# Changes of Polyphenol Oxidase and Peroxidase Activities and Pigment Composition of Some Manufactured Black Teas (*Camellia sinensis* L.)

Pradip K. Mahanta,* Santanu K. Boruah, Hemanta K. Boruah, and Jatindra N. Kalita

Biochemistry Department, Tocklai Experimental Station, Tea Research Association, Jorhat 785 008, Assam, India

The specific activity of two principal enzymes, viz. polyphenol oxidase (PPO) and peroxidase (PO), responsible for formation of brown compounds known as theaflavins was studied in fermented or black teas. PPO and PO activities were assayed via a spectrophotometric method, while the theaflavin group of pigments was analyzed by high-performance liquid chromatography. Although the highest activities of both PPO and PO were observed on rolling, decrease of the former and increase of the latter are the result of mechanical injury, leading to the oxidative polymerization (enzymatic browning) of fermented black tea. Individual pigments were found to be characteristic in different clonal cultivars as well as in black teas manufactured under different conditions of withering and fermentation. The impact of technology on the development of a golden yellow color or on theaflavins production is discussed.

The bulk increase in Indian tea production from about 500 million kilograms in the 1980s to about 700 million kg during the 1990s has been due to the use of high-yielding clonal cultivars and better pest and nutrient management practices. The quality of black tea, which is of no less importance, is influenced by manufacturing techniques and tea-making potentials of green leaf shoots (Kalita, 1992; Mahanta and Baruah, 1992a; McGrath, 1985; Yang and Thseng, 1991). So far as the trade is concerned, bright liquored CTC teas are favored and sold at a higher price than poor liquored teas. Although tasters form an indispensable part of the marketing of teas as a beverage, the technological properties during processing which involves fermentation are important to enhance the consumer's acceptability (Biswas et al., 1975; Cloughley and Ellis, 1980; Hazarika et al., 1984; Spiro and Price, 1986).

The most important step of black tea manufacturing is withering, during which an artificial atmosphere with controlled humidity is maintained for leaf senescence to take place (Baruah, 1977; Mahanta et al., 1988; Ullah et al., 1986). Considerable capital is invested in building and developing improved versions of withering troughs having controlled air flow and desired hygrometric difference. Furthermore, with the introduction of a curl, tear, crush (CTC) machine after withering, the fermented tea industry has now an unusual combination of batch and continuous types of operations running in sequence (Bhuyan et al., 1991; Mahanta, 1988).

Roberts (1962) suggested that theaflavins and thearubigins are formed by the reactions of flavan-3-ols through *o*-quinones generated by polyphenol oxidase (PPO) and peroxidase (PO). Takino et al. (1964) found that a mixture of epicatechin and epigallocatechin oxidized with PPO or alkaline ferricyanide can produce same pigments isolated from fermented tea. Of course, the four major theaflavins, theaflavin, theaflavin 3-monogallate, theaflavin 3′-monogallate, and theaflavin 3,3′-digallate, constituting about 2% of dry weight, are highly desirable for black tea quality and can be used as a biochemical method before releasing a good clone to the industry (Bailey et al., 1991; Coxon et al., 1970; Mahanta and Baruah, 1992b; Takeo and Osawa, 1976). In spite of the abundance of the thearubigins, which contribute about 10–20% by weight of black tea, little progress has been made toward an understanding of the chemical nature of these dark brown products.

It is also generally accepted that enzymatic browning, which is known as the fermentability of a tea cultiver, differs among clones and can be attributed to variations in phenolic contents and enzyme activities which influence the rate of reaction during mechanical maceration (Cloughley, 1980; Harris and Ellis, 1981; Lee et al., 1990; Singleton and Cilliers, 1991; Bhatia and Ullah, 1965). The purpose of the present black tea manufacturing experiment is to study the impact of technology on the fermentability of various clonal cultivars and the role of enzymes contributing to the brown product formation so that a better black tea brew could be readily available to the consumer.

## MATERIALS AND METHODS

**Miniature Manufacture.** CTC black tea was manufactured from a single harvest of clonal shoots of Assamica variety of tea plant grown at the experimental garden of the station during the plucking season of 1989–1991.

(*a*) *Withering Variations.* About 30 kg of freshly plucked clonal leaves (TV 1, TV 2, and TV 18) were divided into three lots and spread over a withering trough; air was blown at 773 $ft^3$/min for a period of 6, 8, and 10 h, and hygrometric difference was maintained at about 6 °F. In a separate lot, 5 kg of green leaf shoots was placed on a hassian cloth for natural withering for about 24 h. After withering, the leaves from each lot were rolled separately in a Little Giant roller for 30 min and then passed through the CTC machine three times.

