enterotoxin from food, and assessing safe exposure to methyl mercury.

Subjects discussed by invited speakers include: the National Dairy Council and a national nutrition policy, milk quality in the public school system, experience in milk dating, surveillance of bulk milk sampling, botulism in canned foods, treatment of milk wastes, preventing problems in the food industry, attacking world food problems, recording temperature of bulk milk, temperature of perishable dairy products in food stores, microbial quality of ground beef and chicken parts, state shellfish program, use of chemicals and water in CIP systems, and conservation of energy in the food industry.

Many of the papers presented at the Annual Meet-

ing will appear in future issues of the Journal of Milk and Food Technology.

BUSINESS MEETING

The annual business meeting of IAMFES was called to order by President E. O. Wright at 10:30 a.m. on Wednesday, August 13. After minutes of the 1973 meeting were approved, the following reports were presented (some were also given to the Executive Board and hence information given earlier in this discussion of the Annual Meeting will not be duplicated here).

(a) Executive-Secretary and Treasurer, given by E. O. Wright; (b) Applied Laboratory Methods Commitee, given by C. N. Huhtanen; (c) Farm Methods Committee, given by M. W. Jefferson; (d) observer at the International Dairy Federation, given by Harold Wainess; (e) Membership Committee, given by Harold Heiskell; (f) Journal Management Committee, given by Dr. W. C. Lawton; (g) Affiliate Council, given by Robert Coe;

(h) Committee on Food Equipment Sanitary Standards, given by Karl Jones (National Sanitation Foundation is to be more concerned with durability and finish of equipment, only hard solder will be permitted, copper tubing for carbonated water will be eliminated);

(i) Committee on Sanitary Procedures, given by D. B. Whitehead (two meetings held during past year; new members are needed);

(*j*) Committee on Baking Industry Equipment, given by Harold Wainess (regulatory agencies can now require that all equipment meets BISSC standards; 34 standards have been developed and the first 10 standards have been revised; standards are available from Ray Walter, Executive-Secretary of BISSC, 521 Fifth Ave., New York, N. Y. 10017);

(k) Representative to Keep America Beautiful National Advisory Council, given by Charles Felix (102 organizations represented at 20th meeting of the Council; KAB is an important force in educating people to recognize the need for personal responsibility in the national quest for a quality life);

(1) Committee on Food Protection, given by Charles Felix (committee has considered food service management programs and revision of the FDA-USPHS Food Service Ordinance and Code);

(m) Committee on Communicable Diseases Affecting Man, given by Richard Swanson (the IAMFES manual on investigating outbreaks of food-borne illness is being revised);

(n) 3-A Symbol Council, given by Dr. K. G. Weckel (council held two meetings; headquarters now at 413 Kellogg Ave., Ames, Iowa 50010; new numbering system has been devised for standards; 167 authorizations are issued; the Council handled six formal complaints during the past year).

The membership voted to amend the by-laws of the Association to update them with current practice as to responsibilities of the Secretary-Treasurer and Executive-Secretary. Specifically, Articles I, II, III, V, and VII were amended by deleting the words "Secretary-Treasurer" and inserting the words "Executive-Secretary" at the appropriate places.

Ray Belknap, chairman of last year's nominating committee announced that David D. Fry was elected second vice-president and that Prof. R. P. March was reelected as Secretary-Treasurer.

W. F. Wilson, Chairman of the *Resolutions Committee*, presented the following resolutions and they were adopted by the membership.

RESOLUTION NO. 1

WHEREAS the IAMFES is dedicated to the general improvement of the quality of life, and,

WHEREAS the tasks of conducting the necessary environmental protection programs requires personnel of particular training, experience, and dedication, and,

WHEREAS such personnel are to be found among all segments of our society, both male and female, and,

WHEREAS fair and equitable compensation is necessary to attract and retain the vital personnel to the sanitarian profession, and,

WHEREAS discriminatory practices of any kind are repugnant and contrary to the moral and legal obligations of all Americans,

Now threfore, be it resolved by the IAMFES in formal convention petition the membership of the International Asso-

ciation of Milk, Food, and Environmental Sanitarians to endorse the several civil rights statutes and declare discrimination on the basis of sex, age, race, creed, national origin, or political affiliation to be repugnant and contrary to the orderly development of society and contrary to the expressed belief and ethical standards of the International Association of Milk, Food, and Environmental Sanitarians. Further, that this resolution be printed in the JOURNAL OF MILK AND FOOD TECHNOLOGY.

