

Quantitative Analysis of Lipids in Plastids by HPLC-FID

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INTRODUCTION

Two laboratories have previously isolated amyloplasts from potato tubers and analyzed their lipid composition using thin layer chromatography (3,5). The purpose of this study was to compare the results of these two previous studies with those obtained using high performance liquid chromatography and flame ionization detection (HPLC-FID).

MATERIALS AND METHODS

Amyloplasts were purified from tubers (cv Russett Burbank) by the method of Fishwick and Wright (3) and lipids were extracted with hexane-isopropanol. The lipid fraction was separated and quantified using an Isco Model 2350 HPLC Pump equipped with an Isco Model 2360 Gradient Programmer, and a Tracor Model 945 Flame Ionization Detector. The column (3 x 100 mm) contained Lichrosorb Si 60, 7 μ m, used at a flow rate of 0.5 mL/min. The ternary gradient system was a modification of two previously published systems (1,2) and was composed of (A) 99% isooctane and 1% tetrahydrofuran, (B) isopropanol, (C) water. Linear gradients were programmed between the following time points; 0 min = 100% A, 5 min = 95% A and 5% B, 10 min = 85% A and 15% B, 15 min = 40% A and 60% B, 33 min = 40% A and 51% B and 9% C. The final composition was held for 15 min and then the system was reequilibrated with 100% A.

RESULTS AND DISCUSSION

From 16 tuber cores (180 g) the total amount of amyloplast lipids obtained were quite low (208 μ g). A 10 μ L sample (25% of the total lipid sample) was separated and quantified by HPLC-FID (Fig. 1). This analysis of the lipids present was similar to those previously published using thin layer

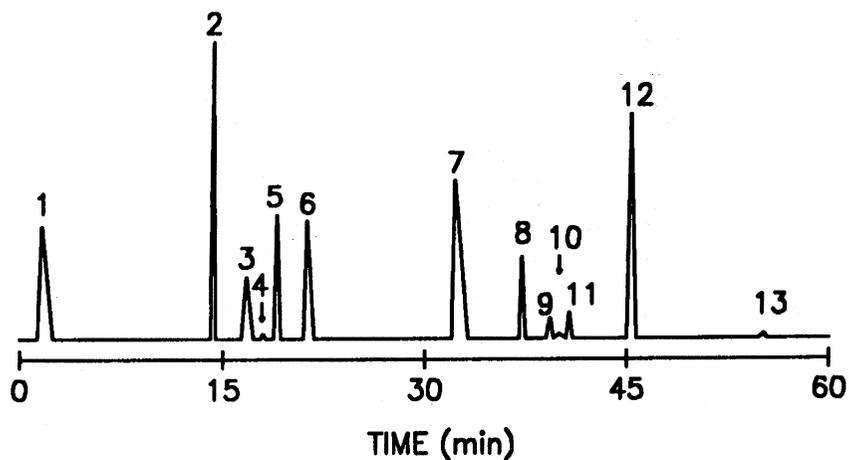


Fig. 1. HPLC-FID analysis of lipid classes in potato tuber amyloplasts. The lipid classes represented by each peak (and corresponding weight % of each peak) were: (1) sterol esters (7.4%), (2) free sterols (11.4%), (3) free fatty acids (9.2%), (4) carotenoids (trace), (5) acylated sterol glycosides (7.6%), (6) monogalactosyl diglyceride (11.9%), (7) digalactosyl diglyceride (25.9%), (8) phosphatidylethanolamine (4.3%), (9) phosphatidylglycerol (2.0%) (10) sulfolipid (trace), (11) phosphatidylinositol (2.0%), (12) phosphatidylcholine (18.4%), (13) lysophosphatidylcholine (trace).

chromatography and various other methods of quantification (3,5). The advantages of this HPLC-FID technique are: a) it is rapid (80 min per sample); b) it is sensitive (minimum limits of detection of each lipid class is 1-2 μg); and c) it is the first chromatographic technique to analyze both polar and nonpolar plant lipids in a single injection. Although detection by FID has previously been used to quantify molecular species of plant lipids (glycerides with the same head group and different fatty acids on the sn-1 and sn-2 positions) (4), this report is the first time it has been employed to quantify lipid classes (i.e. monogalactosyl diglyceride, digalactosyl diglyceride, phosphatidyl ethanolamine or phosphatidylcholine) from plants. We are preparing a comprehensive paper describing the application of this new HPLC-FID system for the quantitative analysis of lipids from other plant tissues.

LITERATURE CITED

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