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Sensitivity of modified DMAB test for lower level detection of urea in milk

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Abstract
Milk is perhaps the most widely adulterated food commodity. There are different adulterants that used in milk like urea, skim milk, washing powder, water, salt, sugar, oil etc. Urea adulteration in milk is very common and frequent in India, as it is the main components of non-protein nitrogen in milk, so external addition of urea increase total nitrogen in milk, so SNF contain use to be increased. As India is an agriculture based country where urea is often used as fertilizer or as a base material for cattle feed formulation. So urea is commonly available in every house hold of dairy farmers.

Wet chemistry based method (DMAB test), suggested by FSSAI is mostly usable methodology for detection of urea in milk. However the maximum detection limit for this method is 0.2%, also for preparation of DMAB reagent ethyl alcohol been recommended, that is some time difficult to procure for sate like Gujarat. So this method should be modify in a view to increase better sensitivity as well as easy availability. In our present research DMAB solution was prepared using isopropanol. Using this standardized said protocol urea could be detected 0.05% (cow), and 0.1% (buffalo) milk respectively.

Keywords: Sensitivity, DMAB, detection, milk

Introduction
Adulteration of food products continues to represent a major anxiety for consumers' point of view. As milk is widely consumed by all section of people, the adulteration of milk and milk products is a major concern to law enforcement agencies. Very common adulterants that used in milk are urea, skim milk, washing powder, water, salt, sugar, oil etc. Among all these adulterants use in milk, very common is urea adulteration in milk. Urea is a small organic water soluble molecule composed of carbon, nitrogen, oxygen, and hydrogen (M.W. 60.06 g/mol) of chemical formula $CO(NH_2)_2$ and is also known as carbamide [1, 2]. It is a natural constituent of natural body fluids including blood (160-420 mg/l), urine (5000-12000 mg/l) and milk (100-450 mg/l) [3]. As urea is the major non protein nitrogen component in milk so addition of urea increase apparent nitrogen so protein use to be increase therefore total SNF of milk use to be increased. As India is an agriculture based country where urea is often use as fertilizer or as a base material for cattle feed formulation. So urea is commonly available in every house hold of dairy farmers.

Urea adulteration is very hazardous from the consumer health point of view, that also leads to carcinogenic effect on human body and it can also lead to gradual impairment of the body [4]. Major problem associated with excess urea happens when kidneys begin to work overtime to rid the body of excess urea, they also excrete large amounts of minerals, the most important of their components is calcium. The high concentration of calcium mixed with uric acid in the kidneys form kidney stones and the body becomes depleted of calcium and draws it out of the bones rendering them weak, the ending result can be osteoporosis [5]. Excess of urea in milk on boiling decomposes to carbonic acid, acetic acid and ammonia, those ammonia-containing calculi might be formed by the partial fermentation of urea in the bladder. Also, it may convert into biuret which causes fall in blood pressure and produces strong irritation in the urinary tract [6].

Numerous methods have been applied for detection of urea adulteration in milk, and all this methods based on spectroscopy method [7], enzyme based biosensor methods [8], paper strip based method [9], wet chemistry based methods [10] etc. All these methods are highly sophisticated, needs highly skill man power or some technologies are patented. So in field level it is not very easy to apply those methods for detection of urea in milk.

Para-Dimethylaminobenzaldehyde (DMAB) based protocol, is recommended by FSSAI (2011) for detection of urea adulteration in milk. However in that said protocol for preparation of DMAB solution DMAB (1.6g) use to be dissolved in 100 ml ethyl alcohol. Therefore sometime the state like Gujarat, where alcohol is restricted; therefore it seems to be problematic for getting methyl, ethyl alcohol or rectified spirit for smaller dairy plant or in chilling plant. So it is tough task for them for ascertain the presence of urea in milk. Considering all the said facts stated above, an attempted been tried to modify the DMAB test for detection of urea adulteration in milk.

Materials and Methods

Collection of milk

Raw Cow and Buffalo milk was collected from local dairy farmers of Amreli, district Gujarat.

Collection of Urea

Urea was used as an adulterants in the present investigation. Urea was purchased from local market of Amreli.

Preparation of adulterated milk samples

For detection of added urea by modified DMAB test, adulterated samples of milk were prepared by addition of urea in both cow and buffalo milk –

Sr. No.	Samples	Adulterants	Level (%)					
1	Cow milk	Urea	0.05	0.10	0.30	0.50	0.80	1.00
2	Buffalo milk	Urea	---	0.10	0.30	0.50	0.80	1.00

Preparation of DMAB (p-dimethyl amino benzaldehyde) solution

DMAB (p-dimethyl amino benzaldehyde) solution was prepared by mixing 1.6 g of DMAB in 100 ml of 70% propan-2-ol (isopropyl alcohol). Thereafter add 10 ml concentrated HCl and mix well and store for further use.

Chromogenic test

First 2 ml milk was taken in a clean and dry test tube; there after 2 ml DMAB solution was mixed and colour was observed. For pure milk sample whitish yellow or faint yellow colour was observed but for adulterated sample a deep yellow colour was observed.

