To what extent is lactose in lactose-free dairy products?

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Introduction

The global prevalence of lactose intolerance has already created a large assortment of commercially available lactose-free food products. Nevertheless, the market for functional food for lactose-intolerant consumers is increasing. In Europe, it is discussed how the lactose content of 'lactose-free' food products will be defined. For example, the working group *Issues of Nutrition* of the *German Society of Food Chemistry* has recommended three categories of food declaration [2], *i.e.* the 'low in lactose' level for food products containing ≤ 1 % lactose, 'very low in lactose' level (≤ 0.1 %) and 'lactose-free' level (≤ 0.01 % of lactose (Lac) and its degradation products galactose (Gal) and glucose (Glc). The latter is the strictest level being discussed. However, the German milk industry has a different opinion about 'lactose-free' food products and proposes a level of ≤ 0.1 % [3], which equals to the 'very low in lactose' level discussed before and is allowed to contain lactose degradation products. In the following, a sensitive and efficient method is demonstrated for screening and quantitation of lactose in lactose-free food.

Results and discussion

Method development and validation

The most streamlined approach for detection and quantitation of lactose in lactose-free dairy products was developed [1]. Separation was performed on HPTLC plates silica gel 60 using *i*-propyl acetate - methanol - water 11:7:2 (v/v/v) up to a migration distance of 60 mm. The separation took 30 min. After derivatization with the *p*-aminobenzoic acid reagent, the fluorescence was measured at 366/>400 nm. For application of volumes up to 250 µL on a rectangular start zone, LODs for lactose in dairy products were obtained down to the 0.04 mg/L range, which is the lowest LOD reported in matrix so far. For 11 different types of dairy products spiked at a lactose content of 0.01 %, the mean recovery rate was 90.5 % ± 10.5 % with a mean repeatability of 1.3 % ± 1.0 % (Table 1, Fig. 1).

Table 1 Recovery rate calculated via standard addition of lactose to dairy products spiked at the strictest lactose value discussed (100 mg/L)

Lactose-free dairy product	#	Mean recovery of lactose (%)	Repeatability (*%RSD*, *n* = 2)
Butter	1	105.3	1.9
Yoghurt	2	87.7	0.7
Milk	3	87.7	0.6
Evaporated milk	5	76.2	3.1
Buttermilk	6	81.2	0.2
Sour cream	7	78.9	2.9
Cream	8	82.8	0.2
Goat cheese	9	103.6	1.4
Cheese	15	102.4	1.2
Cream cheese	16	99.2	0.5
	17	90.3	1.8
Mean ± *%RSD* (n = 11)		90.5 ± 10.5	1.3 ± 1.0

For investigation of the matrix influence on quantitation, the calibration was performed in cheese matrix (1 and 10 µL), previously proven to be lactose-free at these application volumes. Five lactose standard levels, ranged 3 to 120 ng/band, were applied not only solely, but also oversprayed with sample matrix. Calibration curves were equivalent (Fig. 2) and an external standard calibration can be used for routine control of the lactose levels. However, at a 5-times higher matrix load, *e.g.* 50 µL, the calibration curve was shifted by the intrinsic lactose level of the cheese matrix itself. No blank sample was available for such low LODs and in all samples lactose was detected at increased application volumes. The 1:100 wide polynomial working range started at the LOQ of 3 ng/band and showed correlation coefficients r of ≥ 0.9999.

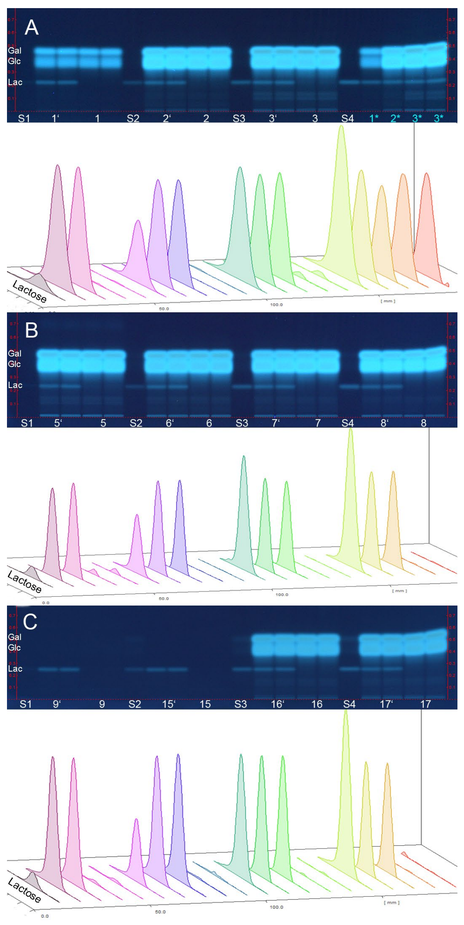

Fig. 1 Determination of the mean recovery rate of lactose (n= 2) in 11 different dairy products (A-C; track # assignments in Table 1)

