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A Thesis

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" Aspects of the Chemistry of Tea -

1. Studies on polyphenol oxidase
2. Chemical composition of thearubigins
3. Effect of age of leaf on chemistry of tea manufacture
4. Chemical changes during the storage of black tea
5. Site of biosynthesis and translocation of theanine

Presented by

CR

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ABSTRACT

Studies on polyphenol oxidase

Polyphenol oxidase, the activity of which is responsible in large measure for the production of theaflavins and thearubigins during tea manufacture, was isolated from tea leaves by adsorption of polyphenols on Polyclar AT, followed by dialysis. The properties of the enzyme and its action during various stages of tea manufacture were studied. The enzyme was found to consist of at least seven enzymes, possibly iso-enzymes, separable by starch gel electrophoresis at pH 8.0 and the optimum temperature for activity of polyphenol oxidase was found to lie between 37°C and 40°C. The enzyme activity was inhibited by high concentrations of the substrate, and by galletannins and ellagitannins isolated from tea shoots and tea bark, as well as by extracts of Terminalia catappa.

The enzyme activity of the intact tea shoots fluctuated slightly during the first stage of black tea manufacture, i.e withering. During the subsequent process of rolling of leaf, the activity increased as a result of a rise in temperature, this rise being due to the rolling pressure. During fermentation there was a rapid decrease in the activity, and there was only a trace of activity in teas which had been "fired", this was completely lost after

storage for one week.

A preliminary study was made of the enzymic oxidation of individual flavanols and other phenolic compounds. The results indicated that there were at least six individual compounds which developed colours during enzymic oxidation. These were l-epigallocatechin, l-epigallocatechin gallate, l-epicatechin gallate, l-epicatechin, d-catechin, chlorogenic acid. It was observed that non-enzymic oxidation of these compounds lead to the development of colours ranging from pink to brown.

Methods were devised for the isolation from fresh tea leaf of l-epigallocatechin, l-epigallocatechin gallate, l-epicatechin gallate, (+) gallocatechin, l-epicatechin and corilagin. In vitro enzymic oxidation of these polyphenols individually and in combination were studied. The reaction products at various stages were identified by paper chromatography, and it was found that theaflavin and its gallate were the main oxidation products.

Chemical composition of thearubigins

Analysis of thearubigin fraction of tea liquor showed that it consisted of at least five distinct complexes, containing mainly proteins, polyphenols, amino acids and sugars. The presence of

theanine, (N-ethyl amide of glutamic acid), in most of these complexes was confirmed using radioactive theanine.

Effect of age of leaf on chemistry of tea manufacture

A qualitative and quantitative study of the relative abundance of polyphenols, amino acids, soluble carbohydrates and caffeine, in different parts of the tea shoots were made. The chemical composition of the different parts, (terminal bud, first leaf, second leaf, third leaf, fifth leaf and tender stem), of tea shoot was found to vary considerably. There was a progressive decrease in enzyme activity, amino acids, caffeine and polyphenols,(with the notable exception of the flavanol glycosides), with increasing age of the plant part. The tender stem had comparatively low levels of polyphenols, and among the amino acids, the amount of theanine was relatively high.

Analysis of black teas manufactured from different parts of the fresh tea shoots showed a direct correlation between the chemical composition of the starting material and the characteristics of the made teas. These findings provided a new approach to the elucidation of the nature of the oxidation products of black tea. The inhibition of enzyme activity by different concentrations of tannins in the different parts of the shoot is discussed.

Chemical changes during the storage of black tea

Chemical changes that occur during the storage of black tea were studied. Analyses were made at weekly intervals for theaflavins and thearubigins, total polyphenols, vanillin reacting polyphenols, and amino acids. Analyses of teas, stored for different periods of time, indicated that freshly manufactured teas contained a high level of epigallocatechin gallate and other unoxidized polyphenols, and that this level decreased on storage. The level of theaflavins was found to first increase, and then decrease, whilst the variation in thearubigin content was inversely proportion to the changes in concentration of total polyphenols and of amino acids.

Site of biosynthesis and translocation of theanine

The use of radiotracer methods established that theanine was biosynthesised in the roots, and translocated therefrom to the growing shoot tip.