RESOLUTION NO. 2

WHEREAS the Florida Association of Milk and Food Sanitarians has displayed all those nationally recognized qualities of Southern Hospitality as hosts of the 1974 annual meeting of the IAMFES, and

WHEREAS all needed facilities for the successful meeting were anticipated and supplied by the Florida Association, and

WHEREAS excellent coordination among the Industry, Educational, and Regulatory members of the Florida Association was accomplished within the highest standard of IAMFES, and

Whereas the 1974 meeting was an outstanding success in attendance, accomplishment, and fellowship of members,

Therefore be it resolved that IAMFES adopt the resolution of gratitude to the Florida Association and further that an appropriate copy of the resolution be sent to the Florida Association as well as being published in the JOURNAL OF MILK AND FOOD TECHNOLOGY.

RESOLUTION NO. 3

WHEREAS the Hilton Hotel of St. Petersburg, Florida was the site of the 1974 IAMFES annual meeting, and

WHEREAS the facilities for the membership's use and personal comfort were outstanding, and

WHEREAS the personnel of the Hilton Hotel did extend themselves individually to provide for all our needs,

Therefore, be it resolved that an appropriate expression of gratitude be sent to the St. Petersburg Hilton Hotel.

H. L. THOMASSON HONORED AS HE RETIRES: E. O. WRIGHT IS NEW • EXECUTIVE-SECRETARY AND MANAGING EDITOR OF IAMFES

THOMASSON RETIRES

Special recognition was given to H. L. "Red" Thomasson at the Awards Banquet of the 61st Annual Meeting of IAMFES. Thomasson, long-time Executive-Secretary and Managing Editor of IAMFES retired January 1, 1974 but continued to serve the Association as a consultant during 1974. Retirement will be complete at the end of 1974 when all headquarters activities of IAMFES will be located in Ames, Iowa.

Dick B. Whitehead, former president of IAMFES, organized the recognition for "Red" and presided for this part of the program at the Awards Banquet. Whitehead began by introducing Mr. and Mrs. Thomasson's four children: Jacqueline, Dan, Peggy, and Michael. Neither of the senior Thomassons knew in advance that their entire family would be present at the banquet. Whitehead then presented



H. L. Thomasson (left foreground) has just received a special award. D. B. Whitehead (right) made the presentation,

"Red" with a special plaque and with approximately \$1300 contributed by the affiliates of IAMFES. These funds are to be used by Mr. and Mrs. Thomasson for a long-delayed foreign trip.

Thomasson was born in Indianapolis, Indiana in 1903. After graduation from high school he worked for a printing firm and a bill collecting agency. In 1924 "Red" enrolled at Franklin College in Franklin, Indiana. He graduated in 1928 with major work in sociology, economics, history, and political science, and with minor work in science. At Franklin College, Thomasson was a member of Phi Delta Theta, Blue Key (honor society), Theta Alpha Phi, and the Interfraternity Council. He also served as Editor-in-Chief of the college yearbook. "Red" and Margaret ("Peg") were married in 1929 in Fort Worth, Texas where Thomasson had accepted a position after graduation from college.

Thomasson held the following positions from 1928 to 1938: salesman for Sellers Supply Co. in Fort Worth; manager of Fertig Dairy in Shelbyville, Indiana; employee of Fertig Dairy Co. in Indianapolis; automobile salesman in Shelbyville; and operator of a clothing store in Shelbyville. In 1938 "Red" accepted a position with the Dairy Division of the Indiana State Board of Health and in 1939 he was put in charge of the Grade A milk program for the entire state. While with the Board of Health, Thomasson obtained adoption of 70 ordinances for Grade A milk. This covered 92% of the urban population of Indiana. His efforts led to eventual adoption of a state-wide Grade A law. Thomasson also was responsible for hiring and training the personnel needed to implement the Grade A milk ordinances.

Thomasson joined IAMFES in 1939 and was elected vice-president in 1949. He served as president in 1951-52. "Red" has continuously served as a member of the Committee on Sanitary Procedures since before he was vice-president of IAMFES.

In 1951 "H. L." became Executive-Secretary and Managing Editor of IAMFES. He established headquarters in Shelbyville. IAMFES was in financial trouble when "Red" became Executive-Secretary. He succeeded in reorganizing the association so it soon was on a sound financial basis. During Thomasson's tenure in office, the number of affiliates grew from 11 to 25 and membership in IAMFES grew accordingly. The *Journal of Milk and Food Technology* became a monthly publication in 1954 (it was bimonthly before) and now approximately 4000 copies are distributed in the U. S. and in 74 other countries.

