AN ANTI-OXIDANT IN TEA

A. S. L. Tirimanna, R. L. Wickremasinghe & K. P. W. C. Perera

Quantitative studies have shown that tea flush is rich in a group of compounds called the tocopherols. One of these compounds, γ-tocopherol, is the most powerful anti-oxidant in the series. The presence of this compound may protect other compounds present in the tea leaf from oxidation during tea manufacture, thereby having a controlling influence on the development and preservation of flavour in the made tea.

Introduction

This paper describes some results of the analysis of tea flush for a group of compounds known as tocopherols, and quantitative determinations of the total tocopherol content. Six tocopherols have so far been found in plants and these in the order of their discovery are α—, β—, γ—, δ—, η—, and ζ— tocopherol. These compounds are all strong reducing agents and are reasonably stable in air, provided the conditions are acidic. The occurrence in made tea of α— tocopherol, commonly known as vitamin E, has been previously reported, (Tirimanna and Wickremasinghe 1966), and evidence has now been obtained that ζ— tocopherol too occurs in appreciable amounts in the tea flush. This finding is of interest because γ— tocopherol is one of the most potent anti-oxidants in the tocopherol series and may, therefore, play a more significant part than α— tocopherol in regulating the oxidizing activity of the quinonoid compounds which appear during the process of tea manufacture.

The mechanism by which this anti-oxidant activity is exerted is due to the ease of conversion of tocopherols to tocopheryl-quinones. Fruton and Simmonds (1960) have postulated the scheme illustrated in Figure 1.

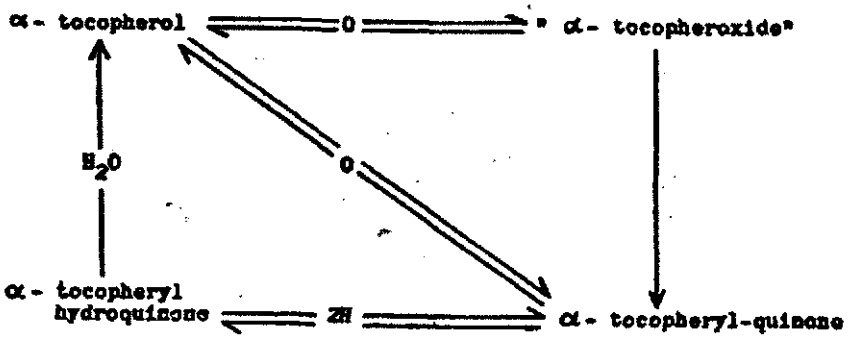

FIGURE 1 — *Interconversions of γ-tocopherol*

It will be seen from Figure 1 that the reversible interconversion of tocopherol and tocopheryl quinone could be a useful method of maintaining the oxidation-reduction balance during the processes of tea manufacture. It is also possible that the degree of protection given by these compounds against oxidation may have a controlling influence on the development and preservation of flavour in tea.

Methods

Twenty g of fresh flush from St Coombs Estate (elevation 4500 feet) were extracted in a Waring blender with 100 ml of acetone, for 15 min. After centrifugation the process was repeated with the residue, using 100 ml of fresh acetone. The combined extracts were evaporated to dryness *in vacuo* and redissolved in ten ml of petroleum ether (BP 40-60°).

Column chromatography

The petroleum ether extract was chromatographed on an acid-washed column of alumina. The compounds were eluted from the column with petroleum ether (BP 40-60°) containing increasing amounts of diethylether. The fractions (5 ml each) were collected in a Radiac fraction collector, and those fractions which gave a positive Emmerie-Engel reaction for tocopherols were combined, evaporated to dryness and redissolved in five ml of absolute ethanol.

Colorimetric estimation

To the ethanolic solution one ml of 0.5% dipyridyl in absolute ethanol, followed by one ml of 0.2% freshly prepared ferric chloride in absolute ethanol were added. Colour development was allowed to proceed for 30 min in the dark and the absorbance was measured at 520 mμ in a Beckmann spectrophotometer. Quantitative measurements were made with reference to a standard curve for pure ∝ — tocopherol.

Thin layer chromatography

A fresh petroleum ether extract of the green leaf was used for thin layer chromatography. The thickness of the silica gel (Merck) layer on the glass plate was 0.25 mm and the plate was activated for 30 min at 110° before use. The solvent used was chloroform.

Results and Discussion

From the quantitative determination of total tocopherol content the total tocopherol content in tea flush was found to be 0.387 mg/g dry weight. This value was obtained after making a correction for the loss of tocopherols during the process of separation on the column. A comparison of the tocopherol content in tea flush with that of other plants is shown in Table 1, from which it is evident that tea flush is rich in tocopherols. However the comparison suffers from the disadvantage that marked seasonal variations in tocopherol content have been reported in plants (Booth and Hobson-Frohock (1961); Green 1958), and the comparative figures used in Table 1 were on young plants (10-13 inches in height), and not on leaves. In the case of tea the figure reported is for flush obtained during the wet season and investigations are in progress of the effect of season on the tocopherol content.

Plant	Concentration of total tocopherols in mgm per gm dry weight
Wheat	0.105
Barley	0.117
Pea	0.127
Maize	0.090
Grass	0.362
*Bean	0.082
Tea (leaf) (Aug. 1966)	0.387

*According to Brown (1953)

Very little information is available on the biosynthesis of tocopherols. Pennock, Hemming & Kerr (1964), studied the tocopherol derivatives in *Hevea* latex and these workers agree with the scheme that tocopherol synthesis could occur by successive methylation of δ—tocotrienol followed by hydrogenation of the side chain. In some plants the rate of synthesis was most rapid after maximum accumulation of carotene and chlorophyll. In sprouting seeds some tocopherols were formed in the dark but light stimulated the reaction (Zakharova 1957). Vil'yams and Grezdeva (1965) were of the view that after destruction of chlorophyll, phytol was a precursor of ∝— tocopherol. Work is also in progress on the quantitative changes of the tocopherols during tea manufacture because it is possible that this may give some information on the synthesis of the tocopherols in the tea plants.

