Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 91 (2005) 771–778
Food Chemistry
www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods

A reliable technique to identify superior quality clones from tea germplasm

S. Joseph Lopez *, Jibu Thomas, P.K. Pius, R. Raj Kumar, N. Muraleedharan

UPASI Tea Research Foundation, Tea Research Institute, Nirar Dam B.P.O., Valparai 642 127, Coimbatore District, TN, India

Received 7 February 2004; received in revised form 17 September 2004; accepted 18 October 2004

Abstract

Variations in the substrate level and enzyme activity of prominent south Indian tea germplasm were studied. The content of polyphenols, catechins (substrates) and polyphenol oxidase (PPO) showed variation, which influenced the final black tea quality. The enzyme PPO occurs in tea shoots and catalyzes the reactions between catechins to form theaflavins in the presence of oxygen. The catechins mainly epicatechin (EC), epigallo catechin gallate (EGCG), epicatechin gallate (ECG) and epigallo catechin (EGC) get mixed with PPO during the oxidation process to form quality constituents like theaflavins (TF), thearubigins (TR) and high polymerized substances (HPS). Theaflavins and their fractions such as simple theaflavin, theaflavin monogallate, (TFMG), theaflavin digallate (TFDG) in black tea are the essential quality constituents that are responsible for the liquor characteristics where as TR and high polymerized substances impart colour to the liquor. As oxidation of macerated leaves proceed through different stages of tea manufacture, a decline in PPO activity, polyphenol and catechin contents were observed. Data revealed that the oxidation reaction was faster during the initial stages of oxidation. During the period, oxygen consumption was higher and declined thereafter. Ratio between the enzyme (PPO) and its substrate (catechins) were used to characterize the quality potential of tea clones. An attempt has also been made to categorize prominent tea clones as high, moderate and average quality clones based on their enzyme substrate ratio. Theaflavin content (oxidation product) of different tea clones suggests that the ratio between PPO and catechins forms an important criterion which determines black tea quality. Results obtained were compared with standard clones of known high quality (CR-6017) and moderate quality (SA-6). The study reveals that the enzyme substrate ratio can be used to identify superior quality clones from the existing tea germplasm.

Keywords: Theaflavins; Catechins; Polyphenol oxidase; Oxidation; Enzyme substrate ratio

1. Introduction

Biochemical constituents such as polyphenols, catechins and polyphenol oxidase (PPO) of tea are intrinsically related to black tea quality. In recent years, knowledge on biochemical components and the changes that take place during oxidation (fermentation) has increased noticeably. Biochemical analysis has been generally constrained to the flush since it is the young crop shoots that are plucked and processed to produce black tea with distinctive aroma (Millin & Rustridge, 1967).

Cut, tear and curl (CTC) type of black tea manufacture involves withering, cutting, oxidation and drying. During withering, a decline in the moisture content and a slight augment in the substrate as well as enzyme levels are noticed. The over all quality and flavour of the final black tea depends on moisture content and the changes in the biochemical constituents that occurs

* Corresponding author. Tel.: +91 4253 235301/3; fax: +91 4253 235302.
E-mail addresses: josephlopez@rediffmail.com, physiol@upasitea-research.org (S. Joseph Lopez).

0308-8146/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2004.10.005

during withering of the leaves. After withering, the leaves pass through "rotor vane", where the leaves are cut into pieces. Sliced leaves are then cut four times in ragged stainless steel rollers revolving oppositely. During oxidation major biochemical changes takes place, leading to the formation of quality constituents and the characteristic flavour of black tea. Thus a crucial stage is influenced by factors such as temperature, humidity, oxygen level, time and finally the biochemical constituents of macerated leaf (dhool). After optimal oxidation of leaves, it is subjected to firing at 120 °C for 10–20 min. During this stage, the enzyme activity is blocked and almost completes removal of moisture results.