(*b*) *Fermentation Variations.* The rolled CTC leaves then were spread on fermenting trays for 1, 1.33, and 1.66 h. Another 15 kg of withered leaves was divided into three lots and passed through the CTC machine after mixing with 0, 2%, and 4% (w/w) lemon peels and then fermented at identical conditions. The fermentation was stopped by heating for 30 min with a blast of hot air at 180–210 °F to obtain black tea with 3% moisture content.

(*c*) *Manganese Nutrition.* Samples were also collected from an experiment on plant nutrition involving Mn and P fertilization in a clone named Cinnamara 33/52. The experiment was carried out with three levels on Mn (0, 10, and 20 kg of Mn/ha as $MnSO_4$) and two levels of P (0 and 80 kg of $P_2O_5$/ha) application as a single dose in soil and was conducted for a complete pruning cycle of 3 years. The plot arrangements were in randomized block design with three replications. The data presented are the average of 3 years of observations. The CTC fermentation time was monitored from the maximum color formation in the miniature factory (Ullah et al., 1979).

0021-8561/93/1441-0272$04.00/0 

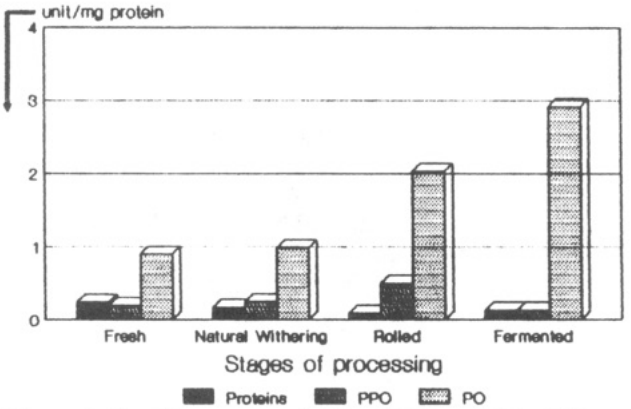


**Figure 1.** Specific enzyme activities of PPO and PO at different stages of manufacture (clone TV 1).

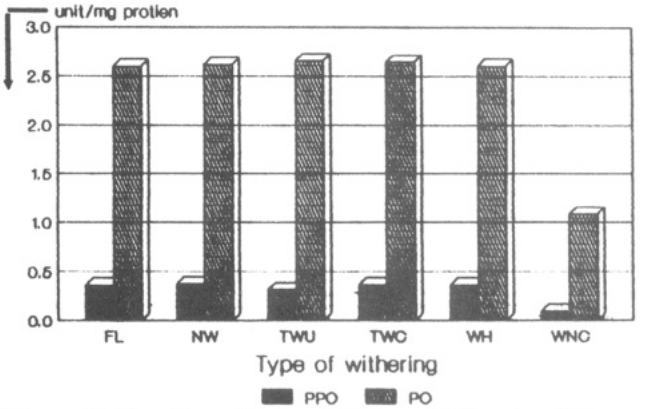


**Figure 2.** Specific activities of PPO and PO under different systems of withering (clone TV 1).

**Enzyme Assay.** The tea leaf shoots (10 g) of Tocklai Vegetative clones (TV 1, 2, 7, 9, 12, 17, 18, and 19) were homogenized in 20 mL of chilled phosphate buffer (pH 5.6) with poly(vinylpyrrolidone) (PVP) in a Kinematica homogenizer for 10 min. The homogenate was then centrifuged at 1200*g* for 15 min. The centrifugate was passed through a small PVP column for complete removal of polyphenols. The eluent was assayed for both PPO and PO (Coggon et al., 1973; Dix et al., 1981). PO was assayed by taking 3 mL of 0.05 M citric acid buffer (pH 5.6), 0.1 mL of *o*-dianisidine (1 μg/mL), 0.2 mL of $H_2O_2$ (1:100 dilution), and 0.2 mL of enzyme extract in a UV spectrophotometer cell at 25 °C in a Backman Model 26 spectrophotometer at 430 nm. The rate of increase in absorbance was recorded every 30 s. PPO assay was also carried out in the spectrophotometer cell, where 2.5 mL of pyrogallol solution [0.05 M pyrogallol freshly prepared in 0.1 M phosphate buffer (pH 5.6)] and 0.5 mL of enzyme preparation were added. Enzyme activity is measured by taking the absorbance at 393 nm at 30-s intervals for 5 min. One unit of enzyme activity is equivalent to the formation of 1 μmol of quinone/min, and the specific enzyme activity is expressed as units per milligram of protein. The amount of protein was estimated by the method suggested by Lowry et al. (1951) using bovine serum albumin (BSA) as standard.

