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Food Chemistry 51 (1994) 251–254
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0308-8146/94/$7.00

Changes in theaflavin composition and astringency during black tea fermentation

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(Received 5 November 1993; revised version received and accepted 25 January 1994)

Both theaflavins and sensory evaluations vary in a quadratic manner with fermentation duration in the process of black tea manufacture. The rate of formation of total (Flavognost) theaflavins varies with clones, and within a clone the individual theaflavins form at different rates with resultant changes in the theaflavin ratios as fermentation progresses. The astringency of black tea also varies with composition and total amounts of the theaflavins, as individual theaflavins have different astringencies. The measurement of total theaflavins may therefore not correctly predict total astringency and hence value of tea.

INTRODUCTION

Fermentation is an important step in the processing of black tea from young tender shoots of *Camellia sinensis* (L). O. Kuntze with most of the chemical reactions associated with the making of black tea occurring during this stage. The reactions are dominated by the oxidation of polyphenols, mainly catechins, to form theaflavins and thearubigins (Sanderson *et al.*, 1976). Thearubigins are polymeric products of polyphenols and other compounds of green tea leaf. Their structures are unknown, but they contribute to the taste (mainly thickness and colour) of black tea. However, no significant relationship has been established between the thearubigin levels and sensory evaluations and/or prices of black tea (Roberts & Smith, 1961). Theaflavins are products of polyphenol oxidase-initiated condensation of a gallocatechin and a simple catechin resulting in a flavanol with benzotropolone ring system. The theaflavins contribute to the astringency (briskness) and brightness of black tea. Several studies have demonstrated a significant relationship between theaflavin levels and sensory evaluations or prices of Malawi (Hilton & Palmer-Jones, 1975; Ellis & Cloughley, 1981; Cloughley, 1981, 1983) and North-East India (Deb & Ullah, 1968) black tea, although such relationships have been less successful for Sri Lanka (Roberts & Fernando, 1981) and Kenya (Owuor *et al.*, 1986) black teas.

Consequently many studies have suggested the use of theaflavins as quality standards (Ellis & Cloughley, 1981; Davies, 1983) and as a means of predicting optimum fermentation time in black tea processing (Takeo, 1974; Cloughley, 1979). The use of theaflavin formation as a means of predicting optimum fermentation, however, proved to be less successful for some black teas (Owuor & Reeves, 1986) and this was attributed to other black tea chemical quality parameters also being important. In all past studies, total theaflavins as measured by the Flavognost method (Hilton, 1973) were used. However, four major theaflavins with different astringencies (Sanderson *et al.*, 1976) are formed during black tea manufacture. Thus, although two black teas could have the same amount of Flavognost theaflavins (Hilton, 1973), if the ratios of the individual theaflavins are different, then the teas would have different astringencies (Sanderson *et al.*, 1976) and hence qualities. Most factors causing variations in the theaflavin ratios have not been identified, although McDowell *et al.* (1991) have shown that the geographical area of production is one such factor. Since the histories of the teas used in this study (McDowell *et al.*, 1991) were not established, the differences could reflect clonal, climatic, agronomic and/or manufacturing differences between producers.

This study was done to find out whether fermentation duration could affect the ratios of the individual theaflavins in black tea, using a clone which during

fermentation develops black tea colour relatively fast or with a clone which develops colour slowly. It is not known whether the differences in fermentation rates, as measured by colour formation, impart variations in the ratios of individual theaflavins and in ultimate astringency of the black teas. In practice, the clones which are slow in colour formation during fermentation are fermented longer. This study was performed to find out whether there are changes in the individual theaflavin ratios due to fermentation duration and/or clones and whether the astringency of a clone which forms colour slowly during fermentation benefits from long fermentation duration.

MATERIALS AND METHODS

Clones S15/10 and 6/8 leaf were plucked from a commercial field planted at Timbilil Estate of the Tea Research Foundation of Kenya, altitude 2180 m above mean sea level and latitude 0° 22" South. The plants were receiving 100 kg nitrogen per hectare per year as NPKS 25:5:5:5 in a single dose. Plucking conformed to normal commercial practice of mostly two leaves and a bud plus minor amounts of three leaves and a bud and some loose leaves. Clone S15/10 used in the experiment is relatively slow in colour formation compared to clone 6/8 which forms colour rapidly during fermentation.

