

Effect of Ultrasound on Disaggregation of Milk Bacteria

The standard plate count for determining bacterial populations in foods is limited in its effectiveness by microorganisms tending to form conglomerates with themselves and with the constituents of foodstuffs. Standardized shaking procedures have been adopted in an attempt to obtain consistent results from sample to sample and locale to locale. A more efficient procedure for breaking up these clumps would be desirable. Ultrasonic waves have been used extensively for disrupting bacterial cells for obtaining cell-free extracts. These cell-rupturing frequencies of ultrasound are associated with high power inputs to the transducer. This paper reports the effects of a lower energy level ultrasound frequency on the disruption of bacterial aggregates in milk.

Experimental Procedure

The ultrasonic generator was the Ultra Clean Model 320 LU produced by the L & R Manufacturing Company, Kearny, New Jersey,¹ and of the type normally used for cleaning certain laboratory and industrial materials. The power output to the transducer, according to the manufacturer's specifications, was in excess of 65 w average at 75 KC (a wave level of approximately 2 cm). All samples were placed in milk dilution bottles and immersed to approximately the fluid level in the bath of cold tap water. Raw milk samples were obtained from a local dairy and represented the bulk tank delivered

¹ It is not implied that the U.S. Department of Agriculture recommends products of companies mentioned to the possible exclusion of others in the same business.

milk from approximately 100 farms. The vacuum foam-dried milk was previously described (1). Pasteurized milk was purchased through a local retailer. Bacteriological methods were as specified in Standard Methods for the Examination of Dairy Products, 11th edition, 1960, published by the American Public Health Association.

Results and Discussion

Table 1 shows the effects of various times in the ultrasonic bath on the numbers of isolatable colonies in raw milk, vacuum foam-dried milk, and in a commercially pasteurized milk. The vacuum foam-dried milk was reconstituted to whole milk solids content with standard phosphate buffer solution. Significant differences in counts were obtained only with raw milk. The optimum time of ultrasonic treatment from this experiment was 4.5 min, with little or no change up to 30 min in the ultrasonic bath. The lack of

TABLE 2
Effect of incubation temperature on ultrasound recovery of bacteria in milk

Ultrasound treatment	Count $\times 10^8$ /ml Incubation temperature		
	2-3 C	27 C	33 C
0	0.8	36	35
30 sec	99	96	23
1.5 min	90	135	36
4.5 min	125	110	37
13.5 min	184	245	62

10^{-1} Dilutions used for ultrasound treatment. Plates at 2-3 C incubated ten days, others two days.

TABLE 1
Effect of ultrasound on plate counts in raw, vacuum foam-dried, and pasteurized milk

Treatment	Raw milk (count $\times 10^8$ /ml)			Vacuum foam-dried milk (count $\times 10^8$ /gm)			Pasteurized milk (count $\times 10^8$ /ml)		
	Replicate plates	Avg	Increase per cent	Replicate plates	Avg	Increase per cent	Replicate plates	Avg	Increase per cent
None									
ultrasound 30 sec	87 62	74	-	125 102	114	-	33 19	26	-
ultrasound 4.5 min	125 85	105	42	100 129	114	0	28 30	29	0
ultrasound 13.5 min	265 235	250	237	144 115	114	0	19 29	24	0
ultrasound 30 min	210 235	222	200	116 146	131	0	28 21	24	0
	190 205	197	166	126 135	130	0	26 23	24	0

Incubation was at 27 C for two days.

TECHNICAL NOTES

TABLE 3
Effect of diluting raw milk on effectiveness of ultrasonic disaggregation

Dilution treated	Duration of ultrasonic treatment	Plate count ($\times 10^3$ /ml)			
		Replicate plates	Avg	Increase (%)	
Raw whole milk	0	360	250	305	-
	30 sec	300	330	315	0
	4.5 min	730	760	745	144
	13.5 min	880	1,060	970	218
10^{-1} Dilution	30 sec	450	440	445	46
	4.5 min	1,000	1,140	1,070	251
	13.5 min	1,160	1,170	1,165	282
10^{-3} Dilution	30 sec	360	370	365	20
	4.5 min	520	480	500	64
	13.5 min	640	540	590	93

Incubation was at 27 degrees for two days. Dilutions made in standard APHA phosphate buffer.

effect in the pasteurized and vacuum foam-dried milk (also pasteurized) indicated that the increase in numbers in raw milk was not caused by dissociation of clumps of thermoturic or spore-forming microorganisms but rather of heat-sensitive bacteria.

This was further borne out by the experiment shown in Table 2. In this experiment the tenfold dilution of milk was treated with ultrasound for various time intervals in a cold-water bath of 20 C, and replicate plates incubated at several temperatures. Very low counts (800 per ml) were obtained at an incubation temperature of 2-3 C with no ultrasound treatment. After 13.5 min of sonication this count rose to 184,000 per milliliter. At 27 C the untreated milk had a count of 36,000 per milliliter, whereas after 13.5 min of ultrasound exposure this count was 245,000 per milliliter. At 33 C the starting count was 35,000 per milliliter, whereas after the 13.5-min treatment interval the count had risen to only 62,000 per milliliter. It was apparent, then, that sonication was of most benefit in the enumeration of the more psychrophilic strains of bacteria.

The protective effect of whole milk on the ultrasonic dissociation of bacteria was indicated from Table 3. The tenfold diluted milk showed an increase of 46% in bacterial counts after 30 sec of treatment, whereas the whole milk showed

no significant increase at this time interval. Longer treatment times showed greater recoveries in both the tenfold dilution and whole milk samples, although the whole milk showed a greater difference from 4.5 to 13.5 min than did the diluted material. Possibly, the colloidal milk proteins or the fat globules absorb or reflect enough of the ultrasound energy to prevent its full impact upon the microorganisms. The lesser recoveries in the thousandfold dilution perhaps indicate a diluting out of bacterial clumps, thus providing fewer points of impact for the sound waves. Microscopic examination by standard milk stains showed no readily visible changes in numbers of free or clumped cells; however, the numbers of microorganisms in the milk samples were too low to properly evaluate microscopically.

C. N. HUHTANEN

Eastern Regional Research Laboratory
Eastern Utilization Research and
Development Division, ARS, USDA
Philadelphia, Pennsylvania

Reference

- (1) Schoppet, E. F., Aceto, N. C., Eskew, R. K., Craig, J. C., Jr., and Holden, T. F. 1965. Continuous Vacuum Drying of Whole Milk Foam. II. Modified Procedure. *J. Dairy Sci.*, 48: 1436.