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Journal of Milk and Food Technology

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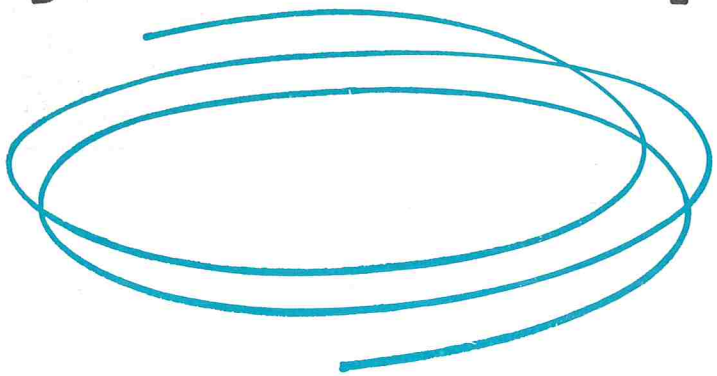

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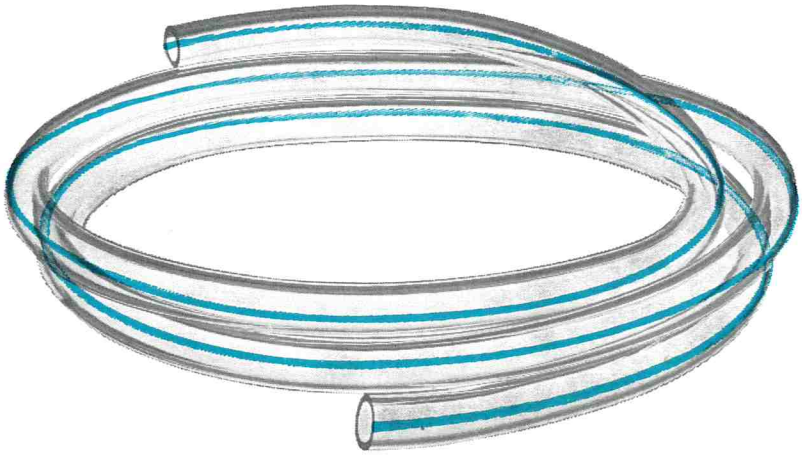

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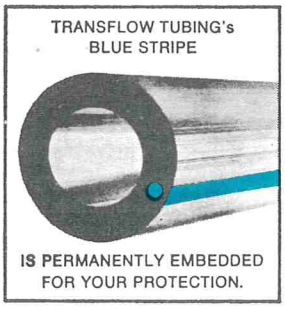

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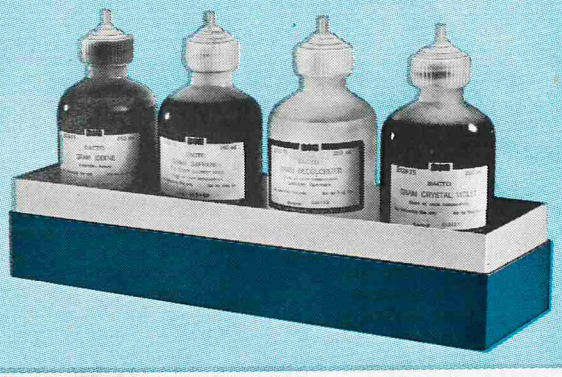

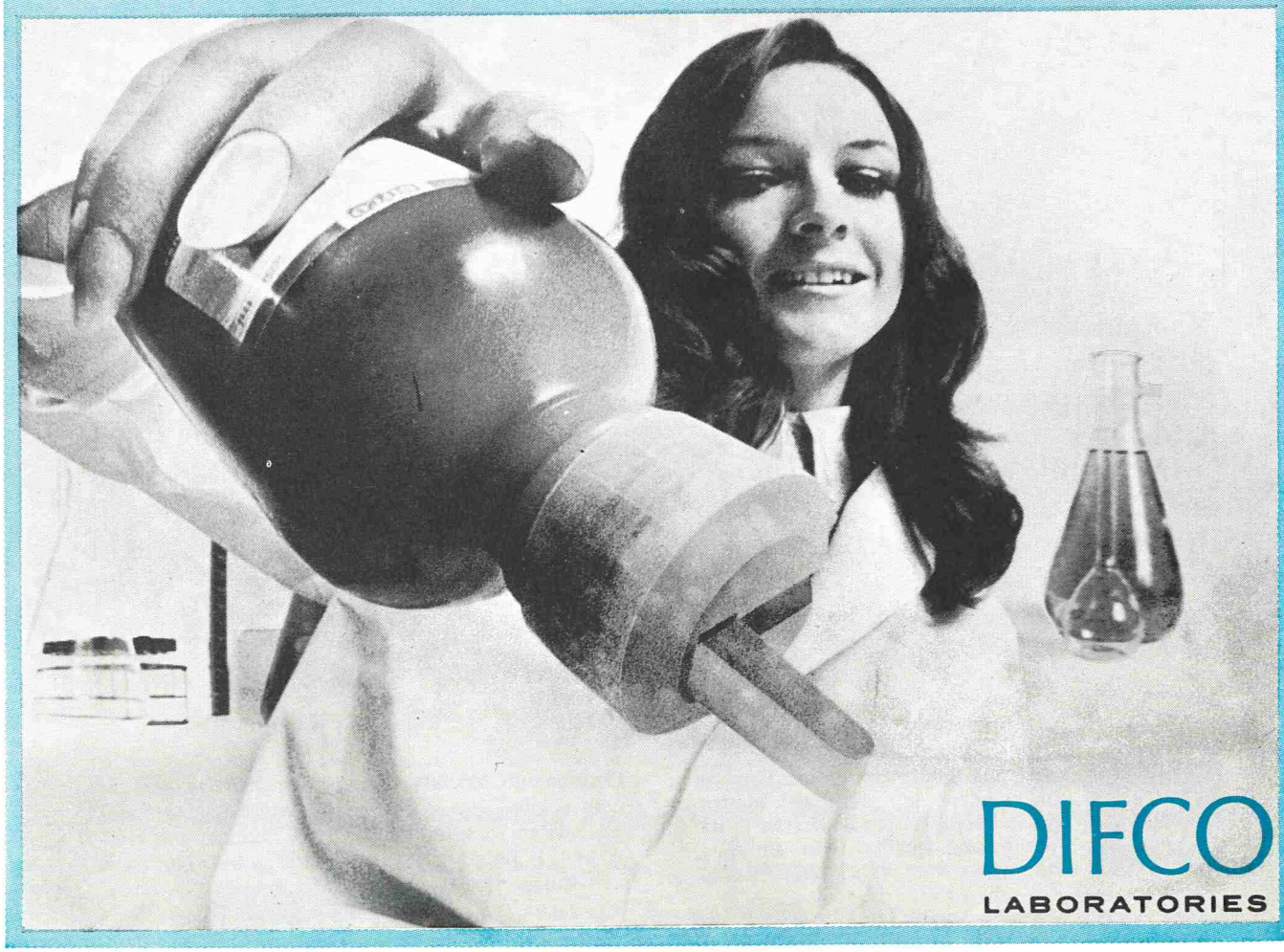

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The Journal of Milk and Food Technology is issued monthly beginning with the January number. Each volume comprises 12 numbers. Published by the International Association of Milk, Food and Environmental Sanitarians, Inc. with executive offices of the Association, 413 Kellogg Ave., P. O. Box 701, Ames, Ia. 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.

Manuscripts: Correspondence regarding manuscripts and other reading material should be addressed to Dr. Elmer H. Marth, Dept. of Food Science, University of Wisconsin, Madison, Wis. 53706.

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Page Charge: Effective January 1, 1969 a charge of $25.00 per printed page will be made for all research papers which are published. See Volume 31, issues 10, 11, or 12 for details.

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Subscription Rates: One volume per year. Individual non-members. Government and Commercial Organizations subscription.
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Official Publication

International Association of Milk, Food and Environmental Sanitarians, Inc.

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

IV. STREPTOCOCCUS LACTIS[1]

D. C. Kulshrestha and E. H. Marth

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(Received for publication May 1, 1974)

Abstract

APT broth inoculated with *Streptococcus lactis* was dispensed into epoxy-lined aerosol cans. Twenty-five milk-associated volatile or non-volatile compounds were added individually to cans to give final concentrations of 1, 10, 100, and 1000 ppm of each compound. The compounds used were formic, acetic, butyric, hexanoic, octanoic, and decanoic acid; formaldehyde; acetaldehyde; propionaldehyde; glyoxal; acetone; 2-butanone; diacetyl; propyl- and hexylamine; furfurol; methanol; methylsulfide; methylsulfone; methanethiol; ethanethiol; acetonitrile; chloroform; ether; and ethylenedichloride. Bacteria were enumerated at intervals during incubation at 30 C.

Fatty acids at 1000 ppm always significantly inhibited S. *lactis*. Decanoic acid was more active than other fatty acids at higher concentrations, whereas formic acid caused more inhibition of S. *lactis* when the acids were tested at a lower concentration (10 ppm). Formaldehyde was most inhibitory of the aldehydes tested. The lowest concentration of aldehydes was only marginally inhibitory. Diacetyl was more inhibitory than acetone or 2-butanone. Acetonitrile (100 ppm), chloroform (10 ppm), ether (10 ppm), and ethylenedichloride (10 ppm) always inhibited S. *lactis*. Lower concentrations of methylsulfone caused minimal growth stimulation early during the incubation, but later this changed to inhibition. Ethanethiol generally was more detrimental than methanethiol, and methylsulfide was less active against S. *lactis* than methanethiol. Amines were more inhibitory than alcohols.

The importance of *Streptococcus lactis* as one of the major acid producing organisms used to manufacture fermented dairy products is well documented. Milk, the raw material for such products, contains a variety of volatile and other compounds *(13)*, some of which may be removed by vacuum treatment. Such compounds could influence the growth of bacteria, including the lactic streptococci, in milk. However, only some reports are available that deal with the influence of a few of these compounds on growth of microorganisms. Other compounds of the type found in milk have not been evaluated for their effect on S. *lactis*.

Tarassuk and Smith *(25, 26)* confirmed the earlier findings of Koestler *(9)* that rancid milk inhibited growth of S. *lactis*. Later other workers *(2, 5-7, 12, 13, 18, 21-24)* also reported that some fatty acids inhibited S. *lactis* or delayed acid production by this organism. Abo-Elnaga et al. *(1)* found that lactic acid bacteria were sensitive to 0.004% (40 ppm) of formalin. Kulshrestha and Marth *(12, 13)* used a disc assay procedure and found that besides fatty acids S. *lactis* was inhibited by aldehydes (formaldehyde, glyoxal, butyraldehyde, and anisaldehyde), diacetyl, and amines (propyl- and hexylamine).

This paper describes results of experiments done to determine how growth of S. *lactis* was affected by some of the volatile or non-volatile compounds associated with milk, when these compounds were added to a culture of the bacterium in an air-tight vessel that was then incubated. A brief report based on these results has been presented *(14)*.

Materials and Methods

Chemicals and procedures used for these experiments were basically as described earlier *(15)* but modified to accommodate S. *lactis*. APT broth (Difco) and APT agar (Difco) were used in place of nutrient broth and plate count agar. A 12- to 16-h old APT broth culture of S. *lactis* C_{10} was used to inoculate APT broth before it was dispensed into cans. The culture was obtained from the culture collection of Department of Food Science, University of Wisconsin, Madison. All incubations were at 30 C. Approximately 1 h was required for broth in cans to attain the incubation temperature.

Results and Discussion

Tables 1 and 2 list results obtained when fatty acids were tested. At 1000 and 100 ppm decanoic acid was most and acetic acid was least inhibitory to S. *lactis*. Generally, with the exception of formic acid, the degree of inhibition was directly related to the chain-length of the acid. Inhibition of S. *lactis* always was significant when 1000 ppm of individual acids were added to the medium, but not early during the incubation when 100 ppm of hexanoic, octanoic, and decanoic acid were tested. At lower (1 and 10 ppm) concentrations, formic acid was more inhibitory to S. *lactis* than were the other fatty acids. Formic, acetic, and butyric acid at 10 ppm always caused minimal but significant inhibition of S. *lactis*.

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

TABLE 1. DIFFERENCES IN POPULATION OF *Streptococcus lactis* IN APT BROTH CAUSED BY ADDED FORMIC, ACETIC, AND BUTYRIC ACID

Chemical	Conc. (ppm)	Differences (%) from control in log of population after hours of incubation				
		2	5	8	11	14
Formic acid[1]	1	(−) 0.7	(−) 0.3	(+) 0.1	(−) 3.7*	(−) 1.1*
	10	(−) 1.4*	(−) 1.5*	(−) 1.0*	(−) 6.0*	(−) 1.6*
	100	(−) 3.8*	(−) 4.7*	(−) 2.5*	(−) 8.6*	(−) 2.4*
	1000	(−)10.6*	(−) 8.5*	(−) 6.6*	(−)12.8*	(−) 6.4*
Acetic acid[1]	1	(+) 1.2*	(+) 1.2	(−) 0.3	(−) 3.4*	(−) 0.3
	10	(−) 0.5*	(−) 2.0*	(−) 1.4*	(−) 2.7*	(−) 0.7*
	100	(−) 1.2*	(−) 2.7*	(−) 2.2*	(−) 5.4*	(−) 1.0*
	1000	(−) 4.5*	(−) 5.1*	(−) 3.4*	(−) 6.0*	(−) 1.8*
Butyric acid[1]	1	(−) 0.2	(−) 0.5	(−) 1.4*	(−) 2.1*	(−) 0.7*
	10	(−) 0.7*	(−) 1.5*	(−) 2.9*	(−) 4.7*	(−) 1.0*
	100	(−) 1.9*	(−) 2.9*	(−) 5.6*	(−) 7.7*	(−) 1.7*
	1000	(−) 5.7*	(−) 6.8*	(−) 7.3*	(−) 8.7*	(−) 2.5*

[1]Control: Log of no./ml: 4.24, 5.92, 7.41, 9.01, and 9.06 at 2, 5, 8, 11, and 14 h, respectively.
*: Population significantly different from control at 5% level.

TABLE 2. DIFFERENCES IN POPULATION OF *Streptococcus lactis* IN APT BROTH CAUSED BY ADDED HEXANOIC, OCTANOIC, AND DECANOIC ACID

Chemical	Conc. (ppm)	Differences (%) from control in log of population after hours of incubation				
		2	5	8	11	14
Hexanoic acid[1]	1	(+) 0.3	(−) 0.5	(−) 0.6*	(+) 0.1	(−) 0.4
	10	(−) 0.3	(−) 0.9	(−) 1.1*	(−) 3.5*	(−) 0.6
	100	(−) 2.0*	(−) 1.4	(−) 4.6*	(−) 7.0*	(−) 1.2*
	1000	(−) 6.3*	(−) 7.5*	(−) 7.3*	(−)11.1*	(−) 6.8*
Octanoic acid[1]	1	(+) 0.5	(+) 0.4	(+) 0.3	(−) 0.2	(−) 0.8
	10	(−) 0.3	(−) 0.5	(−) 1.1*	(−) 4.3*	(−) 1.2*
	100	(−) 1.0	(−) 3.3	(−) 4.8*	(−) 8.3*	(−) 2.1*
	1000	(−) 4.8*	(−) 8.2*	(−)13.1*	(−)19.7*	(−)17.8*
Decanoic acid[1]	1	(−) 1.0	(−) 0.2	(−) 0.1	(−) 0.2	(−) 0.6
	10	(−) 2.0*	(−) 1.2	(−) 2.6*	(−) 4.4*	(−) 0.8
	100	(−) 5.3*	(−) 3.5	(−) 7.0*	(−) 8.7*	(−) 4.8*
	1000	(−)26.6*	(−)42.5*	(−)45.7*	(−)44.7*	(−)33.8*

[1]Control: Log of no./ml: 3.98, 5.77, 7.24, 8.63, and 9.06 at 2, 5, 8, 11, and 14 h, respectively.
*: Population significantly different from control at 5% level.

When only 1 ppm of formic or butyric acid was present, inhibition of *S. lactis* was generally insignificant initially during the incubation. Acetic acid at 1 ppm caused minimal but significant stimulation of growth early during the incubation but later this changed to generally insignificant inhibition. The effect was greatest at 11 h.

Formaldehyde was more detrimental to the growth of *S. lactis* than were acetaldehyde or propionaldehyde (Table 3). Formaldehyde at 1000 ppm apparently inactivated this organism in less than 5 h. With 100 ppm of this compound more than 30% reduction in growth was noted at 2 h; but the inhibitory effect became less pronounced as the incubation progressed. Lower concentrations of formaldehyde (1 and 10 ppm) displayed maximum inhibition at 11 h. Inhibition by formaldehyde was always significant except at 14 h of incubation when 1 or 10 ppm of this compound were tested. Acetaldehyde at 1000 ppm and propionaldehyde at the same concentration were markedly inhibitory to *S. lactis* and the effect increased with the elapse of incubation time. At 100 ppm both acetaldehyde and propionaldehyde were almost equally significantly inhibitory throughout the incubation except at 14 h. When 10 ppm of either aldehyde were tested, both compounds caused significant inhibition early in the incubation; with 1 ppm of either aldehyde the effect was generally al-

ways insignificant.

Of the ketones listed in Table 4 diacetyl was most detrimental and inactivated S. *lactis* in less than 5 h when 1000 ppm of this compound were tested. At 100 ppm the inhibition was quite conspicuous; but was least in magnitude at the end of incubation. At lower (1 and 10 ppm) concentrations diacetyl nearly always was significantly inhibitory. Acetone and 2-butanone at higher concentrations (100 and 1000 ppm) always significantly inhibited S. *lactis*. At 10 ppm both acetone and 2-butanone always were inhibitory, but the effect was significant only during the interval between 5 and 11 h of incubation. These compounds at 1 ppm generally were without any significant effect.

Results obtained when different amounts of acetonitrile, chloroform, and ether were tested are given in Table 5. Chloroform was more inhibitory to S. *lactis* than were the other two compounds, especially at higher concentrations. Chloroform at 1000 ppm inhibited growth of S. *lactis* and the effect increased with time until growth was reduced by more than 22% after 14 h of incubation. When 10 or 100 ppm of chloroform were tested, inhibition was inconsistently significant. Chloroform at 1 ppm was without effect. Acetonitrile (1000 ppm) and ether (100 and 1000 ppm) always were significantly inhibitory to S. *lactis*, although less so than chloroform. Acetoni-

TABLE 3. DIFFERENCES IN POPULATION OF *Streptococcus lactis* IN APT BROTH CAUSED BY ADDED FORMALDEHYDE, ACETALDEHYDE, AND PROPIONALDEHYDE

Chemical	Conc. (ppm)	Differences (%) from control in log of population after hours of incubation				
		2	5	8	11	14
Formaldehyde[1]	1	(−) 1.8*	(−) 1.7*	(−) 3.7*	(−) 5.4*	(−) 0.1
	10	(−) 6.4*	(−) 6.4*	(−) 7.2*	(−) 9.3*	(−) 0.7
	100	(−)31.5*	(−)31.0*	(−)26.2*	(−)21.4*	(−) 8.4*
	1000	(−)63.5*	NG[2]*	NG*	NG*	NG*
Acetaldehyde[1]	1	(−) 0.5	(+) 0.2	(−) 1.4*	–[3]	(−) 0.1
	10	(−) 2.3*	(−) 1.0*	(−) 2.3*	(+) 0.2	(+) 0.1
	100	(−)11.0*	(−) 6.6*	(−) 6.4*	(−) 5.5*	–
	1000	(−)37.0*	(−)53.8*	(−)64.9*	(−)73.7*	(−)79.1*
Propionaldehyde[1]	1	(−) 0.5*	(−) 0.3	–	–	(−) 0.1
	10	(−) 4.6*	(−) 0.5*	(−) 5.4*	(−) 1.2*	(+) 0.6
	100	(−) 9.4*	(−) 7.8*	(−) 6.4*	(−) 6.4*	(−) 0.3
	1000	(−)29.5*	(−)52.8*	(−)59.0*	(−)63.5*	(−)63.8*

[1]Control: Log of no./ml: 4.38, 5.91, 7.09, 8.31, and 8.84 at 2, 5. 8, 11, and 14 h, respectively.
[2]NG: 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 *Streptococcus lactis* IN APT BROTH CAUSED BY ADDED ACETONE, 2-BUTANONE, AND DIACETYL

Chemical	Conc. (ppm)	Differences (%) from control in log of population after hours of incubation				
		2	5	8	11	14
Acetone[1]	1	(+) 1.0	(−) 0.9	(−) 0.3	(−) 0.4*	(+) 0.1
	10	(−) 0.8	(−) 3.5*	(−) 2.9*	(−) 0.6*	(−) 0.1
	100	(−) 1.7*	(−) 4.7*	(−) 2.5*	(−) 1.9*	(−) 0.8*
	1000	(−) 3.2*	(−) 6.6*	(−) 3.9*	(−) 4.2*	(−) 1.7*
2-butanone[1]	1	(+) 1.2	(−) 0.4	(−) 0.7	(−) 0.4*	(+) 0.4
	10	(+) 0.5	(−) 3.3*	(−) 2.0*	(−) 0.8*	(−) 0.2
	100	(−) 1.0*	(−) 4.0*	(−) 3.1*	(−) 2.6*	(−) 0.6
	1000	(−) 4.2*	(−) 4.3*	(−) 4.4*	(−) 4.8*	(−) 2.1*
Diacetyl[1]	1	(+) 0.3	(−) 4.2*	(−) 2.2*	(−) 3.8*	(−) 0.8*
	10	(−) 1.0	(−) 4.9*	(−) 4.0*	(−) 4.8*	(−) 2.2*
	100	(−) 8.5*	(−)12.5*	(−)11.5*	(−)12.1*	(−) 5.1*
	1000	(−)32.6*	NG[2]*	NG*	NG*	NG*

[1]Control: Log of no./ml: 4.02, 5.76, 7.22, 8.41, and 8.99 at 2, 5. 8, 11, and 14 h, respectively.
[2]NG: Less than 10 organisms/ml of test liquid.
*: Population significantly different from control at 5% level.

TABLE 5. DIFFERENCES IN POPULATION OF *Streptococcus lactis* IN APT BROTH CAUSED BY ADDED ACETONITRILE, CHLOROFORM, AND ETHER

Chemical	Conc. (ppm)	Differences (%) from control in log of population after hours of incubation				
		2	5	8	11	14
Acetonitrile[1]	1	–[2]	(+) 0.7	(+) 0.1	(+) 0.1	(+) 0.5
	10	–	(–) 0.5	(–) 0.1	(–) 0.2*	–
	100	(–) 2.7*	(–) 1.9*	(–) 1.1*	(–) 1.0*	(–) 0.1
	1000	(–) 3.4*	(–) 3.9*	(–) 1.9*	(–) 3.6*	(–) 1.8*
Chloroform[1]	1	(–) 0.2	(+) 0.5	–	–	(+) 0.6
	10	(–) 0.5*	(–) 0.2	(–) 0.6*	(–) 0.7*	(–) 0.3
	100	(–) 5.6*	(–) 1.0	(–) 4.5*	(–) 3.1*	(–) 0.3
	1000	(–) 6.1*	(–)15.2*	(–)18.9*	(–)21.9*	(–)22.3*
Ether[1]	1	(–) 1.2*	–	(–) 0.3	(+) 0.1	(+) 0.2
	10	(–) 1.9*	(–) 0.3	(–) 0.7*	(–) 1.0*	(–) 0.9*
	100	(–) 3.6*	(–) 2.7*	(–) 2.9*	(–) 2.5*	(–) 1.9*
	1000	(–) 4.8*	(–) 4.1*	(–) 5.1*	(–) 5.1*	(–) 3.0*

[1]Control: Log of no./ml: 4.13, 5.87, 7.31, 8.36, and 8.94 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 *Streptococcus lactis* IN APT BROTH CAUSED BY ADDED GLYOXAL, ETHYLENEDICHLORIDE, AND METHYLSULFONE

Chemical	Conc. (ppm)	Differences (%) from control in log of population after hours of incubation				
		2	5	8	11	14
Glyoxal[1]	1	(–) 1.0*	(–) 0.7	(–) 0.3	(–) 0.4*	(–) 0.1
	10	–[2]	(–) 4.7*	(–) 2.6*	(–) 4.5*	(–) 0.4*
	100	(–) 8.5*	(–)11.3*	(–)10.8*	(–) 5.9*	(–) 1.4*
	1000	(–)47.1*	(–)67.0*	(–)79.8*	(–)84.6*	NG[3]*
Ethylenedichloride[1]	1	(+) 0.3	–	(–) 0.8*	(–) 0.2	(–) 0.1
	10	(–) 1.5*	(–) 2.8*	(–) 2.2*	(–) 1.3*	(–) 0.4
	100	(–) 2.5	(–) 2.6*	(–) 2.6*	(–) 2.1*	(–) 0.7*
	1000	(–) 5.3*	(–)33.5*	(–)23.9*	(–)11.1*	(–) 1.0*
Methylsulfone[1]	1	(+) 1.5*	(–) 1.0	(–) 0.4	(+) 0.1	(+) 0.1
	10	(+) 1.0*	(–) 2.4*	(–) 0.4	–	(–) 0.3
	100	(+) 1.0	(–) 3.0*	(–) 1.6*	(–) 1.7*	(–) 0.4*
	1000	(–) 1.8*	(–) 4.0*	(–) 2.2*	(–) 2.6*	(–) 0.6*

[1]Control: Log of no./ml: 3.99, 5.76, 7.32, 8.42, and 9.04 at 2, 5, 8, 11, and 14 h, respectively.
[2]–: No difference.
[3]NG: Less than 10 organisms/ml of test liquid.
*: Population significantly different from control at 5% level.

trile at 100 ppm was without significant activity at 14 h. At lower concentrations (1 and 10 ppm) ether generally failed to significantly inhibit S. *lactis*.

