International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2017; 5(6): 1572-1576

Received: 04-09-2017
Accepted: 05-10-2017

Chauhan Mahipalsinh P
Former Scholar,
Dairy Chemistry Department,
SMC College of Dairy Science,
Anand Agricultural University,
Anand, Gujarat, India

Arpita Agnihotri
Former Scholar,
Dairy Chemistry Department,
SMC College of Dairy Science,
Anand Agricultural University,
Anand, Gujarat, India

AI Shaikh
Associate Professor,
Dairy Chemistry Department,
SMC College of Dairy Science,
Anand Agricultural University,
Anand, Gujarat, India

SI Patel
Assistant Professor,
Dairy Chemistry Department,
SMC College of Dairy Science,
Anand Agricultural University,
Anand, Gujarat, India

Dr. KD Aparnathi
Professor & Head,
Dairy Chemistry Department,
SMC College of Dairy Science,
Anand Agricultural University,
Anand, Gujarat, India

Correspondence
AI Shaikh
Associate Professor,
Dairy Chemistry Department,
SMC College of Dairy Science,
Anand Agricultural University,
Anand, Gujarat, India

Evaluation of DMAB test for detection of urea mixed in milk and improvement in its efficacy

Chauhan Mahipalsinh P, Arpita Agnihotri, AI Shaikh, SI Patel and KD Aparnathi

Abstract
Four different categories (carbohydrates, cheaper fats and vegetable oils, non-protein nitrogenous compounds and salts) are used. Amongst the NPN compounds use of urea is most widely reported. DMAB test is one of the most commonly used qualitative tests for detection of urea in milk. For optimization of qualitative tests, different aspects were selected. Different approaches such as selection of medium (milk itself, whey) to perform the test and modification of DMAB reagent by using methanol instead of ethanol in performing the tests to get better results. Depending on the nature of the adulterant and qualitative test to be performed for its detection, a number of approaches were attempted to bring about the improvement in the test. The outcome of the present study suggested a definite improvement in the performance of DMAB tests for detection of urea adulterant in milk. Some of the notable outcomes are ease in judgement due to better differentiation between results of control and test samples, improved sensitivity due to reduction in limit of detection, lowering risk of health hazards as well as environmental pollution due to elimination of corrosive/toxic/poisonous chemical and/or to overcome difficulties in getting some of the prohibited chemicals due to elimination of their use. Moreover, the LoD of urea in whey was 0.06, whereas, in milk was 0.2.

Keywords: Milk, adulteration, urea, qualitative test

Introduction
Milk plays a crucial role in the diet of humans and is considered as one of the essential food commodities. Milk products have high nutritional value and are consumed all around the world, playing a fundamental role in international commerce, thus giving milk a great economic importance [1]. However, individuals aiming higher profits and flouting ethical values renders milk susceptible to several types of adulteration [2]. Recent reports have revealed the use of numerous adulterants in milk intended for either raising the quantity (e.g. water), increasing the compositional values (e.g. urea, sucrose, starch, etc.) or increasing the shelf life (e.g. neutralizers and preservatives) [3].

The most widely practiced approach of adulterating milk is to add water in it and subsequently adding urea to raise solid not fat (SNF). Urea is a normal constituent of milk and amounts to about 55 per cent of the total non-protein-nitrogen in milk [4]. Additionally urea being nitrogenous compound, will give false high level of protein if analyzed by Kjeldahl method [4, 5]. In India, the addition of external urea to the milk is not permitted legally under the FSSA (2006) [4]. However, as per FSSR (2011), the urea content in milk should not be more than 70 mg/100 ml [6-8]. Urea is one of the major ingredients of synthetic milk along with caustic soda, detergent, sugar and foreign fats. Adulteration of natural milk with synthetic milk increases the level of urea and it may causes toxicological hazards [8, 9]. An increase in urea concentration causes renal failure such as acute or chronic urinary tract obstruction with shock, burns, dehydration, and gastrointestinal bleeding [10]. Estimation of urea concentration in milk may serve as a tool for checking the menace of adulteration of natural milk with synthetic milk [8]. There are several methods reported for urea detection and estimation in milk such as chemical method, enzymatic methods, infrared (IR) spectroscopy, etc. [4]

The presence of added urea in milk can be detected using qualitative tests like DMAB (*p*-dimethyl aminobenzaldehyde) test [8, 11-17].

