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Comparison of two HPLC systems and an enzymatic method for quantification of soybean sugars

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Abstract

Successful breeding programs need fast and reliable methods for analyzing sugar composition in new soybean (*Glycine max* (L.) Merrill) lines. The efficiency to quantify the major sugars, including glucose, fructose, sucrose, raffinose, and stachyose, in five soybean lines with two HPLC systems and an enzymatic procedure were compared. Soluble sugars in soybean were extracted with water at a solvent-to-sample ratio of 5:1 at 50 °C for 15 min, and analyzed by high-performance size exclusion chromatography with refractive index detection (HPSEC-RI), high-performance anion-exchange chromatography with pulsed-amperometric detection (HPAEC-PAD), and a raffinose-series oligosaccharides assay procedure. All three methods produced comparable and reproducible results. The HPAEC-PAD method was more sensitive, faster and capable of separating all five major sugars in soybean with improved peak resolution compared with the HPSEC-RI method, and is recommended for soybean breeding programs. The enzymatic procedure required no expensive instrumentation and less sample preparation, but could not quantify individual raffinose and stachyose.

Keywords: Soybean sugars; HPSEC-RI; HPAEC-PAD; Raffinose; Stachyose

1. Introduction

The amount of total soluble sugars in soybean seeds varies among varieties, ranging from 6.2% to 16.6%, and sucrose, raffinose, and stachyose comprise almost 99% of the soybean soluble sugars (Kawamura, 1967). Sucrose is the most abundant sugar in soybean ranging from 3% to 10% and responsible for enhancing the sweet taste of soyfoods (Taira, 1990), whereas stachyose (0.6–5.8%) and raffinose (0.1–1.8%) (Hymowitz & Collins, 1974; Trugo, Farah, & Cabral, 1995) are not digestible. One way to improve the sugar composition of soybean and thereafter its marketability as food and feed is by breeding. For a successful breeding program, breeders need efficient and reliable methods to analyze sugar composition in new soybean lines.

Several methods have been reported for the determination of sugars in soybean and other legumes. The colorimetric method by Dubois, Gilles, Hamilton, Rebers, and Smith (1956) gives a reliable, but only the total sugar content. Paper chromatography (Lineback & Ke, 1975; Pazur, Shadaksharaswamy, & Meidell, 1962; Shallenberger & Moores, 1957) and thin layer chromatography (Tanaka, Thananunkul, Lee, & Chichester, 1975) provide qualitative analysis, but the results are difficult to quantify. Gas chromatography is very sensitive; however, it is laborious due to the need of sugar derivatization (Aman, 1979; Delente & Ladenburg, 1972; Folkes, 1985; Molnar-Perl, Pinter-Szakacs, Kovago, & Petroczy, 1984). High-performance liquid chromatography (HPLC) has become the preferred method because of its simple and efficient separation and quantification of sugars (Black & Bagley, 1978; Ladish & Tsao, 1978; Rabel, Caputo, & Edward, 1976; Reyes, Wrolstad, & Cornwell, 1982). HPLC coupled with refractive index (RI) detection is commonly used in soybean

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and other plants for sugar analysis (Black & Bagley, 1978; Frias, Hedley, & Price, 1994; Johansen, Glitso, & Knudsen, 1996; Kim, Kim, & Hwang, 2003; Knudsen, 1986). RI detection offers a wide linear range for sugar analysis, but is not very sensitive for low concentrations (Lis & Sharon, 1978; Martens & Frankenberger, 1990). More recently, high-performance anion exchange chromatography coupled with pulsed-amperometric detection (HPAEC-PAD) becomes increasingly popular and has been extensively employed for sugar analysis (Cataldi, Campa, Angelotti, & Bufo, 1999; Cataldi, Margiotta, Lasi, & Di Chio, 2000; Frias et al., 1999; Mohamed & Rayas-Duarte, 1995; Rocklin & Pohl, 1983; Townsend, Hardy, Hindsgaul, & Lee, 1988). PAD is highly selective and sensitive because only reactive compounds will give response and at very low concentrations.

Enzymatic analysis has also been routinely used for sugar analysis due to the specificity and sensitivity of enzymes. Maughan, Saghai Maroof, and Buss (2000) used invertase and hexokinase to quantify the sucrose content in 149 soybean varieties. However, little work has been reported to compare HPLC and enzymes for soybean sugar analysis. The objective of this study was to compare three methods, HPAEC-PAD, HPSEC-RI, and enzymes, for quantifying soybean sugars. Their advantages and disadvantages with respect to sample preparation, and sugar quantification and identification were addressed.