The inhibitory activity of glyoxal, ethylenedichloride, and methylsulfone is illustrated by data in Table 6. Glyoxal was very detrimental to growth of S. *lactis* and reduced it by 47% in less than 2 h, when the compound was tested at 1000 ppm. The reduction in population increased with time, leading to essentially complete inactivation of the organism in 14 h. Inhibition caused by 100 ppm of this compound was comparatively less marked but was always significant. Amounts other than 1000 ppm of glyoxal caused greatest inhibition early during the incubation period. At 10 ppm inhibition was significant from 5 h to the end of the incubation. Marginal and generally insignificant inhibition was caused by 1 ppm of glyoxal. Ethylenedichloride generally was more inhibitory than methylsulfone, and greater inhibition usually was noted early in the observation period. Inhibition by 1000 ppm of both methylsulfone and ethylenedichloride always was significant. Ethylenedichloride at 10 and 100 ppm was generally significantly inhibitory, but with 1 ppm the effect was almost always insig-

nificant. Slight stimulation of S. *lactis* was caused by 1, 10, and 100 ppm of methylsulfone early in the incubation. Later 100 ppm methylsulfone was almost always significantly inhibitory, but lower concentrations (1 and 10 ppm) were generally without significant effect.

Results obtained when methylsulfide, methanethiol, and ethanethiol were tested are given in Table 7. Ethanethiol was slightly more inhibitory than methanethiol and methylsulfide was the least effective. Ethanethiol at 100 and 1000 ppm and methanethiol at the same concentrations always significantly inhibited S. *lactis*. Inhibition was more pronounced early during the incubation but declined later. Inhibition by 10 ppm of either thiol was not significant at 2 and 14 h; the effect of 1 ppm of these compounds generally was insignificant. Methylsulfide at 1000 ppm generally was significantly inhibitory except in

TABLE 7. DIFFERENCES IN POPULATION OF *Streptococcus lactis* IN APT BROTH CAUSED BY ADDED METHYLSULFIDE, METHANETHIOL, AND ETHANETHIOL

Chemical	Conc. (ppm)	Differences (%) from control in log of population after hours of incubation				
		2	5	8	11	14
Methylsulfide[1]	1	(+) 0.3	(−) 3.6*	(+) 1.0*	(+) 0.2	(−) 1.1*
	10	(+) 2.7	(−) 5.4*	(−) 2.8*	(−) 0.6*	(−) 0.6
	100	(−) 0.8	(−) 7.3*	(−) 3.4*	(−) 0.7*	(−) 0.2
	1000	(−) 6.0*	(−) 7.3*	(−) 3.6*	(−) 1.7*	(−) 0.8
Methanethiol[1]	1	(−) 0.3	(−) 1.3*	(−) 0.7	(−) 0.2	(−) 0.8*
	10	(−) 0.3	(−) 2.3*	(−) 2.0*	(−) 1.3*	(−) 0.2
	100	(−) 5.2*	(−) 5.2*	(−) 6.0*	(−) 3.0*	(−) 1.3*
	1000	(−) 6.8*	(−) 8.4*	(−)10.6*	(−) 6.4*	(−) 1.7*
Ethanethiol[1]	1	–[2]	(−) 1.5*	(−) 0.6	(−) 0.2	(+) 0.2
	10	(−) 0.3	(−) 2.9*	(−) 1.8*	(−) 0.9*	(+) 0.3
	100	(−) 4.1*	(−) 6.1*	(−) 6.6*	(−) 3.6*	(−) 1.0*
	1000	(−)11.7*	(−) 9.6*	(−)13.7*	(−) 7.3*	(−) 2.4*

[1]Control: Log of no./ml: 3.68, 5.23, 7.16, 8.25, and 9.02 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 *Streptococcus lactis* IN APT BROTH CAUSED BY ADDED FURFURYL AND METHYL ALCOHOL AND PROPYL- AND HEXYLAMINE

Chemical	Conc. (ppm)	Differences (%) from control in log of population after hours of incubation				
		2	5	8	11	14
Furfuryl alcohol[1]	1	–[2]	(−) 1.2*	(+) 0.1	(−) 0.2	–
	10	(−) 0.7	(−) 2.4*	(−) 1.2*	(−) 1.6*	(−) 0.3
	100	(−) 2.1*	(−) 5.3*	(−) 1.9*	(−) 4.0*	(−) 2.1*
	1000	(−) 3.5*	(−) 7.0*	(−) 6.8*	(−) 9.0*	(−) 6.0*
Methyl alcohol[1]	1	(+) 0.2	(−) 0.7	(+) 0.5	–	(+) 0.1
	10	(−) 0.2	(−) 1.7	(−) 0.3	(−) 0.2	(−) 0.4
	100	(−) 1.7*	(−) 4.6*	(−) 0.8*	(−) 3.4*	(−) 2.3*
	1000	(−) 2.4*	(−) 6.7*	(−) 5.5*	(−) 7.4*	(−) 2.9*
Propylamine[1]	1	(−) 0.7	(−) 1.2*	(−) 0.4	(−) 0.2	(−) 0.2
	10	(−) 2.4*	(−) 1.5	(−) 1.1	(−) 2.1*	(−) 0.4
	100	(−) 5.9*	(−) 3.1*	(−) 4.5*	(−) 5.9*	(−) 0.9*
	1000	(−)11.8*	(−)10.9*	(−)13.9*	(−)12.6*	(−) 8.4*
Hexylamine[1]	1	(−) 0.5	(−) 0.3	(−) 0.5	(−) 0.3	(−) 0.1
	10	(−) 1.7*	(−) 1.2	(−) 1.2*	(−) 2.3*	(−) 1.1
	100	(−) 2.4*	(−) 3.9*	(−) 2.9*	(−) 5.3*	(−) 2.9*
	1000	(−) 7.6*	(−) 5.5*	(−)13.7*	(−)15.8*	(−)15.2*

[1]Control: Log of no./ml: 4.24, 5.86, 7.51, 8.71, and 9.20 at 2, 5, 8, 11, and 14 h, respectively.
[2]–: No difference.
*: Population significantly different from control at 5% level.

the terminal stage of incubation, whereas 100 ppm of this compound had a significant inhibitory effect only from 5th to 11th hour of treatment. Slight but insignificant stimulation was caused by 1 and 10 ppm of methylsulfide at 2 h. Methylsulfide at 10 ppm generally was inhibitory but the effect was not significant at 14 h. An irregular pattern of stimulation and inhibition was noted with 1 ppm of methylsulfide.

S. *lactis* was generally inhibited more by amines than alcohols (Table 8). Toward the end of the incubation hexylamine at 1000 ppm was more inhibitory than propylamine at the same concentration. Hexylamine and propylamine (except at 14 h) generally were somewhat more effective later during the incubation. Both amines at 100 or 1000 ppm always were significantly inhibitory to S. *lactis*. Less pronounced and irregularly significant inhibition was caused by 10 ppm of both amines, but 1 ppm of these compounds was essentially without a significant effect. Inhibition always was significant when greater amounts (100 and 1000 ppm) of alcohols were tested. Furfuryl alcohol at 10 ppm always inhibited S. *lactis* but the effect was significant only between 5 and 11 h of incubation. The effect exerted by 1 ppm of furfurol always was insignificant. Methanol at 1 and 10 ppm always was without a significant effect.

S. *lactis* was regularly inhibited by high concentrations of all compounds tested. However, lower concentrations of only some compounds were significantly inhibitory. Milk and milk products, especially different varieties of cheese, contain many of the compounds evaluated in this study. Besides dairy products many natural and processed foods are likely to contain some of these compounds. The influence of these chemicals, at the levels they might be present in different foods, on growth of S. *lactis* is not known. Cheddar cheese flavor is a result of a proper balance between many different volatile compounds. Ripened Cheddar cheese has been reported to contain free fatty acids, ammonia, amines, hydrogen sulfide, aldehydes, ketones, and other volatile compounds *(3, 4, 8, 10, 11)*. It is well recognized that during ripening of Cheddar cheese the total number of bacteria and also of S. *lactis*, used as the starter, decreases. This is particularly true during the later stages of the ripening process. It is possible that initially conditions for organisms are more favorable since the content of such volatile compounds in cheese is not high enough to be detrimental to the organisms. In later stages of cheese ripening as the amount of such volatile compounds increase, their presence may contribute to the demise of some of the bacteria.

Many workers *(2, 5-7, 12, 13, 18, 21-26)* have reported inhibition of S. *lactis* by fatty acids. We confirmed this earlier *(12)* and again in these experiments. Costilow and Speck *(6)* reported that 0.1% (1000 ppm) of capric (decanoic) and caprylic (octanoic) acid inhibited S. *lactis* in milk. Their results are in general agreement with those reported here, although they failed to note inhibition by 1000 ppm of butyric acid and 500 ppm of hexanoic acid when S. *lactis* was grown in homogenized milk. Maxcy and Chandan *(21)* reported that capric acid at 1000 and 500 ppm caused complete and partial inhibition of S. *lactis*, respectively. Their findings that butyric acid was ineffective were not confirmed by us. Results of our experiments generally are in agreement with those of Anders and Jago *(2)* and Nilsson and Willart *(22)*, who reported an increase in inhibitory activity with the chain length of fatty acids up to C_{10} or C_{12}. Less activity of butyric acid than of C_6-C_{10} fatty acids, as observed by Podesta and Bertoldini *(23)*, also was confirmed by our results. Like Pozanski et al. *(24)*, we also found that capric acid inhibited S. *lactis*.

Several opinions have been expressed as to how fatty acids inhibit S. *lactis*. Some workers *(18, 19, 21, 25, 26)* have suggested that lowered surface tension, caused by the added fatty acids, resulted in inhibition of this bacterium. Maxcy and Chandan *(21)* also suggested that the inhibition may be a physical phenomenon of surface tension or a direct toxic effect in which the acids interfere with metabolism of the bacterium. In a later report Maxcy and Dill *(19, 20)* suggested that adsorption of fatty acids at the bacterium:menstruum interface may be critical in the inhibitory process. It also has been suggested by other workers *(2, 5, 6)* that inhibition was caused by a lowering of surface tension and that chain length of the fatty acid was critical. We also observed a reduction in the activity of fatty acids during the terminal stages of incubation as reported by Tarassuk and Smith *(25, 26)*, who suggested that this happened because S. *lactis* utilized the surface tension lowering fatty acids during growth. A more detailed study is needed to determine how bacteria are inhibited or inactivated by fatty acids and as well as by the numerous other volatile compounds that we tested.

The inhibitory effect of these compounds against S. *lactis* in APT broth generally was less pronounced than that observed when *Escherichia coli (15)*, *Salmonella typhimurium (16)*, and *Staphylococcus aureus (17)* were tested in nutrient broth. Many volatile compounds found inactive in our earlier work *(13)* inhibited S. *lactis* in these experiments probably because an air-tight vessel was used to confine the chemical while it was acting on the bacterium.

ADDENDUM

The reader is referred to the Addendum to the first paper in this series (*Escherichia coli*) *(15)* for information on the boiling point and solubility in water of all test chemicals. The initial pH of APT broth was in the range 6.5-6.75 when it contained 1, 10, or 100 ppm of any of the test chemicals. When 1000 ppm of chemicals were present, the pH of APT was between 6.3 and 6.65 except for formic, acetic and butyric acid and propylamine and hexylamine when the pH values were 5.5, 5.9, 6.1, 7.25, and 7.0, respectively.

When the reduction in population of S. *lactis* was approximately 33% or greater, a given chemical at the appropriate concentration was bactericidal. A reduction in population of approximately 0.5 to 32% represents bacteriostatic action by the chemical. Fatty acids were not bactericidal to S. *lactis*, in part, because the highest concentration of the acids failed to reduce the pH as markedly in APT broth as in nutrient broth that was used for other test organisms.

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

V. LEUCONOSTOC CITROVORUM[1]

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(Received for publication May 1, 1974)

ABSTRACT

APT broth inoculated with *Leuconostoc citrovorum* was dispensed into aerosol cans with an epoxy lining. Twenty-five volatile or non-volatile compounds were then added individually 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 and all incubations were at 30 C.

Of the fatty acids tested, decanoic acid was most and butyric acid was least inhibitory to *L. citrovorum*. Formaldehyde was more inhibitory than glyoxal, acetaldehyde. or propionaldehyde. Diacetyl was more inhibitory than acetone or 2-butanone. Acetonitrile, chloroform, and ether, each at 100 ppm, were generally significantly inhibitory especially late in the incubation. Ethylenedichloride and methylsulfone, both at 10 ppm, also generally significantly inhibited *L. citrovorum* except early in the incubation. Ethanethiol was usually more effective than methanethiol, which in turn was more detrimental than methylsulfide. Amines were more inhibitory than alcohols, and even at 10 ppm were generally significantly inhibitory. Methanol was more active against *L. citrovorum* than was furfurol.

Leuconostoc citrovorum is commonly used in conjunction with acid-forming lactic streptococci to produce flavor in certain fermented dairy products. Milk used to culture such bacteria contains many volatile and other compounds *(4)*. How most of these compounds influence the growth of *L. citrovorum* is not known.

Lindsay et al. *(10)* reported that *L. citrovorum* 91404 rapidly utilized acetaldehyde in both acidified and nonacidified milk cultures, and growth of the organism was enhanced when 5 ppm of acetaldehyde were added to nonacidified milk before inoculation. A ripened *L. citrovorum* culture, when added to a single-strain streptococcal culture, decreased its acetaldehyde content *(1, 2)*. Kulshrestha and Marth *(3, 4)* found that a variety of compounds, including aldehydes, amines, fatty acids, and diacetyl, inhibited *L. citrovorum* when tests were done with the disc assay procedure.

This paper describes results of experiments done to further evaluate the influence of some volatile or non-volatile compounds, likely to be found in milk, on *L. citrovorum* during growth in APT broth in an air-tight vessel. A preliminary report including some of these results has been presented *(5)*.

MATERIALS AND METHODS

Procedures and chemicals used for this study were described earlier *(6)*. APT broth (Difco) and APT agar (Difco) served as growth and plating media as they did for our studies with *Streptococcus lactis (9)*. The culture of *L. citrovorum* was obtained from the culture collection of the Department of Food Science, University of Wisconsin, Madison. All incubations were at 30 C. Approximately 1 hr was required for broth in cans to attain the incubation temperature.

RESULTS AND DISCUSSION

Data recorded in Tables 1 and 2 were obtained when various amounts of six different fatty acids were tested. Decanoic acid was most active, and 1000 ppm inactivated *L. citrovorum* in less than 2 h. At the same concentration butyric acid was least effective and caused only about 6% inhibition in the growth of this bacterium. Except at 2 h with butyric acid, all six fatty acids significantly inhibited *L. citrovorum*, when they were tested at 1000 ppm. At 100 ppm formic, acetic, and butyric acid were significantly inhibitory except at 2 h. Hexanoic acid at 100 ppm was always inhibitory but the effect was inconsistently significant. Octanoic and decanoic acid at 100 ppm were generally significantly inhibitory to *L. citrovorum*. Smaller amounts (1 and 10 ppm) of all fatty acids tested were always marginally inhibitory. The effect was usually significant with 10 ppm except at 2 h; whereas it was generally insignificant when 1 ppm of the acids was evaluated. Slight but usually insignificant stimulation was also noted when 1 ppm of some fatty acids was tested.

As is evident from data in Table 3, formaldehyde was more active against *L. citrovorum* than were acetaldehyde or propionaldehyde. When tested at 1000

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

TABLE 1. DIFFERENCES IN POPULATION OF *Leuconostoc citrovorum* IN APT BROTH CAUSED BY ADDED FORMIC, ACETIC, AND BUTYRIC ACID

Chemical	Conc. (ppm)	Differences (%) from control in log of population after hours of incubation				
		2	5	8	11	14
Formic acid[1]	1	(−) 0.6	(−) 0.4	(−) 0.5	(−) 0.3	(−) 1.0*
	10	(−) 1.7	(−) 2.4*	(−) 1.2*	(−) 1.6*	(−) 1.1*
	100	(−) 3.0	(−) 3.7*	(−) 2.3*	(−) 5.8*	(−) 1.5*
	1000	(−) 3.6*	(−)11.9*	(−) 9.1*	(−) 8.1*	(−)11.0*
Acetic acid[1]	1	(−) 0.6	(−) 1.9*	(+) 0.2	(−) 0.5	(−) 0.8*
	10	(−) 0.8	(−) 2.0*	(−) 1.7*	(−) 1.2*	(−) 1.6*
	100	(−) 1.3	(−) 1.5*	(−) 2.5*	(−) 2.5*	(−) 3.0*
	1000	(−) 2.5*	(−) 7.4*	(−) 8.6*	(−) 6.0*	(−) 3.7*
Butyric acid[1]	1	(+) 0.4	–[2]	(+) 0.2	–	(−) 0.5
	10	(−) 0.2	(−) 1.3*	(−) 1.3*	(−) 0.8*	(−) 1.1*
	100	(−) 1.3	(−) 1.7*	(−) 2.2*	(−) 2.1*	(−) 1.2*
	1000	(−) 2.1	(−) 5.9*	(−) 3.0*	(−) 3.3*	(−) 1.6*

[1]Control: Log of no./ml: 5.07, 5.54, 6.03, 6.73, and 7.48 at 2, 8, 11, and 14 h, respectively.
[2]–: No difference.
*: Population significantly different from control at 5% level.

TABLE 2. DIFFERENCES IN POPULATION OF *Leuconostoc citrovorum* IN APT BROTH CAUSED BY ADDED HEXANOIC, OCTANOIC, AND DECANOIC ACID

Chemical	Conc. (ppm)	Differences (%) from control in log of population after hours of incubation				
		2	5	8	11	14
Hexanoic acid[1]	1	(+) 0.4	(−) 0.6	(−) 1.2	(−) 0.2	(−) 0.5
	10	(−) 0.2	(−) 0.4*	(−) 2.7*	(−) 2.2*	(−) 2.7*
	100	(−) 1.5	(−) 2.1*	(−) 4.3*	(−) 5.3*	(−) 4.8*
	1000	(−) 3.7*	(−) 7.6*	(−) 5.9*	(−) 8.0*	(−) 7.3*
Octanoic acid[1]	1	(+) 0.2	(−) 0.2	(−) 0.9	(−) 0.7	(−) 1.0*
	10	(−) 0.6	(−) 1.4*	(−) 2.0*	(−) 1.7*	(−) 1.3*
	100	(−) 2.3*	(−) 0.6*	(−) 2.3*	(−) 4.0*	(−) 4.8*
	1000	(−) 4.8*	(−) 8.7*	(−) 2.8*	(−) 7.0*	(−) 7.4*
Decanoic acid[1]	1	(−) 2.1*	–[2]	(−) 0.2	(−) 0.3	(−) 0.5
	10	(−) 2.7	(−) 1.9*	(−) 0.9*	(−) 1.5*	(−) 2.1*
	100	(−) 5.1*	(−) 3.1*	(−) 1.4*	(−) 6.0*	(−) 4.7*
	1000	NG[3]*	NG*	NG*	NG*	NG*

[1]Control: Log of no./ml: 4.75, 5.16, 5.64, 6.01, and 6.21 at 2, 5, 8, 11, and 14 h, respectively.
[2]–: No difference.
[3]NG: Less than 10 organisms/ml of test liquid.
*: Population significantly different from control at 5% level.

ppm, formaldehyde caused more than 70% reduction in the population of this organism in less than 2 h, and inactivation was complete by 5 h. Acetaldehyde at 1000 ppm was more active than the same concentration of propionaldehyde. Inhibition by both aldehydes was always significant and the effect increased as the incubation progressed. Formaldehyde at 10 and 100 ppm, acetaldehyde at 100 ppm, and propionaldehyde at 100 ppm were always significantly inhibitory to *L. citrovorum;* whereas 10 ppm of acetaldehyde or propionaldehyde did not always significantly reduce growth. The lowest concentration (1 ppm) of the aldehydes tested generally was without any significant effect.

The influence of three ketones on growth of *L. citrovorum* is illustrated by data in Table 4. Diacetyl was most active and 1000 ppm of this compound markedly inhibited *L. citrovorum.* The inhibition increased with time and led to inactivation of this bacterium in 11 h. Acetone and 2-butanone at this concentration were considerably less detrimental; but the inhibition was always significant. Diacetyl at 10

TABLE 3. DIFFERENCES IN POPULATION OF *Leuconostoc citrovorum* IN APT BROTH CAUSED BY ADDED FORMALDEHYDE, ACETALDEHYDE, AND PROPIONALDEHYDE

Chemical	Conc. (ppm)	Differences (%) from control in log of population after hours of incubation				
		2	5	8	11	14
Formaldehyde[1]	1	(−) 0.4	(+) 0.9	(−) 1.2*	(−) 0.2	(−) 0.1
	10	(−) 2.2*	(−) 5.2*	(−) 5.1*	(−) 4.9*	(−) 1.7*
	100	(−) 4.1*	(−) 7.2*	(−)12.4*	(−)12.3*	(−)10.3*
	1000	(−)72.4*	NG*[2]	NG*	NG*	NG*
Acetaldehyde[1]	1	(−) 0.2	(+) 1.4	(−) 0.8	–[3]	(+) 0.5
	10	(−) 1.6*	(+) 0.7	(−) 1.3*	(−) 0.5*	(−) 2.1*
	100	(−) 2.4*	(−) 2.9*	(−) 5.3*	(−) 4.9*	(−) 3.5*
	1000	(−) 4.3*	(−) 6.1*	(−)18.7*	(−)28.7*	(−)36.9*
Propionaldehyde[1]	1	–	(+) 1.3	(+) 0.2	(+) 0.3	(+) 0.7
	10	(−) 1.2*	(+) 1.1*	(−) 1.3*	(−) 0.3	(−) 3.3*
	100	(−) 1.2*	(−) 1.1*	(−) 4.0*	(−) 4.5*	(−) 6.4*
	1000	(−) 1.6*	(−) 5.1*	(−) 9.0*	(−)13.1*	(−)18.1*

[1]Control: Log of no./ml: 4.75, 5.40, 6.03, 6.71, and 7.39 at 2, 5, 5, 8, 11, and 14 h, respectively.
[2]NG: 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 *Leuconostoc citrovorum* IN APT BROTH CAUSED BY ADDED ACETONE, 2-BUTANONE, AND DIACETYL

Chemical	Conc. (ppm)	Differences (%) from control in log of population after hours of incubation				
		2	5	8	11	14
Acetone[1]	1	(−) 0.8	(+) 0.4	(+) 0.7*	(+) 0.3	(+) 0.1
	10	(−) 1.9*	(+) 0.2	(−) 0.7	(−) 0.5	(−) 0.4*
	100	(−) 2.1	(−) 0.7*	(−) 1.3	(−) 2.1*	(−) 1.6*
	1000	(−) 4.1*	(−) 2.1*	(−) 2.5*	(−) 2.7*	(−) 3.1*
2-butanone[1]	1	–[2]	(+) 0.4	(+) 0.8*	(+) 0.3	(+) 0.1
	10	(−) 2.1*	(+) 0.2	–	(−) 0.2	(−) 0.4*
	100	(−) 2.9*	(−) 1.5*	(−) 0.8	(−) 1.7*	(−) 1.4*
	1000	(−) 3.3*	(−) 2.4*	(−) 2.7*	(−) 2.9*	(−) 3.1*
Diacetyl[1]	1	(−) 1.2	(−) 0.2	(+) 0.7*	–	–
	10	(−) 1.9*	(−) 1.5*	(−) 0.7	(−) 1.4	(−) 0.7*
	100	(−) 3.3*	(−) 2.1*	(−) 1.7	(−) 2.7*	(−) 3.1*
	1000	(−)17.9*	(−)29.8*	(−)62.0*	NG[3]*	NG*

[1]Control: Log of no./ml: 4.85, 5.37, 6.00, 6.60, and 7.38 at 2, 5, 8. 11, and 14 h, respectively.
[2]–: No difference.
[3]NG: Less than 10 organisms/ml of test liquid.
*: Population significantly different from control at 5% level.

and 100 ppm was always detrimental to growth of this organism but the effect was inconsistently significant. At 1 ppm this compound was essentially innocuous. At a concentration of 100 ppm, 2-butanone was slightly and generally significantly inhibitory; whereas 10 ppm of this compound generally were inhibitory but the effect was insignificant except in the beginning and at the end of the incubation. At 1 ppm 2-butanone was without significant effect. Acetone at 10 and 100 ppm was inconsistently significantly inhibitory; but 1 ppm of this compound was generally without a significant effect.