During oxidation, PPO interacts with phenolic compounds mainly catechins and their fractions such as Epicatechin (EC), Epicatechin gallate (ECG), Epigallo catechin gallate (EGCG) and Epigallo catechin (EGC) in the presence of oxygen which results in the development of golden yellow theaflavins (theaflavin, theaflavin-3 gallate, theaflavin-3′ gallate and theaflavin-3-3′ gallate) a product of condensation reaction between two molecules of *o*-quinones (Owuor & Obanda, 1998; Madanhire, Whittle, & Khumalo, 1996). To comprehend the formation of quality constituents from potential fresh tea leaves, it is necessary that the enzyme completely converts the available substrates into products. PPO is generally found in association with chloroplast (Kato, Uritani, Saijo, & Takeo, 1976) and in the epidermal cells (Oparin & Shubert, 1950) due to its role in the initial oxidation of catechins to *o*-quinones which later, condenses into quality constituents (Wickramasinge, Roberts, & Perera, 1967). Polyphenols and catechins are mainly localized in the vacuoles and palisade cells of leaves (Mahanta, 1988). During withering, moisture content of the leaves is reduced to about 60%. During cutting and oxidation, the intact cells of tea leaves get ruptured and the substrates (polyphenols and catechins) come in contact with the vacuolar enzyme. This results in the formation of quality constituents of black tea (Selvendran & King, 1976). Since biochemical constituents influence the black tea quality, we have attempted to identify superior quality clones using the bioconstituents of tea leaves prior to black tea manufacture. We report that the enzyme (PPO):substrate (catechin) ratio plays an imperative role in determining the quality potential. We have employed this strategy to characterize the tea clones in terms of their quality potential, besides well known standard tea selections like SA-6 (moderate quality) and CR-6017 (good quality) were used for comparison. We believe that the technique developed will shorten tea breeding endeavors for faster and consistent screening of large seedling germplasm for superior quality traits.

2. Materials and methods

2.1. Materials

Two leaves and a bud were used for biochemical estimations and enzyme assay. Samples were collected for the study from the experimental farm of UPASI TRF, situated at 1050 M above MSL which have a diverse genetic background.

2.2. Biochemical estimations

The estimation of total polyphenols and catechins was carried out in green tea leaves. The ethanol extract of tea shoots was used for determination of total polyphenols and catechins. Total polyphenols were estimated by using Folin phenol Ciocalteu reagent in the presence of sodium carbonate. The absorbance of blue colour developed was measured at 700 nm (Dev Choudhury & Goswami, 1983). Total catechin was estimated using acidified vanillin reagent and the absorbance was measured at 500 nm. (+) Catechin was used as standard (Swain & Hillis, 1959). Total polyphenols and catechins were expressed as % on the basis of dry matter. Catechin fractions in green leaves was analysed by reverse phase HPLC system. Column used for analysis was Luna 5 μM Phenyl Hexyl. Authentic and certified flavanols procured from Sigma-Aldrich, USA were used as reference standards.

2.3. Assay of polyphenol oxidase

PPO was assayed by measuring oxygen uptake coupled to the oxidation of pyrocatechol using a Clark type oxygen electrode (Model 290A; ORION Inc., USA) by modified method of Molla (1992). Unless otherwise stated, the electrode chamber contained 4.5 ml of 100 mM sodium phosphate buffer pH 6.8 and 5 ml of 100 mM pyrocatechol and in a final volume of 10 ml. After the system had equilibrated, 500 μl aliquot of enzyme was injected through a small hole in the vessel cap.

2.4. Black tea manufacturing

Harvested tea shoots (two leaves and a bud) were immediately brought to miniature tea factory at UPASI TRF, spread out on wire trays and withered by passing cool air for 16 h to achieve a 25–30% decrease in fresh weight. Withered shoots were passed through a CTC machine four times. The macerated leaves were spread out in trays and placed in a cooling cabinet for a period of optimum oxidation time at 20 °C. The oxidized *dhool* was dried in a mini fluid bed drier at 107 °C for 20 min, packed in polythene bags and stored until further analysis.

