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DISTRIBUTION OF CAROTENOID PIGMENTS IN TEA LEAVES

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Systematic qualitative and quantitative analysis of carotenoids in tea leaves (*Camellia sinensis* Var. Assamica) at various stages of development has been carried out. The stages examined were the bud, the first leaf and the second leaf, constituting the shoot used for the manufacture of good quality tea, the third and fourth leaves and the mature (seventh and eighth) leaves. The total carotene and xanthophyll contents were found to increase with leaf development to different extents. The analysis revealed the presence of about fifteen carotenoids at each stage.

Tea, a tropical evergreen shrub, is one of the most popular beverages in the world. The bud, the first, and the second youngest leaves, that constitute 'tea flush' are employed for the commercial manufacture of good quality tea. Tirimanna and Wickremasinghe (1971; 1967) have studied the carotenoid pattern in tea flush. So far, however, a systematic qualitative and quantitative carotenoid analysis of tea leaves has not been carried out.

In the present paper, the distribution of various carotenoids at different stages of development has been investigated. The relative positions of leaves on the stem axis decides its stage of maturity. From this point of view, the study of the carotenoid pattern may throw light on carotenogenesis with leaf development.

MATERIALS AND METHODS

The tea leaves obtained during the month of September, from a tea estate near Ooty (South India), were employed in the present experiment. The leaves were classified in accordance with their relative position on the stem, starting from the terminal leaf (the youngest) as the first leaf. The 'mature' leaves referred to are the seventh and eighth leaves. The mature leaves looked healthy and fresh with no signs of senescence.

Isolation, identification and estimation of carotenoid pigments

About 50 g of the weighed fresh leaf material were macerated with 50 ml of methanol in a Waring Blender for 2 min. and filtered. The residue was repeatedly extracted successively with acetone and ether. The total combined extract was washed with water and reduced to dryness *in vacuo*. In order to remove chlorophyll and fat, the resulting crude extract was saponified by refluxing with 10% methanolic KOH (50 ml) at 50-60° C under a stream of N_2 and left overnight at room temperature. Carotenoids were extracted after saponification with peroxide-free ether. The ether layer was washed free of alkali and reduced *in vacuo* to dryness, taken in small amounts of light petrol (40-60° C) and subjected to chromatographic separation. The individual carotenoids were separated on alumina columns by gradient elution with light petrol, light petrol and ether, and light petrol and acetone as described earlier (Subbarayan *et al.* 1965). The individual bands were characterized by their relative absorption spectrum in the visible region, taken on a Cary-14 recording spectrophotometer (Davies 1965). The presence of epoxy groups in the case of carotenoid epoxides was assessed by the ethanolic-HCl test as described by Curl and

Bailey (1954). Final confirmation of identity was established by co-chromatography with authentic samples on thin layers of silica gel and alumina. The individual carotenoids were estimated by their respective $E^{1\%}_{1\,cm}$ values reported earlier (Goodwin 1955).

The dry weights of leaves were estimated by drying a weighed amount of the fresh leaves at 80° C in an oven to a constant weight.

RESULTS AND DISCUSSION

Change in the carotenoid composition with leaf development

Table 1 gives the changes in the carotene and xanthophyll contents as well as the total carotenoid content with leaf development. In the tea plant the leaves are simple, alternate and opposite, and arranged in an acropetal succession—the terminal leaf being the youngest, with an increase in maturity of leaves towards the base. Hence, carotenoid composition of various leaves in relation to its specific placement from the bud reflects the stage of carotenoid biosynthesis.

As seen from Table 1 (*a*) the total carotenoid content increased with increasing leaf maturity, as did the carotene and xanthophyll contents. It could be observed that in each set of leaves, the xanthophyll content far outweighs the carotene content. However, if we consider the relative rates of carotene and xanthophyll synthesis, it is evident that xanthophyll synthesis seems to proceed at an enhanced rate in the younger leaves and slows down subsequently with maturity of the leaves. This is reflected by the ratio of carotene to xanthophyll which in younger leaves is 1:2.5 and drops to 1:1.3 in case of mature leaves. In other words much of the carotene formed in younger leaves is rapidly converted into xanthophyll, and the rate of these oxidative processes seems to slow down with leaf development even though the accumulation of precursor carotene still seems to take place. It appears, therefore, that the formation of carotene and its subsequent conversion to xanthophyll are controlled independently (Goodwin 1971).

While a clear cut increase in carotenoids from the 1st to 2nd leaf and the mature leaves could be observed, the rise from that in the 2nd leaf to that in the 3rd and 4th leaves was not marked. This might be due to the heterogeneity of the sample.