Data in Table 5 were obtained when different concentrations of acetonitrile, chloroform, or ether were tested. Chloroform at 1000 ppm was more detrimental than were ether or acetonitrile at the same concentration. When 1000 ppm chloroform was tested, inhibition generally increased with time and always was significant. At 100 ppm chloroform always was inhibitory but the effect was only significant after 8 h of incubation. Lower (1 and 10 ppm) concentrations of this compound caused limited significant inhibition between 5 and 8 h of incubation; otherwise they were generally ineffective. Ether at 1000 ppm

exerted a limited but always significant inhibitory effect on *L. citrovorum*, and at 100 ppm this compound still remained inhibitory. At 10 ppm ether caused slight but insignificant stimulation of growth in the beginning of the incubation and slight but irregularly significant inhibition in the later stages; 1 ppm of ether was without a significant effect. Acetonitrile was least effective of the three compounds listed in Table 5. At 1000 ppm this chemical always significantly inhibited *L. citrovorum*. Acetonitrile at 100 ppm also was always inhibitory but the effect was significant only late in the incubation. At 10 and 1 ppm acetonitrile was generally without any effect on *L. citrovorum*.

The influence of different amounts of glyoxal, ethylenedichloride, and methylsulfone on growth of *L. citrovorum* is summarized in Table 6. Glyoxal at 1000 ppm was somewhat active and in 2 h caused the population of *L. citrovorum* to decrease by 10%. The activity increased with time so that the bacterium was inactivated in 11 h. A similar concentration of ethylenedichloride and methylsulfone was markedly less active but the inhibition caused by these compounds was always significant and was more evident late in

TABLE 5. DIFFERENCES IN POPULATION OF *Leuconostoc citrovorum* IN APT BROTH CAUSED BY ADDED ACETONITRILE, CHLOROFORM AND ETHER

Chemical	Conc. (ppm)	Differences (%) from control in log of population after hours of incubation				
		2	5	8	11	14
Acetonitrile[1]	1	(+) 1.3	(−) 0.7*	(−) 0.5	(+) 0.5	(−) 0.4
	10	(+) 0.4	(−) 1.3*	(−) 1.5*	(−) 0.3	(−) 1.5*
	100	(−) 0.9	(−) 1.5	(−) 3.1*	(−) 2.1*	(−) 3.9*
	1000	(−) 2.0*	(−) 2.8*	(−) 4.4*	(−) 3.6*	(−) 5.4*
Chloroform[1]	1	(+) 0.9	(−) 0.9*	(−) 0.7*	(−) 0.3	(−) 0.4
	10	(+) 0.2	(−) 1.3*	(−) 2.8*	(−) 1.4	(−) 1.6*
	100	(−) 0.2	(−) 1.5	(−) 6.1*	(−) 2.6*	(−) 5.3*
	1000	(−) 1.3*	(−) 5.7*	(−) 8.4*	(−) 6.0*	(−)10.0*
Ether[1]	1	(+) 1.1	(−) 0.6	(+) 0.3	(+) 0.2	(−) 0.3
	10	(+) 0.4	(−) 2.6*	(−) 1.0	(−) 0.9	(−) 1.6*
	100	(−) 0.9	(−) 3.0*	(−) 4.8*	(−) 3.0*	(−) 5.4*
	1000	(−) 1.3*	(−) 3.9*	(−) 6.7*	(−) 4.7*	(−) 6.2*

[1]Control: Log of no./ml: 4.57, 5.42. 6.08. 6.66, and 7.40 at 2, 5, 8, 11, and 14 h, respectively.
*: Population significantly different from control at 5% level.

TABLE 6. DIFFERENCES IN POPULATION OF *Leuconostoc citrovorum* IN APT BROTH CAUSED BY ADDED GLYOXAL, ETHYLENEDICHLORIDE, AND METHYLSULFONE

Chemical	Conc. (ppm)	Differences (%) from control in log of population after hours of incubation				
		2	5	8	11	14
Glyoxal[1]	1	(+) 0.6	(−) 0.9*	(−) 0.7	–[3]	(−) 1.0*
	10	(−) 0.6	(−) 1.3*	(−) 3.1*	(−) 2.3*	(−) 3.3*
	100	(−) 1.1	(−) 2.2*	(−) 5.8*	(−) 3.9*	(−) 9.4*
	1000	(−) 9.8*	(−)26.2*	(−)54.6*	NG[2]*	NG*
Ethylenedichloride[1]	1	(+) 0.9	(−) 0.7*	(−) 0.8	(−) 0.8	(−) 0.8*
	10	(−) 0.9	(−) 1.5*	(−) 2.2*	(−) 1.8*	(−) 3.0*
	100	(−) 0.2	(−) 1.7*	(−) 5.5*	(−) 6.0*	(−) 7.4*
	1000	(−) 4.5*	(−) 6.7*	(−) 7.3*	(−) 8.3*	(−)11.8*
Methylsulfone[1]	1	–	(−) 0.6*	(−) 0.5	(−) 0.5	(−) 0.3
	10	(−) 1.7	(−) 1.5*	(−) 1.0	(−) 1.5*	(−) 1.0*
	100	(−) 2.1*	(−) 2.0*	(−) 1.7*	(−) 6.4*	(−) 4.8*
	1000	(−) 3.2*	(−) 3.7*	(−) 2.5*	(−) 6.3*	(−) 7.4*

[1]Control: Log of no./ml: 4.69, 5.38, 6.05, 6.66, and 7.35 at 2. 5, 8. 11, and 14 h, respectively.
[2]NG: Less than 10 organisms/ml of test liquid.
[3]–: No difference.
*: Population significantly different from control at 5% level.

TABLE 7. DIFFERENCES IN POPULATION OF *Leuconostoc citrovorum* IN APT BROTH CAUSED BY ADDED METHYLSULFIDE, METHANETHIOL, AND ETHANETHIOL

Chemical	Conc. (ppm)	Differences (%) from control in log of population after hours of incubation				
		2	5	8	11	14
Methylsulfide[1]	1	(−) 1.2*	(+) 0.2	(+) 0.2	(+) 0.7*	(+) 0.3
	10	(−) 1.6*	(+) 0.6	(−) 0.2	(−) 0.2	–[2]
	100	(−) 3.4*	(−) 0.6	(−) 1.2*	(−) 1.5*	(−) 0.2
	1000	(−) 4.1*	(−) 1.2	(−) 1.7*	(−) 2.4*	(−) 1.0
Methanethiol[1]	1	(+) 0.2	(−) 0.2	(+) 0.4	(+) 0.2*	(+) 0.3
	10	(−) 1.2*	(−) 0.2	(−) 0.4	(+) 0.7	(+) 0.2
	100	(−) 1.6*	(−) 1.6*	(−) 1.2*	(−) 0.4	(−) 1.3*
	1000	(−) 3.7*	(−) 5.5*	(−) 3.9*	(−) 0.6*	(−) 1.5*
Ethanethiol[1]	1	–	(+) 0.2	–	(−) 0.2*	–
	10	(−) 1.2*	(−) 0.8	(−) 0.8	(+) 0.2	(+) 0.3
	100	(−) 2.5*	(−) 2.0*	(−) 2.3*	(−) 0.6*	(−) 1.3*
	1000	(−) 3.7*	(−) 7.4*	(−) 3.9*	(−) 0.9*	(−) 2.8*

[1]Control: Log of no./ml: 4.36. 4.89. 5.20, 5.46, and 6.06 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 *Leuconostoc citrovorum* IN APT BROTH CAUSED BY ADDED FURFURYL AND METHYL ALCOHOL AND PROPYL- AND HEXYLAMINE

Chemical	Conc. (ppm)	Differences (%) from control in log of population after hours of incubation				
		2	5	8	11	14
Furfuryl alcohol[1]	1	–[2]	(−) 0.4	(+) 0.4	(−) 0.8	(−) 1.0
	10	(−) 1.8*	(−) 0.8*	(−) 1.1	(−) 2.5*	(−) 2.0*
	100	(−) 2.6*	(−) 1.6	(−) 3.7*	(−) 4.7*	(−) 5.5*
	1000	(−) 3.7*	(−) 7.3*	(−) 9.7*	(−) 9.2*	(−) 9.7*
Methyl alcohol[1]	1	(−) 0.9	–	(−) 0.4	(−) 0.5	(−) 0.6
	10	(−) 3.1*	(−) 1.0*	(−) 1.9*	(−) 3.6*	(−) 1.7*
	100	(−) 2.4*	(−) 2.2	(−) 3.5*	(−) 5.5*	(−) 7.0*
	1000	(−) 6.8*	(−) 8.7*	(−)11.7*	(−)13.0*	(−)14.1*
Propylamine[1]	1	(−) 0.7	(−) 0.4	(−) 3.4*	(−) 1.3	(−) 7.3*
	10	(−) 1.8*	(−) 1.0*	(−) 3.5*	(−) 4.7*	(−) 8.7*
	100	(−) 4.6*	(−) 5.2*	(−) 6.9*	(−)10.0*	(−)11.6*
	1000	(−)21.0*	(−)31.6*	(−)30.2*	(−)28.4*	(−)26.7*
Hexylamine[1]	1	(+) 0.2	(−) 0.8	(−) 3.0*	(−) 1.6	(−) 5.4*
	10	(−) 0.7	(−) 1.8*	(−) 4.2*	(−) 4.0*	(−) 6.2*
	100	(−) 6.4*	(−) 7.1*	(−) 6.2*	(−) 6.6*	(−) 8.7*
	1000	(−)21.0*	(−)33.9*	(−)31.5*	(−)28.9*	(−)25.9*

[1]Control: Log of no./ml: 4.57. 5.04, 5.66, 6.33, and 6.90 at 2, 5, 8, 11. and 14 h, respectively.
[2]–: No difference.
*: Population significantly different from control at 5% level.

the incubation. Glyoxal and ethylenedichloride at 10 and 100 ppm always were inhibitory to *L. citrovorum* but initially the effect was not significant. Inhibition caused by 100 ppm of methylsulfone always was significant; but not so at 10 ppm. At 1 ppm glyoxal, ethylenedichloride, and methylsulfone generally were without effect on *L. citrovorum*.

It is evident from data in Table 7 that ethanethiol was slightly more active than methanethiol; and methylsulfide was least inhibitory of the three compounds. Ethanethiol at 100 and 1000 ppm and methanethiol at 1000 ppm always were significantly inhibitory. Methanethiol at 100 ppm and methylsulfide at 100 and 1000 ppm always were inhibitory but the effect was not always significant. At 1 and 10 ppm the three compounds were without any appreciable effect on *L. citrovorum*.

The inhibitory effect of amines and alcohols tested

is illustrated by data in Table 8. Both propyl- and hexylamine at 1000 ppm were almost equally active, but at 1, 10, and 100 ppm propylamine tended to be somewhat more active against *L. citrovorum* at some stages of growth than was hexylamine. Inhibition caused by propylamine at 10, 100, and 1000 ppm and by hexylamine at 100 and 1000 ppm always was significant. Hexylamine at 10 ppm was always inhibitory but not significantly so early in the incubation. Propylamine at 1 ppm always was inhibitory but not always significantly, whereas hexylamine at 1 ppm was not significantly inhibitory until 8 h of incubation Other than at 1000 ppm, when inhibition was maximum about midway during the incubation, activity of amines generally increased with time. Methanol usually was more detrimental to growth of *L. citrovorum* than was furfurol. Both alcohols at 1000 ppm always were significantly inhibitory, but the inhibition by 100 and 10 ppm of the alcohols was usually significant. At 1 ppm both alcohols tested were generally insignificantly inhibitory.

As observed earlier with other bacteria the higher concentrations of all compounds tested inhibited *L. citrovorum*, but only some compounds caused substantial inhibition when they were tested at lower concentrations. Some compounds such as fatty acids and amines inhibited this bacterium even at concentrations that could be expected in milk. It has been reported *(10)* that 5 ppm of acetaldehyde stimulated growth of *L. citrovorum* in both acidified and nonacidified milk cultures. We also found that acetaldehyde and propionaldehyde at 1 and 10 ppm, and formaldehyde at 1 ppm tended to enhance growth of this organism at least during some phase of the incubation. Only traces of acetaldehyde or formaldehyde occur in raw or mildly heated milk but the amounts may be sufficient to stimulate *L. citrovorum* when it is used together with acid-producing bacteria in the manufacture of fermented dairy products. However, some other compounds in milk, such as fatty acids and amines, tend to inhibit the organism. Thus, the overall effect of all compounds in milk is difficult to assess.

As with *Streptococcus lactis (9)*, the compounds tested tended to be less active against *L. citrovorum* in APT broth than against *Escherichia coli (6)*, *Salmonella typhimurium (7)* or *Staphylococcus aureus (8)* in nutrient broth. Decanoic acid was the most effective fatty acid against both *L. citrovorum* and *S. lactis (9)*. Butyric acid was least active against *L. citrovorum*, whereas the least effective fatty acid against *S. lactis* was acetic acid. Formaldehyde, diacetyl, acetone, 2-butanone, chloroform, ethylenedichloride, methylsulfide, methanethiol, and ethanethiol were slightly more active against *S. lactis (9)* than they were against *L. citrovorum*, but methanol, propyl- and hexylamine, and glyoxal were more inhibitory to *L. citrovorum* than to *S. lactis (9)*.

ADDENDUM

The reader is referred to the Addendum to the first paper in this series (*Escherichia coli*) *(6)* for information on the boiling point and solubility in water of all test chemicals. Initial pH values of APT broth with added chemicals can be found in the Addendum to the fourth paper in this series (*Streptococcus lactis*) *(9)*.

When the reduction in population of *L. citrovorum* was approximately 19% or greater, a given chemical at the appropriate concentration was bactericidal. A reduction in population of approximately 0.4 to 18% represents bacteriostatic action by the chemical.

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

VI. STREPTOCOCCUS THERMOPHILUS[1]

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(Received for publication June 3, 1974)

ABSTRACT

APT broth inoculated with *Streptococcus thermophilus* was dispensed into epoxy-lined aerosol cans. Twenty five volatile or non-volatile compounds likely to be present in milk were added individually to cans to attain concentrations in broth of 1, 10, 100, and 1000 ppm of each compound. Compounds used included formic, acetic, butyric, hexanoic, octanoic, and decanoic acid; formaldehyde; acetaldehyde; propionaldehyde; glyoxal; acetone; 2-butanone; diacetyl; propyl— and hexylamine; furfurol; methanol; methylsulfide; methylsulfone; methanethiol; ethanethiol; acetonitrile; chloroform; ether; and ethylenedichloride. Bacteria were enumerated at intervals during incubation at 37 C.

Of the six fatty acids tested, decanoic acid was most and hexanoic acid was least inhibitory to S. *thermophilus*. At 1000 ppm all fatty acids tested always significantly inhibited S. *thermophilus*. Formaldehyde was more inhibitory to the bacterium than were acetaldehyde, propionaldehyde, or glyoxal. Of the ketones tested, diacetyl was most effective against S. *thermophilus*. Acetonitrile, chloroform, and ether at 10 ppm or more were always inhibitory but significantly so only early during the incubation. At concentrations of 10 and 100 ppm ethylenedichloride and methylsulfone were significantly inhibitory late in the 14-h incubation. Methanethiol was generally more active than ethanethiol, but ethanethiol was more inhibitory than methylsulfide. Amines were always inhibitory and were more active than alcohols.

Streptococcus thermophilus and *Lactobacillus bulgaricus* are used to manufacture yogurt, Swiss cheese, and certain Italian-type cheeses. Incubation to manufacture these fermented foods is at temperatures higher than those used for some other cultured dairy products. S. *thermophilus* functions to produce acid and a proper balance between it and *L. bulgaricus* must usually be maintained for proper fermentation and ripening of the product. Many volatile and other compounds *(3)* are present in milk used to culture these organisms; how these compounds affect growth of S. *thermophilus* has not received much attention. Pozanski et al. *(9)* reported S. *thermophilus* produced 56.6 and 78.8% less acid when 1.5 and 3.0 mM of capric acid, respectively, were added to milk. It has also been reported that the ratio of S. *thermophilus* to *L. bulgaricus* during growth in UHT-treated milk can be maintained between 1.0: 0.5 and 1.0: 3.0; whereas in autoclaved milk the ratio quickly changes and stabilizes at 1 : 20 *(2)*. There are likely to be differences in the kinds and amounts of volatile compounds in milks that received the two heat-treatments. Bottazzi and Vescovo *(2)* found that addition of 5 μg or more of formic acid/ml of UHT-treated milk served to alter the ratio of S. *thermophilus* to *L. bulgaricus* in favor of the latter organism.

This paper summarizes data obtained when some volatile or non-volatile compounds of the types likely to be present in milk were tested for their effect on growth and survival of S. *thermophilus*.

MATERIALS AND METHODS

Procedures and chemicals used for these experiments were the same as described earlier *(4)*. APT broth (Difco) and APT agar (Difco) served as growth and plating media in these experiments just as they were used previously in similar work with *Streptococcus lactis (7)* and *Leuconostoc citrovorum (8)*. The culture used in these experiments was *Streptococcus thermophilus* ST_4, obtained from the Marschall Division, Miles Laboratories, Madison, Wisconsin.

RESULTS AND DISCUSSION

Results obtained when different concentrations of six fatty acids were tested are given in Tables 1 and 2. At 1000 ppm decanoic acid was most and hexanoic acid was least inhibitory to S. *thermophilus*. At this concentration inhibition of the bacterium by decanoic acid increased in magnitude with the elapse of time and was almost 90% at 14 h. When other fatty acids were tested at this concentration, the degree of inhibition generally increased earlier in the incubation, and after 8 h it declined gradually. Formic acid was less inhibitory than was decanoic acid but more so than octanoic acid. Acetic and butyric acid were less inhibitory than octanoic acid but were more so than hexanoic acid. Inhibition by all fatty acids tested at 1000 ppm was always significant. At 100 ppm decanoic and butyric acid always were significantly inhibitory. Formic and acetic acid at 100 ppm and decanoic acid at 10 ppm were generally significantly inhibitory except in the terminal stages

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

TABLE 1. DIFFERENCES IN POPULATIONS OF *Streptococcus thermophilus* IN APT BROTH CAUSED BY ADDED FORMIC, ACETIC, AND BUTYRIC ACID

Chemical	Conc. (ppm)	Differences (%) from control in log of populations after hours of incubation				
		2	5	8	11	14
Formic acid[1]	1	–[2]	–	–	(–) 0.4	–
	10	(–) 0.8	(–) 1.6*	(–) 0.9*	(–) 0.9	(–) 0.1
	100	(–) 1.2*	(–) 2.1*	(–) 6.0*	(–) 3.3*	(–) 0.5
	1000	(–) 3.0*	(–)16.7*	(–)23.5*	(–)17.6*	(–)12.8*
Acetic acid[1]	1	–	(+) 0.2	(+) 0.1	–	(–) 0.2
	10	(–) 0.3	(–) 0.5	–	(+) 0.1	(+) 0.2
	100	(–) 1.2*	(–) 1.8*	(–) 4.8*	(–) 1.2*	–
	1000	(–) 3.5*	(–) 3.9*	(–) 7.8*	(–) 3.5*	(–) 2.0*
Butyric acid[1]	1	–	(–) 0.2	(–) 0.1	(–) 0.2	–
	10	(–) 1.2	(–) 1.3*	(–) 5.2*	(–) 0.8	(–) 0.6
	100	(–) 2.7*	(–) 2.6*	(–) 5.8*	(–) 3.1*	(–) 0.8*
	1000	(–) 3.2*	(–) 3.4*	(–) 7.4*	(–) 4.9*	(–) 2.8*

[1]Control: Log of no./ml: 4.05, 6.10, 7.87, 8.51, and 8.91 at 2, 5, 8, 11, and 14 h, respectively.
[2]–: No difference.
*: Population significantly different from control at the 5% level.

TABLE 2. DIFFERENCES IN POPULATIONS OF *Streptococcus thermophilus* IN APT BROTH CAUSED BY ADDED HEXANOIC, OCTANOIC, AND DECANOIC ACID

Chemical	Conc. (ppm)	Differences (%) from control in log of populations after hours of incubation				
		2	5	8	11	14
Hexanoic acid[1]	1	(+) 0.3	–[2]	(+) 0.1	–	(+) 0.2
	10	–	(–) 0.3	(–) 0.9*	(–) 0.8	(–) 0.1
	100	(–) 0.5	(–) 0.8*	(–) 3.0*	(–) 2.0*	(–) 0.8*
	1000	(–) 1.5*	(–) 1.8*	(–) 5.2*	(–) 3.8*	(–) 2.1*
Octanoic acid[1]	1	–	(–) 0.3	–	(+) 0.1	(+) 0.2
	10	(–) 0.5	(–) 0.7*	(–) 1.3*	(–) 1.0	–
	100	(–) 0.7	(–) 1.3*	(–) 5.8*	(–) 3.2*	(–) 0.6*
	1000	(–) 2.9*	(–) 7.5*	(–)10.2*	(–) 7.7*	(–)10.2*
Decanoic acid[1]	1	(–) 0.7	(–) 0.5	(–) 2.2	(+) 0.1	(+) 0.3
	10	(–) 1.2*	(–) 1.3*	(–) 3.9*	(–) 2.9*	(–) 0.3
	100	(–) 2.0*	(–) 1.6*	(–) 8.7*	(–) 7.8*	(–) 6.4*
	1000	(–)17.2*	(–)50.5*	(–)67.2*	(–)75.8*	(–)88.8*

[1]Control: Log of no./ml: 4.08. 6.16, 7.92, 8.68, and 8.90 at 2, 5, 8, 11, and 14 h, respectively.
[2]–: No difference.
*: Population significantly different from control at the 5% level.

of incubation. At 100 ppm the inhibitory activity of hexanoic and octanoic acid was significant after 2 h of incubation. Formic, butyric, and hexanoic acid at 10 ppm sometimes were significantly inhibitory. Acetic acid at 10 ppm was only marginally inhibitory except late in the incubation when the chemical appeared to be stimulatory; however, the magnitude of the effects were insignificant. At 10 ppm octanoic acid was significantly inhibitory only after 5 and 8 h of incubation. All fatty acids, at 1 ppm, were without a significant effect.

