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SOME OBSERVATIONS ON THE CORRELATION OF POLYPHENOL CONTENT TO THE QUALITY OF TEA CLONES

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Detailed analyses were carried out to determine whether the reported correlation between polyphenol content and quality can be used as a criterion for clonal selection. It was found that theaflavin content showed some correlation but this was not quantitatively related to the polyphenol content or polyphenol oxidase activity. The correlations were not clear enough to be used as an absolute measure of quality.

INTRODUCTION

Fresh green tea leaf is converted to the black tea of commerce by a manufacturing process which primarily depends on the enzymatic oxidation of tea leaf polyphenols to dimeric and polymeric pigments known collectively as theaflavins and thearubigins. There have been numerous studies on the correlation between chemical composition of the leaf and the quality of the black tea produced. These investigations have been extensively reviewed (Sanderson, 1972). As a result of these investigations, it has now become generally accepted that the quality of tea is generally dependant on the chemical composition of the green leaf used for its production provided that process parameters are carefully controlled during manufacture.

The tea plant is propagated vegetatively from single leaf nodal cuttings taken from a parent plant and this method of propagation provides an opportunity of perpetuating uniform genetic material with known desirable characteristics (*ie.* clones). Tea clones are categorised as high, medium and low quality clones depending on the taste characteristics of the black tea manufactured from them (Kirtisinghe, et. al., 1968).

The present process of classifying a new clone involving the propagation of the plant and harvesting of sufficient leaf for a miniature manufacture experiment is a very prolonged operation. Investigations have thus been directed towards finding chemical and physical parameters which may provide a rapid preliminary assessment of the quality potential of a particular clone so that a small quantity of leaves from the parent stock itself could be used to find out whether further field experimentation is worth pursuing.

Sanderson (1964) and Sanderson and Kanapathipillai (1964) attempted to correlate the quality of clones with a number of chemical characteristics such as ash, total flavanols, nitrogen, caffeine and polyphenol oxidase but his results were not fully conclusive. Roberts (1962) attempted to correlate tea quality with the polyphenol present in the leaves and their oxidation products. He found that a number of cases did not exhibit the expected correlation. On the contrary studies by Wickremasinghe (1965) showed that quality could be generally correlated with the quantity of theaflavins which are a group of dimeric flavanols formed during manufacture by the enzymic oxidation and subsequent coupling of the polyphenols.

Hilton and Ellis (1972) and Takeo and Osawa (1974) have reported that the price of tea is determined primarily by its theaflavin content. More recent investigations by Gaffar and Jayaratnam (unpublished results) have shown that there is no obvious correlation between quality potential and polyphenol oxidase activity.

Theaflavin is known to be derived by the oxidation coupling of Epicatechin, Epigallocatechin and their gallates in various combinations (Sanderson, Berkowitz and Graham, 1972) and there have been many investigations aimed at correlating the quantity of individual flavanols present to the theaflavin formed during manufacture of tea. Hilton (1972) and Hilton and Palmer Jones (1973) have reported a high correlation between Epigallocatechin gallate and the quantity of theaflavin formed. Wickremasinghe and Perera (1973) have reported that the tender stems of tea shoots have a greater potential to form theaflavin than young leaves of the same shoot. These results taken together with the detailed analytical data of Bhatia and Ullah (1968) suggested that the relative abundance of individual catechins may be important.

The purpose of the investigation reported in this paper is to further investigate the postulated correlation between theaflavin and individual polyphenols to the quality of tea and to establish whether such analytical data would be a useful index of the quality potential of tea clones.

MATERIALS AND METHODS

Plant Material

Young shoots from clones grown in experimental areas were harvested on the same day, weighed, sealed in polythene bags and stored at −15°C until required for analysis.

Analysis of fresh leaves

A weighed quantity of young shoots (10 g) were immersed in boiling ethanol, heated for 5 min and homogenised in an Atomix blender for 10 min. The homogenate was centrifuged for 10 min at 3000-4000 xg and the supernatant solution recovered. The residue was re-extracted with a convenient volume of 50% Ethanol and the extract combined with the previous solution. The final volume was made up to 300 ml. 15 ml of this extract was evaporated to dryness and dissolved in 25 ml methanol (calculated to contain 0.1 g plant material per ml). This concentrate C1 was used for paper chromatography.

Chromatography

The concentrate C1 was chromatographed on Whatman No. 1 paper buffered with 0.2 N sodium acetate using Butanol: Acetic acid: water 12:3:5 (v/v/v) for 22 h and 2% Acetic acid for 16 h. This method produced a distinct separation of the principal catechin in the extract (K. Sivapalan — personal communication). The polyphenolic spots were located by the fluorescence in UV light after exposure to ammonia vapour.

Elution of compounds

The areas of paper containing the required spots were outlined carefully, cut out with a pointed end, attached to a wick and eluted by descending chromatography in water (16 h). The eluate was made up to a known volume.