The leaf was withered to achieve 70% wither then CTC-macerated (Owuor & Reeves, 1986). The macerated tea 'dhool' was fermented at ambient temperature of 22 to 24°C for various times, i.e. 0, 30, 60, 90, 120, 150 and 180 min, before firing. For each fermentation duration, 1200 g fresh green leaf was used and manufacturing was done in triplicate for each clone. The fired (dried) black teas were subjected to chemical analysis and sensory evaluation (tasting) without sorting.

Total theaflavins were determined by the Flavognost method (Hilton, 1973), while the individual ratios were determined by HPLC (McDowell *et al.*, 1990, 1991; Bailey *et al.*, 1990; Steinhaus & Engelhardt, 1989). For HPLC analysis, liquors were prepared by adding 4 g of black tea to 195 ml deionised water that had just reached the boil and shaking was done for 10 min in a 475-ml capacity Thermos flask. Clean liquor was obtained by filtration through cotton wool. The hot liquor was cooled to room temperature by placing the flask containing the liquor under a cold-water tap (1–3 min). The liquor was diluted (1:1) with double-distilled water prior to HPLC analysis. The liquor was analysed on a Cecil Series 1000 HPLC with a 20-μl sample loop and a Hypersil 5μ ODS Column (25 cm × 4·6 mm). The UV monitor was set at 375 mm and results were recorded and analysed using a JCL600 Cecil data system. Solvent A was 1% aqueous acetic acid and solvent B was acetonitrile. A linear gradient from 8% to 31% solvent B over 60 min with a flow rate of 1·5 ml per minute was used (Bailey *et al.*, 1990; McDowell *et al.*, 1990, 1991). The theaflavin ratios calculated from the HPLC data and the Flavognost (total) theaflavins data were used to calculate the amounts of the individual theaflavins since the molar absorption coefficients of the four theaflavins are similar at 375 nm (Steinhaus & Engelhardt, 1989).

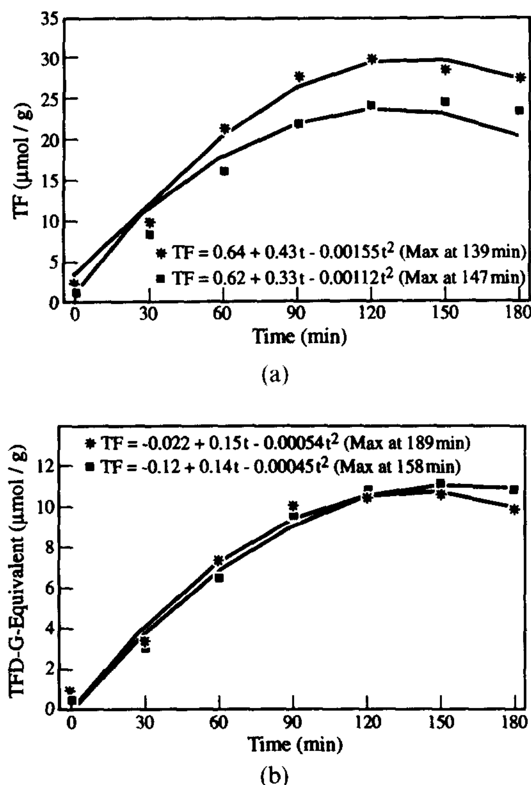


Fig. 1. Changes in (a) total (Flavognost) theaflavins and (b) theaflavin digallate equivalent with fermentation duration. (✱) Clone 6/8; (■) Clone S15/10.

