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Analytical Methods

The use of capillary electrophoresis with contactless conductivity detection for sensitive determination of stevioside and rebaudioside A in foods and beverages

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We would like dedicate this paper to our teacher Professor František Opekar on the occasion of his 70th birthday.

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ABSTRACT

Two electrophoretic methods with contactless conductivity detection have been developed for determination of the content of rebaudioside A and stevioside in samples of sweeteners and beverages prepared from extracts of the plant Stevia rebaudiana Bertoni. The total content of rebaudioside A and stevioside can be determined in a fused silica capillary with an inner diameter of 10 μm and total length of 31.5 cm in optimised background electrolyte with the composition 170 mM H_3BO_3/LiOH (pH 9.0). The combined peak of the two glucosides is characterised by a migration time of 54 s, which completely separates it from EOF. INST coating solution in an amount of 0.5% *v*/*v*, which effectively suppresses the electroosmotic flow, was added to the background electrolyte for mutual separation of rebaudioside A and stevioside. The CE method with suppression of EOF is characterised by complete separation of rebaudioside A and stevioside, LOD is 0.3 mg/L (0.1 μM).

1. Introduction

Extracts from tropical plants of the family Stevia rebaudiana Bertoni are currently used as one of the most widespread natural substitutes for saccharose (Lemus-Mondaca, Vega-Galvez, Zura-Bravo, & Ah-Hen, 2012; Yadav, Singh, Dhyani, & Ahuja, 2011). The sweetness of Stevia is a result of the presence of an extensive group of glycoside substances, which include rebaudiosides, stevioside, steviolbioside and dulcosides. The chemical structure of these substances is based on cyclic diterpene steviol, to which are bonded various numbers of glucopyranose, rhamnopyranose and xylopyranose units; a detailed survey of the chemical composition of steviol glucosides can be found in the articles (Jackson et al., 2009; Morlock, Meyer, Zimmermann, & Roussel, 2014; Zimmermann, 2011). These substances have a sweetening ability that greatly exceeds that of saccharose; for example, rebaudioside A and D have a purely sweet taste without any indication of bitterness and a sweetening ability that is 300–450 times greater than that of saccharose; on the other hand, the sweetening ability of dulcosides is only 100 × greater and they have a markedly bitter to metallic taste (Kinghorn, 2002).

From a practical point of view, stevioside and rebaudioside A (Fig. 1) are the most important of the wide range of various steviol glycosides. These two substances should make an approx. 95% contribution to the overall content of steviol glycosides used in foods and food supplements in order to achieve a purely sweet taste and sensorial feeling comparable to saccharose. The importance of steviol glycosides in the food industry is connected with their almost zero energy value (Lemus-Mondaca et al., 2012). Consequently, they are preferentially used to sweeten beverages for individuals suffering from overweight who are not capable of meeting their daily liquid needs with pure water. In this way, steviol glycosides can be effectively used in regulation of food intake and achieving a feeling of sweetness satisfaction (Carakostas, Curry, Boilea, & Brusick, 2008). Compared to artificial sweeteners of the aspartame type, steviol glycosides do not cause secretion of insulin and, in addition, can be used for individuals suffering from phenylketonuria (Kroger, Meister, & Kava, 2006). For all these reasons, the use of steviol glycosides is becoming increasingly widespread, leading to the requirement of controlling the content of steviol glycosides in foodstuffs to monitor possible falsification.

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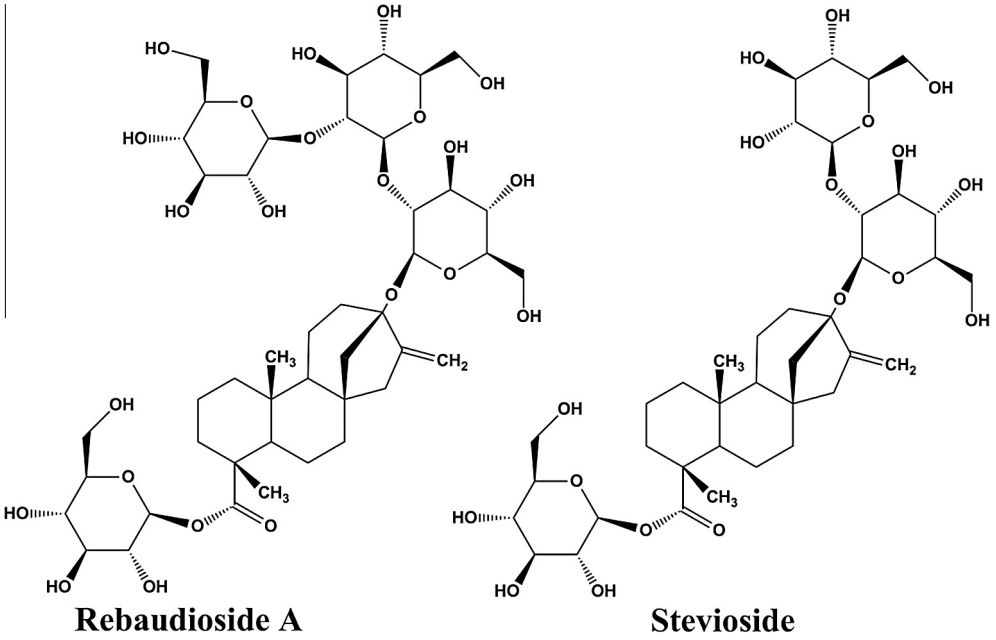

Fig. 1. Structure of the most important steviol glycosides used in the food industry.

