EFFECT OF QUATERNARY AMMONIUM COMPOUNDS ON ACTIVITY OF LACTIC ACID STARTER BACTERIA IN MILK AND CHEESE[1]

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The increased application of quaternary ammonium compounds (QAC) for dairy sanitation purposes has stimulated interest in the effect of various concentrations of these compounds on the growth of lactic acid bacteria in milk. The problem is important from the standpoint of attempts to prevent souring of milk or to reduce bacterial counts prior to delivery to the dairy plant. Another important factor is the effect of QAC on lactic acid bacteria in starters, cheese or other cultured milk products for which such milk might be employed. This study deals with the effect of various concentrations of QAC in milk on representative lactic acid starter bacteria.

DuBois and Dibblee (2) concluded that the presence of added QAC lacked any influence on the bacterial counts of raw or pasteurized milk at concentrations ranging from 1:500 (2,000 ppm.) to 1:25,000 (40 ppm.). It was observed that higher concentrations (200 and 2,000 ppm.) inhibited growth of Gram positive acid-producing organisms but not that of Gram negative types. A *Streptococcus* sp. isolated from milk was inhibited by 200 and 2,000 ppm. but not by 40 ppm. added QAC.

Mull and Fouts (10) reported a reduction in bacterial count when QAC was added directly to milk, but concluded that such compounds would have to be present in low quality milk in concentrations of 200 to 250 ppm. to bring about significant decreases in bacterial count.

Johns and Pritchard (6) reported a slight, but definite, bacteriostatic action when QAC were added to milk in the highest concentration which might hope to escape detection organoleptically, but concluded their preservative effect to be decidedly less pronounced than that of formaldehyde.

Moore (9) found that 25 ppm. of QAC in milk caused partial inhibition and 50 to 75 ppm. effected complete inhibition of commercial lactic acid starter organisms. Barber *et al.* (1) reported some inhibition of *Streptococcus lactis* at 100° F. in reconstituted skimmilk containing as little as 10 ppm. added QAC. Progressive decreases were observed with 25 and 50 ppm. and complete inhibition occurred with 100 ppm. QAC in the milk.

Preliminary work on the effect of QAC in milk on lactic acid starter bacteria has been reported by Miller *et al.* (7). Slight inhibition of acid production was observed when the QAC was present in concentrations as low as 5 ppm.

METHODS

The organisms selected for this study included a single strain lactic culture, *Streptococcus cremoris* (R-6), a mixed strain commercial lactic culture widely

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used in the manufacture of cottage and Cheddar cheese, and two Swiss cheese starter organisms, *Lactobacillus lactis* (39a) and *Streptococcus thermophilus* (C-3). The QAC preparations employed were as follows: Alkyl dimethyl benzyl ammonium chloride, para di-isobutyl phenoxy ethoxy dimethyl benzyl ammonium chloride, methyl dodecyl benzyl trimethyl ammonium chloride and a detergent sanitizer containing para di-isobutyl phenoxy ethoxy dimethyl benzyl ammonium chloride. For purpose of comparison, a commercial sodium hypochlorite also was included in the trials. Concentration of QAC stock solution was established by the titration method of Harper *et al.* (4), and concentration of hypochlorite solution by titration with sodium thiosulphate.

QAC or hypochlorite from the stock solution was added to 50-ml. quantities of sterile reconstituted skimmilk to provide added concentrations in the milk of 0, 5, 10, 15, 20, 25, 30, 40, 50, 75 and 100 ppm. of the compound under study. A concentration of 200 ppm. available chlorine as hypochlorite also was used. It was recognized that the hypochlorite in the above concentrations in milk dissipates rapidly due to reaction with organic matter and thus, the above concentrations do not necessarily represent final content of available chlorine in the milk. The quaternary compounds are more stable in milk than the hypochlorites, and the quantities of QAC as added in this experiment can be detected by a method reported in a subsequent paper (8).

