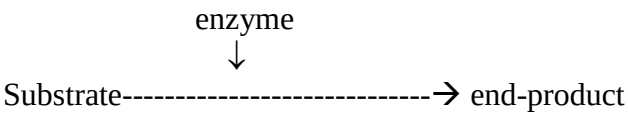
WEBER SCIENTIFIC

Testing Milk Pasteurization

A number of "rapid" tests have been developed for determining the effectiveness of the pasteurization process. They are all based upon the enzyme Alkaline Phosphatase (ALP). ALP very specifically cleaves the monophosphoric ester bond that is found in a wide variety of substrates and then releases a phosphate radical. Acid Phosphatase (ACP) is another enzyme that is also found in nature. ALP, like most other enzymes, is a protein so it heat-labile (sensitive to destruction or de-activation by heat). ALP is produced by the cow and deposited in her milk. ALP is also produced by bacteria. ALP is de-activated at approximately the same temperature that destroys the harmful and unwanted bacteria in milk. Testing for pasteurization efficiency by measuring the ALP activity is much faster than culturing the milk for bacterial levels (usually 1-2 days to get results).

The principle behind testing ALP is quite simple:



The enzyme (ALP) converts the substrate (monophosphoric ester bond) and releases an end-product (phosphate radical). The liberated phosphate radical might contain a color indicator and you can compare the test color to a color standard to indicate the level of enzyme activity. Of course, the end-product is a function of the substrate you use. Visualization (colorimetric) of the end-product is the basis of the Scharer Rapid Phosphatase Test. A number of substrates have been used as ALP test methods have been improved such as - phenyl phosphate, p-nitrophenyl phosphate, phenolphthalein diphosphate, monophosphorylated benzothiazole derivative (MBZ) and 1,2 dioxetane (DXE).

As a visual test the Scharer Test has its limitations in accuracy and detection limit due to color interferences. This test is no longer recognized as an official test. Still, it has been a popular, low cost, rapid test that requires no specialized equipment. If desired, one can use a spectrophotometer to measure the color in Scharer tubes. More accurate methods use the principles of fluorescence and chemiluminescence. If you expose the end-product of the MBZ to a certain wavelength of light the end-product will fluoresce - that is, it emits light at a higher wavelength. The fluorimeter quantifies the fluorescence and this has the advantages of being able to detect very low levels of ALP and that no other colors will interfere with this measurement. In the chemiluminescence method when the ALP cleaves off the phosphate radical a very short-term chemical reaction occurs which produces a burst of light (think of a lightning bug). This light is detected using a scintillation counter (also used to measure radioactivity).

The problem with all ALP tests is the potential for false-positives and false-negatives. In some situations the ALP can actually become re-activated as the milk product is stored. Another false-positive occurs due to the ALP in the bacteria (remember, milk is not sterile!)

John Beck
8/10/06