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Original Article

Development of Indigenous Alkaline Phosphatase Kit for the Detection of Milk Quality

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ABSTRACT

Milk is a profoundly nutritious food that provides the favorable environment and nutrition for the growth development of large number of microorganisms. Microbiological quality assurance techniques could be usually utilized as a speedy strategy to survey the microbiological nature of crude and pasteurized milk. **Objective:** To develop indigenous rapid kit for determination and differentiation of milk quality, microbial presence, pasteurized and unpasteurized milk. **Methods:** Some 14-milk raw and pasteurized milk samples were collected from different geographical areas of Lahore and different brands of pasteurized milk. The colorimetric indigenous alkaline phosphatase milk quality detection kit was prepared for 200 reactions was developed. Alkaline phosphatase kit was tested at different temperature and volume of milk. **Results:** Results showed that a wide range of milk that bought from local stores and nearby market with exorbitant cost milk types shown no difference in milk quality in terms of presence of microbes. Moreover, different effect of pasteurized milk was observed after affirm test the variety stayed blue and not changed. **Conclusions:** This indigenous kit is test is quick monetary strategy that can be utilized for identification of milk quality on the basis of microbial presence, therefore, pasteurized or unpasteurized milk can be tested in field as well.

INTRODUCTION

Standard practices for status of milk items are fundamentally founded on the warm inactivation energy of the endogenous milk chemical, soluble phosphatase [1]. Quality milk ought to have a sweet and clean flavor with no trailing sensation [2]. Endogenous milk ALP manifests a slightly higher heat resistance than the pathogenic microflora upon which pasteurization time and temperature requirements are based. Hence, ALP activity is recognized as best available method of choice for the rapid validation of milk product pasteurization [3]. These imperfections of milk smell might be characterized by; consumed microbial and enzymatic processes [4]. The crude milk might go about as numerous destructive microbes prompting different illnesses, like undulant fever, *Salmonellosis*, Looseness of the bowels and Tuberculosis with microbes count under a predetermined cutoff [5]. The time span of usability of purified milk can be impacted by enormous number of substantial cells in crude milk [6]. Expanded physical cell numbers are emphatically corresponded with an intensity stable protease and of lipoprotein lipase in newly created milk. Exercises of these catalysts can enhance those of bacterial hydrolases, consequently shortening the chance to decay [7]. Methylene blue reduction depends on the way that the variety bestowed to drain by the expansion of a color, for example, methylene blue will vanish pretty much rapidly

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when the expulsion of the oxygen from milk and the development of decreasing substances during bacterial digestion makes the variety vanish [8, 9]. The alkaline phosphatase is naturally occurring enzyme of milk but it degrades at the temperature of pasteurization and can indicate that milk has been pasteurized adequately and is free from microbial contamination [10]. Furthermore, this indigenous alkaline phosphatase milk quality detection can distinguish the milk quality by colorimetric differentiation of pasteurized milk from unpasteurized milk with microbial contamination. Each kit is sufficient to conduct 300 colorimetric reactions at room temperature within 15 minutes at Lab, home or in field.

METHODS

A sum of fourteen examples containing seven raw milks from local markets of Lahore and seven pasteurized milk samples of known brands from hyper market all tested simultaneously. All milk samples were kept in a fridge at 4ºC before moving to the research center under chilled conditions. Reagent I was preparing with Methylene blue powdered 1.5g, 95% ethyl alcohol in distilled water. Reagent II was prepared with 10% Potassium hydroxide filtered and 1:20 solution was prepared. For optimization different concentrations and temperatures were tested. These solutions present in the kit are labelled as reagent I and Reagent II, and final ethylene blue concentration per reaction be achieved 0.005% in milk test sample control sample. Therefore, to perform alkaline phosphatase-based test from it 50µl of Reagent I, 15µl of Reagent II in 20ml of control milk sample or test milk sample and incubate for 15 minutes at room temperature (30-35ºC). Each kit has Reagent I, 15ml and Reagent II, 5ml and can be used to perform 300 reactions.

