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RESEARCH ARTICLE

Performance evaluation of strip based test for detection of urea adulteration in milk

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Abstract: Milk samples spiked with various concentrations of urea ranging from 0.02, 0.04, 0.06 and 0.08% was used to check the urea detection ability of the developed strip. The LoD in each type of milk viz. raw, pasteurized and boiled was found to be 0.07% urea. Further, 121 (nos.) milk samples (cow, buffalo and mixed milk) were analyzed with the MilkoScan FT120 and developed strip for urea content. The urea content of the milk sample obtained using MilkoScan was compared with the colorimetric result of the strips. Both these results were in alignment. Moreover, the data of urea content obtained from MilkoScan and strip were used to calculate sensitivity, specificity and overall accuracy of the strip which were found to be 90.90, 92.00 and 90.09%, respectively. The strip performance was also checked for its effectiveness spectrophotometrically using 10 mixed milk samples. The developed strips were found to be stable when stored at room temperature (30-40°C) and refrigeration temperature (7±2°C) up to six months.

Key words: Milk, urea, adulteration, strip, detection, performance

Introduction

Milk adulteration is one of the serious issue faced by the dairy industry, particularly in the developing countries. Milk is adulterated with many adulterants including urea. Urea is one of the common adulterants being reported in milk samples collected from various cities of India (Hanford et al. 2016; Roy et al. 2017). Urea is common adulterant found in milk to mask the developed acidity, to increase milk whiteness, to show apparent increase in protein content (Azad and Ahmed, 2016). The official wet chemistry based method for detecting urea in milk as recommended by FSSAI is labour-intensive, requiring specific glassware and reagents. The estimated average time of this analysis is about 15 min per sample. Wet chemistry methods are disadvantageous as it requires chemical preparation and hence becomes difficult to use at the field level. In contrary, dry chemistry based methods do not require chemicals and are easy to use at remote locations. IDF has also specified enzymatic method for determination of milk urea content using differential pH (ISO 14637, IDF 195: 2004). Several workers have recommended strip based methods for detection of urea in milk (Kumar et al. 2000; Panchal, 2013; Gautam, 2016; Luther et al. 2017). Thus, a low cost, sensitive urea detection strip can be a useful quality control tool for dairy industry.

There are several strip based tests available for detection of urea adulteration in milk, albeit with minimal published data on validation. Validation of the strips for urea detection is quite necessary as urea is one of the naturally occurring substance in milks of many species. The Limit of Detection (LoD) of the urea detection strip was determined using spectroscopic method, while sensitivity, specificity, overall accuracy was determined using FTIR method.

Savaliya et al. (2022) developed a strip-based test for detection of urea adulteration in milk. This study is contemplated to evaluate the performance of the developed strip-based test for the detection of urea adulteration in raw, pasteurized and boiled milk. The criteria were sensitivity, specificity and overall accuracy of the developed strip for detection of urea in milk. The effect of storage of urea added milk on the performance of the test was also evaluated. The FTIR method was selected as it is routinely used in the industry with the advantages of faster results, good sensitivity and simplicity. Further, if the strip performance is comparable to the results of the FTIR, it would be easy to convince the users to adopt a simple, rapid, sensitive and cheap alternative to high end instruments. Urea is natural constituent of milk,

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however a maximum limit for urea concentration in milk is set by FSSAI as 700 ppm (FSSR, 2011).

Materials and Methods

Milk samples

Pooled samples of cow and buffalo milk were procured respectively from the Livestock Research Station and Reproductive Biology Research Unit of Anand Agricultural University, Anand, Gujarat. Some of the milk samples were also procured from the local farmers for certain components of the study. Mixed milk samples were prepared by mixing cow and buffalo milk in equal proportions.

In order to study the effects of milk processing, the milk samples were pasteurized and boiled at the laboratory.

Total 121 milk samples of cow, buffalo and mixed milks were analyzed using both MilkoScan FT120 and developed strip.

