NOTE

Improved Characterization of Sorghum Tannins Using Size-Exclusion Chromatography

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Sorghum (*Sorghum bicolor* L. Moench) is a native grass species of the arid and semi-arid regions of Africa (Kimber 2000). Sorghum is a genetically diverse crop and contains some genotypes that have a pigmented testa and therefore contain tannins (Rooney et al 1980; Rooney and Miller 1982). Such lines have the dominant B1_B2_ genes and are the only sorghum types with tannins (Blakeley et al 1979), despite common misperceptions that all sorghums have tannins or that the presence of tannins is linked to seed color (Boren and Waniska 1992). Due to the high antioxidant activity, sorghum tannins have recently been examined for potential as a nutraceutical, especially cancer prevention (Awika and Rooney 2004).

Generally, tannins which are polymers, are characterized on the basis of their monomeric composition, and their degree of polymerization, both of which can impact their functionality (Asquith et al 1983). Many analytical methods have been used to study their composition such as reversed phase (RP) HPLC (Putman and Butler 1989; Prior and Gu 2005), normal-phase (NP) HPLC (Gu et al 2002; Awika et al 2003), size-exclusion chromatography (SEC) (Williams et al 1983; Karchesy et al 1988; Kennedy and Taylor 2003), capillary electrophoresis (CE) (Bae et al 1994; Cifuentes et al 2001; Bonoli et al 2004), and mass spectrometry (Cheynier and Fulcrand 2003; Kruger et al 2003).

It is generally agreed that sorghum tannins are composed of monomeric flavan-3-ols. Thus, recent research has focused on the degree of polymerization of these subunits, with one limitation being how well the largest polymers (i.e., a high degree of polymerization [DP]) can be resolved. Recently, NP-HPLC has resolved tannins to a DP of >10 and has significantly improved the separation and characterization of tannins from a number of sources, including sorghum (Hammerstone et al 1999; Gu et al 2002). While NP-HPLC was capable of excellent resolution, run times were lengthy (40 min) and NP-HPLC must be conducted in the total absence of water, which complicates switching instruments between types of HPLC such as RP-HPLC.

Thus, the objectives of this study were to investigate the use of improved SEC procedures for analyzing sorghum tannins that required no special sample preparation (such as derivatization, tannin purification, etc.) and that would provide information on the M_w distribution of the tannins.

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MATERIALS AND METHODS

Sorghum Cultivars and Processing of Sorghum

Seven high-tannin lines with varying levels of tannin content (Shanqui Red, Ajabsido, Koro Kollo, IS8525, Sumac, SC103-12E, and SC599) were grown over two years at the Kansas State University Ashland Bottoms Research Farm, Manhattan KS. All sorghum samples were mechanically cleaned by a Clipper office tester (A.T. Ferrell Co., Bluffton, IN) to remove unwanted material; glumes were removed by hand when necessary. Samples were then decorticated using a tangential abrasive dehulling device (TADD) (Venebles Machine Works, Saskatoon, Canada) equipped with an 80-grit abrasive disk. Samples were decorticated until 20% of the grain (by weight) was removed. Bran fractions from the decortication process were collected by aspiration and stored at –20°C until use.

Analytical Procedures

Tannin content of the bran fractions was determined using the modified vanillin hydrochloric acid (MV-HCl) (Price et al 1978). Tannins from a tannin-containing sorghum hybrid (A2TX623 X SC103-12E) were extracted from whole grain and purified according to the method of Hagerman and Butler (1980). This purified tannin extract was used as the analytical standard for chromatographic methods.

Tannin composition was analyzed using the SEC method described by Kennedy and Taylor (2003). Samples were extracted using the same extraction procedure as for the MV-HCl method. After extraction, samples were filtered with a 0.45-µm polypropylene syringe type filter (Phenomenex, Torrance, CA). Filtered samples were then analyzed by SEC (1100 HPLC, Agilent, Palo Alto, CA) using two PLgel columns, 300 × 7.5mm, 5 µm, 500 × 100 Å, (Varian, Palo Alto, CA) connected in series with a mobile phase consisting of N, N-dimethylformamide with 1% (v/v) glacial acetic acid, 5% water, and 0.15*M* lithium chloride (v/v) with a flow rate of 1 mL/min. A diode array detector monitored the elution at 280 nm. To determine the DP of the tannins separated by SEC, purified procyanidin standards (B1 and B2) (Sigma St. Louis, MO) were separated under the same conditions.