Large number of qualitative test are reported for detection of various adulterants in milk, Moreover, very wide variations are found in procedure reported for a particular test of a given adulterant. However, it appears from the survey of literature that no attention has been paid on

systematic work for improving performance of the qualitative tests suggested for detection of adulterants in the milk. There is ample scope to improve performance of these qualitative tests by optimizing different parameters of the procedures. Therefore, the present study was carried out with the objective to establish a comparative appraisal between various procedures reported for DMAB test for the detection of urea in milk.

Materials and methods

Milk sample

Raw milk samples were procured from dairy farm of Anand Agricultural University, Anand. Milk Samples were prepared by spiking the raw milk with urea at the suitable level for a particular test.

Chemicals and reagents

- DMAB (Loba Chemie Pvt. Ltd.), Trichloroacetic acid (Loba Chemie Pvt. Ltd.), Ethyl Alcohol (Shree Madhi Vibhag Khand Udyog Sahakari Mandli., Madhi, Surat), Methanol (S D Fine-Chem Ltd., Mumbai), Lactic acid (Loba Chemie Pvt. Ltd., Mumbai), Hydrochloric acid (S D Fine-Chem Ltd., Mumbai), Citric acid (S D Fine-Chem Ltd., Mumbai), Acetic acid (Spectrochem (P) Ltd., Vadodara) and Urea (SD Fine Chemicals Ltd., Mumbai).

Test procedures used for detection of adulterants in milk

DMAB test

A) Test reported by Bector *et al.* (1998) [12]

In a test tube 5 ml of the suspected milk sample was taken. To it, 5 ml of the 1.6% w/v DMAB reagent was added and mixed well. In another test tube 5 ml of control milk sample (known to be free from added urea) and 5 ml of DMAB reagent were taken and mixed. The presence of urea was confirmed by appearance of deep yellow colour as compared to control sample, which gave a pale yellow colour due to presence of natural urea content. Same procedure was also reported by various authors [13-18].

B) Test reported by Sharma *et al.* (2012) [8]

Equal quantity of milk and 24% TCA was taken in a glass stoppered test tube. Contents were mixed and filtered with Whatman No. 42 filter paper. In another test tube 3 ml of filtrate was taken and 3 ml of 1.6% DMAB reagent was added. The occurrence of distinct yellow colour indicated the presence of added urea in milk sample whereas slight yellowish colour developed in control sample.

Results and discussion

The qualitative and/or quantitative variations are reported for DMAB test, urease test and phenol test used for detection of urea in milk. Therefore, variations within different procedures were evaluated to check their effect on the test results.

From the several test reported for detection of urea in milk DMAB test is most widely used method.[19] To perform the test, 5ml of samples of milk and its respective control were taken and 5ml of DMAB reagent was added and mixed well. The presence of urea was confirmed by the formation of a deep yellow colour in the sample as compared to the control, which gave a pale yellow colour due to presence of natural urea. The 1.6% DMAB reagent was prepared by dissolving 1.6g of DMAB in small amount of ethanol (95%, v/v), 10ml of concentrated HCl is added subsequently and volume was made up to 100ml with ethanol.[19] Three approaches were attempted to modify the test for detection of urea adulteration in the milk. Replacement of ethanol with methanol, evaluation of whey as a medium to perform the test and selection of acid to prepare the whey.

Replacement of ethanol with methanol in preparation of reagent

Since ethanol is costly and prohibited item when compared with methanol, which is relatively cheaper and easily available as a routine chemical; it was thought as replacement. Thus, replacement of ethanol with equivalent amount of methanol was tried for the preparation of DMAB reagent. The sample of milk was prepared by adding 0.21g urea per 100ml of milk. The DMAB test was performed for adulterated sample and control sample of milk (known to be free from urea adulteration) by taking in test tubes. In the first set, the test was performed using DMAB reagent prepared in ethanol. Whereas, in second set, methanol was used for the preparation of DMAB reagent. The result obtained are presented in Plate1.