"Red" devoted much time during the last 23 years to improving the knowledge of sanitarians and others so they could more effectively improve the nation's



H. L. Thomasson (center) and his family at the Awards Banquet.



E. O. Wright, executive-secretary of IAMFES and managing-editor of the *Journal of Milk and Food Technology*.

public health. His efforts have not been in vain! We salute you, "Red," for a job well-done and wish you well during your years of retirement.

WRIGHT IS NEW EXECUTIVE-SECRETARY

Earl O. Wright, Professor of Food Technology at Iowa State University, retired from his position at the University and became Executive-Secretary and Managing Editor for IAMFES effective January 1, 1974. Wright has served as Executive-Secretary during the past year and will assume complete responsibilities as Managing Editor on January 1, 1975 when publication of the *Journal of Milk and Food Technology* will be moved from Indiana to Ames, Iowa.

Earl is a native of Wisconsin; he was born and reared on a farm near Bloomington, Wisconsin and later graduated from the Bloomington High School. In 1941 Wright received the B.S. degree in Agriculture from the University of Wisconsin-Platteville. After graduation from the University, Earl taught vocational-agriculture and general science in high school. He then served in the U. S. Army for 3.5 years during World War II. After his return from service, Wright became the County Extension Director for Lincoln and Clark counties in Wisconsin. Later Earl enrolled at the University of Wisconsin-Madison and in 1954 received the M.S. degree in Dairy and Food Industries (now Food Science). While at the University in Madison Wright also served as Extension Specialist and Instructor in the Department of Dairy and Food Industries.

In 1954 Earl joined the staff of Iowa State University in Ames and served there until December 31, 1973 when he retired as Professor of Food Technology Extension. Wright was responsible for initiating the bacteriological testing program for manufacturing grade milk in Iowa. He also was instrumental in writing minimum standards for manufacturing grade milk in the state.

Wright spent much time in training fieldmen and food plant personnel in proper laboratory methods; he advised plants on selection of equipment, processing problems, product development, and quality control. Earl also prepared a monthly newsletter for the dairy and food industry of Iowa and wrote a monthly column that appeared in *Hoard's Dairyman* for 8.5

years. Additionally he published scientific articles in the *Journal of Milk and Food Technology* and elsewhere and prepared numerous bulletins, circulars, and leaflets that were published by Iowa State University.

Earl has served on committees of IAMFES and also on its Executive Board, having just completed his term of office as president. He has also been active in the American Dairy Science Association by serving on several committees and as Chairman of the Business and Industry Section. Wright has served on the Executive Board of the National Conference of Interstate Milk Shipments. He currently is secretarytreasurer of the 3-A Sanitary Standards Symbol Administrative Council.

Earl is a member of Gamma Sigma Delta and Epsilon Sigma Chi. He is listed in Who's Who in the Midwest and in American Men and Women of Science. His alma mater, the University of Wisconsin-Platteville, has honored him with the Distinguished Alumnus Award.

Earl's wide background in the food and dairy industry, his familiarity with the science and practice of food and dairy hygiene, and his organizational and administrative experience make him eminently qualified to fill the vacancy created when H. L. "Red" Thomasson retired as Executive-Secretary and Managing Editor of IAMFES.

IAMFES HONORS SCHILLING, THOMASSON, WECKEL, LUCHTERHAND, MARCH, AND THE WASHINGTON AFFILIATE

Each year IAMFES honors several members and one affiliate organization for meritorious service. Honors given to individual members are in the form of four awards designated as the Citation Award, Honorary Life Membership, Sanitarians Award, and the University-Industry Award. The Shogren Award is given annually to the affiliate organization with the most outstanding program during the past year. Recipients of awards are selected by the Committee on Awards and Recognition. In 1974 the committee consisted of O. M. Osten, chairman; R. A. Belknap, E. Gadd, B. Luce, and O. Majerus.

CITATION AWARD TO JOHN C. SCHILLING

The Citation Award is given annually to a member of IAMFES who has contributed substantially to the growth, advancement, and status of the Association. This award, in 1974, went to John C. Schilling.

