Theaflavin Pigment Formation and Polyphenol Oxidase Activity as Criteria of Fermentation in Orthodox and CTC Teas

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Depending upon the condition of traditional method of black tea manufacturing, polyphenol oxidase (PPO) activity pigment profiles together with a possible mechanism that could operate during the polyphasic conditions of tea processing were studied simultaneously in three types of fermented leaves. Theaflavins, the most desirable pigments having a benztropolone moiety, and unstable *o*-quinones, which are generated by PPO, were analyzed by high-performance liquid chromatography. Furthermore, the oxidation rates of two methods of black tea processing, orthodox and curl, tear, crush, were monitored in an oxygraph fitted with a Clark-type electrode, and the role of technology on the quality of the black tea beverage is discussed.

INTRODUCTION

The most widely used form of tea is fermented or black tea and is manufactured from the immature leaf shoots of *Camellia sinensis* (L). Orthodox and curl, tear, crush (CTC) are the two principal categories of black teas, processed through withering–rolling–fermentation and firing stages (Harries and Ellis, 1981; Mahanta, 1988). The conventional orthodox method of tea processing is now being replaced gradually by CTC techniques which give larger numbers of cups per kilogram and better color, strength, and quality compared to orthodox teas, and it has been continuously reviewed by an FAO (1989) committee working on tea. However, in the tea trade the market value depends purely on the quality of the brewed liquor and the texture or appearance of the made tea, where trained tea tasters are indispensable. The phenomenon of creaming is of considerable technological importance in connection with the evaluation of the quality of black tea brew. When a hot tea infusion is cooled, an insoluble precipitate appears, which is known as the cream (Roberts, 1962; Wood and Roberts, 1964; Bhuyan et al., 1991).

Disruption of the cells in the tea leaf shoots during the rolling and cutting operations in the tea manufacturing processes results in the release of oxidoreductase enzymes like polyphenol oxidase (PPO) and peroxidase (PO), enabling them to interact with phenolic compounds of tea leaves to cause reactions that produce the well-known golden-yellow coloration in fermented teas. The exposure of the rolled or cut surfaces to air is known as fermentation in black tea manufacture. The outstanding feature of tea fermentation is the formation of golden-yellow theaflavin, a product of condensation reaction between two molecules of *o*-quinone, one derived from epicatechin (dihydroxy) and the other derived from epigallocatechin (trihydroxy) which are probably generated by PPO (Coggon et al., 1973; Kato et al., 1976; Raymond-Miller et al., 1990). However, the greater part of the *o*-quinones formed during tea fermentation appears to combine with amines, phenols, amino acids, peptides, and proteins to give more intense colored products of diverse structures known as thearubigins (Brown et al., 1969; Dix et al., 1981; Roberts, 1962; Rouet-Mayer et al., 1990; Sekiya et al., 1984). Tea cream contains theaflavins and thearubigins besides caffeine, but there is a lack of detailed information (Cloughley and Ellis, 1980; Seshadri and Dhanaraj, 1988).

Color attribute is no doubt of considerable importance in the tea industry, where measurements of pigments and oxygen consumption were essential in the optimization of processing parameters of manufactured tea (Matheis et al., 1987; Roberts and Smith, 1961). The measurement of the extinction coefficient at 380 nm for theaflavins and at 460 nm for thearubigins is a useful and widely used method, but it does not allow for many products that are produced during tea fermentation and which may interfere with the analysis (Cattell and Nursten, 1977). However, the expediency of improving the commercial processing technique to produce greater cuppage through higher water solubles and better creaming properties is one of the main objectives of tea manufacture. The present black tea manufacturing experiment was designed to study the pigment profile using HPLC and PPO activity to assess the optimum fermentation conditions in various processed leaf samples.

MATERIALS AND METHODS

Tea Manufacturing. Flushing tea shoots from Tocklai vegetative clone (TV1) comprising an epical bud and terminal two or three leaves were harvested from the Experimental Garden and manufactured at the Minisature factory of the Research Station according to the following methods.

(a) *Rotorvane (RV)–CTC Manufacture.* Rotorvane is a preconditioner where the leaf is passed through a cylindrical barrel with internal vanes and a rotating Archimedes screw. Freshly harvested tea shoots of about 75% moisture content (unwithered) were passed through a rotorvane machine. Next, the rotorvaned leaf shoots were fed through the CTC machine, where the tea leaf shoots were macerated between stainless steel rollers with edged surfaces which rotate opposite to each other at a speed ratio of about 1:10.

(b) *Roll–CTC Manufacture.* The leaves were spread over hessian cloths to a thickness of about 5 cm for 18–20 h under normal atmospheric condition to obtain normal withered leaf containing 68–70% moisture. The leaves were then rolled for 30 min in a Little Giant roller, where tea leaf shoots were subjected to a circular motion by a vertical metal cylinder on a large brass table, fitted with series of ribs and buttons, and then passed twice through a CTC machine.

