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## Research Note

# A Simple and Rapid Colorimetric Method for Lactose Determination in Milk

*ABSTRACT*

*A simple and rapid technique for the estimation of lactose in fluid milk and whey, utilizing trichloroacetic acid (TCA) as a precipitating agent, is outlined. The color development is based on the reaction of picric acid with lactose in the presence of phenol, sodium hydroxide and sodium bisulfate. This method can be completed within 10 min. There is no significant difference ($p \leq 0{\cdot}05$) between the results obtained by the proposed method and those obtained by the IDF method.*

## INTRODUCTION

Lactose is the major carbohydrate of milk and its concentration represents about 40, 50 and 70% of the solids in whole milk, skim milk and whey respectively (Nickerson *et al.*, 1975; Wolfschoon-Pombo *et al.*, 1983). Lactose levels vary from one product to another, depending on the conditions under which the product has been manufactured and processed. In addition, the levels of the lactose may also vary in the same product category depending on the treatment to which that product has been subjected. Therefore there is a need for a simple and quick method to measure the lactose content in milk and its products.

Numerous methods have been developed to quantitatively determine lactose content in milk and its products (Wolfschoon-Pombo *et al.*, 1983). In general, lactose can be determined either gravimetrically (AOAC, 1975) or by titration with sodium thiosulfate (FIL, 1974). Both enzymatic or polarimetric methods may be used in lactose determination (AOAC, 1975).



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These methods, however, require extensive time, sophisticated equipment, corrosive reagents and a considerable amount of glassware. Teles *et al.* (1978) developed a rapid quantitative colorimetric method, suitable to determine lactose in the presence of sucrose, ethanol and acetate without any interference in the reaction. Wolfschoon-Pombo *et al.* (1983) reported that Teles' procedure presented a great variation among duplicate determinations even when performed by a skilled technician. This variation (SD) was found to be about $\pm 0{\cdot}08$ with data of Teles *et al.* (1978).

The method proposed here was developed to minimize the effects of some of the disadvantages described above in the paper.

## MATERIALS AND METHODS

### Standard lactose solutions

A series of lactose solutions was prepared with the following concentrations: 2·0, 2·5, 3·0, 3·5, 4·0, 4·5, 5·0, 5·5, 6·0, 6·5% of anhydrous lactose in distilled water. A standard curve was prepared for this method by plotting absorbances against the corresponding lactose concentrations.

### Statistical analysis

The data were analyzed by linear regression, according to the method of Steel & Torrie (1960). Correlation coefficients were also calculated.

### Reagents

The following stock solutions were prepared in distilled water: 1% phenol, 5% sodium hydroxide, 1% picric acid and 1% sodium disulfite ($Na_2S_2O_5$). A working solution (two days shelf life) was prepared by mixing one volume of phenol, two volumes of sodium hydroxide, two volumes of picric acid and one volume of fresh sodium disulfite solution thoroughly (Teles *et al.*, 1978). The working solution was diluted 1:1 with distilled water.

24% trichloroacetic acid (TCA) was prepared in distilled water. The proposed method will depend mainly on TCA as a total protein precipitating agent as well as modified Teles' reagent by replacing sodium bisulfite ($NaHSO_3$) with sodium disulfite.

### Procedures

Five ml of TCA was added to 5 ml of fluid milk or whey sample and mixed thoroughly. The sample was then filtered through Whatman no. 40 filter

paper and 2 ml of the clear filtrate was diluted to 100 ml with distilled water. A 1·0 ml aliquot of diluted filtrate was transferred to a 15 ml tube fitted with a screw cap. A 2·5 ml aliquot of diluted working solution was added to the tube contents and mixed thoroughly. The tube was stoppered and immersed to a depth of 4 to 6 cm in a violently boiling water bath for exactly 2·5 min. Then the tube was immediately cooled under cold tap water and 7 ml of distilled water was added to the tube contents and mixed thoroughly. The absorbance at 520 nm was measured against a blank containing all reagents except that milk was replaced by distilled water. Volumes of fat and protein in the samples were corrected as described by Grimbleby (1956).