2.5. High performance liquid chromatography analysis

For catechin fractions, 2 g of dried green tea leaf was extracted with 10 ml of 70% methanol at 70 °C for 10 min. The extract was centrifuged at 6000 rpm for 10 min and the supernatant was collected and was made up to 10 ml with 70% methanol. The extract was then filtered through a 0.2 μ Millipore filter and was injected into HPLC (HP 1100 series). The chromatographic conditions were as follows: injection volume: 10 μL; column: Phenomenex LUNA 5 μ Phenyl-Hexyl 250 × 4.60 mm; column temperature: 35 °C; Mobile phase – solvent A: acetonitrile/acetic acid/water (18:4:178, v/v); solvent B: acetonitrile/water (160:40, v/v); flow rate: 1 mL min^{-1} (ISO/CD, 14052–2).

For theaflavin fractions, two grams of black tea was extracted with 100 mL of demonized water for 15 min. The extract was centrifuged at 6000 rpm for 10 min. The extract was filtered through 0.2 μ filter membrane and was injected into HPLC (HP 1100 series). The chromatographic conditions were as follows: injection volume: 20 μL; column: HP hypersil ODS 5 μm 2.1 × 200 mm; column temperature: room temperature; mobile phase – solvent A: 100% acetonitrile; solvent B: acetonitrile/acetic acid (196:4); flow rate: 1.2 mL min^{-1} (Bailey, Mc Dowell, & Nursten, 1990).

3. Results and discussion

3.1. Variation in bioconstituents in clones

Green tea leaves contains high levels of polyphenols, mainly catechins. Several earlier studies have shown that catechin levels could be related to black tea quality. However, it was necessary to quantify the individual catechin fraction and relate it to the amount of theaflavin formed. Green leaf plucking standard (2/3 leaves and bud) and plucking interval influenced the biochemical constituents of manufactured tea to an extent. Variations in substrate (polyphenols and catechins) and enzyme (PPO) levels in prominent tea clones are listed in Table 1. The substrate level in most of the clones was almost the same. UPASI-12 had the highest content of polyphenol (30.31%) where as UPASI-20 had the lowest (25.54%). On the other hand, catechin content was the highest in UPASI-10 (21.35%) and least in UPASI-5 (17.31%). Catechins and their fractions take part in the enzymatic oxidation during oxidation in the presence of PPO which leads to the production of black tea quality constituents such as theaflavins. UPASI-17 registered highest amount of EGCG (242.36 ppm) which is the major fraction which contributes to TF. ECG was high in UPASI-14 (24.49 ppm) where as EGC was found to be more in UPASI-7 (Table 2). Certain climatic factors were also found to influence the biosynthesis of catechins in tea shoots. Thanaraj and Seshadri (1990) reported that among the different shoot components, bud exhibited the highest content of catechins and polyphenols and normally leaves contained more substrates than internodes and coarse leaves. The formation of theaflavins occurred only with appropriate combination of catechin fractions mainly from EGC, EC, ECG and EGCG.

Table 1
Substrate and enzyme levels of clones

Clone	Polyphenol (%)	Catechin (%)	PPO activity[a]
UPASI released clones			
UPASI-1	28.97	19.97	14.07
UPASI-2	27.81	18.94	14.37
UPASI-3	30.13	21.02	20.56
UPASI-4	26.65	20.65	17.13
UPASI-5	29.01	17.31	13.01
UPASI-6	28.09	20.29	13.93
UPASI-7	28.53	18.05	15.43
UPASI-8	25.84	18.13	14.22
UPASI-9	30.21	20.40	19.25
UPASI-10	27.23	21.35	18.62
UPASI-11	27.27	20.46	16.05
UPASI-12	30.31	19.20	17.95
UPASI-13	25.72	18.16	13.90
UPASI-14	29.04	19.68	17.92
UPASI-15	29.37	20.35	19.07
UPASI-16	25.83	18.38	14.74
UPASI-17	29.01	19.88	18.97
UPASI-18	26.73	20.73	13.81
UPASI-19	26.96	17.39	13.42
UPASI-20	25.54	19.83	17.12
UPASI-21	26.91	18.97	13.36
UPASI-22	29.59	20.79	20.25
UPASI-24	26.08	21.18	14.91
UPASI-25	26.41	19.77	14.79
UPASI-26	27.15	20.34	15.39
UPASI-27	28.65	19.16	14.82
Estate selections			
CR 6017	28.24	20.48	19.29
SA-6	26.97	19.81	10.13
SE	1.18	0.46	1.36
C.D. at $P = 0.05$:	3.28	1.28	3.78

[a] μmole oxygen/min/g fresh weight.