Milks containing the various added concentrations of germicide were inoculated with 1 per cent of the respective cultures and incubated as follows: Commercial lactic culture, 37.8° C.; *S. cremoris,* 37.8° C.; *S. thermophilus,* 48° C.; and *L. lactis,* 44° C. After 6 hr. incubation, the titratable acidities were determined with 0.1 *N* NaOH to phenolphthalein end point. Incubation at temperatures near the maximum for the organisms involved has been employed as an activity test in other studies to more closely simulate manufacturing conditions to which the lactic acid organisms are subjected (3, 5).

The experiment also was repeated using sterile whole milk instead of skimmilk to determine whether or not the presence of butterfat might affect the bacteriostatic action of the various germicides. Trials also were carried out using an incubation period of 16 hr. at the following temperatures for the respective organisms: Commercial lactic culture, 21.1° C.; *S. cremoris,* 21.1° C.; *S. thermophilus,* 37° C.; and *L. lactis,* 37° C.

Triplicate experimental lots of Cheddar cheese were made with milk containing 0, 5 and 10 ppm. alkyl dimethyl benzyl ammonium chloride. The commercial lactic culture was used for these trials. One lot of cheese was made with 20 ppm. in the cheese milk. Effect of the QAC was followed by acidity measurement at various stages of manufacture.

RESULTS

Results of the trials are shown in tables 1 and 2. Each value in the table represents the average of three experiments run on separate days. The values obtained in the three different experiments were practically identical throughout. Titratable acidities of inoculated milks were slightly lower than the uninoculated

TABLE 1

Effect of various quaternary ammonium compounds and a sodium hypochlorite on acid development of different lactic acid starter bacteria during a 6-hr. incubation period at temperatures near their maximum

Culture	Incub. temp.	Germicide[a]	Titratable acidity developed in sterile skimmilk containing the following ppm. concentration of germicide:[b]											
			0	5	10	15	20	25	30	40	50	75	100	200
	(°C.)		(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
Commercial Lactic culture	37.8	QAC 1	0.40	0.29	0.24	0.22	0.19	0.19	0.18	0.17	0.17	0.16	0.16	
		QAC 2	0.40	0.32	0.24	0.22	0.20	0.19	0.18	0.18	0.17	0.16	0.16	
		QAC 3	0.39	0.32	0.25	0.21	0.19	0.18	0.18	0.18	0.17	0.17	0.16	
		DS	0.40	0.31	0.24	0.22	0.19	0.18	0.18	0.17	0.17	0.16	0.16	
		NaOCl	0.38	0.37	0.37	0.37	0.36	0.36	0.35	0.31	0.30	0.24	0.21	0.16
S. cremoris (R-6)	37.8	QAC 1	0.34	0.32	0.30	0.26	0.23	0.19	0.18	0.17	0.17	0.16	0.16	
		QAC 2	0.35	0.33	0.28	0.25	0.22	0.19	0.18	0.17	0.17	0.16	0.16	
		QAC 3	0.36	0.33	0.30	0.27	0.25	0.21	0.19	0.17	0.17	0.16	0.16	
		DS	0.34	0.33	0.29	0.25	0.21	0.19	0.17	0.17	0.17	0.16	0.15	
		NaOCl	0.34	0.34	0.33	0.33	0.33	0.32	0.32	0.30	0.28	0.26	0.19	0.17
S. thermophilus (C-3)	48.0	QAC 1	0.34	0.30	0.29	0.27	0.25	0.24	0.22	0.19	0.17	0.15	0.14	
		QAC 2	0.31	0.29	0.27	0.27	0.24	0.24	0.21	0.19	0.16	0.15	0.14	
		QAC 3	0.32	0.28	0.27	0.26	0.24	0.23	0.23	0.19	0.17	0.15	0.14	
		DS	0.32	0.30	0.27	0.25	0.23	0.23	0.22	0.18	0.16	0.15	0.14	
		NaOCl	0.31	0.31	0.31	0.30	0.30	0.29	0.28	0.27	0.25	0.24	0.23	0.15
L. lactis (39a)	44.0	QAC 1	0.30	0.29	0.26	0.23	0.20	0.20	0.20	0.18	0.18	0.18	0.16	
		QAC 2	0.31	0.28	0.28	0.26	0.23	0.21	0.19	0.19	0.18	0.17	0.16	
		QAC 3	0.32	0.30	0.27	0.24	0.21	0.21	0.19	0.19	0.18	0.17	0.17	
		DS	0.30	0.29	0.26	0.23	0.20	0.19	0.19	0.18	0.18	0.17	0.16	
		NaOCl	0.29	0.30	0.30	0.30	0.29	0.29	0.28	0.28	0.26	0.25	0.23	0.18

