Residual Alkaline Phosphatase Activity in Milks Subjected to Various Time-Temperature Treatments

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ABSTRACT

Milk is routinely tested for proper pasteurization. The Scharer and Fluorophos methods, among others, test for residual alkaline phosphatase (ALP) activity to assure proper pasteurization. Until recently there were no tests available to accurately detect residual ALP activity levels below the U.S. legal limit of 1 µg of phenol or 350 mU of ALP per liter of milk. The new Fluorophos method can detect accurately residual ALP activity levels as low as 10 mU/liter.

The Fluorophos method was used to investigate residual ALP activity levels in several fluid milk products. The milk products were thermally processed under various time and temperature protocols below, at, and above current U.S. Food and Drug Administration-mandated heat treatments for fluid milk and milk products. The data established values for residual ALP activity in milks pasteurized under high-temperature short-time (HTST) and low-temperature long-time (LTLT) treatments. The mean ALP activities for whole, 2% lowfat, 1% lowfat, skim, half and half, and chocolate-flavored milks thermally processed at the legal minimum HTST pasteurization treatment are 169.7 ± 12.3, 145.2 ± 9.3, 98.6 ± 8.9, 72.5 ± 4.2, 38.4 ± 4.6 and 157.3 ± 6.5 mU/liter, respectively. The mean ALP activities generated at the legal minimum LTLT pasteurization treatment are 81.8 ± 4.8, 66.4 ± 5.9, 56.4 ± 2.1, 39.1 ± 3.9, 35.0 ± 1.2 and 91.3 ± 7.7 mU/liter, respectively. The values for all milks pasteurized at the legal minimum heat treatment were significantly below the current legal cutoff for residual ALP activity of 350 mU/liter of milk or milk product.

Key words: Milk, alkaline phosphatase, milk pasteurization, pasteurization, Fluorophos

The first commercial pasteurizer in the United States was installed in New York City in 1907 to eliminate the presence of pathogens in finished milk products (11). Since 1907 there has been a need to determine if a milk product received an adequate pasteurization heat treatment. Several test methods were developed over the years which utilized the heat-sensitive enzyme alkaline phosphatase (ALP); which is native to milk, as an indicator for the degree of heat treatment a milk product received. These methods include those of Kay and Graham (8), Scharer (21, 22), Sanders and Sager (19), Aschaffenburg and Mullen (4), Kosikowsky (10), Kleyn and Lin (9), and the Fluorophos method (17).

Pasteurization indicators provide many benefits to a dairy plant. The use of alkaline phosphatase tests in hazard analysis critical control point (HACCP) systems enables the dairy plant to detect errors that may occur during processing. Errors such as faulty equipment or improperly operated pasteurizers are uncommon, yet they can lead to underpasteurized milk or possible contamination by raw milk.

Repairs to a pasteurizer often require breaking a tamper-evident seal put in place by a state regulatory agency. ALP tests are beneficial in that they allow a dairy plant to continue to operate in the interim before the state can time and reseal the pasteurizer. Tests such as the Advanced Instruments Fluorophos method produce a printout as documentation to verify that milk has been adequately pasteurized.

The most recent method accepted by the Association of Official Analytical Chemists (2, 3) for the determination of ALP in milk is the Advanced Instruments Fluorophos method. This method is the most rapid, accurate, sensitive and precise test currently available (5, 17). The Fluorophos test method can detect less than 10 mU of ALP activity per liter of pasteurized milk, while the Scharer Method is accurate to 500 mU/liter (equivalent to 1 µg of phenol per ml of milk). The current U.S. legal cutoff value for residual ALP activity in pasteurized milk is 1 µg of phenol per ml reported by the Scharer method (21, 22) or 350 mU/liter reported by the Fluorophos method (15).

No data are currently available for residual ALP activity levels in pasteurized milk and milk products for the minimum temperature-time specifications listed in the Pasteurized Milk Ordinance (PMO) (15) or the various combinations of temperature and time used by the dairy industry. According to Black et al. (5), residual ALP activity may vary widely in commercial pasteurized milk. Therefore, a series of controlled experiments was designed to determine the degree of variability in ALP activity levels in pasteurized milk.

