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Analytical Methods Section

Determination of lactose by an enzymatic method

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A new enzymatic method for the determination of lactose has been developed. The method is based on the hydrolysis of lactose by a β-galactosidase enzyme of industrial application and the subsequent determination of the liberated glucose by means of an enzymatic kit designed for clinical use. The method has good linearity between lactose concentrations and absorbance; the *Y*-intercept was $n = -0{\cdot}0095$, the gradient was $m = 0{\cdot}0584$, and the correlation coefficient was $r = 0{\cdot}9994$; the coefficient of variation was 6·5% for 2% lactose and 5·0% for 7% lactose. The limit of detection (LOD) was 0·15 (w/v) and the limit of quantification (LOQ) was 0·50% (w/v). The method requires simple equipment and is of low cost.

INTRODUCTION

The determination of lactose is of considerable interest in several fields of application, for example, in milk and dairy products, in the control of the whey effluents from dairy industries or in the industrial reuse of whey. Among the classic methods for determining lactose we can cite gravimetric, polarimetric and infrared methods (Horwitz, 1980); several colorimetric methods (Nickerson, *et al.*, 1976; Feitosa *et al.*, 1978); the electrochemical method (Lundback & Olsson, 1985; Pilloton and Mascini, 1990); a cryoscopic method (Frank & Christen, 1984); chromatographic GLC and HPLC methods (Dunmire & Otto, 1979; Li & Schuhmann, 1981; Kowalski & Giesecke, 1986; Harvey, 1988, Ball, 1990) have great interest but require expensive instrumentation and accessories. Enzymatic methods are a useful option for the analysis of lactose, especially in the food field, e.g. Essig and Kleyn (1983) and Kleyn and Trout (1984).

In the present work has been developed an enzymatic method for lactose determination, based on the colour test Peridochrom of Boehringer-Mannheim, GmbH Barcelona, Spain (Trinder, 1969).

MATERIALS AND METHODS

Enzyme. A liquid commercial preparation, Lactozym 3000 L, type HP, with a β-galactosidase (EC 3.2.1.23), of activity 3000 LAU ml^{-1} from *Kluyveromyces fragilis* was supplied by Novo Nordisk Bioindustrial, S.A., Madrid, Spain.

Lactose. Lactose monohydrate powder (Merck) quality DAB, Ph Eur, BP, USP.

Buffer. A buffer solution of citrate 0·1 M, pH = 6·6, was used.

Kit for glucose determination. The glucose was measured by the colorimetric method GOD-PAP (Peridochrom) of Boehringer-Mannheim GmbH, catalogue number 676543.

In this method a reaction solution of the following composition was prepared: buffer tris-phosphate 180 mM; phenol 11 mM; 3,4-dichlorophenol 2·1 mM; fatty-alcohol-polyglycol ether 0·24 mM; 4-aminophenazone 0·8 mM; peroxidase (POD) 0·9 U ml^{-1}; glucose oxidase (GOD) 15 U ml^{-1}. The following reactions take place:

$$\text{glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} \text{gluconate} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{phenol} \xrightarrow{\text{POD}} \text{4-(}p\text{-benzoquinone monoimino)-phenazone} + 4H_2O$$

Methodology

First, the lactose is hydrolysed by the β-galactosidase and an equimolecular mix of glucose and galactose is produced. Then the glucose is measured by the test Peridochrom.

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The sample for analysis must have a lactose concentration similar to that of the milk. In other cases, adequate dilutions must be prepared.

The sample was diluted 1 : 100 in distilled water. The diluted sample (80 μl) was placed in a spectrophotometer cuvette, then distilled water (20 μl), citrate buffer (200 μl) and lactozyme (50 μl) were added. The mixture was gently shaken and incubated for 20 min at 25°C, to allow lactose hydrolysis. Afterwards, Peridochrom solution (2 ml) was added and incubated again for 45–60 min at 25°C. Then the developed colour was measured at 510 nm in a Beckman DU-65 spectrophotometer, using as blank a solution containing all the components except lactose.

When it was necessary, deproteinization was accomplished by Carrez procedure (according to Essig and Kleyn, 1983): 1 ml of milk sample was placed in a 100 ml flask; and also 30 ml of distilled water, 2·5 ml of Carrez I solution (3·60 g $K_4[Fe(CN)_6] \cdot 3H_2O$ per 100 ml), 2·5 ml of Carrez II solution (7·20 g of $ZnSO_4 \cdot 7H_2O$ per 100 ml) and 5 ml of 0·1 M NaOH. The mixture was shaken and the solution allowed to come to room temperature. It was diluted to 100 ml with distilled water and was mixed. Finally it was filtered and the filtrate taken for assay.

