A new method for free glucose and lactose determination in milk.

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Scope:

α-Lactose is the principal constituent of milk and an important factor in the assessment of milk quality and composition. Moreover, lactose intolerance [1] has been raising during the last decades and bring about an increasing demand for lactose-free milk. There is a need for methods for product and process control through glucose and lactose determination [2]. A new method based on differential pH-metrics [3] was developed which enables the determination of both glucose and lactose content in milk and dairy products by means of two coupled enzymatic reactions.

Materials and methods.

The lactose and glucose determinations were performed with Microlab®EFA, a fully automated instrument provided with software dedicated to differential pH measurements available from Eurochem SpA Ardea -Italy.

Reagents: buffer pH 8.0 Tris (20 mmol L^{-1}), ATP2Na (13 mmol L^{-1}), Na_3PO_4 12 H_2O (8 mmol L^{-1}), NaOH (2 mmol L^{-1}), $MgCL_2$ $6H_2O$ (10 mmol L^{-1}), Bronopol (0.01 % w/w), KCl (100 mmol L^{-1}) and Triton x100 (2 % w/w) [4].

Enzymes: Hexokinase 300 U/ml; β-galactosidase 1500 U/ml stored in 50% glycerol, preservatives.

Calibrator: D-glucose 50 mM and α-lactose 100 mM in water solution with stabilisers and preservatives.

The repeatability and the reproducibility interval of the pH-metric method was estimated by means of an international ring test. Five laboratories were provided with equipment and chemicals for the analysis of 10 samples which were prepared by CECALAIT. Each laboratory analysed the samples in duplicate and repeated the procedure twice. The pH metric data were processed by CRA - Gembloux according to IDF Standard 135B:1991.

The samples were also sent to 38 other laboratories for lactose determination by a large scoop of methods: enzymatic (Boehringer), HPLC and others. The miscellaneous data from the 43 laboratories were treated by CECALAIT. For each sample, a rough average value was calculated. For each laboratory, the mean deviation d and the standard deviation Sd was computed and set out in a graph.

Results:

Eurochem ascertained linear response of the Microlab®EFA in the range 5 to 150 mM for both free glucose and lactose.

The repeatability and reproducibility intervals of the pH-metric results (IDF 135B:1991) were respectively less than 0.08 and 0.12 g lactose.H2O per 100 ml milk (table 1).

The standard deviation of the pH-metric results between laboratories was < 0.05 g lactose.H2O per 100 ml milk.

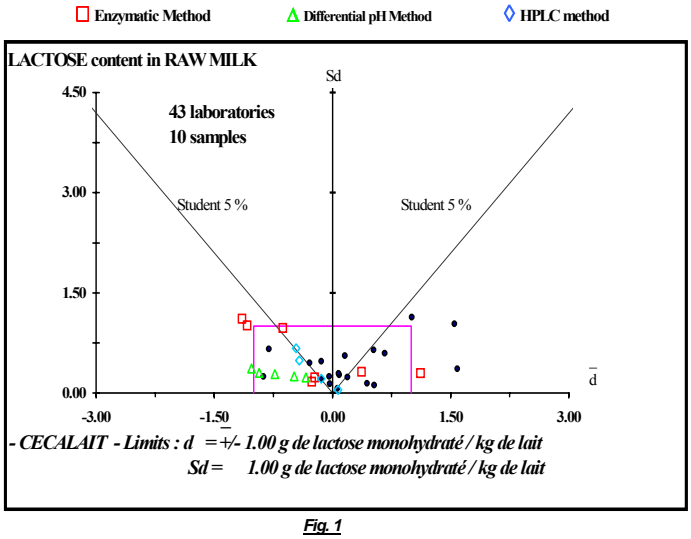

Fig. 1

The comparison of the pH metric method with the other ones is The comparison of the pH metric method with the other ones is shown in Fig. 1. The average results obtained with the pH metric, HPLC and enzymatic (Boehringer) methods are within 0.10 g lactose.H2O per 100 ml.