RESULTS

Different raw milk samples were obtained from local market from the geographical surroundings of PCSIR Lahore GPRS coordinates were recorded are provided in Table 1. Six brands of Pasteurized and local raw milk were purchased form the market. All the samples were kept at 4ºC until test was performed.

Table 1: GPRS (latitude and longitude) coordinates of Raw and Pasteurized Milk Collection Point

Raw Milk Lab-code	GPRS (latitude and longitude) Coordinates of Collection Point	Pasteurize Milk Lab-code	GPRS (latitude and longitude) Coordinates of Collection Point
003/RGenED	31.51487537347517, 74.29841999799864	001/PGenED	31.519949932041296, 74.32124632771942
004/RGenED	31.52402130647496, 74.29095272846344	001/PGenED	31.520160287134495, 74.32077962337347
005/RGenED	31.519265533052256, 74.299621627579	001/PGenED	31.50729903119769, 74.35290653751485
006/RGenED	31.50828974736308, 74.27902226334393	001/PGenED	31.493835698803636, 74.35756882586983
007/RGenED	31.523948142563718, 74.29138188188496	001/PGenED	31.491827805621572, 74.30963266819869
008/RGenED	31.519411868152233, 74.30339817768876	001/PGenED	31.52109773342149, 74.31925612872693

Test was performed for optimization kit based on concentration of Methylene Blue in 10ml sample with successive increase in temperature to evaluate the time required for reduction of methylene blue. It was observed that increase in temperature speeds up reaction up till certain level as graphically described in given in Figure 1.

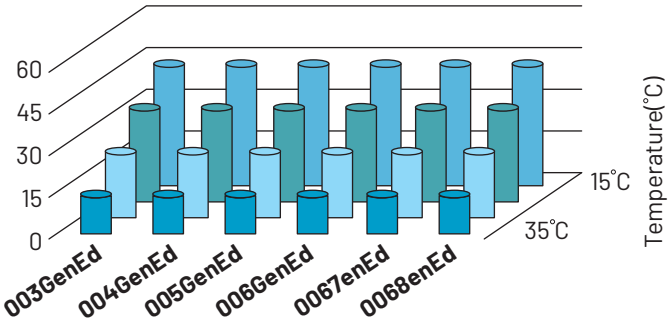

Figure 1: Optimization for Temperature for Detection of Milk Quality through Indigenous Developed Kit

The quality of milk was assessed as on the basis of alkaline phosphatase enzyme in raw milk pasteurized milk is used for the detection of pasteurized milk or food that whether they are pasteurized for write time and temperature [11]. The alkaline phosphatase enzyme is naturally present in raw milk but it is degraded at high temperature in limited time of pasteurization which makes milk free from pathogen Table 2.

Table 2: Observation of Time required for Colorimetric Detection of Milk Quality

Sr. No.	Initial Time	Final Time	Raw Milk Lab-code	Inference	Pasteurized Milk Lab-code	Inference
All tests were performed at 35°C						
1.	09:00	4:00	003/ RGenED	Colour Change after 13 min.	001/PGenED	No Colour Change
2.	09:00	4:00	004/ RGenED	Colour Change after 14 min.	001/PGenED	No Colour Change
3.	09:00	4:00	005/ RGenED	Colour Change after 15 min.	001/PGenED	No Colour Change
4.	09:00	4:00	006/ RGenED	Colour Change after 12 min.	001/PGenED	No Colour Change
5.	09:00	4:00	007/ RGenED	Colour Change after 13 min.	001/PGenED	No Colour Change
6.	09:00	4:00	008/ RGenED	Colour Change after 12 min.	001/PGenED\	No Colour Change

The test determines the quality of milk is adequately pasteurized and free from contamination. The colorimetric test make is feasible for the detection of milk quality. The

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kit developed makes it possible to determine the milk quality in lab, home and even outside with optimized temperature and concentration are provided with the kit instructions in simplest manner Table 3.

