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Streamlined analysis of lactose-free dairy products

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ABSTRACT

Functional food for lactose-intolerant consumers and its global prevalence has created a large market for commercially available lactose-free food products. The simplest approach for detection and quantitation of lactose in lactose-free dairy products was developed. A one-step sample preparation was employed and the resulting 10% sample solution was directly subjected to the chromatographic system. LODs down to 0.04 mg/L were obtained for dairy products by application volumes up to 250 μL on a rectangular start zone, which is the lowest LOD reported in matrix so far. The highly matrix-robust, streamlined approach was demonstrated for a broad range of dairy products, even with high fat and protein contents. The mean recovery rate for 11 types of dairy products spiked at the strictest lactose content discussed (0.01%) was 90.5 ± 10.5% (n = 11). The mean repeatability for 11 dairy products spiked at the 0.01% level was 1.3 ± 1.0% (n = 11). It is the simplest approach with regard to sample preparation at low running costs (0.3 Euro or 0.4 USD/analysis) and fast analysis time (3 min/analysis). It enabled an efficient product screening, and at the same time, the quantitation of lactose in relevant samples. This streamlined analysis is highly attractive to the field of food safety and quality control of lactose-free dairy products, for which a limit value for lactose is expected soon in the EU. This methodological concept can be transferred to other challenging fields.

1. Introduction

The triple A in food industry, *i.e.* availability all over the world at any place and time, added value (functionality), and affordability, is still the pacesetter [1]. Functional food for lactose-intolerant consumer and its global prevalence has created a large market for commercially available lactose-free food products. Lactose-intolerant individuals have a deficiency of the enzyme lactase, and thus, lactose is not completely catabolized into its monosaccharide units glucose and galactose. Lactose is the major disaccharide found in milk and milk products. The various animal milks contain mostly up to 5% lactose, but for example horse and ass milk contain even up to 7% lactose [2]. 'Lactose-free' milk and milk products are mostly produced by breaking down lactose into glucose and galactose by enzymatic hydrolysis with ß-galactosidases (often labelled as lactase on the food). However, the resulting milk products might contain varying amounts of residual lactose. Lactose intolerance varies widely among individuals with lactose maldigestion and the threshold of lactose is highly individual [3]. Additionally, especially in the newborn screening, the analysis of galactose is of interest as some individuals suffer from the genetic metabolic disorder galactosemia. Such individuals do not tolerate lactose and additionally galactose. For example, 'lactose-free' milk beverages, in which lactose is enzymatically hydrolyzed to glucose and galactose and from which the latter is not subsequently removed, are not suitable for patients with galactosaemia.

Hence, it is discussed in Europe, 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 (LChG) has recommended three categories of food declaration [4], *i.e.* the *low in lactose* level for food products of ≤1% lactose, *very low in lactose* level (≤0.1%) and *lactose-free* level (≤0.01% of lactose and its degradation products). The latter is the strictest level being discussed, which permits that these food products can safely be used in the dietetic management of patients even with galactosaemia, as such food products are also indicated to be 'free' of galactose. From the point of view of nutrition and of consumers with different lactose thresholds, this 3-level categorization is rational for finding adequate food products on the market.

However, the German milk industry has a different opinion about *lactose-free* food products and proposes levels ≤0.1% [5], which equals to the *very low in lactose* level discussed before and is allowed to contain lactose degradation products. The production of lactose-reduced milk by hydrolysis of lactose is harmonized and regulated by EU law [6], whereas the production of other lactose-reduced products adhere to national law, which regulates that a dairy is only allowed to add the ß-galactosidase on the authority

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of the respective German ministry (BMELV) [7]. The final *lactose-free* product tastes sweeter if compared to the standard product as the breakdown products are more intense in their sweetness. The production of *lactose-free* products ≤0.01% of lactose, but not galactose-free, is patented and the application of the technology would require a license [8]. This technology involves a 3-step based membrane filtration followed by re-combination: first, ultrafiltration (UF) of the milk, secondly nanofiltration (NF) of the UF permeate, thirdly reverse osmose (RO) for concentration of the NF permeate to a salt, and fourthly rearrangement, *i.e.* combination of the RO and UF retentate. By doing so, half the lactose is separated out of the milk without any other changes to the milk's composition. The subsequent addition of lactase splits the remaining lactose content into glucose and galactose. The final product gains the same organoleptic characteristics as standard milk and completely complies with consumer expectations. With regard to galactosaemia patients, the further absence of galactose would require additional milk treatment steps. Thus, the German milk industry advocacy of the ≤0.1% lactose level is understandable as the production of the strictest *lactose-free* level (≤0.01% of lactose and its degradation products according to the LChG working group) generates expensive extra costs and might not have an adequate high consumption rate.

