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Thermal Inactivation of Phosphatase by the AOAC-V Method

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ABSTRACT

Thermal inactivation of phosphatase in raw whole milk was determined by the AOAC-V method. D-values in duplicate experiments at temperatures of 63.3, 66.1, 68.9, and 71.7°C were 332.0 and 338.0 s, 91.6 and 90.2 s, 31.4 and 27.6 s, and 9.9 and 8.9 s, respectively. The Z_D -value was 5.39°C. Results confirmed phosphatase activity in milk heated at 71.7°C for 15 s and the need to heat raw whole milk for at least 19 s at 72.2°C to obtain a negative test by the AOAC-V.

A recent study (W. H. Stroup, R. W. Dickerson, and J. T. Tierney, unpublished data) of the efficacy of pasteurization was conducted using a slug flow heat exchanger (*13*) and a commercial scale pasteurization unit. The commercial scale unit operated at an average temperature of 72.2°C (162°F) for 19 s to ensure that no particle of milk was pasteurized below 71.7°C (161°F), as required by the Grade "A" Pasteurized Milk Ordinance (*5*). Portions of the same milk were heated at 71.7°C (161°F) for 15 s in the slug flow heat exchanger. The test for phosphatase activity in the heated milk was positive in 70% of runs using the AOAC-V method for the slug flow heat exchanger. The average μg phenol/ml was 1.11 ± 0.39 in 23 runs. Under the conditions listed above, the commercial pasteurization unit gave a negative test result for phosphatase.

The phosphatase test determines "improper pasteurization on the basis of residual phosphatase activity" (*9*). Criteria for the test are based on thermal resistance of phosphatase, as reported in the late 1940s and early 1950s, using the AOAC I and II methods (*3*) developed by Sanders and Sager. Phosphatase thermal resistance studies published before 1948 did not consider corrections for inactivation of phosphatase activity during the time milk was heated to test temperature or cooled to ambient temperature (*8*). In addition, some studies omitted the initial concentration of phosphatase activity in the milk tested.

Thermal inactivation studies by Hetrick and Tracy (*6*), Lear and Foster (*7*), and Sanders and Sager (*11,12*) included appropriate thermal corrections; they provide the basis for assuming that the thermal process has met or exceeded Public Health Service pasteurization (*9*) standards [62.8°C (145°F) for 30 min or 71.7°C (161°F) for 15 s] when a negative phosphatase test result occurs. A negative result, however, can occur with a blend of overheated milk and a trace of raw milk. Therefore, the positive results observed by Stroup et al. (unpublished data) of thermal inactivation of raw whole milk at 71.7°C for 15 s was cause for concern. The AOAC-V method (*1*) used in the present report is a modification of the Scharer rapid method (*2*). The study was designed to compare the thermal inactivation of phosphatase activity with results reported previously (*6,7,11,12*) and to determine the effectiveness of the methods as indicators of pasteurization.

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MATERIALS AND METHODS

Milk

Raw whole milk (about 3.25% milk fat) was obtained from individual cow's milk from a local dairy farm on the day of the thermal inactivation test and held at 4°C.

Test for residual phosphatase

The determination for residual phosphatase was performed by the AOAC-V method given in secs 16.115 - 16.126 of the AOAC Official Methods of Analysis (*1*).

Thermal resistance studies

To determine thermal resistance of phosphatase, 1.5-ml amounts of raw whole milk were dispensed into 13 x 100 mm borosilicate glass tubes. The tubes were then sealed and heated in a water bath at four temperatures between 63.3 and 71.7°C. Duplicate tubes were withdrawn at six intervals for each temperature and immediately cooled in ice water to 4.4°C. Duplicate unheated tubes were prepared at each temperature and used to determine initial concentration. The heating experiments were repeated twice. Heating and cooling curves for raw whole milk were prepared for each final heating temperature from data obtained with thermocouples sealed in the tubes containing raw whole milk. Temperature corrections were computed (*14*) to compensate for phosphatase inactivation during heating and cooling of the tubes. Duplicate 0.5-ml portions of the contents of each tube, with or without dilution, using milk heated to 95°C for 1 min, were tested for residual phosphatase activity expressed as μg phenol/ml.

Statistical methods

Thermal inactivation rates were determined from a linear regression of $\log_{10}$ (μg phenol/ml) versus heating times (*4*) at each of four (63.3, 66.1, 68.9, and 71.7°C) temperatures. Tests showed no significant influences (*4*) on the estimate of slopes. The least-square estimate of slope was calculated and is the rate of thermal inactivation at constant temperature. Estimates of the

D-value were obtained by taking the absolute value of the inverse of the slope. Linear regressions of the $\log_{10}$ D-value versus temperature were computed, and the Z_D-value was computed by taking the absolute value of the inverse of the slopes.

RESULTS AND DISCUSSION

Results for inactivation of phosphatase measured by the μg phenol/ml are summarized in Table 1. The inactivation was observed to be first order and the D-value was computed for each experiment. Since the milk test samples were obtained at different times, the initial concentrations (C_0) in μg phenol/ml were recorded, and the estimate of total process time from C_0 to 1.0 μg phenol/ml was computed as (D $\log_{10}C_0$). A negative test was defined as < 1.0 μg phenol/ml. The time to inactivate phosphatase at 71.7°C required an average of 26.4 s, thus confirming the observation (*14*) that milk heated to 71.7°C for 15 s would be positive (i.e., concentration > 1.0 μg phenol/ml).

