

THE MECHANISM OF OPERATION OF CLIMATIC FACTORS IN THE BIOGENESIS OF TEA FLAVOUR

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The relative amounts of chlorophyll, leucine, α -alanine and α -ketoisocaproic acid in tea leaves, and those of carotenoids, β -ionone, dihydroactinidiolide, theaspirone, β -amyrin, acetate, trans-2-hexenal and phenylacetaldehyde in processed black tea have been found to vary with climatic conditions, and indicate the mechanism of flavour development in tea. These variations are related to the degree of CO₂ fixation, and the extent of intra- as compared with extra-chloroplastidic reactions. Evidence is discussed that extra-chloroplastidic biogenesis of terpenoid compounds from leucine leads to the development of tea flavour, whereas intrachloroplastidic fixation of CO₂, and the consequent derivation of terpenoids from acetate results in the production of teas without flavour. The leucine pathway terpenoid biogenesis operates in conditions of climatic stress which limit CO₂ fixation in the leaf, whereas the acetate pathway predominates in conditions of vigorous growth.

INTRODUCTION

The delicate and economically valuable flavour of tea (*Camellia sinensis*, var, *assamica*) is a seasonal characteristic, and the reasons for its ephemeral nature have long been sought to be understood. In Sri Lanka (Ceylon), there are two well-defined seasons during which Ceylon tea flavour is outstanding. These are Jan/Feb in the Dimbula District, and July/Aug/Sept in the Uva District. The characteristics common to both of these seasons are cool (t 20°) dry, windy, bright, cloudless days, followed by cold (t 6-10°), clear nights. In these climatic conditions growth is slow, and 2 consecutive weeks of such weather are generally necessary before flavour becomes manifest. Rain, even slight, arrests the development of flavour, and than a further continuous period of suitable, dry weather is necessary for flavour to reappear. The famed 'first' and 'second flush' Darjeeling flavour is also confined to that short period of time when the bushes are recovering from their winter dormancy, and here again growth is slow, and the weather bright, cool and dry.

The foregoing observations and other characteristics of tea aroma are all explicable by an extension of the findings of Goodwin¹ that the terpenoids of photosynthesizing organisms and leaves are normally derived from acetate, and that the relative amounts of the different terpenoids depend on whether intra-or extra-chloroplast reactions predominate. This was established as being due to the compartmentalization of enzymes within and outside the chloroplast, and the impermeability of the chloroplast membrane to mevalonic acid. Goodwin's results together with additional reactions which are now proposed as occurring in the tea leaf, are depicted in Table 1, and this extended scheme provides an explanation for the effect of climatic factors on the biogenesis of tea flavour.

RESULTS AND DISCUSSION

Before applying in scheme of reactions shown in Table 1 to an explanation of tea flavour, it is convenient to categorize into three groups, some of the observed differences between "flavoury" and "non-flavoury" teas. Group 1 comprises those observations which are partly responsible for reduced CO₂ fixation by the tea leaf

in "flavoury" weather. Group 2 indicates the alternate route of terpenoid biogenesis that comes into operation in these conditions, whilst Group 3 indicates some of the consequences of the reduced CP_2 fixation and alternate route for the biogenesis of terpenoids. These are summarized in Table 2, and the cause of effect of all these differences fits into the scheme of reactions shown in Table 1 on the basis that tea flavour is due to a reduction in the amount of CO_2 fixation within the chloroplast, and the enhanced biogenesis of terpenoid compounds from leucine rather than from acetate.

TABLE 2 — Differences in tea during "flavoury" and "non-flavoury" seasons

	"Flavoury" season	"Non-flavoury" season	Reference
1 Leaf stomata	Closed	Open	2,3
Chlorophyll	Low	High	Table 3
Appearance of processed tea	Brown	Black	5
Mite infestation (Ceylon)	Yes	No	6,7
Greenfly infestation (Darjeeling)	Yes	No	8
2 Acetate	Low	High	4
Leucine	Low	High	10
α -Ketoisocaproic acid	High	Low	11
α -Alanine	Low	High	12
Carotenoids	Low	High	Table 4
β -Ionone	High	Low	17,18
Theaspirone			
Dihydroactinidiolide			
Rhodoxanthin	High	Low	6,7
β -Amyrin	++	++	Table 4
3 Volatile compounds of low boiling point (e.g. acetate and <i>trans</i> -2-hexenal)	Low	High	4,19,20,21
Phenylacetaldehyde	Low	High	21

Group I

The bright, dry, cloudless days, desiccating wind and low temperature necessary for flavour production, cause a reduction in CO_2 assimilation,^{2,3} due to the individual and combined effects of (a) closure of stomata, (b) disorganization of the chloroplast and (c) reduction of chlorophyll content (Table 3). In these conditions formation of acetate would be low⁴ and there would be only slight terpenoid synthesis via the acetate pathway shown in Table 1. The brown appearance of processed "flavoury" teas is a reflection of the reduced content of chlorophyll, as it has been shown that blackness of tea is dependent on the content of pheophytin, derived from chlorophyll during the "firing" stage of black tea manufacture⁵.