(c) *Orthodox Manufacture.* The tea leaf shoots were withered as in the roll–CTC process described above except that the leaves were spread only to a 2-cm thickness on the hessian cloths so that the moisture content of the tea leaf shoots was reduced to about 60–65%. The withered leaves were rolled in the Little Giant roller for 60 min for orthodox teas.

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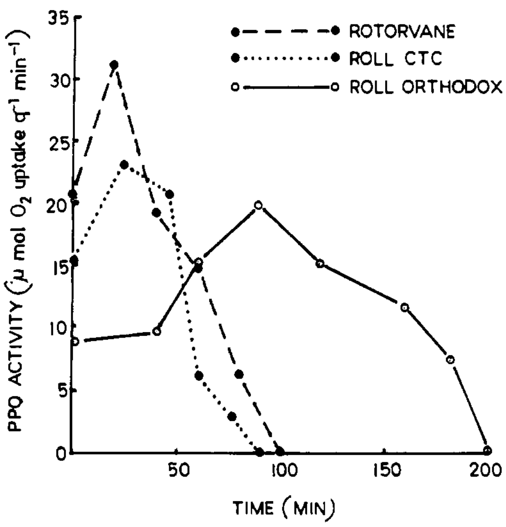

Figure 1. Effect of process variables on oxygen uptake by tea leaf PPO.

The macerated leaves produced under the three different machine treatments were then exposed to air (fermentation), resulting in the formation of bright coppery brown pigments in tea. The fermentations were terminated when maximum color was ascertained (determined visually) by heating the fermented tea leaf material with a hot blast of air at about 90 °C for 20 min.

Measurement of Oxidation Rates. For routine analysis, 3 g of fermenting leaf material was homogenized (Kinematica tissue homogenizer) at different time intervals in a glass vessel, containing 0.05 M phosphate buffer at pH 5.6; 0.3 mL of suspension was added to the cell of an oxygraph (Gilson Model 5/6), fitted with a Clark electrode in which 1.5 mL of air saturated with 0.05 M phosphate buffer (pH 5.6) was present. The oxygen consumption was followed at 30 °C for 3 min. Oxidation rates were expressed as the amount of green leaf (dry weight) which catalyzes the consumption of 1 μmol of O_2/min.

HPLC Analysis of Tea Brew. Three grams of tea leaf was infused in 300 mL of boiling water for 10 min, first extracted with 50 mL (×3) of chloroform to remove caffeine and then further extracted with 25 mL (×3) of ethyl acetate to obtain black tea pigments. The vacuum-dried compounds were dissolved in 20 mL of HPLC solvent B, and 20 μL was injected in a LKB-HPLC single-pump system. Chromatographic conditions were as follows: column, Lichrosorb RP-18 (C_{18}, 4.6 × 150 mm), particle size 5 μm; mobile phase, (A) 10% acetone in 0.5% acetic acid, (B) 60% acetone in 0.5% acetic acid; flow rate, 0.5 mL/min; wavelength monitored, 380 nm; sensitivity, 0.16 aufs. Gradient:

time, min	% A	% B
0	85	15
15	20	80
25	0	100
35	100	0

RESULTS AND DISCUSSION

Optimum Fermentation and Polyphenol Oxidase Activity. The rapid uptake of oxygen in the minced tea leaf shoots appears to be almost due to enzymic oxidation of polyphenols which converted into quinones, with the concomitant reduction of two oxygen atoms to water (Rzepecki and Waite, 1989; Wood and Roberts, 1964). To measure the oxygen uptake after rolling/cutting, a polarographic method was undertaken using a Clark-type electrode. Quick and accurate measurement of oxygen partial pressure was recorded in three types of minced leaves, (a) rotorvane–CTC, (b) roll–CTC, and (c) roll–orthodox, as shown in Figure 1. In the roll–CTC teas the oxygen uptake tends to attain the maximum earlier than in roll–orthodox and later than in rotorvane–CTC. In both types of CTC processes the oxidation reaction was found to progress faster and to be completed earlier than in the orthodox process. In roll–orthodox teas the cell injury will be milder than in CTC, where crush and cut should have more drastic effects on the release of particulate and soluble PPO inherent in the leaf shoots to catalyze primary reaction during fermentation (Kato et al., 1976; Dix et al., 1981).

