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Short communication

Simultaneous determination of galactose, glucose, lactose and galactooligosaccharides in galactooligosaccharides raw materials by high-performance anion-exchange chromatography with pulsed amperometric detection

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ABSTRACT

This study describes a method for the simultaneous determination of galactose, glucose, lactose and galactooligosaccharides (GOS) in GOS raw materials (GOS syrups and powdered GOS) by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD). The GOS raw materials were extracted with phosphate buffer. The extract was then treated with β-galactosidase to hydrolyze GOS and lactose. The total amounts of galactose and glucose released from GOS and lactose were determined in the treated solution. Free galactose, glucose and lactose were determined in the initial solution. The glucose in a β-galactosidase solution was also determined. The content of GOS in GOS raw materials was calculated by the increment of galactose and glucose after GOS were hydrolyzed, and the glucose and galactose also released from lactose were taken into consideration. The validated method has been successfully applied to determine the content of galactose, glucose, lactose and GOS in GOS syrups and powdered GOS.

1. Introduction

Gibson and Roberfroid (1995) first defined prebiotic as “a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health”. This definition has been revised several times over the years, and the most recent version states that a dietary prebiotic is “an ingredient selectively fermented that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health” (Gibson et al., 2010). According to this concept, the commercially available GOS ingredients are likely prebiotics. It has been demonstrated that GOS are non-digestible (Krasaekoopt & Watcharapoka, 2014), modulate the intestinal microbiota (Bruno-Barcena & Azcarate-Peril, 2015) and the immune system (Sangwan, Tomar, Ali, Singh, & Singh, 2015), and have a beneficial effect on barrier function (Beleli, Antonio, Dos Santos, Pastore, & Lomazi, 2015) and mineral absorption (Dos Santos, Tsuboi, Araujo, Andreollo, & Miyasaka, 2011).

Recently GOS have been defined as “a mixture of those substances produced from lactose, comprising between 2 and 8 saccharide units, with one of these units being a terminal glucose and the remaining saccharide units being galactose and disaccharides comprising 2 units of galactose” (Torres, Goncalves, Teixeira, & Rodrigues, 2010). The synthesis of GOS is carried out by β-galactosidase (EC3.2.1.23) from lactose by glycosyl transfer of a D-galactose residue on to lactose or oligosaccharide products of the galatosyltransferase reaction or monosaccharie products of lactose hydrolysis (Lu et al., 2012). This process yields a mixture of transgalactosylated oligosaccharides with different degrees of polymerisation (DP) and different glycosidic linkages (Osmana, et al., 2014). Structural analyses of the oligosaccharide components in GOS demonstrate the presence of several types of glycosidic linkages including β-(1, 6), β-(1, 4), β-(1, 3), and β-(1, 2). Linkages between galactosyl units, the efficiency of transgalactosylation, and the composition of a GOS mixture depend upon the source of enzymes and the conditions used in the reaction (Rodriguez-Colinas, Fernandez-Arrojo, Ballesteros, & Plou, 2014; Guerrero, Vera, & Illanes, 2013; Gosling, Stevens, Barber, Kentish, & Gras, 2010; Van Leeuwen, Kuipers, Dijkhuizen, & Kamerling, 2016). GOS have been widely used in infant formulas, beverages, baked goods, yoghurts, desserts, nutrition

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bars, sugar confectioneries, soups, sauces, meal replacement shakes and baby foods for their nutritional advantages and functional properties (Lamsal, 2012).

Studies on GOS mostly focus on their function (Montilla, Megias-Perez, Olano, & Villamiel, 2015), synthesis (Guerrero, Vera, Acevedo, & Illanes, 2015; Srivastava, Mishra, & Chand, 2016), purification (Pruksasri, Nguyen, Haltrich, & Novalin, 2015; Guerrero, et al., 2014) and the identification of their individual components (Wieneke, Klein, Geyer, & Loos, 2007; Martinez-Villaluenga, Cardelle-Cobas, Corzo, & Olano, 2008; Cardelle-Cobas, Martínez-Villaluenga, Sanz, & Montilla, 2009; Coulier et al., 2009; Hernandez-Hernandez, Calvillo, Lebron-Aguilar, Moreno, & Sanza, 2012; Van Leeuwen, Kuipers, Dijkhuizen, & Kamerling, 2014). There are few studies on the quantitative analysis of GOS in raw materials. To accurately determine GOS is difficult, especially in samples with high concentrations of free lactose. The lack of standards for GOS is a significant disadvantage in the identification and quantification purposes. One of the most commonly-used GOS quantitative methods is the AOAC 2001.02 method (De Slegte, 2002). In this method, GOS content is calculated as GOS = $k \times G_g$, where G_g is the galactose released from GOS and the k factor is defined as: $k = (180 + 162n)/180n$, where n is the average number of galactose moieties in the GOS molecules. Regrettably, how the n factor should be calculated (or measured) is not described in the AOAC method. Provided the n factor is known, one can obtain the k factor, and invoke the AOAC 2001.02 method to calculate GOS content in food samples. Thus an accurate estimation of the n factor, and consequently the k factor is needed for the AOAC method. We have found that the k factor varies among manufacturers and even batches. Ideally, each manufacturer should determine the k factor, and then provide it for their customers. There are different approaches to calculating the n factor, such as the *SEC* method, the LC-MS method and so forth, whereas few could be adopted to solve the problem of interference. Although manufacturers could supply appropriate k factors for their customers, such a solution would not be applicable for supervision organizations and third party detection institutions since they are unlikely to have access to this information. On this account, we modified the AOAC 2001.02 method to determine the GOS content of GOS raw materials, through taking the formula for calculating the n factor into the formula for calculating GOS content. This modified method could spare the extra time needed to separately determine the n factor (and consequently the k factor). As a result, we can acquire the GOS content of GOS raw materials by one formula. But this does not mean that with this modified method the k factor could be dispensed with. At least for the present, it is necessary to determine the k factor if we want to determine GOS in selected foods by the AOAC 2001.02 method. Another weakness of the AOAC 2001.02 method is that it is unsuitable for the determination of the GOS content in infant formulas, which has aroused much concern from research institutions. Several studies (Ruiz-Matute, et al., 2012; Austin, Benet, Michaud, Cuany, & Rohfritsch, 2014) reported on the determination of the GOS content in foods, but the quantitative analysis was carried out by external calibration using standard solutions of other sugars, or several GOS ingredients as standards which are not so rigorous.

