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**Recent advances in analytical techniques for the
determination of lactose**

Siyue Jia, Xiaodong Dong*

College of Medicine, Hebei University, Baoding 071000, China

*Corresponding Author: xddong@hbu.edu.cn

Abstract

Lactose is disaccharide which is composed of glucose and galactose, and is the only saccharide synthesized by mammals and is enriched in mammalian milk. Lactose plays multiple roles in the health of neonates, by stimulating the growth of selected beneficial bacteria in the gut, and participating in the development and growth of newborns. Lactose concentrations may be indicative of abnormalities and be used for clinical diagnosis. Thus, the establishment of a simple and rapid method for the determination of lactose with high selectivity and sensitivity is of great significance to dairy industry and public health. In this article the studies of detection methods for lactose in recent years are reviewed.

Keywords: lactose; milk; determination; detection.

1. Introduction

Lactose, a disaccharide found in milk and dairy products, is the only source of carbohydrates in milk, with concentrations ranging from 4.4 to 5.2%. Lactose concentration is a basic marker for the evaluation of milk quality and the detection of abnormalities [1-3]. Effective control of lactose levels in dairy products is also important for the cheese industry in order to control the fermentation processes. In addition, lactose is a basic parameter in wastewater control and veterinary medicine [4-6]. The determination of lactose is also clinically relevant. An excessive amount of lactose in blood indicates gastrointestinal malignancy. Lactose control in food products is, besides economic reasons, especially important in lactose reduced or lactose-free products for the 75% of adult worldwide suffering from lactose intolerance [7-9]. Therefore, the quantification of lactose is an important challenge in many areas. In this paper, the attributes of different analytical technique for the determination of lactose in recent years are reviewed.

2. Analytical Methods

2.1. HPLC method. High-performance liquid chromatography (HPLC) is a powerful tool that enables the separation of complex mixtures into individual components, and is a highly sensitive and reproducible analytical technique. In recent years, HPLC has been combined with many sensitive detection techniques and has experienced continuous improvement of stationary phases, which have improved its sensitivity and specificity. HPLC is currently widely used for the analysis of drugs and dosage forms with respect to quality control, quantitative determination of active ingredients and impurities, monitoring drug blood concentration in patients, and bioequivalence assessment [10,11].

Silveira *et al.* [12] adopted HPLC method for determination of lactulose and lactose content in commercial samples of the ultrahigh temperature and sweetened condensed milk. The used HPLC method showed a good resolution of the analytes evaluated. The analyzed ultrahigh temperature milk samples presented levels for lactulose in accordance with the limit recommended by the International Dairy Federation.

There was no significant variation in lactulose concentration for sweetened condensed milk samples. However, one sweetened condensed milk sample showed lactose level lower than the established values (10–12%).

Wang *et al.* [13] developed and validated a simple method for the determination of glucosamine and lactose in milk-based formulae by HPLC with refractive index detector. Samples were cleaned up just by protein precipitation before HPLC analysis. Separation was achieved with a Shodex Asahipak NH2P-50 column. The method showed good linearity, sensitivity, precision and recovery, and proved very simple and rapid for routine analysis since separation was completely achieved at 10 min.

2.2. Electrochemical method. Electroanalytical techniques are a promising alternative for the determination of organic molecules in complex matrices, because they deliver lower cost and analysis time, high selectivity, and high sensitivity [14-16]. Enzyme-based amperometric biosensors have been found to constitute versatile analytical devices with high selectivity that can be operated by unskilled personnel. Accordingly, they can be envisaged as serious competitors for conventional techniques, representing an attractive alternative for small industries [17-19].

Tasca *et al.* [20] described a new third-generation amperometric biosensor for lactose determination. The biosensor was based on the highly efficient direct electron transfer between cellobiose dehydrogenase (CDH) from *Phanerochaete sordida* (PsCDH) and single walled carbon nanotubes (SWCNT). The SWCNTs were surface modified with aryl diazonium salts of p-phenylenediamine (NH₂-PD) and deposited on the top of a glassy carbon (GC) electrode. The PsCDH NH₂-PD/SWCNT-GC biosensor showed very efficient DET and exhibited an extraordinary high current density of 500 $\mu\text{A cm}^{-2}$ in a 5 mM lactose solution at pH 3.5. The biosensor had a detection limit for lactose of 0.5 μM , a large linear range from 1 to 150 μM lactose and a high sensitivity. It showed also a fast response time, good reproducibility and good stability. In addition, it was easy, simple to manufacture, and cheap because a low amount of enzyme was required and highly selective, as no significant interference was observed. For these reasons, it could represent a valid alternative to HPLC measurements for lactose determination in milk and dairy products.

Gursoy *et al.* [21] developed the lactose biosensor based on surfactant doped polypyrrole. Galactose oxidase and b-galactosidase was coimmobilized in polypyrrole matrix during electropolymerization process with the presence of sodium dodecylbenzene sulphonic acid as surfactant. The response of the enzyme electrode was measured by CV in the range of 20.1 to 1.0 V versus Ag/AgCl which was due to the electro-

oxidation of enzymatically produced H₂O₂. The effect of lactose concentration was investigated. Response time of biosensor was found to be 8–10 s and the upper limit of the linear working portions was found to be 1.22 mM lactose concentration with a detection limit of (2.6×10^{-6} M). The apparent Michaelis–Menten constant was found to be 0.117 mM lactose. The effects of interferents were determined.

2.3. Other methods. In addition to these main approaches mentioned above for lactose detection, still a few special techniques with high sensitivity have been applied. Srivastava *et al.* [22] developed a novel method for quantification of lactose in mammalian milk through high-performance thin-layer chromatography and determination by a mass spectrometric technique. Weber *et al.* [23] proposed the detection of dissolved lactose employing an optofluidic micro-system. Li *et al.* [24] developed a raman spectroscopy-based approach for the rapid determination of lactose in milk using crystal violet as an internal standard. Soldatkin *et al.* [25] proposed the development of conductometric biosensor array for simultaneous determination of maltose, lactose, sucrose and glucose.

3. Conclusion

One of the most significant needs in the dairy industry is lactose monitoring, which is required for maintaining specified lactose levels during the production steps and in the final milk products. Lactose plays an important role in the formation of the neural system and the growth of skin (texture), bone skeleton and cartilage in infants. It also prevents rickets and saprodonia [26,27]. Therefore, the precise determination of lactose is important for industry and public health. This review has highlighted the significant developments in rapid and alternative techniques for the detection of lactose in recent years. We believe the direct and rapid online monitoring of lactose in milk, dairy products and some biological samples using simple analytical devices with a high performance is still the future effort.

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