

PARTIAL PURIFICATION OF CHLOROPHYLL EXTRACTED FROM SPINACH LEAVES
BEFORE CHROMATOGRAPHIC SEPARATION AND ISOLATION

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An effective method for partial purification of chlorophylls using dioxane and washing with 80% (v/v) aqueous methanol before chromatography was studied for the preparative purpose. The chromatographic analysis revealed that the preparation of chlorophylls thus obtained did not contain any leaf yellow pigments which disturbed the isolation of chlorophylls a and b from one another in column chromatography.

Interest in chlorophyll studies now arises in many diverse areas of investigation. However some difficulties still remain in preparation of chlorophylls for the studies. One of the difficulties exists in preventing the chlorophyll preparations from contamination of leaf yellow pigments. Therefore we aimed to eliminate from crude extracts any yellow pigments, which disturb the separation of chlorophylls from one another in a column, before chromatographic separation of chlorophylls a and b. Recently we reported on "dioxane method", a simple method for partial purification of chlorophylls from plant materials, using dioxane¹⁾. Chlorophyll was precipitated selectively in a spherocrystalline form by adding dioxane and water to a crude chlorophyll solution obtained by extracting plant materials by acetone, leaving the bulk carotenoids. However, yellow pigments (xanthophylls), which was located ahead of chlorophyll a zone in a powdered sugar column, equilibrated and developed with 0.5% (v/v) isopropanol in petroleum ether, prevented the purification of chlorophyll a to high purity, because it still contaminated the chlorophyll preparation partially purified using dioxane. By washing the partially purified chlorophylls with 80% (v/v) aqueous methanol according to the method of Strain and Svec²⁾, the xanthophylls were also removed from the partially purified chlorophylls obtained by the dioxane method.

This letter is concerned with the improved dioxane method for a partial purification of chlorophylls from spinach leaves before chromatography.

Spinach leaves (100 g fresh weight) were homogenized for 3 min in a Waring blender with 500 ml of acetone. The green juice obtained were filtered through a pad of cotton to remove coarse debris and the filtrate was centrifuged at 10,000g for 5 min to remove insoluble materials. The deep green supernatant solution was employed in the partial purification of chlorophyll by the dioxane method¹⁾. The chlorophyll preparation, twice precipitated from acetone-dioxane solution by drop-wise addition of water, was dissolved into 500 ml of methanol containing 125 ml of petroleum ether (b.p. 20-40°) and then 250 ml of distilled water was added into the solution. The upper petroleum ether layer was washed with 80% (v/v) aqueous methanol for several times to eliminate the remaining yellow pigments from the solution according to the method of Strain and Svec²⁾. After washing with the aqueous methanol for several times, the petroleum ether solution containing chlorophylls a and b was dried in a vacuum desiccator and dark-green microcrystals were obtained. The partially purified chlorophyll thus obtained was separated on a sugar column according to the method of Perkins and Roberts³⁾. The chromatographic analysis showed that all the leaf yellow pigments except for carotenes, unadsorbed on the column, had been eliminated from the partially purified chlorophyll (dioxane method) by washing with 80% (v/v) aqueous methanol before chromatography.

The procedure described is recommended as a routine method for preparation of chlorophyll in laboratory from spinach leaves.

References

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