**High-Performance Liquid Chromatography (HPLC) Analysis of Tea Brew.** For routine analysis of tea brew, 2 g of black tea was infused for 10 min in 200 mL of boiling water and filtered. From 100 mL of the infusion, caffeine was separated with chloroform (25 mL × 4 washings), and then theaflavins were extracted with ethyl acetate (25 mL × 3 extractions) and dried under vacuum. Chromatographic conditions for HPLC analysis of tea liquor were as follows: mode, gradient (%B max, 100; %B min, 0; time, 35 min); injection volume, 30 μL; mobile phase, 40% acetone in 0.5% acetic acid for solvent B and 10% acetone in 0.5% acetic acid for solvent A; flow rate, 0.5 mL/min; sensitivity, 0.16 AUF; wavelength, 380 nm; integration, LKB 2220 recording integrator.

## RESULTS AND DISCUSSION

**Polyphenol Oxidase and Peroxidase Activities during Processing.** The oxidation of phenolic compounds by ubiquitous polyphenol oxidase (PPO) and peroxidase (PO) enzymes with or without $H_2O_2$ has been spectrophotometrically estimated while assaying the enzyme activities in tea leaf shoots. Figure 1 shows the specific activity, units per milligram of protein, of PPO and PO during different stages of black tea manufacture, such as fresh, withered, rolled, and fermented leaves. On withering, there is a slight change in enzyme activity, which may be responsible for plant senescence essential in producing changes in organic soluble solids in black tea (Mahanta and Baruah, 1989; Saijo and Takeo, 1974; Leshem et al., 1981; Tremoliers and Bieth, 1984). Furthermore, hydroxylation of monophenols and oxidation of *o*-diphenols to *o*-quinones have been realized in the withering stage of black tea manufacture. After rolling, both PPO and PO activities were found to have increased; PO activity, in particular, continued to rise with the progress of fermentation. This indicates that while the PPO active sites might be blocked, PO does not suffer from such an inactive effect. Dix et al. (1981) emphasized that leaf peroxidase and $H_2O_2$ are produced during the processing, which could act as potent inhibitors for PPO. Thus, PPO activity could be differentiated from PO activity in tea fermentation (Tsushida and Takeo, 1981; Takeo and Kato, 1972; Gregory and Bendall, 1966). This is quite in agreement with the finding that peroxidase affects deterioration, while polyphenol oxidase promotes primary oxidation products of desired character. With prolonged fermentation when the enzymic reaction due to PPO becomes inactivated, substantial PO-induced reaction can still take place in the presence of oxygen. A longer reaction time resulted in an increased polymerization forming protein–phenol complexes through combined enzymic reactions (Piffaut and Metche, 1991; Sekiya et al., 1984; Pierpoint, 1985). Thus, ultrastructural changes undergone during tissue injury can play an indispensable role in transforming ortho diphenolic compounds into quinones, which in turn could favor formation of compounds such as theaflavins and thearubigins of special organoleptic importance (Kato et al., 1987; Spencer et al., 1988; Roberts, 1962).

Improvement in black tea quality lies in the effective design of withering troughs, where commencement of senescence takes place associated with softening of the tissue (Okolie and Ugochukwu, 1988; Ullah et al., 1986). The specific activities of PPO and PO from fresh leaves (FL) and naturally withered (NW), trough withered uncovered (TWU), trough withered covered (TWC), withered under humid atmosphere (WH), and withered under nitrogen and calcium chloride (WNC) leaves are shown in Figure 2. The trough withered leaves show low PPO activity in uncovered samples, where heating (about 35 °C) appears to deactivate the enzyme, while both PPO and PO activities were found to increase in the samples withered/stored under humid/covered conditions. Therefore, it is clear that the process of senescence would take place even under inert nitrogen, humid, or dehumid (presence of $CaCl_2$) atmospheres after the harvest of tea leaves. The stabilities of both PPO and PO have been strongly affected in leaves during storage under nitrogen and calcium chloride (WNC) for survival of fermentation (Tremolieres and Bieth, 1984; Weng et al., 1991). Saijo and Kuwabara (1967) have already shown that teas manufactured in nitrogen atmosphere do not possess

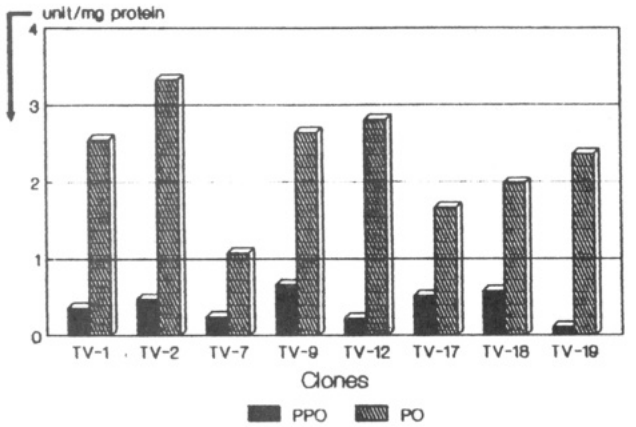


**Figure 3.** Clonal variation of PPO and PO specific activity.

typical black tea aroma, and very poor enzyme activity may be the probable reason.