## RESULTS AND DISCUSSION

### Standard curve

The standard curve presented in Fig. 1 was based mainly on five trials with three replicate determinations, using ten different lactose concentrations. This curve was found to conform to the requirements of Beer's Law. The regression equation of the standard curve is $Y = 0{\cdot}070X - 0{\cdot}0229$, where $Y$ = absorbance at 520 nm and $X$ = percentage lactose (g/100 ml). The slope and intercept were 0·070 and −0·0229 respectively. The slope represents the rate at which perceived absorbance increases with increasing lactose concentration.

The standard curve was linear over the range of 2·5 to 6·5% lactose. The correlation coefficient ($r$) between the lactose concentration and absorbance was 99·6.

### The time for color development

The absorbance at 520 nm increased with increasing boiling time up to 2·5 min. However, extending the boiling time from 2·5 to 4 min did not produce any significant increase in absorbance, as shown in Fig. 2. These results confirmed that the time needed to develop the color reaction at the temperature of boiling water was 2·5 min.

### Effect of the presence of TCA

The absorbance of lactose solutions at 520 nm was increased significantly ($p \leq 0{\cdot}05$) by the presence of TCA (Fig. 3). These results confirmed that the TCA used in the precipitation of proteins in milk samples did not exert any inhibitory effect on the development of color.

