Journal of Chromatography, 234 (1982) 494–496
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,241

Note

High-performance liquid chromatography separation of soybean isoflavones and their glucosides

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(Received July 27th, 1981)

Soybeans are known to contain several isoflavones (daidzein, glycitein, genistein) and isoflavone glucosides[1] (daidzin, glycitein-7-β-O-glucoside, genistin) which have been reported to have estrogenic[2], antifungal[3] and antioxidant[4] activity. The quantitative determination of soybean isoflavones has been reported by gas–liquid chromatography (GLC)[1], and more recently high-performance liquid chromatography (HPLC)[5,6,7] has been used.

Carlson and Dolphin[5] separated soybean isoflavone aglycones present in an alcohol extract on a μPorasil column. The isoflavone glucosides were determined after hydrolytic treatment with aqueous acid. In a paper by West *et al.*[6] only two isoflavone aglycones from soybean extracts were separated.

This communication reports the development of a procedure for the separation of the naturally occurring soybean isoflavone glucosides and isoflavone algycones using HPLC with mild solvents and reversed phase packing that may be less likely to catalyze decomposition compared to other packings.

MATERIALS AND METHODS*

A Waters Assoc. (Milford, MA, U.S.A.) HPLC system was used, comprised of a WISP 710A, M-45 solvent delivery system, Model 660 solvent flow programmer, Model 450 variable-wavelength detector, with a DuPont Zorbax ODS 25 × 0.46 cm I.D. column protected by a 2-cm Corasil C_{18} guard column. The solvent flow-rate was 1 ml/min and the absorption was measured at 262 nm. Solvents were spectral grade, and distilled water was deionized before use. All solvent ratios are on a volume basis.

Genistein and daidzein were obtained from ICN Pharmaceuticals, Life Science Group (Plainview, NY, U.S.A.). *n*-Butyrophenone was purchased from Aldrich (Milwaukee, WS, U.S.A.). Coumesterol was purchased from Pfaltz and Bauer (Stamford, CT, U.S.A.). Genistin and daidzin were synthesized from the aglycones by the pro-

* The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

0021-9673/82/0000–0000/$02.75

cedure of Zemplén and Farkas[8] and were a gift from Dr. L. C. Wang of our laboratory. Purity of the synthesized products determined by HPLC was found to be 99.2 and 94.9%, respectively. Genistin and daidzin were also isolated by preparative HPLC from an alcoholic extract of hexane-defatted soybean meal. Mixtures that contained genistin and daidzin; daidzin and glycitein-7-β-O-glucoside; daidzin, genistin and glycitein-7-β-O-glucoside and the relative proportion of each in the mixtures were generously supplied by Dr. A. Bondi fo the Hebrew University of Jerusalem.

Separations were carried out with an aqueous methanol gradient from 25 to 50% using either curve 6 (linear) or curve 8 (concave) on the 660 gradient programmer. Solvent A contained 15% methanol and solvent B was 65% methanol. By using these combinations of solvents, formation of bubbles in the system was avoided.

RESULTS AND DISCUSSION

Since glycitein, the aglycone, was not available, the mixture of three isoflavone glucosides obtained from Dr. Bondi, which contained naturally occurring concentrations of the glucosides, was hydrolyzed with 2 *N* sulfuric acid in ethanol for 2 h by refluxing. The hydrolyzate was cooled and extracted with diethyl ether, and the ether layer was taken to dryness. By chromatographing both the mixture of natural occurring glucosides and the prepared aglycones, the retention times of the aglycones could be determined and compared with those of their corresponding glucosides.

Fig. 1 shows the separation of soybean isoflavones as observed in the current method. The glycosides eluted first and were followed by the isoflavone aglycones in the same relative order. The eight peaks are daidzin, glycitein-7-β-O-glucoside, genistin, daidzein, glycitein, genistein, coumesterol and *n*-butyrophenone. The methanol

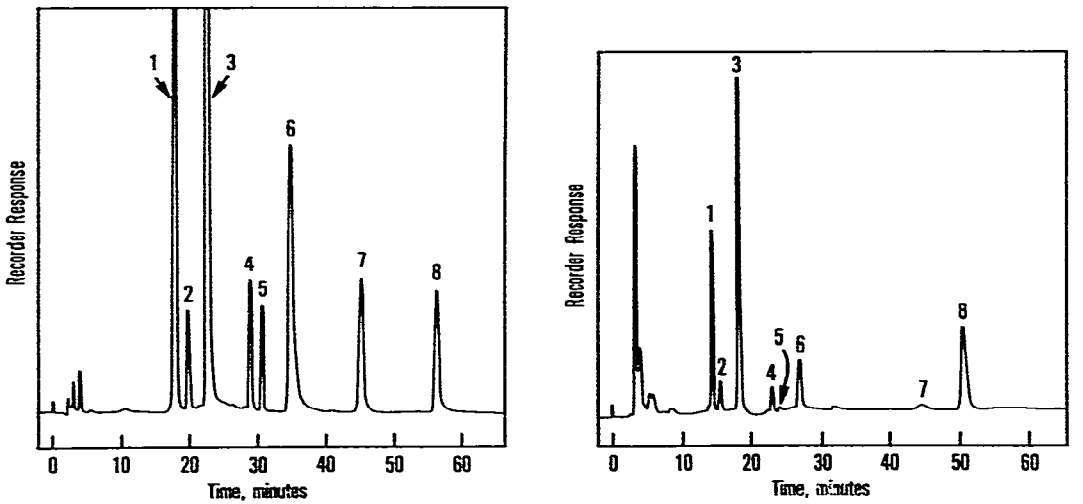

Fig. 1. HPLC elution diagram of daidzin (1), glycitein-7-β-O-glucoside (2), genistin (3), daidzein (4), glycitein (5), genistein (6), coumesterol (7) and *n*-butyrophenone (8), using a linear gradient of methanol from 25 to 50% in 20 min.

Fig. 2. HPLC elution diagram of an aqueous methanolic extract (80%, reflux) of hexane-defatted soybean meal with added *n*-butyrophenone as a standard. Peaks as in Fig. 1.

gradient ran from 25 to 50% in 20 min, and then the solvent remained at 50% for the duration of the separation.

The elution position of coumesterol was of interest, since it is present in soybeans[9] and has physiological activity. However, the concentration is so low in soybean meal that a peak corresponding to coumesterol was not detected in most of my samples.

The elution of *n*-butyrophenone is shown because it was found to be useful as an internal standard in the quantitative determination of soybean isoflavones.

Fig. 2 shows an elution pattern of a hot 80% methanolic extract of hexane-defatted soybean meal. Peaks 1, 2, 3, 4 and 6 were collected from several analysis and their spectra were measured. Each isolated peak had the typical peak at 258–262 nm and shoulder in 320 nm area.

Soybean meal contains at least three isoflavone glucosides (peaks 1, 2 and 3), and the meal also contains at least three isoflavone aglycones, peaks 4, 5 and 6). However, only five of these peaks are present in measurable amounts.

The amounts (mg/100 g) of isoflavones in hexane-defatted soybean meal from Amsoy soybeans, 1979 crop, have been found to be: daidzin, 62; glycitein-7-β-O-glucoside, 18; genistin, 127; daidzein, 48; glycitein, trade; genistein, 40.

This analytical procedure is being applied to the quantitative determination of these biologically active components in soybeans and soybean products, and the results will be reported elsewhere.

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