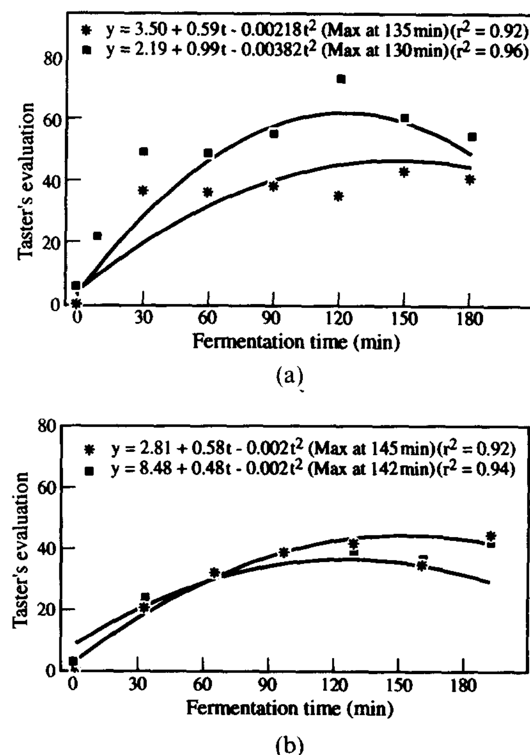
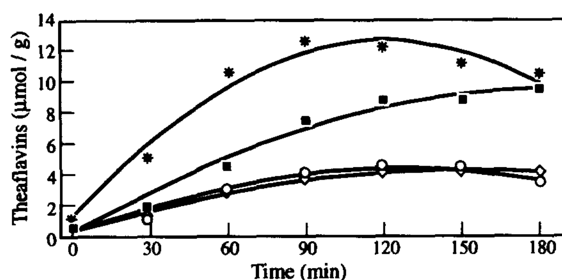


Fig. 2. Changes in clonal black tea sensory evaluation due to varying fermentation time (min.). (a) Clone 6/8; (b) clone S15/10; (✱) taster A; (■) taster B; t = min.

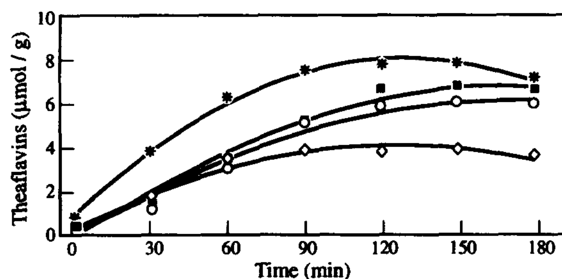
Sensory evaluation was done by two professional tasters and scores were based on briskness, brightness, infusion, thickness, flavour and overall quality on a scale of 0 to 10 for Taster A and 0 to 20 for Taster B.

RESULTS AND DISCUSSION

The theaflavins as measured by the Flavognost method (Hilton, 1973) followed a quadratic pattern. The maximum levels were reached after 139 and 147 min for clones 6/8 and S15/10, respectively (Fig. 1(a)). This pattern is similar to those obtained in previous studies (Cloughley, 1979; Owuor & Reeves, 1986). Indeed, clone S15/10 which is normally considered a slow fermenter had maximum theaflavins formed 8 min later than clone 6/8. Sensory evaluations also changed in a quadratic manner with peaks after 135 min and 145 min for Taster A and 130 and 142 min for Taster B for clones 6/8 and S15/10, respectively (Fig. 2). Thus, even by sensory evaluations, clone S15/10 was a slow fermenter. The different theaflavins formed at different rates and the rates also varied with the clones (Fig. 3). This caused variations in the theaflavin composition (Fig. 4) as fermentation progressed. Thus, the observed variations in theaflavin ratios due to country of origin (McDowell *et al.*, 1991) could also be caused by climatic and genetic variations and/or fermentation duration.



(a)



(b)

Fig. 3. Production of individual theaflavins with fermentation time in (a) clone 6/8 and (b) clone S15/10. (✻) TF; (■) TF-3-G; (◇) TF-3'-G; (○) TF-3-3'-G.

Maximum production points (min)

	6/8	S15/10
TF	121	130
TF-3-G	189	175
TF-3'-G	152	128
TF-3-3'-G	128	172

Earlier, Sanderson *et al.* (1976) had demonstrated that the individual theaflavins have different astringencies. Thus theaflavin-3,3'-digallate is 6·4 times more astringent than theaflavin (TF), while theaflavin-3-gallate and theaflavin-3'-gallate (TFMG) are 2·22 times more astringent than theaflavin. Using the data, Thanaraj and Seshadri (1990) developed an equation to normalise the contribution of the various theaflavins to be equivalent to that of theaflavin-3,3'-digallate (TFDG). In their equation:

$$\text{TFDG equivalent of total TF (\%)} = \text{TF}\,(A/6{\cdot}4 + B/2{\cdot}22 + C)\,/\,100$$

where TF = Total theaflavins content and A, B, C denote the percentage of TF, TFMG and TFDG, respectively. We re-examined this equation and found that it does not correctly calculate the TFDG equivalent of total TF, since TFMG converts to:

