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Plant Lipid Biochemistry, Structure and Utilization

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Plant lipid class analysis by HPLC

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SYNOPSIS

Lipid extracts from corn coleoptiles and from apple mesocarp were separated and quantified by HPLC with either a) a flame ionization detector, or b) an evaporative light scattering detector, and the results compared.

INTRODUCTION

Until recently, thin layer chromatography was the method of choice for the separation of plant lipid classes, and quantification, when necessary, was performed by scraping the silica gel from the plates and quantifying analytes by a secondary method. Recently Demandre et al, 1985, reported an HPLC system for the separation of plant lipid classes, but quantification was not achieved due to the limitations of UV detection of lipids. Christie et al, 1985, developed a similar HPLC method for the quantitative analysis of mammalian lipids which employed an evaporative light scattering detector. We have recently reported a technique to separate and quantify plant lipid classes by HPLC with flame ionization detection (Moreau et al, 1990). In this study, we are now reporting comparable studies performed with our previous chromatographic system and an evaporative light scattering detector.

METHODS

Corn seedlings were germinated in the dark on moistened filter paper for 5 days and the coleoptiles were harvested. Granny Smith apples were purchased locally. Lipids were extracted and analysed by HPLC with flame ionization detection as previously described (Moreau & Asmann, 1989; Moreau et al, 1990). An evaporative light scattering detector (Varex Universal HPLC Detector, Varex Co., Burtonsville, MD, USA) was then attached to the system, in place of the FID, and the same samples were injected. The temperature of the detector was set at 40 °C and N₂ (25 psi) was used as a nebulizing gas.

RESULTS AND DISCUSSION

Analysis of lipids in corn coleoptiles

When the lipid extract from corn coleoptiles was analyzed by both HPLC-FID and HPLC-ELSD thirteen peaks were resolved (Fig. 1). The identity of each of the peaks was confirmed by cochromatography with lipid class standards as described

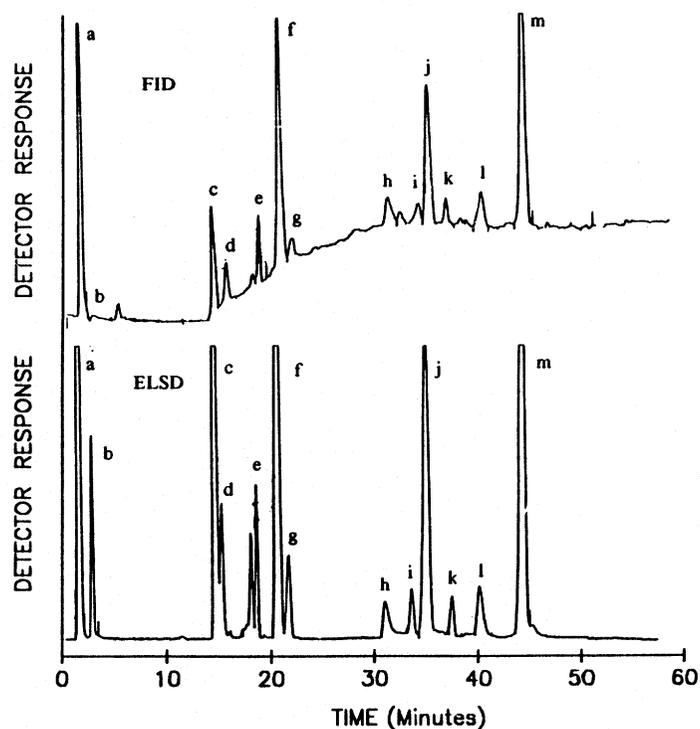


Figure 1. Analysis of lipid classes in corn coleoptiles by HPLC with a flame ionization detection (FID) or an evaporative light scattering detector (ELSD). Each injection contained 125 μg of lipid. The lipid classes were identified as: a) sterol esters, b) triacylglycerols, c) sterols, d) free fatty acids, e) acylated sterol glycoside, f) monogalactosyl diacylglycerol, g) sterol glycoside, h) digalactosyldiacylglycerol, i) cardiolipin, j) phosphatidylethanolamine, k) phosphatidylglycerol, l) phosphatidylinositol, and k) phosphatidylcholine.