[a] QAC 1 = para di-isobutyl phenoxy ethoxy dimethyl benzyl ammonium chloride; QAC 2 = methyl dodecyl benzyl trimethyl ammonium chloride; QAC 3 = alkyl dimethyl benzyl ammonium chloride; DS = detergent sanitizer (para di-isobutyl phenoxy ethoxy dimethyl benzyl ammonium chloride); NaOCl = commercial sodium hypochlorite.

[b] Titratable acidity of uninoculated control = 0.18 per cent.

TABLE 2

Effect of various quaternary ammonium compounds and a sodium hypochlorite on acid development of different lactic acid starter bacteria during a 16-hr. incubation period at normal temperatures

Culture	Incub. temp.	Germicide[a]	Per cent titratable acidity developed in sterile skimmilk containing the following ppm. concentration of germicide:[b]											
			0	5	10	15	20	25	30	40	50	75	100	200
	(°C.)		(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
Commercial lactic culture	21.1	QAC 1	0.79	0.77	0.70	0.63	0.48	0.37	0.32	0.23	0.21	0.18	0.16	
		QAC 2	0.82	0.78	0.74	0.68	0.61	0.43	0.34	0.24	0.21	0.18	0.16	
		QAC 3	0.79	0.72	0.67	0.57	0.48	0.39	0.32	0.23	0.20	0.17	0.17	
		DS	0.79	0.75	0.72	0.63	0.46	0.34	0.30	0.23	0.19	0.16	0.15	
		NaOCl	0.79	0.80	0.80	0.80	0.79	0.79	0.78	0.78	0.78	0.71	0.72	0.63
S. cremoris (R-6)	21.1	QAC 1	0.71	0.68	0.65	0.59	0.53	0.30	0.26	0.21	0.19	0.17	0.16	
		QAC 2	0.72	0.69	0.67	0.64	0.59	0.48	0.35	0.27	0.22	0.17	0.16	
		QAC 3	0.71	0.68	0.63	0.58	0.48	0.32	0.26	0.20	0.18	0.17	0.16	
		DS	0.69	0.67	0.63	0.58	0.43	0.30	0.25	0.21	0.18	0.16	0.15	
		NaOCl	0.71	0.71	0.72	0.72	0.71	0.70	0.71	0.69	0.70	0.68	0.67	0.59
S. thermophilus (C-3)	37.0	QAC 1	0.74	0.65	0.61	0.59	0.59	0.52	0.49	0.44	0.28	0.18	0.14	
		QAC 2	0.72	0.71	0.68	0.65	0.65	0.62	0.54	0.42	0.27	0.20	0.14	
		QAC 3	0.69	0.62	0.61	0.56	0.56	0.52	0.48	0.39	0.27	0.16	0.15	
		DS	0.73	0.71	0.64	0.60	0.53	0.48	0.42	0.36	0.25	0.16	0.14	
		NaOCl	0.72	0.72	0.71	0.71	0.71	0.70	0.68	0.67	0.65	0.59	0.55	0.40
L. lactis (39a)	37.0	QAC 1	0.78	0.77	0.69	0.67	0.60	0.52	0.49	0.42	0.28	0.15	0.14	
		QAC 2	0.77	0.76	0.72	0.68	0.63	0.59	0.54	0.46	0.31	0.24	0.14	
		QAC 3	0.75	0.67	0.64	0.61	0.58	0.54	0.50	0.44	0.32	0.16	0.14	
		DS	0.77	0.74	0.70	0.63	0.57	0.52	0.48	0.46	0.31	0.15	0.12	
		NaOCl	0.75	0.75	0.75	0.75	0.76	0.75	0.75	0.74	0.72	0.65	0.63	0.46

