A rapid paper chromatographic method for detection of anionic detergent in milk

Amit K. Barui · Rajan Sharma · Yudhishthir S. Rajput · Smita Singh

Revised: 15 December 2012 / Accepted: 17 January 2013 / Published online: 29 January 2013

© Association of Food Scientists & Technologists (India) 2013

Abstract A paper chromatographic method for the detection of adulteration of anionic detergent in milk is described. The method is based on the complexing of anionic detergent with methylene blue dye and separation of complex from free dye using simple paper chromatographic method. Since complexing of detergent is with dye, visualization is direct without involvement of subsequent detection of complex. The method is simple and results are available in 10 min. The method is sensitive to detect 0.1 % (w/v) labolene (laboratory grade detergent) or 0.01 % (w/v) sodium dodecylbenzene sulfonate (pure anionic detergent) in milk. The method can be adopted at quality control laboratories in dairies for ascertaining the quality of milk.

Keywords Anionic detergent · Paper chromatography · Milk adulteration · Milk quality · Methylene blue dye · Dye-detergent complex

Introduction

India has emerged as the highest milk producer in the world (Anonymous 2009). However, as a large proportion of milk produced in India is processed by unorganized sector, the quality of such milk and milk products is far from satisfactory. Chemical quality of milk is linked with the hidden desire of unscrupulous milk producers/suppliers for making

more profit. Milk has been reported to be adulterated with chemical substances (Arora et al. 2004; Souza et al. 2011). White colored milk-like preparation, produced by blending a well-designed assortment of urea, detergent, vegetable oil, neutralizers, sugar and water (Bansal and Bansal 1997; Paradkar et al. 2000a, b, 2001; Jha and Matsuoka 2004; Amrutkar 2008) is reported to be added into milk. Reports have been received that large quantities of such spurious and fatal product have found their way into the market. Detergent is believed to be one of the essential components of such milk-like preparation and is foreign to pure milk (Paradkar et al. 2000a, b, 2001; Sadat et al. 2006). Detergent is added to emulsify foreign fat from non-milk origin, which is added as a substitute for milk fat (Paradkar et al. 2001; Barui et al. 2012). Anionic detergents are used most widely in such milk-like preparations due to their low cost and ease in availability (Barui et al. 2012). Several attempts have been made to detect anionic detergent in such milk samples. Paradkar et al. (2000b) have proposed a method to detect anionic detergents in milk, based on the extraction of a detergent-methylene blue complex in chloroform and further quantified it by measuring the absorbance values of the extracted layer at 653 nm. Using this method a blank value of 0.52 was obtained for pure milk samples, which was considered as too high for blank reading. Jha and Matsuoka (2004) have described a method based on near-infrared spectroscopy (NIRS) and the method can predict the content of adulterants only up to 10 % (v/v) level. The alternate current conductance of pure and adulterated milk is different and using this principle, 25 % (v/v) adulteration can be detected (Sadat et al. 2006). Therefore, it becomes essential to develop a simple and sensitive rapid method for detecting presence of anionic detergent in milk. In present communication, a paper chromatographic method for detection of detergent in milk is described.

A. K. Barui · R. Sharma (✉)
Dairy Chemistry Division, National Dairy Research Institute,
Karnal 132001, India
e-mail: rajansharma21@gmail.com

Y. S. Rajput · S. Singh
Animal Biochemistry Division, National Dairy Research Institute,
Karnal 132001, India

Materials and methods

Materials

Pooled or individual raw cow and buffalo milk samples collected from the cattle yard of the Institute in clean glass bottles were brought to the laboratory and used for analysis. Methylene blue (Sigma Aldrich Inc. St. Louis, USA), sodium dodecylbenzene sulfonate (SDBS, Sigma Aldrich Inc. St. Louis, USA) and Chloroform (Merck Specialties Pvt. Ltd., Mumbai, India) used during the course of the study were of analytical grade. Labolene—a commercial detergent (Thermo Fisher Scientific India Pvt. Ltd., Mumbai, India) was used in the present study for preparation of adulterated milk samples. Filter paper (Whatman No. 1, circular diameter 18.5 cm, GE Healthcare, Ahmedabad, India) was used for paper chromatographic separation. Other chemicals were purchased from SD Fine-Chem Ltd., Mumbai, India.

