

THE SEPARATION AND IDENTIFICATION OF VITAMIN E IN BLACK TEA

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The occurrence of vitamin E in black tea is reported for the first time and the methods used for its separation and identification are described. It is suggested that the anti-oxidant properties of Vitamin E may play a part in controlling the oxidizing activity of quinonoid compounds formed during tea manufacture.

Introduction

Several vitamins, *viz* B1, B2, folic acid, pantothenic acid, nicotinic acid and 'P' have been detected in black tea (Tea Board, India 1957). All of these vitamins are soluble in water and contribute to the nutritional value of tea. Apart from these water-soluble vitamins, another group of vitamins referred to as 'fat-soluble', are also of importance in human nutrition. Vitamins A, D, K and E are included in this group and previous work (Tirimanna & Wickremasinghe 1965), has shown that tea contains β -carotene which is converted in the human system to vitamin A. A closer study of the 'fat-soluble', fraction has now revealed that appreciable amounts of vitamin E are also present, and this paper is a brief description of the evidence on which this identification was based. The presence of other compounds related to vitamin E was also detected but these have not yet being fully identified.

Materials & methods

Black tea from St Coombs Estate (elevation 4500 ft above mean sea level), manufactured the previous day was used in this study.

Extraction procedure

Forty grammes of fresh black tea were extracted in a Waring blender for 30 minutes with 150 ml of acetone. The suspension was centrifuged and the residue was re-extracted with the same solvent. The combined extracts were evaporated in vacuo and the residue dissolved in 15 ml of petroleum ether (40°-60°). This solution was subsequently used for column chromatography.

The saponification procedure used in the study of the carotenoids, terpenes and sterols of black tea (Tirimanna & Wickremasinghe 1965 ; 1966) was omitted in this case, as vitamin E and other related tocopherols were unstable to alkali treatment. The unsaponified material could not be analysed directly by thin layer chromatography as the chlorophylls interfered in the separations in most of the solvent systems used. These difficulties were overcome by using column chromatographic techniques for separation, followed by analysis of the different fractions by thin layer chromatography.

Column chromatography

Acid alumina was prepared as follows : 100 g of commercial alumina (BDH) was mixed with 400 ml of N HCl, the mixture being continually stirred for one hour. The suspended particles were decanted and the treated alumina was washed with water, until the washings were feebly acidic to litmus. Finally it was oven-dried for one hour at 100°C. A column (25 cm in length and 2.5 cm in diameter), was

prepared with the treated alumina suspended in petroleum ether (40°–60°C). The compounds were eluted from the column with petroleum ether (40°–60°C) which contained increasing amounts of acetone (upto 10%). Acetone was preferred to commonly used benzene as it was less toxic. Fractions, of 10 ml each, were collected in a LKB RadiRac fraction collector (Sweden). The fractions which gave a positive Emmerie-Engel reaction were subsequently subjected to thin layer chromatography.

Thin layer chromatography

Shandon TLC equipment was used. The thickness of the silica gel (Merck Darmstadt, Germany) layer on the glass plate was 0.25 mm. Co-chromatography was carried out using an authentic sample of DL- α -tocopherol, (vitamin E), obtained from Mann Research Laboratories Inc. New York.

Results

Black tea extracts were found to contain a substance which had Rf values identical with those of the authentic sample of vitamin E in five different developing solvents (Table 1).

TABLE 1 — *Identity of Rf values of compound in black tea and vitamin E*

Solvent system	Rf value
Hexane/diethyl ether 3 : 7 v/v	0.96
Benzene/Methanol 10 : 1 v/v	0.86
Chloroform	0.76
Cyclohexane/diethyl ether 80 : 20 v/v	0.66
Petroleum ether 40,60°/benzene 1 : 1 v/v	0.40

Apart from the evidence of Rf values, the identification of the compound in the black tea extract as vitamin E was confirmed by the following colour reactions : Pink colour with Emmerie-Engel reagent (Stahl 1965), light violet which changed to brown with Sonnenschein reagent (Stahl 1965), blue with phosphomolybdic acid reagent (Stahl 1965), chocolate brown with Tollen's reagent (Dawson *et al* 1959) and light blue with potassium ferricyanide reagent (Dawson *et al* 1959).

The other compounds present in the black tea extract gave similar colour reactions but differed from each other and vitamin E in their Rf values. These are probably chemically related to vitamin E, but their identity is, at present, unknown.

Discussion

Vitamin E takes part in photosynthetic reactions (Dilley & Crane 1963) and is also known to act as a co-factor of the enzyme, lipase, in the hydrolysis of long chain unsaturated glycerides (Ory & Altschul 1962). It has also been reported that the growth rate of plants is inversely proportional to vitamin E content (Booth & Hobson-Frohock 1961) ; it would therefore, be of some interest to study the seasonal variations of this vitamin in tea plants. Work is now in progress on the quantitative estimation of vitamin E and this is being done first to find out whether there are any differences in vitamin E content during the withering, fermenting and firing stages of tea manufacture. The results may prove to be interesting because vitamin E has been used as an antioxidant and as an antibrowning agent for the preservation of foods (Kihara & Inoue 1965). It is, therefore, possible that vitamin E plays a part in controlling the oxidizing activity of quinonoid compounds formed during tea manufacture.

Summary

- 1 — Vitamin E has been shown to occur in extracts of black tea.
- 2 — The importance of vitamin E in plants is discussed.

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