The PPO and PO activities of some clonal cultivars released to the tea industry of North East India were found to vary greatly among the different cultivars as shown in Figure 3. Clone TV 9 showed the highest PPO activity among the eight different cultivars, followed by TV 18, TV 17, and TV 19, which had the lowest activity that could be related to the degree of browning/fermentation. It is accepted that peroxidases are involved in lignification, hormone metabolism, and response to stress, whereas polyphenol oxidases mediate reduction of molecular oxygen and thus help in growth and development of a plant (Takeo and Baker, 1974; Takeo and Kato, 1972). On the other hand, the plant enzymes, including peroxidase, can oxidize substrates used in the assay of polyphenol oxidase and may be the same proteins responsible for the development of color, odor, and taste of some foods such as cider, tea, and cocoa. However, hydrogen peroxide can be generated endogenously by a number of intracellular systems, possibly evolving sufficient substrate for peroxidase activity (Miller et al., 1990; Sherman et al., 1991). In any case, peroxidase activity is quite high compared to polyphenol oxidase activity in all cases (Figures 1–3), and with the control of peroxidase activity during manufacture one can enhance quality in manufactured tea.

**Pigment Composition of Different Product Categories.** A reversed phase gradient elution HPLC method has been standardized for routine determination of theaflavins and *o*-quinones, where the majority of *o*-quinones may condense to give a thearubigin type of polydispersed group of polymer complexes. The analysis of thearubigins becomes difficult due to limited solubility (Mahanta and Baruah, 1992b). The results of the analyses of theaflavin pigments of Orthodox black tea of Darjeeling and Assam CTC black teas, which control the briskness and brightness of the liquor, are shown in Figure 4. Interestingly, almost equal amounts of theaflavins were found to be present in Orthodox black teas of Darjeeling and Assam CTC teas. However, the larger amount of terpenoids in Darjeeling tea as compared to that in Assam tea has been suggested to be the reason for the greater flavor and aroma in Darjeeling tea (Mahanta et al., 1988; Takeo, 1983).

Figure 5 shows variations in the ethyl acetate soluble HPLC pigment profiles of CTC black teas manufactured under different withering conditions of clone TV 18. Thus, senescence patterns vary under natural and trough withering conditions affecting pigments in black teas. Greater amounts of soluble organic compounds, especially theaflavins and thearubigins, could be obtained only when the

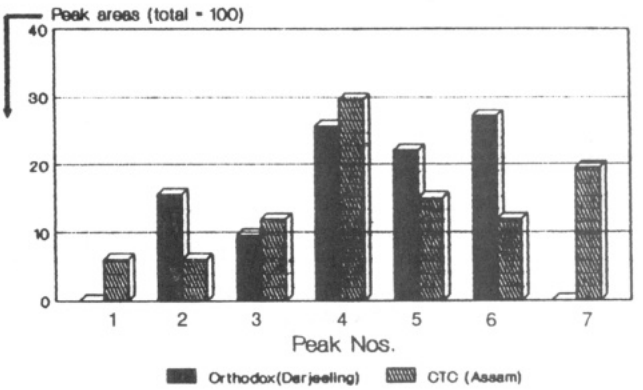


**Figure 4.** Ethyl acetate soluble pigment profile of black tea in HPLC monitored at 380 nm.

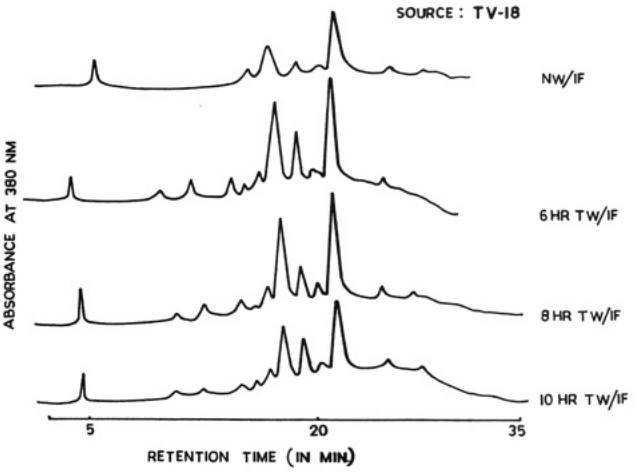


**Figure 5.** Ethyl acetate soluble HPLC pigment profile of black teas manufactured under different withering conditions. NW, natural wither; TW, trough wither with 1 h of fermentation time.

leaves were withered optimally. The aeration appears to help oxidoreductase enzymatic reactions of catechins in transforming ortho diphenolic compounds into theaflavin and thearubigin complexes.