Result & Discussion

Urea is a normal constituent of milk and urea content in natural milk varies from 20 mg/100 ml to 70 mg/100 ml. Addition of external urea in milk increase apparent protein as total nitrogen in milk used to be increased. According to FSSAI (2011), it is recommended that the concentration urea of more than 70 mg /100ml of milk considered as added external urea, hence it would be considered as an adulteration of milk. Urea is one of the major ingredients of synthetic milk along with caustic soda, detergent, sugar and foreign fat.

For detection of added urea by modified DMAB test, adulterated samples of milk were prepared by addition of urea in both cow and buffalo milk. The prepared samples of milk subjected to modified DMAB test. Results obtained (Level of detection-LoD) for milk are presented in Plate A and B, respectively.

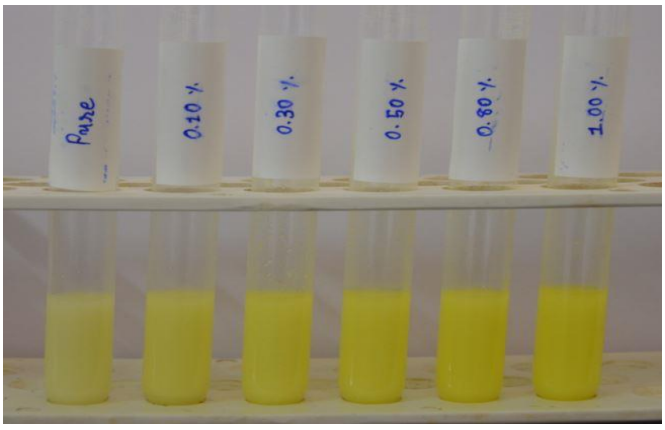

Plate A: LoD of modified DMAB test performed in buffalo milk

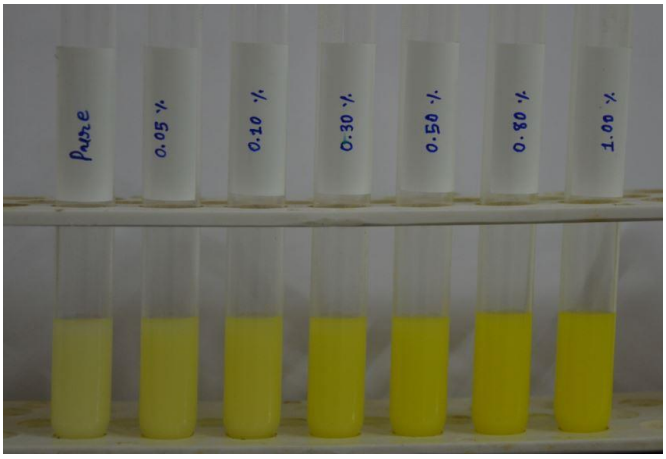

Plate B: LoD of modified DMAB test performed in cow milk

It is evident from the Plate A that noticeable change in colour (yellow colour) of milk was observed up to 0.1g of urea per 100ml of buffalo milk, when compared with the colour obtained in control sample of buffalo milk. The clear-cut distinction between colour of control sample and that of the adulterated sample of milk appeared from 0.1g urea per 100 ml buffalo milk. Hence, 0.1g of added urea considered as a LoD for detection in buffalo milk.

It is also evident from the Plate B that noticeable change in colour (yellow colour) of milk was observed up to 0.05g of urea per 100ml of cow milk, when compared with the colour obtained in control sample of cow milk. The clear-cut distinction between colour of control sample and that of the adulterated sample of milk appeared from 0.05g urea per 100 ml cow milk. Hence, 0.05g of added urea considered as a LoD for its detection in buffalo milk.

The addition of urea to milk can be detected by using para-dimethylaminobenzaldehyde (DMAB). This method is based on the principle that urea forms a yellow complex with DMAB in a low acidic solution at room temperature. In this modified method 70% propan-2-ol (isopropyl alcohol) been used for preparation of DMAB solution.

Ethanol and methanol is prohibited item in Gujarat, so it was thought to prepare DMAB reagent as replacement with 70% propan-2-ol (isopropyl alcohol) which is cheaper and easily available. Thus, replacement of ethanol with equivalent amount of propan-2-ol (isopropyl alcohol) was tried for the preparation of DMAB reagent. Chauhan *et al*. [11] reported to detect 0.2% added urea in milk by modified DMAB based protocol.

Hence in this current protocol para-dimethylaminobenzaldehyde (DMAB) been used that is

cheaper and easily available and also this protocol is more sensitive than earlier reported [10, 11] different modified DMAB test protocol. It was also observed that for this said protocol added urea could be detected in case of cow milk 0.05% and buffalo milk 0.1% respectively. This might be due to that concentration of urea in cow milk is more than buffalo milk; hence in cow milk LoD is lower than buffalo milk.

For checking the efficacy of this said modified DMAB test protocol we repeated this protocol fifty times for both cow and buffalo milk respectively and observed the same results.

Conclusion

Detection of urea in milk by DMAB test is mostly recommended and usable test in for detection of urea in milk. Here DMAB reagent was prepared by replacing of ethanol with 70% propan-2-ol (isopropyl alcohol) which is cheaper and easily available. Using this said simple protocol, urea could be detected in case of cow milk 0.05% and buffalo milk 0.1% respectively. Same results were observed after 50 trials. Hence this simple test could be recommended for regular quality control lab for detection of urea in milk specially in Gujarat like state where alcohol is restricted.

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