It remains exciting how the decision of the EU commission about lactose limit values will be made in the near future. Not only for food production the strictest *lactose-free* level is challenging, but also for food analysis. Many currently available analytical methods for sugar analysis, like polarimetry, mid-infrared detection, photometry/fluorometry, and gravimetry, do not allow the differentiation between carbohydrates and are not suitable for measuring lactose in food products with diverse sugars. Enzymatic assays [9–12] and liquid column chromatography in combination with diverse universal detectors, *e.g.*, refractive index detector (RID), evaporative light-scattering detector (ELSD), and corona charged aerosol detector (CAD), miss the required capability of detection for the strictest level of 0.01% discussed [13]. For example, using HPLC–CAD, the limit of quantitation (LOQ) of lactose in lactose-reduced low-fat milk was estimated to be 0.02% [14]. However, recently a high-performance anion-exchange chromatography with pulsed amperometric detection was reported, for which the method's detection limit (LOD) for the lactose standard solution was 0.12 mg/L (0.000012%) using a 65-min gradient [15]. Also a capillary electrophoresis method with electrochemical detection reported a LOD of 0.1 mg/L (0.00001%) for the lactose standard solution at a separation time of 24 min [16]. Such methods would allow the control of the strictest level discussed (100 mg/L or 0.01%). Nevertheless, food control demands fast, cost-effective and at the same time matrix-robust analytical methods with regard to the varying sample matrices of the meanwhile large assortment for 'lactose-free' products. The following study describes, to our knowledge, the simplest approach for accurate and robust determination of lactose in 'lactose-free' dairy products with a one-step sample preparation followed by high-performance thin-layer chromatography and fluorescence detection (HPTLC–FLD) after selective derivatization.

2. Materials and methods

2.1. Materials

D(−)-fructose, D(+)-maltose-1-hydrat, D(+)-mannose and sucrose (all ≥ 98%) as well as acetonitrile (≥99%), acetone, butanol, *i*-propanol, methanol, *i*-propylacetate and ethyl acetate (all ≥ 99%) as well as aniline (≥99.9%), diphenylamine (≥98%), *p*-aminobenzoic acid (≥99%) and *o*-phosphoric acid (85%) were obtained from Fluka Sigma Aldrich, Seelze, Germany. D(+)-lactose-1-hydrat (Ph. Eur.) were delivered by Roth, Karlsruhe, and D(+)-glucose and D(+)-galactose (GPR Rectapur) by vwr, Darmstadt, Germany. Acetic acid (≥99.8%), sulphuric acid (98%), boric acid (≥99.9%), 2-naphthol, ninhydrin (both per analysis) and HPTLC plates silica gel 60, 20 cm × 10 cm, were provided by Merck, Darmstadt, Germany. Alternatively, plates with indicator F_{254} can be used. Distilled water was produced by Heraeus Destamat Bi 18 E (Thermo Fisher Scientific, Schwerte, Germany) and deionized water by a Synergy System (Millipore, Schwalbach, Germany). All food samples were purchased at local stores.

2.2. Extraction of 'lactose-free' food samples

2.5 g of each sample were dissolved with 8 mL distilled or deionized water in a 25-mL volumetric flask. For cheese and chocolate samples, 70 °C hot water was used. The sample was stirred for 10 min with a magnetic stir bar on the magnetic stirrer. After removal of the stir bar by rinsing with methanol (and cooling down), the flask was filled up to the mark with methanol. This resulted in a final concentration of 100 mg/mL in methanol–water 2:1, v/v. An aliquot of each sample was centrifuged (13.000 g, 5 min) and stored at 4 °C until analysis.

2.3. Standard (mixture) solutions

For mobile phase optimization, two methanolic sugar mixture solutions were prepared (100 ng/μL each), which contained fructose, galactose and glucose (mix 1) and lactose, maltose, sucrose and mannose (mix 2). For analysis of dairy products, aqueous stock solutions of lactose, glucose and galactose (100 ng/μL each) were diluted 1:33 together in mixture 3 (mix 3; 3 ng/μL) and 1:100 in mixture 4 (mix 4; 1 ng/μL) using methanol. For spiking of dairy products, an aqueous lactose solution (3 μg/μL) was used and its 1:300 methanolic dilution for calibration (10 ng/μL).

2.4. Application

The solutions were sprayed-on as 8, 11 or 15 mm bands or 15 mm × 3 mm rectangles with a track distance of 9, 12 or 16 mm, respectively, using the Automatic TLC Sampler 4 (ATS 4, CAMAG, Muttenz, Switzerland). The distance to the lower edge was set at 8 mm. The dosage speed was investigated as follows: 150 and 250 nL/s (bands) as well as 250 to 1000 nL/s (rectangles) with and without heating nozzle at 60 °C. For calibration, 1, 5, 10, 15 μL (3–60 ng/band), 1, 10, 20, 30, 40 μL (3–120 ng/band) or 1, 30, 60, 100 μL (3–300 ng/band) were sprayed-on using mix 3, or 1, 5, 10, 15 μL (10–150 ng/band) using the diluted lactose solution. 1 to 250 μL sample solution were applied depending on the application zone geometry (8, 11, 15 mm bands or 15 × 3 mm rectangles). Overlapped application (overlap of 50%) was performed by application of 16 mm bands each of a sample (2.5 μL) and standard solution (50 μL of mix 3). After application the plate was dried for 30 s in a stream of warm air, or automatically in the Automated Developing Chamber ADC 2 (CAMAG).

2.5. Chromatography

Mobile phase development was carried out on cut plates (5 × 10 cm) in the Twin Trough Chamber (10 × 10 cm, CAMAG) with varying mobile phase combinations and ratios. Food analysis was performed using *i*-propyl acetate–methanol–water 11:7:2 (v/v/v/v) as mobile phase up to a migration distance of 60 mm. The separation took 30 min in the Twin Trough Chamber 20 × 10 cm or ADC 2. The relative air humidity was 40 ± 10% and the room temperature 23 ± 5 °C during the whole study.