Chemicals and instruments

The chemicals and reagents used were: β-mercaptoethanol (AR grade, Loba Chemie (P) Ltd., Mumbai, India); Ethanol (Shree Madhi Vibhag Udhyog Sahakari Manadali Ltd., Surat, India); Phenol red (AR Grade, Central Drug House Ltd., New Delhi); Urea (Allied Chemicals Corporation, Vadodara , India); Trichloroacetic acid (TCA)(Merck Specialities (P) Ltd., Mumbai, India); *p*- Dimethyl amino benzaldehyde (AR Grade, SRL Pvt. Ltd., Mumbai, India); Potassium dihydrogen orthophosphate (KH_2PO_4): (AR Grade, Qualigens Fine Chemicals, Mumbai, India); Dipotassium hydrogen phosphate (K_2HPO_4): (AR grade, Loba Chemie Pvt. Ltd., Mumbai, India) ; Chromatography paper (GE Healthcare, UK Ltd.); Soybean seeds (Anand Agricultural University, Anand); The UV-1800 model spectrophotometer from SHIMADZU, Japan were used for spectroscopic analysis and MilkoScan FT120 (FOSS), Denmark was used for rapid urea determination.

Methods for determination of urea in milk

The urea detection strips were prepared using the method as described by Savaliya et al. (2022).

In order to determine LoD of the developed strip, at first the natural urea content of the raw milk was determined spectrophotometrically using the DMAB method (FSSAI, 2016). The standard curve was prepared for the determination of urea content (Supplementary Fig. 1). The urea level in milk was then adjusted to 0.05, 0.06, 0.07, 0.08, 0.09 and 0.1% (natural + spiked urea) using extraneous urea.

The quantitative results of urea in milks obtained using MilkoScan FT120 was used for comparison with strip results for sensitivity, specificity and overall accuracy of the developed strip for urea detection.

Simultaneously, the appearance of colour on the strip was also observed subjectively for positive or negative results of the strip test.

For determination of the response time, the colour developing on the strips was noted at different time interval of 1 to 10 min.

Results and Discussion

Determination of LoD of strip

For determination of LoD, 3 different types of milk samples viz. raw, pasteurized (heated at 75°C for 15 s) and boiled milk were used. The developed strips were dipped separately for 10-15 s in control and urea spiked milk samples. Subsequently, these strips were kept on a white paper sheet for observation of the developed colour.

In case of raw milk samples (Fig. 1), the strips dipped in the milk sample containing natural urea (control sample) turned yellow. The strips dipped in milk samples containing 0.05 and 0.06% urea produced different shades of orange colour. However, different shades of pink colour in the strips were observed for milk samples containing ≥0.07% urea. The change in colour of the strips used for control sample and milk containing extraneous urea could be differentiated at all levels, the minimum noticeable level to be differentiated being 0.07%. Thus, for raw milk, the LoD of the developed strips was considered to be 0.07%.

In case of pasteurized and boiled milk (Fig. 1), identical colour change in strips to that of raw milk samples (both control and urea spiked milk samples) were observed. Hence, for pasteurized and boiled milk samples, the LoD of the developed strips was considered to be 0.07%.

The results of the performance of the strip-based test indicated that the developed strip can be effectively used for detection urea in raw, pasteurized and boiled milk with the LoD of 0.07%.

Gautam (2016) and Panchal (2013) have indicated the LoD of their developed strips as 0.08 and 0.09% urea in milk respectively. Luther et al. (2017) have reported the LoD of paper card for urea as 0.07%. Panchal (2013) checked the effect of boiling of milk on the performance of urea detection strips using 0.25% externally added urea and did not observe significant effect on the colour intensity of the strips.