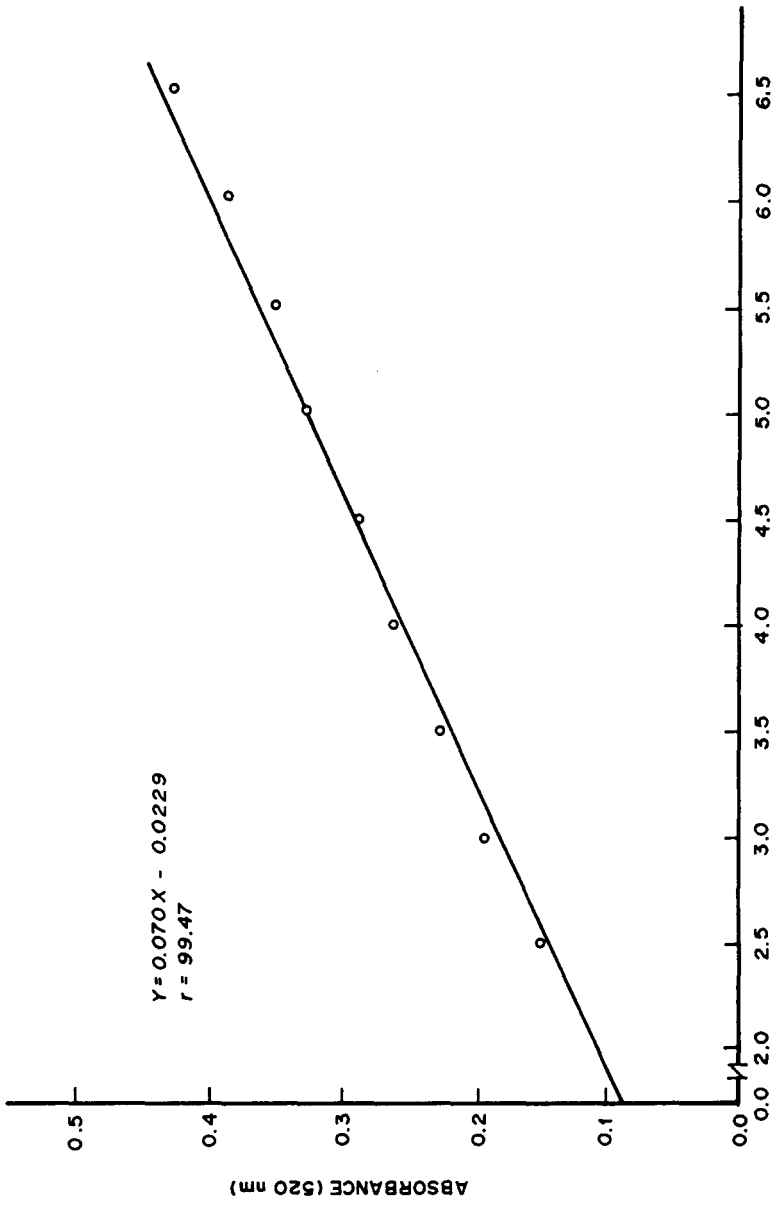


**Fig. 1.** Calibration curve for standard lactose solution.

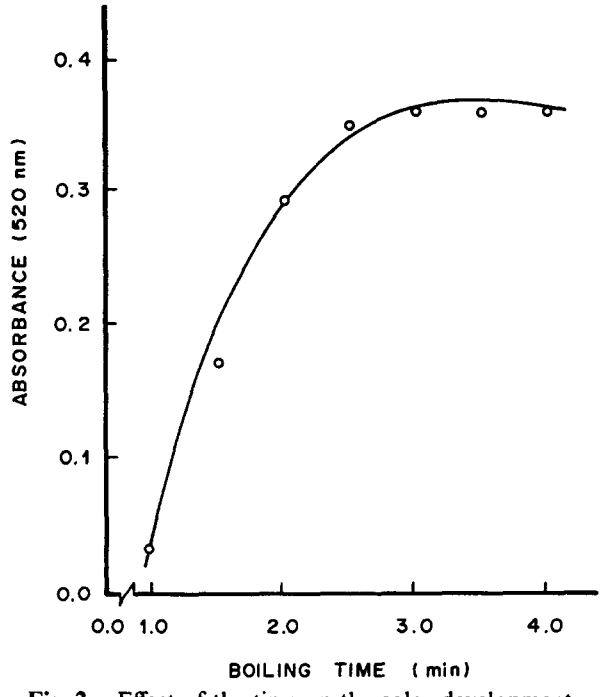


**Fig. 2.** Effect of the time on the color development.

## Recovery of lactose added to milk

The influence of the component of milk on the efficiency of recovery of lactose added to skim milk samples was tested using the proposed method. The results obtained are shown in Table 1. The consistently high recovery rate of approximately $99 \pm 2{\cdot}86\%$ at different increments of added lactose indicates a highly quantitative determination of lactose in milk samples

**TABLE 1**
Recovery of Lactose Added to Skim Milk[a]

| *Sample* | *Theoretical value (mg/ml)* | *Average lactose found (mg/ml)* | *Recovery (%)* |
|---|---|---|---|
| 1. Skim milk | $47{\cdot}2 \pm 0{\cdot}27$ | $47{\cdot}2 \pm 0{\cdot}27$ | — |
| 2. +05 mg lactose[b] | $52{\cdot}2 \pm 0{\cdot}27$ | $52{\cdot}1 \pm 0{\cdot}18$ | $98{\cdot}0 \pm 3{\cdot}58$ |
| 3. +10 mg lactose | $57{\cdot}2 \pm 0{\cdot}27$ | $56{\cdot}2 \pm 0{\cdot}30$ | $99{\cdot}1 \pm 3{\cdot}00$ |
| 4. +15 mg lactose | $62{\cdot}2 \pm 0{\cdot}27$ | $62{\cdot}1 \pm 0{\cdot}36$ | $99{\cdot}0 \pm 2{\cdot}04$ |
| Average | | | $99{\cdot}0 \pm 2{\cdot}86$ |

[a] Five replicates for each concentration.
[b] Anhydrous lactose.