RESULTS AND DISCUSSION

The stability of the colour formed was studied by a sequence of determinations for lactose concentrations in the range of the method, 1–8%. The results are shown in Fig. 1. As can be observed, the colour was adequately stable for 45 min in all the samples, and the measured colour remained stable for periods as long as 75 min. After considering the data, an incubation time of between 45 and 60 min is suggested.

The linear standard curve was made by triplicate sampling for each concentration of lactose and taking the average value of the absorbance. Adequate colour development was established at 60 min. Figure 2 shows the results of absorbance *versus* anhydrous lactose

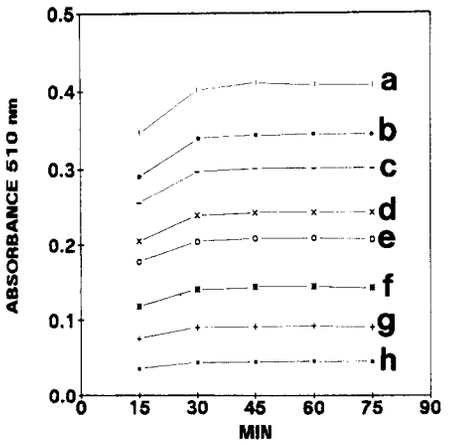

Fig. 1. Change in the final absorbances (2nd reaction) with time at 510 nm. Lactose concentrations (g per 100 ml): a = 8; b = 7; c = 6; d = 5; e = 4; f = 3; g = 2; h = 1.

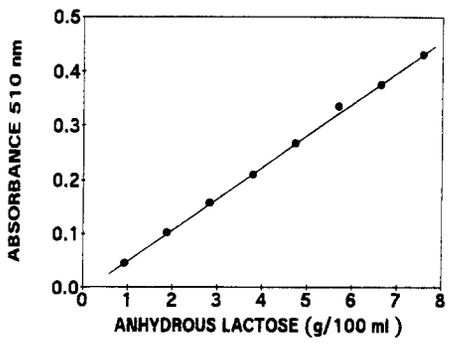

Fig. 2. Standard curve for determining anhydrous lactose.

concentration in g per 100 ml. The Y-intercept was $n = -0{\cdot}0095$, the gradient was $m = 0{\cdot}0584$, and the correlation coefficient was $r = 0{\cdot}9994$.

The precision of the method was determined using the coefficient of variation of the measurements corresponding to lactose monohydrate concentrations of 2 and 7%. Ten cuvettes of each of the two concentrations were prepared and processed as for the samples used in the determination of the standard curve. For the samples of 2% lactose, an average value of the absorbance of 0·0942 with a standard deviation of 0·006 088 was obtained, with a coefficient of variation (CV) of 6·5%. In the samples containing 7% lactose, the respective values were: average = 0·3364; standard deviation = 0·016 788; CV = 5%.

The method was evaluated by calculation of the limit of detection (LOD) and the limit of quantitation (LOQ). For this purpose, ten blank cuvettes of the reaction mix minus lactose were prepared; the usual procedure was followed but, in this case, the colour wash measured at 510 nm using distilled water as a reference black. The equivalent lactose concentrations were obtained from the absorbance values by means of the standard curve previously determined. The standard deviation (σ) of the equivalent lactose concentrations was 0·050 47; thus, the limits (% (w/v)) are

$$\text{LOD} = 3,\ \sigma = 0{\cdot}15;\ \text{LOQ} = 10,\ \sigma = 0{\cdot}50$$

Taking these results into account the present method can be very useful for different analytical purposes, especially for milk and dairy products.

This method has been applied to lactose determination in goats' milk (from the Spanish 'Murciano-Granadina' breed) and the results are shown in Table 1. The milk samples were taken towards the end of June; the analytical values were (% (w/v)): fat 3·96; protein

Table 1. Determination of lactose (monohydrate %(w/v)) content in goats' milk

	Whole milk			Milk without protein	
NIR	Samples	Mean	(Difference)	Samples	Mean
	4·55			4·75	
	4·70			4·84	
4·47	4·45	4·57	(+2%)	4·74	4·78

3·52; total solids 12·64; non-fat solids 8·59; and lactose 4·47. The device used was the near infrared (NIR) Milko-Scan 133-B (A/S N. Foss Electric, Hillerød, Denmark). As shown in Table 1, the method was used without deproteinization (whole milk) and with deproteinization (Carrez). The whole-milk sample showed a value closer to the NIR result, with a deviation of only 2%. Therefore, it is possible to apply the method without the necessity of deproteinization. This allows a considerable saving of time and reagents.

This method has been recently used in the determination of lactose in cheese and in the control of lactic bacteria in fermenters (Marín *et al.*, 1990). To the general advantages of enzymatic methods, low cost can also be added.

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