Determination of response time of strip

The developed strips were evaluated for determining the time required to obtain noticeable colour change between control and urea spiked milk. The total urea content in the pooled raw milk samples were adjusted to 0.05, 0.06, 0.07, 0.08, 0.09 and 0.1% (natural + spiked urea) levels (Supplementary Fig. 2). The strips

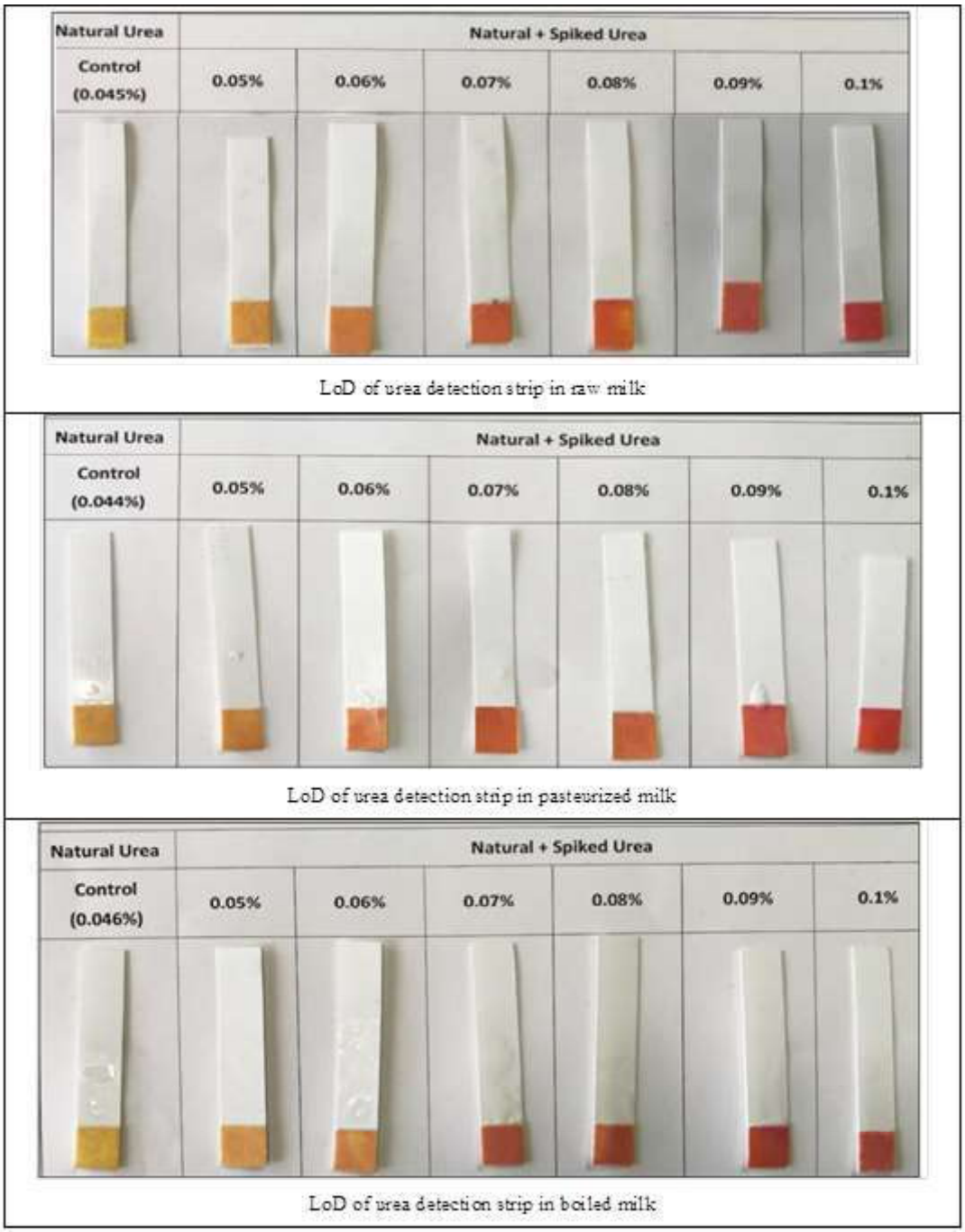

Fig. 1 LoD of urea detection strip in raw, pasteurized and boiled milk

were simultaneously dipped in all the samples of milk and kept for 10-15 s. Subsequently, the strips were kept on the white paper sheet and observed for colour development. The images of the treated strips were taken at the interval of 1 min. The change in colour development between strips used for control and urea spiked milk samples was observed. Up till 2 min. a visually noticeable colour change between control and urea spiked milk samples was not observed. Three minutes onwards the colour change developed between control and urea spiked samples was significantly clear till 5 min of time. Hence, the response time of the strip was considered as 3-5 min. It was also observed that after 6 min. the colour of the strips started to fade. This can lead to misinterpretation of the test result, so the result should be recorded within 3-5 min.

Repeatability of developed strip for urea detection

For checking the repeatability of the developed strip for urea detection in milk, 20 different mixed milk samples were analyzed. The natural urea content in these milk samples was estimated

Fig. 2 Repeatability of developed strip for urea detection