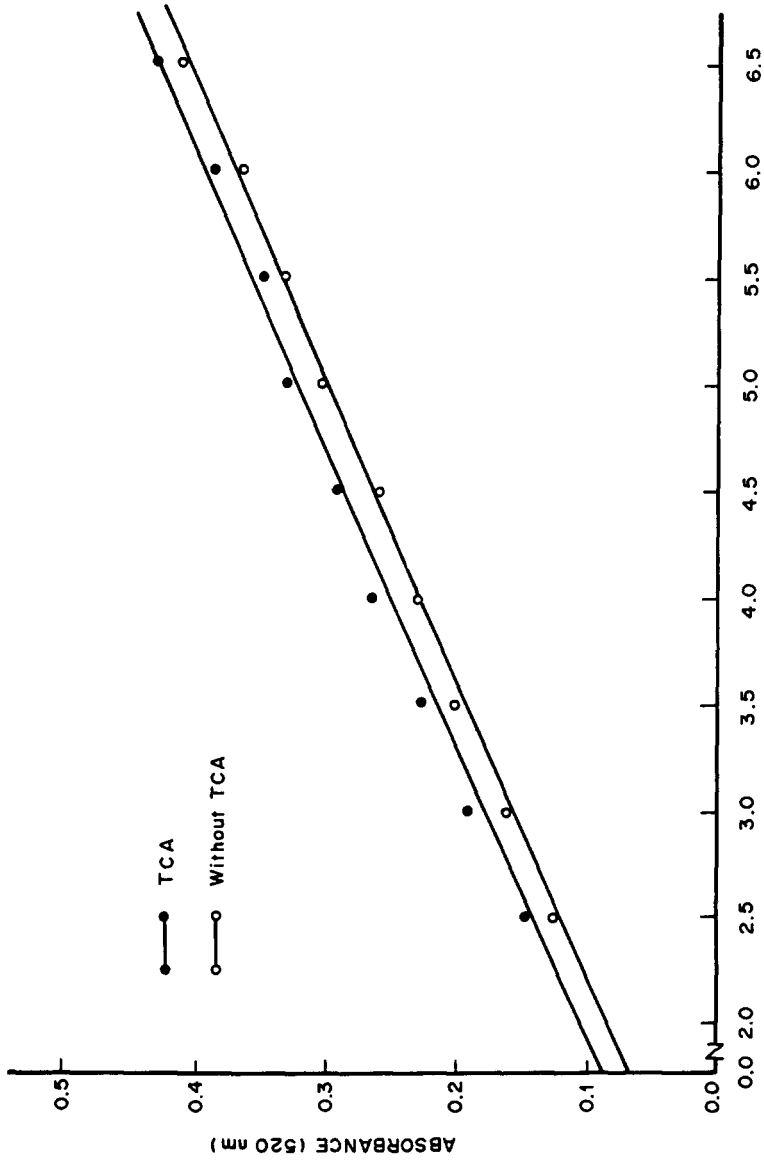


**Fig. 3.** Calibration curves for standard lactose solutions; effect of eliminating TCA from the reagents.

using the proposed method, with no interference of the other components of milk.

## Repeatability

The repeatability of the proposed method was determined by measuring the difference between duplicate analyses. Twenty samples of milk and whey were analyzed in duplicate (data not shown). The lactose concentration in milk ranged from 4·60 to 4·85% and in whey from 4·10 to 4·30%. Average differences between duplicate samples of milk and whey were $0{\cdot}016 \pm 0{\cdot}011$ and $0{\cdot}011 \pm 0{\cdot}009$ respectively. This showed that the proposed method was suitable for measuring lactose in milk samples.

## Comparison of methods

Ten samples of both milk and whey were analyzed in duplicate to compare the proposed method with the FIL method. The results are shown in Table 2. The average lactose content of milk samples, determined using the proposed method, is not significantly different ($p < 0{\cdot}05$) from that determined using the FIL method. Similarly, there is no significant difference ($p \leq 0{\cdot}05$)

**TABLE 2**
Comparison of Proposed Method and FIL-IDF for Determination of Lactose in Milk and Whey Samples[a]

| *Samples* | *Lactose in milk* (%) | | *Lactose in whey* (%) | |
|---|---|---|---|---|
| | *FIL-IDF method* | *Proposed method* | *FIL-IDF method* | *Proposed method* |
| 1 | 4·75 | 4·67 | 4·29 | 4·25 |
| 2 | 4·58 | 4·55 | 4·22 | 4·25 |
| 3 | 4·72 | 4·62 | 4·28 | 4·17 |
| 4 | 4·68 | 4·75 | 4·26 | 4·21 |
| 5 | 4·74 | 4·67 | 4·24 | 4·25 |
| 6 | 4·64 | 4·62 | 4·28 | 4·20 |
| 7 | 4·58 | 4·53 | 4·20 | 4·22 |
| 8 | 4·60 | 4·71 | 4·23 | 4·25 |
| 9 | 4·73 | 4·70 | 4·28 | 4·25 |
| 10 | 4·68 | 4·67 | 4·23 | 4·20 |
| Average | 4·69[b] | 4·67[b] | 4·25[c] | 4·24[c] |
| SD | 0·047 | 0·042 | 0·031 | 0·039 |

[a] Average of duplicate.
[b,c] Means with the same superscripts are not significantly different ($p \leq 0{\cdot}05$).

between the results using the proposed method and those using the FIL method for measuring lactose in whey samples. These results demonstrate that the proposed method may be used in measuring lactose content in milk and whey samples.

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