Table 1. Enzymatic pH- metric determination of lactose in milk
Preliminary ring test March 2002 – lactose results g/100 ml

First session

	Lab A		Lab B		Lab C		Lab D		Lab E	
Samples	**R. 1**	**R. 2**	**R. 1**	**R. 2**	**R. 1**	**R. 2**	**R. 1**	**R. 2**	**R. 1**	**R. 2**
Milk 11	5,13	5,25	5,24	5,25	5,26	5,33	5,18	5,22	5,21	5,22
Milk 12	5,07	5,11	5,17	5,20	5,22	5,18	5,12	5,15	5,12	5,15
Milk 13	5,03	5,11	5,10	5,10	5,14	5,09	5,07	5,11	5,07	5,10
Milk 14	4,98	5,02	5,03	5,02	5,05	5,06	4,97	5,01	5,01	5,04
Milk 15	4,91	4,94	4,97	4,99	5,00	5,01	4,94	4,95	4,97	4,97
Milk 16	4,86	4,91	4,95	4,94	4,97	4,99	4,90	4,91	4,93	4,94
Milk 17	4,87	4,86	4,87	4,93	4,91	4,95	4,88	4,89	4,90	4,91
Milk 18	4,84	4,89	4,90	4,92	4,89	4,91	4,85	4,87	4,88	4,89
Milk 19	4,81	4,84	4,81	4,89	4,87	4,89	4,82	4,85	4,84	4,87
Milk 20	4,78	4,81	4,84	4,87	4,86	4,89	4,79	4,83	4,81	4,85

Repeatability r = 0.027 * 2.8 = 0.076 g lactose per 100 ml milk
Reproducibility R = 0.037 * 2.8 = 0.104 g lactose per 100 ml milk

Second session

Samples	**R. 1**	**R. 2**	**R. 1**	**R. 2**	**R. 1**	**R. 2**	**R. 1**	**R. 2**	**R. 1**	**R. 2**
Milk 11	5,16	5,22	5,24	5,27	5,30	5,37	5,19	5,21	5,22	5,24
Milk 12	5,10	5,13	5,21	5,20	5,15	5,18	5,14	5,13	5,13	5,16
Milk 13	5,03	5,06	5,11	5,12	5,12	5,10	5,08	5,09	5,09	5,09
Milk 14	4,94	5,00	5,06	5,05	5,06	5,04	4,99	4,98	5,03	5,03
Milk 15	4,91	4,96	5,01	5,01	5,01	5,03	4,97	4,96	4,96	4,97
Milk 16	4,93	4,90	4,97	4,97	4,97	4,97	4,89	4,89	4,93	4,93
Milk 17	4,90	4,83	4,96	4,91	4,98	4,97	4,83	4,82	4,90	4,91
Milk 18	4,89	4,87	4,94	4,90	4,90	4,90	4,87	4,87	4,88	4,85
Milk 19	4,81	4,83	4,86	4,84	4,88	4,87	4,84	4,85	4,84	4,85
Milk 20	4,84	4,79	4,88	4,85	4,85	4,86	4,77	4,80	4,82	4,81

Repeatability r = 0.020 * 2.8 = 0.056 g lactose per 100 ml milk
Reproducibility R = 0.041 * 2.8 = 0.115 g lactose per 100 ml milk

Discussion:

The actual available methods for glucose and lactose determination in milk and dairy products require a sample pre-treatment before the determination itself and glucose and lactose has to be determined in a distinct way. The differential pH method doesn't require sample pre-treatment and enables the determination of both sugars in the same run. The results are comparable to classic and reference methods, with even better repeatability and reproducibility. The method will be examined more in detail and an extensive ring test will be organised for the international standardisation of the method.

Conclusions:

The differential pH is a promising alternative to measure the lactose and glucose content in milk and milk products because it is rapid (130 seconds), specific (enzymatic reaction), user friendly and roughly cheap.

References:

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