(DMAB method, FSSAI, 2016) and found to vary between 0.025 to 0.053%. Furthermore, these milk samples were spiked with external urea to adjust the total urea content to 0.07% (LoD). The milk samples without spiked urea (only natural urea) were used as a negative control.

As per Fig. 2, the milk sample no. 1-5, 10-15, 21-25 and 31-35 containing only natural urea gave negative test when checked using the developed urea detection strip, while, milk sample no. 6-10, 16-20, 26-30 and 36-40 (containing external urea to a level of 0.07%) gave positive test when checked using the developed urea detection strip. Based on these observations, it can be inferred that the developed strip can detect urea in all 20 milk samples containing 0.07% urea (i.e. pink colour). However, milk samples containing natural urea alone developed yellow to orange colour when checked using the strip. These observations ascertained the repeatability of the developed urea detection strip.

Reproducibility of the developed strip

The performance of the developed strip for urea detection was checked against FT120 milk analyzer and spectrophotometer in order to determine reproducibility, accuracy, sensitivity and specificity (Supplementary Fig. 3(A), 3(B), 3(C), 3(D)). For better understanding of the performance of the test, the results of the strip is portrayed in text of different colours (Supplementary Table 1).

Total 121 milk samples including cow, buffalo and mixed milk were analyzed using both MilkoScan FT120 and developed strip. However, 10 samples got soured and could not be tested on the MilkoScan FT120 but were analyzed with strips. The urea content