Schilling graduated from the University of Missouri-



John Schilling (left) receives the Citation Award from O. M. Osten,



C. K. Luchterhand (left) receives the Sanitarians Award from O. M. Osten,



Professor R. P. March (left) receives the University-Industry Award from O. M. Osten.

Rolla in 1943 with a B.S. degree in Chemical Engineering. He then joined the St. Louis Health Division where he has served as Public Health Engineer (Community Sanitation and Rat Control), Dairy Plant Supervisor in the Milk Control Section, and Chief of the Milk Control Section. At present, John is the Assistant Health Commissioner in the Bureau of Environmental Health Services of the St. Louis Health Division.

John is a member of IAMFES, the Missouri Association of Milk, Food, and Environmental Sanitarians, the Missouri Public Health Association, the Missouri Mastitis Council, and the National Conference on Interstate Milk Shipments. Schilling is a member of the IAMFES Committee on Sanitary Procedures and was Chairman of the Local Arrangements Committee in 1968 when the annual meeting of IAMFES was held in St. Louis.

Additionally, John has served as Chairman of the Sanitation Section of the Missouri Public Health Asso-

ciation and as a Director of the Missouri Mastitis Council. At present Schilling is Vice Chairman of the National Conference on Interstate Milk Shipments and also is serving on that group's Executive Board.

HONORARY LIFE MEMBERS-H. L. THOMASSON AND K. G. WECKEL

H. L. Thomasson

H. L. Thomasson has been a member of IAMFES since 1939 and has served the Association as Executive Secretary and Managing Editor from 1951 until his retirement at the end of 1973. During 1974 "Red" has continued to serve IAMFES on a parttime basis and has been responsible for many of the details of journal publication. A complete biography of Thomasson appears in a separate article elsewhere in this issue of the *Journal*.



Dr. L. O. Luedecke (left) is about to receive the 1974 Shogren Award that went to the Washington Milk Sanitarians Association. O. M. Osten made the presentation.



E. O. Wright (left) is about to receive the Past-President's Award from O. M. Osten,

K. G. Weckel

Dr. K. G. Weckel is a native of Canton, Ohio where he was born in 1905. He attended the University of Wisconsin-Madison where he majored in Dairy Industry and received the B.S., M.S., and Ph.D., degrees in 1931, 1932, and 1935, respectively. Weckel continued on at the University of Wisconsin where he became Assistant Professor in 1935, Associate Professor in 1941, and Professor in 1945.

Ken has had a long and continuing interest in the welfare of IAMFES. He was president of the Wisconsin Association of Milk and Food Sanitarians in 1945 and 1947 and was president of IAMFES in 1951. Weckel has served on numerous committees of IAMFES and has been a member of the Editorial Board of the *Journal of Milk and Food Technology* since 1945.

Some of Dr. Weckel's other achievements and appointments include: (a) secretary of the Wisconsin Dairy Technology Society for many years; (b) consultant to the Wisconsin Alumni Research Foundation since 1936; (c) chairman of the National Conference on Interstate Milk Shipments, 1953-1955; (d) chairman, first National Mastitis Conference, 1961; (e) member, Food Technology subcommittee of the National Research Council; (f) consultant to the Public Health Service Sanitary Engineering Center, 1957-1960; (g) member of the Gross Committee of the Public Health Service, 1961; and (h) chairman of the Wisconsin Section of the Institute of Food Technologists, 1960. In addition Weckel is or was a member of the Grade A Milk Ordinance Advisory Council, Refrigeration Research Foundation, National Confectioners Education and Research Foundation, and the Wisconsin Food Advisory Council. Ken also is a registered sanitarian in Wisconsin.

Dr. Weckel holds memberships in the following professional and related societies: Alpha Zeta, Phi Sigma, Sigma Xi, Phi Tau Sigma, IAMFES, American Dairy Science Association, Institute of Food Technologists, American Chemical Society, and the American Candy Technologists.

SANITARIANS AWARD TO CLARENCE K. LUCHTERHAND

This award consist of a plaque and \$1,000. It is given annually to a member of IAMFES who has made outstanding contributions to the field of public health during the preceding 7 years. Funds for the award are provided jointly by the Pennwalt Corporation, Klenzade Products (Economics Laboratory), and the Diversey Corporation. The 1974 recipient of the Sanitarians Award is C. K. Luchterhand, Chief of the Section on Milk Certification, Wisconsin Department of Health and Social Services.



Dr. K. G. Weckel is about to receive an Honorary Life Membership Award.



