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Factors affecting Quality, Strength and Colour of Black Tea Liquors

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Abstract Total and vanillin—reacting polyphenols, total amino-acids, caffeine and polyphenoloxidase activity were higher in young than in mature leaves. The tender stem contained a high level of amino-acids, especially theanine and glutamine, but polyphenol oxidase activity, and contents of polyphenols and caffeine, were relatively low.

Black teas manufactured from leaves of differing maturity, and from tender stems, showed marked differences in organoleptic properties, and in theaflavin and thearubigin contents. The reasons for the differences in quality, strength and colour are ascribed to the proportions of different polyphenols, polyphenoloxidase activity, caffeine, theanine, theogallin and compound G 36 contents of the raw material, from which the black tea was processed.

1. *Introduction*

Black tea of the best quality is made from carefully plucked "flush", comprising the apical bud and two adjacent leaves, (termed first and second leaves), with included stem. There is a marked reduction in quality of the black tea when the raw material used for processing includes the more mature leaves of the tea shoot, but the reasons for this loss of quality are not precisely known. The purpose of this study is to evaluate the nature of the chemical compounds responsible for the differences in quality, strength and colour of the brews derived from black teas processed from tender stem and leaves of differing maturity.

Earlier studies of polyphenols in Assam tea[1] and in Malawi tea[4] grown at Cambridge, England, had shown that the levels of catechin gallates, gallic acid, theogallin and compound G 36 (unidentified, but found to yield gallic acid on hydrolysis) were maximal in the flush and decreased as the leaf matured. On the other hand, the levels of myricetin glycosides and epigallocatechin were markedly higher in mature leaves, whilst deri-

vatives of the flavones, apigenin and luteolin, were detected in mature leaves only. The young stem contained catechins, gallocatechins and gallic acid, together with myricetin glycoside and rhamnoglucoside, and two other unidentified flavanols; compound G 36 was absent from the stem, but chlorogenic and p-coumarylquinic acids were more concentrated in the stem than in the leaves.

In the present investigation on Ceylon tea, the stem, apical bud, first, second, third and fifth leaves were analysed quantitatively for total and vanillin - reacting polyphenols, and semi-quantitatively for some of the individual polyphenols. Analyses were also made of total amino-acids, caffeine, soluble reducing sugars and sucrose, and polyphenoloxidase activity. In addition, the different tissues were processed to black tea, and these black teas were assessed organoleptically, and analysed for theaflavins and thearubigins.

2. *Results and Discussion*

2.1 Polyphenols occur at maximal concentration in the first leaf, and show a steep fall as the leaf matures, and the amount of polyphenols in the stem is relatively low. (*Figure* 1) Semi-quantitative studies, made by inspection of 2-dimensional chromatograms sprayed with diazotized p-nitroaniline, gave results in agreement with those of previous workers[1-4]. The steep decrease in polyphenols with leaf age was observed to be due to the progressively lower contents of flavanol gallates in the more mature leaves; the relatively higher level of flavanols in these leaves may be due to reduced esterification[4] or to the effect of a tannase, which causes hydrolysis of the gallates originally present. Flavanol glycosides were more abundant in mature leaves than in young leaves, and although the stem contained relatively low levels of theogallin and other polyphenols, the proportions of p-coumarylquinic and chlorogenic acids were high. The high proportions of these depsides would not, however, be shown in Figure 1, as neither of the reagents used (Folin Ciocalteau and vanillin), forms coloured products with these polyphenolic compounds.

2.2 Amino-acids There was a progressive decrease in amino acid concentration as the leaf matured, but the tender stem contained a high level of amino-acids (*Figure* 2) Paper chromatographic analyses revealed that the high levels of amino-acids in the apical bud and tender stem were due to the abundance of glutamine and theanine in these tissues. The first and second leaves contained relatively less theanine than the third and fifth leaves, but the higher level of total amino-acids in the younger leaves was due mainly to their higher contents of α- alanine and glutamic acid.