Data from this study allowed calculation of a mean value of ALP activity in milks pasteurized below, at, and above the U.S. Food and Drug Administration-mandated...
minimum temperature and time using the Advanced Instruments Fluorophos method. Products evaluated were whole milk (3.4% milkfat), 2% and 1% low-fat milks, skim milk (<0.5% milkfat), chocolate-flavored milk (1.4% milkfat), and half and half (10.5% milkfat).

MATERIALS AND METHODS

Pooled raw milk was obtained from the University of Wisconsin–Madison dairy plant. Products evaluated were standardized to give the appropriate fat content for half and half (10.5%), chocolate-flavored milk (1.4%), whole milk (3.4%), 2% low-fat milk, 1% low-fat milk, and skim milk (<0.5%). Chocolate-flavored milk contained the added ingredients of 22 kg of chocolate powder mix and 110 kg of sugar per 378.5 liters of milk. The products were held at a temperature below 5°C (40°F) prior to and following processing. Each experiment used between 375 and 800 liters of raw milk.

The experiment was designed to establish a range of values for residual ALP activity in milks pasteurized under various time and temperature treatments. All milks were pasteurized using both a high-temperature short-time (HTST) pasteurizer (Junior Paralow model #21734; APV-Crepaco, Chicago, IL) and a low-temperature long-time (LTLT) pasteurizer (Damrow, Inc.; Fond du Lac, WI). The time-temperature heat treatment experiments consisted of two protocols. The milks in triplicate lots were heat treated under each protocol for both the HTST and the LTLT pasteurizer.

The first protocol involved heat treating milks for a constant time while varying the temperature. The HTST constant-time protocol subjected whole, 2%, and 1% low-fat and skim milks to heat treatments of 68°C (155°F), 70°C (158°F), 72°C (161°F), 74°C (165°F) and 75°C (167°F) for 15 s. The LTLT constant-time protocol subjected whole, 2%, and 1% low-fat and skim milks to heat treatments of 60°C (140°F), 61°C (142°F), 63°C (145°F), 64°C (147°F) and 66°C (150°F) for 30 min. Chocolate-flavored milk and half and half were heat treated 2°C (5°F) higher at each temperature interval for both the HTST and LTLT constant-time protocols. The temperature treatment was higher for chocolate-flavored milk due to the added sugar and increased viscosity and for half and half due to its higher fat content as specified in the PMO (15).

In the second protocol milks were heat treated for various times while keeping the temperature constant. The HTST variable-time protocol subjected whole, 2% low-fat, 1% low-fat, and skim milks to a heat treatment of 72°C (161°F) for 0, 5, 10, 15, 20, and 25 s. The LTLT protocol subjected whole, 2% low-fat, 1% low-fat, and skim milks to a heat treatment of 63°C (145°F) for 0, 5, 10, 15, 20, 25, 30, 35, and 40 min. Chocolate-flavored milk and half and half were heat treated 2°C (5°F) higher at each time interval for both the HTST and LTLT constant-temperature protocols.

High-temperature short-time general procedure

The HTST regenerative plate pasteurizer used for this research operated in compliance with the PMO in that every particle of milk or milk product was continuously subjected to the proper temperature for the required length of time in properly operated and maintained equipment (15). The HTST experiments each required 400 to 600 liters of milk. The indicating thermometer was calibrated using a National Institute of Standards and Technology (Gaithersburg, MD) (NIST) thermometer.

The pasteurizer was timed by using a conductivity probe and a timer (Milk Tester; Lumenite Electric Co., Inc., Franklin Park, IL). The apparatus was obtained from the Wisconsin Department of Agriculture, Trade and Consumer Protection. The salt-injection point on the pasteurizer was located after the plate heater but before the holding tube. The terminal conductivity probe was located at the end of the holding tube but before the sampling device and diversion valve.

Timing was set for 15 s at 72°C (161°F) for whole milk, 2% low-fat milk, 1% low-fat milk and skim milk while for chocolate-flavored milk and half and half timing was set for 15 s at 74°C (167°F). The pasteurizer was retimed for each product to adjust for variations in flow rates. The timing was set by first timing a weighed amount of water going through the pasteurizer. Second, a milk product was timed to determine the time to deliver an equal weight of product. The two times were then inserted into the equation shown below to determine flow time with water which equated to 15 s for a given milk product (15). Next, the pasteurizer timing was set to the nearest tenth of a second based on a calculated flow time.