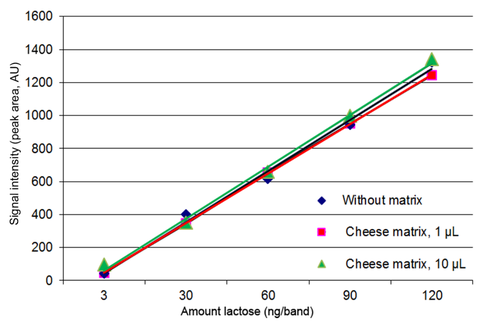

Fig. 2 Comparison of the external calibration curve with the calibration in cheese matrix (1 and 10 µL) showing determination coefficients R^2 ranged 0.9928 - 0.9999

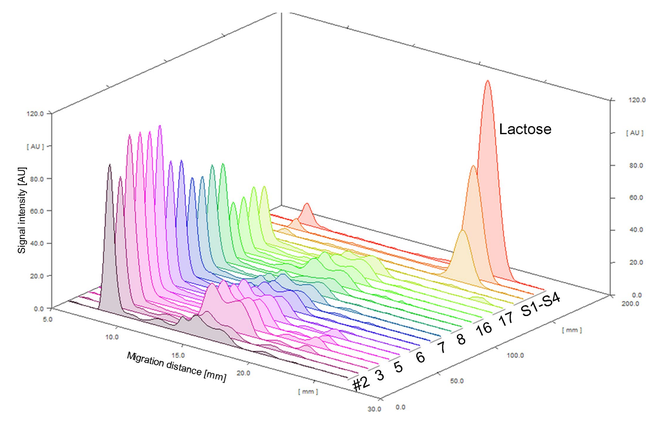

Fig. 3 3D-graphic of densitograms recorded at UV 366/>400 nm for quantitation of lactose-free dairy products via external standard calibration (S1-S4, 1-100 ng/band) showing that milk #3 and evaporated milk #5 contained 0.007 % and 0.006 % lactose, respectively.

Sample analysis

A broad range of 'lactose-free' labelled dairy products, even with high fat and protein contents, was investigated for their lactose content. After dissolution, 1 µL of the resulting 10 % food sample solution was directly applied. This allowed to control the LOD level of 0.001 %, which is below the strictest lactose level discussed so far (Fig. 3). In each 'lactose-free' sample lactose was detected when higher sample volumes were applied. Three out of the 17 commercially available 'lactose-free' dairy products contained lactose above the selected level of 0.001 %. In the goat cheese #10 0.064 % lactose was detected, in the milk #3 it was 0.007 % and in the evaporated milk #5 the content found was 0.006 %. The repeatabilities of the sample results (*%RSD*, *n* = 3) obtained at this low mg/L-level were below 10.1 %.

Efficiency of the method

Parallel screening of 20 samples (tracks) was performed. The running costs were low (0.3 Euro or 0.4 USD/analysis) and analysis time was fast (3 min/analysis). If the instruments of the HPTLC workings station are used in a 30-min interval shift, the sample throughput is calculated to be 300 samples per 8-hour day. If compared to current HPLC methods for lactose determination in milk products, gradient times of 24 to 65 min were reported [4, 5], which allows a sample throughput of 22 to 60 samples per 24-hour day. However in general, more effort was spent on sample preparation, and fortunately, this was kept simple when HPTLC was used. To conclude, simple sample preparation (dissolution), selective and good detectability, robustness with regard to varying matrix, low running costs, fast analysis time at the 10 mg/L level as well as good performance data are advantages.

Conclusions

The developed, streamlined method is highly attractive to the field of food safety and quality control of lactose-free products, as a limit value for lactose is expected soon. In all investigated dairy samples, lactose was found, however, only one goat cheese contained lactose above the strictest lactose level discussed so far. The efficacy of advanced production technologies for lactose-free dairy products can be investigated by this method thanks to its very low detectability of lactose.

References [1] G. Morlock, L. Morlock, C. Lemo, J. Chromatogr. A 1324 (2014) 215–223. [2] Working group *Issues of Nutrition*, Position paper of the *German Society of Food Chemistry* with regard to the statement *lactose-free* and *low in lactose*, Lebensmittelchemie 59 (2005) 45-46. [3] Background information on lactose, paper of the German Milk Industry Association, 30th March 2011, Berlin. [4] ESA, Dionex, Application Note 2011 (without year). [5] Thermo, Application Note 248, 2010.

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