In the search for cultivars with improved black tea manufacturing potentials, enzyme activities and enzymic browning products have been taken as methods for evaluating quality since the inception of tea research (Bhatia and Ullah, 1965; Ullah, 1985; Robertson, 1983). Variations in the content of catechins and oxidation rate during fermentation result in the variation in the pigment pattern in teas manufactured from different clones and hence cause differences in the taste and aroma of the brewed tea. The levels of pigment profiles of black teas manufactured from different clonal tea leaf shoots such as TV 1, TV 2, and TV 18 are illustrated in Figure 6. From the figure it is clear that pigment profile composition varies depending upon cultivar type, viz. TV 2 (*Assamica*), TV 1 (*Assamica-China* hybrid), and TV 18 (*Cambod*) (Bezbaruah and Dutta, 1977; Hazarika et al., 1984; Mahanta and Hazarika, 1985).

Moreover, once the leaf shoots are rolled, they are susceptible to different fermentation conditions, especially fermentation time. The HPLC pigment profiles with respect to 6, 10, and 24 h normally withered leaf shoots (TV 1) when fermented for 1, 1.33, and 1.66 h are shown in Table I. The maximum amount of theaflavins appears to have been produced in teas fermented for 1.33 h. Theaflavin levels in black teas, for instance, fermented for 1 and 1.66 h are lower, indicating that at optimum fermentation maximum development of theaflavins as well as quinones takes place to enhance the quality of tea brew.

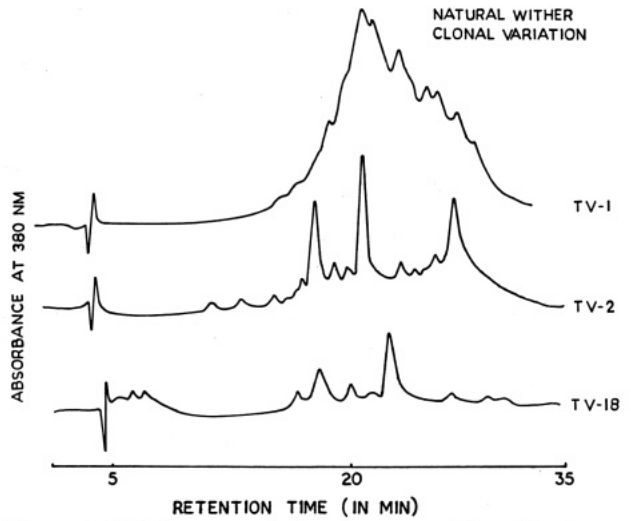


**Figure 6.** Ethyl acetate soluble pigment profiles of black teas manufactured from various clonal cultivars.

**Table I. Pigment Profile Analysis of Different Black Teas Fermented for Various Time Intervals (Clone TV 1)**

| | | normal temperature: 31 °C | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | natural wither, | 6 h TW | | | 10 h TW | | |
| peak | RT | 1.33 h | 1 h | 1.33 h | 1.66 h | 1 h | 1.33 h | 1.66 h |
| 1 | 20 | 0.54 | 1.49 | 0.53 | 0.54 | 3.68 | 6.39 | 0.72 |
| 2 | 21 | 1.10 | 3.23 | 3.27 | 2.50 | 5.91 | 6.03 | 2.26 |
| 3 | 23 | 3.64 | 2.84 | 2.62 | 2.49 | 5.67 | 7.47 | 2.63 |
| 4 | 24 | 5.12 | 4.94 | 5.90 | 4.70 | 6.09 | 22.55 | 3.88 |
| 5 | 26 | 1.31 | 12.18 | 16.67 | 13.06 | 19.87 | 5.26 | 10.50 |
| 6 | 27 | 3.48 | 5.30 | 7.50 | 5.96 | 4.29 | 10.12 | 2.85 |
| 7 | 28 | 7.88 | 6.39 | 8.70 | 5.96 | 5.51 | 15.49 | 3.32 |
| 8 | 29 | 6.34 | 10.81 | 12.50 | 8.09 | 8.33 | 10.24 | 4.76 |
| 9 | 30 | 2.26 | 56.14 | 4.50 | 3.97 | 22.40 | 14.30 | 7.64 |
| 10 | 32 | 10.79 | | 68.90 | 50.48 | 13.04 | 31.78 | 8.51 |
| 11 | 34 | | | | | 22.31 | | 10.01 |
| total peak areas | | 42.46 | 103.32 | 131.09 | 97.75 | 117.10 | 130.13 | 57.08 |

**Table II. HPLC Profiles of Tea Samples Treated with Lemon Peels**

| retention time, min | profile peak areas[a] | | | |
|---|---|---|---|---|
| | control | sample A | sample B | sample C |
| 12 | 233840 | 842510 | 281290 | 168440 |
| 17 | 77947 | 32430 | 127860 | 58690 |
| 22 | 554860 | 461750 | 2474800 | 3564100 |
| 26 | 1054500 | 911410 | 3195800 | 1987200 |
| 31 | 249680 | 2894400 | 1002500 | 4647900 |
| total | 2170827 | 5142500 | 7082250 | 10426330 |

[a] Sample A, 1% lemon peel; sample B, 2% lemon peel; sample C, 4% lemon peel; control, no lemon peel.