In Table 1 (*b*) the distribution of individual carotenoids in each set of leaves is depicted. About sixteen different carotenoids are detected in each set of leaves. β—carotene is found to form the bulk of hydrocarbon carotenes being as much as 90% of total carotenes whereas lutein forms the bulk of the xanthophyll, being 82% of the total xanthophyll in the 1st leaf. Among the carotenes, phytofluene, α—carotene, β—zeacarotene, —carotene occur only in small amounts. It could be speculated that is of α—carotene could be the probable precursor of lutein, yet it is β—carotene which constitutes the bulk of the carotene in leaves. This may be due to the conversion of most of the synthesized α—carotene to lutein, whereas very little of β—carotene might be getting hydroxylated to the corresponding xanthophylls like zeaxanthin, violaxanthin, *etc.*, as indicated by the relative amounts detected.

Of particular interest is the detection of monohydroxy xanthophylls like cryptoxanthin and its epoxides. It is probable that the formation of diepoxy-dihydroxy xanthophylls like violaxanthin, luteoxanthin, *etc.*, may proceed by sequential steps involving monoepoxy, monohydroxy xanthophylls as the probable intermediates,

TABLE 1 —*Carotenoid analysis of tea*

Table 1 (a)

Carotenoids	1st leaf	2nd leaf	3rd and 4th leaf	Mature	Reference and source
Total	25.42*	35. 8	41. 3	126.08	
Hydrocarbons	6.91	10.72	10.11	53.68	
Xanthophylls	17.51	23.80	30.24	72.20	
Hydrocarbon: Xanthophyll	1:2.5	1:2.22	1:2.99	1:1.34	
β — Carotene: Lutein zeaxanthin	1:2.3	1:3.28	1:3.20	1:1.37	

Table 1 (b)

Phytofluene	Traces	Traces	Traces	Traces	Tomatoes (Koe and Zechmeister, (1953)
β — Carotene	0.15 (0.59)	0.24 (0.66)	0.15 (0.36)	0.23 (0.18)	Authentic (Hoffmann La-Roche)
β — Carotene	6.24 (24.52)	6.72 (18.74)	8.02 (19.40)	49.86 (39.38)	Authentic (Hoffmann La-Roche)
β — Zeacarotene	0.38 (1.49)	3.52 (9.82)	1.56 (3.77)	0.61 (0.48)	—
— Carotene	0.14 (0.55)	Traces	Traces	0.64 (0.50)	—
Mutatochrome	Traces	0.16 (0.44)	0.18 (0.43)	1.47 (1.16)	Synthesized (Karrer and Jucker, 1945)
Aurochrome	Traces	0.08 (0.22)	0.20 (0.48)	0.87 (0.68)	Synthesized (Karrer and Jucker, 1945)
Cryptoxanthin	0.53 (2.10)	0.20 (0.55)	1.05 (2.54)	1.20 (0.94)	Oranges (Subbarayan and Cama, 1965)
Cryptoxanthin — 5,6 — monoepoxide	0.72 (2.83)	0.12 (0.33)	0.16 (0.38)	0.10 (0.07)	Synthesized (Karrer and Jucker, 1946)
Cryptoxanthin — 5,6 — diepoxide	Traces	Traces	1.01 (1.08)	0.78 (0.61)	Synthesized (Karrer and Jucker, 1945)
Cryptoxanthin — 5,8 —diepoxide	Traces	Traces	0.12 (0.29)	0.6 (0.47)	—
Lutein and zeaxanthin	14.4 (56.59)	22.08 (64.39)	26.02 (62.96)	68.34 (53.98)	Gul Mohr (Jungalwala and Cama, 1962)
Lutein epoxide	Traces	0.46 (1.28)	0.52 (1.25)	0.7 (0.55)	—
Violaxanthin	1.53 (6.01)	0.48 (0.50)	0.46 (1.11)	0.04 (0.03)	*T. stans* (Premachandra *et al.*, 1974)
Luteoxanthin	0.24 (0.94)	0.16 (0.44)	0.15 (0.36)	0.18 (0.14)	*T. stans* (Premachandra *et al.*, 1974)
Neoxanthin	0.09 (0.35)	0.30 (0.83)	0.75 (1.80)	0.26 (0.20)	—

* — Values indicate mgs./100 gms. dry wt.
The values in brackets indicate/of the total carotenoids.

instead of it being a simultaneous, single step conversion from β—carotene. There appears, however, to be a general tendency towards an increase in epoxy carotenes as the leaves mature. However, as could be seen from Table 1 (*b*), the amounts of minor carotenoids and xanthophylls, at each stage of leaf development are too small to arrive at definite conclusions as regards the absolute biosynthetic pathways based on their relative quantitative distribution.

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