

STUDIES ON THE QUALITY AND FLAVOUR OF TEA—4 OBSERVATIONS ON THE BIOSYNTHESIS OF VOLATILE COMPOUNDS

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The transformation of the amino-acid, L-leucine, to volatile compounds requires, among other factors, the presence of a leucine transaminase, coenzyme A and manganese. All three of these have been shown to be present in tea flush, and it has also been found that coenzyme A and manganese levels show seasonal and clonal variations. St Coombs flush differs from Uva flush in having a lower content of manganese and a slightly higher content of coenzyme A.

Previous investigations (Wickremasinghe & Swain 1965) have indicated that the concentrations of the amino-acid, L-leucine, was lower in flavoury teas than in non-flavoury teas, and it was suggested, that L-leucine could be one of the precursors of some of the compounds contributing to tea flavour (Wickremasinghe 1967). Evidence in support of this suggestion was obtained when it was found that radioactive volatile compounds, carotenoids and mevalonic acid were detected in black tea manufactured from flush which had been fed with radioactive leucine (Wickremasinghe & Sivapalan 1966; Wickremasinghe 1967). The results of this experiment provided indubitable evidence that leucine was transformed to volatile compounds and that one of the intermediate compounds in the transformation sequence was probably mevalonic acid. The known transformation sequence required the participation of several enzymes, coenzymes and activators, all of which should occur simultaneously at the levels of activity optimal for the smooth operation of the reaction sequence.

The results are here reported of the studies of three of the entities required for the transformation of L-leucine to mevalonic acid and volatile compounds—these are an enzyme, transaminase, a coenzyme, known as coenzyme A, and an activator, manganese. All three of these are present in significant amounts in tea flush and lend support to the proposed hypothesis. The results are also reported of seasonal variations in the coenzyme A and manganese levels of different clones of tea growing at St Coombs and in estates in the Uva Province.

METHODS AND MATERIALS

Detection of transaminase

Transaminase was extracted from tea flush by grinding with polyclar AT and sand. The Polyclar AT was added to remove polyphenolic material (Sanderson 1964). The enzyme was extracted into phosphate buffer, pH 7, after which it was dialysed against distilled water at 4°C for 20 hours. The dialysed enzyme was then incubated at 37°C for 2½ hours with reaction mixtures consisting of combinations of single keto-acids (pyruvic, α -ketoisocaproic, oxaloacetic or α -ketoglutaric acid), and amino-acids (L- α -alanine, L-valine, L-leucine, L-glutamic acid or L-aspartic acid). The reaction mixtures were analysed by paper chromatography in order to find out whether transamination had occurred between the different combinations of keto-acid and amino-acid.

Estimation of Coenzyme A

The methods of estimation of Coenzyme A, (CoA), was essentially the pigeon liver acetylation technique described by Kaplan and Lipman (1948). This method

could not be directly applied to tea flush on account of the high concentration of polyphenolic compounds, which inhibited the activity of the transacetylase of pigeon liver. The inhibitory compounds were removed according to the technique given by Sanderson (1964) by treatment with Polyclar AT. Using this modified technique, the levels of CoA in tea flush were determined at about four weekly intervals in different clones, in order to find out whether there were any seasonal and clonal variations in CoA content.

Estimation of Manganese

Manganese was estimated by the formaldoxime method (Bradfield 1957).

Estimation of Iron

Iron was estimated by the catechol method.

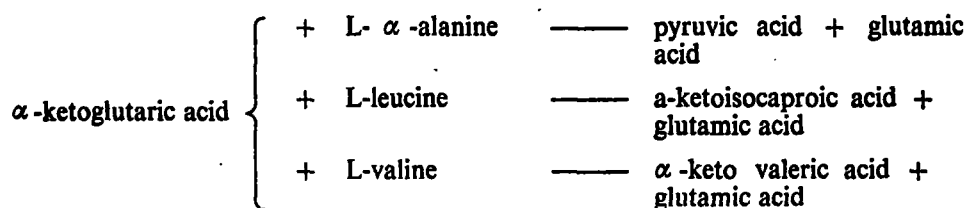
Materials

The experimental material used was clonal tea flush.