The equation to determine flow time is \[ H = \frac{DMW}{W} \] where \( H \) is holding time wanted for milk, 15 s; \( D \) is average specific gravity for milk; the values used were 1.032 for whole, 2% low-fat, 1% low-fat and skim milks, 1.050 for half and half, and 1.052 for chocolate milk; \( W \) is average time to deliver set weight of water; \( Mw \) is average time to deliver an equal weight of milk; and \( T \) is the time to set pasteurizer with water.

HTST experiments were initiated with a warm-up period of approximately 5 min. During the warm-up period water was flowing through the pasteurizer at the first testing temperature. Once the pasteurizer was warmed and maintaining proper temperature, the holding tank was drained of water and the milk product was introduced. Next, the pasteurizer was operated until all milk diluted during the changeover was purged from the system. Once the pasteurizer was purged and the temperature held constant the experiment was commenced.

All sampling for constant time–variable temperature procedures was at the sampling valve located in close proximity to the indicating thermometer and diversion valve, a distance of 35 cm. Sampling for variable time–constant temperature procedures also was performed at the same sampling valve. One exception was for zero time, when milk was sampled at the valve located at the beginning of the holding tube where the injector for the timing equipment was located.

Sampling for each procedure interval was in triplicate. The process involved four steps. First, the indicating thermometer was monitored until the appropriate temperature held constant. At proper temperature the sampling valve was fully opened to flush any accumulated milk residue. Following the 2 to 3 s of flushing, two 50-ml vials (Snap Caps; Stuart W. Johnson & Co., Lake Geneva, WI) were positioned in the stream of milk. The vials, holding 10 to 20 ml of milk, were then quickly closed and vigorously shaken in an ice water bath for 30 to 40 s to cool. The same procedure was repeated using one vial to complete the triplicate sampling. Two samples taken at the same time were considered separate samples since milk was continuously flowing and thus provided two sampling points. All three samples could not be collected together since the handling was cumbersome, causing time delays between sampling, closing, and cooling. Rapid cooling was essential for uniform determinations.

The HTST constant time–variable temperature experiments were initiated at the lowest sampling temperature. This was done because the pasteurizer was better able to control changes to increased temperature than to decreased temperature. Sampling followed pasteurizer warmup and complete flushing with milk. Once samples for a given set point were taken, the milk product...
temperature was increased and the pasteurizer was allowed to equilibrate.

The HTST variable time–constant temperature experiments required time-interval differences of 5 s. To accomplish the 5-s intervals, the pasteurizer timing was set for 15 s through three sections of holding tube, so that flow through each section of holding tube took place in 5 s. The different lengths of holding tube were timed by using water to assure that each addition of a portion of holding tube allowed the proper holding time. This arrangement allowed for a quick change in holding time without retiming the pasteurizer before each variable change.

The holding time for milk was changed following each sampling interval. Upon completion of sampling, the pasteurizer was changed over from milk to water. Next, the length of holding tube was changed while the pasteurizer was still operating, in order to maintain the temperature of the system. Finally, the pasteurizer was switched back to milk and allowed to purge prior to another sampling.

Low-temperature long-time general procedure

The LTLT variable time–constant temperature experiments were carried out in two 1,136-liter (300-gal) commercial dairy vats (Steriliner Steel and Tube Products Co., Milwaukee, WI). The constant time–variable temperature experiments were carried out in a 189-liter (50-gal) commercial vat (Damrow, Inc.; Fond du Lac, WI). All of the vat pasteurizers met the PMO requirement that every particle of milk or milk product be continuously subjected to the proper temperature for the required length of time in properly operated and maintained equipment.

Sampling for all LTLT experiments was carried out in the same manner. Each given interval in an experiment was sampled in triplicate. First, three sampling vials were simultaneously hand dipped into the vat, removing 10 to 20 ml of milk per vial. Then the vials were closed quickly and vigorously shaken in an ice water bath for 30 to 40 s to cool.

With the LTLT constant time–variable temperature experiments, each temperature treatment needed approximately 100 liters of milk heat treated for 30 min. The time of heating started following a warming period as the product was brought to a given temperature. A warming period of approximately 5 min was consistent for all products. The product temperature was maintained for the required 30 min, and then samples were collected. Next the product was pumped to a holding tank to be cooled. The vat pasteurizer was cooled and cleaned between each interval of an experiment.