Estimation of flavanols

The eluates were used for the estimation of flavanol content by the Vanillin reagent method described by Swain and Hillis (1959). The standard curve was based on l-catechin.

Theaflavin formation

A miniature process was developed for the fermentation of tea leaves on a micro scale. The young shoots were ground in a motor with half their weight of sand and spread out for fermentation on a petri dish. The petri dishes were placed in a closed chamber whose atmosphere was maintained saturated with water by having a gentle stream of water saturated air passing through the chamber. Fermentation was allowed to proceed for 2 h. The process of fermentation was stopped by plunging the fermented material into boiling water and allowing it to stand for 10 min without heating. This method of controlled fermentation was found to provide better correlation of data than the drying and extraction of such small quantities of leaf.

The extract was recovered by centrifugation or filtration and used for the quantitative determination of theaflavins by thearubigins and thearubigin polymers (Takeo, 1974).

RESULTS

The results of quantitative analysis of Epigallocatechin, Epigallocatechin gallate, Epicatechin gallate and Epicatechin in clones of different quality potential are shown in Table 1. Detailed analysis of these results revealed that there was no statistical correlation between the quantities of individual polyphenols and the quality ranking of the clones. The total flavanol content also showed no correlation to clonal quality ranking.

TABLE 1 — *Concentration of individual flavanols in clones arranged according to quality potential*

	Clones	*Concentration (mg-^{1}g flush)*			
		EGC	*EGCG*	*ECG*	*EC*
High Quality	777	53.2	124.3	61.2	46.9
	1294	13.9	22.4	9.8	5.1
	1526	23.3	23.3	11.8	5.7
	2142	8.3	16.7	5.1	9.0
	DT 1	15.9	37.7	22.1	5.3
	N 2	38.6	30.5	11.8	6.8
	TC 9	5.2	14.9	5.9	2.4
	DK 19	12.3	11.3	7.4	3.9
Medium Quality	2023	8.2	16.9	6.6	1.5
	2024	3.6	12.8	11.2	4.1
	2027	8.4	23.4	6.1	2.5
	K 150	27.1	49.1	9.6	10.0
	NAY 3	20.7	45.6	9.7	8.5
	2025	8.0	52.9	18.7	3.9
	N	18.3	21.6	7.6	2.0
	KEN 16/3	13.7	15.1	7.0	3.0
Low Quality	2026	12.3	34.1	3.6	2.0
	2043	9.1	25.1	14.3	5.5
	PA 22	27.8	38.3	7.0	2.8
	TRI 740	23.8	28.8	9.0	9.2
	2016	9.9	59.9	11.6	2.9
	MT 18	17.5	22.4	8.6	2.6
	CH 13	12.0	18.9	9.6	7.3
	DT 95	15.6	21.3	5.6	1·8

The ratios of Epicatechin (EC) to Epigallocatechin gallate (EGCG), EC to EGC, ECG to EGCG did not show any statistical correlation to either quality or theaflavin formation.

Table 2 shows the theaflavin formed by the different clones under the described experimental conditions. In this instance there is some correlation between the quantity of theaflavin formed and the quality potential of a clone. The high quality clones have a high mean value as compared to low quality clones.

TABLE 2— *Formation of theaflavins during manufacture (Theaflavins expressed in arbitrary spectrophotometric units)*

High quality clones	*TF*	*Medium quality clones*	*TF*	*Low quality clones*	*TF*
777	0.323	2023	0.250	2026	0.247
1294	0.302	2024	0.269	2043	0.184
1526	0.258	2027	0.261	PA 22	0.142
2142	0.284	K 150	0.309	TRI 740	0.275
DT 1	0.279	NAY 3	0.320	2016	0.223
N 2	0.312	2025	0.222	MT 18	0.291
TC 9	0.282	N	0.290	CH 13	0.243
DK 19	0.262	KEN 16/3	0.278	DT 95	0.243
Mean	0.288		0.275		0.228

DISCUSSION

The concentration of theaflavin formed during fermentation shows some correlation with the quality classification of tea clones but the significance of this correlation and its usefulness as an absolute measure of quality will become apparent only if threshold values can be determined for different climatic and other conditions. Theaflavin formation however seems to be controlled by a number of undetermined factors in addition to the parent polyphenol concentration and the oxidase activity neither of which have shown a significant correlation to the quality potential of clones.

The results obtained in the present investigation are at variance with the data reported by Hilton (1972) and Hilton and Palmer Jones (1973), whose sampling and analysis are probably based on a very special set of conditions which cannot be used as a basis for generalisations. It is also necessary to note that the quality classification of clones is carried out on the tasters evaluation of the tea manufactured from each clone and this classification cannot be regarded as absolute but only as a guide to the choice of clones. Our findings would lead to the general conclusion that the quality of tea is determined by a complex combination of chemical parameters which cannot be determined by the analysis of isolated compounds, however precise the analysis.

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