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A SIMPLE COLORIMETRIC METHOD FOR THE DETERMINATION OF UREA IN MILK

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INTRODUCTION

In recent years, several reports have provided convincing evidence that the level of urea in cow milk plays a significant role in regulating its heat stability (Muir and Sweetsur, 1976, 1977, 1978; Holt *et al.*, 1978; Muir *et al.*, 1979; Fox *et al.*, 1980). It has been suggested that the routine measurement of urea in cow milk during its use in the manufacture of some dairy products, such as sterilized milk, would help solving problems arising as a result of variations in the heat stability of milk (Muir and Sweetsur, 1978). Hence, estimation of urea in milk becomes a necessary step. The existing method for urea estimation in milk involves the conversion of urea to ammonia by urease and which is subsequently determined colorimetrically (Muir and Sweetsur, 1976). Although the method is precise and sensitive, it requires the use of special purity urease which is difficult to procure in many dairy plants and laboratories in our country. Moreover, contamination by small amount of atmospheric ammonia would give misleading results. This prompted us to develop an alternate method, equally sensitive and precise for estimation of urea. The present paper describes such a colorimetric method for urea estimation in milk, based on the modification of the microanalysis of urea in blood (Wootton, 1974).

MATERIALS AND METHODS

The individual milk samples were collected from cows and buffaloes maintained at the Institute herd. Urease was purchased from Sigma Chemical Co., U.S.A. All other reagents used in this study were procured locally and were of analytical grade.

METHODS

a) *Reagents :*

i) *Acid reagent* — For the preparation of acid reagent, 2.5 mg of ferric chloride was dissolved in 45 ml distilled water to which 80ul of phosphoric acid solution was added. The volume of the mixture was then made up to 250 ml with 9 N H_2SO_4.

ii) *Mixed colour reagent* — The mixed colour reagent was prepared by mixing up 16.75 ml each of diacetyl monoxime (0.125%) and thiosemicarbazide (0.313%). The volume of the mixture was then made up to 250 ml with distilled water.

b) *Preparation of milk serum :*

To 3 ml of cow or buffalo milk 3 ml of 10% trichloroacetic acid was added. The precipitated protein was filtered through Whatman No. 1 filter paper. An aliquot of 0.2 ml of the filtrate was diluted to 5 ml with distilled water and 1.0 ml of the diluted filtrate was used for urea estimation.

c) *Estimation of urea :*

Two ml of standard urea solutions containing 2.5 to 20 *ug* of urea were used for preparing the standard curve. For milk

samples, to 1.0 ml of diluted filtrate from milk as in (b), 1.0 ml of water was added. Two ml of mixed acid reagent followed by 2.0 ml of mixed colour reagent were added to each tube. The tubes were kept in boiling water bath for 20 min, cooled to room temperature and the developed pink colour was read at 520 nm.

RESULTS AND DISCUSSION

Preparation of standard curve :

As the first exercise, a standard curve using pure solution of urea (2.5 to 20) was prepared. From Fig 1 it is apparent that urea concentration and the developed colour follow a linear relationship, thereby obeying the Beers Law. This curve was then utilized for the estimation of urea in milk samples.

Precision and sensitivity of the method :

To find out whether the natural soluble components other than urea of milk are responsible for the developed pink colour by this method it was necessary to prepar the milk filtrate free from urea. This wa achieved by preparing a urea-depleted mil sample by incubating 0.2 ml of diluted mil (1:9 with 0.1 ml of urease solution (1 units/ml. in phosphate buffer, 0.05M, pl

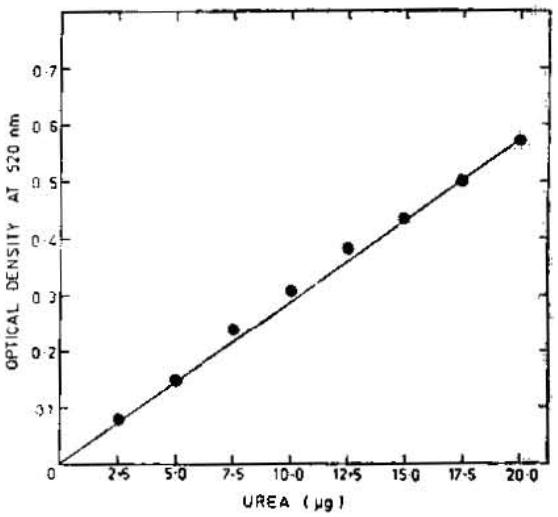

Fig. 1

Standard curve for urea showing the linear relationship with concentration

TABLE 1

Recovery of added urea in cow and buffalo milk

Sample No.	Initial urea level (mg/100ml)	Added urea level (mg/100ml)	Final estimated urea level (mg/100ml)	Recovery (%)
1	27.72	15.00	42.70	99.86
2	32.46	30.00	62.58	100.40
3	37.42	45.00	82.51	100.20
4	12.36	60.00	72.42	100.10
5	17.78	75.00	92.64	99.81
6	18.83	90.00	108.02	99.10

6.5) at 37°C for 10 min. The trichloroacetic acid filtrate was obtained from this urea-depleted milk and subjected to colour development. It was found that such filtrate after treatment with the reagents did not give any absorbance at 520 nm. Thus the interference of milk components other than urea in this method was ruled out. This further strengthens the method as the developed colour was due to urea only.

Accuracy of the method :

To assess the accuracy of the method, recovery experiment was conducted using known quantity of urea added to cow and buffalo milk samples. The levels of urea were then estimated in these samples. Table 1 shows the values of urea obtained by this method, indicating an excellent recovery. Hence it could be concluded that this simple method for estimation of urea in milk is an accurate one.

Urea levels in cow and buffalo milk :

The range alongwith the average values for urea levels in 20 samples each of cow and buffalo milk are shown in Table 2. In general the urea level in buffalo milk (17 mg/100 ml) was lower than in cow milk (31 mg/100 ml). Hence, the possibility of the lower heat stability in buffalo milk due to the lower level of innate urea cannot be ruled out due to correlation between heat stability and urea level as observed in case of cow milk (Muir and Sweetsur, 1978).

TABLE 2

Urea level in cow and buffalo milk

Species*	Range	Average
	—(mg/100ml)—	
Cow	18.69-48.35	31.85±9.26**
Buffalo	12.02-26.64	17.302±3.94

*No. of samples analysed in each case was 20.

**Mean ± S.D.

These results provide convincing evidence on the use of this colorimetric method for the estimation of urea in milk as it satisfies all the requirements of such procedure. Recently, in another investigation (Ganguli and Sharma, 1980) it was observed that the added urea (in solid form) in milk cannot be quantitatively determined either volumetrically or gravimetrically although it is supposed to give an equivalent increase in either SNF or total solids. The recovery in volumetric method using Richmond's formula is about 70% whereas gravimetrically it is even poor (40%). This further demands the use of a nondestructive method for urea estimation in milk. The present colorimetric method overcomes such drawbacks. Work is now in progress on the applicability of the method for urea estimation in milk from other species, in different milk products and to evaluate the impact of feed-urea on the milk-urea level.

SUMMARY

A simple colorimetric method for the estimation of urea in milk has been developed. The urea-depleted milk did not respond to the colour development, thus showing the method as precise and sensitive. The recovery of known amount of added urea to milk by this method was nearly 100% suggesting good accuracy. Estimation of urea in cow and buffalo milk by this method led to the conclusion that urea level in buffalo milk is considerably lower than in cow milk.

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