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RAPID COMMUNICATIONS

Improved Method for Gas Chromatographic Analysis of Genistein and Daidzein from Soybean (*Glycine max*) Seeds

Keywords: *Gas chromatography; daidzein; genistein; neutralized; Glycine max*

Soybean seeds are a chief source for two naturally occurring important isoflavones, genistein and daidzein. Normally within seed tissues these molecules are found as conjugated glycosides, that is, daidzin and genistin (Coward et al., 1983; Farmakalidas and Murphy, 1984; Wang et al., 1990), necessitating the use of high-performance liquid chromatography (HPLC) as the analytical tool to identify and quantify the different molecular species. Under circumstances when only the total isoflavonoid aglycon quality and quantity are of importance, gas chromatography (GC) is a suitable method of analysis (Bankova et al., 1992; Creaser et al., 1989; Furuya, 1985; Schmidt et al., 1994). Previously this laboratory (Fenner, 1996) reported a modification of the method of Wang et al. (1990) for the GC analysis of total isoflavanoid aglycons from soybean seeds that utilized low-temperature treatment. During those analyses concern was raised regarding the likely presence of inorganic acid used during processing with respect to instrumentation and sample degradation when samples are stored overnight. This paper describes a further modification/improvement of our previous procedure that does not require low-temperature treatment and results in reduced sample turnover rate, improved instrument performance, and better sample recovery.

MATERIALS AND METHODS

In screw-capped tubes, 75 μg of biochanin A (an internal quantitative standard) was added to 50 mg of finely powdered soybean meal. Samples were hydrolyzed in screw-capped tubes with 0.5 mL of HCl (1 M) for 2 h in a water bath at 98−100 °C. After hydrolysis, samples were allowed to cool and neutralized with NH_4OH (2 M) followed by the addition of 3 mL of ether and vortexing. Following centrifugation to pellet tissue debris, 1 mL aliquots of ether were removed and dried under nitrogen in small glass vials. The dried sample was converted to trimethylsilyl ether (TMSE) derivatives with pyridine (25 μL) and bis(trimethylsilyl)trifluoroacetamide (BSTFA) (50 μL) at 60 °C for 5 min. One microliter of the derivatized samples was analyzed directly on a Varian 3500 gas chromatograph on either an SPB-1 fused silica capillary column (Supelco Inc., Bellefonte, PA) or a DB-1 (J&W Scientific, Folsom, CA) column, both 30 m × 0.32 mm i.d., 0.25 μm. The GC conditions were as described previously (Fenner, 1996). Isoflavonoids were identified by comparison of retention times to those of authentic genistein and daidzein (Sigma Chemical Co., St. Louis, MO) and biochanin A (Aldrich Chemical Co., Inc., Milwaukee, WI). Quantification of genistein and daidzein was performed using response factors calculated on the basis of biochanin A.

RESULTS AND DISCUSSION

In our efforts to expedite the capability to efficiently isolate and quantify isoflavonoid aglycons from soybean seeds, the original procedure utilizing low-temperature treatment has been further optimized (Fenner, 1996). Figure 1 shows well-resolved peaks on the GC chromatogram of soybean isoflavonoids utilizing the modified method outlined. The principal modifications, that is, neutralizing the acid-hydrolyzed material with 2 M ammonium hydroxide (to pH 6.5−7) and subsequent partitioning into ether, save valuable time when these analyses are performed compared to the previous overnight treatment. Additionally, we noticed an increase in instrument performance using this procedure. During previous experiments, our concern with regard to possible residual acid was well founded because we noticed poorer injector performance over the injection of several samples. No such decrease in performance was seen over similar or greater numbers of injections using the current method. Furthermore, biochanin A was a superior internal standard compared to quercetin for soybeans. Quercetin, a flavonol compound, elutes at 35 min using the analytical protocol described here, whereas

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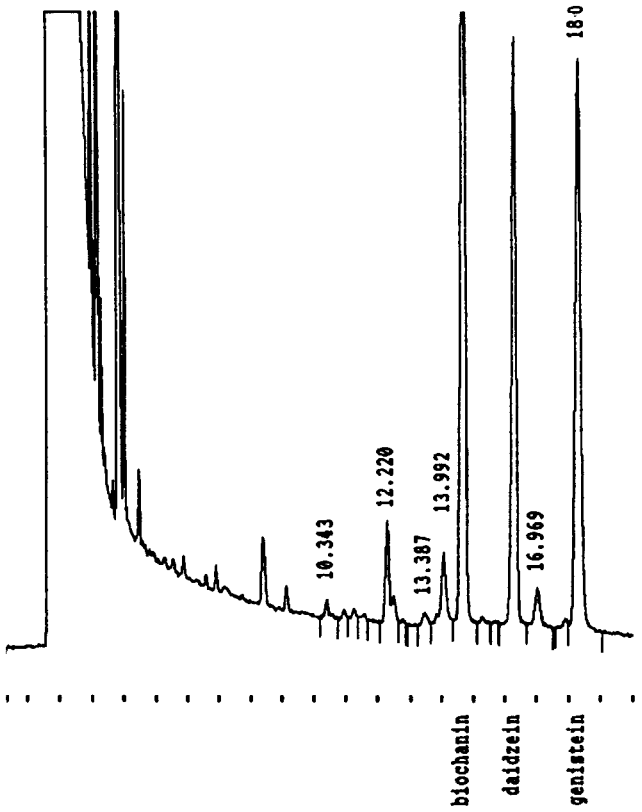

Figure 1. Gas chromatogram of TMSE derivatives of isoflavonoid aglycons from soybean seeds after neutralizing acid-hydrolyzed samples with 2 M ammonium hydroxide and partitioning in ether. Biochanin A was used as internal standard.

biochanin A elutes at ~14 min. Biochanin A, a 4′-methoxy-5,7-dihydroxy isoflavone, is more closely related in structure to daidzein and genistein than quercetin. Thus, for quantitative purposes, biochanin A should be more representative of isoflavonoids during extraction and derivatization procedures. This is supported by data from two identical samples extracted using our current and previous methods, which yielded 1680 and 1051 μg/g of seed tissue, respectively. The new method also enabled a reduction in sample size from 200 to 50 mg and a reduction in derivatizing time from 30 to 5 min. In this study, after hydrolysis of 50 mg of seed material, only 1 mL aliquots of the ether extract (of three) were used for analyses; thus, it is possible to begin with even smaller seed samples. This procedure therefore offers an advantage when seed material may be limited and time is of the essence.

This procedure does not replace HPLC for the analysis of conjugated isolavonoid glycosides. However, this method is an alternative when analysis of only the total isoflavonoid aglycon content is required. Because of its speed and simplicity, this modified method should be of great value to laboratories where sample throughput rates are extremely high, as would be the case when used in conjunction with breeding programs.

ABBREVIATIONS USED

HPLC, high-performance liquid chromatography; GC, gas chromatography; TMSE, trimethlysilyl ether; BSTFA, bis(trimethylsilyl)trifluoroacetamide; FID, flame ionization detection.

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