Representatives of firms that fund awards are in attendance at the Awards Banquet.

Luchterhand was born in 1914 on a farm near Chilton, Wisconsin. He attended the University of Wisconsin-Madison from 1933-1935 and again in 1937 when he enrolled in the dairy short course. Additionally, Clarence has enrolled in short courses at the University of Michigan and at Michigan State University.

After the financial difficulties of the Great Depression caused Luchterhand to leave the University, he found employment with the Carnation Company and remained with this organization until 1942. In 1942 Clarence became an inspector with the Wisconsin Department of Agriculture and in 1944 he moved to the Wisconsin State Board of Health as a milk sanitarian. Luchterhand held this position until 1951 when he was named Chief of the Section on Milk Certification which now is in the Wisconsin Depart-



The Executive Board of IAMFES at the Awards Banquet. Top row from left to right: P. J. Skulborstad, president, and Mrs. Skulborstad; H. E. Thompson, president-elect, and Mrs. Thompson; Prof. R. P. March, secretary-treasurer; D. D. Fry, second vice-president, and Mrs. Fry; E. O. Wright, junior past-pesident, and Mrs. Wright. Bottom row from left to right: Dr. H. V. Atherton, first vice-president; W. F. Wilson, senior past-president, and Mrs. Wilson; O. M. Osten, senior past-president during 1973-1974 and now retired from the Board, and Mrs. Osten; R. Cole, chairman of the Council of Affiliates for 1973-1974; Dr. E. H. Marth, editor of the Journal of Milk and Food Technology, and Mrs. Marth.

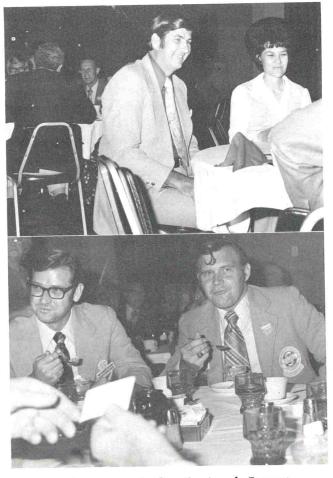
ment of Health and Social Services.

Clarence has devoted the major part of his professional career to promoting the dairy program in Wisconsin and to working with the dairy industry so trade barriers could be eliminated and so that milk could be moved freely without costly duplication of inspection. Because of Luchterhand's leadership capabilities in directing the Grade A milk program in Wisconsin, he was able to obtain the needed cooperation among communities and local health departments to permit free movement of milk among various municipal jurisdictions. Luchterhand also was instrumental in developing a program to eliminate unsafe water from farms that produce Grade A milk. This program is now being applied to all farms in Wisconsin.

For some years Clarence has been chairman of the Joint Committee on Education that is made up of representatives from the Wisconsin Association of Milk and Food Sanitarians and the Wisconsin Environmental Health Association. In this capacity Luchterhand provided the leadership needed to develop a 4-year degree program in Environmental and Public Health at the University of Wisconsin-Eau Claire.

Luchterhand is a member of IAMFES, the Wisconsin Association of Milk and Food Sanitarians, the Wisconsin Conference on Milk and Food, the Wisconsin Public Health Association, the National Conference on Interstate Milk Shipments, the Wisconsin Environmental Health Association, and the National

Environmental Health Association. Clarence has received the "Sanitarian of the Year" award from



Some of the persons attending the Awards Banquet.

both the Wisconsin Association of Milk and Food Sanitarians and the Wisconsin Environmental Health Association and also has served both groups as president.

Luchterhand has served on numerous state and national committees. One of these is the IAMFES Committee on Sanitary Procedures of which he has been a member since 1955, is a past chairman, and currently is vice-chairman. Other important current committee assignments include: secretary of the Sanitarians Registration Council for Wisconsin and chairman of the Committee on Public Health for Paper and Plastic Containers, Syracuse University.

UNIVERSITY-INDUSTRY AWARD TO PROFESSOR RICHARD P. MARCH

The University-Industry Award was instituted in 1973 to recognize significant contributions to food safety and sanitation by an IAMFES member in industrical or academic work. The award is funded by the Milking Machine Manufacturers Council of the Farm and Industrial Equipment Institute and consists of a plaque and \$1,000. The second recipient of the award is Richard P. March, Professor of Food Science at Cornell University, Ithaca, New York.