RESULTS AND DISCUSSION

SEC of Sorghum Tannins

Figure 1 shows the SEC chromatograms of purified tannins and tannins extracted directly from whole grain and isolated bran of the tannin-containing sorghum hybrid A2TX623 X SC103-12E. Overall, the chromatogram of the tannins extracted directly from bran was similar to purified tannins. Whole grain samples showed contamination from other constituents in the sorghum, especially in the 16–18 min elution range. This trend was observed in all 14 samples. Thus, tannins can be extracted directly from bran without the need of a lengthy purification process using Sephadex LH-20, a widely used stationary phase in liquid chromatography. For this reason, tannins extracted directly from bran fractions were then used for analysis in the remainder of the study.

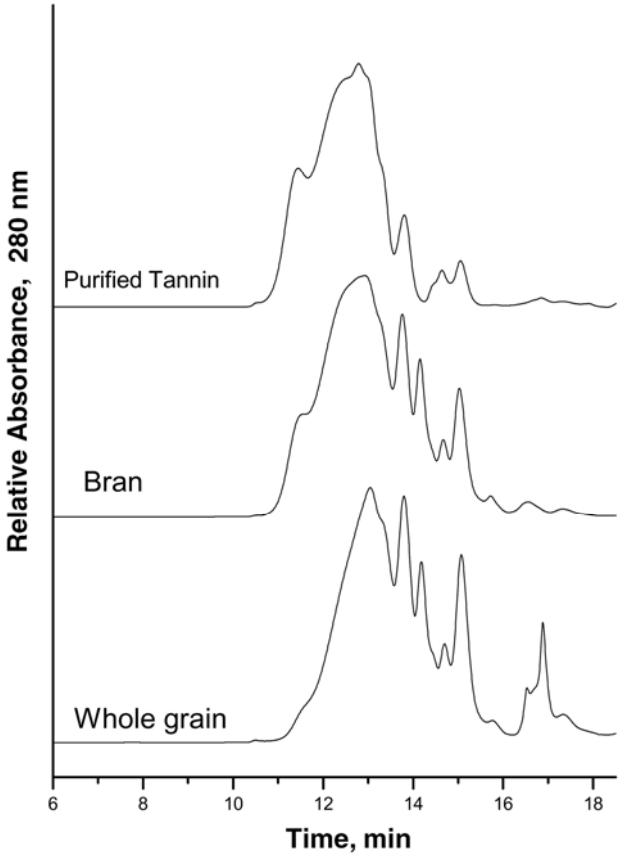

Fig. 1. Typical SEC chromatograms of tannins extracted from sorghum hybrid A2TX623 X SC103-12E.

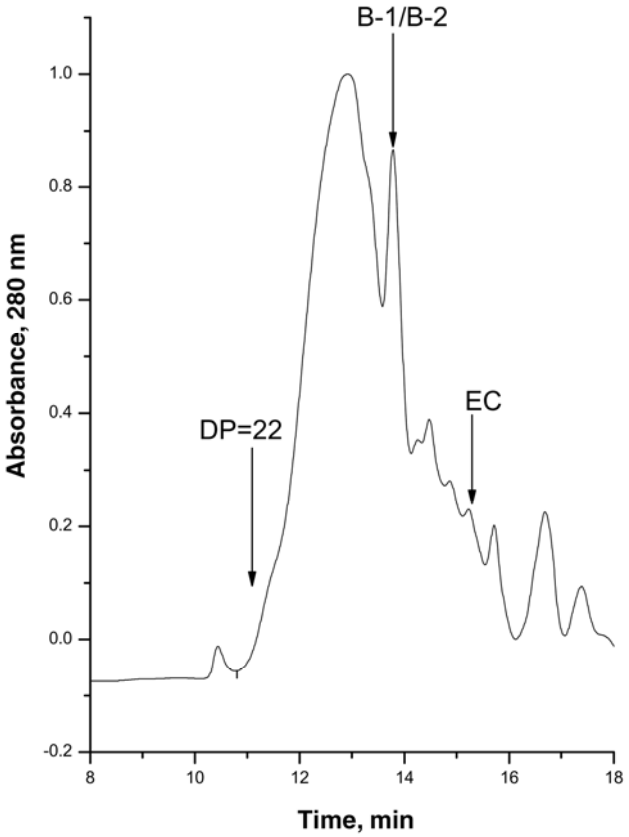

Fig. 2. SEC chromatogram of tannin extract from bran of sorghum line Ajabsido showing approximate elution times of procyanidin B-1, B-2, and epicatechin standards (EC).