Table II shows the differences in pigment contents of lemon peel treated and untreated tea leaf samples. The presence of lemon could enhance PPO/PO activities in the production of desirable theaflavins has been successfully monitored by HPLC pigment profile analysis. Lemon peel, a common tea additive, appears to have changed the equilibrium toward soluble theaflavins as compared to teas of normal manufacture for better quality (Jackson and Lee, 1988; Piffaut and Matche, 1991).

In another manufacturing experiment, the effects of different levels of Mn (0, 10, and 20 kg/ha) and phosphate (0 and 80 kg $P_2O_5$/ha) on fermentability, theaflavin content, and taster's grading were studied. The hypothesis that manganese activates enzymic oxidation has been proved; fermentation of leaves receiving Mn fertilization (10 and 20 kg/ha) was found to be faster than that of the untreated samples. At the same time, improvement of quality of

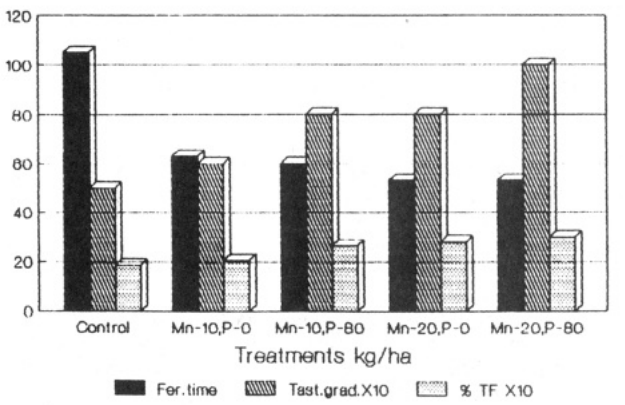


**Figure 7.** Effect of Mn and P on the reduction of fermentation time and quality of CTC black tea.

black tea was also observed as shown in Figure 7. The quality parameters are expressed as taster's grading and theaflavin content, the conventional method of monitoring tea quality in laboratory. Taster's grading is an arbitrary value given by recognized tea tasters on the basis of the organoleptic tastes of tea decoction. The higher the theaflavin content, the better is the quality of the tea. It was found that Mn-treated leaf shoots (at the rate of 10 kg/ha) reduced the normal fermentation time of 105 min to 65 min, which may be of particular interest with respect to Mn fertilizing schedule in tea fields (Kalita, 1992).

ACKNOWLEDGMENT

We are grateful to Dr. B. C. Barbara, Director, Tocklai Experimental Station, Tea Research Association, for his encouragement and permission to publish the results.

LITERATURE CITED

Bailey, R. G.; Nursten, H. E.; McDowell, I. Comparative study of the reversed phase high performance liquid chromatography of black tea liquors with special reference to the thearubigins. *J. Chromatogr.* **1991**, *542*, 115–128.

Baruah, T. C. Common problems with withering trough. *Two Bud* **1977**, *24*, 35–37.

Bezbaruah, H. P.; Dutta, A. C. Tea germplasm collection at Tocklai Experimental Station. *Two Bud* **1977**, *24*, 22–30.

Bhatia, I. S.; Ullah, M. R. Quantitative changes in the pigments during the processing of tea leaf and their relation to liquor characters of made tea. *J. Sci. Food Agric.* **1965**, *16*, 408–416.

Bhuyan, L. P.; Tamuly, P.; Mahanta, P. K. Lipid content and fatty acid composition of tea shoots and manufactured tea. *J. Agric. Food Chem.* **1991**, *39*, 1159–1162.

Biswas, A. K.; Sarkar, A. R.; Biswas, A. K. Biological and chemical factors affecting the valuations of N.E. Indian plain teas. III. Statistical evaluation of the biochemical constituents and their effects on color, brightness and strength of black teas. *Two Bud* **1975**, *22*, 159–172.

Cloughley, J. B. The effect of temperature on enzyme activity during the fermentation phage of black tea manufacture. *J. Sci. Food Agric.* **1980**, *31*, 920–923.