RESULTS AND DISCUSSION

Transaminase

On incubating L-leucine with α -ketoglutaric acid, it was found that transamination occurred, resulting in the formation of α -ketoisocaproic acid and glutamic acid. It was found that the optimal pH for transamination was in the alkaline range, and proceeded more rapidly at pH 8.0 than at pH 5.8. The keto-acids, pyruvic and oxaloacetic acid could not replace α -ketoglutaric as acceptor of the amino group of L-leucine. In addition to L-leucine, the other amino-acids of those tested which underwent transamination were L- α -alanine and L-valine. Here too it was only α -ketoglutaric acid which acted as amino group acceptor, the other keto-acids tested being ineffective. Of the amino-acids tested the transamination occurred best with L- α -alanine, the least with L-valine, whilst L-leucine was intermediate in its reactivity. These results show that tea flush contains a transaminase enzyme system capable of effecting the following reactions:



It has been suggested earlier that L-leucine may be transformed during manufacture to some of the volatile compounds responsible for the flavour of tea. The first step in this transformation is the production of α -ketoisocaproic acid, and in this connexion it has been found, that the amount of this keto acid in withered leaf was higher in the flavoury season than during the nonflavoury season (Wickremasinghe 1964). The detection of a transaminase system capable of converting L-leucine to α -ketoisocaproic acid is, therefore, further evidence in support of the proposed scheme for the biosynthesis of compounds contributing to tea flavour. It is also evident that high concentrations of L- α -alanine would be detrimental to the development of flavour, because this amino-acid would be expected to transaminate preferentially with α -ketoglutaric acid, and so reduce the amount of this keto-acid available for reaction with L-leucine.

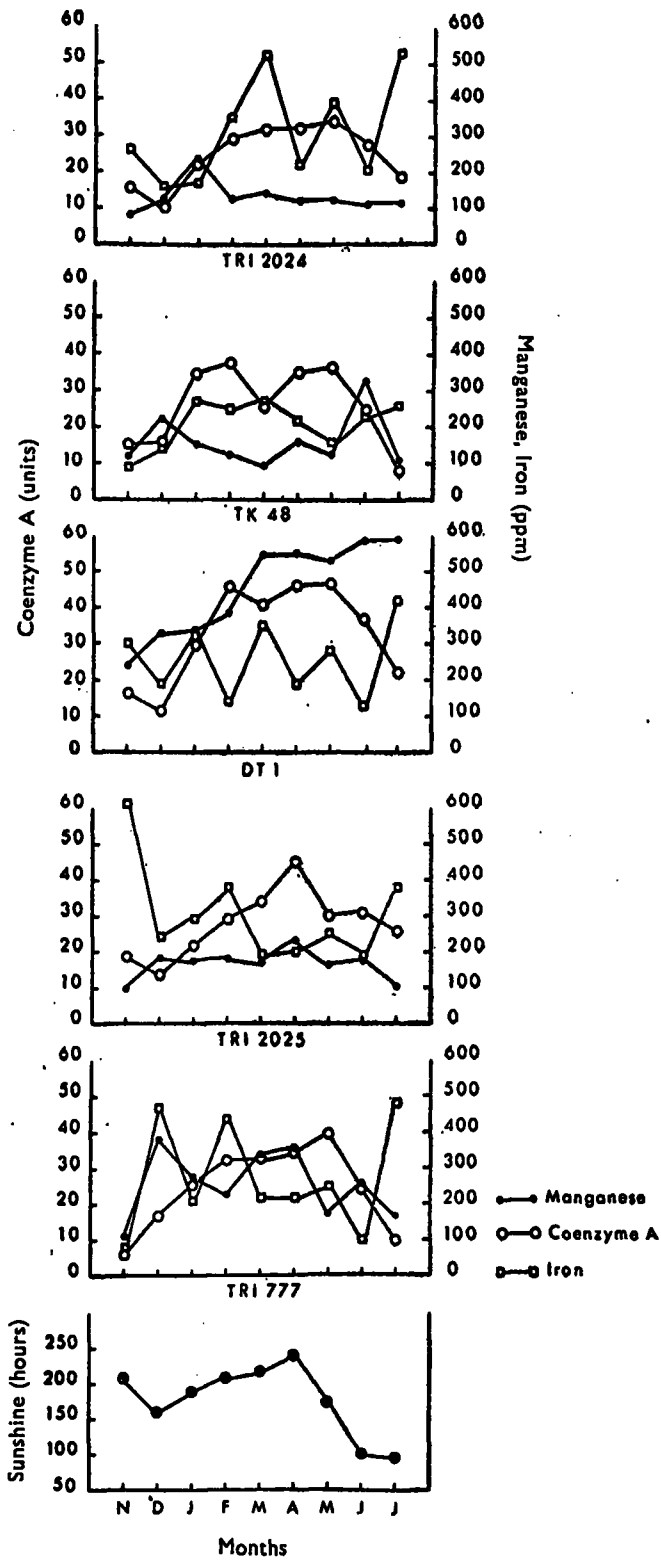


FIGURE 1—Seasonal variations in coenzyme A, manganese and iron in five clones growing at St Coombs