As illustrated by data in Table 3, formaldehyde was more detrimental to S. *thermophilus* than was acetaldehyde, and propionaldehyde was less inhibitory than was acetaldehyde. Formaldehyde at 1000 ppm reduced the population of S. *thermophilus* by more than 80% in less than 2 h and completely inactivated the organism in 5 h. Acetaldehyde at 1000 ppm caused progressively greater inhibition that terminated in inactivation of the organism after 14 h of incubation. Inhibition by propionaldehyde, when tested at 1000 ppm, increased from less than 5% at 2 h to about 50% at 5 h and then slowly declined later in the incubation. Inhibition by 1000 ppm of any of the aldehydes always was significant. When 100 ppm of these compounds were tested, formaldehyde always was significantly inhibitory to S. *thermophilus* and was most active late in the incubation. Acetaldehyde and propionaldehyde at the same concentration were most active about midway during the incubation, and inhibition by these compounds was significant except early in the incubation. At 10 ppm all three compounds were inhibitory; the extent of inhibition was insignificant at the beginning and end of the observation period. A somewhat similar effect was noted with 1 ppm of formaldehyde, whereas 1 ppm of acetaldehyde or propionaldehyde failed to inhibit the streptococcus.

Table 4 lists results obtained when acetone, 2-butanone, or diacetyl were tested. Diacetyl was most and acetone was least inhibitory to S. *thermophilus*. At 1000 ppm diacetyl reduced the population by about 65% within 5 h; and the organism was inactivated in 8 h. Inhibition by acetone or 2-butanone, at 1000 ppm, although significant was much less than that of diacetyl; maximum activity by these chemicals occurred midway through the incubation. Diacetyl at 100 ppm always significantly inhibited S. *thermophilus*, whereas less but generally significant inhibition occurred with acetone at 100 ppm, 2-butanone at 100 ppm, or diacetyl at 10 ppm. Acetone at 1 and 10 ppm was without significant effect, whereas 2-butanone at 10 ppm was significantly inhibitory only late in the incubation. At 1 ppm the three ketones tested nearly always failed to significantly affect growth of S. *thermophilus*.

The influence of different concentrations of acetonitrile, chloroform, and ether on growth of S. *thermophilus* is illustrated by data in Table 5. Inhibition by acetonitrile at 1000 ppm was always significant and was greatest early in the incubation. At 100 ppm this compound caused limited and often nonsignificant inhibition of S. *thermophilus*. Even less inhibition, that was significant only early in the incubation, occurred with 10 ppm of acetonitrile. With 1 ppm of acetonitrile only marginal and generally insignificant inhibition or stimulation of S. *thermophilus* was observed. Chloroform at 10, 100, and 1000 ppm always was significantly inhibitory to S. *thermophilus*. Generally inhibition by chloroform increased progressively for 5 h and then declined later in the incubation. When 1 ppm of this compound was tested, slight but significant inhibition occurred early in the incubation; the effect became insignificant later. Ether was generally less inhibitory than chloroform. At 1000 ppm ether was

TABLE 3. DIFFERENCES IN POPULATIONS OF *Streptococcus thermophilus* IN APT BROTH CAUSED BY ADDED FORMALDEHYDE, ACETALDEHYDE, AND PROPIONALDEHYDE

Chemical	Conc. (ppm)	Differences (%) from control in log of populations after hours of incubation				
		2	5	8	11	14
Formaldehyde[1]	1	–[2]	(–) 1.6*	(–) 1.6*	(–) 0.6*	(–) 0.3
	10	(–) 0.8	(–) 2.3*	(–) 2.2*	(–) 2.0*	(–) 1.0
	100	(–) 4.2*	(–)33.3*	(–)37.0*	(–)38.8*	(–)36.2*
	1000	(–)81.7*	NG[3]*	NG*	NG*	NG*
Acetaldehyde[1]	1	(–) 0.5	(–) 1.3*	(–) 0.3	(–) 0.1	(–) 0.3
	10	(–) 0.5	(–) 1.8*	(–) 0.9*	(–)3.3*	(–) 0.6
	100	(–) 2.4	(–) 5.4*	(–) 8.0*	(–) 7.7*	(–) 0.9*
	1000	(–) 7.9*	(–)48.2*	(–)64.7*	(–)88.1*	NG*
Propionaldehyde[1]	1	(–) 0.5	(–) 0.8	(+) 0.1	(–) 0.1	(–) 0.1
	10	(–) 1.1	(–) 2.4	(–) 0.3*	(–) 3.3*	(–) 0.5
	100	(–) 2.4	(–)11.4*	(–)12.4*	(–) 6.9*	(–) 1.6*
	1000	(–) 4.7*	(–)49.0*	(–)62.0*	(–)60.6*	(–)46.1*

[1]Control: Log of no./ml: 3.82, 6.16, 7.64, 8.41, and 8.86 at 2, 5, 8, 11, and 14 h, respectively.
[2]–: No difference.
[3]NG: Less than 10 organisms/ml of test liquid.
*: Population significantly different from control at the 5% level.

TABLE 4. DIFFERENCES IN POPULATIONS OF *Streptococcus thermophilus* IN APT BROTH CAUSED BY ADDED ACETONE, 2-BUTANONE, AND DIACETYL

Chemical	Conc. (ppm)	Differences (%) from control in log of populations after hours of incubation				
		2	5	8	11	14
Acetone[1]	1	(+) 1.1	(+) 0.3	(+) 0.3	(–) 0.1	(+) 0.1
	10	(+) 0.3	(–) 1.0	–[2]	(+) 0.1	(+) 0.2
	100	(–) 0.5	(–) 2.2*	(–) 4.9*	(–) 0.4*	(–) 0.3
	1000	(–) 1.6*	(–) 3.3*	(–) 6.0*	(–) 1.1*	(–) 1.1*
2-butanone[1]	1	(–) 0.3	(+) 1.0*	(+) 0.1	–	(–) 0.5
	10	(–) 0.8	(–) 0.8	(–) 0.9*	(–) 0.7*	(–) 1.0
	100	(–) 1.3	(–) 1.5*	(–) 4.1*	(–) 2.0*	(–) 1.5*
	1000	(–) 2.6*	(–) 8.7*	(–) 9.3*	(–) 4.8*	(–) 2.2*
Diacetyl[1]	1	(–) 2.4	(–) 0.3	(–) 0.7	(–) 1.1*	(–) 0.6
	10	(–) 3.7*	(–) 1.2	(–) 3.2*	(–) 3.2*	(–) 1.1*
	100	(–) 4.8*	(–)12.2*	(–)13.5*	(–) 8.6*	(–)10.5*
	1000	(–) 7.4*	(–)64.8*	NG[3]*	NG*	NG*

[1]Control: Log of no./ml: 3.79, 6.00, 7.63, 8.40, and 8.92 at 2, 5, 8, 11, and 14 h, respectively.
[2]–: No difference.
[3]NG: Less than 10 organisms/ml of test liquid.
*: Population significantly different from control at the 5% level.

TABLE 5. DIFFERENCES IN POPULATIONS OF *Streptococcus thermophilus* IN APT BROTH CAUSED BY ADDED ACETONITRILE, CHLOROFORM, AND ETHER

Chemical	Conc. (ppm)	Differences (%) from control in log of populations after hours of incubation				
		2	5	8	11	14
Acetonitrile[1]	1	(+) 1.0*	(−) 0.2	(+) 1.8*	(+) 0.3	(−) 0.2
	10	(−) 4.2*	(−) 2.4*	(−) 0.2	(−) 0.1	(−) 0.2
	100	(−) 5.4*	(−) 4.0*	(−) 0.9	(−) 2.9*	(−) 1.1
	1000	(−) 6.2*	(−) 8.1*	(−) 4.2*	(−) 3.9*	(−) 1.4*
Chloroform[1]	1	(−) 1.5*	(−) 0.4*	(+) 0.6	(−) 0.3	—[2]
	10	(−) 2.0*	(−) 2.2*	(−) 2.1*	(−) 3.0*	(−) 0.9*
	100	(−) 5.2*	(−) 5.5*	(−) 3.3*	(−) 3.9*	(−) 1.6*
	1000	(−) 5.9*	(−)10.7*	(−) 5.4*	(−) 6.2*	(−) 2.8*
Ether[1]	1	(−) 1.5*	—	(+) 0.2	(+) 0.1	(−) 0.1
	10	(−) 2.7*	(−) 0.6*	(−) 1.4	(−) 0.4	(−) 0.7
	100	(−) 4.4*	(−) 2.8*	(−) 1.7	(−) 2.7*	(−) 0.8
	1000	(−) 5.9*	(−) 6.3*	(−) 3.6*	(−) 4.1*	(−) 0.9*

[1]Control: Log of no./ml: 4.05, 5.44, 6.65, 8.03, and 8.90 at 2, 5, 8, 11, and 14 h, respectively.
[2]—: No difference.
*: Population significantly different from control at the 5% level.

TABLE 6. DIFFERENCES IN POPULATIONS OF *Streptococcus thermophilus* IN APT BROTH CAUSED BY ADDED GLYOXAL, ETHYLENEDICHLORIDE, AND METHYLSULFONE

Chemical	Conc. (ppm)	Differences (%) from control in log of populations after hours of incubation				
		2	5	8	11	14
Glyoxal[1]	1	(−) 1.1	(−) 2.4*	(−) 1.0	(−) 0.6*	(−) 0.1
	10	(−) 2.4*	(−) 4.7*	(−) 1.6*	(−) 3.0*	(−) 0.8*
	100	(−) 2.9*	(−) 8.0*	(−) 5.2*	(−) 5.3*	(−) 2.4*
	1000	(−)24.5*	NG[2]*	NG*	NG*	NG*
Ethylenedichloride[1]	1	(−) 0.3	(+) 0.4	(−) 0.3	—[3]	(+) 0.1
	10	(−) 0.8	(−) 0.5	(−) 0.6*	(−) 0.6*	(−) 1.2*
	100	(−) 2.4	(−) 0.2	(−) 0.9	(−) 0.5*	(−) 4.7*
	1000	(−) 5.8*	(−) 2.2*	(−) 1.5	(−) 5.3*	(−) 5.6*
Methylsulfone[1]	1	(−) 1.1	(+) 0.5	(−) 1.3	(−) 0.1	(+) 0.2
	10	(−) 2.1*	(+) 0.2	(−) 1.3*	(−) 0.5*	(−) 1.1*
	100	(−) 2.1	(−) 0.7	(−) 2.1*	(−) 1.7*	(−) 2.1*
	1000	(−) 3.2*	(−) 3.6*	(−) 3.4*	(−) 5.9*	(−) 2.7*

[1]Control: Log of no./ml: 3.79, 5.52, 6.80, 8.25, and 8.95 at 2, 5, 8, 11, and 14 h, respectively.
[2]NG: Less than 10 organisms/ml of test liquid.
[3]—: No difference.
*: Population significantly different from control at the 5% level.

consistently significantly inhibitory, whereas this was not true when 100 ppm of this compound were tested. Ether at 10 ppm was always inhibitory but the extent was generally not significant. At 1 ppm this compound had essentially no significant effect on *S. thermophilus*.

Inhibition of *S. thermophilus* by glyoxal, ethylenedichloride, and methylsulfone is illustrated by data in Table 6. Glyoxal at 1000 ppm inactivated *S. thermophilus* within 5 h. Although the degree of inhibition decreased as the concentration of glyoxal was reduced, inhibition by 10 and 100 ppm of this compound was always significant. When 1 ppm of glyoxal was tested, inhibition usually was non-significant. Ethylenedichloride at 1000 ppm usually was significantly inhibitory. Lower concentrations (10 and 100 ppm) of this compound were consistently but not always significantly inhibitory. At 1 ppm, ethylenedichloride had no significant effect on *S. thermophilus*. Methylsulfone at 1000 ppm always was significantly inhibitory to *S. thermophilus*, whereas 100 ppm of this compound caused inhibition that was only significant late in the incubation. At 10 ppm limited but usually significant inhibition of *S. thermophilus* was observed; at 1 ppm the effect was always without significance.

As illustrated by data in Table 7, methanethiol inhibited *S. thermophilus* slightly more than did ethanethiol; methylsulfide was the least inhibitory of these three compounds. Methylsulfide at 1000 ppm was generally significantly inhibitory to *S. thermophilus*. Limited inhibition, that was significant only midway during the incubation, occurred when 100 ppm of this compound were tested. Smaller amounts (1 and 10 ppm) of methylsulfide had no significant effect on growth of *S. thermophilus*. Inhibition of *S. ther-*

mophilus by 1000 ppm methanethiol was substantial and was significant at and beyond 5 h of incubation. Although inhibition occurred regularly with 10 or 100 ppm of methanethiol, the extent was significant only as the incubation progressed beyond 5 to 8 h. At 1 ppm this compound caused limited inhibition that was generally insignificant. Ethanethiol was slightly less inhibitory than was methanethiol. At 1000 ppm ethanethiol was regularly significantly inhibitory. Lower concentrations (10 and 100 ppm) of ethanethiol continued to be inhibitory but the extent was only significant late in the incubation. Ethanethiol at 1 ppm had no significant influence on growth of *S. thermophilus*.

Data depicting the influence of amines (propyl- and hexylamine) and alcohols (furfurol and methanol) on growth of *S. thermophilus* are summarized in Table 8. As with other organisms tested *(4-8)*, amines were more inhibitory to *S. thermophilus* than were alcohols. Both amines were regularly significantly inhibitory when tested at 1000 ppm. At 100 ppm both propyl- and hexylamine were generally significantly inhibitory except early or late in the incubation. Hexylamine at 1 and 10 ppm was not always significantly inhibitory. With 10 ppm of propylamine inhibition was not significant in the beginning and at the end of the incubation, whereas when 1 ppm of this compound was tested, limited but significant inhibition occurred during the first 8 h of treatment. Generally significant inhibition occurred when 100 and 1000 ppm of furfurol or methanol were tested. Both alcohols always inhibited *S. thermophilus* when they were tested at 10 ppm, but significantly so only from 5 to 11 h of incubation.

TABLE 7. DIFFERENCES IN POPULATIONS OF *Streptococcus thermophilus* IN APT BROTH CAUSED BY ADDED METHYLSULFIDE, METHANETHIOL, AND ETHANETHIOL

Chemical	Conc. (ppm)	Differences (%) from control in log of populations after hours of incubation				
		2	5	8	11	14
Methylsulfide[1]	1	(+) 0.8	–[2]	–	(+) 0.3	(–) 0.4
	10	(–) 1.6	(–) 0.2	(–) 0.3	(–) 0.1	–
	100	(–) 0.8	(–) 1.0	(–) 1.3*	(–) 1.3*	(–) 0.9
	1000	(–) 2.5	(–) 4.3*	(–) 3.4*	(–) 4.1*	(–) 2.2*
Methanethiol[1]	1	(–) 0.3	(–) 1.0*	(–) 0.3	(–) 0.3	(–) 1.1
	10	(–) 0.6	(–) 1.8*	(–) 1.3*	(–) 1.7*	(–) 1.5*
	100	(–) 1.1	(–) 2.1	(–) 2.3*	(–) 3.9*	(–) 2.3*
	1000	(–) 1.9	(–) 5.7*	(–) 9.1*	(–)14.6*	(–)15.9*
Ethanethiol[1]	1	(–) 0.6	–	–	(+) 0.1	(+) 0.6
	10	(–) 0.8	(–) 0.6	(–) 2.9*	(–) 1.4*	(+) 0.1
	100	(–) 1.1	(–) 1.2	(–) 2.3*	(–) 3.5*	(–) 0.5
	1000	(–) 4.1*	(–) 7.0*	(–) 9.4*	(–)10.5*	(–)15.2*

[1]Control: Log of no./ml: 3.66, 4.88, 6.18, 7.62, and 8.56 at 2, 5. 8, 11, and 14 h, respectively.
[2]–: No difference.
*: Population significantly different from control at the 5% level.

TABLE 8. DIFFERENCES IN POPULATIONS OF *Streptococcus thermophilus* IN APT BROTH CAUSED BY ADDED FURFURYL AND METHYL ALCOHOLS AND PROPYL AND HEXYLAMINE

Chemical	Conc. (ppm)	Differences (%) from control in log of populations after hours of incubation				
		2	5	8	11	14
Furfuryl alcohol[1]	1	–[2]	–	(–) 0.3*	(+) 0.1	(–) 0.2
	10	(–) 0.5	(–) 2.1*	(–) 1.3*	(–) 1.6*	(–) 0.3
	100	(–) 1.5	(–) 3.7*	(–) 5.6*	(–) 5.2*	(–) 0.9*
	1000	(–) 2.3*	(–) 7.5*	(–) 7.8*	(–) 7.6*	(–) 2.7*
Methyl alcohol[1]	1	–	(–) 0.2	(+) 0.2	–	–
	10	(–) 0.3	(–) 1.7*	(–) 0.6*	(–) 1.4*	(–) 0.6
	100	(–) 0.8	(–) 2.6*	(–) 5.5*	(–) 5.7*	(–) 0.7*
	1000	(–) 1.5	(–) 5.8*	(–) 6.7*	(–) 7.6*	(–) 3.2*
Propylamine[1]	1	(–) 1.0*	(–) 0.9*	(–) 0.6*	(–) 0.3	(–) 0.2
	10	(–) 1.8	(–) 2.6*	(–) 1.6*	(–) 2.5*	(–) 0.8
	100	(–) 2.1	(–) 8.0*	(–) 4.8*	(–) 7.1*	(–) 1.9*
	1000	(–) 2.6*	(–)16.4*	(–)12.0*	(–)10.2*	(–) 4.1*
Hexylamine[1]	1	(–) 0.3	(–) 1.5*	(–) 1.6*	(–) 0.4	(–) 0.1
	10	(–) 0.8	(–) 2.6*	(–) 1.7*	(–) 2.7*	(–) 0.7
	100	(–) 0.8	(–) 5.0*	(–) 6.4*	(–) 7.1*	(–) 2.6*
	1000	(–) 2.6*	(–)11.6*	(–)11.5*	(–)11.6*	(–) 3.8

[1]Control: Log. of no./ml: 3.89, 5.37, 6.41, 7.92. and 8.98 at 2, 5. 8, 11, and 14 h, respectively.
[2]–: No difference.
*: Population significantly different from control at the 5% level.

Methanol and furfurol at 1 ppm generally were without any significant effect.

It is evident from the foregoing results that S. *thermophilus* was significantly inhibited by the higher concentrations (100 and 1000 ppm) of all compounds that were tested. Most of the compounds, other than fatty acids and possibly amines, generally caused insignificant inhibition of this bacterium when the chemicals were tested at the lower concentrations (1 and 10 ppm). All the compounds used in these experiments were tested individually by adding them to APT broth. Hence it is difficult to assess the synergistic or antagonistic effect of these compounds on growth of S. *thermophilus* in milk or milk products such as yogurt or Swiss and Italian cheeses. As mentioned earlier, it has been reported that the ratio of S. *thermophilus* to *L. bulgaricus* changed in favor of the latter when formic acid was added to UHT-treated milk *(2)*. Our results suggest that formic acid probably inhibited S. *thermophilus* and thus caused the imbalance in the proportion of organisms. It may also be possible that formic acid adversely influenced growth of both organisms but was more inhibitory to S. *thermophilus* than to *L. bulgaricus*. It has been reported that growth of *Lactobacillus lactis* is stimulated by formic acid *(1)*. Hence another possibility might be that stimulation of *L. bulgaricus* together with inhibition of S. *thermophilus* caused the ratio of these bacteria to change in favor of *L. bulgaricus* when formic acid was added to UHT-treated milk. The reason for this shift in the ratio needs further investigation.

As noted with *Streptococcus lactis (7)* and *Leuconostoc citrovorum (8)*, inhibition of S. *thermophilus* in APT broth by test compounds was less pronounced than that of *Escherichia coli (4) Salmonella typhimurium (5)*, and *Staphylococcus aureus (6)* in nutrient broth. Inhibition of S. *thermophilus* was generally greater than that of S. *lactis (7)* when thiols, methylsulfone, acetonitrile, or 2-butanone were tested; whereas methylsulfide, ethylenedichloride, glyoxal, and chloroform were less active against S. *thermophilus* than against S. *lactis (7)*. With the exception of ethylenedichloride, methylsulfone, amines, and alcohols, all compounds tested were either equally or slightly more detrimental to growth of S. *thermophilus* than to that of *L. citrfovorum (8)*.

ADDENDUM

The reader is referred to the Addendum to the first paper in this series (*Escherichia coli*) *(4)* for information on the boiling point and solubility in water of all test chemicals. Initial pH values of APT broth with added chemicals can be found in the Addendum to the fourth paper in this series (*Streptococcus lactis*) *(7)*.

When the reduction in population of S. *thermophilus* was approximately 25% or greater, a given chemical at the approprite concentration was bactericidal. A reduction of approximately 0.3-24% represents bacteriostatic action by the chemical.

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METALLURGY AND PROCESSING OF STAINLESS STEEL FROM MILL TO FINISHED PRODUCT[1]

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(Received for publication June 7, 1974)

ABSTRACT

This paper discusses the relation of "Materials" to 3-A Standards; "Passivity" defined; corrosion resistance of stainless steels; "Free Iron"; "Austenite" defined; analyses; corrosion resistance data; corrosion phenomena; carbide precipitation affect; Galvanic Series; least "noble" (anodic), "active," and sacrificial; most "noble" (cathodic), "least active," and least readily sacrificial; welding processes; stainless steel finishes and surface finishes for sanitary food equipment; 3-A Standards resume and explanation of their intent: cleanliness for bacteriological and anti-corrosion reasons.

The 3-A Standards under "Materials" cover several possible materials to be used as "product contact surfaces" for dairy and food equipment, dependent on the "conditions of intended use" and always with the controlling criteria that they be "corrosion-resistant, non-toxic, relatively non-absorbent, resistant to normal cleaning and bactericidal solutions, readily cleanable, relatively insoluble, relatively resistant to abrasion, and shall not adversely affect the product." These criteria cover a gamut of materials for specific use purposes, such as stainless steel of the A1S1-300 series or corresponding AC1 types, which are the most widely used metals; or equally corrosion resistant metals; or heat resistant glass piping.

For specific applications the historic "dairy metal" which under the 3-A Fittings Standard 08-09 is designated as "optional metal alloy," one of which is designated as "nickel silver" or more properly "leaded nickel bronze;" the hardened A1S1-400 series stainless; chromium or equally corrosion-resistant non-toxic plating; specific silver and tin-lead solders; rubber and rubber-like materials conforming to the applicable provisions of the 3-A Standard Serial #18-00; plastic materials conforming to the applicable provisions of the 3-A Standard Serial #20-00; carbon and/or ceramic materials; all with controlling criteria, may be used. I shall confine this discussion mainly to a consideration of the A1S1-300 series stainless steels and their application in the dairy and food industry.

[1]Presented in part at a monthly meeting of the Wisconsin Dairy Technology Society, Waukesha, Wisconsin, January 8, 1974

STAINLESS STEEL

"Stainless Steel" is the commonly applied name for many iron-base alloys that contain at least 10.5% chromium and sometimes other alloying elements in various amounts. Twelve per cent minimum chromium is generally accepted as the amount to maintain "passivity." "Passivity" is defined as a condition in which a piece of metal, because of an impervious covering of oxide or other compound, has a potential much more positive (cathodic) and therefore less readily attacked than where the metal is in the active state.

The most noteworthy property of stainless steels is their superior resistance to corrosion. According to widely accepted theory, this resistance results from the presence of a thin, impervious film of chromium oxide that develops quickly upon exposure to oxidizing environments—even such as air. When subjected to reducing chemicals, the inherent corrosion resistance is impaired. Normally, the oxygen in air is sufficient to restore resistance if the reducing conditions are intermittent.

This development of the impervious chromium oxide film is what is known as "passivation." As applied to stainless steel, it is the exposing of a clean surface to an oxidizing medium to form, or to reinforce, the chrome oxide surface, which is what makes "Stainless" stainless. As previously stated it is generally accepted that it takes at least 12 to 14 per cent of chromium in the base metal to produce passivity.

