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# Inhibitory Effect Of Heavy Metal On Enzymatic Regulations Of Alkaline Phosphatase Enzyme In Raw Milk

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***Abstract:*** ***A study was carried out to examine the competence of alkaline phosphatase enzyme upon addition of mercuric chloride in raw milk. A set of 16 samples of raw milk were used in the study to check out the effect of alkaline phosphatase activity in the presence of mercuric chloride. The Lovibond comparator method for the determination of ALP activity in milk was used to verify the completeness of pasteurization process of milk. It was observed that upon addition of mercuric chloride in raw milk, it exhibits complete inhibition of ALP enzyme which can be utilize as a key tool for false negative reaction of unpasteurized or partially pasteurized milk.***

***Keywords: Heavy metals, enzymatic regulations, raw milk, enzyme inhibition***

***Abbreviations: ALP-Alkaline phosphatase enzyme, ppm-parts per million, Hg-mercury***



## I. INTRODUCTION

Alkaline phosphatase (ALP) is the most important enzyme in dairy industry. Activity of this enzyme is related to the quality of the process of pasteurization. During the heat treatment, its activity decreases about 500- fold (1). ALP is a membrane– bound glycoprotein, common in animal tissues and microorganisms. Due to time-temperature combination of pasteurized milk, it is generally used as an indicator of the effectiveness of pasteurization(2). The quality of dairy products depends on milk quality from which the products are made. Contamination or addition of raw milk to pasteurized milk can cause the risk of pathogen contamination of the product. Wrong process of pasteurization can influence the final product by denaturation of whey proteins, inactivation of enzymes, destruction of beneficial bacteria, or chemical changes, which can affect an increase or decrease in substrate availability for bacteria and enzymes (3,4).

Mercury is considered a highly toxic contaminant. The toxicity of mercury depends on its chemical form methyl mercury being the most hazardous metal and stable form of mercury that has been attributed to the suffering of most avian and mammalian predators at the top of contaminated tropics. Industrial wastes and sewage water form the chloroalkali industry are a major source of mercury pollution(12,13). Their concentrations in animal organs and their milk concentrations may increase very fast, although their excretion through milk is very low. Their ecosystem accumulation (water-soil-plant-animal) makes them very toxic and leads to undesirable consequences for live organisms (14, 16). In the local studies, cadmium, lead, copper, zinc, iron, chromium and manganese are found at toxic levels in soils and vegetables. Certain studies guide to hypothesize that fodders grown on such soils will also accumulate these heavy metals, and animals reared on contaminated fodder will contain heavy metal residues in edible tissues, such as milk. The general public eating such contaminated edible products may accumulate toxic levels of heavy metals(17,18).

Because of that, the heat treatment of milk for consumption and production of dairy products should be limited. The methods of determination of ALP in milk and dairy products are based on colorimetric reactions in which para-nitro phenyl ortho phosphate disodium salts are used as substrates.

## II. SCOPE OF THE STUDY

The present study was conducted to evaluate the inhibitory effect of heavy metal mercury on alkaline phosphatase enzyme present as natural component in raw milk and document its effect on dairy performances. Data should be collected regarding Inhibition of ALP enzyme at different $HgCl_2$ concentration. The goal of the present study is also to clarify the importance of enzyme inhibition theory by heavy metals and its application and awareness in dairy industries.

## III. MATERIALS

- ✓ Mercuric Chloride (Powder), Qualigenes, Catalogue No. 25284
- ✓ Waterbath, ( EIE Instruments Pvt. Ltd.)
- ✓ Para Nitro phynyl phosphate di sodium salts
- ✓ (SRL Cat. No.88485) (144816) (4264-83-9)
- ✓ Tintometer No. 302279, Lovibond 2000, MK.II No. 142000
- ✓ ALP buffer-Dissolve 1.5g of sodium bicarbonate (Merck, Cat. No. MH9M59199) and 3.5g of sodium carbonate (Merck, Cat. No. 1.93611.0521) in 1 liter distilled water. pH of the solution was 10.2-ALP buffer substrate-Dissolve 1.5g of disodium p-nitrophenyl phosphate in 1 liter distilled water. This solution is stable in a refrigerator at 4ºC or less for 1 month.

The samples of raw milk used in the present study were collected from the market.

For comparison, negative control and positive control samples were also run during the experiment.

## IV. METHODS

The study was conducted using 16 raw milk samples which were selected to check the inhibitory effect of mercuric chloride on ALP enzyme. Data were collected regarding ALP inhibition and reproductive parameters. 16 samples of raw milk were treated with different concentration of mercuric chloride including ranges from 20 ppm to 100 ppm. The AP test was conducted for all the samples followed by analyzing alkaline phosphatase concentration by Lovibond comparator and visual appearance. Positive and Negative control samples were also analyzed parallel with the experiment.