Coenzyme A, manganese and iron

The further conversion of α -ketoisocaproic acid to volatile compounds requires the participation of coenzyme A, (CoA), and manganese, as well as several other factors which remain to be studied. Fig. 1 illustrates the results obtained for the CoA, manganese and iron contents of five clones growing at St Coombs. The analyses were made during the period November 1966 to July 1967. The sunshine graph for this period is also included in Fig. 1. The results indicate that there are seasonal and clonal variations in the levels of CoA, manganese and iron. The curve also shows that the level of CoA is generally higher in January to April than during the other months. In the case of manganese, no obvious trend is evident, but it will be seen that DT 1 and TRI 777 have a higher content of this element than TRI 2024, TK 48 or TRI 2025. This is of some interest, because DT 1 and TRI 777 are considered two of the best clones for flavour. In the case of iron content, too, clonal differences are evident, and it was observed that the proportion of iron to manganese was lower in the flavoury clones than in the non-flavoury clones. This observation may be of some significance in view of the effect of the iron/manganese ratio on the oxidation reduction potential, and the conclusion of other workers (*eg* Srivasta, Agrawal & Jafri, (1965) working on sugar cane plants), that this ratio plays an important part in plant metabolism.

The results of studies in three Uva estates are shown in Fig. 2. The analyses for CoA and manganese (but not iron), were made over a period of 3 months, July to September 1967. The results show that manganese levels of flush growing in Uva are much higher than those at St Coombs. Whereas the levels at the latter did not exceed 250 ppm, those in Uva reach more than 1000 ppm. The levels of CoA, however, tend to be somewhat lower in the Uva estates than at St Coombs. It is, perhaps, noteworthy that flush from Uva Highlands Estate had a high level of CoA together with a high level of manganese. As mentioned earlier, however, an optimum level of manganese and CoA are not the only factors required for the development of flavour during manufacture.

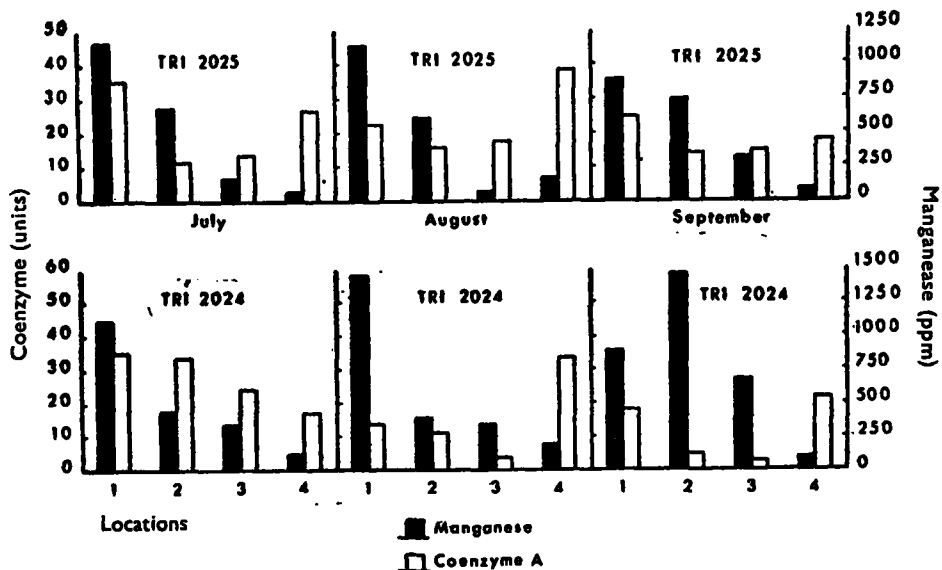


FIGURE 2—Variations in Coenzyme A and manganese during July to September in two clones growing at three locations in the Uva Province and at St Coombs

Locations : 1—Uva Highlands, Bandarawela
2—Neluwa, Bandarawela
3—St James, Hattela
4—St Coombs

Summary

- 1 — Tea flush contains a transaminase system, capable of effecting the transformation of leucine to α -ketoisocaproic acid.
- 2 — Seasonal and clonal variations occur in coenzyme A and manganese contents of tea flush.
- 3 — Coenzyme A and manganese levels may play a part in the development of tea flavour.

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