The only advantage there might be in immersing the stainless steel in nitric acid (20%), a highly oxidizing reagent, would be to remove any "free" iron that might be present on the surface. This free iron can come from scratching with a carbon steel instrument, or plate, or from carbon steel filings or chips. None of these, however, should be involved in the fabrication of stainless steel.

An equally effective treatment is that with dilute phosphoric or nitric acid as outlined in the "3-A Accepted Practices for Permanently Installed Sanitary Product—Pipelines and Cleaning Systems" Serial

#60500, as follows: "Upon completion of welded pipeline installation and prior to use all interior line and weld areas shall be subjected to circulation of a cleaning solution of 0.5% to 1.0% alkalinity at a minimum of 160 F for 30 min, followed by an adequate post rinse, followed by circulation of a 0.5% minimum and 1% maximum phosphoric or nitric acid solution at 150-180 F for 10 min to clean all interior surfaces of ferric impurities. This treatment shall be followed by an adequate rinse." An "adequate rinse" is one that assures removing all of the previously used solution from the system.

Phosphoric acid will remove both ferric impurities and the colored oxide, if present, within the tube, that would result from weld temperatures when exposed to air. This oxide color can be minimized by use of inert gas backup of the weld during welding and while the weld area cools down below oxidizing temperatures. This colored oxide, if present, on or adjacent to the weld has no deleterious effect on the stainless steel, since it is itself "passive." Nitric acid will remove the ferric impurities but will not remove the colored chrome oxides, if present. It may take several cycles of Cleaned-in-Place, with the normal phosphoric solutions used, to remove the colored oxides, but as we stated, they have no deleterious effect anyway.

Passivation, or "pickling," (the words should not ordinarily be used interchangeably) has no beneficial effect on the metallurgical structure of a weld on austenitic stainless steel, either of the weld metal itself, or of the adjacent heat affected zone, so it should not be considered as some magic "potion" for a stainless steel assembly, as some people wrongly believe.

This present discussion will be primarily concerned with that class of stainless steels which contain at least 6.0% of nickel in addition to chromium. These alloys generally contain 16 to 26% chromium, 6 to 22% nickel and sometimes other elements added to develop specific properties. In identifying these alloys, chromium is listed first, nickel second. For example 18-8 Chromium-Nickel Stainless. AISI Types 304 and 316 are included in this class.

Contrary to the straight chromium stainless steels, the "18-8" or chromium-nickel alloys are not hardenable by heat-treatment. In their manufacture, the phase transformation is suppressed and Austemite is retained, regardless of a fast or slow rate of cooling from high temperatures. This is why these alloys are frequently referred to as "Austenitic Chromium Nickel Stainless Steel." "Austenite" is a solid solution of one or more elements in face centered cubic iron. Unless otherwise designated (such as Nickel-Austenite), the solute is generally assumed to be carbon.

Changes in mechanical properties can be effected only by "cold working" (by rolling or otherwise working the metal at normal temperatures). Steels fabricated by this cold-working method possess very high strength. Usually, Austenitic steels are non-magnetic but certain alloys do become appreciably magnetic through cold-working. Cast Austenitic steels, particularly those containing molybdenum (such as AISI-316) may be appreciably magnetic because of deliberate composition adjustment resulting in the presence of delta ferrite which is promoted to compensate for or mitigate micro-shrinkage. This does not adversely affect corrosion resistance, nor does it promote "rusting."

AISI Type 304 stainless steel is the basic 18% chrome–8% nickel type which is intended for relatively mild corrosion resistance. AISI Type 316 stainless steel is another "18-8" type containing 2 to 3% molybdenum for superior resistance to pitting and to most kinds of corrosion particularly in reducing and neutral solutions. The specific chemical content of these two basic Austenitic steels is as follows:

	Type 304	Type 316
Carbon (maximum)	0.08%	0.08%
Manganese (maximum)	2.0	2.0
Phosphorous (maximum)	0.045	0.045
Sulphur (maximum)	0.03	0.03
Silicon (maximum)	1.0	1.0
Chromium	18-20	16-18
Nickel	8-12	10-14
Molybdenum	—	2-3

AISI Type 317 is an "18-8" stainless steel similar to type 316 except that it contains 3-4% molybdenum, giving it moderately better corrosion resistance in some applications, such as high concentrations of acetic anhydride and hot acetic acid.

Carpenter type 20 is a 29% nickel, 20% chromium steel with copper and molybdenum. It is a special alloy intended specifically for resistance to sulfuric acid.

AISI 400 Series alloys should more properly be designated as "stain resistant" rather than "stainless" steel. These alloys are generally used in trim and decorative applications and their corrosion resistance characteristics are not relative to the scope of this discussion.

Corrosion resistance data are available in many commercial publications such as the various steel company's handbooks. For more specific data, an excellent source is *A Guide to Corrosion Resistance*, published by Climax Molybdenum Division of American Metal Climax, Inc., 1270 Avenue of the Americas, New York, New York, in cooperation with The In-

ternational Nickel Co., Inc. This guide lists actual test results of average corrosion rates in inches of penetration per year, covering alloy types 304, 316, 317, Carpenter 20, and "Ni-O-Nel" exposed to an extremely wide range of media. Factors such as type of test (field or laboratory), average temperatures, duration of exposure, aeration of solutions, and degree of agitation are presented along with detailed chemical compositions of media.

"Ni-O-Nel" or Inconel 825, is a 42% nickel, 21.5% chromium steel with copper and molybdenum, developed to meet more severe corrosion and stress-corrosion conditions than can be handled by the stainless steels but where Nickel-base alloys are not needed.

We repeat that chromium is the only element you can add to alloy with steel to make iron stainless. The minimum quantity, as we stated previously, is from 12 to 14%. Therefore, when you reduce the amount of chromium present by any means you reduce the stainless property. We stress this point at this time particularly because of some of the effects that welding, and particularly, poor welding techniques, may have on stainless steels.

CORROSION

Galvanic bi-metal attack

First of all, what is it? It is the coupling of two metals or alloys, separated in the electromotive series, in an electrolyte so that current flows from one to the other. Carbide precipitation in a weld, or in a not properly solution-annealed piece of stainless steel, causes the grain boundaries to lose chromium and this area then becomes less noble (anodic) and is subject to attack. Grains can actually be plucked out if precipitation is severe. The area also may rust due to become less noble. It is an area relationship where the less noble area is small with respect to the great area of more noble metal and it, (the less noble) therefore, becomes sacrificial.

Metal under stress becomes anodic (less noble) and, therefore, any stressed areas as, for example, in or adjacent to a weld can become sacrificial and may, therefore, corrode. It (stressed metal) is a physical phenomenon rather than a chemical one such as is carbide precipitation. The entire alloy, therefore, corrodes as one metal.

Chemical attack

Porosity lends itself to attack, by cell formation, by deposits which make the under-lying area less noble (therefore sacrificial), and therefore the chain of attack is continued. Chemicals also form a stronger electrolyte through ionization, which accelerates attack on stressed or carbide precipitated areas. An acidic electrolyte is particularly active, since the corrosion products are soluble, whereas with an alkaline electrolyte, even though it is also ionized, the corrosion products are insoluble.

Typical galvanic series

The least "noble" (anodic) and therefore the most "active" metal as they appear in the galvanic series will become sacrificial to the more noble metal. The greater the distance between two of them the greater the expected activity.

Anodic end (least noble and most active or most readily sacrificial) to Cathodic End (most noble and least active and least readily sacrificial): 1 – Magnesium, 2 – Magnesium alloys, 3 – Zinc, 4 – Galvanized Steel, 5 – Aluminum alloy 5052H, 6 – Aluminum alloy 3004, 7 – Aluminum alloy 3003, 8 – Aluminum alloy 1100, 9 – Aluminum alloy 6053, 10 – Alclad (pure aluminum on alloy), 11 – Cadmium, 12 – Aluminum alloy 2017, 13 – Aluminum alloy 2024, 14 – Mild steel, 15 – Wrought iron, 16 – Cast iron, 17 – Chromium iron (active), 18 – Ni-resist, 19 – 13% Chrome stainless, type 410 (active), 20 – 50-50 Tin-lead solder, 21 – 18-8 Stainless steel type 304 (active), 22 – 18-8-3 Stainless steel, type 316 (active), 23 – Hastelloy "C", 24 – Lead-tin solders, 25 – Lead, 26 – Tin, 27 – Muntz metal (60-40 copper-zinc brass), 28 – Manganese bronze, 29 – Naval brass, 30 – Nickel (active), 31 – Inconel (active), 32 – Hastelloy "B", 33 – Cartridge brass (70-30 copper-zinc), 34 – Admiralty brass, 35 – Aluminum bronze, 36 – Red brass (85-15 copper-zinc), 37 – Copper, 38 – Silicon bronze, 39 – Cupro-nickel (70-30), 40 – Titanium, 41 – Silver solder, 42 – Nickel (passive), 43 – Inconel (passive), 44 – Monel (nickel-copper), 45 – Chromium iron (passive), 46 – 18-8 Stainless steel, type 304 (passive), 47 – 18-8-3 Stainless steel, type 316 (passive), 48 – Silver, 49 – Gold, 50 – Platinum (least active).

"Galvanic corrosion" is corrosion associated with the current of a galvanic cell which consists of two dissimilar conductors in an electrolyte or two similar conductors in dissimilar electrolytes. Where the two dissimilar metals are in contact with each other, the resulting reaction is referred to as "couple action."

Contact and crevice corrosion

This results from product being trapped in porosity, slivers, cracks, folds, mismatch, on the surface itself, or in the case of a weld in areas of lack of penetration or skips in the weld melt.

Stress corrosion and corrosion fatigue

This is again caused by stress in a weld or some other surface area, as for example adjacent to a weld,

either due to shrinkage, to applied mechanical cold-working of the surface, or to excessive heat applied in welding.

We have referred to "welding" and I believe a brief discussion of the subject with reference to stainless steel is in order.

WELDING PROCESSES AVAILABLE

We would like to list these in the order which we would consider best effects the ultimate criterion of the best physical properties and corrosion resistance of the weld and the adjacent areas:

Inert gas-shielded tungsten arc (TIG)

We would consider it best for direct butt fusion welding up to a thickness of 0.180 inch (7 GA.). A non-consumable tungsten electrode, in a blanket of inert shielding gas, either helium or argon, or a mixture of both, which also transfers and maintains the arc, eliminates oxidation, and permits a variation of welding current with speed of travel of the torch along the seam being welded. The result is a better control of distortion, the heat-affected zone, and stress concentration in the weld and adjoining areas. This method results also in the most uniform cross-section, free of spatter, and is especially useful in the reduction of time required to produce a smooth surface where welds must be ground. Helium gives better penetration with less applied heat (arc voltage and amperage) than does argon. Therefore, you can butt-weld heavier gages of material with helium than you can with argon. Modification of the above method to include hand feeding of filler wire into the melt.
This is useful where matching of the sections to be butt-welded is difficult; for heavier sections where the adjoining sections must be chamfered to effect full penetration, or where straight fusion may cause undesirable undercuts such as in a corner.

Inert Gas–Shielded Metal Arc Welding (normally called "MIG welding") This is the most logical choice for joining heavy gage sheets. It is usually used for gages 1/4 inch or heavier, but it also works well on lighter gages.

A stream of the same inert gas surrounds a continuously fed consumable wire and protects the melt in the same manner as described for the first process. The filler wire which effects and maintains the arc can be the same as the base metal since there is no alloy loss due to atmospheric action. It can also be a modified composition if desired. This process requires more skill in manual operation and may require more in the way of automation than the first process. (Useful also on aluminum).

Shielded metal arc welding

There are no plate thickness limits. A coated electrode is used and its coating forms a fusible slag that flows over the weld, protecting it from air and replacing any aloys that may still be lost. When cool, the slag can be flaked off. Much of the success of this process depends on the operator's skill. Multiple passes with small electrodes (e.g. 1/16) are preferable to a single pass with a heavy electrode (e.g. 1/8 or 3/16). This minimizes the heat effect.

Where one or more weld beads are laid upon another, it is imperative that all the slag be removed from the previous bead if trapping of slag is to be avoided.

Coating

Coating can be either of the Lime or Titania type. Lime Coating effects a penetrating and cutting type of bead. Titania effects a smooth and not so cutting a type of bead. There is, therefore, more chance of undercutting on the edges of a Lime protected bead, whereas the Titania bead will be more or less free of this characteristic and, due to its smoothness, will be easier to clean up and polish. In any welding it is desirable, that to effect the best in ultimate properties, that the weld be made as rapidly as possible with the least amount of generated heat.

Cell formation

With the increased use of "In-Place" or "Mechanical" cleaning it is particularly important to avoid any cell formation that will result in deleterious corrosion effects which may not be detected or which, when they are detected, have progressed to a point approaching failure which cannot be corrected by counter measures.

The criteria of a welded joint from the "3-A Accepted Practices for Permanently Installed Sanitary Product–Pipelines and Cleaning Systems" under "Fabrication" Paragraph 5 states: "Welded joints shall be smooth and free from pits, cracks, inclusions, or other defects."

STAINLESS STEEL FINISHES AND SURFACE FINISHES FOR SANITARY FOOD EQUIPMENT

The most lucid explanation of the development of stainless steel plate, sheet, or strip and their finishes is incorporated in a paper recently presented by J. H. McConnell, Staff Metallurgist and G. N. Douglas, Specification Metallurgist, of U.S. Steel Corporation at the Design Engineering Technical Conference of the ASME at Cincinnati, Ohio, on September 11, 1973.

The points pertinent to our discussion describe the molten stainless steel being cast into ingots of some 10 to 15 tons in weight and 15 to 30 inches thick or continuously cast into slabs of 5 to 8 inches in thickness.

Stainless steel slabs—whether rolled or cast—have surface imperfections that must be removed. Slivers, cracks, and folds are characteristics of the highly alloyed compositions and they are removed by arbitrarily conditioning—usually by grinding—100% of the surfaces.

The slabs are then reheated and hot rolled to either plates or sheet coils. Stainless plates are then annealed and quenched, flattened, sheared to size, grit blasted, and pickled to remove all rolling and heat treating oxide. Most stainless plate is sold in this condition which is described as a hot rolled annealed and pickled finish.

Stainless plate is 3/16 inch thick and heavier and the reduction to attain the desired gage generally is by hot working. These two factors—the relatively heavy gage and the type of reduction—are not conducive to good surface density. If it is necessary to polish plate product considerable grinding using successively finer abrasives is usually required.

On the other hand, to produce sheets, the slabs are rolled to coils 1/8 to 1/4 inch thick. They are then continuously annealed and cleaned. Again, a surface inspection frequently will reveal an incidence of light imperfections that are removed either by strip grinding 100% of the surface or additional trips through the annealing and cleaning line. Once they are conditioned, the coils are then cold reduced as much as 50 to 65%. This cold working is the primary operation in developing a dense smooth surface. When annealed and cleaned, the product is in the 2D finish condition. This treatment provides a soft product used primarily for forming deep drawn articles. When temper rolled the product is described as 2B finish. Temper rolling is a very light cold rolling operation that extends the material only about 0.5% in length. This is usually done using highly polished rolls that result in a significant improvement in appearance, density, and flatness. Even though this 2B finish is not used for many sanitary requirements, it has the wide-spread general trade acceptance because of its versatility. *(a)* It has the flatness suitable for paneling. *(b)* It is soft enough for forming and light drawing operations. *(c)* It has a pleasing appearance. *(d)* It can be readily polished when required.

It follows then that a 2B finish is used to make polished sheets. A No. 3 finish stainless sheet is made by polishing with belts impregnated with 100 grit abrasive. No. 4 finish stainless sheets are made by intermediate grinding and finishing with 150 or 180 grit belts. This is one of the more popular polished finishes used widely in restaurant, kitchen, beverage, and dairy equipment.

If very thin (under approximately 0.035) sheets or strips are being made, it becomes necessary to repeat the cold reduction and the annealing and cleaning to make a light gage 2D finish. This can also be temper rolled to develop a 2B finish. Polishing practices are the same as with heavy gage material.

The significant point is that the second cold reduction required to make light gages further improves the surface density. This product unquestionably has less pits and is easier to polish than thicker product.

To recapitulate, three levels of surface density have now been established: *(a)* A heavy gage hot rolled plate finish. Considerable work would be required to polish this surface to a fine finish. *(b)* Heavy gage cold reduced sheets. The surface density is immeasurably improved by some 60% cold reduction. *(c)* Double cold reduced sheet. This is typical of a more dense product and because of this, it is the easiest to polish. A special finish is developed by bright annealing. In this process the annealing is done under a controlled atmosphere to prevent any oxidation and thereby obviate the need for acid pickling. The product leaving the furnace has the same bright appearance as the product entering the furnace.

Returning now to the subject of surface density, it is important to establish whether the term "2B finish" actually describes a finish or a process. In making the very heavy gage sheets the maximum cold reduction attainable is only 25% because of the limitations in producing the hot roll coil stock. Conversely, some very light gages require three heavy reduction cycles. Obviously then, the 2B finish made with 25% reduction cannot have the same appearance, or density, or polishability as the 2B made with three 50% cold reductions. The appearance and density of 2B varies throughout the entire range of gages because of various amounts of cold reduction. Thus, the term 2B more accurately describes the way product is made rather than the finish, per se.

Now, whereas the rolled finish does not provide consistent uniformity, a polished finish does offer excellent uniformity regardless of the prior processing. The size of the abrasive grit is the primary factor in developing a polished finish, although it must be acknowledged that speeds, feeds, and lubricants have a secondary effect. With hot rolled annealed and pickled plate, considerable preparatory grinding is required before using the final polishing abrasive. And, at the other extreme, a one-step polishing operation with no preparation would probably be sufficient on very light gage sheets. The important feature is that both plates and sheets, when polished, would have the same finish.

Another advantage of polishing is, it highlights

small pits that cannot be seen in the 2B finish. They can then be easily removed to provide a pit-free surface for the most critical sanitary requirements.

These characteristics of polished finishes—uniformity, easy pit detection, pleasing appearance, repairability—are the very important reasons for its widespread acceptance by sanitary authorities in the food and beverage industry.

Over the past years major improvements have been made in the surface quality of stainless steel rolled finishes. Light gages made by multiple reduction may be pit free but this is probably not the case for heavy gage material made with a minimum of cold reduction. There is little likelihood that this difference in density can ever be eliminated. This is why the producer cannot guarantee pit-free 2B finish and this is why the polished finish—with reliable uniformity—has become a standard with nearly all regulatory authorities in the food, dairy, and beverage industry."

IN CONCLUSION

Earlier we made reference to 3-A Standards a short resumé and explanation of what they are may be enlightening. 3-A and E-3-A Sanitary Standards and Accepted Practices represent criteria for cleanability of dairy processing and egg processing equipment, respectively.

They are formulated by the cooperative effort of industry and regulatory groups as represented by the Dairy Industry Committee, International Association of Milk, Food, and Environmental Sanitarians, U. S. Public Health Service, U. S. Department of Agriculture, Dairy and Food Industries Supply Association, and the Institute of American Poultry Industries. The intent of the Standards and Accepted Practices is the promotion and the preservation of the public health. 3-A and E-3-A Standards and Practices are published in the *Journal of Milk and Food Technology*. There are 27 3-A and nine E-3-A published Sanitary Standards and six 3-A and three E-3-A Accepted Practices to date.

For the sanitarian, adoption and application of uniform design and construction principles has made equipment inspection much more readily effected and allows the application of these same principles to food handling equipment not covered by a 3-A Standard. This atmosphere is attributable to the pyramiding mutual cooperation, trust, and respect generated by and emanating from the participating Committees.

Additionally, we cannot stress too strongly that systems must be kept clean both for bacteriological and for anti-corrosion reasons. We do not mean parts of a system but rather the whole complete system.

"General cleanability" we believe is easily evaluated and we do not believe there is any significant difference in this respect between a true #4 Finish and a 2-B Finish. It is, however, with respect to "specific cleanability" that we become concerned. These are the areas of pits, folds and crevices that become evident during polishing to a #4 Finish by visual examination and may not be readily evident on a 2-B Finish surface which may appear to have been thoroughly cleaned but which may have deposits in these areas that will develop trouble.

TEMPERATURE PROFILES DURING COMMERCIAL PASTEURIZATION OF MEAT FROM THE BLUE CRAB

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(Received for publication April 18, 1974)

Abstract

Crab meat is pasteurized to inactivate most of the spoilage microorganisms, thereby extending its shelf life under refrigeration, but the process is not intended to achieve commercial sterility. As a first step in determining if spores of *Clostridium botulinum* would survive the process, crab meat temperatures were measured during commercial pasteurization. Tests were done in commercial establishments in Florida, South Carolina, North Carolina, Virginia, and Maryland. Cans of crab meat were instrumented with thermocouples, and heating curves were recorded during normal commercial processing. Water bath temperatures were reasonably constant among the different processors, ranging from 186 to 189 F. Holding times were more variable and ranged from 92 to 150 min. Lethality of the lowest thermal process was estimated to be equivalent to 18 min at 180 F.

The blue crab is found in abundance along the Gulf and East coasts of the United States, where it is harvested commercially. The Chesapeake Bay has one of the heaviest concentrations, and almost 100 million lb. of live crabs are taken annually in Virginia and Maryland *(6)*. Live crabs are steam-cooked in retorts at about 250 F to denature the meat so it can be removed from the shell.

Most crab meat is hand-picked, hand-packed, and ready-to-eat without further processing. Occasionally, however, because of a large supply of dwindling demand, the processor pasteurizes the crab meat in sealed cans to extend its shelf life by inactivating most of the spoilage organisms. *Clostridium botulinum* types B and E have been found in fresh-packed blue crab meat and type F has been found in the pasteurized product (D. A. Kautter, personal communication). Survival of type E spores and subsequent toxin formation has been detected in pasteurized Dungeness crab meat stored at 40 F *(4)*.

Because of concern for the possibility of survival of botulinum spores in pasteurized crab meat, the Food and Drug Administration is conducting studies to determine the thermal resistance of type E and type F spores in blue crab meat. When combined with heating curves from commercial production, these thermal death time data will permit the identification of microbial lethality of the process used to pasteurize crab meat.

The objective of this work was to determine the thermal history of crab meat during commercial pasteurization as a step toward determining whether spores of *C. botulinum* will survive in the pasteurized crab meat.

MATERIALS AND METHODS

Products

Commercially-harvested blue crabs were cooked and picked in commercial processing plants. Crab meat was packed directly into the can used for pasteurization. The meat was packed in the can in "dry" or "as picked" condition; no water or brine was added. The meat was usually pasteurized the same day it was picked, but not later than the following day, after overnight refrigerated storage. All tests were done during June, July, and August 1972. The different types of meat are identified as follows: *(a)* Backfin lump. White meat of the backfin muscle, usually picked as one piece. *(b)* White flake meat. White meat from the skeletal muscles in the body of the crab, plus small pieces of the backfin muscle that did not come out as one piece. *(c)* Claw meat. Darker meat from the claws of the crab.

Nearly all cans were 4-1/16 inches in diameter and 3-1/16 inches high (401 × 301), with a net weight of 1 lb. The one exception was a can that was 3-7/16 inches in diameter and 4-9/16 inches high (307 × 409), with a net weight of 1 lb. (there is less than 4% difference in the volumes of the two cans).

Instrumentation

Internal meat temperatures in the can were measured with Type C-1 rigid thermocouples manufactured by O. F. Ecklund, Cape Coral, Florida. Thermocouples were installed so that the temperature-sensitive tip was located at the geometric center of the can (Fig. 1). Water temperature outside each instrumented can was measured with number 20-gauge, nylon insulated, duplex wire thermocouples. For one test that was done to measure the temperature profile inside a can of crab meat, number 24-gauge, flexible thermocouples were used (Type C-4, O. F. Ecklund). All thermocouples were copper-constantan.

Temperatures were recorded with a 12-point, strip-chart recorder (Model 760, Bristol Co., Waterbury, Conn.). Before each test, the thermocouples were placed in a water bath and checked for proper calibration with a precision, mercury-in-glass thermometer. Adequate electrical grounding was not pre-

sent in all plants, and it was sometimes necessary to run a common ground wire to the water bath and recorder to achieve proper temperature calibration.