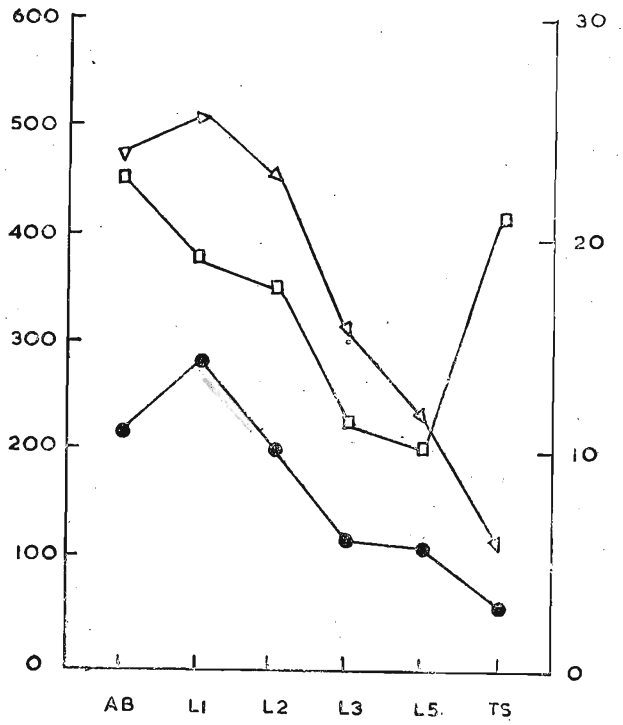

Figure 1. Polyphenol and amino-acid contents of apical bud, leaves of different age, and tender stem of tea shoot.—Δ—Δ, total polyphenols;— ● — ●, vanillin—reacting polyphenols ;— □ — □, total amino-acids ; AB — apical bud; L_1, L_2, L_3, L_5—first, second, third, and fifth leaves respectively; TS — tender stem.

Autoradiographic studies[16] have shown that theanine is biosynthesized in the roots of the plant, and translocated therefrom to the growing shoot tip. The relative amounts of theanine in the different leaves suggest that translocation ceases after a certain level of theanine is attained in the leaf, and that this accumulated theanine is not translocated out of the leaf after it attains maturity. The exact role of theanine in the tea plant has not been clearly defined, but it is possible that this most abundant amino-acid of tea may have a function in protecting the enzymes of the tea leaf from inactivation by polyphenolic and other compounds or elements. Thus, the $-\underset{\underset{O}{\|}}{C}-\underset{\underset{C_2H_5}{|}}{N}-H$ group in theanine could form hydrogen bonds with polyphenols and may, in this way, prevent the formation of insoluble enzyme-polyphenol complexes, having an activity less than that of the free enzyme. The hydrogen bond formed between phenols and N-substituted amides has been described as one of the strongest types of hydrogen bond[3].

2.3. Caffeine The percentage of caffeine in mature leaves was less than that in young leaves, and was lowest in the tender stem (*Figure* 2). It is possible that caffeine, together with polyphenols, sugars, and amino-acids (other than theanine), may be some of the substances translocated from the mature leaves to the growing shoot tip[12]. A possible role for caffeine in the tea plant may be similar to that of theanine, *viz.* the prevention of insolubilization of enzymes by polyphenols. Evidence in favour of this hypothesis is the finding[5] that caffeine splits the insoluble complexes formed between tannin and enzymes, with no loss in activity of the regenerated enzyme, and the observation (*unpublished*) that polyphenols were dissociated from insoluble gelatine-tea polyphenol complexes by the addition of caffeine.