[a] QAC 1 = para di-isobutyl phenoxy ethoxy dimethyl benzyl ammonium chloride; QAC 2 = methyl dodecyl benzyl trimethyl ammonium chloride; QAC 3 = alkyl dimethyl benzyl ammonium chloride; DS = detergent sanitizer (para di-isobutyl phenoxy ethoxy dimethyl benzyl ammonium chloride); NaOCl = commercial sodium hypochlorite.

[b] Titratable acidity of uninoculated control = 0.18 per cent.

control. This may be explained by the neutralizing effect of the cation of the respective germicide compounds added to the milk.

Table 1 shows the effect of the various germicides on growth and acid production of the starter organisms at temperatures near their maximum. Slight inhibition of acid production by 5 ppm. of the respective QAC or the detergent sanitizer was evident in the case of each culture. The degree of inhibition increased progressively with an increase in the concentration of the compound. Inhibition was almost complete with 25 to 30 ppm. of QAC in the milk. In general, the degree of inhibition of the organisms used was similar. The results suggest that *S. thermophilus* showed slightly greater resistance to bacteriostatic effect of the QAC than the other organisms. All titratable acidities, including the control, were somewhat low in the case of *L. lactis* because the culture was not fully activated following removal from the stock culture; however, the results are representative of the comparative inhibition of different concentrations and compounds against this type of organism. The effect of the QAC and detergent sanitizer on acid production in general was about the same. Complete inhibition apparently occurred with the sodium hypochlorite at a concentration between 100 and 200 ppm.

The presence of the milk fat did not greatly affect the bacteriostatic action of the QAC. When the experiment, as shown in table 1, was repeated with whole milk instead of skimmilk, identical results were obtained and therefore the data have not been included in this report.

Other studies have shown that the inhibitory effect of QAC in milk is not materially reduced by pasteurization of the milk after QAC addition, and in some trials inhibitory effect by QAC in milk has been apparent after more severe heat treatment, such as sterilization at 121.1° C. for 15 min.

Table 2 shows the inhibition by the various compounds at incubation temperatures normally employed for the different cultures. The relative effect of all types of germicides was less pronounced at the lower incubation temperatures than at near-maximum temperatures. With one exception, complete inhibition by the QAC present was not effected until the concentration reached 50 to 75 ppm. As much as 200 ppm. sodium hypochlorite did not completely inhibit growth at the normal incubation temperature of the respective cultures. The *S. cremoris* and commercial lactic culture appeared to be more susceptible to bacteriostatic effect of the QAC than *S. thermophilus* or *L. lactis*. The methyl dodecyl benzyl trimethyl ammonium chloride appeared less bacteriostatic on a ppm. basis against the lactic acid bacteria studied than the other QAC preparations used.

The effect of alkyl dimethyl benzyl ammonium chloride on acid production during manufacture of Cheddar cheese is shown in table 3. Since only two experimental vats were available for a single trial, it was necessary to include a 0 ppm. control vat for each concentration of QAC studied. Thus on 1 day, one vat contained 0 and the second, 5 ppm., and on the next day, one contained 0 and the second, 10 ppm. QAC. The entire experiment including 0, 5 and 10

TABLE 3

Effect of added quaternary ammonium compound in cheese milk on acid development during manufacture of Cheddar cheese

