Indian Journal of Chemical Technology
Vol. 7, May 2000, pp. 146-147

Notes

A rapid estimation of urea in adulterated milk using dry reagent strip

Hemant Kumar *, Ashok Kumar, Poonam Kumari, S Jyotirmai & N B Tulsani

Centre for Biochemical Technology, Delhi University Campus, Mall Road, Delhi 110 007, India

Received 27 July 1999; accepted 23 March 2000

This article describes development and optimization of a visually evaluable dry reagent strip technique for semi-quantitative estimation of urea in adulterated milk. It is based on (i) urease reacting with urea to liberate ammonia and carbon dioxide and (ii) liberated ammonia reacting with a specific chromogen to change color of the strip from light yellow to magenta, which is visible with naked eyes. The technique is versatile as (i) it is used single step-working reagent to complete the reaction within 30 s at room temperature, (ii) gives different shades of color from yellow to magenta, depending upon concentration of urea present in the milk, (iii) this strip can measure urea concentration as low as 0.1g/L and (iv) this dry reagent strip is stable up to one year at room temperature .

Adulteration is a global problem, especially with edibles when these are mixed with unhygienic materials for making more profit. Milk is one of the essential nutrients *perse* for human and animal consumption as well as through other dairy products. The main constituents of milk include proteins, carbohydrates, vitamins, minerals and water. Taking into consideration a huge demand of milk, milk sellers either add water to milk or make synthetic milk. However, the addition of water dilutes milk resulting into decrease in specific gravity. For maintaining the specific gravity, an alternative route of milk adulteration is adapted. Addition of urea, detergent and oils to milk are found to be lucrative due to their low cost. This practice of adulterating the milk is increasing day by day, particularly in metro cities of India and in developing countries. Recently, there were reports of mustard oil being adulterated with argemone, polybromide and even with mobil oil and as a result of which several people were affected and died in Delhi [1-2]. Similarly, there were reports of adulteration of milk and other dairy products in many parts of India[3-4].

Milk adulteration can be of two types; one, by making synthetic milk and second by dilution of milk by water. Synthetic milk is produced by blending urea, cooking oil, detergent, caustic soda, sugar, salt and skimmed milk powder in water to make more profit[5]. It does not contain natural milk and hence is devoid of essential nutrients. In second type, natural milk, is adulterated diluting with water, and addition of substances such as urea, fat, sugar, neutralizers, salt, hydrogen peroxide, etc. to maintain desired viscosity and specific gravity of milk. Concentration of urea in natural milk varies from 0.2 –0.7 g/L. In adulterated milk, its concentration is about twenty times more. The added adulterants are thus harmful for the consumers and methods for their detection are available [6-8]. Therefore, urea is a chief component whose concentration can play a key role to differentiate whether the milk is natural or adulterated.

Here, development of a dry reagent strip for instant detection of urea in adulterated milk is being reported. This strip is reliable, easy to handle and economical . The technique developed being useful for the detection of adulterated milk is discussed in the present article.

Experimental Procedure

Materials - Tris (hydroxymethyl) aminomethane, glutaraldehyde and β-mercaptoethanol were purchased from M/s Sigma Chemical Company U.S.A. Adhesive used was from Vamicol, PSV, India. Milk and plastic sheets were purchased from local market. Urea, acetic acid and chromogen were obtained from M/s S.D. Fine Chemicals, India. All solutions were prepared in triple distilled water from quartz distillation apparatus.

Purification of urease enzyme—Urease was purified from Arhar dal (*C. cajan*) and assayed[9]. It had specific activity of 1200 units/mg protein.

Preparation of substrate—(i) Standard urea solutions, ranging from 0.02-14g/L were prepared in water to obtain color gradation of biostrip.

*For correspondence

Table 1—Color chart of the urea dry reagent strip

S.No.	Urea in Milk (g/L)	Color of the Strip
1	Zero-0.20	Yellow
2	0.2-0.70	Peach
3	0.7-1.20	Reddish Brown
4	1.2-1.70	Pink
5	1.7-14.00	Magenta

(ii) Different concentrations of urea ranging from 0.02-14 g/L were also prepared in milk from Mother Dairy New Delhi.