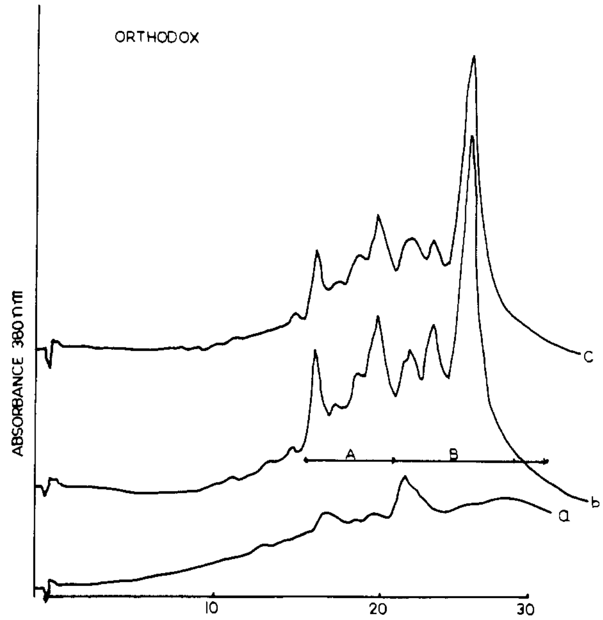

Figure 2. Pigment profiles of ethyl acetate soluble part of conventional orthodox fermentation: (a) just after rolling; (b) optimum fermentation; (c) overfermentation.

The particular time at which maximum oxygen uptake occurs during fermentation was taken as the optimum fermentation of the injured leaf. Subsequently, at this particular point of manufacture the enzymic autoxidation can be controlled by a hot blast of air to stop fermentation. With prolonged fermentation, black tea quality may deteriorate; taste will be "flat", and appearance will be dull (Hazarika et al., 1984; Mahanta and Hazarika, 1985). The shift of optimum fermentation time from unwithered (fresh leaf) to hard withered leaf is well marked. Thus, the mechanical modification of conventional orthodox processing to the CTC method was a breakthrough in the tea industry which reduced fermentation time by half (Figure 1).

Theaflavin and *o*-Quinone Analysis by HPLC. The fascinating problem of structural correlation between the natural polyphenols present in green leaf and the theaflavins, the bentropolone derivatives, established the genuine constituents of black tea, but little is known about the structure of the thearubigins (Brown et al., 1969; Coxon et al., 1970; Takino et al., 1964). In any case, it has been shown that *o*-quinones formed by polyphenol oxidase react more discriminatingly and are incorporated into proteins and phenols during tissue injury (Cilliers and Singleton, 1991; Pierpoint, 1985; Roberts, 1962; Rouet-Mayer et al., 1990). The success of HPLC as an analytical method for determining and comparing pigment profiles in various process teas during fermentation relies on the fact that although all of the individual theaflavins and the polyquinone adducts have similar extinction coefficients at around 380 nm, they could be separated and quantified. Furthermore, theaflavins, being stable compounds, could be compared with authentic spectra from tea brew extract used in the present HPLC analysis (Bailey et al., 1990; Hoeflar and Coggon, 1976; Robertson and Bendall, 1983).

Figures 2–4 show the ethyl acetate soluble pigments

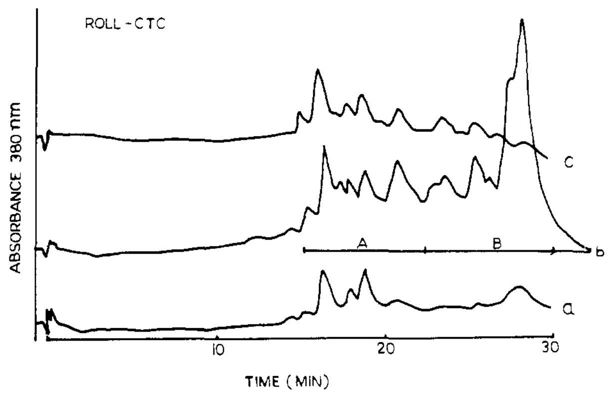

Figure 3. Pigment profiles of ethyl acetate soluble part of roll–CTC fermentation: (a) just after rolling; (b) optimum fermentation; (c) overfermentation.

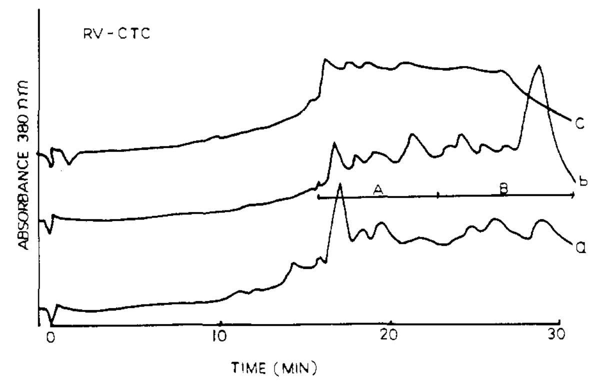

Figure 4. Pigment profiles of ethyl acetate soluble part of rotorvane–CTC: (a) just after rolling; (b) optimum fermentation; (c) overfermentation.