March received the B.S. degree in Dairy Industry from the University of Massachusetts in 1944. After service in the U. S. Marine Corps from 1944 to 1946, March enrolled at Cornell University and in 1948 received the M.S. degree in Dairy Industry from that institution. In 1947 March was appointed Instructor of Dairy Science at Cornell University. He remained at Cornell and was advanced to Assistant Professor in 1951, Associate Professor in 1955, and Professor in 1965.

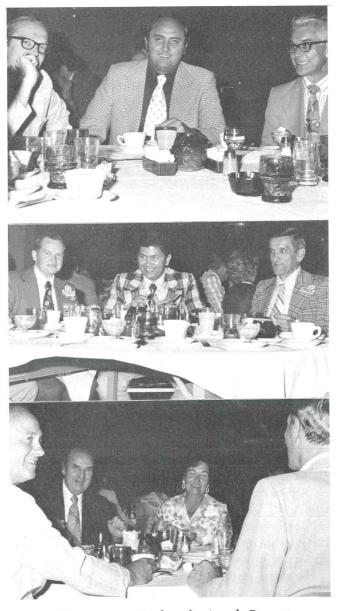
Professor March is a member of IAMFES, the New York State Association of Milk and Food Sanitarians, the Finger Lakes Sanitarian Association, the Connecticut Milk and Food Sanitarians Association, the American Dairy Science Association, the National Mastitis Council, the Central New York Section of the Institute of Food Technologists, and Epsilon Sigma Phi.

Important assignments of Professor March include: (a) appointment by Governor Nelson Rockefeller as a research consultant to study the economic impact that a national sanitation act would have on New York's dairy industry, 1962-1963; (b) executive-secretary of the New York State Association of Milk and Food Sanitarians, 1957-present; (c) secretary-treasurer of IAMFES, 1970-present; (d) chairman, Northeast Dairy Practices Council, 1969-present; (e) secretary of the New York State Association of Milk and Food Sanitarians Council of Affiliates, (f) secretary of Cor-

nell's Food Science Department Planning Committee, and (g) secretary (1956-1962) and chairman (1963-1964) of the IAMFES Council of Affiliates. In addition March has served on numerous state and national committees.

Awards received by Professor March include: (a) the Dr. Paul B. Brooks Memorial Award, (b) the Emmet R. Gauhn Award (both from the New York State Association of Milk and Food Sanitarians), (c) a citation from the New York State Association of County Agricultural Agents, and (d) a College of Agriculture Travel Award.

Professional achievements of Dick March are legion. He has cooperated with health departments in his state to provide training in sanitation to dairy plant



Some of the persons attending the Awards Banquet.

fieldment. Efforts by March have resulted in reducing and eliminating differences in regulations governing production, processing, and distribution of milk and milk products that once existed among states in northeastern U. S. One of March's greatest achievements has been to develop adequate means of communication so that various groups and individuals in his state and region are informed about changes in regulations and technology associated with milk production, processing, and distribution. Evidence of Dick's efforts at communication is the fact that he authored or coauthored more than 100 bulletins, circulars, and other articles. March's great concern about a safe food supply, especially of milk and milk products, is amply demonstrated by his professional accomplishments and by his involvement with professional societies that are dedicated to the achievement of this goal.

Shogren Award to the Washington Affiliate

The Shogren Award was developed by the Affiliate Council of IAMFES to annually recognize the affiliate organization with the most outstanding program. A questionnaire is submitted annually to the secretary of each affiliate organization. Completion of the questionnaire serves to enter the organization in the competition and provides the information used by the Committee on Recognition and Awards to select the winner. This year the award went to the Washington Milk Sanitarians Association. Dr. L. O. Luedecke, secretary of the affiliate organization, accepted the award.

The major factors considered by the committee in selecting the affiliate organization to receive this award include: number of members, activities of the affiliate during the year, percentage of members that also belong to IAMFES, number of papers by members that appeared in the *Journal of Milk and Food Technology*, number of members that serve on IAMFES committees, number of members that attend the annual meeting of IAMFES, and preparation and circulation of a newsletter.

OTHER AWARDS

Several other awards are usually given at the Awards Banquet. This year H. L. Thomasson received a special award in recognition of his many years of service to IAMFES as its executive-secretary and managing editor. Details of this award appear in a separate article in this issue of the *Journal*.

Charles W. Felix, editor of *Environment News* Digest, presented the traditional gavel to incoming President P. J. Skulborstad. The Past-President's Award went to Earl O. Wright and was presented to him by O. M. Osten.