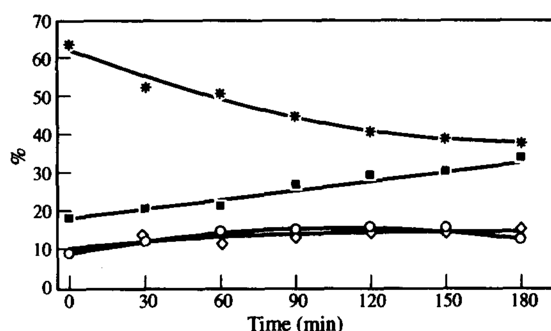
$$\text{TFDG equivalent as TFMG} \times \frac{2{\cdot}22}{6{\cdot}4}$$

not as TFMG/2·22.

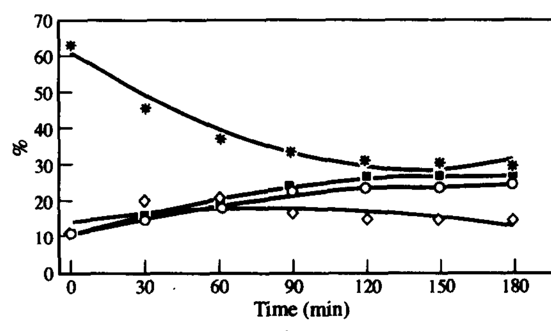
The equation:

$$\text{TFDG equivalent of total TF } (\mu\text{moles/g}) = \text{TFDG} + \text{TF}/6{\cdot}4 + \text{TFMG} \times 2{\cdot}22/6{\cdot}4$$

was found to be more accurate. The TFDG equivalent of total TF variation with fermentation time of the two clones is presented in Fig. 1(b). It is noted that although clone S15/10 had overall lower total (Flavognost) theaflavins compared to clone 6/8 (Fig. 1(a)), the difference in their astringencies measured by the TFDG equivalent was not large. This was mainly due to the



(a)



(b)

Fig. 4. Changes in the proportion of individual theaflavins with fermentation duration. (✻) TF; (■) TF-3-G; (◇) TF-3'-G; (○) TF-3,3'-G.

higher concentration of TFDG in clone S15/10 compared to the proportion in clone 6/8 (see Figs 3 and 4). In terms of quality determination, the TFDG-equivalent data are more relevant than the Flavognost theaflavin data since they take into account the astringency contribution of the individual theaflavins. Although it was observed that the Flavognost theaflavins of the two clones were different, with the difference increasing at long fermentation times such that clone S15/10 had considerably lower theaflavins than clone 6/8, when the TFDG-equivalents of total theaflavins data were used, at short fermentation time, clone 6/8 had only marginally higher amounts of the TFDG-equivalent than clone S15/10. However, after long fermentation durations clone S15/10 had a higher TFDG-equivalent of total theaflavins. Thus after long fermentation duration, clone S15/10 possesses higher astringency than clone 6/8, despite its low total (Flavognost) theaflavins. Using the TFDG-equivalent of total theaflavins, clone S15/10 and 6/8 maximum astringencies were reached after 156 and 139 minutes, respectively. Thus, measured by the TFDG-equivalent of the total theaflavins, clone S15/10 reaches its maximum astringency and hence optimum fermentation time (Cloughley, 1979) later than clone 6/8.

The results have other implications. In the previous experiments where regression had been done between total theaflavins and prices or sensory evaluations, although there were instances where significant relationships were obtained (Hilton & Palmer-Jones, 1975; Cloughley, 1981, 1983; Ellis & Cloughley, 1981; Davies, 1983) and others where the relationships were less significant (Roberts & Fernando, 1981; Owuor *et al.*, 1986), the results presented here suggest a need to look at these relations again with the knowledge that the individual theaflavins have different astringencies, thus imparting different briskness to black tea. Such a study is currently being undertaken.

Similarly, in clonal selection, there are clones which had been neglected as they were classified as of poor quality due to their low total (Flavognost) theaflavins. There is need to re-examine the clones, as their astringency levels could be higher. Again some of the clones rejected as having low TF, could in fact have been selected, if their optimum fermentation time had been established. The clones could be slow fermenting.

ACKNOWLEDGEMENT

This paper is published with the permission of the Director of TRFK. The authors acknowledge useful comments from the senior Scientific Staff of TRFK.

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