Fig. 3 Comparative performance of urea detection strip with spectroscopic method

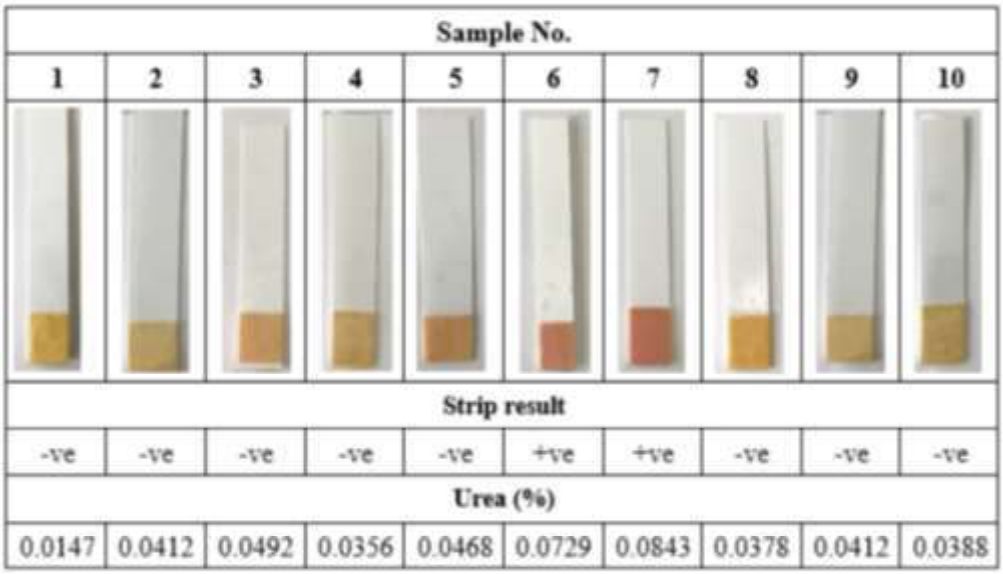

Table 1: Computation of sensitivity, specificity and overall accuracy of strip

Total number of samples analyzed: 111		
Strip	MilkoScan FT120 (Yes)	MilkoScan FT120 (No)
Positive test (yes)	10	8
Positive test (no)	1	92
a= True positive sample	10	
b= False positive sample	8	
c= False negative sample	1	
d= True negative sample	92	
Sensitivity of strip (%)	a / (a+c) =	90.90
Specificity of strip (%)	d / (b+d) =	92.00
Overall accuracy of strip (%)	(a+d) / (a+b+c+d)=	90.09

of the milk sample obtained using MilkoScan FT120 was compared with the colorimetric result of the strips.

The data obtained by analyzing the milk samples both using strip and MilkoScan FT120 were utilized for calculation of sensitivity, specificity and overall accuracy of the developed strip according to the formula given by Cleophas et al. (2012).

Accuracy can be defined as test's ability to show which milk is true positive or true negative with respect to parameters being tested. For the qualitative test the minimum level of desired accuracy is 50%, however as can be seen from the Table 1 the value of the overall accuracy obtained for the developed strip was 90.09%. Sensitivity is defined as the ability of test to detect true positive. In the developed strip value of sensitivity was found to be 90.90%. Specificity is defined as the ability to detect true negative sample. For the developed strip specificity was found to be 92.00%.

Performance of strip against spectrophotometric method

The strip performance was evaluated spectrophotometrically using 10 mixed milk samples. Milk samples were analyzed using the urea quantification method given by the FSSAI (2016). These samples were subsequently, analyzed using a developed urea detection strip. The results obtained by both methods were compared. The strips used for milk samples with urea content <0.07% did not give pink colour. Thus, the result was considered negative. Samples containing ≥0.07% urea showed pink colour

on the developed strip, indicating positive results. The performance of the strip was validated by spectrophotometric method (Fig. 3).

Storage stability of strip at different storage temperatures

The developed strips for urea detection were packed in air tight plastic container with desiccant and stored at two different temperatures viz. room temperature (30-40°C) and refrigeration temperature (7±2°C). The strips were evaluated every 15 days for their performance with respect to their response time and LoD. Pooled mixed milk samples spiked with 0.07% urea were used to check the stability of the strips. At room temperature and refrigeration temperature, all the strips had the response time of 3-5 min with LoD values of 0.07%. The results indicated the stability of the strips and was found to be satisfactory both at room and refrigeration temperature, since, there was no change in response time and LoD.

Conclusion

Milk samples (raw, pasteurized and boiled) were spiked with extraneous urea (0.02, 0.04, 0.06 and 0.08%) for LoD determination of the developed strip using spectrophotometric method and was found to be 0.07% urea. Further, 121 (nos.) milk samples (cow, buffalo and mixed milk) were analyzed with the MilkoScan FT120 for urea content and the results were compared with that of the developed strip. Both the results were in alignment. These data were used to calculate sensitivity, specificity and overall accuracy and were found to be 90.90, 92.00 and 90.09%, respectively. The strip performance was also checked for its effectiveness spectrophotometrically using 10 mixed milk samples. The developed strips were found to be stable when stored at room temperature 30-40°C) and refrigeration temperature (7±2°C) for 120 days. The developed urea detection strip can be used conveniently at field, household and plant level to quickly detect adulteration urea in milk. This can be of great help in ensuring food safety and quality of milk. In general, it can be concluded that using inexpensive material and simple technology, we can produce milk urea detection strip with required accuracy, specificity and sensitivity.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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