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Dairy Foods Science Notes

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ALKALINE PHOSPHATASE TESTING FOR MILK PASTEURIZATION

What Is Alkaline Phosphatase?

Alkaline Phosphatase (ALP) is an enzyme naturally present in all raw milks, which is used as an indicator of proper milk pasteurization. Complete pasteurization will inactivate the enzyme to below levels which are detectable by conventional methods. Because the heat stability of ALP is greater than that of pathogens which may be present in milk, the enzyme serves as an indicator of product safety. However, the failure to detect ALP activity does not guarantee that the product is pathogen free.

How Has Phosphatase Activity Been Determined?

The classic test for the determination of alkaline phosphatase activity is the Scharer Rapid Phosphatase Test. The enzyme cleaves a phosphate group from the substrate, disodium phenyl phosphate, liberating phenol which then reacts with a color producing compound to give a blue color. The more enzyme activity present the more phenol is liberated giving a deeper color blue. The intensity of the blue is then read visually or with a spectrophotometer. Results of this procedure are expressed as micrograms of phenol per mL of milk. A value of greater than 1 microgram is indicative of improper pasteurization. The sensitivity allows for the detection of approximately 0.1 % raw milk contamination in pasteurized milk or the equivalent (i.e. under-pasteurization).

What Are the New Instrumental ALP Test Systems and Why Are They Better Tests?

The Fluorophos ALP Test System (Advanced Instruments, Inc.) and the Charm ALP/PasLite (Charm Sciences, Inc.) are relatively new instrumental procedures for detecting alkaline phosphatase activity in pasteurized milk products. An inert substrate containing a phosphate group is converted to either a fluorescent or a light-generating compound when the phosphate group is cleaved by ALP. The level of fluorescence or light as read by the systems instrument is proportional to the activity of ALP in the sample. These systems are much more rapid, simple and sensitive than the colorimetric method, and allow for the detection of much lower levels of residual ALP. The results are expressed as milliunits of ALP per liter. A level of 500 milliunits/liter is approximately equivalent to 1 microgram of phenol/mL as detected in the Scharer Method. Most plants milk is pasteurized well above the legal minimum, and will generally have instrument readings of less than 20 milliunits/liter. When pasteurized milk is contaminated with raw milk or if the milk is under-pasteurized, readings will increase proportionately. This is illustrated in the following table of results from a collaborative study (Rocco, R.M. 1991. J. AOAC 73:842), which used the Fluorophos ALP system to detect different levels of added raw milk to pasteurized product. Similar results should be found with the Charm ALP systems.

% Added Raw Milk	Homogenized Milk	Skim Milk	Chocolate Milk	1/2 & 1/2 Cream
	Milliunits per liter of ALP			
0.00 %	12	12	10	8
0.05 %	256	262	262	156
0.10 %	494	508	521	327
0.20 %	960	995	1020	610

Keep in mind that the level of ALP in raw milk is variable. ALP activity for different levels of added raw milk may vary depending on the source of raw milk, though the values in the table are good estimates of what might be found. ALP is associated with the cream or the fat globules of the milk. When milk is separated after pasteurization, the cream may have a higher ALP activity than cream that is separated and then pasteurized. The half & half cream reported in the table was pasteurized after separation.

Instrumental ALP Testing; How Can The Results Be Used?

Routine testing of processed fluid milk products for levels of residual ALP using the Fluorophos and the Charm systems will support other procedures that ensure proper pasteurization. Though the cut off level of ALP is 350 milliunits/liter, the levels common in pasteurized milk are often less than 20 due to the higher than required pasteurization temperatures commonly used. This ALP value may vary depending on the processing conditions and the source of raw milk so it is important that each plant establishes a baseline level of what would be considered normal ALP values for the specified processing conditions. Once a baseline ALP level is established, routine monitoring can be used to detect processing deficiencies. If ALP activity of a sample is higher than what is normally seen in the baseline or in previous samples, it should sound an alarm. **Higher ALP activity may indicate serious deficiencies in the pasteurization process.**

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