Cloughley, J. B.; Ellis, R. T. The effect of pH modification during fermentation on the quality parameter of central African black teas. *J. Sci. Food Agric.* **1980**, *31*, 924–934.

Coggon, P.; Moss, G. A.; Sanderson, G. W. Tea catechol oxidase: isolation, purification and kinetic characterisation. *Phytochemistry* **1973**, *12*, 1947–1955.

Coxon, D. T.; Holmes, A.; Ollis, W. D.; Vora, V. C. The constitution and configuration of the theaflavin pigments of black tea. *Tetrahedron Lett.* **1970**, *60*, 5237–5240.

Dix, M. A.; Fairley, C. J.; Millin, D. J.; Swaine, D. Fermentation of tea in aqueous suspension. Influence of tea peroxidaes. *J. Sci. Food Agric.* **1981**, *32*, 920–932.

Gregory, R. P. F.; Bendall, D. S. The purification and some properties of the polyphenoloxidase from tea, (*Camellia sinensis*. L.). *Biochem. J.* **1966**, *101*, 569–581.

Harris, N.; Ellis, R. T. Black tea manufacture. Effect on leaf structure of different processing systems. *Ann. Appl. Biol.* **1981**, *99*, 359–366.

Hazarika, M.; Chakravarty, S. K.; Mahanta, P. K. Studies on thearubigin pigments in black tea manufacturing systems. *J. Sci. Food Agric.* **1984**, *35*, 1208–1218.

Jackson, L. S.; Lee, K. Chemical forms of iron, calcium, magnesium and zinc in black, oolong, green and instant black tea. *J. Food Sci.* **1988**, *53*, 181–184.

Kalita, J. N. Some aspects of chemistry in the production of tea in relation to mineral nutrition of Boron and Manganese under field and green house conditions. Ph.D. thesis submitted to Dibrugarh University, 1992.

Kato, M.; Okamoto, J.; Omori, M.; Obata, Y.; Saijo, R.; Takeo, T.; Hasegawa, T.; Suzuki, T. Effect of microbial polyphenol-oxidase on the color of infused black tea. *Nippon Nogeikagaku Kaishi* **1987**, *61*, 599–601.

Lee, C. Y.; Kagan, V.; Jawaorski, A. W.; Brown, S. K. Enzymatic browning in relation to phenolic compounds and polyphenol oxidase activity among various peach cultivars. *J. Agric. Food Chem.* **1990**, *38*, 99–101.

Leshem, Y. Y.; Wurzburger, J.; Grossman, S.; Frimer, A. A. Cytokinin interaction with free radical metabolism and senescence: Effects on endogenous lipoxigenase and purine oxidation. *Physiol. Plant* **1981**, *53*, 9–12.

Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265–275.

Mahanta, P. K. Color and flavor characteristics of made tea. In *Modern Methods of Plant Analysis*; Linskens, H. F., Jackson, J. F., Eds.; Springer Verlag: Berlin, 1988; Chapter 8, 221–295.

Mahanta, P. K.; Baruah, S. Relationship between process of withering and aroma characteristics of black tea. *J. Sci. Food Agric.* **1989**, *46*, 461–468.

Mahanta, P. K.; Baruah, S. Changes in pigments and phenolics and their relationship with black tea quality. *J. Sci. Food Agric.* **1992a**, *59*, 21–26.

Mahanta, P. K.; Baruah, H. K. Theaflavin pigment formation and polyphenol oxidase activity as criteria of fermentation in Orthodox and CTC teas. *J. Agric. Food Chem.* **1992b**, *40*, 860–863.

Mahanta, P. K.; Hazarika, M. Chlorophylls and degradation products in Orthodox and CTC black teas and their influence on shade of color and sensory quality in relation to thearubigins. *J. Sci. Food Agric.* **1985**, *36*, 1133–1139.

Mahanta, P. K.; Baruah, S.; Owuor, P. O.; Murai, T. Flavor volatiles of Assam CTC black teas manufactured from different plucking standards and Orthodox teas manufactured from different altitudes of Darjeeling. *J. Sci. Food Agric.* **1988**, *45*, 317–324.

McGrath, S. P. The effects of increasing yields on the macro and micro element concentrations and offtakes in the grain of winter wheat. *J. Sci. Food Agric.* **1985**, *36*, 1073–1083.

Miller, A. R.; Kelley, T. J.; Mujer, C. V. Anodic peroxidase isoenzymes and polyphenoloxidase activity from cucumber fruit: tissue and substrate specificity. *Phytochemistry* **1990**, *29*, 705–709.

Okolie, P. N.; Ugochukwu, E. N. Changes in activities of cell wall degrading enzymes during fermentation of Cassava (Manihot esculanta crantz) with *Citrobacter freundii*. *J. Sci. Food Agric.* **1988**, *44*, 51–61.