3.2. Polyphenol oxidase activity of green leaves during oxidation

PPO is the major enzyme involved in tea oxidation, it catalyses the crucial initial reaction during tea oxidation. Orthodiphenols are oxidized to form their corresponding quinones which are then spontaneously transformed to more complex oxidation products. PPO activity of UPASI-3 was found to be more (20.6 μmole oxygen consumed/min fresh weight) and UPASI-5 registered less activity (13.0 μmole oxygen consumed/min/g fresh weight) (Table 1). PPO shows a reverse trend in crop shoots; internodes exhibited more PPO activity when

Table 2
Catechin fractions in different tea cultivars

Clone	S. Cat.[b]	Catechin fractions[a]			
		EGC[b]	EC[b]	EGCG[b]	ECG[b]
UPASI-1	45.78	33.23	135.4	204.15	21.36
UPASI-2	35.49	95.28	99.10	201.69	21.94
UPASI-3	26.04	88.18	71.95	212.99	14.76
UPASI-4	31.25	75.62	59.02	156.37	14.65
UPASI-5	114.35	49.35	49.37	146.82	18.16
UPASI-6	78.03	97.02	80.33	152.01	14.94
UPASI-7	29.4	148.56	100.97	211.36	22.06
UPASI-8	27.32	76.82	74.74	157.12	14.87
UPASI-9	77.7	121.69	32.03	100.17	11.00
UPASI-10	145.29	84.67	40.98	189.33	14.25
UPASI-11	64.37	110.36	38.21	114.39	21.39
UPASI-12	161.27	85.05	60.53	111.06	13.89
UPASI-13	38.9	86.35	56.21	128.73	15.99
UPASI-14	207.72	123.6	73.57	181.58	24.49
UPASI-15	230.59	69.8	65.10	197.46	13.37
UPASI-16	61.35	76.19	72.32	122.8	23.64
UPASI-17	194.18	114.84	70.80	242.36	17.49
UPASI-18	112.05	120.88	42.39	128.31	21.36
UPASI-19	49.58	86.22	66.74	138.58	17.58
UPASI-20	83.26	68.46	73.64	145.69	16.34
UPASI-21	128.49	59.07	58.9	173.67	21.06
UPASI-22	198.26	116.35	86.64	205.64	19.46
UPASI-24	32.99	59.87	73.69	109.57	13.59
UPASI-25	86.24	76.21	49.28	113.67	17.85
UPASI-26	97.83	46.29	47.61	154.39	20.85
UPASI-27	135.08	94.51	56.38	156.27	15.28
CR 6017	215.35	80.68	94.36	225.64	16.14
SA-6	52.89	97.43	77.23	126.35	13.27

EGC, epigallo catechin; EC, epicatechin; EGCG, epigallo catechin gallate.
[a] S. Cat., simple catechin; ECG, epicatechin gallate.
[b] Unit: ppm.

compared to leaves. Changes observed in PPO activity during withering were mainly due to time duration and temperature (Sanderson, 1972).

3.3. Change in the substrate level during tea processing

A gradual decline was observed in the polyphenol and catechin content during oxidation (Fig. 1). During tea oxidation, the amount of oxygen consumed for the development of derived products (theaflavins) also played a crucial role. Theaflavins and their fractions (Table 3) in black tea is the essential quality constituent which are responsible for the liquor characteristics such as taste, strength, briskness, astringency and colour (Wickramasinge et al., 1967). There are also reports that the digallate equivalent of theaflavins (DGETF) derived