Water bath method

The water bath method described by Tatro *(6)* was used in all plants that we visited. Steam was introduced into the bottom of the bath through a steam distributor. Most cylindrical baths were equipped with an "X"-type distribution pipe with steam exit holes placed so as to create a swirling type of agitation. The vessel was filled with water to a level that covered the uppermost cans of crab meat with several inches of water.

Water bath temperature was maintained constant with a temperature- controlled, steam-flow valve. In some plants, air was introduced into the bottom of the water bath to improve circulation. With the exception of one rectangular tank, the pasteurizing vessels were vertical cylindrical tanks.

Test procedure

All tests were done in commercial processing plants. Cans of crab meat (1 lb., net weight) were placed in metal baskets in a preferred pack. Cans were packed one layer at a time, and those instrumented with thermocouples were placed in selected positions: the geometric centers of the bottom, middle, and top layers of cans, and the outside edge of the top layer.

Depending on the supply of meat to be pasteurized, loads ranged from 56 to 325 cans per basket. When the basket was filled, a lid was attached to keep the cans submerged in the water bath (without the lid, cans would float to the surface).

Baskets were then hoisted over the pasteurizing bath, thermocouples were connected to the temperature recorder, and the basket was lowered into the bath. Some processors turn on the steam supply after the basket is lowered into the bath, and others have the bath at processing temperature before it is lowered. With the latter method, immersion of the cold product drops the water bath temperature less than 10 F, and the steam supply heats it back to processing temperature in less than 5 min. In either event, timing of the process does not start until the water bath has been heated to the required processing temperature.

After being held in the water bath for the proper time, the cans of crab meat were transferred to an ice-cooled or refrigerated bath of about the same capacity as the hot water bath. Cooled cans of meat were then placed in refrigerated storage.

RESULTS

Tests were done in five processing plants located in Florida, South Carolina, North Carolina, Virginia, and Maryland – the major states producing pasteurized crab meat. Holding times for the pasteurization process were as brief as 92 min and as long as 150 min. Despite the wide range in holding times, water bath temperature was reasonably constant between 186 and 189 F (Table 1).

Heating curves for individual cans in the same water bath were almost identical (Fig. 2). Two of the cans were at room temperature before immersion in the water bath, and the temperature data were so closely grouped that one curve sufficed for both cans. The remaining two cans were refrigerated prior to immersion in the water bath, and one curve was also sufficient for the refrigerated cans. During the last 22 min of the holding time, the difference in temperature at the geometric center of four instrumented cans was less than 2 F, despite a variation of 20 F in initial meat temperature between the refrigerated cans and the ones at room temperature.

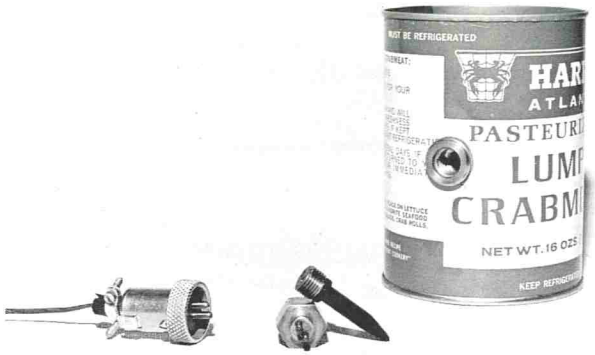

Figure 1. Instrumented can of crab meat showing thermocouple, packing gland, and connector.

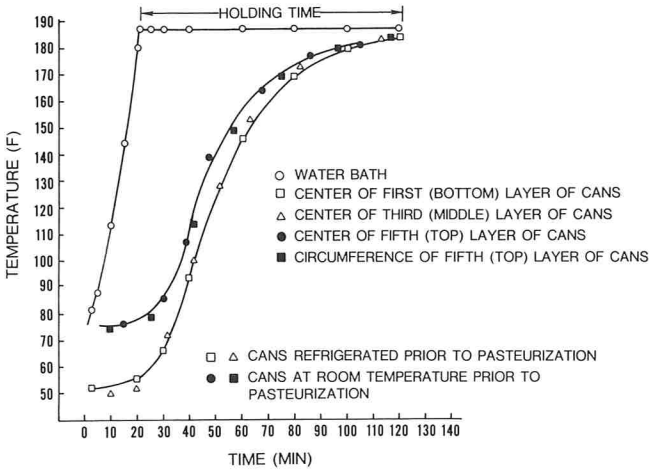

Figure 2. Heating curves at the geometric center of cans of crab meat during pasteurization in the water bath (plant located in Florida).

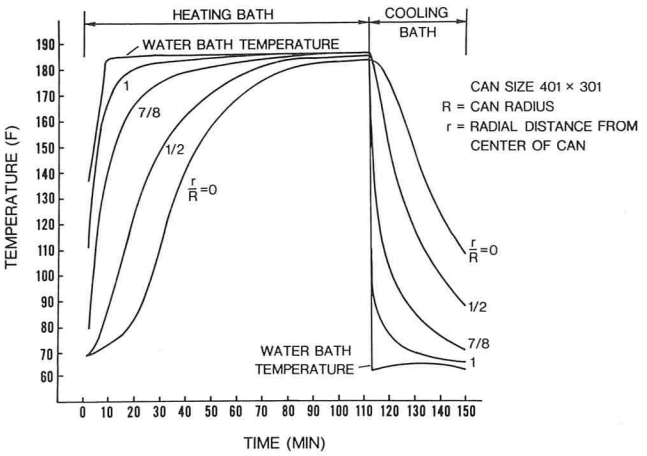

Figure 3. Temperature distributions in one can of backfin lump meat during pasteurization in the water bath (plant located in Florida).

TABLE 1. COMPARISON OF CRAB MEAT PASTEURIZATION PROCESSES USED IN FIVE STATES

Plant location	Florida	South Carolina	North Carolina	Virginia	Maryland
Minimum bath temperature (F)	187	189	186	188	189
Holding time (min)	100	150	120	92	115
Minimum meat temperature at end of holding (F)	184	188.5	183	182	184
Time above 180 F (min)	22	67	21	6	21
Variation in bath temperature between 5 locations (F)	<1	2	<1	3	3
Variation in meat temperature between 5 cans at end of holding (F)	<1	2	<1	1	3
Type of meat in cans[a]	WF	BL	BL	WF, BL	C, WF, BL
Total load in pasteurizer (lb)	214	130	56	113	325
Can size	401 × 301	307 × 409	401 × 301	401 × 301	401 × 301
Steam distributor	Yes	Yes	No	Yes	Yes
Air used to improve circulation	Yes	No	No	Yes	No
Maximum cooling bath temperature (F)	85	44	52	50	50
Holding time in cooling bath (min)	58	91	36	78	75

[a]WF = white flake meat, BL = backfin lump meat, C = claw meat.

As demonstrated by the small temperature difference between meat and water bath near the end of the pasteurizing process, meat temperature is primarily governed by water bath temperature, and long periods are required for even slight additional increases in meat temperature. Yet some processors do hold the product for long periods to achieve increases in meat temperature as small as 1 F. A more direct and easier method of raising meat 1 F is to raise the water bath temperature 1 F.

A 401 × 301 can holds 1 lb. of crab meat, and a computation of density yields a value of 6.1 lb./gal. A comparison with the density of water (8.3 lb./gal) suggests that the meat is densely packed and is heated by conduction heat transfer. If so, meat near the wall of the can would be heated quickly and meat at the center would be heated slowly, leading to the possibility of product damage because of uneven heat treatment. This concerns most processors, since they believe that high temperatures tend to discolor or darken the meat in the vicinity of the can walls. Consequently, the temperature profile across a single can was measured by installing four flexible thermocouples (number 24-gauge) along a radius of the can. There was considerable difference between the heat treatment received by meat at the inside surface of the can and that at the center of the can (Fig. 3). The temperature of meat 1/4 inch away from the can wall was above 184 F for 41 min, but the temperature of meat at the center of the can was above 184 F for only 6 min. Furthermore, the volume of meat located in the last 1/4 inch of can radius, near the wall, represents 23% of total can volume. One solution to the problem is to use smaller cans. In vigorously agitated water baths, heating and cooling rates vary as the square of thickness *(3)*, and if the shortest dimension between the geometric center and wall of the can is reduced by a factor of 2, heating and cooling times will be reduced by a factor of 4.

The water bath used in the tests done in South Carolina was a rectangular tank, approximately 8 ft long, 4 ft wide, and 3 ft high, and it held several baskets simultaneously. The baskets were smaller than those used in other plants, and they held only 130 cans of crab meat. Baskets were moved in and out of the bath on a schedule determined by the availability of picked crab meat. During our tests on one basket, four additional baskets were lowered into the bath at various times. The lowering of a full basket of cans into the bath had no measurable effect on bath temperature. This was primarily due to a large steam capacity and a temperature controller with a high sensitivity and speed of response.

The South Carolina plant also uses a different size can (307 × 409), but the heating curves were almost identical to those of the Florida plant (Fig. 2). The time required to heat the 307 × 409 can to 170 F was 62 min. After the water bath of the Florida plant reached 187 F, the time required for the slowest heating 401 × 301 can to reach 170 F was 60 min. The data also compare favorably with those of Littleford *(5)* for 307 × 409 cans of crab meat. In a 190 F bath, the time required for the temperature at the center of the can to reach 170 F was 65 min *(5)*.

The plant in Virginia had the shortest holding time as well as the lowest thermal process. The center temperature of the slowest heating can of crab meat was above 180 F for only 6 min (Table 1). To evaluate the total lethal effect of the process, thermal death time data are needed, and very few

data have been published for botulinum spores in seafoods. The z-value of type E spores in fish paste is 14 F *(1)*, and assuming this value for crab meat, we estimated the lethality of the process used in the plant in Virginia to be equivalent to 18 min at 180 F.

The Food and Drug Administration is now conducting studies to determine the thermal resistance of type E and type F spores in blue crab meat, and these data combined with the temperature-time data presented here, will permit identification of the microbial lethality of the process used to pasteurize crab meat.

ACKNOWLEDGMENTS

Many people contributed to this work. Plant Managers gave generously of their time and placed more than ample additional resources at our disposal. State Health Officers worked in close cooperation, and the sum of these efforts resulted in meaningful data from commercial systems, during commercial operations. In particular, we are grateful for the assistance and cooperation of F. Allhands, R. Benton, C. Burroughs, D. Clem, J. Colcolough, L. Crab, A. Daigle, J. D'Alfonso, R. Dawson, E. Dedeke, G. Dennis, G. Duet, B. Duplantis, Howard, J. Howe, D. Johnson, T. Jones, L. Kauffman, V. Keys, D. Estlack, G. Flick, E. Greene, E. Griffis, F. Hobbs, I. W. King, C. Kitchel, J. Krakosky, B. Korzen, R. Lancaster, H. Lupton, E. Martin, A. Montgomery, R. Price, Mrs. N. Rodgers, G. Rose, C. Rudolph, D. Smith, L. Sterling, M. Tatro, C. Tolley, N. Travis, J. Tomas, S. Waskiewicz, C. Wiley, and W. Woodward.

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A Research Note

INCIDENCE AND VIABILITY OF *CLOSTRIDIUM PERFRINGENS* IN GROUND BEEF

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(Received for publication May 6, 1974)

Abstract

The incidence of *Clostridium perfringens* in 95 ground beef samples obtained from a retail store in Denver, Colorado was 47.4%. Although viability was not reduced after 24 h at −20 C, greater than 90% of the organisms usually could not be detected after frozen storage over a 4-month period.

Clostridium perfringens is widely distributed in the intestinal contents of man and animals, in sewage, and in soil, and foods frequently become contaminated from these sources. Several studies have shown a rather high incidence of *C. perfringens* contamination in raw meats. Hall and Angelotti *(3)* showed that 58% of veal, beef, chicken, lamb, and pork cuts sampled from retail stores were positive for *C. perfringens*. Strong et at. *(4)* showed only a 16.4% contamination in raw meat, poultry, and fish. This note documents the incidence, and viability after frozen storage, of *C. perfringens* in 95 samples of ground beef obtained from a retail store in Denver, Colorado.

Materials and Methods

Five 1-lb. samples of ground beef were randomly selected each week, over a period of 19 weeks. The experimental procedure consisted of aseptically placing 25 g beef in 225 ml of sterile phosphate buffered distilled water *(1)* and blending for 2 min at high speed in sterile stainless steel blender cups. A 1-ml aliquot was placed in duplicate petri dishes and pour plates prepared using sulfite–polymixin B–sulfadiazine agar (BBL 11580) and incubated anaerobically for 24 h at 37 C. All pure black colonies were counted as presumptive *C. perfringens*, and 20% were picked to a nitrate-motility medium (BBL 11342) if the count was greater than 10. All colonies were picked if the count was 10 or less. All nitrate-positive, motility-negative colonies were confirmed as *C. perfringens*.

After initial sampling, the remainder of each pound of ground beef was frozen at −20 C. The five samples each week were numbered from 1 to 5. If positive, the first sample was recultured in 24 h, the second in 1 month, the third in 2 months, the fourth in 3 months, and the fifth in 4 months. Additional platings were not made if *C. perfringens* was not isolated from the initial sample.

Results and Discussion

Forty-five of the 95 samples of ground beef were found to contain *C. perfringens*, an incidence of 47.4%. Table 1 shows that the largest number of samples had *C. perfringens* counts in the range of 5 to 100/g. The highest number detected was 640/g. These results indicate that *C. perfringens* is present in ground beef obtained in the Denver, Colorado area and, at times, in quite high numbers. Although no procedures were carried out to indicate the nature of toxin production nor the degree of pathogenicity, the presence of *C. perfringens* is not without concern. Three is always the possibility that heat-resistant spores may be present which are capable of surviving cooking temperatures and converting into the vegetative state if the cooked ground beef is not properly refrigerated.

Probably of greater concern is the possible contamination of kitchen and kitchen personnel with *C. perfringens* by handling the raw ground beef. In a

Table 1. Range of counts of *C. perfringens* in ground beef

No./g ground beef	No. samples
0	50
5-100	33
101-200	6
201-300	4
301-400	0
401-500	1
501-600	0
601-700	1

Table 2. Viability of *C. perfringens* in 12 samples per group of ground beef held at −20 C

Plating time	Sample group 1	2	3	4	5
	(No./g)				
Initial	38	46	105	43	66
24 hours	52				
1 month		3			
2 months			9		
3 months				9	
4 months					2
% Viability	100	7	8	14	3

study by Bryan and Kilpatrick (2), 20 of 67 swabs from raw boneless beef used in a quick-order roast beef restaurant were positive for *C. perfringens*. Further contamination of the premises and personnel was detected from 5 of 14 swabs of the meat slicer, 6 of 15 thermal pins, and 4 of 10 hand washings by kitchen employees. It is conceivable, then, that a contaminated raw product can increase the chances of recontaminating a cooked product or other food products in the kitchen indirectly either by the contaminated hands of kitchen personnel or by kitchen utensils or equipment.

Data on viability of *C. perfringens* in ground beef at −20 C are shown in Table 2. Freezing for 24 h did not reduce the number of viable organisms. Since the samples were held at refrigeration temperatures (maximum of 24 h) before testing, perhaps the surviving organisms were also able to withstand freezing temperatures for 24 h. However, viability was decreased to 7, 8, 14, and 3% after 1, 2, 3, and 4 months, respectively. This indicates that *C. perfringens* in ground beef is quite susceptible to the effects of frozen storage.

ACKNOMLEDGEMENTS

We thank Specialist Fifth Class Jack J. Jorgensen for technical assistance.

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HAZARD ANALYSIS OF *CLOSTRIDIUM PERFRINGENS* IN THE SKYLAB FOOD SYSTEM

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(Received for publication May 1, 1974)

Abstract

The Skylab Food System presented unique microbiological problems because food was warmed in null-gravity (diminished convection) and potentially diminished conduction due to poor surface contact), and because the heat source was limited to 69.4 C (to prevent boiling in null-gravity in the approximately one-third atmosphere total pressure). For these reasons, the foods were manufactured using critical control point techniques of quality control coupled with appropriate hazard analyses. One of these hazard analyses evaluated the threat from *Clostridium perfringens*. Samples of food were inoculated with *C. perfringens* and incubated for 2 h at temperatures ranging from 25 to 55 C. Generation times were determined for the foods at various temperatures. Results of these tests were evaluated taking into consideration: food-borne disease epidemiology, the Skylab food manufacturing procedures, and the performance requirements of the Skylab Food System. Based on this hazard analysis, a limit for *C. perfringens* of 100/g was established for Skylab foods.

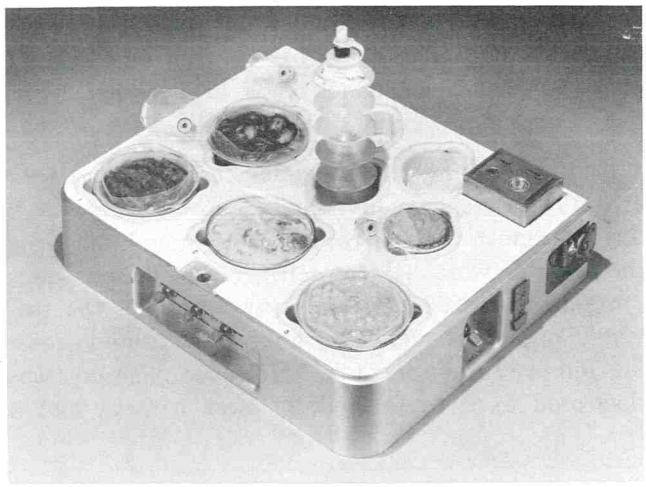

Figure 1. The Skylab food warmer-serving tray.

The Skylab manned space flight program presented unique problems involving the microbiological safety of foods. The unmanned Skylab spacecraft was launched into earth orbit in May of 1973. This unmanned vehicle contained most of the foods for life support of the nine astronauts who later rendezvoused with it and lived in it for over 500 man-days during the next 10 months. The Skylab food supply had to have long-term stability and safety and yet accurately and adequately provide nutrition for the astronauts during their epic expeditionary voyages.

All the Skylab foods, other than beverages, were packed in drawn-aluminum cans fitted with full-panel pullout lids. At meal time, cans were assembled into meals and inserted into a food warmer-serving tray (Fig. 1). In this way, the astronauts warmed their food and consumed it (by use of conventional tableware) directly from the opened cans that were held in the warmer-serving tray. This tray provided the first capability to heat foods during a U.S. space flight. The heaters were electrical resistance wires designed to heat to a maximum of 69.4 C. Higher temperatures had to be avoided to prevent food from boiling and expelling particles in null gravity. Boiling would have occurred near 72.2 C in the Skylab that was approximately one-third atmosphere total pressure.

The food warmer-serving tray was designed so that frozen food (-23.2 ± 5.5 C) could be heated to 65 ± 3.3 C within 2 h under null gravity conditions. The design also provided for holding the food at 65 ± 3.3 C until consumed. These design criteria were established to prevent multiplication of potential pathogenic microorganisms. The watt density design calculations of the food tray assumed heat transfer only by conduction because convection currents were expected to be minimal in null gravity (radiant-heat transfer was ignored as being insignificant). The groundbased time temperature and percent heater-on-time relationships for the Skylab heating-serving tray are presented graphically in Fig. 2. Groundbased testing with the complete absence of convection currents in foods is impossible. The possibility always remained, therefore, that heating of food in null gravity would be slower than that indicated by groundbased testing. Such a condition could result from poor contact between the food and its container during weightless flight. Therefore, before flight there was considerable uncertainty as to the actual heating times in null gravity, and

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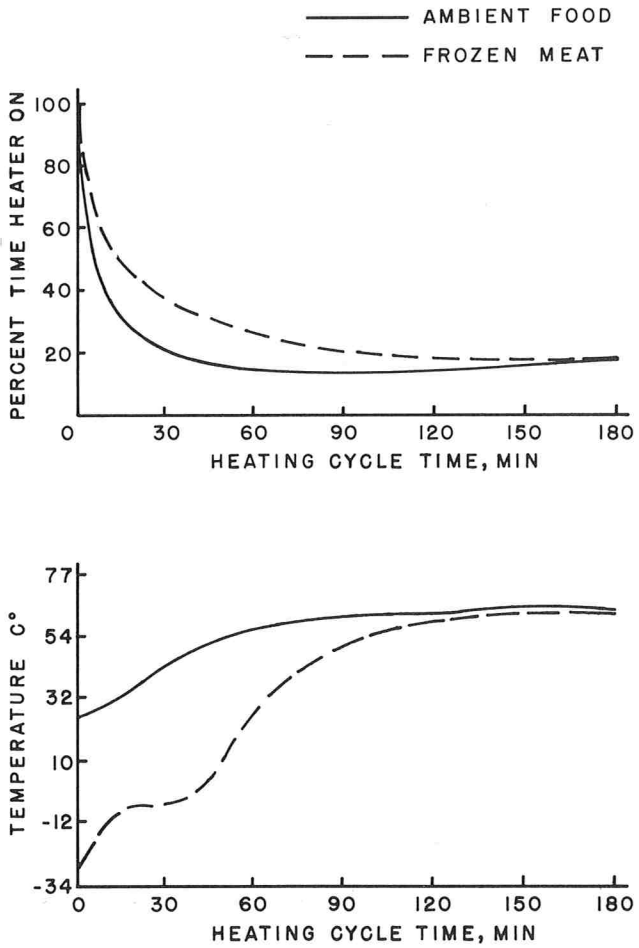

Figure 2. Time-Temperature relationships in Skylab food heating-serving tray.

there was no definitive means to test these heating characteristics before flight. Skylab food was, therefore, manufactured under a uniquely rigid quality control program that incorporated a critical control point system of production and test standards established after appropriate hazard analyses *(2, 6, 8)*. One of the hazard analyses conducted involved evaluation of the potential for multiplication of *Clostridium perfringens* in the foods during food warming times.

C. perfringens was chosen as the test organism for hazard analysis because of its importance in food poisoning, ubiquitous nature, heat tolerance, and known capability to multiply in foods. The growth rates of *C. perfringens* in the Skylab food heating profile was judged critical to the hazard analyses of food handling procedures and crew safety. A test plan was devised to determine the rate of growth of *C. perfringens* using foods selected to represent three categories – "all" meat items, half meat-half vegetable items, and "all" vegetable items. Growth rates were measured over a range of potential temperature profiles that foods could have been subjected to during the Skylab flight while meals were in preparation. The tests measured the spore germination, outgrowth, and vegetative cell proliferation at various temperatures for periods up to a maximum of 2 h. This was the maximum time for heating in Skylab because of automatic timing of heater operations which was implemented to reduce power consumption.

MATERIALS AND METHODS

Skylab foods used for this study were freeze-dehydrated turkey rice soup and mashed potatoes. Crew preparation procedures for these foods required addition of hot water (55 C), mixing, and heating in the Skylab food warmer-serving tray for periods up to 2 h. Turkey rice soup and mashed potatoes were flight food items produced in accordance with Skylab specifications *(7)*. Turkey rice soup was composed of freeze-dried diced turkey meat, 45.58%; freeze-dried rice, 17.09%; parsley flakes, 0.29%; and soup base, 37.04%. The soup base was composed of dried chicken broth, 49.04%; waxy maize starch, 50.36%; and white pepper, 0.60%. The turkey meat, rice, parsley flakes, and soup base were individually weighed into portions, packaged in rehydratable packages and flushed with nitrogen three times before vacuum sealing. Mashed potatoes were composed of potatoes, 75.54%; whole milk, 19.59%; butter, 4.55%; salt, 0.31%; and antioxidant (20% BHA, 20% BHT, 60% corn oil) 0.01%. Potatoes and other ingredients were mashed and then freeze-dried. The freeze-dried mashed potatoes were packaged in the same manner as the turkey rice soup. The third "food" used was cooked meat medium (Difco). Sterile cooked meat was tempered for 2 h and other foods 1/2 h before inoculation. Sterile cooked meat was reconstituted according to manufacturer's directions. Other foods were diluted 1:10 with sterile deionized water, mixed, and transferred to a sterile 500 ml Erlenmeyer flask. Flasks were plugged with cotton, covered with aluminum foil, and placed in a water bath that had been preset at the incubation temperature.