Preparation of dry reagent strip—Urease (10 mg, specific activity of 1200 units/mg protein) was dissolved in 9.0 mL of 25mM tris-acetate buffer, *p*H 5.5. It was mixed thoroughly with 1 mL aliquot of 0.5 percent chromogen solution. Stability of urease was increased by adding 100µL of β-mercaptoethanol. Cross-linking reagent (0.01% glutaraldehyde) was added to the above solution. The mixture was then centrifuged at 10,000 g for 5 min. The supernatant was immobilized on Whatman No 1 paper and dried at 30°C in a humidity free chamber. After complete drying, color of the paper changed from white to light yellow. It was cut into several pieces of 5mm width.

The plastic sheet (1mm thickness) was cut into size of 9 x 90 cm. The immobilized pieces of Whatman paper were pasted onto the plastic sheet using non-reactive adhesive Vamicol (diluted 1:10 with water). The sheets were dried in a humidity free chamber for 4 h and cut into 0.5 x 9.0 cm pieces in such manner that one end of the strip has an enzymatic pad and the other is used for handling. These strips were packed into bottles containing silica gel bags as desiccant and stored at room temperature for almost one year.

Results and Discussion

In routine manner urea is estimated by standard chemical and enzymatic methods [10-14]. These methods require lab space, spectrophotometer and trained personnel, while in case of urea strip person without training can check urea concentration in the milk.

The urea dry reagent strip gives different shades of colour depending upon concentrations of urea added to milk. The colour ranges from light yellow to magenta (Table 1). The normal value of urea present in milk, as reported earlier, is less than 0.70 g/ L [10-14]. Control (yellow colour of biostrip) showed normal value of urea present in natural milk, whereas adulterated milk showed different shades of color from reddish brown to dark pink, depending upon the concentration of urea added. Response time of the strip was 30 s.

In this strip, immobilized urease converts urea into ammonia and carbon dioxide after dipping into milk samples. The liberated ammonia then reacts with dye and changes the color of the strip. Reading is taken after 30s to visualize a change in the colour and then compared with the colour chart printed on the bottle. The efficacy of biostrip was carried out using the standard solution of urea in milk and comparing the values obtained by various reported methods [10-14].

The results obtained with urea biostrip are comparable with other routine methods. The enzyme used from other sources also showed similar results in urea strip. The strips are stable for more than a year at room temperature, if stored under humidity free conditions. Thus, biostrip technique is useful for qualitative and semiquantitative estimation of urea present in adulterated milk. The strips can be used by dairy inspectors. Being visual assessment this technique does not require any specialized training or sophisticated equipment.

Acknowledgement

The authors are thankful to Prof. S. K. Brahmachari, Director, Centre for Biochemical Technology for encouragement and providing necessary facilities to carry out the work.

References

1 *The Hindustan Times*, Newspaper from Delhi, August 28 (1998) 5
2 *Navbharat Times*, Newspaper from Delhi, August 27 (1998) 3
3 Sharma S, *The Hindustan Times*, Newspaper from Delhi, July 6 (1996) 4
4 Choudhary M R, *Navbharat Times*, Newspaper from Delhi, Sept. 28, (1997) 3
5 Bansal P & Bansal N, *Current Sci*, 73 (11), (1997) 904
6 *ISI Handbook of Food Analysis*, Part XII, Manak Bhawan, New Delhi, 17
7 *Dairy products*, Official Methods of Analysis of AOAC International, 16th Ed. 2, 33
8 Varley H, *Practical Clinical Biochemistry*, 4th Ed., (CBS Publishers, Delhi, India), 1969
9 Malhotra O P & Rani I, *Ind J Biochem Biophys*, 6, (1969) 15
10 Singhal O P, Bector B S & Ram M, *NDRI Annual Report*, (1993) 81
11 Harrison G A, *Chemical Methods in Clinical Medicine*, 3rd Ed. Churchill, London (1947)
12 Chaney A L 7 Marbach E P, *Clin Chem*, 8 (1962) 132
13 Natelson S, *Microtechniques of Clinical Chemistry for the Routine Lab.*, edited by Thomas C C, (Springfield, Illinois), 381
14 Kumar A, Kumar H, Tulsani N B & Joshi A P, *Ori J Chem*, 14 (2), (1998) 189