ASSOCIATION AFFAIRS

ANNUAL MEETING OF WASHINGTON AFFILIATE

The Washington Milk Sanitarians Annual State Meeting at Spokane, September 10th was both interesting and informative. Reports were submitted by the chairmen of the various committes and copies of the conclusions will be sent to the corresponding committees of International.

At the Annual business meeting Dr. Lloyd Luedecke of Washington State University reported on the International Meeting and was re-elected Secretary-Treasurer of Washington Milk Sanitarians Association. Clayton Gustafson, Washington Department of Agriculture, was named President-Elect. D. J. Crawford, Director, Packaging Laboratories, Ex-Cell-O Corporation, climaxed the afternoon meeting by bringing us up to date on asceptic packaging of milk and dairy products.

We were fortunate to have Earl Wright with us throughout the meeting and Earl was very helpful answering our questions and discussing 3A standards



Washington Milk Sanitarian's President Jim Shoemake, congratulates new president, Fred Froese.



Earl Wright addressing the Washington Milk Sanitarians Association at the annual banquet at Spokane, September 10, 1974. W.M.S.A. Secretary-Treasurer, Lloyd Luedecke, left; Past President, Jack Salvadalena and Fred Froese, incoming President, next to podium.

committee work. President Jim Shumake presided over the afternoon session and turned the gavel over to New President Fred Froese at the evening banquet. Earl Wright was our guest speaker at the



Ben Luce introducing guest speaker, Earl Wright at the Washington Affiliate Annual Meeting. Jim Shoemake, President, right. Jack Salvadalena, Past President, left.

banquet and discussed programs and future plans of the International Association and affiliate contribution to the future of International. Earl's encouraging and inspirational address was a fitting climax to a very successful Annual Meeting.

NOTICE

Eeach year IAMFES recognizes outstanding contributions and performance by it's members.

The success of this program is dependent not only on those organizations who so generously support the monetary aspects of these awards, but it is equally dependent on your individual help in furnishing the Awards Committee with appropriate information and names of potential award winners.

Will you please give serious thought to the following Awards, which will be considered for presentation at our 1975 IAMFES Annual Meeting.

- 1. The Sanitarian's Award of \$1000 to a state or Federal sanitarian, who, during the past seven years has made outstanding contributions to the health and welfare of his community.
 - 2. Educator-Industry Award of \$1000 to a Uni-

versity or Industry employee who has made outstanding contributions to food safety and sanitation.

- 3. *The Citation Award* to a member who has given outstanding service to IAMFES in fulfilling it's objectives.
- 4. The Shogren Award to the affiliate organization that has the best statewide or regional program.
- 5. Honorary Life Membership to those members who have given long and outstanding service to IAMFES.

Please contact Walter F. Wilson, Chairman of the IAMFES Recognition and Awards Committee, County Los Angeles Health Dept., 313 N. Figueroa St., Los Angeles, Ca. 90012.

WENDELL CARR

Wendell I. Carr, 58, director of the Dairy Division of the state Department of Agriculture, died at Medical Center Hospital in Burlington after a short illness.

In 1942, he became a milk inspector with the Department of Agriculture and was promoted to senior inspector before becoming director of the dairy division.

Mr. Carr was president of the Vermont Dairy Industry Administration in 1968. He also was secretary of the national association of state Departments of Agriculture.

He was district chairman of the Long Trail Council, Boy Scouts of America, of Burlington while living in that city and was manager of a Little League farm team and deacon of the First Congregational Church there. Wendell was a long time member of International Committee on Dairy Farm Methods.

Born Dec. 31, 1916, in Calais, he was the son of Ivon and Ruth (Converse) Carr. He attended the East Montpelier grade school and Montpelier Seminary.

During World War II, he served aboard the USS Panamint as a fireman.

AVI PUBLISHING COMPANY LAUNCHES MAJOR FOOD REFERENCE WORK

Dr. Donald K. Tressler, President of the Avi Publishing Company, announces the publication in December, 1974, of the ENCYCLOPEDIA OF FOOD TECHNOLOGY, the first comprehensive reference covering all phases of food technology ever issued. Drs. Arnold H. Johnson and Martin S. Peterson are the editors. Contributors include leading food technologists and scientists from the United States and many foreign countries.

