Determination of Lactose Concentration in Milk Serum by Refractometry and Polarimetry

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Abstract

The research had in view to evaluate and compare two instrumental techniques used for the determination of milk lactose. Refractometric and polarimetric measurements were carried out on milk serum obtained after precipitation of casein by two different methods: by acidification of milk to its isoelectric point (E1), and by using copper sulphate and potassium ferrocyanide (E2). The average lactose content measured by refractometry was 5.469±0.256 g% for method E1 and 5.852±0.218 g% for method E2. The obtained average lactose values measured by polarimetry were higher both for E1 (5.613±0.253 g%) and E2 (5.910±0.224 g%) methods, due to the interference with other optically active components. The experimental data revealed a high correlation between the results obtained by refractometry and polarimetry (r=0.8712) when casein precipitation was performed by potentiometric titration until pH=4.6, at 25°C with 2N acetic acid (method E1).

Keywords: lactose, milk, polarimetry, refractometry

1. Introduction

Milk is the most nutritionally complete food that can be found in nature, containing vitamins (principally thiamine, riboflavin, panthothenic acid and vitamins A, B_{12} and D), minerals (calcium, sodium, phosphorus, potassium, and trace minerals), proteins (which include all the essential amino acids), carbohydrates (mostly lactose), and lipids (fats).

Lactose is the only carbohydrate that mammals synthesize. It is synthesized in the mammary glands.

Lactose is the major carbohydrate in the milk of most species. Lactose is a disaccharide composed of the monosaccharides D-glucose and D-galactose, joined in a ß-1,4-glycosidic linkage. The chemical name for lactose is 4-0-ß-D-galactopyranosyl-D-glucopyranose. It is essentially unique to milk, although it has been identified in the fruit of certain plants. Of the mammalian species where information is available, only some marsupials have an alternative sugar other than lactose, and those sugars are generally trisaccharides of glucose and galactose [1].

Lactose accounts for about 54% of the total solids-not-fat content of whole milk and about 30% of its calories (about 9% of the calories of 2% reduced fat milk) [2]. The lactose content of milk varies among mammals. Cow's milk contains about 4.8% lactose, whereas human milk has 7% lactose [3]. The higher concentration of lactose in human milk explains why lactose is used to enrich breast milk substitutes or infant formula. The lower lactose content of cheeses is due to the removal of lactose-rich whey and the conversion of lactose to lactic acid by select microorganisms in cheese-making [4].

In addition to lactose, milk contains other carbohydrates in small amounts, including glucose, galactose and oligosaccharides [5]. Lactose is an important osmotic regulator of lactation [6, 7] and the process of synthesis of

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lactose is responsible for drawing water into the milk. Lactose is synthesized in the Golgi lumina. The Golgi membrane is impermeable to lactose, so water must be drawn into the lumen to restore osmotic balance [8].

As a carbohydrate source in milk, lactose is by weight the most abundant of the milk solids. Early methods of lactose analysis in milk are based on the determination of lactose by difference, an indirect method in which lactose is calculated by subtracting the protein, fat, and ash contents from the total solids content [9].

Currently available analytical methods for the detection of lactose include mid-infrared detection, fluorometry, photometric methods, polarimetry, gravimetric detection, differential pH techniques, oxidation-reduction titration, gas-liquid chromatography, high pressure liquid chromatography and enzymatic assays [10, 11].

The objective of this study is to evaluate and compare two instrumental techniques used for the determination of milk lactose. Both methods are based on the measuring of physical properties, the refractive index in refractometry and the specific rotation in polarimetry.

Refractometry is a technique of measuring how light is refracted when it passes through a given substance. The amount by which the light is refracted determines the refractive index. The relation between the refractive index and the concentration depends on the solvent and solute, temperature, and wavelength. Refractive index (RI) is normally determined at 20°C with the D line of the sodium spectrum. The refractive index of whole milk is 1.3440 to 1.3485. Water has the highest refractive index, followed by lactose and other minor constituents [12].

Polarimetry measures the extent to which a substance interacts with plane polarized light (light which consists of waves that vibrate only in one plane). Lactose is an optical active compound, which has chiral centers (with 4 different groups attached to it.). Polarimetry measures optical rotation by visually matching the intensity of split fields, using visible light from the sodium D-line at 589 nm.

2. Materials and methods

The experiment was made on 20 raw milk samples collected from dairy cows. For the methods to be successful, one must remove all optically active compounds except for the lactose (for instance the proteins). The solution must be transparent enough for the polarized light to pass through readily. Therefore the isolation of oligosaccharides from milk requires delipidation and deproteinization [13]. The measurements were carried out on milk serum samples, obtained according to two different procedures: (E1) the precipitation of casein at the isoelectric pH (4.6) with 2N acetic acid solution, and (E2) the precipitation of casein with cupric sulphate and potassium ferrocianyde. For the polarimetric determination the milk serum must be colorless, because the measurements cannot be done even at small remanence of the cupric sulphate.

The refractive index of a sample relative to the refractive index of water was measured with a refractometer Krüss DR301-95. The instrument was blanked with distilled water before each series of measurement. All readings were made at room temperature (approximately 20°C).The values of the refractive indices measured are converted in lactose concentrations [14].

In the clarified filtrate at 20°C, the angle of rotation of polarised light was measured in a Carl Zeiss Jena polarimeter. The content of lactose was calculated from the equation: $[\alpha]_D^{25} = 100 \cdot \frac{\alpha}{l \cdot c}$

where: $[\alpha]_D^{25}$ =+55.4° [15]

α=angle of rotation

l=length of the polarimeter tube (dm)

c=concentration (g%)

3. Results and discussion

We obtained a clear milk serum by potentiometric titration to pH=4.6 using 2N acetic acid, avoiding dilution of the sample considerably. The values of the refractive index are not found in the table [10] when the samples are too diluted. The procedure with copper sulphate and potassium ferrocyanide was more laborious. Because milk serum was colored light blue, it was required discoloration with Zn powder. The method is indicated mostly in polarimetry, the samples being diluted. This procedure needs a greater milk volume to fill the polarimetric tube.