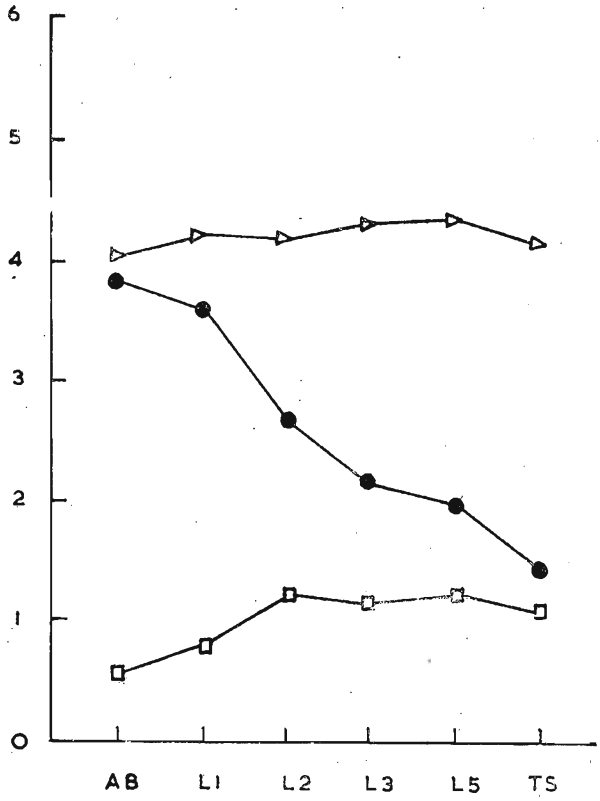

Figure 2. Caffeine and soluble sugar contents of apical bud, leaves of different age and tender stem of tea shoot.— ● — ● caffeine;—Δ—Δ,sucrose;— □ — □ , reducing sugars. AB, L_1, L_2, L_3, L_5, TS, as in Figure 1.

2.4. Soluble carbohydrates There were only small variations in the amounts of soluble sugars and sucrose in young and in mature leaves, although the amounts were higher in the latter (*Figure* 2). This higher

level is maintained in spite of the possible translocation of sugars from these leaves to the young leaves, and suggests that the mature leaves are more actively photosynthetic than the younger leaves. Translocation of photosynthetically formed material from mature leaves to the growing apex of tea plants has been demonstrated earlier.[12]

2.5. Polyphenoloxidase activity was found to decrease as the leaf matured, and to be comparatively low in the tender stem (*Figure* 3). The high enzyme activity in young leaves is in accord with the finding[17] that polyphenoloxidase, localized in the leaf epidermal cells, was of equal activity in both the upper and lower epidermis in young leaves, but

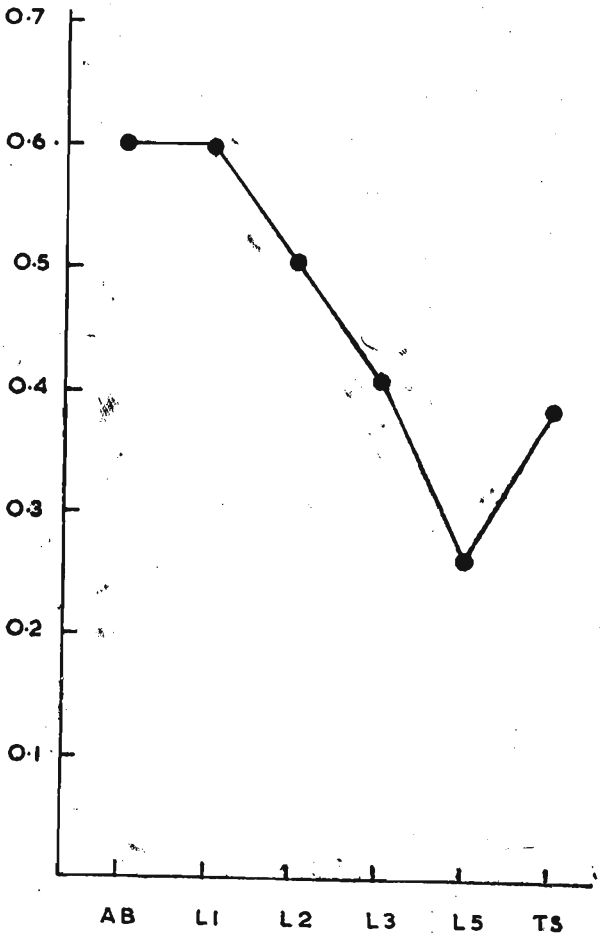

Figure 3. Production of material absorbing at 380 mμ (theaflavins) by enzyme extracts of apical bud, leaves of different age, and tender stem. (Polyphenoloxidase activity). AB, L_1, L_2, L_3, TS, as in Figure 1.

