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Development of Strip for Detection of Urea Adulteration in Milk

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India is the world's largest milk-producing country, with a per capita availability of 471 grams of milk per day & 239.30 million tonnes in 2023-2024. As a source of essential nutrients and often referred to as the "ideal food," milk is high in demand. Unfortunately, this high demand has tempted unethical individuals to add hazardous substances to milk for greater profit.

One of the major adulterants frequently added to milk is **urea**. Urea is added to artificially increase the milk's total solids, heat stability, and whiteness. While urea is naturally found in milk, a maximum limit of 700 parts per million (ppm) is set by FSSAI. Adulteration with urea, however, can lead to several human health issues.

The Need for Simple Detection Methods

Current methods for detecting urea adulteration, such as spectrophotometric, infrared, and enzymatic techniques, can often be costly, time-consuming, and impractical for use in rural and field-level settings. To overcome this constraint, researchers have developed a simple, low-cost, and sensitive chromatographic paper-based test strip for detecting urea in milk.

How Does the Strip Work?

The innovative strip utilizes the power of natural enzymes:

1. **Preparation:** A chromatographic paper strip is dipped for 10 minutes in a mixture of a supernatant (from coarse-ground soybean powder soaked overnight in water) and an acid-base indicator.

2. **The Principle:** The strip uses **urease**, an enzyme naturally present in soybean, to break down any urea present in the milk.
3. **The Reaction:** The breakdown of urea by urease produces **ammonia**, which consequently raises the pH of the milk sample.
4. **Color Change:** This rise in pH causes the acid-base indicator on the strip to change color, signaling the presence of urea.

Key Features of the Developed Strip

After screening various indicators, **Phenol Red** was found to be the most effective for the final strip preparation, especially in the presence of -mercaptoethanol. The finalized strip offers several benefits:

- **Limit of Detection (LoD):** The strip can detect a minimum of **0.07% urea** in milk. This is more sensitive than some previously developed strips which reported LoD values of 0.08% and 0.09%.
- **Response Time:** The color change on the strip is visible within **3 to 5 minutes**.
- **Stability:** The strip maintains its stable and reproducible results for **6 months** when stored at ambient temperature.
- **Ease of Use:** It is a cost-effective and highly convenient test.

Recommended Testing Protocol

The recommended protocol for using the developed strip is straightforward:

1. Collect a well-mixed, fresh milk sample.
2. Hold the strip by the end opposite the test area and immerse the test area in the milk for **10–15 seconds**.
3. Shake off excess milk. Blotting the strip should only be done if the milk has high fat content or separation has occurred.
4. Observe the color change on the strip within **3 to 5 minutes**.

Note- This strip is detecting adulteration of neutraliser also

If colour change occurs Within 10 sec conclude to milk added neutraliser discrimination of each adulteration depends upon time taken to colour change

Interpretation of Results:

- A **yellow or orange** colour indicates a **negative** result (no extraneous urea).
- Different **shades of pink** color indicate a **positive** result, signifying the presence of urea.

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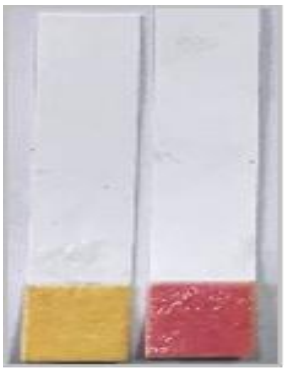
- This simple, sensitive, and portable strip-based test is a significant development for quick and easy detection of urea adulteration, particularly for routine analysis at the field & household level.
- **Interference:** Presence of neutralizer and pH higher than the normal can also give positive results.
- **Limit of detection:** 0.07% urea.

Control 0.05% 0.06% 0.07% 0.08% 0.09% 0.1%



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