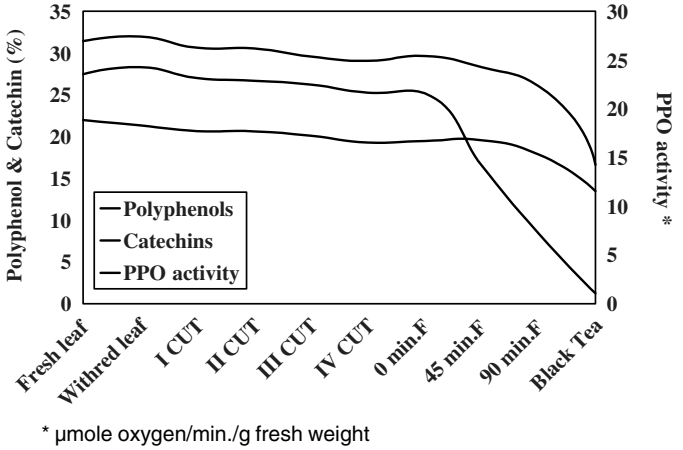

Fig. 1. Enzyme activity and substrate level during tea processing.

Table 3
Relative distribution of theaflavin fractions in different clones

Clone	STF	TF 3 gallate	TF3′ gallate	TF3-3′ gallate	DGETF[a]
UPASI-1	3.26	56.38	2.97	37.39	0.47
UPASI-2	2.61	54.97	3.52	38.90	0.58
UPASI-3	3.01	52.96	2.75	41.28	0.87
UPASI-4	2.86	59.34	3.64	34.16	0.64
UPASI-5	2.68	53.69	3.42	40.21	0.48
UPASI-6	3.05	62.14	3.56	31.25	0.68
UPASI-7	3.12	53.14	2.89	40.85	0.59
UPASI-8	3.28	51.97	3.65	41.10	0.58
UPASI-9	2.68	59.54	2.48	35.30	0.75
UPASI-10	3.56	61.58	3.64	31.22	0.71
UPASI-11	2.58	55.87	2.78	38.77	0.64
UPASI-12	3.64	58.64	3.91	33.81	0.74
UPASI-13	2.08	52.97	2.58	42.37	0.55
UPASI-14	2.15	60.58	2.46	34.81	0.69
UPASI-15	3.64	54.69	2.57	39.10	0.79
UPASI-16	2.05	56.31	3.26	38.38	0.59
UPASI-17	2.64	61.99	3.48	31.89	0.82
UPASI-18	3.47	52.36	2.58	41.59	0.70
UPASI-19	3.64	55.97	3.97	36.42	0.64
UPASI-20	3.29	51.78	2.64	42.29	0.59
UPASI-21	2.56	53.67	3.85	39.92	0.54
UPASI-22	3.24	51.68	2.49	42.59	0.80
UPASI-24	2.69	59.41	2.47	35.43	0.57
UPASI-25	3.57	61.64	3.62	31.17	0.67
UPASI-26	2.75	49.76	3.19	44.3	0.49
UPASI-27	3.19	52.38	3.28	41.15	0.53
CR 6017	2.91	51.62	2.86	42.61	0.76
SA-6	3.52	61.24	2.74	32.5	0.41

[a] DGETF, digallate equivalent of theaflavin.

from TF fractions influences the black tea quality (Sarma, 1999).

3.4. Formation of theaflavins during tea processing

Data revealed that the oxidation reaction was faster during the initial stages of oxidation. A rapid uptake of oxygen in the minced tea leaves appears to be due to the enzymatic oxidation of catechins (Rzepecki & Waite, 1989). The time at which maximum oxygen uptake occurs was taken as the optimum oxidation of the cut dhool. Prolonged oxidation resulted in deterioration of black tea quality and it was observed that TF and *o*-quinones reached a maximum at optimum oxidation time and showed a decline in quality if the processed dhool was under oxidized or over oxidized (Mahanta & Hazarika, 1985). PPO activity was in good agreement with TF formation during oxidation. TF in internodes were found higher than leaves due to the impact of PPO activity (Thanaraj & Seshadri, 1990). To maximize