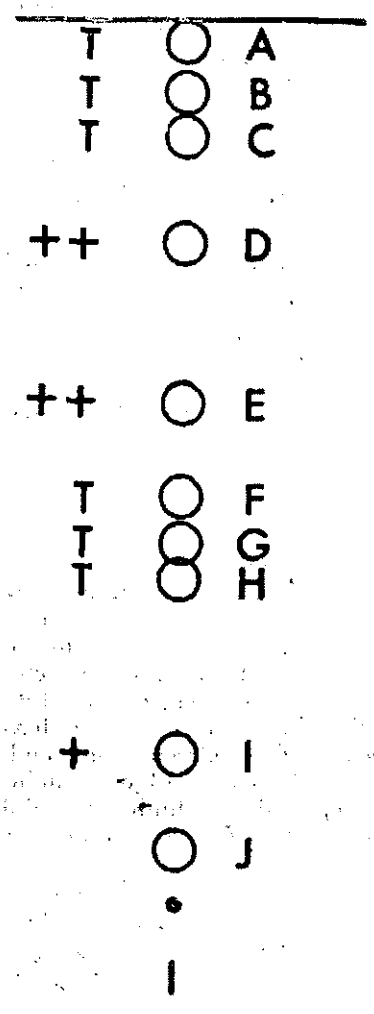

FIGURE 2—*Separation of tocopherols : A – β –carotene (a carotenoid)* (0.98)–*B –tocopherol derivative* (0.92)–*C –tocopherol derivative* (0.87)–*D – ∝ –tocopherol* (0.75)–*E – γ – tocopherol* (0.57)7 – *F – phaeophytins a and b.* (0.46)–*G – tocopherol derivative* (0.41) – *H – tocopherol derivative* (0.37) – *I – Lutein (a carotenoid)* (0.17) – *J – unidentified* (0.06) *The figures in brackets are the Rf values– The approximate concentrations are indicated by the following : T – a trace, + – fair concentration and ++ – appreciable concentration– The solvent used was chloroform.*

The separation of the tocopherols of tea flush is shown in Figure 2. Compounds B and C occurred in trace amounts and gave a weak Emmerie-Engel reaction for tocopherols, although a strong pink colour was observed after 24 hr at room temperature. These compounds also gave a violet colour after treatment with Sonnenschein reagent (Stahl 1965), and are probably esters of tocopherol.

Compounds D and E were both present in appreciable amounts and constituted the major proportion of the tocopherol fraction of tea flush. Compound D has been shown to be α— tocopherol (Tirimanna and Wickremasinghe 1966), and compound E has now been identified as γ— tocopherol on the basis of its Rf value and characteristic violet colour on treatment with diazotized O-dianisidine reagent (Dawson 1959), and the green colour with antimony pentachloride reagent (Stahl 1965). The presence or absence of β— tocopherol could not be shown, as it had identical Rf values as that of γ— tocopherol in the solvent chloroform and moreover β— tocopherol does not give a colour with diazotized 0. dianisidine reagent. Compound G is probably related to γ— tocopherol as similar colour reactions were given compound H found in trace amounts was unidentified although it gave a positive Emmerie-Engel reaction.

Summary

1 – The total tocopherol content in tea flush has been determined by a colorimetric method.

2 – Tea flush is rich in tocopherols.

3 – Appreciable amounts of γ— tocopherol are found in tea flush.

References

BOOTH, V. H. & HOBSON-FROHOCK, A. (1961). The α-tocopherol content of leaves as affected by growth rate. *J. Sci. Fd Agric.* **12** : 251-256.

BROWN, F. (1953). The tocopherol content of farm feeding-stuffs. *J. Sci. Fd Agric.* **4** : 161-165.

DAWSON, R. M. C., ELLIOT, D. C., W. H. & JONES, K. M. (1959). "*Data for Biochemical Research*" Oxford, Clarendon Press, 262 pp.

FRUTON, J. S. & SIMMONDS, S. (1960). '*General Biochemistry*'. New York, John Wiley and Sons Inc. 777 pp.

GREEN, J. (1958). The distribution of tocopherols during the life-cycle of some plants. *J. Sci. Fd Agric.* **9** : 801-812.

PENNOCK, J. F. ; HEMMING, F. W. & KERR, J. D. (1964). Identification estimation of Tocotrienols in *Hevea* latex. *Biochem. Biophys. Res. Comm.* **17.**

STAHL, E. (1965). ' *Thin layer chromatography* ', Berlin, Springer-Verlag. 553 pp.

TIRIMANNA, A. S. L. & WICKREMASINGHE, R. L. (1966). The separation and identification of vitamin E in black tea. *Tea Quart.* **37** : 229-231.

VIL'YAMS, V. V. & GRUZDEVA, E. Yu. (1965). *Dokl. T. S. Kh. A.* 109. Part 1. 147-51.

ZAKHAROVA, M. P. T. & VSESOYUZ, N. (1954). *Issledovatel. Vitamin Inz.* **5** : 159-63. *Quoted, Chem. Abstr.* **51** : 7515.

(*Accepted for publication—20th November 1966*)