FACT AND SPECULATION IN THE CHEMISTRY AND BIOCHEMISTRY OF BLACK TEA MANUFACTURE

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It is matter of speculation as to how the first cup of tea was brewed. One can conjecture that bruising of the leaf was accidental and the results so gratifying that it became an established practice. However, although tea has been drunk for more than 5000 years, it is only within the last fifty years that the chemical and biochemical reactions that occur during the processing of tea are, with increasing tempo, beginning to be understood. The starting materials for these reactions lie in the tea flush, and it is therefore first necessary to know what these constituents are and then follow the transformations of these compounds to the products which give black tea its characteristic properties. Plant constituents may be broadly classified into nitrogenous and non-nitrogenous substances ; the nitrogenous compounds of tea include proteins, amino-acids, chlorophylls, caffeine and other purine and pyrimidine compounds, whilst some of the non-nitrogenous compounds are the carbohydrates, carotenoids, keto-acids, polyphenols, terpenes, tocopherols and minerals. We have a great deal of knowledge regarding many of these groups of compounds but it has not, as yet, been possible to evolve a comprehensive scheme of the changes that occur during tea manufacture in order to show the interplay between the different isolated bits of information available. This paper is an attempt to evolve such a scheme which will accommodate the established facts, although some speculation has been necessary at points where insufficient data is available at the present time.

Polyphenols and the quality and colour of tea liquors

Perhaps the most characteristic feature of the chemical composition of tea is the quantity and variety of polyphenolic compounds. These were worked out in great detail by Dr E. A. H. Roberts, who separated and identified the majority of the polyphenols of tea flush, and also followed the changes that these compounds undergo during black tea manufacture. He showed that epigallocatechin and its gallate were the main polyphenols of tea, and that these were enzymically oxidized to theaflavins during fermentation. Other workers subsequently provided evidence that epicatechin gallate too was oxidized, and it was also found that theogallin and the chlorogenic acids decreased during manufacture, suggesting that these compounds too may play a part in the fermentation process. However, the possible role of these compounds was not considered by Roberts in the scheme he proposed for tea fermentation, although the postulated transformations of epigallocatechin and its gallate to theaflavins have been fully substantiated. Roberts and his co-workers obtained evidence that there was some correlation between theaflavin content and the quality of tea, and to account for the " colouriness " of tea liquors, it was proposed that this was due to the further oxidation of the theaflavins to an ill-defined group of compounds, designated as the " thearubigin " fraction. However, it was emphasized that the evidence for this mode of origin of the thearubigins was purely circumstantial, and some doubt of its correctness arose when Vuataz and Brandenberger found that their thearubigin fraction contained substances related to humic acids, and these results were, therefore, completely at variance with those of Roberts. Hence, although the group of thearubigins are responsible for the greater part of the colour and strength of tea liquors, and are also among the most abundant of the constituents of tea, the chemistry of this group of compounds was obscure.

Investigations were therefore carried out at the Tea Research Institute of Ceylon to obtain information of the chemical nature of the thearubigins, and with this end in view, it was considered desirable to identify certain polyphenolic compounds appearing on our chromatograms which had not been characterized by other workers. It was found that there were seven such compounds and at the present time, three of these have been identified as chebulinic acid, corilagin and glucogallin respectively ; some of the chemical properties of the remaining four compounds have been determined, but their exact identification has not been made as yet.

Recent analyses of the thearubigin fraction of tea carried out by the TRI have shown that this consists of at least five complexes. The most coloury complex was found to contain mainly corilagin, theanine and protein, although the possible presence of other compounds or elements has not yet been excluded. The remaining three less coloured complexes also contained, corilagin and theanine together with theogallin, chlorogenic acid, and chlorogenic acid plus epicatechin respectively. This elucidation, at least in part, of the chemical structure of the thearubigins explains the coloury nature of the liquors of low-grown teas as these contain more corilagin and theanine than high-grown teas. At the same time, the formation of these complexes accounts for the hitherto unexplained decrease during manufacture of theanine, theogallin and chlorogenic acids.

Dr G. W. Sanderson described an elegant method for freeing extracts of tea flush from polyphenolic inactivators by the use of insoluble substances having an affinity for polyphenols. Using this technique he was able to show that the polyphenolase of tea was, in fact, a soluble enzyme and so resolved a long standing controversy in tea biochemistry. Apart from the usefulness of this technique for future studies of tea enzymes, it is noteworthy that all the insoluble absorbents used by Sanderson had a grouping identical with that found in theanine, an amino-acid which is of unique occurrence in tea. It is tempting to speculate that, this amino-acid too is used by the plant to prevent or reduce the inactivation of its enzyme systems by polyphenols.