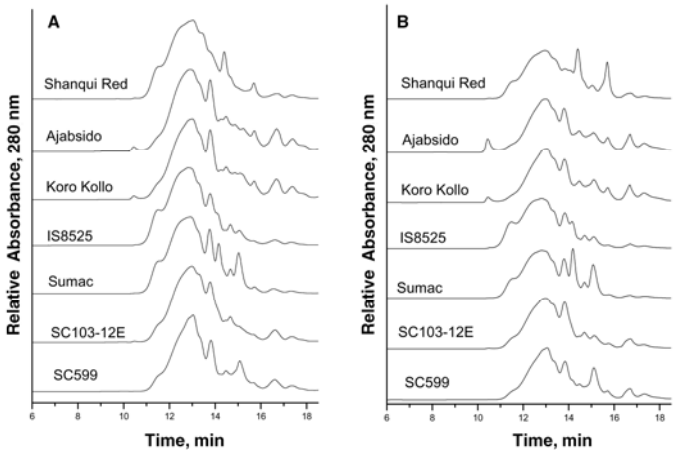

Fig. 3. Normalized size-exclusion chromatograms of tannin extracts from seven tannin cultivars grown over two years at the same location. **A,** 2003; **B,** 2004.

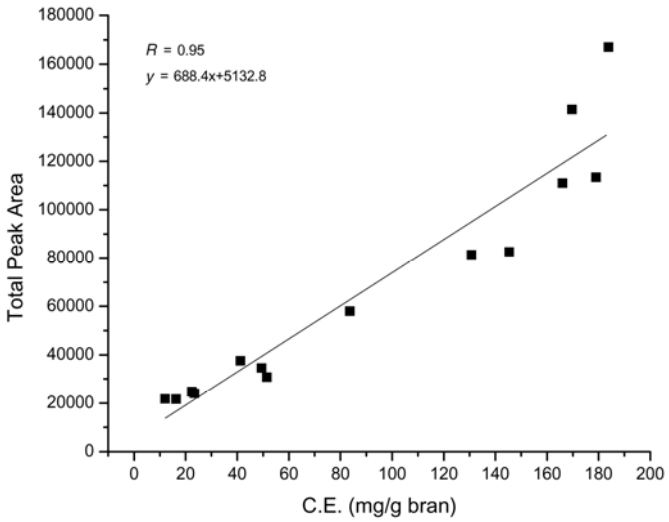

Fig. 4. Correlation between total peak area measured on SEC and tannin content of 14 tannin-containing sorghums measured using modified vanillin HCl method. Tannin content is reported as catechin equivalents (C.E.).

Figure 2 shows a typical SEC chromatogram of tannins isolated from bran of the sorghum line Ajabsido with the elution times of procyanidin standards B-1 and B-2 indicated on the chromatogram. These standards elute at ≈13.5 min. Thus this time can be associated with DP 2 and provides a good estimation of the molecular size distribution on the tail end of the chromatogram. The standard epicatechin (a monomer) eluted at ≈15 min. As these elution times are similar to the times found by Taylor et al (2003), a tentative estimation for the elution of tannins with a DP 22 as reported by Taylor et al (2003) is also shown in Fig. 2. However, further research with purified sorghum tannins or mass spectrometry should be conducted to verify this result.

Comparison of Diverse Tannin-Containing Sorghum Lines

Tannins from diverse sorghum lines were analyzed by SEC to further test the ability of the SEC method to discriminate differences in the tannin molecular weight distribution and composition. Figure 3 shows normalized chromatograms of tannin extracts from seven high-tannin cultivars grown over two years at the same location. Considerable variation in the peak distribution was seen across the cultivars with the main differences in the 13–16 min elution range. Analysis of the total peak area from the SEC chromatograms showed strong correlations with the tannin content from seven tannin-containing lines used in this study grown

over two years at the same location as analyzed by the MV-HCl method (Fig. 4). Thus, this method could be used to determine tannin content as well as M_w distribution. It should be noted that extracts from non-tannin sorghum lines may have peaks in the region of the chromatogram where the lower M_w material elutes (14–18 min). This method should not be used to determine the presence or absence of tannins in a given sorghum sample.

CONCLUSIONS

Sorghum tannins can be efficiently analyzed by SEC and provide good information on the molecular weight distributions. SEC allows for an easy and rapid measurement of the M_w distribution with results comparable to normal-phase HPLC. Analysis of the bran fractions of sorghum cultivars gives a clearer and more accurate analysis of the distribution than analysis of the whole grain. These bran fractions are very similar to the purified tannin extract when analyzed by SEC. Sorghum tannins can also be fractionated in a similar fashion as tannins found in other species of plants, with differing DP fractions being separated. The analysis of peaks and their correlation to tannin content indicates that SEC could be used as a rapid and automated method for measuring tannin content in sorghum similar to the MV-HCl method. SEC does not need a color reaction to form, which is very time-dependent in the MV-HCl method. At the same time, SEC can also provide information on the molecular weight distribution and DP of the tannins. Future research of the DP and monomeric composition using catalysis and mass spectrometry is need to determine more relationships between the sorghum tannin composition and the tannin content as well as further characterization of the tannin.

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