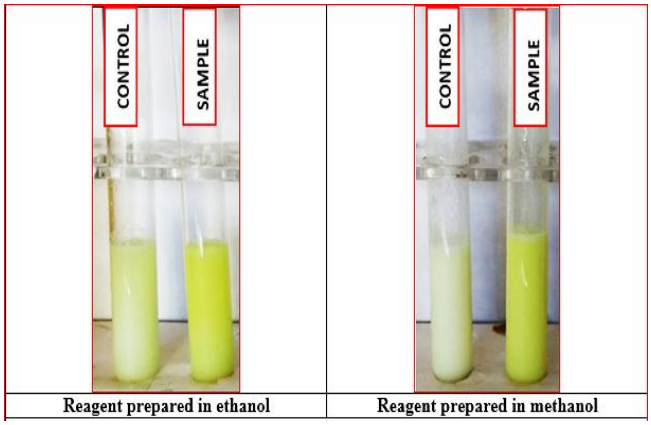

Plate 1: Comparison of DMAB test performed using reagent prepared in ethanol and methanol

It is evident from the Plate 1 that when ethanol was used in preparation of reagent the control sample had light yellow colour, whereas, when methanol was used in preparation of reagent the control sample was having vary faint yellow colour. The adulterated sample of milk in both the cases was dark yellow in colour. Thus the distinction between control and adulterated sample of milk was better in case of DMAB reagent prepared in methanol. Therefore, it was inferred that ethanol can be very well replaced by methanol in preparation of DMAB reagent for using detection of added urea in milk.

Evaluation of whey as a medium for performance of the test

The possibilities were also explored to evaluate the use of whey as a medium for performing the DMAB test for detection of urea adulteration in milk. The adulterated sample of milk was prepared by adding 0.2g of urea per 100ml of milk followed by heating to near boiling and cooling to room temperature. The test was performed in whey obtained by adding acetic acid solution in the adulterated sample of milk, as well as in the whey obtained from the pure sample of milk as a control. For comparing the performance of the test in whey with that of the milk, simultaneously, the test was also performed in the pure sample of milk as well as the adulterated sample of milk. The difference in performance of test when performed in milk and that in whey is presented in Plate 2.

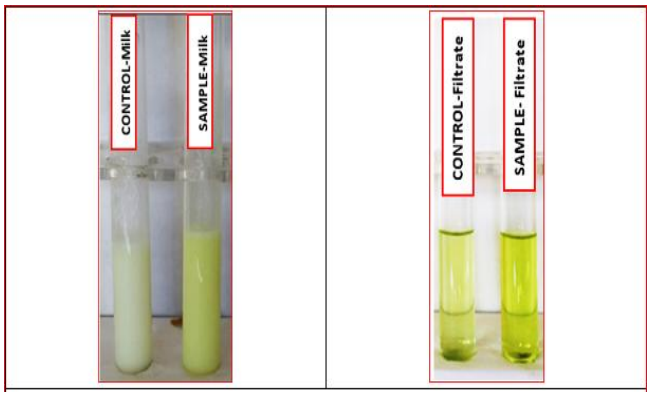

Plate 2: Effect of different medium on performance of DMAB test

The results presented in the Plate 2 indicates that the performance of the test in case of whey was relatively better compare to that in case of milk. There was clear-cut distinction in colour between the control and the adulterated sample when whey was used as a medium to perform the test. Whereas, in case of milk the distinction between control sample and adulterated sample was relatively poor.

The possible reason for better performance of DMAB test in whey compare to milk might be attributed to removal of possible masking effect of colloidal casein. It appears from the literature that no study has been reported so far about comparative appraisal of performance of DMAB test in whey and milk.