decreased in activity, first in the upper, and then in the lower epidermis as well, as the leaf matured. If one of the roles of polyphenoloxidase and polyphenols in young tea leaves is to produce quinones and other compounds, which confer protection against invading pathogens and pests,[17,19] it would seem that this mode of protection loses its importance as the leaves mature and become resistant, as a result of morphological and chemical changes. The occurrence of such changes is indicated by the increased content of ethanol-insoluble material in mature, as compared to immature leaves.[13]

2.6 Theaflavin formation is highest in the tender stem and lowest in the fifth leaf (*Figure* 4). The latter observation is to be expected in view of the low polyphenol content and low polyphenoloxidase activity of the fifth leaf. The finding that the tender stem surpasses the apical bud in theaflavin formation is unexpected, because the apical bud contained a level of polyphenols and enzyme activity which were higher than that of the stem. The apparently anomalous result may be explained by assuming that the stem contains the *optimal* levels[18] and proportions of the different tea polyphenols and enzyme, which are necessary for the rapid formation of theaflavins, and that this reaction goes to completion due to the low levels or absence of compounds which interfere with or inhibit the enzyme oxidation reaction. *e. g.* intermediates in the formation of theaflavins, and other inhibitory polyphenols - one of which may be compound G 36. It is also possible that the high level of theanine in the stem contributes towards protecting the polyphenoloxidase from inactivation or insolubilization by the oxidation and polymerization

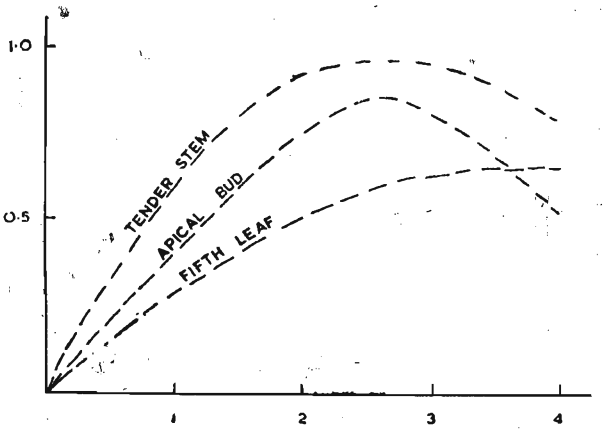

Figure 4. Theaflavin formation by tender stem, apical bud, and fifth leaf. Material macerated and extracts for analysis made in boiling water after different periods of fermentation.

products, and it is noteworthy that the rate of theaflavin formation does not fall steeply after reaching a maximum, as in the case of the apical bud.

2.7. Black teas manufactured from the different tissues showed marked differences in organoleptic characteristics (*Figure* 5), as assessed by professional tea tasters, and in theaflavin and thearubigin content (*Figure* 6).

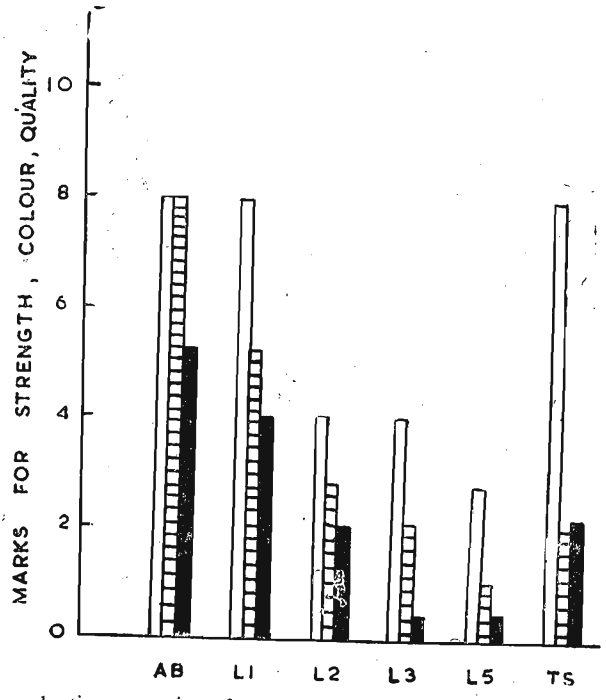

Figure 5. Organoleptic properties of processed black teas. ▬, quality ; ═, strength ; □, colour. AB, L_1, L_2, L_3, L_5, TS as in Figure 1.