## V. RESULTS & DISCUSSION

The results indicated that the inhibition of alkaline phosphatase depend on the content of the bioavailable pool of heavy metals. It is evident from the table 1 that enzyme activity decreased with an increased concentration of active forms of heavy metals.

| Tube no. | Concentration of $HgCl_2$ (ppm) | Lovibond Disc No. | Visual Appearance | Time Exposure (h) |
|---|---|---|---|---|
| 1 | 0 (raw milk, positive control) | 42 | Yellow | 00:30 |
| 2 | 20 | 6 | Faint Yellow | 3:00 |
| 3 | 40 | 0 | white | 24 |
| 4 | 60 | 0 | white | 24 |
| 5 | 80 | 0 | white | 24 |
| 6 | 100 | 0 | white | 24 |
| 7 | 0 (pasteurized milk, negative control) | 0 | white | 24 |

*Table 1: Effect of mercuric chloride on ALP enzyme at different concentration*

Additionally, the activity of ALP enzymes varied throughout the experiment in line with the concentration of the bioavailable pool of heavy metals. The enzyme tested showed highest sensitivity to the concentration of active forms of mercuric chloride at 20 ppm. At the rate of 20 ppm of mercuric chloride exposure partial inhibition was found after 3 hours but as the concentration of heavy metals increased it exhibits complete inhibition of ALP enzyme (Picture 1,2).

*Figure 1: Alkaline phosphatase Enzyme Inhibition by mercuric chloride resulting in to no color change*

*Figure 2: Positive Control. Alkaline phosphatase Enzyme produces yellow color upon active participation to degrade Para nitro phenyl ortho phosphate in to para nitro phenol*

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Taking into consideration our current knowledge suggesting that mercury is not an essential element for living organisms, it was expected to obtain a stronger relation between this metal and enzyme activity. Some elements works as cofactor and some elements works as inhibitor for enzyme (figure 3).

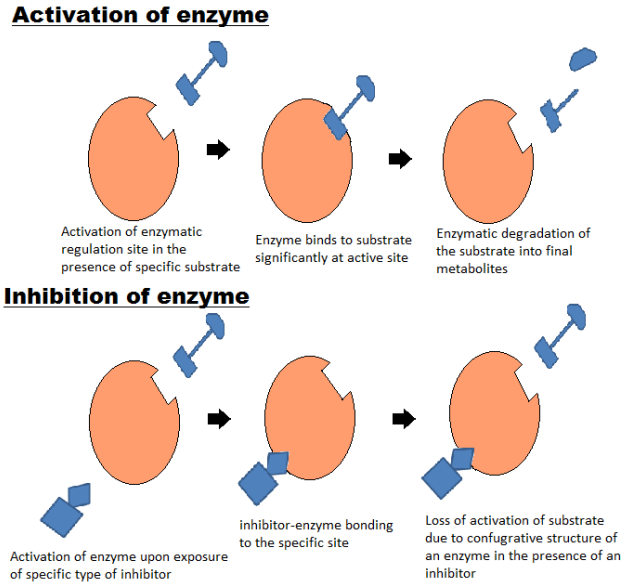


*Figure 3: Schematic diagramme of ALP Inhibition by mercuric chloride*

As mercury is strongly attached to the enzymatic functional groups, their regulations deemed significantly lower. This pattern was specifically noticeable in concentration gradient study. The better solubility of mercuric chloride compounds and their affinity to the milk phase ensured their high reactivity with the ALP.

## VI. CONCLUSION

It is evident from the study, that the activity of raw milk enzyme was strongly inhibited by heavy metals especially mercury. Weak correlation was found between the very trace amount of metal content and enzyme activity. Alkaline phosphatase exhibited the higher sensitivity toward mercury. Dairy Industry is strongly reliable on ALP detection test as this is the key tool to check the pasteurization process. But if the raw milk contains mercury element in high concentration than the permissible limit than it may give the false negative results. Thus further investigation is in demand for future to make the milk much safer for consumers.

This study indicates that heavy metal mercury decreases the efficiency of alkaline phosphatase enzyme and gives negative result. It indicates that Hg works as a inhibitor for alkaline phosphatase enzyme. So if by contamination with water or from any other source Hg comes in the milk it may be chances that it may gives false negative result.

## VII. SUMMARY OF STUDY

Hg works as an inhibitor for alkaline phosphatase enzyme. Mercury decreases the rate of reaction of ALP with the p-nitro phenyl ortho-phosphate present in ALP substrate resulting in to no yellow color development which might leads to false negative result.

## VIII. FUTURE ISSUE

India is richest country in milk production in Asia, as quality parameters are becoming more stringent day by day with the government support for the public benefits,

Further research is needed for the dairy industry to comply legal standard strictly on the basis of presence of heavy metals.

More detailed investigations on influence of heavy metals on enzymatic action and proteins as well as disturbance in the physical properties of milk are required on this aspect and if significant results are achieved, the dairy industries will prosper and benefit by research work on own fit.

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