Pierpoint, W. S. Phenolics in food and feed stuffs: The pleasures and perils of vegetarianism. *Annu. Proc. Phytochem. Soc.* **1985**, *25*, 427–451.

Piffaut, B.; Metche, M. Properties of peroxidase and polyphenol-oxidase in natural complexes from walnuts (*Juglens regia*) and in active DL-DOPA copolymers. *J. Sci. Food Agric.* **1991**, *57*, 493–506.

Roberts, E. A. H. Economic importance of flavanoid substances: tea fermentation. In *Chemistry of Flavanoid Compounds*; Geissman, T. A., Ed.; Pergaman Press: Oxford, U.K., 1962; pp 468–512.

Robertson, A. Effects of catechin concentration on the formation of black tea polyphenols during in vitro oxidation. *Phytochemistry* **1983**, *22*, 897–903.

Saijo, R.; Kuwabara, Y. Volatile flavor of black tea. Part I. Formation of volatile components during black tea manufacture. *Agric. Biol. Chem.* **1967**, *31*, 389–396.

Saijo, R.; Takeo, T. Induction of peroxidase activity by ethylene and indole-3-acetic acid in tea shoots. *Agric. Biol. Chem.* **1974**, *38*, 2283–2284.

Sekiya, J.; Kajiwara, T.; Monma, T.; Hatanaka, A. Interaction of tea catechins with proteins: formation of proteins precipitate. *Agric. Biol. Chem.* **1984**, *48*, 1963–1967.

Sherman, T. D.; Vaughn, K. C.; Duke, S. O. A limited survey of the phylogenic distribution of polyphenoloxidase. *Phytochemistry* **1991**, *30*, 2499–2506.

Singleton, V. L.; Cilliers, J. J. L. Characterization of the products on nonenzymic autoxidative phenolic reactions in a caffeic acid model system. *J. Agric. Food Chem.* **1991**, *39*, 1298–1303.

Spencer, C. M.; Martin, Y. R.; Gaffney, S. H.; Goulding, P. N.; Magnolato, D.; Lilley, T. H.; Haslam, E. Polyphenol complexation—some thoughts and observations. *Phytochemistry* **1988**, *27*, 2397–2409.

Spiro, M.; Price, W. E. Determination of theaflavins in tea solution using the flavognost complexation method. *Analyst* **1986**, *111*, 331–333.

Takeo, T. Effect of clonal specificity of monoterpene alcohol composition of tea shoots of black tea aroma profile. *Jpn. Agric. Res. Q.* **1983**, *17*, 120–124.

Takeo, T.; Baker, J. E. Changes in multiple forms of polyphenol oxidase during maturation of tea leaves. *Study Tea* **1974**, *76*, 59–62.

Takeo, T.; Kato, Y. Tea leaf peroxidase. Isolation and partial purification. *Study Tea* **1972**, *44*, 52–59.

Takeo, T.; Oosawa, K. Photometrical analysis and statistical evaluation of black tea infusion. *Bull. Natl. Res. Inst. Tea, Jpn.* **1976**, *12*, 125–181.

Takino, Y.; Imagawa, H.; Harikwa, H.; Tanaka, A. Studies on the mechanism of the oxidation of tea leaf catechins. Part III. *Agric. Biol. Chem.* **1964**, *28*, 64–71.

Tremoliers, M.; Bieth, J. G. Isolation and characterization of the phenoloxidase from senescent leaves of black poplar. *Phytochemistry* **1984**, *23*, 501–505.

Tsushida, T.; Takeo, T. The hydroxilation of p-coumarate to caffeate by tea enzyme. *Study Tea* **1981**, *60*, 29–33.

Ullah, M. R. Chemical criteria for classification and selection of cultivated tea populations of NE India. *Two Bud* **1985**, *32*, 21–24.

Ullah, M. R.; Bajaj, K. L.; Chakraborty, S. "Factory floor fermentation test"; Advisory Leaflet 12; Tocklai Experimental Station, Jorhat, India, May 1979; pp 1–6.

Ullah, M. R.; Bordoloi, A. K.; Gogoi, N. Hastening of leaf senescence in the processing of green tea leaf to black tea. *J. Sci. Food Agric.* **1986**, *37*, 1042–1044.

Weng, Z.; Hendrick, M.; Maesmans, G.; Gebruers, K.; Tobback, P. Thermostability of soluble and immobilized horse radish peroxidase. *J. Food Sci.* **1991**, *56*, 574–578.

Yang, S. S.; Thseng, F. S. Interrelationships among the yield, fermentation ability and agronomic characters in tea plants. *Proc. Int. Symp. Tea Sci. Jpn.* **1991**, 414–418.

Received for review June 5, 1992. Accepted October 29, 1992.