The topics range from the technology of products (apples, cherries, pineapples, citrus fruit, potatoes, etc.) to the technology of processing (freeze drying, canning, dehydration, radiation preservation of food, microwave preservation, and includes new processes such as those used to produce fabricated foods. Also covered are food spoilage factors: oxidation, microbial contamination, loss of color and flavor, and other deteriorative influences.

This authoritative one-volume reference work will provide the technologist, the student, the food executive, and informed layman with succinct answers to the numerous questions that arise in connection with processing, storing, and marketing foods.

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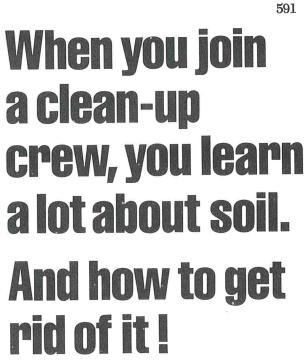
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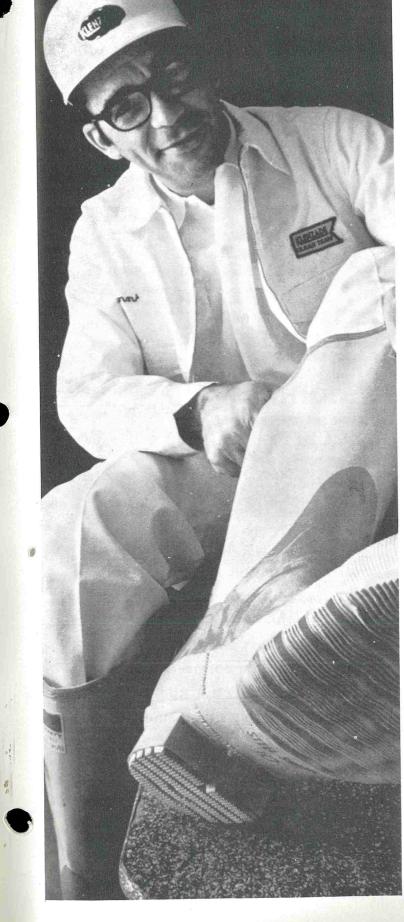
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Public health officials who work with NSF as trustees, consultants or committee members are well aware that the awarding of the NSF seal to a manufacturer is only the *beginning*. Permission to place the seal on a product is granted only after it has passed evaluation tests confirming that the product does equal or exceed the requirements of an NSF standard.

The manufacturer's right to con-

tinue placing this seal on his product must be *continuously earned*.

A member of the NSF field staff appears at the factory, and no one knows when he's coming. He chooses samples of the product at random—for on-thespot examination, or for testing in the NSF laboratory at Ann Arbor. The product must demonstrate that it continues to qualify for the NSF seal through adherence to its standard.



National Sanitation Foundation—an independent non profit, non governmental organization dedicated to environmental quality. NSF Building, Ann Arbor, Mich. 48105. (313) 769-8010. Government officials and professional workers concerned with public health and environmental quality are invited to write for the NSF Publications List. It is free. It lists all NSF standards and criteria as well as listings, reports and NSF literature.





Dairy authorities speak out on better cow milking



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

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

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

LABOR TAKES A BIG BITE

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

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

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

THE OPERATOR IS A BUSY MAN

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

SOME RESEARCH RESULTS

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

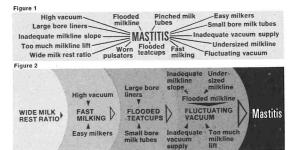
- Automatic unit take-off significantly reduced the number of guarters infected with mastitis.
- 2. Automatic unit take-off reduced udder irritation as evidenced by lower CMT scores.
- The men operating the automatic take-off units reduced their walking distance in the parlor by more than 25%.

Dr. Nelson Philpot, leader of this study, says one should not expect miracles. Automatic take-off units do not make a poor operator better. They do, however, allow a conscientious operator to do an even better job on more cows. According to dairymen using this equipment, proper maintenance and proper operation of equipment is even more important with non-automated systems. The ability, cooperative attitude and location of your local serviceman should become a primary factor in deciding whether to install this more sophisticated and expensive equipment.

MASTITIS PREVENTION NOT SIMPLE

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

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



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

AUTOMATIC TAKE-OFFS A COMMON SIGHT?

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

- *For our purposes, the labor figures include all dairy chore labor, feeding labor, and the raising of offspring. Field labor isn't included.
- **In 250-cow and 500-cow herds, we assume the existence of a parlor and a free-stall barn with mechanized feeding and waste handling.

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