TABLE 1. *Summary of D-values for phosphatase inactivation.*

Temperature (°C)	Initial concentration (μg phenol/ml)	Estimated total process[a] (s)	D-value (s)	Mean (s)	Coefficient of variation (%)
63.3	735.8	951.8	332.0	335.4	1.4
	723.6	966.5	338.0		
66.1	735.8	262.6	91.6	90.9	1.1
	753.3	259.5	90.2		
68.9	632.1	87.9	31.4	29.5	9.1
	626.5	77.2	27.6		
71.7	643.0	27.8	9.9	9.4	7.5
	626.5	24.9	8.9		
Z value			5.39°C		

[a]Total process = D $\log_{10}$ (initial concentration).

Three studies (*6,7,11,12*) have presented data on the thermal inactivation of phosphatase. Sanders and Sagers (*11*) used their own procedures (i.e., AOAC Method II), to study phosphatase inactivation in several products, including raw whole milk. The total time to inactivate phosphatase from an average initial concentration of 2200 μg phenol/ml milk to an end point concentration of 4 μg phenol/ml (*12*) was 2250 s at 61.7°C (143°F), 1800 s at 62.1°C (143.7°F), 24 s at 71.1°C (160°F), and 15 s at 72.1°C (161.8°F).

Hetrick and Tracy (*6*) also used the Sanders and Sager (*1,10*) method; however, they used an end point of 1 μg phenol/ml. From an average initial concentration of 2230 μg phenol/ml, they reported times to inactivation at [65.6°C (150°F), 68.3°C (155°F), 71.1°C (160°F), and 73.9°C (165°F)] and expressed the total time of inactivation versus temperature in an equation. Lear and Foster (*7*) reported the time to inactivate an initial concentration of phosphatase to 0.5 μg phenol/ml at 60°C at one-degree intervals up to 71°C. They did not report an initial concentration. Their method of analysis was the New York City Department of Health Test (*3*), i.e., AOAC Method-I (*1*), which is now a surplus AOAC method and no longer in use. The milk was incubated for 1 h at 37 to 45°C (41° preferred).

Results at 71.7°C from these studies were compared (Table 2). The initial (C_0) and end point (C_e) concentrations given were presented in earlier publications (*6,7,11,12*). The number of logarithmic cycles of inactivation was estimated, and estimated D-values (the inverse of the rate of enzyme inactivation) were computed as D = (total time of activation)/($\log_{10}C_0$ - $\log_{10}C_e$) in s. Because Hetrick and Tracy (*6*) used 1 μg phenol/ml for the end point rather than 4 μg phenol/ml as required by the AOAC-II method, their total time of inactivation was recalculated. The summary of methods, estimated total times, and end points are also shown (Table 2).

The results of the AOAC-I method reported by Lear and Foster (*7*) indicate that the phosphatase activity in raw whole milk would be negative after a 71.7°C, 15-s process. The AOAC-II and AOAC-V methods (*1*) would yield positive results after the minimal Pasteurized Milk Ordinance process (*5*). Sanders and Sager (*11*) reported that inactivation of phosphatase required 15 s at 72.1°C. The difference in end points for these methods is probably due to the different times and temperatures. AOAC methods II and V yield observed positive tests at 71.7°C for 15 s. Although all three tests would be expected to yield negative results at 72.2°C for 19 s, the AOAC-V method may yield an infrequent positive result at this time and temperature. These conditions represent the minimum conditions used by many commercial pasteurization units. Most units, however, operate above 76°C for 19 s, thereby ensuring that the phosphatase test would be negative. The plant operator should be aware that positive phosphatase results are possible when the time-temperature conditions fall below 72.2°C for 19 s. These positives can be the result of variation about the expected end point.

TABLE 2. *Comparison of published phosphatase inactivation test results.*

Parameter	Hetrick and Tracy (*6*) Computed[a]	Hetrick and Tracy (*6*) Equation[b]	Lear and Foster (*7*)	Sanders and Sager (*11,12*)	Current study (71.7°C)
AOAC method	II	II	I	II	V
Initial concentration (C_0) μg phenol/ml	2100	2230	--[c]	2200	634.8
End point (C_e) μg phenol/ml	1	1	0.5	4	1
($\log_{10}C_0$-$\log_{10}C_e$)	3.322	3.348	--	2.740	2.803
Estimated D-value (s)	7.0	8.3	--	6.9	9.4
Z-value (°C)	4.94	5.00	4.90	4.85	5.39
Total time (s) to inactivate C_0 to end point C_e	19.0[d]	22.8[d]	15.1[d]	18.4[d]	25.5[d]
C_e	4	4	0.5	4	1

[a]Computed from data in Table 2 (*6*).
[b]Calculated from equation (*6*).
[c]Initial concentration was not given.
[d]Estimated by linear regression.

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