In this paper, we describe a method to accurately determine galactose, glucose, lactose and GOS in GOS raw materials simultaneously by HPAEC-PAD. The flow chart for analyzing GOS in raw materials is shown in Fig. 1. This method shortens the analysis time and requires fewer instruments. For via this method there is no necessity to determine galactose, glucose and lactose in GOS raw materials by HPLC and to determine GOS in GOS raw materials by HPAEC-PAD, respectively (GB 5009.8-2016 National Food Safety Standards). The validated HPAEC-PAD method can be applied to determine the content of galactose, glucose, lactose and GOS in GOS syrups and powdered GOS by a modified formula without predetermining the k factor. The result can be utilized to evaluate the efficiency of the synthesis and purification of GOS raw materials.

2. Experimental

2.1. Reagents and materials

Galactose reference standard (≥98.5% purity), glucose reference standard (≥99.0% purity) and lactose monohydrate reference standard (≥98.5% purity) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). β-galactosidase originating from *Aspergillus oryzae* (10.3 units/mg solid) was purchased from Sigma-Aldrich (Steinheim, Germany).

50% sodium hydroxide solution (50–52% in H_2O, eluent for ion chromatography) and sodium acetate (bioUltra, ≥99.0% purity) were bought from Sigma-Aldrich (Steinheim, Germany). Monopotassium phosphate (analytical grade), dipotassium phosphate trihydrate (analytical grade), acetonitrile (analytical grade) were purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, PR China). Other chemicals in use were all of analytical grade. Ultrapure water was obtained from Millipore System (Bellerica, USA).

Injection vials were obtained from Thermo Scientific (Sunnyvale, USA). Water phase injection filters (PES, polyether sulfone) were purchased from Shanghai ANPEL Scientific instrument Co. Ltd. (Shanghai, PR China). GOS raw materials were kindly provided by Baolingbao Biology Co. Ltd. (Yucheng, PR China) and Quantum Hi-Tech Biological Co. Ltd. (Jiangmen, PR China).

2.2. Preparation of standard stock and working solutions

The standard stock solution of galactose (1 mg/mL) was prepared by dissolving 100 mg galactose in 100 mL water. The standard stock solution of glucose (1 mg/mL) was prepared by dissolving 100 mg glucose in 100 mL water. The standard stock solution of lactose (1 mg/mL) was prepared by dissolving 105.3 mg lactose monohydrate in 100 mL water.

The standard stock solutions of galactose, glucose and lactose were then diluted with water to get intermediate combination solution concentrations of 1, 5, 10, 20, 25 μg/mL for calibration purposes. The working solutions were prepared daily and the stock solutions were permanently stored at 4 °C.

2.3. Reagent preparation

Phosphate buffer solution (0.2 mol/L pH 6) was prepared by dissolving 22 g monopotassium phosphate and 6 g dipotassium phosphate trihydrate in 1000 mL water (pH was adjusted to 6) and prepared just before use. β-galactosidase solution (500 U/mL) was prepared by suspending approximately 5000 U β-galactosidase in 10 mL phosphate buffer solution. Suspension was stirred well and prepared just before use. Acetonitrile solution (20%, V/V) was prepared by diluting 200 mL acetonitrile with water to 1 L.

2.4. Instrumentation and conditions

Analyses were carried out using an HPAEC-PAD Dionex ICS-5000 system (Sunnyvale, USA) equipped with a DP-5 gradient pump, an AS-1 auto-sampler and an ED DC-5 electrochemical detector (pulsed amperometry) including a detection cell with a 1.0 mm diameter gold electrode and a pH-Ag/AgCl combination reference electrode. The pulse setting for the amperometric detector was the sugar four potential wave form, as named by Dionex. The time program of the detector is given in Fig. 2. A Dionex CarboPac™ PA-20 analytical column (150 mm × 3 mm I.D.) and a Dionex CarboPac™ PA-20 guard column (50 mm × 3 mm I.D.) (Sunnyvale, USA) were used at a flow rate of 0.45 mL/min at a column temperature of 30 °C. The injection volume was 10 μL. Three eluents were prepared in plastic bottles that were carefully purged using helium. The eluents consisted of water (A), 250 mM NaOH (B) and 100 mM NaOH containing 400 mM sodium acetate (C). Analyses were performed under the gradient mode and the gradient program is