Quantitative changes in polyphenols

Quantitative studies of the changes in polyphenols during black tea manufacture showed the expected increase of the oxidized and condensed polyphenols at the expense of the unoxidized and partially oxidized polyphenols present in tea flush. However, there appeared to be an optimal value in respect of the level of unoxidized and partially oxidized polyphenols and it is postulated that the basis of quality resided in this group of compounds. This group included the theaflavins which, as mentioned earlier, have been shown to play a part in determining quality, but the evidence is strong that the theaflavins cannot by themselves account wholly for this desirable property of tea. It is possible that the level of theaflavins is a good indication of the degree of enzyme activity and that the functioning of enzyme systems is necessary for the formation of the theaflavins and other compounds which are developed during manufacture.

The studies of seasonal and clonal variations in polyphenol content carried out by the TRI recently, showed that rainfall did have some effect on the amounts of this group of compounds, but the magnitude of the changes were not of statistically significant proportions in all the clones studied. The interclonal relationships indicated that there might be an optimal level of polyphenols and amino-acids which favoured the development of quality — values higher or lower than this optimum detracted from the maximum development of quality.

Anti-oxidants of tea

Analysis of tea has shown the presence of compounds which could act as anti-oxidants, and possibly control the activity of oxidizing agents such as the quinonoid compounds produced by the oxidation of polyphenols. The carotenoids and tocopherols of tea have been the groups of compounds which have been studied so far. Apart from their anti-oxidant activity, the carotenoids are of interest because they include Vitamin A, and are also capable of giving rise to odoriferous compounds of the nature of β – ionone, which is responsible for the aroma of violets as well as the off-flavours that develop during the storage of carrots. The tocopherols include Vitamin E and are also of interest in that a negative correlation has been reported between growth rate and tocopherol content. This latter finding may be significance in the light of the observation that quality of tea is greater under certain conditions where growth rate is slow.

Considering first the results of studies of the carotenoids, methods for their extraction were worked out by the TRI and the constituent carotenoids separated by chromatographic methods. Tea flush was found to contain 14 carotenoids, and of these 11 have been identified. Quantitative estimations showed that there was an appreciable increase in total carotenoids during withering, followed by a fall in concentration during the subsequent stages of manufacture. The reason for the fall during fermentation and firing has not been ascertained as yet, but it is of interest that recent work has led to the identification of α – and β – ionones among the volatile compounds of tea. Regarding the tocopherols, tea flush has been found to contain appreciable amounts of this class of compounds. Here too methods of extraction and separation were worked out by the Institute and the presence of α – tocopherol (vitamin E) in tea demonstrated. The α – isomer was also detected and this compound, although not possessing Vitamins E activity, is of interest because it is one of the most potent anti-oxidants in the tocopherol series.

Carbohydrates of tea

The carbohydrates are the primary food reserves of plants and also the starting point for the biogenesis of cell constituents, *eg* polyphenols, organic acids, amino acids *etc.* Investigations of the carbohydrates of tea carried out by the Institute have shown that the main soluble sugars in tea shoot tips are glucose, fructose and sucrose, whilst the insoluble fraction included many complexes containing sugars in their structure ; among these was a polysaccharide similar to, but not identical, with starch. The qualitative picture of the carbohydrates of tea roots was similar to that in the shoot tips, with the exception that starch was present in the roots, and quantitatively, roots contained more carbohydrate than shoot tips.

Blackness of tea

Comparative analyses of extracts of black and brownish teas showed that the main difference between the two lay in the concentrations of the breakdown products of chlorophyll. Black teas showed a preponderance of phaeophytins over phaeophorbides, whilst the latter predominated in brownish teas. Phaeophytin is a black compound derived by the direct removal of the magnesium atom of chlorophyll, whilst phaeophorbide is a brownish compound derived from enzymically hydrolysed chlorophyll. The enzyme which effects this hydrolysis is known as chlorophyllase and is of universal occurence in green leaves. It is therefore evident that brownish teas will result if the conditions of processing favour the activity of chlorophyllase. It is also obvious that the total chlorophyll content in the flush will contribute to the degree of blackness, (or brownishness), of the made tea; quantitative determinations have shown that low-grown flush contains a higher level of chlorophyll than high-grown flush, and this could be one of the reasons for the extreme blackness of teas made from the former.

The colour of tips is also probably dependent on the chlorophyll content of the bud at the time of plucking. It is postulated that silver tip is produced from buds which have not developed chlorophyll, golden tip from buds containing a small amount of chlorophyll and black tip from "mature buds" which have a high level of chlorophyll. On this basis it would seen that shorter intervals between plucking rounds would favour the production of teas with a content of silver tip.