(Moreau et al, 1990). The chromatograms obtained with the two detectors contained essentially the same number of major peaks. Most of the peaks in the HPLC-ELSD chromatogram were slightly larger than in the HPLC-FID chromatogram. The most significant difference between the two chromatograms of lipids in corn coleoptiles was the shape of the baseline. In the HPLC-FID chromatogram the baseline was stable for the first 15 minutes, increased gradually up to about 30 minutes and then remained elevated for the remainder of the analysis, with a small amount of noise. The baseline of the HPLC-ELSD chromatogram was flat for the entire run and had very little noise.

Analysis of lipids in apple mesocarp

When the lipid extract from apple mesocarp was analyzed by both HPLC-FID and HPLC-ELSD ten peaks were resolved (Fig. 5). Less total lipid was deliberately injected in this analysis than in the previous one in order to observe how the geometry of the peak shapes varied with sample size. A comparison of the HPLC-FID and HPLC-ELSD chromatograms obtained for apple mesocarp revealed the same types of trends described in the previous section.

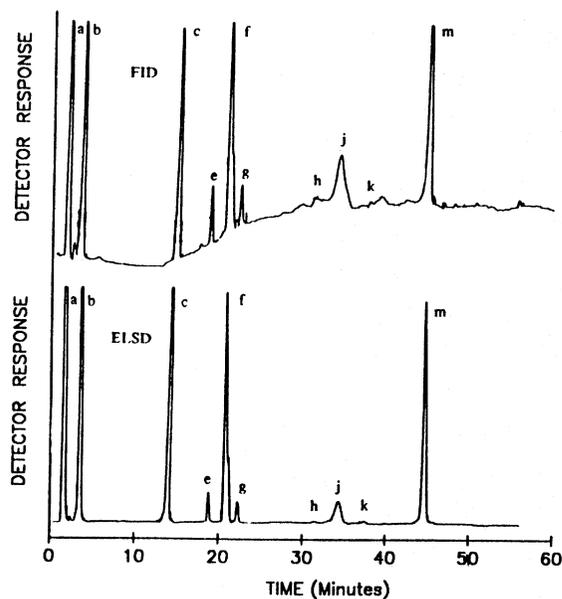


Figure 2. Analysis of lipid classes in apple mesocarp by HPLC with a flame ionization detector (FID) and an evaporative light scattering detector (ELSD). Each injection contained 50 μg of total lipid. The lipid classes were identified as indicated by the symbols in Fig. 1.

Comparison of the two detectors for HPLC analysis of plant lipid classes

Although the mechanism of detection is very different for the two types of detectors, it can be seen that the actual shape and size of the individual peaks obtained by each detector were quite similar. In our hands, the minimum limits of detection for both detectors was about 1 μg . The mass vs signal response for the FID was very linear for each lipid class so far examined in the range of 1 to 200 μg . With the ELSD the mass vs signal response was quite linear in the range of 10 to 200 μg , but was parabolic in the range of 1 to 10 μg . With the ELSD, larger signals were obtained with peaks that elute as a sharp peak than with peaks which were broad (see the phosphatidylethanolamine peaks in both chromatograms). A major disadvantage of the FID was that the baseline was not stable and there were some problems with noise. We have recently begun to use the FID with a reversed phase ternary gradient system of methanol-acetonitrile-water and have observed a more stable baseline. Although the initial cost of both detectors was similar, the FID has required more maintenance time and expense.

This paper is intended for the reader's information only and does not constitute an endorsement of a particular product by the U.S. Department of Agriculture over others that may be commercially available.

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