Selection of acid to prepare the whey

As better results were obtained for detection of urea in whey as compared to milk itself; further studies were carried out using whey as a medium for detection of urea. Different acids were evaluated as coagulating agent to carry out the test. Four commonly used acids viz. lactic acid, acetic acid, citric acid and hydrochloric acid (HCl) were tried. In case of acetic acid and HCl 10% concentration was used; whereas, in case of citric and lactic acid 5% concentration was used. In both the cases *i.e.* control and adulterated sample, 20 ml milk was taken in a flask and coagulated by adding the aforementioned acids drop by drop till visible coagulation. The content was filtered using Whatman No. 1 filter paper. The whey thus obtained was used as medium for performing the test. The test was performed in whey samples and result obtained are presented in Plate 3.

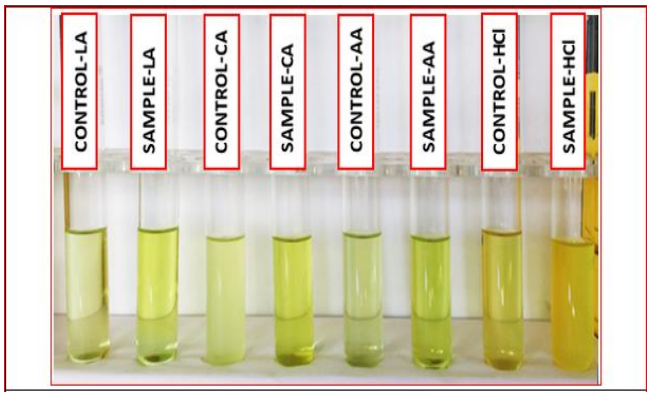

Plate 3: effect of different coagulating acids on performance of DMAB test

It is evident from the Plate 3 that the best differentiation between control and adulterated sample was obtained, when citric acid was used as coagulating agent. The results obtained in case of lactic acid and acetic acid were almost similar, however, control sample was having slight yellow colour when compared with that of the citric acid. The intensity of light yellow colour formed in adulterated sample was relatively low in case of acetic acid and HCl. Therefore, use of citric acid is suggested to obtain whey for performing DMAB test for detection of urea adulteration in milk.

The better performance of citric acid might be attributed to its chelating action towards divalent cation like Ca^{+2}, thereby, reducing the turbidity of the reaction mixture [20].

LoD of DMAB test to detect urea using in milk and whey as a medium

For comparison of LoD of milk and whey as a medium to detect urea by DMAB test, adulterated samples of milk were prepared by addition of urea at the rate of 0.00, 0.01, 0.02, 0.04, 0.06, 0.08, 0.1, 0.2 and 0.3g per 100ml milk. For detection in milk urea was added at the rate of 0.00, 0.01, 0.02, 0.04, 0.06, 0.08, 0.1, 0.2 and 0.3g per 100ml milk. Whereas, for detection in whey, the urea was added in milk at the rate of 0.00, 0.01, 0.02, 0.04, 0.06, 0.08 and 0.1g per 100ml milk, followed by coagulation of milk using citric acid and filtration to obtain the whey. The prepared samples of milk and whey were subjected to DMAB test. Results obtained for milk and whey are presented in Plate 4A and 4B, respectively.

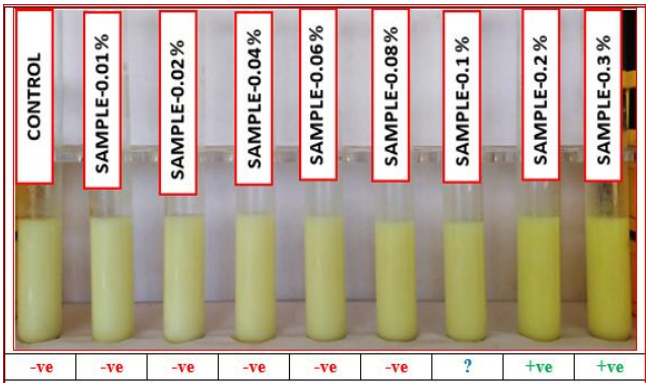

Plate 4A: LoD of DMAB test performed in milk

It is evident from the Plate 4A that no noticeable change in colour of milk was observed up to 0.08g of urea per 100ml of milk, when compared with the colour obtained in control sample of milk. Some distinction in colour of control sample and that of the adulterated sample of milk started from 0.1g urea per 100ml milk, however, the result appeared to be doubtful. The clear-cut distinction between colour of control sample and that of the adulterated sample of milk appeared from 0.2g urea per 100 ml milk. Hence, 0.2g of added urea might be considered as a LoD for its detection in milk.

