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Development of Disposable Activated Bombyx Mori Silk Fabric Biostrips for Pasteurization Test of Milk

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***Abstract*:-- Pasteurization of Milk and milk products are essential because non-pasteurized milk has alkaline phosphatases (ALP) which cause intra-abdominal bacterial infections after drinking. Therefore, milk industries test the milk after pasteurization using various chemical methods including boiling. Here, objective of this work is to detect the presence of alkaline phosphatase in the pasteurized milk by using disposable dry-reagent biostips of sodium nitrate treated/ sodium chloride treated activated Bombyx mori silk fabric which are prepared by immobilization of bromocresol green and p-nitophenol phosphate which gave the instant color change from light blue to green if sample is not pasteurized and test is called positive (ALP +ve). This test was observed to carry out any ambient temperature conditions and prepared activated silk strips can be stored up to 7-8 months in non-humid conditions which might be considered cost effective and rapid biosensor employed for the qualitative estimation of alkaline phoshatase in the milk samples or milk products.**

***Keywords*:-- Alkaline Phosphatase, Pasteurization, P-Nitophenol Phospahate, Biostrips, Activated Silk Fabric**

I. INTRODUCTION

Alkaline phosphatase (ALP) is an enzyme naturally present in raw milk which causes intra-abdominal bacterial infection after consumption and pasteurization test has been reported in raw milk and milk products [1]. Previously, silk fibers had been chosen in the form of woven fabric because of its biocompatibility and antimicrobial properties for the immobilization of lipase from Candida sp. and alkaline phosphatase as a novel and inexpensive matrices [2, 3]. Hence to exploit these properties of silk fabric, the activation was done by using sodium nitrate/sodium chloride for improved immobilization of of bromocresol green (chromogen) and p-nitophenol phosphate (substrate) which gave positive (+ve) with the instant color change from light blue to green if sample is not pasteurized/boiled well which means that alkaline phosphatase is still present in the sample. As well as, this test can be out any ambient temperature conditions and prepared activated silk biostrips can be stored up to 7-8 months in non-humid conditions which might be considered cost effective and rapid biosensor to perform qualitative estimation of alkaline phosphatase in the milk samples or milk products being a more potent pasteurization test.

II. MATERIALS & METHODS

The Mixture of 2mg of the Bromocresol green dye and 2mg of p-nitrophenol phosphate in 10 ml of 1 M Tris-HCl buffer (pH 8) solution was immobilized on uniform strips of Bombyx mori silk fabric which was activated via their pretreatment with sodium nitrate/sodium chloride and dried at 30°C for 1h in a humidity-free chamber for 2-4 hours [3,4].

Evaluation of Pasteurization Test in Chosen Milk Samples These prepared biostrips were blue colored and dipped once in various milk samples chosen milk samples e.g., boiled or unboiled raw milk (cow Milk & buffalo Milk) and Mother Diary milk sample. Change in color was started to observe within 5 to 10 seconds after dipping from light blue to green if alkaline phospatase (ALP) might be present in samples and test was found positive (+ve). If no change in the color was observed which gave negative test (-ve) test that indicate the denaturation of alkaline phosphates is done well in chosen milk samples.

Color change:

Original strips color ---------- After dipping in chosen milk samples

(Light Blue) (Light Blue)…… ALP -ve test

(Green)…… ALP +ve test

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III. RESULTS & DISCUSSION

The light blue color was changed to light green when the dried strips were dipped in raw unboiled milk samples of cow and buffalo milk samples which are available in the market for local consumption. Thus, we were able to observe distinguish the qualitative detection of alkaline phosphatase (ALP) in chosen milk samples such as raw milk (unboiled) and pasteurized milk samples (Table 1). Thus, it was found the color change from light blue to green in non-pasteurized/ raw unboiled milk samples such as local milk of cow milk and buffalo because of presence of alkaline phosphatase. And no change in color was observed in chosen pasteurized milk samples such as Mother dairy Milk sample because complete denaturation of alkaline phosphpatase was done in this sample by boiling or UV method employed by the respective milk company (Table 1). These results were found be pretty comparable with the previous observations of employed dry-reagent biostrips which has been used for quantitative estimations of various chemicals and biomolecules [4,5,6]. As well as, these disposable activated silk strips of activated Bombyx mori silk fabric were found to be stable at room temperature for up to 7 to 8 months when stored under humidity-free conditions.

Table 1: Qualitative Observations of Pasteurization Test in Chosen Milk Samples by Using Disposable Activated Silk Fabric.

S.No.	Chosen Milk Samples	Observed Pasteurization Test
1.	Raw Cow milk	Green (ALP +ve)
2.	Boiled Cow Milk	Light blue (ALP -ve)
3.	Raw Buffalo Milk	Green (ALP +ve)
4.	Boiled Buffalo Milk	Light blue (ALP -ve)
5.	Mother Dairy Milk	Light blue (ALP -ve)

IV. CONCLUSION

Hence, this developed method using disposable activated silk fabric biostrips was found to be very easy, safe, economical and quick biosensor method which do not require any sophisticated instrument and can be visualized by naked eyes. The color change of biostrips depends upon the presence or absence of alkaline phosphatase (ALP) in chosen milk samples which are available in market for consumption. So, this method might be considered safer and more cost effective over other previously employed methods for quantitative estimations of various chemicals/biomolecules which were noted more expensive, cumbersome and require many chemicals to detect alkaline phosphatase in pasteurized milk.

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