Table 3: Parameter for Milk Quality Assessment

Sr. No.	Decolouration Time (hours)	Quality of Milk
Temperature, 35°C		
1.	Less than 2 hours	Poor
2.	In between 2 to 5 hours	Fair
3.	Between 6 to 8 hours	Good
4.	More than 7 Hours	Excellent

DISCUSSION

Milk is important complete nutrition in natural form as milk contains fat, protein, starches, minerals, nutrients and other essential nutrient [12]. However, it is profoundly affected by bacterial pollution and subsequently becomes unable to drink [13]. A portion of these microbes that fill in milk, during the development of metabolites, may cause an unsuitable tangible modification, like off flavor, scent, and change in surface or appearance, named as deterioration [14]. These microorganisms such as presence of coliform microbes ought to be unambiguous waste milk, some microorganisms may likewise bring out alteration in milk without on any tactile changes. The microbial nature of crude milk is significant for the development of dairy items and it additionally impacts their timeframe of realistic usability [15, 16]. Methylene blue regularly is utilized as a speedy strategy to evaluate the microbiological nature of crude and purified milk [17, 18]. This test depends on the way that the blue shade of the color arrangement added to the milk get decolorized when the oxygen present in the milk get depleted because of microbial movement. The colorimetric assay provided clear and distinguishable results, enabling both qualitative and semi-quantitative assessment of alkaline phosphatase levels. Comparative analyses with established laboratory methods showed a strong correlation, validating the accuracy and reliability of the kit. Furthermore, the indigenous kit exhibited excellent stability, shelf life, and reproducibility, making it a suitable tool for routine milk quality analysis. Moreover, the cost-effectiveness of the indigenous kit ensures its accessibility to a wide range of users, including small-scale dairy farmers and processors. This democratization of milk quality analysis contributes to the overall improvement of food safety standards and consumer confidence. Furthermore, the development of indigenous kits reduces dependence on imported products and promotes local innovation and economic growth [19, 20]. In this study, the tests were performed for milk from general store and involved optimize temperature for colourimetric detection of quality as displayed All milk types that show no difference in colour in stipulated time, considered to be great quality of the milk. The test affirms the outcomes of Methylene Blue into the milk tests and distinguish the quality involving time as displayed. The raw or crude milk samples from market change colour with limited time shows their compromised quality which could overly affect the human health. This indigenous alkaline phosphatase milk quality detection can distinguish the milk quality by colorimetric differentiation of pasteurized milk from unpasteurized milk with microbial contamination. Each kit is sufficient to conduct 300 colorimetric reactions at room temperature within 15 minutes at Lab, home or in field. Moreover, the UHT treated milk was found to be free from coliform bacterial tests and has brilliant quality for human consumption. Future research directions could involve the further optimization and refinement of the kit to enhance its sensitivity, specificity, and user-friendliness. Exploring the integration of digital technologies, such as smartphone-based applications for result interpretation and data management, could also extend the functionality and accessibility of the kit. Additionally, efforts should be made to promote awareness and adoption of the indigenous kit among dairy industry stakeholders through training programs and knowledge dissemination initiatives.

CONCLUSIONS

In conclusion, the quality of milk is of paramount importance for ensuring public health and consumer satisfaction. Among various quality indicators, alkaline phosphatase (ALP) has been recognized as a reliable marker for evaluating milk freshness and detecting potential contamination. This research article presents the development of an indigenous alkaline phosphatase kit for the detection of milk quality. The kit aims to provide a cost-effective, rapid, and user-friendly solution for dairy industry stakeholders to assess milk quality in both laboratory and on-site settings.

Authors Contribution

Conceptualization: SM
Methodology: RE, BB, SA, IP
Formal Analysis: YS, NA
Writing-review and editing: NA, QS, SR, IP, SHA

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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