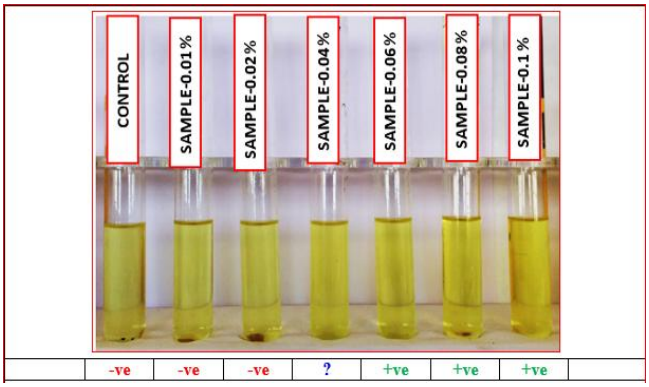

Plate 4B: LoD of DMAB test performed in whey

It is evident from the Plate 4B that no noticeable change in colour of whey was observed up to 0.02g of urea per 100ml of milk, when compared with the colour obtained in control sample of whey. Some distinction in colour of control sample and that of the adulterated sample of milk started from 0.04g urea per 100ml milk, however, the result appeared to be doubtful. The clear-cut distinction between colour of control sample and that of the adulterated sample of milk appeared from 0.06g urea per 100ml milk. Hence, 0.06g of added urea might be considered as a LoD for its detection in whey.

Sharma *et al.* (2012) [8] reported LoD of 0.2g urea per 100 ml of milk by using DMAB test in milk. In present study LoD of 0.2g urea per 100ml of milk was obtained when applied in milk. When DMAB test was applied in whey, LoD of 0.06g urea per 100ml of milk was obtained. Therefore, on performing the test in milk, LoD of urea obtained in presence study was in agreement with that reported in literature. However, LoD obtained in whey could not be compared with the literature, since no such work is reported in whey.

The comparison of results presented in the Plate 4A and 4B indicates that performance of the test in case of whey was relatively better compared to that in case of milk. There was clear-cut distinction in colour between the control and the adulterated sample when whey was used as a medium to perform the test, starting from minimum addition of urea. Thus, the test became more sensitive when performed in using whey as a medium, which is clearly evident from drastic reduction in LoD of the test.

Summary and conclusion

DMAB test is the most commonly reported method for detection of added urea in milk. For performing the test, 5ml of DMAB reagent is added to 5ml of samples of milk and mixed well. The presence of urea is confirmed by the formation of a deep yellow colour in the adulterated sample as compared to the control. DMAB reagent is prepared by dissolving 1.6g of *para*-Dimethylaminobenzaldehyde (DMAB) in small amount of 95 per cent ethanol, 10 ml of concentrated HCl is added and the volume is made up to 100 ml with the ethanol.

To modify the test, work was carried out to explore possibility for replacing ethanol with methanol, evaluating whey as a medium to perform the test and selection of acid to obtain whey. The distinction between control and adulterated sample of milk was better in case of DMAB reagent prepared in methanol, compared to ethanol. Thus, ethanol can be replaced by methanol in preparation of DMAB reagent for detection of added urea in milk. Similarly, better differentiation was obtained when whey was used as a medium to conduct the test in place of milk. Amongst four commonly used acids (lactic acid, acetic acid, citric acid and HCl) for coagulating milk to obtain whey, the best differentiation between control and adulterated sample was obtained using citric acid. LoD of DMAB test to detect urea using milk and whey as a medium was 0.2 and 0.06g per 100 ml of milk respectively. Thus an improvement in the limit of detection (LoD) was achieved when whey was used as medium.