Methods

Sample preparation

Stock solution of Labolene (10 %, w/v) and SDBS (1 %, w/v) was prepared and kept in refrigerator (4 °C). These stock solutions were used for spiking milk samples to obtain various Labolene or SDBS concentration. Milk samples were repeatedly inverted to allow uniform mixing of detergent.

Dye solution

Methylene blue dye was prepared by dissolving 20 mg dye in 100 ml double-distilled water. The dye solution is stored in brown bottle at room temperature.

Paper chromatography method

I. Preparation of Paper

A circle of 3 cm diameter was drawn (with pencil) on Whatman paper No. 1 (diameter 18.5 cm). A circular hole of 5 mm diameter was made with the help of punching machine (generally used for paper punching) at the center of filter paper.

II. Preparation of wick

A paper wick of about 3 cm height was prepared from a rectangular (15×4 cm) Whatman No. 1 sheet. Repeated cut (distance between cut 1 mm; cut height 2.5 cm) were made in above said rectangular sheet at one side (lengthwise) and then the sheet is rolled in a way so that diameter of rolled sheet is less than diameter of central hole of circular Whatman filter paper. The cut portions are pulled outward. This device is referred as wick.

Fig. 1 Differential mobility of free dye and dye-detergent complex. Five microliter of methylene blue and 5 μ l mixed solution (methylene blue dye and detergent; 0.1 % sodium dodecylbenzene sulfonate, w/v) were applied at indicated point and subjected to paper chromatographic separation

III. Sample loading and chromatographic run

Chloroform was poured up to 1 cm height in a glass petri-plate (diameter 14 cm) and covered with a lid to allow equilibration of solvent in vapour phase. One milliliter of milk was mixed with 1 ml dye solution, using vortex mixer for 15 s. Five microliters of mixed contents was spotted on the circle drawn (using graphite pencil) on the filter paper. The spot was dried using hot air blower. Then, the wick was inserted at the center of the filter paper. Filter paper containing wick was placed in chloroform pre-saturated petri-plate such that edge of filter paper rests on edge of a petri-plate and wick rests at the bottom of petri-plate. The mobile phase (chloroform) was allowed to run for 4 min. Then the filter paper was air dried. The number of blue spots appeared on the filter paper were noted and photographed.

Results and discussion

Anionic detergents carry hydrophobic moiety at one end and negative charge on other end. In contrast, cationic dyes carry

Fig. 2 Paper chromatographic separation of methylene blue dye-detergent complex; methylene blue dye added to milk and methylene blue dye added to milk spiked with 0.1 % (w/v) labolene. Dye-detergent complex moves faster than free dye

Fig. 3 Sensitivity of paper chromatographic method for detecting labolene in milk. Milk samples were spiked with (c) 0.05 %, (d) 0.10 %, (e) 0.20 %, (f) 0.60 % (w/v) labolene. Dye alone (a) and pure milk (b) were used to know the mobility of free dye alone and in pure milk

positive charge. Therefore, anionic detergent and cationic dye make complexes instantaneously (Pedraza et al. 2007). This leads to change in polarity of dye. In the present experiment, a method has been developed to separate dye-detergent complex from free dye. Dye-detergent complex is more non-polar as compared to free dye and therefore in partition chromatography dye-detergent complex will move fast when chloroform (non-polar solvent), is used as mobile phase (Fig. 1). There was clear separation of free dye from that of dye-detergent complex ($n=58$) (Fig. 1). In fact, there were two well separated spots, other than the spot corresponding to free dye. One of this spots is eclipse shaped while front running spot is diffused. When concept was validated in milk sample spiked with 0.1 % (w/v) labolene, there was one additional spot (eclipse shaped) while diffused spot visible with SDBS (Fig. 1) was not observed (Fig. 2). The labolene is laboratory grade detergent having about 21 % (w/w) active ingredients (Barui et al. 2012) while purity of SDBS was nearly 100 % (w/w). It appears that absence or presence of diffused spot is dependent on the concentration of active ingredients in commercial or analytical preparation. The intensity of the additional spots observed in milk samples spiked with detergent was dependent on the concentration of labolene (Fig. 3) or SDBS (Fig. 4). Since dye is colored, no additional steps are required for visualization of spot. The limit of detection (LOD) was determined by spiking milk samples with 0.05, 0.10, 0.20 and 0.6 % (w/v) labolene ($n=3$) or 0.01, 0.04, 0.08, and 0.10 % (w/v) SDBS ($n=3$); the results are shown