The LTLT variable time–constant temperature experiments were carried out in two identical 1,136-liter (300 gal) vat pasteurizers. For each experiment, 757 liters (200 gal) of product were heated. Time zero was set to occur after a warming period necessary to bring the product up to temperature. The warming period for all products was approximately 20 min. Sampling began at time zero and continued every 5 min for 40 min. A constant product temperature was maintained throughout the experiment.

Time and temperature data for all pasteurization experiments were documented using a chart recorder (for HTST treatment, a recorder from ABB Kent-Taylor Inc., Rochester, NY; for LTLT treatment, from Anderson Instrument Co., Fultonville, NY). A vat thermometer standardized using a NIST thermometer was used to monitor product temperature throughout experiments.

Product analysis

All milk samples were analyzed for residual ALP activity on an Advanced Instruments Fluorophos apparatus (Advanced Instruments, Inc., Norwood, MA; Model FLM 200), which consisted of a fluorometer, printer, and programmable calculator. All equipment and reagents used for analysis were supplied by Advanced Instruments. The manufacturer’s written procedures (1) were followed to obtain printouts of residual ALP activity. Calibration of the Fluorophos apparatus was required for each product tested. The calibration ratios used to calculate ALP activity for whole, 2% low-fat, 1% low-fat, skim, half and half, and chocolate-flavored milks were 114.2, 108.7, 123.0, 131.5, 75.0 and 103.7, respectively.

Control samples were tested with each batch of samples to ensure that the reagents and the samples were of good quality. Negative and positive controls were analyzed for whole, 2% low-fat, 1% low-fat, skim, half and half, and chocolate-flavored milks. An additional interference control sample was analyzed for all batches of chocolate-flavored milk. A negative control required 30 ml of raw milk to be heated for 4 min in 100°C water. This sample when tested should read less than 10 mU of activity per liter. A positive control was produced by adding 5 µl of raw milk to 25 ml of the negative control. After the positive control was well mixed, it was assayed to verify that there was approximately 500 mU of activity per liter. An interference control was prepared by adding 75 µl of well-mixed chocolate-flavored milk to 2.0 ml of Calibrator A from Advanced Instruments, Inc. The control was prewarmed to 38°C in the heating block for 5 min. The control tested after 5 min, should read less than 10 mU of activity per liter.

The SAS statistical program (20) was used to generate the mean values and standard errors for each of the products in the various processes; t-tests were performed (α = 0.05) to establish statistical differences between product and protocol values.

RESULTS AND DISCUSSION

The mean values for the HTST constant time–variable temperature experiments for whole milk, 2% low-fat milk, 1% low-fat milk, and skim milk are in Figure 1. The values for chocolate-flavored milk and half and half are in Figure 2. The HTST variable time–constant temperature graphs are similar to the constant time–variable temperature graphs. Subsequent reference to ALP activity values generated at minimum pasteurization times and temperatures will be a combined mean from both HTST protocols.

![FIGURE 1. ALP activity (log mU/liter) of whole, 2% low-fat, 1% low-fat, and skim milks pasteurized for 15 s at various temperatures.](image-url)
At present there is little published data (18) that can be compared to the data presented herein. A few studies have used the Fluorophos method to evaluate milks that were commercially pasteurized (5, 7, 17). However, none gave the exact temperature and time at which the milk was pasteurized. In a published collaborative study (18), the mean ALP activity values for commercially pasteurized whole milk and skim milk were 11.7 mU/liter and 11.9 mU/liter, respectively. In general, most dairy plants pasteurize at higher temperatures and hold for longer times than minimum legal heat treatments to ensure that milk will be properly pasteurized and have an extended shelf life. Therefore, the collaborative study data were compared to the higher heat treatment data shown in Figure 1. From Figure 1, whole milk and skim milk pasteurized at 75°C (167°F) for 15 s yielded mean ALP activity values of 22.9 and 13.0 mU/liter, respectively. Comparison of data suggests that the collaborative study data were generated under elevated time-temperature treatment. Rocco published ALP activity values for skim milk commercially pasteurized for 16 s at various temperatures (18). In Figure 3 the inactivation curve from the published skim milk data is compared to the present skim milk data from Figure 1. Even though the published heating time was 1 s longer than that shown in Figure 1, the inactivation curves were similar.