The tea tasters' scores for quality, colour and strength showed a progressive decrease for the teas made from the apical bud to the fifth leaf, and this is in accord with the chemical composition and polyphenoloxidase activity of the various tissues. The processed stems showed the highest level of theaflavin, and had a fair score for quality which was, however, less than that for the processed apical buds, suggesting that theaflavin content *per se* is not responsible for quality. Furthermore, the processed stem also contained the highest thearubigin content of all the black teas, but colour and strength were less than that of the teas from apical bud or from the first leaf. The reasons for these findings may be the comparatively low level of theogallin, and lack of compound G 36, in the stem; theogallin has been reported[2] to be one of the factors affecting the cash

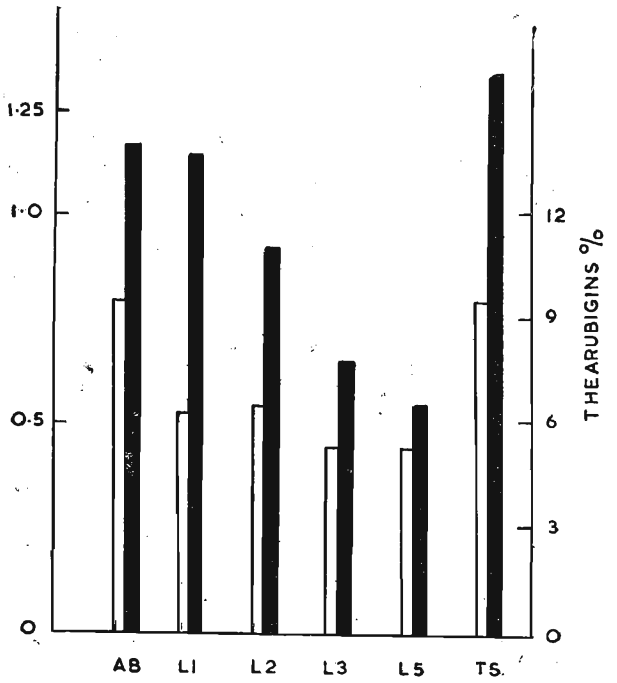

Figure 6. Theaflavin and thearubigin contents of processed black tea. ■, theaflavins ; □, thearubigins ; AB, L_1, L_2, L_3, L_5, TS, as in Figure 1.

valuation, briskness and quality of black teas. It is also possible that the high level of theanine in the stem may prevent the organoleptic perception of strength. Theanine has been found to be incorporated as part of the thearubigin complex[8] and a high level of theanine may result in an alteration of the relative proportions of the constituents of this complex, and so alter its contribution to strength and colour.

3. *Conclusions*

The high quality of black teas made from the apical bud and young leaves may be regarded as being due to the high level of polyphenols and polyphenoloxidase activity, which results in the oxidation of polyphenols to compounds responsible for quality. The oxidation reaction is favoured by the high levels of theanine and caffeine, which protect the enzyme from insolubilization by the oxidation products. Black teas made from tender stems were also of high quality in spite of the low polyphenol content and low polyphenoloxidase activity in this plant material, and it is suggested that quality was here due to the correct balance of polyphenols and enzyme,

protection of the enzyme by the comparatively high level of theanine, adequate caffeine, and the absence of compound G 36. In mature leaves the low polyphenol content together with low polyphenoloxidase activity and low caffeine content resulted in the production of teas of low quality.

The contents of theaflavins and thearubigins were high in black teas derived from the apical bud and stem, and both were rated high for quality. Apical bud black teas were rated high for colour and strength as well, but stem black teas were scored much lower for these characteristics, suggesting that the thearubigins of these two teas were different, possibly due to the relatively high theanine, low theogallin and absence of compound G 36 in the stem.