eluted at 380 nm from three methods of tea processing, orthodox (Figure 2), roll–CTC (Figure 3), and rotorvane–CTC (Figure 4), and at three stages of fermentation, just after rolling (a), optimum fermentation (b), and overfermented leaves (c). The peaks have been broadly classified into two groups, group A and group B. The first group of peaks (group A) was found to be stable, while the rest of the peaks disappear, indicating that these compounds undergo further polymerization. The stable group of compounds compares well with authentic theaflavins, and the less stable compounds have been assigned to be *o*-quinones. It is observed that theaflavins and *o*-quinones reach a maximum at optimum fermentation and show a lower value if the processed leaf material is under- or overfermented. The depletion of the pigment profile (especially the group B peaks) in overfermented tea is an indication that the *o*-quinones polymerize or react with one another and other available substrates in the presence of a second enzyme or autoxidize to produce heterogeneous thearubigin pigments. These types of polyquinone adducts have characteristic absorbance and are routinely used to monitor fermentation in tea manufacture (Mahanta, 1988; Rouet-Mayer et al., 1990; Rzepecki and Waite, 1989).

During CTC–rolling, development of most of the group A pigments takes place, while group B forms during the fermentation stage. In the case of conventional orthodox processing most of the pigments are formed during the fermentation stage only. Thus, the conventional method of orthodox manufacture demonstrated that oxidation reactions which may utilize the *o*-quinones during fermentation are rather slow as compared to CTC processing. A remarkable resemblance of oxygen uptake and development of various classes of pigments during fermentation

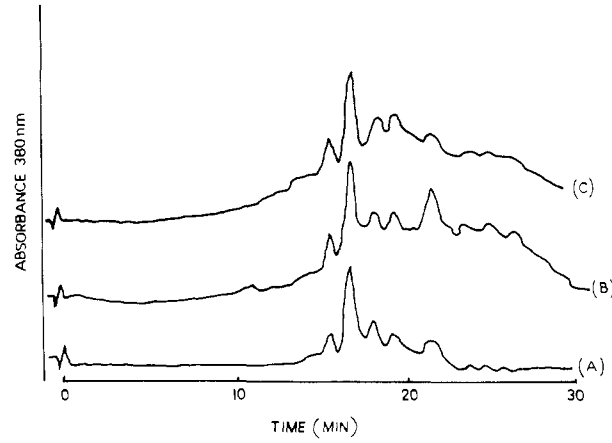

Figure 5. HPLC analysis of theaflavins and related pigments of black tea liquors monitored at 380 nm: (A) rotorvane–CTC; (B) roll–CTC; (C) roll–orthodox.

has been observed. It is presumed that since the group B compounds are reactive electrophilic quinone intermediates and can undergo nucleophilic attack, producing compounds of diverse spectral characteristics and molecular weights, the thearubigins will fail to show distinct peaks in HPLC (Cilliers and Singleton, 1991; Roberts and Smith, 1961). Comparison of fermented leaves of the three manufacturing systems shows that all theaflavin peaks are present in all types of teas but in different proportions. Although the area under the *o*-quinone peak rose and then fell, the secondary products, the so-called thearubigins, might increase concomitantly depending upon the type of machine used during manufacture. However, we observe virtually no degradation of the group A pigments, but group B pigments do undergo significant change throughout the tea manufacturing process (Figures 2–4).

When the desired fermentation level has been reached, the leaves are dried to reduce the moisture to about 3%. The pigment profiles of black tea brew manufactured separately from (A) rotorvane–CTC (B) roll–CTC, and (C) roll–orthodox are shown in Figure 5. During the firing stage, the group B pigments diminish considerably, which further supports the unstable nature of group B compounds. The less stable compounds may undergo transformation at the initial temperature enzymatically and then nonenzymatically to the thearubigin group of compounds (Dix et al., 1981). Thus, the degree of wither and type of maceration will determine the rate of oxidation in the various fermented teas; the influence of the significant difference in pigment composition in the different types of black teas has already been felt (Hazarika et al., 1984; Mahanta and Hazarika, 1985).

From Figure 5 of made teas, it is clear that group A, the theaflavins, and group B compounds are higher in properly withered, roll–CTC leaf than in nonwithered or less withered rotorvane–CTC or in hard withered orthodox teas. The similarities and intensities of the peaks are the first indication of oxidation reaction and mechanical modification that could be instrumental in such variation in the product quality. Therefore, the unwithered black teas, manufactured by rotorvane–CTC processes, shall make the brewed liquor diminished in color, a characteristic of black tea quality. This type of thin liquor/low creaming can be assigned to low amounts of compounds like group A and group B. On the other hand, the withered roll–CTC leaf can produce more pigment than fresh leaf rotorvane–CTC or orthodox black teas.

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