Variables that influence ALP activity levels in heat treated milks are the point of sampling, type of pasteurizer, location of the indicating thermometer relative to the sampling valve, and rate of cooling of the sample. The location of the sampling point is critical in obtaining valid ALP activity values. Products should be sampled at the exact point where the physical timing of the pasteurizer ends. Therefore, when a product is pasteurized for 15 s it must be sampled directly following this holding time. However, some reported experiments with skim milk have sampled at an outlet valve, which added more time to the heat treatment (18). These data illustrate the extreme importance of the location of the indicating thermometer in respect to the point of sampling. The indicating thermometer must be located physically as close as possible to the sampling point to ensure that the sample product was collected at the proper temperature. It was not given where the sampling point was located and how product was cooled (18).

The current U.S. legal maximum residual ALP activity allowed in pasteurized milk is 350 mU/liter. The mean values for whole milk (169.7 ± 12.3), 2% low-fat (145.2 ± 9.3), 1% low-fat (98.6 ± 8.9), and skim milk (72.5 ± 4.2 mU/liter) pasteurized at the minimum legal heat treatment of 72°C (161°F) for 15 s (15) suggest that the actual ALP activity values in minimally pasteurized milks are far below the legal limit. These mean values were at least 150 mU/liter below the legal limit. The difference was large enough to allow for milk to be underpasteurized, yet fall below the 350 mU/liter cutoff. In fact, a collaborative study found that pasteurized milk with 0.05% raw milk added gave values of 256.2 and 262.3 mU/liter for whole milk and skim milk, respectively (18).

The values given previously for milks pasteurized at the legal minimum heat treatment of 72°C (161°F) for 15 s (15) were plotted to compare products (Figure 4). The trend for products with a lower fat content to have less residual ALP activity was apparent. Whitney (24) reported that 30 to 40% of ALP is concentrated in the cream while the balance is dispersed throughout the skim milk. On the basis of Whitney’s findings, pasteurized skim milk would contain less residual ALP activity than pasteurized whole milk. Data in Figure 4 support this theory.

The values for chocolate-flavored milk and half and half at the minimum pasteurization heat treatment of 74°C (166°F) for 15 s were 157.3 ± 6.5 and 38.4 ± 4.6 mU/liter, respectively. These values fell far below the legal cutoff of 350 mU/liter.

Historically, chocolate-flavored milk has been difficult to accurately analyze for phosphatase activity due to the effects of chocolate constituents on the activity of the enzyme (13) and observation of color changes. Apparently,
chocolate constituents have an inhibitory effect and quench the enzyme or the product formed by the enzyme, such as that which fluoresces yellow in the Fluorophos method (12, 14, 16, 17). Other researchers have found that sugar increases the heat stability of ALP (6, 19). In theory, it is possible that the portion of ALP that is not inhibited by chocolate constituents may be heat stabilized by sugar. This would lead to higher activity values compared to those of unflavored milk of the same fat content. The value for chocolate-flavored milk (157.3 ± 6.5 mU/liter) is higher than the values from Figure 1 for whole (22.9 ± 0.5), 2% low-fat (19.8 ± 0.4), 1% low-fat (18.2 ± 0.6), and skim milks (13.0 ± 1.8 mU/liter) pasteurized at a similar high heat treatment. While the effects of chocolate-flavored milk constituents are not completely understood, the Fluorophos method can accurately detect residual ALP in chocolate-flavored milk where other methods fail.

Half and half requires a higher heat treatment than whole milk due to the higher proportion of milkfat, which has been suggested to protect ALP and microorganisms (15). The higher heat treatment is sufficient to inactivate the required amount of ALP. The value for properly pasteurized half and half of 38.4 ± 4.6 mU/liter fall far below the maximum allowable value of 350 mU/liter.

The data in Figure 1 illustrate a typical inactivation curve observed in this research. The reaction rate is first order initially but as the severity of the heat treatment increases, the curve levels off, generating another first-order reaction rate. Data shown by the curve illustrate that some ALP survives most pasteurization treatments, which has been observed previously (19). The different reaction rates can be explained by the differences in ALP location within the milk system. About 30 to 40% of ALP is located in the fat-globule membrane which acts as a protective coating against a heat treatment (23). Thus, the enzyme in milkfat and skim milk acts as two separate entities, one that is more heat resistant than the other, which generates two separate reaction rates during heat inactivation. Thus under normal pasteurization times and temperatures, the inactivation rate of ALP decreases directly with the amount of residual ALP and indirectly with the severity of heat treatment.