in Figs. 3 and 4 respectively. The LOD for labolene and SDBS was 0.1 and 0.01 %, respectively. Diffused spot was clearly visible at higher concentration of labolene (Fig. 3e) corresponding to 0.20 % (w/v) concentration. Further, LOD of commercial detergent will depend on the extent of active ingredients present in commercial preparation. The test results are available in 10 min. No false positive or false negative results were observed during the course of study. The method can be adapted in quality control laboratories to check the purity of milk as also, it helps to detect added synthetic milk (usually contains detergent) to natural milk.

Conclusions

The chromatographic method is based on the difference in the solubility of free dye and dye-detergent complex. In case of pure milk, only one spot is visualized. However, in case of adulterated milk, one or more additional spots were observed. The fast moving diffused spot was visible at relatively higher concentration of detergent, perhaps due to complexing of more than one detergent molecule with one molecule of methylene blue dye. No additional reagents were required for visualizing the spots. The intensity of spot depends on the amount of anionic detergent present in the sample. The method has been found to be sensitive to detect 0.1 % (w/v) of labolene or 0.01 % (w/v) of SDBS in milk.

Fig. 4 Sensitivity of paper chromatographic method for detecting sodium dodecylbenzene sulfonate in milk. Milk samples were spiked with (c) 0.01 %, (d) 0.04 %, (e) 0.08 %, (f) 0.10 %

(w/v) sodium dodecylbenzene sulfonate. Dye alone (a) and pure milk (b) were used to know the mobility of free dye alone and in pure milk

Acknowledgment This work was supported by National Agricultural Innovation Project (NAIP), Indian Council of Agricultural Research, India and authors sincerely acknowledge the financial support.

References

- Amrutkar S (2008) Synthetic milk-white poison. *Dairy Plan* 4:32–34
- Anonymous (2009) Food and agricultural commodities production—Commodities by countries. <http://faostat.fao.org/site/339/default.aspx>. Accessed 05 February 2012
- Arora S, Sharma V, Raj D, Ram M, Kishore K (2004) Status of milk adulteration in some states of North India. *Indian J Dairy Sci* 57:65–66
- Bansal P, Bansal N (1997) Synthetic milk—genesis, current status and options. *Curr Sci* 73:904–905
- Barui AK, Sharma R, Rajput YS (2012) Detection of non-dairy fat in milk based on quantitative assay of anionic detergent using azure A dye. *Int Dairy J* 24:44–47
- Jha S, Matsuoka T (2004) Detection of adulterants in milk using near infrared spectroscopy. *J Food Sci Technol* 41:313–316
- Paradkar MM, Singhal RS, Kulkarni PR (2000a) An approach to the detection of synthetic milk in dairy milk: 1. Detection of urea. *Int J Dairy Technol* 53:87–91
- Paradkar MM, Singhal RS, Kulkarni PR (2000b) An approach to the detection of synthetic milk in dairy milk: 2. Detection of detergents. *Int J Dairy Technol* 53:92–95
- Paradkar MM, Singhal RS, Kulkarni PR (2001) An approach to the detection of synthetic milk in dairy milk: 3. Detection of vegetable oil and sodium bicarbonate. *Int J Dairy Technol* 54:34–35
- Pedraza A, Sicilia MD, Rubio S, Pérez-Bendito D (2007) Assessment of the surfactant–dye binding degree method as an alternative to the methylene blue method for the determination of anionic surfactants in aqueous environmental samples. *Anal Chim Acta* 588:252–260
- Sadat A, Mustajab P, Khan IA (2006) Determining the adulteration of natural milk with synthetic milk using ac conductance measurement. *J Food Eng* 77:472–477
- Souza SS, Cruz AG, Walter EHM, Faria JAF, Celeghini R, Ferreira M, Granato D, Sant'Ana AS (2011) Monitoring the authenticity of Brazilian UHT milk: a chemometric approach. *Food Chem* 124:692–695