The experimental data revealed differences between lactose values measured in milk serum obtained by method E1 and E2 (Table 1). The average lactose content measured by refractometry was 5.469±0.256 g% for method E1 and 5.852±0.218 g% for method E2. The obtained average lactose values measured by polarimetry were higher both for E1 (5.613±0.253 g%) and E2 (5.910±0.224 g%) methods, due to the interference with other optically active components. The lactose values were higher for E2, as the lactose content was calculated by extrapolation.

There is only a moderate positive correlation (r=0.7179) between the lactose content measured by refractometry, using method E1 and E2 for obtaining the milk serum (Figure 1). We also observed a moderate positive correlation (r=0.7412) between lactose values measured by refractometry and by polarimetry for method E2 (Figure 2), which suggests inefficient removal of interfering optically active components.

Table 1. The lactose values obtained by refractometry and by polarimetry

Sample	Refractometric method		Polarimetric method	
	Lactose, g% for E1	Lactose, g% for E2	Lactose, g% for E1	Lactose, g% for E2
1.	5.35	5.94	5.52	5.82
2.	5.4	5.94	5.58	6.18
3.	5.85	6.24	5.88	6.27
4.	5.8	5.94	5.98	5.98
5.	5.8	5.92	5.71	5.59
6.	5.35	5.64	5.45	5.50
7.	5.6	6.04	5.88	5.92
8.	5.35	5.84	5.45	5.75
9.	5.4	5.74	5.45	5.71
10.	5.25	5.84	5.59	5.82
11.	5.9	6.14	6.14	6.14
12.	5.35	6.05	5.88	6.37
13.	5.35	6.04	5.75	6.07
14.	5.85	5.94	5.75	5.88
15.	5.24	5.6	5.45	5.88
16.	4.95	5.44	4.88	5.88
17.	5.25	5.44	5.45	5.75
18.	5.4	5.74	5.45	6.18
19.	5.35	5.64	5.60	5.98
20.	5.6	5.94	5.27	5.98
Xm±DS	5.469±0.256	5.852±0.218	5.613±0.253	5.910±0.224

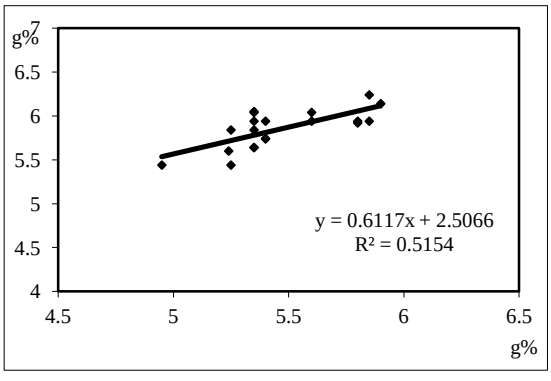

Figure 1. Correlation between the lactose content measured by refractometry, using method E1 and E2 for obtaining the milk serum

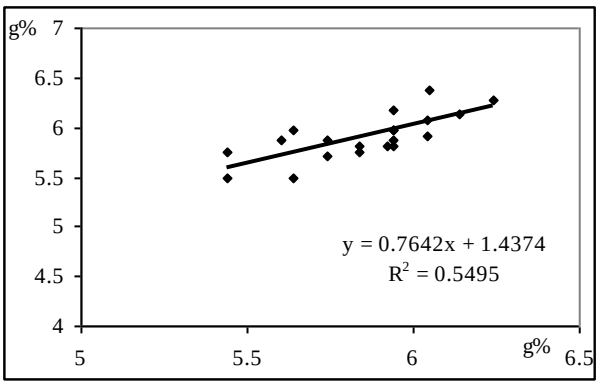

Figure 2. Correlation between lactose values measured by refractometry and by polarimetry, using method E2

The experimental data revealed a high correlation between the results obtained by refractometry and polarimetry (r=0.8712) (Figure 3) when casein precipitation was performed by potentiometric titration to pH=4.6, at 25°C with 2N acetic acid (E1).

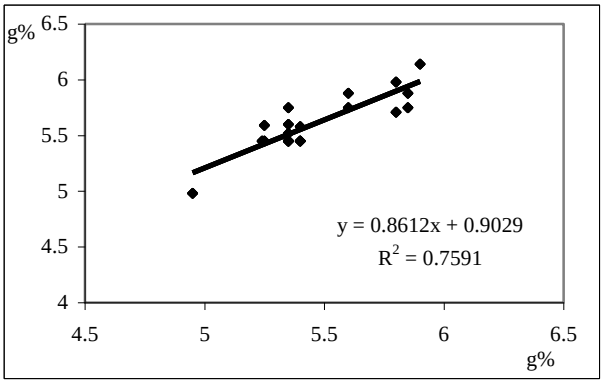

Figure 3. Correlation between lactose values measured by refractometry and by polarimetry, using method E1

4. Conclusions

Lactose assay by refractometry is simple and rapid, and has the advantage to need smaller milk samples.

Determination of milk lactose by polarimetry is time-consuming because of extensive sample preparation. Polarimetry needs greater volume of colorless milk serum, more difficult to obtain, and has interferences from other optically active components.

When using method E2 for delipidation and deproteinization, the milk serum was not free of interfering optically active components.

The best casein precipitation procedure is by potentiometric titration until pH=4.6, at 25°C with 2N acetic acid.

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