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RESEARCH COMMUNICATION
EXTENT OF NON-ENZYMIC OXIDATION IN BLACK TEA PROCESSING

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Non-enzymic oxidation of tea polyphenols resulted in the formation of both theaflavins and thearubigins to the extent of about 20% of that found after enzymic oxidation. While elevated 'fermentation' temperatures favoured non-enzymic formation of theaflavins and thearubigins, extended fermentation periods gave contradictory results.

INTRODUCTION

Enzymic oxidation or fermentation of dhools has been studied extensively (Robertson and Bendall, 1933 ; Robertson, 1933a ; Robertson, 1933b) and reviewed thoroughly (Sanderson, 1972 ; Stagg, 1974 ; Wickremasinghe 1978 ; Bokuchava and Skoboleva, 1930) but possible non-enzymic oxidation effects have not been investigated.

The only report on the antioxidant action of three common tea polyphenols (Lea and Swoboda, 1957) showed that epicatechin gallate and epigallocatechin gallate were more effective than catechin at preventing the autoxidation of unsaturated long chain fatty acid methylesters.

This preliminary investigation was undertaken with a view to study whether non-enzymic oxidation of tea polyphenols resulted in the formation of theaflavins and thearubigins in the first instance and whether altered conditions of fermentation had any noticeable effects on such formations.

MATERIALS AND METHODS

Young tea shoots, consisting of two leaves and a bud, of clone TRI 2025 were harvested from a nearby plot and withered at room temperature for 16 h by spreading them out thinly in the laboratory. Withered flush was divided into two lots one of which was minced in a food grinder. After two passes, the dhools were thinly spread for enzymic-fermentation at room temperature. The second lot was steamed in a Buchner funnel through which live steam was passed for 10 mins. The steamed shoots were pressed firmly between two wads of blotting paper to remove excess moisture and subject to the same mincing action as the first lot and spread thinly. After fermenting for 2 or 3 h the dhools were dried in an oven with air circulation for about 30 min. at about 85°C.

Fermentations at elevated temperature were carried out in an incubator set at 34°C with free access to air. The other parameters were maintained at the same values as before.

Extractions of the dried products were carried out by weighing accurately a 5 g sample into a conical flask and adding 100 ml of boiling distilled water after which the flask was maintained on a steam bath for 5 mins. The aqueous extracts were filtered through cotton wool, diluted suitably and used for theaflavin, thearubigin and flavonol estimation.

Theaflavins (TF) and thearubigins (TR) were estimated by the method of Robert and Smith (1961, 1963) while vanillin reacting flavonols were estimated by the method of Swain and Hillis (1959).

Chromatographic analysis of the products was carried by paper-partition chromatography. Ethanolic extracts of the products were spotted on Whatman No. 1 paper and developed in the first direction for 16 h with n-Butanol: Acetic acid: water (12:3:5) after which the papers were dried and then developed in the 2nd direction with 2% acetic acid for 4 h. Spots were visualized by spraying a solution of diazotised para nitraniline.

RESULTS AND DISCUSSION

TABLE 1—*TF, TR and Flavonol levels of black tea made after oxidation at* 24°C *and* 34°C *(average of two determinations)*

	TF (μ*mol g*$^{-1}$)		*TR* (*% by weight*)		*Flavonols* (*mg g*$^{-1}$)	
Enzymic oxidation ..	24°C	34°C	34°C	34°C	24°C	34°C
2h fermentation ..	118.0	102.8	5.37	6.23	11.10	10.63
3h fermentation ..	95.4	105·4	6.28	6.95	6.25	6.88
Non-enzymic oxidation						
2h fermentation ..	10.0	20.8	1.17	2.21	25.44	19.94
3h fermentation ..	8.1	13.6	0.96	1.96	37.63	19.00

The TF, TR and flavonol levels of black tea made after oxidation at 24°C and 34°C is presented in Table 1.

From Table 1 it is evident that non-enzymic oxidation produces about 10% of the theaflavin content produced by enzymic oxidation and about 20% of the thearubigin content. With a 10°C increase in reaction temperature the theaflavin content of the non-enzymically oxidized tea samples increased to about 20% of that produced by enzymic oxidation. The thearubigin content increased to about 35%.

In normal tea fermentation it is an accepted fact that an increase in fermenting temperature generally enhances the thearubigin levels more than the theaflavin levels with increased fermenting time (Robertson, 1983a). This trend however, seems changed in non-enzymic oxidation where both the theaflavin and thearubigin levels are decreased by increased 'fermenting' times (Table 1). An explanation by way of an equilibrium situation becomes untenable, at the higher temperature, due to the very slight corresponding increase in the flavonol levels.

Paper-partition chromatography confirmed the formation of both theaflavins and thearubigins in the tea, resulting from non-enzymic oxidation. This was a significant finding both from the academic and practical point of view. The formation of theaflavins as condensation products of quinones has been established (Takino *et al.*, 1964 ; Sanderson, Berkowitz and Co, 1972). However, there is an uncertainty about the mechanism of formation of the thearubigins (Wickremasinghe, 1978, although their structural identity has been established (Brown *et al.*, 1969).

There is no doubt (Robertson, 1983a) that the quinones, which result after the polyphenoloxidase mediated oxidation of tea polyphenols, are the precursors of both theaflavins and thearubigins. However, the fact that both theaflavins and thearubigins can form even after non-enzymic oxidation, poses the relevant question whether their precursors, the quinones, can be formed even in the absence of the enzyme. The antioxidant action of tea polyphenols (Lea and Swoboda, 1957) proves that autoxidation to quinones is possible in the presence of promoting substances like hydroperoxides. Tea leaf lipoxygenase is known to catalyze the formation of hydroperoxides (Hatanaka, *et al.*, 1982) starting from the withering stage, which in turn gives rise to trans-2-hexenal. However, when these hydroperoxides are formed within a receptive polyphenolic matrix like that of tea interspaced with chlorophylls and their breakdown products, they may well promote quinone formation. The levels of ascorbic acid and heavy metal ions such as copper and manganese which are found to be present in a fairly high level in tea (Stagg and Millin, 1975) may also influence it due to their combined pro- or anti-oxidant activities, reported in other food matrices (Kanner and Budowski, 1978 ; Kanner, Mendel and Budowski, 1976).

From a practical standpoint, non-enzymic oxidation may contribute to fermentation if the flush is largely from poor fermenting clones, the standard of leaf is low, leaf has been subject to bad post-harvest handling.

Further investigations are being carried out with a view to examining the effects of clones, mode of manufacture and leaf standard on non-enzymic oxidation.

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