4. *Experimental*

4.1 **Plant material.** Shoots were obtained from tea bushes (*Camellia sinensis* L. var. *assamica*, clone TRI 2023), which had been allowed to grow freely for three weeks after being plucked. Five hundred shoots were separated into apical bud, first, second, third and fifth leaves, and tender stem included by the bud, first and second leaves. A portion of each material was extracted for analyses of chemical constituents, and the remainder processed to black tea by conventional methods.

4.2 **Extracts** for polyphenols, amino-acids, and soluble sugars were made by macerating the material (10g) for 5 min. with 80% (v/v) aqueous ethanol (50 ml), and boiling the suspension obtained for 5 min. The suspension was filtered through glass wool, and the residue re-extracted for 10 min. periods with boiling 80% (v/v) aqueous ethanol (twice, 50 ml portions) Extracts were pooled and made up to 100 ml.

4.3 **Polyphenols.** Total and vanillin—reacting polyphenols were estimated colorimetrically, according to the methods of Swain and Hillis[15] using Folin — Ciocalteau and vanillin reagents.

4.4 **Amino-acids** were estimated with ninhydrin, according to Yemm and Cocking[20].

4.5 **Caffeine** was estimated by the A.O.A.C. method, described by Pearson[7].

4.6 **Soluble reducing sugars and sucrose.** The polyphenols were first removed by Polyclar AT[11]. Reducing sugars were determined colorimetrically, using Somogyi[14] and Nelson[6] reagents. Sucrose was determined after hydrolysis with invertase (β-D- fructofuranoside fructohydrolase 3.2.1.2.6.). Invertase (0.01 ml BDH suspension stabilized with glycerol) was added to 1.0 ml. solution containing sucrose (20-80 mg), and the hydrolysis allowed to proceed for 1 hour at 27°C. The reducing sugars were then estimated as above.

4.7 **Theaflavins and thearubigins** were determined spectrophotometrically, as described by Roberts and Smith[9].

4.8 **Polyphenoloxidase extraction** was carried out in a cold room at 4° C by grinding the tissue (20 g), frozen at —20° C, Polyclar AT (10 g), acid-washed sand (4g) and

0.5 M phosphate buffer, pH 5.8 (60 ml)[10], The homogenate was filtered through muslin, and Polyclar AT (1 g) was added to the filtrate to remove any residual polyphenols, and the suspension re-filtered. The filtrate was centrifuged at (3000 r.p.m. for 10 min. at —5° C.) The supernatant, which still contained traces of chlorogenic and p-coumarylquinic acids, was used as the enzyme extract.

4.9 **Polyphenoloxidase activity** was determined spectrophotometrically by measuring the amount of material absorbing at 380 mμ ; enzyme extract (0.5 ml). (equivalent t o the enzyme present in 1 g. of fresh tissue) was incubated (20 min. at 37° C), with a mixture of epigallocatechin gallate and epicatechin gallate (18 mg.) dissolved in 0.5 M phosphate buffer, pH 5.8 (2,5 ml). The mixture of gal lates was obtained by elution of the appropriate spots from 2-dimensional chromatograms, developed first in butanol—acetic acid—water (6: 1: 2 v/v) and then in acetic acid (6% v/v), and estimated with reference to a standard curve prepared by using varying concentrations of D-catechin.

4.10. **Polyphenols and amino-acids.** Semi-quantitative assessments of polyphenols were made by visual inspection of 2—dimensional chromatograms developed in butanol—acetic acid—water (6:1:2, v/v) and acetic acid (6%, v/v), and sprayed with diazotized p-nitroaniline. For amino-acids the chromatograms were developed first in the same butanol—acetic acid—water solvent, and then in phenol (500 g. in 125 ml. water), followed by spraying with ninhydrin (0.4% w/v) in ethanol, (95% v/v) containing $CoCl_2.6H_2O$ (0.2% w/v)

4.11. **Processing of black teas.** The various tea shoot entities (250 g) were withered for 18 hours, macerated in miniature tea rollers, and allowed to ferment for 2½ hr. Fermentation was arrested by 'firing' for 21 min. at 80°C and the black teas stored for 2 weeks before organoleptic assessment and analyses for theaflavins and thearubigins, were made.

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