2. Materials and methods

2.1. Materials

Seeds of five soybean lines, namely Hutcheson, Camp, SS-516, MFL-552, and 03CB-14, grown in Fayetteville, Arkansas in 2002 were provided by the Department of Crop, Soil and Environmental Sciences at the University of Arkansas, Fayetteville, AR. Fifteen grams of each variety were ground in a mill for 20 s (Knifetec 1095, Foss, Hoganas, Sweden) and the ground meal was screened through a 150-μm sieve (W.S Tyler, Mentor, OH) and used for sugar extraction. The moisture content of each ground meal was determined according to Approved Method 44-31 (AACC, 2000). Glucose, fructose, melibiose, sucrose, raffinose, stachyose, and maltoheptaose were obtained from Sigma Chemicals Co. (St. Louis, MO). A raffinose-series oligosaccharides enzymatic assay procedure (RSO 8/98) was purchased from Megazyme (Megazyme Intl Ireland Ltd, Wicklow, Ireland). All other chemicals were ACS grades.

2.2. Extraction and purification of soluble sugars in soybean meal

Soluble sugars were extracted by the procedure previously optimized (Giannoccaro, Wang, & Chen, 2006). One gram sample spiked with an internal standard, which was used to check recovery and to assure an accurate quantification of sugars (Black & Bagley, 1978; Li, Schuhmann, & Wolf, 1985), and 5 mL of distilled water were placed in a 50-mL centrifuge tube. Melibiose was used as the internal standard for a HPAEC-PAD system, while maltoheptaose was used as the internal standard for a high-performance size exclusion chromatography with RI detection (HPSEC-RI) system. Different standards were chosen for the efficiency of each individual analysis. The tube was capped, placed horizontally, completely immersed in a water bath at 50 °C, and shaken at 200 rpm for 15 min. After the extraction, the tube was centrifuged at 20,000*g* for 10 min and 2 mL of the supernatant were pipetted into another centrifuge tube.

The same extract purification procedure was applied to both systems, which followed the method of Black and Glover (1980) with modifications. Three millilitres of acetonitrile was slowly added into the centrifuge tube containing the supernatant with constant shaking to precipitate the residual protein, and then the tube was left at room temperature for 30 min. The tube was centrifuged at 1500*g* for 10 min, and 1 mL of the clear supernatant was pipetted into a 1.7-mL microcentrifuge tube and brought to completely dryness using a heat block at 80 °C for 60 min. For the HPAEC-PAD system, the residue was re-dissolved in 1 mL of 90 mM NaOH, quantitatively transferred to a 100-mL volumetric flask, and brought to volume with 90 mM NaOH. Ten millilitres of the diluted solution was filtered through a 0.2-μm membrane (HT Tuffryn Nylon) followed by a cartridge (OnGuard II RP, Dionex, Sunnyvale, CA) to remove residual lipids, surfactants, hydrocarbons, and high molecular-weight carboxylic acids (Kadnar, 1998; Wicks, Moran, Pittman, & Hodson, 1991) prior to injection. For the HPSEC-RI system, the residue was re-dissolved in 1 mL of 0.1 M $NaNO_3$ containing 0.2% NaN_3, quantitatively transferred to a 10-mL volumetric flask, and brought to volume with 0.1 M $NaNO_3$ containing 0.2% NaN_3. The diluted solution was further purified as previously described prior to injection.

2.3. Separation and quantification of soluble sugars by HPAEC-PAD and HPSEC-RI

The HPAEC-PAD system (Dionex DX500) consisted of a GP-50 gradient pump, ED40 electrochemical detector, a CarboPac PA-10 pellicular anion-exchange resin column (250 × 4 mm i.d.) preceded by a CarboPac PA-10 guard column (50 × 4 mm i.d.) and an AminoTrap column (30 × 3 mm i.d.) (Dionex, Sunnyvale, CA). Samples were injected via an AS40 automated sampler with a 25-μL sample loop, and sugars were eluted with 90 mM NaOH at a flow rate of 1 mL/min. The mobile phase, 90 mM NaOH, was prepared by diluting carbonate-free 50% (w/w) NaOH solution in distilled water, which was previously filtered with a 0.45-μm membrane and degassed with a sonicator (Zenith Inc, T800-2H, Norwood, NJ) for 30 min. The HPSEC-RI system consisted of a 515 HPLC pump with