ASPECTS OF THE BIOCHEMISTRY OF TEA MANUFACTURE

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I have chosen as my subject, a few of our recent laboratory findings, which have helped us to obtain a clearer understanding of the biochemical processes that occur during the manufacture of black tea. The findings are of fundamental interest, but have proved useful not only for an understanding of miscellaneous practical observations, but also for a more rational approach to current problems and the design of future experiments.

The first of these is concerned with the determination of the site in the leaf of the enzyme system which is responsible for the development of an important part of the quality and colour of tea liquors. It has been known for a long time that this enzyme sytsem, known as polyphenol oxidase, is spatially separated from its substrates, which it oxidizes to coloured compounds. This is, of course, the reason why intact tea leaves do not turn brown unless they are damaged by rolling or some other form of maceration. Although the fact of spacial separation was self-evident, the precise site ot the polyphenol oxidase of the tea leaf was not known with any degree of certainty. Experiments were, therefore, designed to determine the location of this enzyme and, in brief, these consisted of staining sections of leaves with a flourescent substance which had an affinity, which was specific for polyphenol oxidase.

It was found that the polyphenol oxidase is localized in the outer or epidermal layer and in the vascular bundles of the leaf. Other investigators, working in the Botany Department of the University of Ceylon, have shown that the substrates of this enzyme, the polyphenols, are situated in the palisade layer of the leaf and we, therefore, now have a clear picture of the separation in space of enzyme and substrate. From the practical point of view, it can now be readily understood why disruption of the cellular structure of the leaf is necessary to initiate fermentation. Furthermore, it is also possible to adduce reasons for some obrervations and practices in tea manufacture, *eg* one of the contributory reasons for the 'greenishness' of teas manufactured from unwithered or under-withered leaf is the complete oxidation of polyphenols. This may be due to part of the enzyme being retained in intact epidermal cells, in a state in which it is not available for fermentation. The reason for this could be the turgidity of the epidermis which encourages the formation of flakes of leaf, containing cells which have escaped disruption. In properly withered leaf, on the other hand, the flaccid nature of the epidermis makes it likely that light conventional rolling breaks down the separating walls between the individual epidermal cells, and so result in the production of an outer sac of enzyme. On rupture of the cells whether in conventional or unorthodox rollers, all of the available enzyme is released for fermentation. This may be one of the reasons for the beneficial effect of a light, preconditioning roll in rotorvane manufacture.

The importance of polyphenol oxidase of tea is, as stated before, for the production of part of the quality and colour of tea liquors. Several enzymes, although of a completely different nature to polyphenol oxidase, are responsible for the development of flavour. In this instance, in addition to enzymes, other substances such as coenzymes and enzyme activators, as well as a fine balance between substrates and several other factors, are necessary for the production of flavour. It is likely that flavour is due to a complex containing many compounds, arising by different biochemical routes, but our studies have been concerned mainly with the products of

the metabolism of the amino acid leucine. Our interest in this particular amino acid arose from the observation that the leucine content of flavoury, black tea samples was less than that of non-flavoury samples, and this suggested that leucine may have been converted to compounds which were at least partly responsible for flavour. In order to test this possibility an experiment was carried out on Delmar Estate, Halgranoya, where radioactive leucine was fed to tea flush collected during the flavoury season last year. The treated flush was processed to black tea on a miniature, laboratory scale and samples obtained after withering, fermenting and firing, were analysed. The results showed that leucine did, in fact, undergo transformation to steam-volatile compounds during manufacture, and that one of the three compounds in the fired tea sample had the properties of a terpene alcohol, which class of compound could make an useful contribution to flavour. Further, the analysis of the withered and fermented tea samples provided an indication of the steps by which leucine was transformed to terpenes and this has formed the basis of a theory of flavour development which is being published in *The Tea Quarterly*.

From what I have said so far, it will be seen that enzymes play an important part in the development of quality and flavour and it is, therefore, evident that any compounds which inhibit enzyme activity would have an adverse effect on these desirable properties of tea. We, therefore, carried out a careful examination of high- and low-grown teas for the presence of enzyme inhibitors and found that there were at least seven such substances — of these three have been fully identified, and the remaining four have been partly characterized as being capable of inhibiting enzymes.

The concentration of these substances was high in low-grown teas and suggested the possibility that this observation could be responsible for the lack of quality in such teas. It is relevant in this connexion that the non-fermenting clones, TRI 9 and TK 48, were found to have an exceptionally high content of one of the inhibitors. High-grown tea also contained these inhibitory compounds, but at a concentration which was below that in low-grown — *eg* in flush from the highest field of Radella Estate, the amount of these inhibitors was negligible. I should like to mention at this point that these inhibitory substances are not entirely without use as they enter into the composition of the complexes responsible for the colouriness of tea liquors and their high concentration in low-grown teas is one of the reasons for the coloury nature of the liquors.

On the one hand, therefore, we have the enzymes, co-enzymes, *etc* tending to promote the development of quality and flavour, whilst on the other are the inhibitory substances favouring the production of ' colouriness ' at the expense of these characteristics. An added complication is the effect of caffeine, and possibly of theanine, in antagonizing the activity of the inhibitors, but I shall not discuss this effect in any detail, and mention it only to draw attention to the fact that there are probably many other factors to be unravelled before the biochemistry of tea manufacture is fully understood.