Of the analytical methods known to date, steviol glycosides in Stevia leaves have been determined by desorption electrospray ionization mass spectrometry (Jackson et al., 2009), near infrared reflectance spectroscopy (Hearn & Subedi, 2009), high-performance thin-layer chromatography with densitometric quantification (Jaitak, Gupta, Kaul, & Ahuja, 2008) and electrospray ionization mass spectrometry (Morlock et al., 2014). However, HPLC techniques using an NH_2 column (Kitada, Sasaki, Yamazoe, & Nakazawa, 1989; Pól et al., 2007), RP18C column (Bililign, Moore, Tan, & Leeks, 2014; Bovanova, Brandsteterova, & Baxa, 1998) and newly also an HILIC column (Ahmed & Dobberstein, 1982; Zimmermann, Woelwer-Rieck, & Papagiannopoulos, 2012) hold a very dominant position in the analysis of foodstuffs. HPLC was also recommended in 2010 by the World Health Organisation as a reference method for determining steviol glycosides in foodstuffs (Tada et al., 2013). A difficulty encountered when using HPLC analysis of weakly absorbing glycosides lies in finding sensitive detection techniques. When using UV detection, measurements are carried out at short wavelengths around 190 nm (Makapugay, Nanayakkara, & Kinghorn, 1984; Wolwer-Rieck, Tomberg, & Wawrzun, 2010) or the sensitivity is increased by derivatisation of glycosides using p-bromophenacyl bromide (Kitada et al., 1989). Consequently, HPLC is combined with all types of mass analysers, which simultaneously ensure the selectivity of the determination (Gardana, Scaglianti, & Simonetti, 2010; Pól, Hohnová, & Hyotylainen, 2007; Wolwer-Rieck et al., 2010; Zimmermann et al., 2012).

The first experiments with analysis of steviol glycosides were performed using capillary electrophoresis (CE) (Liu & Li, 1995). The separation electrolyte was based on sodium tetraborate with addition of organic solvent and the capillary zone electrophoresis (CZE) technique was employed (Dacome et al., 2005; Liu & Li, 1995; Liu, Ong, & Li, 1997); in one case sodium dodecyl sulphate (SDS) was added to the sodium tetraborate and separation was performed by the micellar electrokinetic chromatography (MECK) technique (Mauri, Catalano, Gardana, & Pietta, 1996). In the newest CE study, rebaudioside A and stevioside were separated using addition of modified beta-cyclodextrin to phosphate buffer (Ayyappa et al., 2015). Detection was performed using low-sensitivity UV detection without derivatisation of steviol glycosides (Ayyappa et al., 2015; Dacome et al., 2005; Liu & Li, 1995); CZE analysis of extract from stevia leaves employed sensitive MS detection (Mauri et al., 1996). The sensitivity of the CE determinations described to date is low and this technique can be used only for determination of steviol glycoside in concentrated stevia extracts; as far as we have been able to determine, foodstuffs have not yet analysed. This communication describes the development and use of highly effective electrophoretic separation combined with sensitive contactless conductivity detection (C^4D) (Kubáň & Hauser, 2009, 2013) for the determination of rebaudioside A and stevioside in sweeteners and beverages commonly available in the commercial network. Compared with HPLC, CE has a number of advantages such as short analysis time, simple sample preparation based on only dilution and, last but not least, miniaturisation of the whole analytical process including minimal reagent consumption (Bergamo, da Silva, & de Jesus, 2011; Tůma, Málková, Samcová, & Štulík, 2011), which is currently in accord with the concept of green chemistry.

2. Materials and methods

2.1. Chemicals and BGE preparation

All the chemicals employed were of analytical purity: stevioside (Sigma), rebaudioside A (Sigma), rebaudioside B (Sigma), lithium hydroxide (Fluka), boric acid (Sigma), polyethylene glycol (PEG M_r 8000, Fluka), polyvinyl alcohol (PVA, Fluka), acetonitrile (ACN, Sigma), INST coating solution (Biotaq, U.S.A.). Deionized Milli-Q water (18.2 MΩ cm, Millipore) was used to prepare the background electrolytes (BGE) and 1 mg/mL stock solutions of rebaudioside A, rebaudioside B and stevioside, which were stored in a refrigerator at 4 °C until the analysis. Stock solutions of 20% *m*/*v* PEG and 5% *m*/*v* PVA were prepared by dissolving the solid substances in water (PEG 8000 in cold water) and then used at room temperature as an additive for BGE preparation. In preparing the BGE, the appropriate amount of H_3BO_3 was dissolved in deionized water and then solid LiOH was added to the solution until the required pH was attained. Titration with solid LiOH is important