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Note

Analysis of isoflavones in Bengalgram by high-performance liquid chromatography

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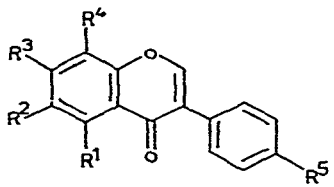
Isoflavones are almost totally confined to the family *Leguminosae* and characteristically to the sub-family *Papilionoidae*[1], which includes a high number of food crops[2]. These compounds are not found in dormant seeds but are present in germinated seeds, presumably synthesised during germination[3]. They are of interest due to their diverse biological properties (anti-fungal, anti-oxidant, anti-haemolytic, oestrogenic[4–6] as well as their importance in chemotaxonomy. Moreover the oestrogenic type isoflavones[6], formononetin (I) and biochanin A (II), common in Bengalgram (*Cicer arientanum*) have been shown to lower significantly serum cholesterol levels in experimental animals[7,8], whereas daidzein (III), and genistein (IV), the principal isoflavone in soya (*Glycine max.*), would appear not to have this property[9,10].



(I) Formononetin: $R^1=R^2=R^4=H; R^3=OH; R^5=OCH_3$
(II) Biochanin A: $R^1=R^3=H; R^2=R^4=OH; R^5=OCH_3$
(III) Daidzein: $R^1=R^2=R^4=H; R^3=R^5=OH$
(IV) Genistein: $R^1=R^3=R^5=OH; R^2=R^4=H$

Previous quantitation of these compounds has been carried out by thin-layer chromatography, gas–liquid chromatography of the trimethylsilyl ethers[11] and gel filtration.

Our work on the beneficial and/or detrimental properties of minor constituents in the human diet has dictated a need for a rapid method of analysis of these compounds in various foods under differing conditions of storage and processing. A facile and rapid method for the determination of these compounds serves as a basis for this report.

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EXPERIMENTAL

A 50-g sample of Bengalgram (commercial variety) was germinated for 72 h. The germ was filtered, dried at 50°C under vacuum and milled (UDY cyclone sample mill, Tecator, CO, U.S.A.). A 10-g sample of the flour was extracted with 85% aqueous methanol (3 × 150 ml) at 60°C. The methanolic extract was evaporated under vacuum and taken up in 70% aqueous ethanol (50 ml) and extracted with hexane (3 × 30 ml). The alcoholic phase was evaporated and the residue taken up in water (10 ml) and extracted with ethyl acetate (3 × 3 ml). The combined ethyl acetate extract was dried (magnesium sulphate), filtered, and made up to constant volume (10 ml). The instrument used was an Applied Chromatography System Model 750 gradient chromatograph. Injection was carried out via a Rheodyne injection valve, Model 7120 (20-μl loop). Detection was made by ultraviolet absorption (Cecil CE 212 detector) at a wavelength of 280 nm. Peak areas were measured using a Hewlett-Packard HP 3390A integrator. Chromatographic columns (10 cm × 5 mm) of Spherisorb-5-ODS were packed in our own laboratory. Methanol was of HPLC grade obtained from Rathburn (Wakeburn, Great Britain). Separations were carried out by using a mobile phase of methanol–water (1:4) initial concentration, rising to (3:2) at 5% min^{-1}. A flow rate of 2 ml min^{-1} was used.

Authentic samples of isoflavones were obtained from Apin Chemicals, Cardiff, Great Britain.

RESULTS AND DISCUSSION

HPLC offers a high degree of separation of the isoflavones as illustrated in Fig. 1. A typical chromatograph for a Bengalgram extract is shown in Fig. 2.

A rapid gradient was employed to obtain the minimal time of analysis (*ca.* 10 min) with adequate separation of the components of interest. The conditions employed were quite sufficient for the method of extraction and food analysed. Although, work with other products may necessitate slightly less severe gradients should interference from other peaks become apparent.

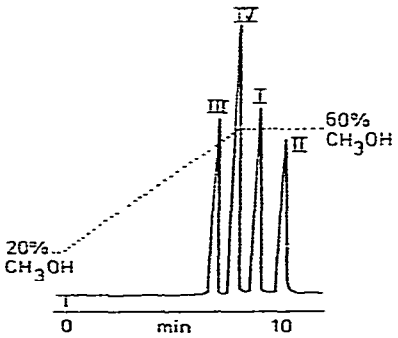

Fig. 1. Chromatogram of isoflavone standards. Solvent gradient, 20 – 60% methanol in water. Detector, × 1. All isoflavones 0.50 mg ml^{-1}.

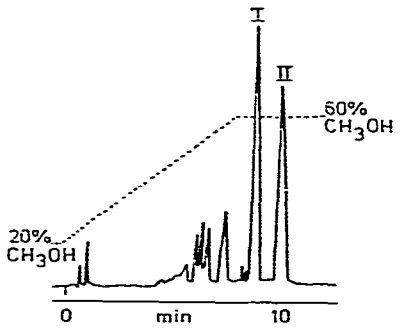

Fig. 2. Chromatogram of Bengalgram extract. Solvent gradient, 20 – 60% methanol in water. Detector, × 1.

Isoflavone concentrations present in the germ as determined by HPLC are shown in Table I. These are in the correct region, although variations are likely to occur with variety and conditions of germination.

TABLE I

ISOFLAVONE CONCENTRATION OF BENGALGRAM

N.D. = Not detectable.

Isoflavone	*Concentration (mg/100 g germ)*	*Literature*[8] *(mg/100 g germ)*
Biochanin A	71	98.6
Formononetin	77	44.1
Genistein	N.D.	N.D.
Daidzein	N.D.	5.1

ACKNOWLEDGEMENT

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