C. perfringens S-89, obtained from the Food and Drug Administration (Food Microbiology Branch, Cincinnatti, Ohio), was used in all of the generation time determinations. Stock cultures were maintained in cooked meat medium and transferred at monthly intervals. Stock cultures were transferred to 10 ml cooked meat medium 24 h before each determination. Two milliliters of the 24-h old culture were inoculated into 198 ml of either sterile cooked meat, turkey rice soup, or mashed potatoes that had been tempered to the test temperature. Incubation temperatures were 25, 30, 35, 40, 45, 50, and 55 C. The liquid level of the flask was submerged 5 cm below the liquid level of the water bath. One milliliter aliquots were removed at 0, 20, 40, 60, 80, 100, and 120 min exposure. Flask contents were mixed by swirling before each sampling. Care was taken to prevent the contents from reaching 5 cm above the liquid level during mixing. One milliliter aliquots were transferred to 9 ml of freshly boiled and cooled (4 C) thioglycolate medium (Difco). These 1:10 dilutions were maintained at 4 C until the end of each experiment (120 min) and then surface plated on duplicate SFP agar *(12)* plates. Plates were placed into GasPak anaerobic jars (BBL) and incubated at 37 C for 24 h. Total vegetative cell counts were done on each sample. The number of generations (n) and generation time (g) was calculated for each incubation time.

TABLE 1. GENERATION TIME[a] (g) OF *Clostridium perfringens* IN DIFFERENT FOODS AT VARIOUS TEMPERATURES

Incubation time (t) (Minutes)	25 C		30 C		35 C		40 C		45 C		50 C		55 C	
	n[b]	g[a]	n	g	n	g	n	g	n	g	n	g	n	g
Cooked meat														
20	0.14	143	–	–	2.07	10	0.86	23	–	–	0.71	28	–	–
40	–[c]	–	–	–	0.50	80	0.26	154	–	–	0.35	114	–	–
60	0.44	182	–	–	1.45	41	–	–	0.29	207	0.35	171	–	–
80	–	–	–	–	2.28	35	0.16	500	0.13	615	–	–	–	–
100	–	–	0.48	208	3.01	33	–	–	–	–	–	–	–	–
120	0.14	857	0.33	364	4.36	28	–	–	–	–	–	–	–	–
Turkey rice soup														
20	1.03	19	–	–	–	–	–	–	–	–	–	–	–	–
40	0.63	63	–	–	–	–	0.02	2000	–	–	–	–	–	–
60	0.29	207	–	–	–	–	0.11	545	–	–	–	–	–	–
80	–	–	0.18	444	–	–	0.36	222	–	–	–	–	–	–
100	0.35	286	–	–	–	–	–	–	–	–	–	–	–	–
120	1.28	94	–	–	–	–	–	–	–	–	–	–	–	–
Mashed potatoes														
20	2.10	10	–	–	2.07	10	0.37	55	1.21	16	–	–	–	–
40	3.56	11	–	–	1.90	21	–	–	2.31	17	–	–	–	–
60	3.07	20	–	–	2.07	29	–	–	2.35	26	–	–	–	–
80	4.00	20	–	–	1.68	48	–	–	2.40	33	–	–	–	–
100	3.59	28	–	–	1.54	65	0.44	227	2.26	44	–	–	–	–
120	2.98	40	–	–	1.42	85	0.47	255	1.86	65	–	–	–	–

[a] g (generation time) $= \frac{t}{n}$, where n is the number of generations at time, t *(9)*.

[b] n (number of generations) $= 3.3 \log_{10} \frac{y}{x}$, where y is the number of organisms present at time, t, and x is the number of organisms present initially *(9)*.

[c] = No growth detected.

RESULTS AND DISCUSSION

Three categories of foods were utilized in this analysis of the hazard from *C. perfringens* in Skylab foods. The three categories represented an "all" meat food, a food composed of approximately half meat and half vegetable, and an "all" vegetable item. Cooked meat medium was selected as the optimal growth support medium and representative of the microbial growth supporting potential of the Skylab foods that were largely composed of meat. Skylab turkey rice soup was used as a characteristic half meat-half vegetable product, and Skylab mashed potatoes represented the non-meat foods.

The media as well as the incubation temperature influenced the growth rate of *C. perfringens* (Table 1). Significant increase in the number of generations was observed in cooked meat only at 35 C and the average time for the population to change 1 log cycle (generation time) over the 2-h period was 28 min. Others have shown that *C. perfringens* does grow at higher temperatures *(3)*. In the turkey rice soup, significant generation was seen only at 25 C (average generation time, 94 min), and in the mashed potatoes at 25, 35, and 45 C (at which average generation times were 40, 85, and 65 min, respectively). Decreases in the number of organisms occurred at 55 C in the cooked meat, at 45, 50, and 55 C in the turkey rice soup and at 50 and 55 C in the mashed potatoes. Brown and Twedt *(3)* reported greater than 99% reduction of *C. perfringens* on roast beef in less than 6 h at 53.3 C.

In a single test, *C. perfringens* was incubated in the turkey rice soup at 35 C for 28 h. A total of 8.7 generations were produced with a generation time of 193 min. This observation indicates that, although the organism did not multiply in the 2 h hazard time tested, it cannot be concluded that longer times will not allow growth to occur. The hazard analysis conducted here was limited to the 2 h time range because of the limits of heating foods in the Skylab mission. Most of the cultures were probably in a temporary lag or phase of slow development during the 2 h incubation period. Brown and Twedt *(3)* reported growth of *C. perfringens* in roast beef chucks up to 12 h at 51.1 C followed by a reduction in viable cells at 18 h.

An upper limit for *C. perfringens* was established for Skylab foods after hazard analysis which included

TABLE 2. SKYLAB FOODS WHICH WERE WARMED PRIOR TO EATING AND IN WHICH *Clostridium perfringens* WAS JUDGED TO BE A POTENTIAL HAZARD

Rehydratable foods
Sausage Patties
Cream of Tomato Soup
Potato Soup
Turkey Rice Soup
Chicken and Rice
Chicken and Gravy
Pork and Scalloped Potatoes
Beef Hash
Veal and Barbecue Sauce
Spaghetti and Meat Sauce
Macaroni and Cheese
Frozen foods
Filet Mignon
Pork Loin and Dressing
Lobster Newburg
Prime Rib of Beef

the evaluation of their generation times and the following factors: epidemiology of food-borne diseases, nature of each Skylab food in regards to its potential to support growth of *C. perfringens*, Skylab food processing procedures, microbiological data of comparable space foods, quality control standards enforced during manufacture *(8)*, recommended limits established by public health organizations *(10, 11)*, problems associated with null gravity heating and refrigeration, and proposed crew handling procedures. The specification limit of not greater than 100 *C. perfringens*/g was established after review of the above parameters and assuming that: *(a)* a dose of 10^6 viable organisms would be required to cause symptoms, *(b)* no lag phase would occur in multiplications of the organisms, *(c)* a generation time would be at least 20 min, *(d)* any one Skylab meal would not contain more than 100 g of contaminated food (foods involved are generally entrees or rehydratables which contain about 30 g per serving, dry weight). Predicted on these assumptions, it would require that food be held 2 h or longer under optimum conditions for growth of *C. perfringens* before a population could be built up sufficient to cause a clinical episode. The limit of 100 *C. perfringens*/g was, therefore, set for the Skylab foods listed in Table 2. These standards proved to be adequate to protect the Skylab astronauts. The Skylab was inhabited for over 500 man-days without any evidence of food-associated illness. Routine testing of Skylab foods utilized the sample plan and method for *C. perfringens* as specified by Heidelbaugh, et al *(6)*. This procedure employed SFP agar *(12)* as used in the hazard analysis tests reported here. This medium is less selective than other media *(5)* for *C. perfringens*. This non-selectivity may have somewhat increased the conservativeness of the test limit. All of the Skylab foods examined for *C. perfringens* (listed in Table 2) proved to have less than 10 *C. perfringens*/g.

Foods are commonly exposed to temperatures that support microbial growth during preparation under a variety of food service operations (including space flight, commercial restaurant, institutions, vending machines, and at home). Bryan and Kilpatrick *(4)* isolated *C. perfringens* from 11 of 36 samples of sliced roast beef, cooked to an internal temperature of 68.4 C. Fortunately, food must either be grossly contaminated and/or grossly mishandled for it to produce a safety hazard from *C. perfringens*. The recommended temperature for holding potentially hazardous foods in food service operations, in vending machines, and aboard aircraft is 60 C *(10, 11)*. Various investigators *(1, 3, 4)* have suggested that the holding temperature of 60 C could be reduced for roast beef without endangering the public health from growth of staphylococci, salmonellae or *C. perfringens*. Results of the hazard analyses conducted for Skylab substantiate Brown and Twedt's *(3)* suggestion relative to *C. perfringens*. However, the same degree of safety is not provided as the serving temperature is lowered to 54 C. Allowance must always be made for inadvertent errors in maintaining time-temperature profiles during preparation and serving.

The findings of these hazard analyses suggested that an upper limit of 100/g for *C. perfringens* was adequate for Skylab foods. This limit was predicted on a sampling plan and enumeration of the organisms by a stipulated procedure *(6)*. This limit merits consideration for any food that is manufactured under quality control procedures comparable to those used for Skylab foods *(7, 8)*. Hazard analysis indicates that this limit provides safety for such foods even when they are exposed to temperatures between 25 and 55 C for up to 2 h.

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AMENDMENT TO 3-A SANITARY STANDARDS FOR MULTIPLE-USE PLASTIC MATERIALS USED AS PRODUCT CONTACT SURFACES FOR DAIRY EQUIPMENT

Serial # 20-06

Formulated by

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

The "3-A Sanitary Standards for Multiple-Use Plastic Materials Used as Product Contact Surfaces for Dairy Equipment, Serial # 20-00 are hereby amended as indicated in the following:

Section I. Standards for Acceptability, Sub-paragraph (2):

Add the following material to the list of Generic Classes of Plastics:

Cross-linked polyester resins 0.20 0.20 0.20 (vinyl ester-styrene copolymer)

These standards shall become effective Nov. 15, 1974.

3-A SANITARY STANDARDS FOR CENTRIFUGAL AND POSITIVE ROTARY PUMPS FOR MILK AND MILK PRODUCTS

Serial #02-06

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 developments. Milk pump specifications heretofore or hereafter developed which so differ in design, material, construction, or otherwise, as not to conform with the following standards, but which in the manufacturer's or fabricator's opinion are equivalent or better may be submitted for the joint consideration of the IAMFES, USPHS, and DIC at any time.

A.

SCOPE

A.1

These standards cover the sanitary aspects of centrifugal and positive rotary pumps for milk and milk products.

A.2

In order to conform to these 3-A Sanitary Standards, centrifugal and positive rotary pumps shall comply with the following design, material and fabrication criteria.

B.

DEFINITIONS

B.1

Product: Shall mean milk and milk products.

B.2

SURFACES

B.2.1

Product Contact Surfaces: 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.2.2.

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

B.3

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.

B.4

Engineering Plating: Shall mean plated to specific dimensions or processed to specified dimensions after plating.[1]

C.

MATERIALS

C.1

All product contact surfaces shall be of stainless steel of the AISI 300 series[2] or corresponding ACI[3] 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.)

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[1]QQ-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.

[2]The 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.

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

C.1.2.
Rotors, drive shafts, drive pins and rotor pins may be made of metal covered with an engineering plating of nickel, chromium or an equally corrosion-resistant, non-toxic material.

C.1.3.
Pump impellers or rotors, and cases or stators, which operate in conjunction with a metallic counterpart, may be made of, or covered with, rubber or rubber-like materials or plastic materials. Rubber or rubber-like materials and plastic materials used for pump impellers or rotors, and cases or stators, 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 and rubber-like materials and plastic materials may be used for O-Rings, seals and parts used in similar applications.

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.
Pump impellers or rotors, and cases or stators, which operate in conjunction with a metallic counterpart and the sealing faces of rotary seals may be covered with a ceramic material. 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.1.8.
Single service sanitary type gaskets may be used.

C.2
All materials having a product surface(s) used in the construction of a pump designed to be used in a processing system to be sterilized by heat and operated at a temperature of 250°F or higher shall be such that they can be (1) sterilized by saturated steam or water under pressure at a temperature of at least 250°F and (2) operated at the temperature required for processing.

C.3
Non-product contact surfaces shall be of corrosion-resistant material or material that is rendered corrosion-resistant. If coated, the coating used shall adhere. 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
The minimum thickness of engineering plating shall be 0.0002-inch for all product contact parts except that when the parts that are to be plated are other than stainless steel, the minimum thickness of the engineering plating shall be 0.002-inch.

D.3
Rubber or rubber-like materials and plastic materials having product contact surfaces that are a coating or covering 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 and residual adhesive, if used, shall conform to the criteria in C.1.5 or C.1.6.

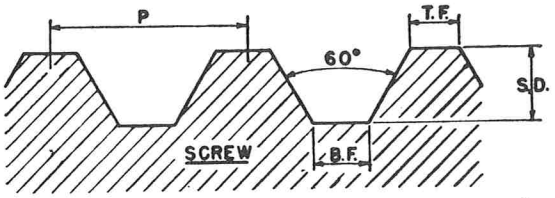
D.4
All product contact surfaces shall be easily accessible for cleaning, either when in an assembled position or when removed. Removable parts shall be readily demountable.

D.5
Pumps designed to be mechanically cleaned shall be fabricated so that all product contact surfaces of the pump can be mechanically cleaned.

D.6
There shall be no threads on product contact surfaces except where necessary for attaching the impeller to the shaft. In such case(s) the thread shall conform to the following drawing known as the "brass valve stem thread." The threaded angles shall be not less than 60 degrees and with not more than 8 threads to the inch, nor less than

5/8 inch major basic diameter. The length of the nut shall not exceed three-quarters of the thread basic major diameter and the nut shall be of the open type.

BRASS VALVE STEM THREAD



P = PITCH	$P = \frac{1}{T.P.I.}$
S.D. = SINGLE DEPTH	S.D. = .381 x P
T.F. = TOP FLAT	T.F. = .280 x P
B.F. = BOTTOM FLAT	B.F. = .280 x P
T.P.I. = TH'DS PER INCH	

D.7

Gaskets having product contact surfaces 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.8

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

D.8.1

Where for space or functional reasons it is impossible to have a radius of 1/4 inch. When for functional reasons the radius must be less than 1/32 inch, in such applications as flat sealing surfaces, the product contact surface of this internal angle must be readily accessible for cleaning and inspection.

D.8.2

The minimum radii 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.8.3

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.9

Inlet and outlet 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

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.

D.11

The shaft seal(s) shall be of packless type, sanitary in design with all parts readily cleanable.

D.12

Pumps designed to be used in a processing system to be sterilized by heat and operated at a temperature of 250°F or higher shall comply with the following:

D.12.1

Be of such construction that all product contact surfaces can be (1) sterilized by saturated steam or water under pressure at a temperature of at least 250°F and (2) operated at the temperature required for processing.

D.12.2

Pumps to be used in such a processing system not designed so that the system automatically is shut down if the product pressure in the system becomes less than that of the atmosphere and cannot be started until the system is resterilized shall have a steam or other sterilizing medium chamber surrounding (1) the shaft(s), (2) the portion of the inlet and outlet connection adjacent to the product and (3) the pump cover.

D.12.3

The connection(s) on the steam or other sterilizing medium chamber(s) for the steam or other sterilizing medium lines shall be such that the line(s) can be securely fastened to the connection(s). The line(s) shall be connected in a manner that they may be disconnected to allow the sterilizing chamber(s) to be inspected and cleaned if necessary.

D.13

The means of supporting pumps shall be one of the following:

D.13.1

With legs: Legs shall be adjustable, smooth with rounded ends, and have no exposed threads. Legs made of hollow stock shall be sealed. Legs shall be of sufficient length to provide a clearance between the lowest part of the base, pump, motor or drive and the floor no less than:

D13.1.1.

Four inches on pumps with legs designed to be fixed to the floor or pumps having a horizontal base area of more than one square foot.

D.13.1.2.
Two inches on pumps having a horizontal base area of not more than one square foot and not designed to be fixed to the floor.

D.13.2
Mounted on the drive.

D.13.3
Mounted on a slab or island: The base of the pump shall be such that it may be sealed to the mounting surface (See Appendix, Section H.)

D.13.4
Mounted on a wall or column: If the pump is to be sealed to a wall or column, the base shall be such that it may be sealed to the mounting surface.

D.14
The motor, pump, and drive shall be mounted so that all non-product contact surfaces are easily accessible for cleaning and drain freely.

D.15
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.16
Non-product contact surfaces shall be smooth, free of pockets and crevices, readily cleanable and those to be coated shall be effectively prepared for coating.

D.17
Pumps to be used as the timing device in a high-temperature short-time pasteurizing system shall be provided with an easily accessible or externally visible means of sealing the drive mechanism that will, when a seal(s) is in place, prevent the operation of the pump at a greater capacity than that which gives legal holding time.

D.18
Pumps shall have an information plate in juxtaposition to the name plate containing a statement that the pump *is* or *is not* designed for mechanical cleaning or the statement shall appear on the name plate.

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.

F.

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 ASTM[4] 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.

H.

SLABS OR ISLANDS

When a pump is designed to be installed on a slab or an island, the dimensions of the slab or island should be such that the pump base 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 top of the pump base is not less than 4 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 pump base and upper edges of the slab or island should be sealed.

These Standards are effective Feb. 12, 1975, at which time the "3-A Sanitary Standards for Pumps for Milk and Milk Products, Revised, Serial #02-03" and the amendments to it are rescinded and become null and void.

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

EXAMINATION OF OFFICIAL MEASUREMENT TOLERANCES FOR ONTARIO'S FARM MILK TANKS

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(Received for publication May 20, 1974)

Abstract

A random sample of 72 farm milk tanks was check-calibrated to determine whether official tolerances for estimates of milk weights were realistic. The overall calibration error for 859 calibration check values was + 3.12 lb. Sixty-nine percent of all values exceeded the official tolerances and this was reduced to 23% when the calibration check values for each tank were reduced by an amount equal to the mean calibration check error for the tank. Under these circumstances the official tolerances were approximately equal to one standard deviation of errors. When the tolerances were doubled (approximately two standard deviations), the percentage of the calibration check values which were excessive was reduced to 4.9%. It was concluded that the official tolerances were too small and should be doubled in size, giving a tolerance range of 6 to 16 lb. (0.5 to 0.18%) for a range of tank capacity from 1200 to 9000 lb.

More than 13,000 refrigerated farm milk tanks in Ontario are used for storing about 80% of the 5 billion lb. of milk sold off farms annually. This milk is purchased from producers by the Ontario Milk Marketing Board on the basis of a measurement using a gauge rod. A tank calibration chart is used to convert the measurement to a weight equivalent. The measurement is no more accurate than the accuracy of the manufacturer's calibration of the tank. After the tank is installed other factors may also affect the accuracy of the measurement including levelness of tank and structural defects that allow tank capacity to change with time *(3)*. The purpose of this study was to determine whether estimates of weights of milk in Ontario's farm milk tanks were within limits of official tolerances *(4)*.

Materials and Methods

A random sample of 72 farm tanks was selected from the counties of Wellington, Waterloo, Oxford, and Perth in southwestern Ontario. The procedure for checking calibration of a farm tank was to add increments of about 200 lb. of water from a portable delivery container positioned above the farm tank. This container was filled with water from a supply carried in a tank truck. After each addition of water the water temperature was recorded and two separate measurements were made with the gauge rod dusted with Bon Ami powder. The average measurement was converted to pounds of milk and was recorded with the corresponding weight of milk found on the tank calibration chart. This procedure is hereafter referred to as a calibration check. Successive increments of water were added until the tank was filled to capacity. A total of 859 calibration checks were made on the 72 tanks.

The delivery container was previously carefully calibrated by filling and emptying the tank six times with distilled water to determine the net weight of water. The Toledo scale used for these weighings was checked and adjusted with standard 50-lb. weights by the Department of Weights and Measures just before use. Weighings were done at five different water temperatures ranging from 38 to 57 F. The range of net weight of water for the 30 determinations was 200.0 to 200.2 lb. Variations at the various temperatures were not significantly different so an overall average weight of 200.068 lb. was used as weight of water delivered.

To convert weight of water to volume the following conversion factors were used: 1 lb. = 0.45359 kg; volume in liters = kg ÷ apparent wt. 1 liter distilled water in kg; Imperial gallon = liters ÷ 4.54596 *(1)*; Imperial gallons per delivery $= \frac{200.068 \times 0.45359}{4.54596 \times \text{apparent wt.}}$

A table of apparent weights of water at 0.9° intervals from 32 to 68 F was used in making the conversions. The table was calculated by the formula:

$$\text{Apparent weight} = \frac{D}{1 + 0.00106 + 0.0012\ (1\text{-}D)}$$

where D is the absolute density in grams per milliliter. This was derived from a table for reductions of weighings in air to vacuo *(2)*.

The estimated volume of water added was converted after each addition to pounds of milk using the official conversion factor 10.32 × Imperial gallons *(4)*.

The random sample included tanks of 17 manufacturers that were arranged in six groups. Groups A, B, C, and D each contained tanks of a single manufacturer and numbered 18, 13, 7, and 6 tanks, respectively. Of the remaining 28 tanks, 13 had a capacity of 1800 lb. or less and were placed in Group E. The remainder were placed in Group F. "Over-calibration" means tanks containing less milk than the calibration chart indicated. "Under-calibration" means the opposite.

Results and Discussion

A summary of check calibration data is presented in Table 1. The individual calibration check error range and the tank average error range indicated that there were considerable differences between actual amounts of milk in some tanks and amounts being determined by the gauge rods. The overall average error for the tanks in each group indicates that producers in Group A were paid for an average of 8.9 lb. of milk less than was in the tanks. In all other groups the average error was positive indicating that producers were being over-paid. Forty-six tanks were over-calibrated and 26 were under-calibrated. The overall calibration error for all

TABLE 1. SUMMARY OF CHECK-CALIBRATION DATA INDICATING INDIVIDUAL CHECK POINT AND TANK AVERAGE ERROR RANGES, AND OVERALL AVERAGE ERROR

Tank group	Tank capacity range (00's lb.)	No. of tanks	Individual check-point error range (lb.)	Tank average error range (lb.)	Overall average error (lb.)	Tanks check calibrated	
						Over	Under
A	12 – 38	18	–53 to 19	–42 to 9	–8.9	3	15
B	18 – 52	13	– 6 to 30	– 2 to 20	9.0	11	2
C	15 – 30	7	–18 to 42	– 6 to 31	10.9	5	2
D	16 – 30	6	– 7 to 22	0.2 to 12	6.9	6	0
E	12 – 18	13	–16 to 21	–11 to 17	2.6	8	5
F	20 – 52	15	–13 to 37	– 2 to 31	7.8	13	2

TABLE 2. ERRORS IN BULK TANK CALIBRATION AFTER ADJUSTMENT OF CALIBRATION CHECK VALUES TO ZERO AVERAGE ERROR

Tank capacity (lb.)	Number of tanks	Total No. check points	Max. error (lb.)	Min. error (lb.)	Standard deviation of errors	Official tolerance (lb.)
1200	4	24	1	–2	0.9	3
1400	5	35	7	–7	3.3	3
1600	9	72	8	–10	3.7	4
1800	5	45	6	–15	3.4	4
2000	12	120	9	–13	3.0	4
2200	7	77	17	–13	5.1	4
2400	2	24	11	–7	3.8	4
2600	11	143	18	–21	5.3	4
3000	4	60	19	–15	5.7	5
3200	3	48	11	–9	4.5	5
3400	1	17	6	–13	5.0	5
3600	1	18	10	–13	6.5	5
3800	3	57	11	–16	5.0	5
4200	2	42	10	–8	4.1	5
5000	1	25	8	–11	5.9	6
5200	2	52	11	–23	5.8	6

calibration checks for all tanks was +3.12 lb. Sixty-nine percent of all calibration check values exceeded tolerances allowed by the Regulations of the Milk Act *(4)*. Reasons for this unsatisfactory situation were not clear. Even though no attempt was made during the survey to ascertain condition of the tanks in respect to levelness or structural defects, it was assumed that these were reasons for some of the errors. Also, methods of calibrating tanks might introduce serious errors. There is no common method of calibration used by manufacturers of tanks sold in Ontario. If methods are such that the gauge rod reading for a given volume of water does not duplicate the reading for a similar volume of milk, then an error is introduced. In addition, it is believed by the authors that the gauge rod reading for a given volume of milk may vary depending upon such factors as temperatures of gauge rod and milk, humidity of air, condition of gauge rod surface, composition of milk at liquid-metal interface as well as human error in reading the gauge rod. Therefore, it was considered possible that the large percentage of calibration check values which exceeded the official tolerances was not only due to condition and levelness of the tank but also due to official tolerances being too small to be practical under field conditions.