Amino-acids of tea and a theory of flavour development

The amino-acids occupy a central position in plant metabolism and have been extensively studied by several workers. More than twenty amino-acids have been detected in tea and their changes during manufacture carefully followed by workers in the TRI and elsewhere. A striking increase in total amino-acid content was observed to occur during withering, and investigations of individual amino-acids showed that the increase was most spectacular in respect of the leucines, valine, serine, glutamic acid, glutamine, threonine and phenylalanine. A peptidase enzyme capable of accounting for the increase in total amino acids was characterized. The increase during withering was followed by decreases on fermenting and firing, which suggested that amino-acids were possibly metabolized during the latter stages of manufacture. On feeding labelled glycine to tea flush, it has been found that essentially all the amino-acids were labelled within three hours of introducing the labelled amino-acid. It was also noted that caffeine became highly labelled during withering, providing evidence that this alkaloid is a product of the metabolism of amino-acids. Furthermore, as was pointed out earlier in this paper, theanine combines with tannin and other compounds to form thearubigins and this reaction could account at least in part, for the observed decrease of theanine during manufacture. Amino-acids also interact with polyphenols and sugars to give rise to aldehydes which could contribute to the bouquet of tea, and also to form brown products which could contribute to the colour of tea liquors. It is probable that the amino-acids undergo several other reactions during manufacture but our recent investigations have been concerned mainly with the metabolism of leucine. Interest in this particular amino-acid arose from the observation that its concentration was less in flavoury teas than in non-flavoury teas. A possible reason for this finding could be that leucine may have been converted to compounds which contributed to at least a part of tea flavour. To test this possibility, radioactive leucine was fed to tea flush collected during the flavoury season, and it was found that this amino-acid was converted to steam-volatile compounds during manufacture, and that some of these compounds had the properties of terpenes, which could contribute to tea flavour. The biochemical mechanism by which leucine was converted to terpenes was also studied and it was found that the first step involves transamination with one or more keto-acids to form α – keto-iso-caproic acid. The keto-acids in tea flush and the changes they undergo during manufacture were investigated and, it was found that the amounts of α – keto iso-caproic acids increased during withering and then decreased during fermentation, and also that the production of this keto-acid reached a high level in flavoury weather conditions and was especially high in flavoury clones *eg* DTI.

Based on these observations, the theory is proposed that development of flavour is dependent, in the first instance, on conditions which are suitable for the formation of α -keto-iso-caproic acid from leucine by transamination with appropriate keto-acids, *eg* α – keto-glutaric acid. In conditions of bright weather, it may be expected that there would be an accumulation of keto-acids in the leaf due to a rapid rate of carbon dioxide fixation leading to formation of glucose, which is one of the precursors of keto-acids. If at the same time weather conditions are dry, the absorption and transport of nitrogenous material from the soil would conceivably be retarded, and hence the high level of keto-acids produced by the bright weather conditions would not be reduced as a result of their conversion to amino-acids by amination.

Both of these speculations, *viz* high level of keto-acids and low level of total amino-acids in flavoury weather conditions have been borne out in quantitative studies of these two groups of compounds. However, although the level of *total* amino-acids is low, it is possible that the amount of leucine *relative* to that of the other amino-acids is higher than at other times of the year, and this relatively higher amount of leucine confers on it an advantage over the other amino-acids in the competition for transamination with the available keto-acids. In this connection evidence has been obtained that tea flush does, in fact possess an enzyme system capable of effecting transamination between leucine and α – keto-glutaric acid. One of the products of this reaction has been identified as α –keto-iso-caproic acid, and hence there is no doubt that tea flush is equipped with the enzymes and substrates required for the first step in the transformation of leucen to terpenes. In the subsequent conversions of α – keto-iso-caproic acid, the key intermediate is mevalonic acid, because this acid is the most important precursor of the terpenes, carotenoids and sterols of plants. The separation and identification of radioactive mevalonic acid after feeding flush with radioactive leucine, therefore, afforded evidence in strong support of the theory being outlined. The further evidence for the conversion of mevalonic acid to terpenes was provided by the detection of radioactivity in the steam volatile fraction, and the observation that the major part of this radioactivity could be traced to a compound having the properties of a terpene. Some additional evidence in support of this theory was provided by the observed increase in the amounts of coenzyme A and manganese in tea flush during periods of flavoury weather, and also by the detection of a phosphatase system, all of which are involved in the pathway of conversion of leucine to terpenes. The enhancement of flavour by cold nights following bright, dry days is possibly due partly to a further retardation in the transport of nitrogenous material from the roots to flush, and partly to the effect of cold in encouraging the preservation and activity of those enzyme system which catalyse the formation of essential oils, (composed of terpenes), which enable plants to better withstand the cold conditions.

The work described above which form part of the investigations carried out during the past two years has thrown some light on the biochemical mechanisms responsible for the development of quality and flavour during the manufacture of black tea, and also forms the basis of investigations carried out by the TRI for the production of a high quality, flavoury instant tea, and modifications in the manufacture of flavoury, black tea.