Reference

1. Astrid Hilding-Ohlsson, Jonathan A. Fauerbach, Natalia J. Sacco, M. Celina Bonetto, and Eduardo Cortón. Voltamperometric Discrimination of Urea and Melamine Adulterated Skimmed Milk Powder. Sensors (Basel), 2012; 12(9):12220–12234.
2. Souza SS, Cruz AG, Walter EHM, Faria JAF, Celeghini RMS, Ferreira MMC, *et al.* Monitoring the authenticity of Brazilian UHT milk: A chemometric approach. Food Chemistry. 2011; 124:692–695.
3. Sukumaran MK, Hemanth Singuluri. Milk Adulteration in Hyderabad, India - A Comparative Study on the Levels of Different Adulterants Present in Milk. Indian Journal of Dairy Science. 2015; 68(2):190-193.
4. Paradkar MM, Singhal RS, Kulkarni PR. An approach to the detection of synthetic milk in dairy milk: 1. Detection of urea. Int. J. Dairy Technol. 2000; 53:87–91.
5. Sadat A, Mustajab P, Khan IA. Determining the adulteration of Natural milk with synthetic milk using ac conductance measurement. J. Food Eng. 2006; 77(3):472–477.
6. Trivedi UB, Lakshminarayana D, Kothari IL, Patel NG, Kapse HN, Makhija KK, *et al.* Potentiometric biosensor for urea determination in milk. Sensor Actuat B-Chem. 2009; 140(1):260–266.
7. Mishra GK, Mishra RK, Bhand S. Flow injection analysis biosensor for urea analysis in adulterated milk using enzyme thermistor. Biosens Bioelectron. 2010; 26(4):1560–1564.
8. Sharma R, Rajput YS, Barui AK, Laxmana NN. Detection of Adulterants in Milk - A Laboratory Manual, Edition-1. NDRI Publication No: 88/2012. National Dairy research Institute, Karnal-132001., 2012.
9. Shaikh AI, Parmar SC, Raj D. Validation of methods for detection of adulterants, neutralizers and preservatives in khoa and burfi. Ind J Dairy Sci, 2011; 64(5):400-405.
10. Tiwari A, Aryal S, Pilla S, Gonga S. An amperometric urea biosensor based on covalently immobilized urease on an electrode made of hyperbranched polyester functionalized gold nanoparticles. Talanta. 2009; 78:1401–1407
11. BIS. Methods of test for dairy industry, Part- I: Rapid examination of milk, Bureau of Indian standard, Manak Bhavan, 9 Bahadur Shah Zafar Marg, New Delhi - 110002, 1960
12. Bector BS, Ram M, Singhal OP. Rapid platform test for the detection/determination of added urea in milk. Indian Dairyman. 1998; 50(4):59-62.
13. Dairyforall. Tests to detect adulteration in milk. Cited from http://www.dairyforall.com/milk-adulteration.php (05-10-2014), 2006.
14. Draaiyer J, Dugdill B, Bennett A, Mounsey J. Milk Testing and Payment Systems Resource Book - A practical guide to assist milk producer groups. FAO. 2009, 38-43.
15. NDDB. Methods for detection of common adulterants in milk and milk products. National Dairy Development Board. 2009; 83:1-36. http://www.dairyknowledge.in/sites/default/files/technews_83.pdf cited on 24-11-2015.
16. FSSAI. Manual of methods of analysis of foods (milk and milk products) - A Laboratory Manual. Food safety and standards authority of India, Ministry of health and family welfare, Government of India, New Delhi, 2012.
17. Kamthania M, Saxena J, Saxena K, Sharma DK. Milk adulteration: Methods of detection &remedial measures. Int. J Engg Tech Res. 2014; 1:15-20.
18. Dixit S. Test yourself – detect adulteration in milk. Cited from http://face-cii.in/sites/default/files/dr_sitaram_dixit_-1.pdf (18-10-2014), 2012.

19. Food Safety and Standards Authority of India. Manual of methods of analysis of foods (milk and milk products) - A Laboratory Manual. Food safety and standards authority of India, Ministry of health and family welfare, Government of India, New Delhi, 2015.
20. Kaushik P. Use of natural organic and synthetic chelating agents for efficient phytoremediation. Int. J. Enhanced Res. Sci. Technol. Engg. 2015; 4(6):99-101.