To estimate the magnitude of tolerances that might be practical, each calibration check value for a given tank was reduced by an amount equal to the mean calibration check error for that tank. This resulted in 23% of the calibration checks still exceeding the official tolerance. It is worth noting (Table 2) that official tolerances for tanks were approximately equal to one standard deviation of errors. When the tolerances were doubled (approximately two standard deviations), the percentage of the calibration check values that were excessive was reduced from 23% to 4.9%. The statistics describing the distribution of errors are presented in Table 2 where tanks have been rearranged into groups according to capacity. Official tolerances are also included.

It is apparent that the condition of Ontario farm tanks is such that most of them cannot meet the stand-

ards, in respect to estimation of milk contained therein, as required by the Regulations made under the Milk Act *(4)*. Some portion of the measurement errors can probably be overcome by periodic re-calibration of the farm tanks. However, it is also apparent that the official tolerances are too restrictive and are not realistic for practical operations. A doubling of the tolerances seems to be reasonable and would mean a range of 6 to 16 lb. (0.5 to 0.18%) for a range in tank capacity from 1200 to 9000 lb.

ACKNOWLEDGEMENT

This study was supported in part by the Dairy Industry Branch of the Ontario Ministry of Agriculture and Food, Ontario Milk Marketing Board, Ontario Dairy Processors Council, and Automotive Transport Association.

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ASSOCIATION AFFAIRS

NOMINATIONS FOR OFFICES OF IAMFES, INC.–1975-1976

(Notice to membership—ballots will be mailed to paid up members as of December 31, 1974)

FOR SECOND VICE-PRESIDENT AND SECRETARY-TREASURER

HOWARD HUTCHINGS

Howard Hutchings is director of Sanitation and Safety for the South Dakota State Health Department. In this position, he supervises the inspection and licensing of all food, lodging and campground establishments in the State. The safety program includes radiation safety and mine inspection.

Howard considers himself a grass roots food sanitarian. He has a B.S. in Food Technology and a M.S. in Public Health, both from the University of Missouri at Columbia. He started his public health career as a part-time health inspector at Boonville, Missouri. Three years later he became a full-time inspector for the Columbia City Health Department.

While working as food sanitarian for the City of Columbia he started a weekly health department radio show, created a grocery store-church inspection program and began enforcement of a previously dormant city food code.

In 1960 he established the first campus sanitation program at the University of Missouri and spent seven years as their sanitarian. His M.S. thesis was based on his food handler educational programs at the University in which he documented the importance of proper training.

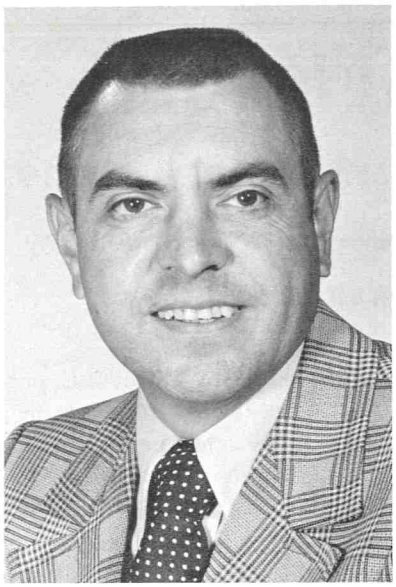
He has held his current position since 1967. In South Dakota, he has established a public school food service inspection program, plan review for all new or remodeled establishments, developed a campground law and regulation and a recent food salvage law. While practicing a fair, but firm, enforcement program he feels training and cooperation is still the key to functional public health. He lectures widely at high schools, colleges and training workshops.

He is an active member of I.A.M.F.E.S., serving on the Food Protection Committee, Food Equipment Committee and is currently secretary of the Affiliate Council. He has served as secretary-treasurer of the

South Dakota Environmental Health Association for several years. He is a charter member of the Restaurant Management Advisory Committee, and the College of Nursing Advisory Committee, both at South Dakota State University. He is also a member of the State Advisory Council, South Dakota Easter Seal Society for Crippled Children and Adults. He holds a Lifetime Membership in PTA and is presently president of the Pierre Jr. High PTA.

Howard is active in local church work and has served as Sunday School teacher, director and as lay Baptist minister.

Howard, his wife Peggy and their two sons all enjoy sports, hunting, fishing and life in general.



LEON TOWNSEND

Leon Townsend is presently assistant Milk Branch manager, Bureau of Health Services, Department for Human Resources, Frankfort, Kentucky. Prior to this he has held the following positions with the Kentucky Department of Health: Grade A Survey Officer from 1962 to 1972, delegate to the 1969 IMS Conference at Denver, Colorado, and Director, Manufacturing Milk Program from 1972 to 1974. As director of this program, Leon was instrumental in bringing the Manufacturing Milk Program into compliance with the USDA recommended standards. Leon was also employed as a Senior County Sanitarian in Hancock and Henderson Counties for four years before beginning his career with the State in 1962.

He was born July 2, 1935 in Henderson County, Kentucky, obtained his elementary and high school education in Kentucky, and has a B.S. degree in Agriculture and Biology from Western Kentucky University.

Active in IAMFES activities for the past twelve years, Leon has been in regular attendance at their annual meetings. He served as IAMFES affiliate secretary in 1970 and again in 1973 and 1974, and has been Kentucky's affiliate council representative for ten years. Presently, he is chairman of the Dairy Farm Methods Subcommittee on Antibiotics, Pesticides, and other Adulterants, and a member of the 1974-75 awards committee.

Leon has also continued his education, completing additional college credit courses in Agricultural Engineering, and has attended many environmental health workshops.

As secretary-treasurer of the Kentucky Association of Milk, Food, and Environmental Sanitarians for the past ten years, the association has increased its membership from 75 to over 250. The Kentucky Association has submitted members to International, for the Outstanding Sanitarians Nominations on four occasions, and have won the IAMFES Outstanding Sanitarian Award on all four occasions.

The Kentucky Association also won the C. B. Shogren Award as the Outstanding IAMFES affiliate in 1973.

Leon's hobbies are golf and baseball. He has been married seventeen years to Elsie C. Townsend and they have three children: Jeff, age 16, Brenda, age 14, and Jill, age 9. Their home is 110 Tecumseh Trail, Frankfort, Kentucky.

SECRETARY-TREASURER

RICHARD PELL MARCH

Richard P. March is a professor in the Department of Food Science at the New York State College of Agriculture, Cornell University, Ithaca, New York.

Until 1965, he devoted 75% of his time to extension work as a specialist in milk quality and fluid milk handling and processing, and the balance of his time in research and teaching courses in fluid milk processing and quality control. At present, extension accounts for 95% of his time with 5% for research activities.

He was raised in Massachusetts, majoring in dairy industry at the University of Massachusetts, receiving a B.S. degree in 1944. After a tour in the U. S. Marine Corps, he entered the Graduate School at Cornell University to major in dairy industry, receiving an M. S. degree in 1948.

Professor March taught a one-year program in dairy manufacturing until its termination in 1951, at which time he was promoted from instructor to assistant professor. He became an associate professor in 1955, and full professor in 1965. In 1965 he also because department extension leader and is still serving in this capacity.

He is active in the New York State Association of Milk and Food Sanitarians, serving as secretary-treasurer from 1957 and executive secretary since 1967, secretary of the Dairy Industry Equipment Committee from 1952-57, secretary of the Farm Practices Committee from 1955-62, and secretary of the Council of Affiliates since 1952. He is a member of the International Association of Milk, Food, and Environmental Sanitarians, serving as Secretary-Treasurer since 1970, a member of their Farm Methods Committee from 1959-65, secretary of their Council of Affiliates in 1961, and chairman in 1962 and 1963.

In both the State and International Associations he has served as chairman of a number of subcommittees including the Uniform Milkhouse Plans for the Northeast, Milk Transfer Systems, Sediment Testing, and Training Programs for Bulk Tank Truck Operators, and co-chairman of the Northeast Committee on Uniform Guildlines for Loose Housing Systems. In 1963 he was the recipient of the New York State Association's Dr. Paul B. Brooks Memorial Award for outstanding contributions to the organization; in 1972 he received the Emmet R. Gauhn Memorial Award for outstanding service to the State Association.

Professor March has been chairman of the Northeast Dairy Practices Council, an 11 state organization, since its inception in 1969.

BARNUM NASCO CONSULTANT

HAROLD BARNUM

Harold Barnum, formerly Chief of Milk Sanitation at Denver, Colorado, has joined Nasco as a consultant for the company's milk sampling products operation.

Barnum was instrumental in the development of Nasco's Whirl-Pak Sampling Bag, a sterile throw-away container for milk samples. More than 50 million Whirl-Pak Bags were used in the dairy and food industries last year.

After 22 years with the Denver Department of Health and Hospitals, Barnum retired in August of 1969. Since that time he has worked as a milk sanitation consultant. He has served in many dairy organizations, including positions as President of the International Association of Milk and Food Sanitarians, Chairman of the National Conference on Interstate Milk Shipments, and Executive Secretary of the Dairy Products Improvement Institute. Barnum received the National Sanitarians Award in 1957.

According to Phil Niemeyer, Head of Nasco's Whirl-Pak operation, "Mr. Barnum will be working with Nasco for the continued improvement of milk sampling products, and in the development of training programs for milk haulers and other dairy personnel."

Nasco International, Inc. operates a mail order division to serve agriculture and education, and a manufacturing division that produces defense items and plastic balls for industry and recreation. Besides the headquarters in Fort Atkinson, other plants are operated at Antigo and Waukesha, Wisconsin; Modesto, California; Chatham, New Jersey; and Guelph, Ontario, Canada.

LETTER TO IAMFES MEMBERS

Dear Friends:

This issue of the Journal will be the 254th and final issue put out by me. I must admit that, while it has been quite a task many times, I have some twinges of regret as I put this issue to bed for my last time.

I most sincerely want to thank all the IAMFES members who have been so kind to me these many years and all the affiliates who always made me feel at home and welcome at their meetings.

If there is ever anything I can do for you please let me know, it will be my pleasure. Thanks for the privilege of serving you these past twenty-three and a half years.

Sincerely,
H. L. "Red" Thomasson
Ex-Exec.-Sec'y. and Mg. Ed.

NATIONAL SANITATION FOUNDATION ANNOUNCES TWO PROMOTIONS

President Robert M. Brown of NSF has announced the promotion of two staff executives to vice presidential positions. Tom S. Gable is vice president, customer services. Dr. Nina I. McClelland is vice president, technical services. Brown's announcement followed approval of the appointments by the NSF board of trustees executive committee at their semi-annual meeting.

TOM S. GABLE

Mr. Gable's responsibilities include administration of NSF's U.S. and foreign customer service organization and all programs of standards, evaluation and listing for food service equipment, plastics, swimming pool equipment and special catagories. He is a graduate in sanitary engineering from Michigan State University and holds a master's degree in public health from The University of Michigan.

Before joining NSF in 1958 he served in executive positions in public health engineering in Allegan, Michigan, Flint, Michigan, Lincoln, Nebraska, and Allegheny County, Pennsylvania. Since 1958 he has been a resident lecturer in environmental health at the School of Public Health, The University of Michigan. He is a fellow of the American Public Health Association, secretary of the APHA engineering and sanitation section 1966-1969, and chairman in 1971. He is a member of the National Environmental Health Association, the International Association of Milk and Food Sanitarians, Delta Omega and other environmental and public health organizations.

DR. NINA I. McCLELLAND

Dr. McClelland is responsible for administration of water and wastewater programs along with research and development in these fields. She is also in charge of testing laboratory programs for good service equipment, plastics, swimming pool equipment and special categories. These include physical, chemical, microbiological and radiological testing services.

She received her bachelor's and master's degrees in science from the University of Toledo, her MPH and PhD degrees from The University of Michigan.

After five years as chemist-bacteriologist with the department of health, Toledo, Ohio, she served as chief chemist of the Toledo wastewater treatment plant from 1956 to 1963 with responsibility for the industrial waste program of the city. Following her doctoral program at The University of Michigan she joined the staff of NSF in 1968 as director of the

water research program. She is a lecturer at The U of M School of Public Health, a technical consultant to Ann Arbor Science Publishers and a member of Sigma Xi. She is program chairman, division of environmental chemistry of the American Chemical Society, a fellow and committee member of the APHA, chairman of the corrosion committee of the American Water Works Association, a member of NEHA and the Water Pollution Control Federation.

EDUCATION COURSES IN ENVIRONMENTAL HEALTH

The University of Wisconsin-Eau Claire will present several continuing education courses in Environmental Health during the 1974-75 period at several Wisconsin cities. The schedule is as follows:

Eau Claire—Environmental Health 111, Community Hygiene I Aug. 30-Dec. 6, 1974.

Waukesha—Environmental Health 111, Community Hygiene I Aug. 22-Dec. 12, 1974.

Wausau—Environmental Health 111, Community Hygiene I Aug. 23-Dec. 6, 1974.

Eau Claire—Environmental Health 112, Community Hygiene II Jan. 10-May 9, 1975.

Waukesha—Environmental Health 112, Community Hygiene II Jan. 15-April 24, 1975.

Wausau—Environmental Health 112, Community Hygiene II Jan. 17-April 25, 1975.

Fond du Lac—Food-Borne Disease Control 301 Aug. 21-Dec. 6, 1974.

Madison—Food-Borne Disease Control 301 Aug. 23-Dec. 13, 1974.

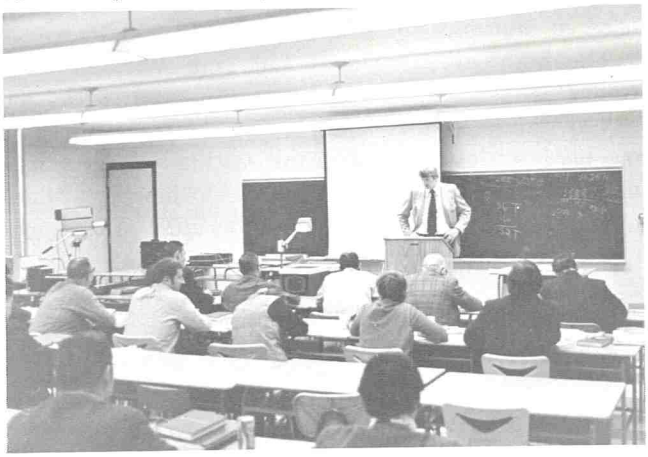
Fond du Lac—Food-Borne Disease Control 302 Jan. 24-May 2, 1975.

Madison—Food Borne Disease Control 302 Jan. 17-April 25, 1975.

The Community Hygiene Course has as its objectives to introduce the sanitarian to the various problems of the various areas of municipal and rural sanitation. Food-Borne Diseases is more specific to the handling of food and food products.

Class in Environmental Health III, Community Hygiene (Madison) 1973-74. Instructor - Dr. Walter Gojmerac, Professor of Entomology. University of Wisconsin-Madison.

Class in Environmental Health III, Municipal and Rural Sanitation (Fond du Lac) 1973-74.



Class in Environmental Health III, Community Hygiene (Fond du Lac) 1973-74. Instructor - Dr. Robert Nelson, Assistant Professor, University of Wisconsin-Eau Claire.

These courses were developed through the efforts of the Joint Committee on Education for the Wisconsin Environmental Health Association and the Wisconsin Association of Milk and Food Sanitarians, and through the cooperation of the University of Wisconsin-Eau Claire. Arrangements were made with the Center for Disease Control in Atlanta so as to maintain a cross-certification with CDC's educational (HEW) program. An objective in the continuing education courses is to upgrade the sanitarian's educational background. The courses are presented on Friday afternoons, approximately every three weeks. This is so implemented that the full-time working sanitarian will have two weekends for preparation for the lessons.

Requests for information about the courses and registration forms should be submitted to Dr. John Gerbirich, Director of Allied Health, University of Wisconsin-Eau Claire, Eau Claire, Wisconsin 54701.

ZERO INTRODUCES AN ACCURATE, MODERATELY-PRICED VACUUM CONTROLLER FOR MILKING SYSTEMS DESIGNED FOR RAPID RESPONSE TO VACUUM LEVEL CHANGE

Realizing the need for an accurate, moderately-priced vacuum controller–Zero Manufacturing Company of Washington, Missouri is introducing their Model 110 Zero Vacuum Controller for use on milking systems for 3-10 horsepower suppliers.

Designed for rapid response to vacuum level change–it will maintain a setting at ± 0.5 inches of mercury from no load to full load operation with an adjustment range from 9 to 22 inches of mercury.

A single unit will efficiently handle pump capacities up to 100 c.f.m. ASME, or they can be used in multiples for larger systems.

The body of the Model 110 Zero Vacuum Controller is constructed of cast aluminum with 300 series stainless steel and brass components. The mechanism design is a pivot system–which eliminates the need for springs, axles or hinges. Maintenance is kept at a minimum because of the materials used and specific design. The Model 110 Zero Vacuum Controller is patented.

NATIONAL CONFERENCE ON INTERSTATE MILK SHIPMENTS

1975 Conference

The 1975 meeting of the Conference is scheduled to be held at the Chase-Park Plaza hotel, St. Louis, Missouri, May 11-15. Hotel management has given us a flat rate of $23.00 single and $28.00 twin. Indoor parking will be available for hotel guests at the rate of $1.00 per day, with in/out privileges. Early in March you will receive pre-registration forms and hotel reservation cards. Please pre-register since it will make it easier for both you and the secretary when you get to St. Louis. Plan now to attend this important meeting.

Personnel Changes

Chairman John C. Schilling presented his resignation from that office to the Executive Board during their meeting in St. Louis on February 6, 1974. Schilling stated that the demands of his regular position as Ass't. Health Commissioner, City of St. Louis, had grown so greatly that he could no longer devote the necessary time to the conference activities. The Board voted a unanimous resolution of commendation for a job well done. Schilling was replaced as chairman by H. H. Vaux, who was vice-chairman. Schilling was unanimously elected to the office of vice-chairman to replace Vaux.

Russell Wright, Alabama State Board of Health, replaced Alex Pais, Maryland State Board of Health, on Council II.

Constitution Revision Committee

Chairman Vaux has appointed a Constitution Revision committee to update the constitution. The committee consists of: Dick Stedman, Ken Weckel, M. W. Jefferson, Jim Kennedy, Sam Rich, and Don Race as chairman. Race presented a progress report at the August meeting of the Board, indicating that the committee's work would involve (1) a general updating of the constitution, (2) updating the procedure used for the selection of Board members, (3) modifying the procedures used in the selection and certification of official delegates.

Local Arrangements Committee

Chairman Vaux has appointed John Schilling chairman of this committee with the following members: Vernon R. Cupps, St. Louis Health Division; Raymond Lange, St. Louis County Health Department; George L. Stemmler, Rutledge Advertising Agency, representing the dairy industry; Mrs. Dorothy Schilling, Ladies Activities.

Program Committee

John F. Speer, Jr., Program committee chairman has announced the following committee members: John Adams, National Milk Producers Federation; M. W. Jefferson, Virginia Department of Agriculture; Shelby Johnson, Kentucky Department of Human Resources; Berry E. Gay, Jr., Illinois Department of Public Health.

Speer states that, while no firm decision has been made regarding the exact program format, the following important items my be discussed and resolved: (1) revision of the Pasteurized Milk Ordinance, (2) recommended changes in the IMS constitution, including amendments to selection and certification of voting delegates, (3) several amendments to conference procedures, (4) evaluation of the *Methods of Making Sanitation Ratings* document in view of recent developments regarding check ratings. Revision will include discussion of allocation of points.

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JOURNAL OF MILK AND FOOD TECHNOLOGY

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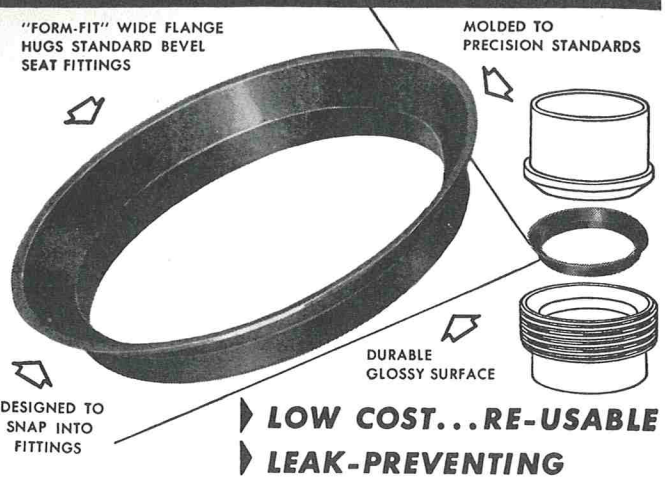

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Dairy authorities speak out on better cow milking

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

Rapid cooling can help maintain high quality of raw milk

The two most important things a dairyman can do to insure milk quality at his dairy are:

1. Put the cleanest possible milk into the bulk tank.
2. Cool it as quickly and efficiently as possible.

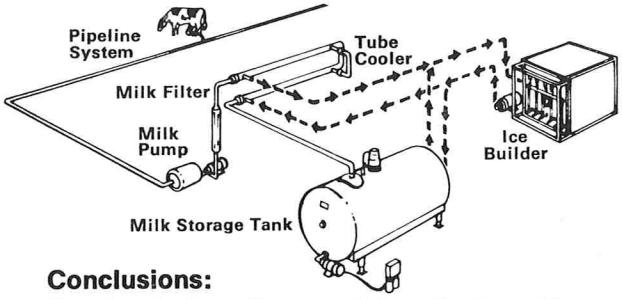
Much has been said about the necessity of sanitary milk handling through the entire milking operation. However, even under the most sanitary conditions, milk from a healthy cow will contain several hundred to several thousand bacteria per milliliter. Certain strains can cause undesirable conditions such as rancidity or other off-flavors unless their growth is retarded.

Follow the rules

The best way to retard bacterial growth is by cooling milk as rapidly as possible, without freezing it.

1. *Milk must be cooled promptly.* Delays result in bacterial growth. Some of the bacteria in milk can multiply in as little as 20 to 30 minutes if the milk is warm.
2. *Cooling should be rapid,* so further appreciable bacterial growth does not occur during the cooling process. Care must always be exercised so that milk does not freeze.
3. *Milk must be cooled to and maintained at a safe temperature.* Cooled milk must be held at a 40° F. and preferably 36-38° F. This temperature must be maintained throughout the storage period. When freshly drawn milk is added to milk already in the bulk tank, the rise in temperature of the initial milk must be minimal and the temperature of all milk in the tank must be rapidly reduced to 36-38° F. (Again, milk must not be frozen in the process.)
4. *Raw milk should not be stored for excessive periods* and should be moved from the bulk tank to the tank truck under conditions which preclude additional microbial contamination.

The refrigerated bulk cooling tank is the most widely used device to cool milk on the farm today. However, it is limited in its ability to meet some of the demands outlined above. New equipment available makes it possible to "pre-cool" milk on the way to the tank. Instant coolers using chilled water from an ice-builder help make rapid cooling practical on the farm. This type of cooling also eliminates the possibility of freezing milk.



Conclusions:

Even though all conditions needed for effective rapid cooling are met, some bacteria can still grow in refrigerated milk. Two points already discussed bear repeating. Be certain that good sanitary practices are followed during production of milk to insure that few bacteria of the kind able to grow at refrigeration temperatures are present. Do not hold raw milk refrigerated for excessive periods.

The successful marketing of milk depends on everyone doing his part at each step along the way. Even though you, as an individual dairyman, may not benefit *directly* from each of your efforts to improve sanitation and milk handling, you most certainly will benefit *indirectly* through greater total consumer acceptance of milk and dairy products thanks to fewer flavor problems and the absence of other negative factors caused by improper handling.