Tea pests may play a contributory role by damaging the chloroplast, and so further interfering with intra-chloroplastidic CO_2 fixation. In Ceylon, infestation by mites (*Oligonychus coffeae* Nietner—the red spider mite, *Brevipalpus californicus* Banks—the scarlet mite, *Hemitarsonemus latus* Banks—the yellow mite) reaches a

Reduced CO₂ fixation in the chloroplast would, therefore, appear to be favourable for the production of tea flavour, and the slow growth which has been observed to accompany flavour production is partly due to a retardation of intrachloroplastidic reactions. It is noteworthy that at least a fortnight of the appropriate weather conditions are necessary before closure of the stomata, decrease of chlorophyll content, and disorganization of the chloroplast combine together to induce flavour, and the immediate abolition of flavour development in the tea leaf by rain may be ascribed to the rapid recovery of the CO₂ fixation mechanism as soon as stress conditions are removed. In Darjeeling, the valuable "first" and "second flush" teas are those obtained at a time when the tea bush is awaking from a winter dormancy, i.e. at a time when the chloroplasts are in an immature state of development, and terpenoids are probably derived by a route which does not involve CO₂ fixation. Here too a complete disappearance of flavour follows the onset of the monsoon rains.

Group 2

In weather conditions favourable for growth, freshly plucked tea flush does not contain detectable free leucine, but the content of this amino acid increases remarkably during the artificial "withering" stage of manufacture⁹. It was found¹⁰ that the amount of leucine in "flavoury" black tea was relatively less than that in a black tea without flavour, and it was suggested¹¹ that leucine was converted, during processing, to compounds which contribute to tea flavour. Evidence in support of this suggestion was the identification of a leucine- α -ketoglutarate transaminase in tea leaves¹² and the detection of labelled mevalonic acid, steam volatile compounds and carotenoids,¹¹ during the processing to black tea, of tea leaves which had been fed through the stem with ¹⁴C leucine. The intermediary compounds shown in Table 1 for the transformation of α -ketoisocaproic acid to mevalonic acid are based on the results of previous investigators¹³ on leucine catabolism.

Leucine, as pointed out earlier, is normally not detectable in "unwithered" freshly plucked tea flush, but the dry "flavoury" weather conditions result in what is referred to as "natural wither" of the flush even before it is plucked from the bush, and release of leucine by protein hydrolysis is probably one of the reactions which occur during "natural wither". It was also observed that α -ketoisocaproic acid production was greater during the "flavoury" than "non-flavoury" season, and was especially high in clones with a reputation for flavour¹¹—it is noteworthy that the growth rate of these clones was at all seasons much slower than that of other "non-flavour" clones. The observation that the amount of α -alanine during the flavour season is low¹² would favour leucine transamination, by abolishing competition by α -alanine for the α -ketoglutarate available for transamination. The assumption is made that the leucine pathway to terpenoids is extrachloroplastidic, and that it is suppressed, or of secondary importance, in conditions favourable to growth when the intrachloroplastidic acetate pathway is predominant.

TABLE 4 — Carotenoid and *B*-amyryn contents of "flavoury" and "non-flavoury" Black teas

Tea plantation	"Flavoury" black tea		"Non-flavoury" black tea	
	Carotenoids	β -Amyryn	Carotenoids	β -Amyryn
1	36	+	54	++
2	26	+	62	++
3	27	++	68	++
4	44	++	67	++
5	40	++	63	++

Black tea samples obtained from five different plantations during and after the flavour season. Carotenoids determined colorimetrically by reference to a standard curve using β -carotene. Relative amounts of β -amyryn estimated by visual inspection of thin layer chromatograms.

The amounts of carotenoids and β -amyryn in samples of "flavoury" and "non-flavoury" black teas are compared in Table 4. Three samples of "flavoury" teas were found to contain as much β -amyryn as the corresponding "non-flavoury" teas, but carotenoid contents were invariably much lower in the former. Reference to Goodwin's scheme of reactions in Table 1 indicates that, the formation of triterpenes in extrachloroplastidic, whilst that of carotenoids is intrachloroplastidic. The results shown in Table 4 do, therefore, lend support to the assumption that, in weather conditions conducive to the production of tea with flavour, the route of biosynthesis of terpenoids is extrachloroplastidic. Early investigations with *Phycomyces blakesleeanus* had also indicated that leucine was carotenogenic^{14,15} and with regard to the observed similar effect of ¹⁴C-leucine in tea leaves it is presumed that this extrachloroplastidic production of carotenoids is quantitatively less than that produced via CO₂ fixation from acetate in normal conditions. Moreover, the high light intensity experienced in conditions of "flavoury" weather could cause an alteration in the composition of carotenoids.¹⁶ e.g. with respect to rhodoxanthin content. This difference in carotenoid composition, apart from attracting mites,^{6,7} would also influence tea flavour in view of the findings that β -ionone,^{17,18} dihydroactinidiolide, theaspiron and other volatile compounds, the relative amounts of which are known to affect black tea flavour, are derived by oxidative degradation of the carotenoid compounds present.