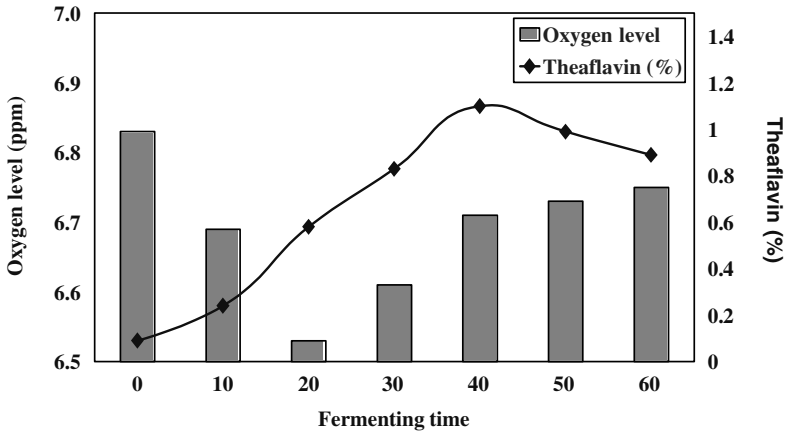

Fig. 2. Oxygen level and TF formation during tea processing.

Table 4
Categorization of clones according to enzyme substrate ratio

High quality	*E*/*S* ratio[a]	Moderate quality	*E*/*S* ratio[a]	Average quality	*E*/*S* ratio[a]
UPASI-3	0.978	UPASI-7	0.855	UPASI-26	0.757
UPASI-22	0.974	UPASI-4	0.830	UPASI-5	0.752
UPASI-17	0.954	UPASI-16	0.802	UPASI-25	0.748
UPASI-9	0.944	UPASI-8	0.784	UPASI-1	0.705
UPASI-15	0.937	UPASI-11	0.784	UPASI-21	0.704
UPASI-12	0.935	UPASI-27	0.773	UPASI-24	0.704
UPASI-14	0.911	UPASI-19	0.772	UPASI-6	0.687
UPASI-10	0.872	UPASI-13	0.765	UPASI-18	0.666
UPASI-20	0.863	UPASI-2	0.759		

[a] Enzyme/substrate ratio (PPO/catechins).

the TF content in the manufactured tea, it was essential to manufacture black tea on a pilot scale to optimize oxidation experiments in order to find out the maximum TF formation period (Cloughley, 1980). The amount and rate of TF fractions and ratios varied according to the oxidation time. Each TF fraction reached its maximum at different oxidation time. Prolonged oxidation enhanced the formation of theaflavin monogallate (TFMG) and theaflavin digallate (TFDG) while short time oxidation increased the formation of TF and TFDG. PPO activity in the oxidizing dhool was affected by factors like temperature, pH and amount of oxygen and availability of substrates (Roberts, 1962). PPO activity assayed at different stages of tea processing indicated a gradual decline in the enzyme activity (Fig. 1). Theaflavin formation coincided with maximum oxygen consumption during tea processing (Fig. 2).

3.5. Selection of superior quality clones using enzyme substrate ratio

Screening of superior quality clones from large tea germplasm is a time consuming and tedious job. Manufacturing of black tea from individual bush as each bush yields only 100–200 g of fresh leaves per plucking round makes it difficult to manufacture black tea. In order to overcome this constrain, use of biochemical constituents in green leaves as reliable parameters to asses the quality potential of bushes are developed. Data revealed that biochemical constituents of green leaves influenced the quality of black tea. Ratio between PPO and catechin which is the major contributing factor for the formation of quality constituents was found to be higher in UPASI-3, UPASI-9, UPASI-12, UPASI-15 and UPASI-22 when compared to other clones (Table 4). Magoma, Wachira, Obanda, Imbuga, and Agong (2000) reported that catechin content in tea plants can be used as a reliable parameter to identify quality clones. Quality of black tea is correlated to total polyphenol content, catechins, and enzyme activity (PPO) in tea shoots (Roberts & Wood, 1950; Mayer & Harel, 1991; Biswas, Biswas, & Sarkar, 1971).