Operation	Time	Change in per cent titratable acidity with following concentrations of QAC:			
		Control 0 ppm.	QAC 5 ppm.	Control 0 ppm.	QAC 10 ppm.
	(*hr.: min.*)	(%)	(%)	(%)	(%)
Received milk	0:00	0.17	0.17	0.17	0.17
Added starter	0:15	0.18	0.18	0.17	0.17
Added rennet	1:15	0.19	0.19	0.18	0.18
Cut curd	1:45	0.12	0.12	0.13	0.13
Began cooking	2:00	0.12	0.12	0.13	0.13
Steam off	2:30	0.14	0.14	0.14	0.14
Drained whey	3:30	0.16	0.16	0.17	0.16
Packed curd	3:45	0.21	0.21	0.23	0.18
Cheddared	4:00	0.27	0.26	0.30	0.23
	4:15	0.34	0.31	0.40	0.27
	4:30	0.39	0.37	0.45	0.30
	4:45	0.44	0.42	0.52[a]	0.33
	5:00	0.52[a]	0.48		0.36
	5:15		0.52[a]		0.40
	5:30				0.48
	5:45				0.54[a]

[a] Milling time.

ppm. was run three times on successive days. The results were highly consistent through all three experiments and table 3 represents a typical trial. A concentration of 5 ppm. QAC in the cheese milk delayed milling time about 15 min. over the control with no QAC. A delay in milling time of 45 to 60 min. occurred with 10 ppm. QAC in the cheese milk. In an additional trial with 20 ppm. alkyl dimethyl benzyl ammonium chloride added to the cheese milk, the acidity of the whey reached only 0.20 per cent during a 7.5-hr. manufacturing period.

DISCUSSION

The minimum concentration of 5 ppm. QAC added to milk in these studies caused very slight inhibition of four different lactic acid starter cultures. If excessive quantities of QAC should enter the milk supply, through intentional adulteration or accident, definite inhibition of lactic acid starter bacteria may occur. The effect of higher concentrations of QAC (5 to 10 ppm.) would vary with the manufacturing conditions employed for a dairy product. For example, the degree of inhibition of the lactic acid bacteria might be more pronounced in manufacture of Cheddar cheese than in cottage cheese or buttermilk. Presumably, the near-maximum growth temperature employed in Cheddar cheese manufacture accentuates the bacteriostatic effect of the germicide on lactic starter organisms. The delay of 45 to 60 min. in milling time caused by 10 ppm. QAC is considered significant. Such an extension in time would be detrimental to economical operation of a cheese plant. A concentration of 5 ppm. QAC in the cheese milk might not cause a noticeable delay in acid development in such a product. Another problem suggested but not investigated in these

studies is the possible effect of QAC in the cheese milk on rate of ripening and final flavor of the cheese.

Results of these studies emphasize that a delay in souring of raw milk may be accomplished by 20 ppm. of QAC. Unless a milk grader is familiar with the bitter flavor contributed to milk by most quaternary compounds, he may fail to detect such a quantity in the milk. However, concentrations as low as 5 ppm. QAC in milk can be detected by a modified eosin indicator titration method developed in the course of these studies (8).

SUMMARY AND CONCLUSIONS

A study was carried out on the effect of three QAC products (quaternary ammonium compounds), a QAC containing detergent sanitizer and a sodium hypochlorite on acid development by four types of lactic acid starters in sterile skimmilk and whole milk.

A mixed-strain commercial lactic culture, *S. cremoris, L. lactis* and *S. thermophilis* was slightly inhibited by 5 ppm. of each of the QAC compounds added to the milk.

The inhibition of acid development was nearly complete in all cultures with 25 to 30 ppm. of QAC in the milk when the organisms were incubated at temperatures near their maximum. At incubation temperatures normally used for culturing the organisms, 50 ppm. QAC in the milk effected nearly complete inhibition.

Milling time was delayed 15 min. by presence of 5 ppm. and 45 to 60 min. by 10 ppm. QAC in the manufacture of experimental Cheddar cheese.

Results obtained suggest the necessity of employing farm and plant sanitizing procedures that will avoid contamination of milk with inhibitory concentrations of QAC.

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