Values generated following the HTST variable time–constant temperature protocols produced underpasteurized milk with residual ALP activity values that still were below the legal cutoff. The 1% low-fat milk (337.1 ± 73.3), skim milk (279.2 ± 18.6), chocolate-flavored milk (263.7 ± 5.1), and half and half (45.3 ± 5.9 mU/liter) were heat treated for 5 s less than the legal minimum pasteurization settings. These data support the findings of Rocco (18) in that pasteurized milk containing small amount of raw milk can still be considered legally pasteurized by the current standard of not more than 350 mU of ALP activity per liter of product.

The mean values for the LTLT constant time–variable temperature experiments for whole milk, 2% lowfat milk, 1% lowfat milk, and skim milk are illustrated in Figure 5 and those for chocolate-flavored milk and half and half are illustrated in Figure 6. The trends seen in the LTLT data are similar to those of the previously mentioned HTST data. Lower fat products generally had lower residual ALP activity values; as the severity of the heat treatment increased, the inactivation curve decreased. More importantly, whole (81.8 ± 4.8), 2% lowfat (66.4 ± 5.9), 1% lowfat (56.4 ± 2.1), skim (39.1 ± 3.9), and chocolate-flavored milks (91.3 ± 7.7), and half and half (35.0 ± 1.2 mU/liter) pasteurized at the minimum legal time and temperatures had residual ALP values significantly below the legal cutoff of 350 mU/liter.

The LTLT variable time-constant temperature experiments lacked the precision seen in the constant time–variable temperature experiments because temperature control was more difficult with the 1,136-liter (300 gal) vat compared to the 189-liter (50-gal) vat used for the latter experiments. However, all of the trends previously mentioned also were seen in the variable time–constant temperature results. The most important result was that whole

FIGURE 4. ALP activity (mU/liter) of whole, 2% lowfat, 1% lowfat, and skim milks pasteurized at 72°C (161°F) for 15 s (data from HTST constant time–variable temperature experiment).

FIGURE 5. ALP activity (log mU/liter) of whole, 2% lowfat, 1% lowfat, and skim milks pasteurized for 30 min at various temperatures.
(43.5 ± 0.5), 2% lowfat (57.1 ± 10.3), 1% lowfat (38.4 ± 8.0), skim (58.0 ± 13.3), and chocolate-flavored milks (96.1 ± 9.5), and half and half (35.3 ± 5.8 mU/liter) pasteurized at the minimum legal time and temperatures had residual ALP activity values significantly below the legal cutoff of 350 mU/liter.

Underpasteurized whole (190.8 ± 1.3), 2% lowfat (261.1 ± 79.5), 1% lowfat (139.8 ± 4.3), skim (282.5 ± 78.0), and chocolate-flavored milks (147.0 ± 11.8), and half and half (36.6 ± 6.8 mU/liter) heat treated for 10 min less than the minimum legal pasteurization treatment produced ALP activity values below the maximum legal cut off of 350 mU/liter. It should be noted that the preheating time necessary for products to reach the required temperature was 20 min.

Milks thermally processed at the minimum legal pasteurization treatment for both HTST and LTLT protocols produced residual ALP activity values below 225 mU/liter, which is significantly lower than the current cutoff for residual ALP of 350 mU/liter. This suggests that the current cutoff of 350 mU/liter or 1 µg of phenol is not an accurate assessment value for properly pasteurized milks.

Half and half produced significantly lower residual ALP activity values than did chocolate-flavored milk treated similarly. Half and half and chocolate-flavored milk properly pasteurized using both HTST and LTLT protocols produced values significantly below 350 mU/liter. Half and half and chocolate-flavored milk heat treated for 5 s less than the required 166°F (74°C) for 15 s minimum produced ALP activity values below the legal cutoff. Again, the ability of underpasteurized half and half and chocolate-flavored milk to produce values below 350 mU/liter suggests the maximum allowable residual ALP in pasteurized milk products should be lowered.

From data developed in this study, milks with lower fat contents had lower residual ALP activity values than products with higher fat contents. This result was most apparent when residual ALP activity in skim milk was compared to residual ALP activity in whole milk.

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