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Note

Removal of chlorophyll pigments from plant neutral lipids

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Chlorophyll pigments, which are present in all green plants, co-extract with lipids into chloroform–methanol, which is the most popular solvent mixture used for the extraction of lipids. Nichols[1] and Allen *et al.*[2] have used countercurrent distribution, and Nichols[3] has used silicic column chromatography, to separate total leaf lipids into non-polar or neutral and polar fractions. Chlorophyll pigments were eluted mostly into the non-polar, neutral lipid fraction with chloroform, and these pigments had hindered further analysis of the neutral lipid classes. Nichols[3] has suggested thin-layer chromatography as a means of obtaining a small amount of purified neutral lipids devoid of chlorophyll pigments, but gave no further information regarding the solvent system to be used.

During the course of our study of the lipid composition of young leaves we have developed a simple and rapid column chromatographic method to remove chlorophyll pigments from plant neutral lipids.

MATERIALS AND METHODS

Young cassava (*Manihot esculenta*, Cratz) leaves were obtained from the experimental farm of the University of Agriculture, Serdang, Selangor, Malaysia. The leaves were macerated and extracted with 20 volumes of chloroform–methanol (2:1, v/v) mixture and washed with 0.2 volume of diluted salt solution[4]. The lower chloroform fraction was evaporated to dryness on a rotary evaporator. The total lipids thus obtained were dissolved in chloroform and applied to a acid-treated Florisil column[5] and separated into non-polar neutral lipid and polar lipid fractions using chloroform and methanol, respectively.

The non-polar neutral lipids contaminated heavily with chlorophyll pigments were applied to a short glass column of 1.2 cm I.D. which contained 1.5 g of a mixture of activated charcoal and Celite 545 (2:1, w/w). The final height of the column was 4.5 cm. Chloroform was used to elute all the neutral lipids. Carotenoids were also eluted with the chloroform fraction but chlorophyll pigments were retained in the column. Methanol would elute all the chlorophyll pigments retained in the column.

RESULTS AND DISCUSSION

Authentic neutral lipid standards were put through the activated charcoal–Celite column as described. It was found that 50 ml of chloroform was sufficient to elute all the neutral lipids from the column and the recovery for each of the

neutral lipid standards, namely, monopalmitin, dipalmitin, tripalmitin, palmitic acid, cholesterol and cholesterol stearate was almost 100%. For a 1.5 g charcoal–Celite column, as much as 1 g of tripalmitin can be eluted in 50 ml of chloroform without any decrease in recovery. Measurements were made of the absorption spectrum of chlorophyll pigments from 400 nm to 700 nm before and after the neutral lipid fraction obtained from the acid-treated Florisil column were put through the charcoal–Celite column. These showed that practically all the chlorophyll pigments were retained in the column (Fig. 1).

Fig. 1. Spectra taken before (A) and after (B) the neutral lipid mixture in chloroform was put through the activated charcoal–Celite column.

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