Group 3

Predominance of the leucine pathway for mevalonate and terpenoid production over that using acetate would afford an explanation of the well substantiated finding that the proportion of volatile compounds of low boiling point^{14,19,20} is greater in "non-flavoury" than in "flavoury" black teas. A high proportion of *trans*-2 hexenal, in particular, has been identified as deleterious to tea flavour.²¹ This aldehyde is derived by oxidation of linolenic acid,²² and it will be seen from Table 1 that the precursor of this fatty acid is acetate. The proportion of *trans*-2-hexenal will, therefore be reduced if the leucine pathway for production of terpenoids predominates over the acetate pathway. Furthermore it has been suggested²³ that acetate is significantly utilized for terpenoid biosynthesis by senescing chloroplasts, whilst in healthy chloroplasts, acetate is preferentially used for fatty acid synthesis. This implies that metabolism of acetate inside a subnormal chloroplast will be directed towards increased terpenoid synthesis, and reduced linolenic acid production, both of which would enhance the flavour of the tea.

The observation²¹ that "flavoury" black tea contain less phenylacetaldehyde than "non-flavoury" teas also indicates that intrachloroplastidic CO₂ fixation is taking place at a reduced level in the tea leaf in conditions of "flavoury" weather. Phenylacetaldehyde in black tea is derived, by Strecker degradation of phenylalanine,²⁴ which is itself derived via shikimic acid, from the sugars²⁴ formed by intrachloroplastidic photosynthesis. Any reduction in the formation of sugars would, therefore, be reflected in a reduction of the amount of phenylacetaldehyde. These are only facets of the accumulating evidence that tea flavour is due to the *relative* proportions of a number of volatile compounds rather than to the absolute level of any particular group of compounds. In this connection it is of interest that on-going experiments in this laboratory indicate that the terpenoids formed on feeding tea leaves with ¹⁴C acetate and ¹⁴C leucine respectively, are qualitatively similar, but show a disparity in their amounts relative to each other.

EXPERIMENTAL

Comparative amounts of chlorophyll in leaves of different tea clones at different seasons. "Flush"* (10g sample) plucked during "non-flavoury" weather conditions (July) from separate plots of 7 different tea clones (DT 1, TRI 777, TRI 2024, TRI

26, CH 13, CVB1 and PA22) was extracted with 25 ml 80% aq. acetone by macerating for 3 min in a Waring Blendor. The residue was re-extracted with 3 x 20 ml aliquots of aqueous acetone. Each batch of 4 chlorophyll extracts were pooled suitably diluted with acetone, and the absorbance read at 665 nm. The procedure was repeated in "flavoury" weather conditions (February of the following year), on "flush" plucked from the identical bushes of the 7 different clones.

*Terminal bud and two adjacent leaves, with included stem, which is the unit plucked from the tea bush for black tea manufacture.

Estimation of carotenoids. Samples (10g) of black tea, obtained during the "flavoury" and "non-flavoury" season, from 5 different tea plantations were macerated for 2 mins with 200 ml of CHCl_3 -MeOH (8:1), and stood overnight at 20°C. After filtration, the residue was washed with 100 ml of the CHCl_3 -MeOH mixture. The pooled filtrates were evaporated to dryness, and the dry residue extracted successively with EtOH (10.5 and 5 ml) and finally with 80% aq. EtOH (10ml). The alcohol extracts were saponified,²⁶ the ether extracts of the saponified extracts taken to dryness, and the dry residue taken up in CHCl_3 (10ml). The absorbance of this solution was measured, and the carotenoid content determined by reference to a curve, using pure β -carotene as standard.

Semi-quantitative determination of B-amyirin. B-amyirin in the above chloroform extracts was separated by TLC, the spots visualized by chlorosulphonic acid-HOAc spray reagent,²⁷ located with reference to an authentic marker, and determined semi-quantitatively by visual inspection 2 μ l of extracts were spotted in duplicate on activated silica gel plates, and developed in diisopropyl ether, CH_2Cl_2 and benzene-MeOH (20-1) solvents respectively.

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