Table 5
Analysed and predicted content of theaflavins in clones

Clones	TF% analysed	TF% predicted
UPASI-1	1.22	1.25
UPASI-2	1.36	1.36
UPASI-3	1.87	1.79
UPASI-4	1.43	1.50
UPASI-5	1.21	1.34
UPASI-6	1.25	1.22
UPASI-7	1.51	1.55
UPASI-8	1.44	1.41
UPASI-9	1.69	1.72
UPASI-10	1.55	1.58
UPASI-11	1.47	1.41
UPASI-12	1.65	1.70
UPASI-13	1.43	1.37
UPASI-14	1.59	1.65
UPASI-15	1.73	1.71
UPASI-16	1.49	1.44
UPASI-17	1.71	1.74
UPASI-18	1.26	1.18
UPASI-19	1.47	1.38
UPASI-20	1.52	1.56
UPASI-21	1.27	1.25
UPASI-22	1.67	1.78
UPASI-24	1.31	1.25
UPASI-25	1.29	1.34
UPASI-26	1.37	1.35
UPASI-27	1.44	1.39
CR 6017	1.69	1.72
SA 6	0.75	0.87

Results obtained in the present study with regard to high and poor quality clones, substantiated the earlier findings of Thanaraj and Seshadri (1990). Theaflavin content (derived from catechins and determine liquor characteristics) of different tea clones suggests that the ratio between PPO and catechin is an important criteria (Table 5), which determine the tea quality than the ratio between polyphenols and catechins as reported earlier by Ramakrishnan, Raj Kumar, Marimuthu, and Joseph Lopez (2002).

Quality analysis of black teas revealed that the clones which, recorded higher values of enzyme substrate ratio showed significantly higher levels of theaflavins. Results

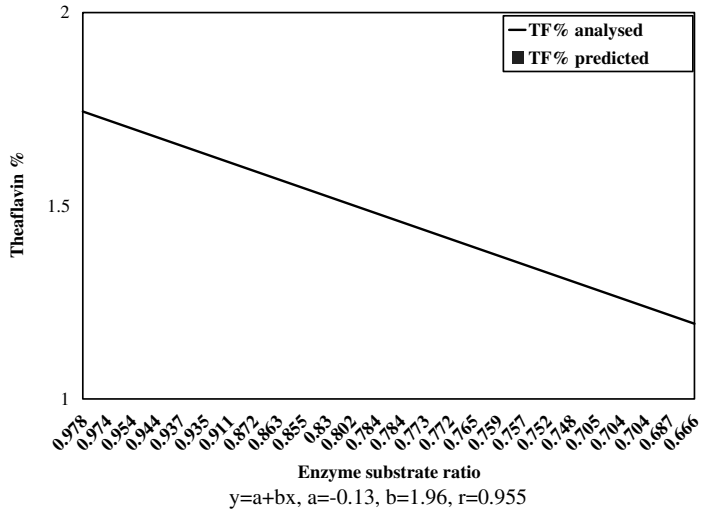

Fig. 3. Relation of analysed and predicted content of TF with enzyme substrate ratio.

were compared with standard high quality (CR-6017) and poor quality (SA-6) clone, which indicated that the enzyme substrate ratio had a direct correlation with black tea quality. A strong correlation was observed between an increase in enzyme substrate ratio and an increase in theaflavin content of all clones fitting the regression equation $y = a + bx$, where y = theaflavin content, x = enzyme substrate ratio, $a(-0.13)$ and $b(1.96)$ are regression constants. Coefficient value ($r = 0.955$) was highly significant at 1% probability (Fig. 3). UPASI tea clones have been categorised into high, moderate and average quality on the basis of enzyme substrate ratio (Table 4) and the same procedure can be used as a reliable parameter to screen large progenies of unknown tea germplasm of unknown prominence for high quality.

Acknowledgements

The first author thank the Director, UPASI Tea Research Foundation for his constant encouragement in this study. The first author thank Tea Board, Government of India, Kolkata for financial assistance under the IX Five Year Plan Project.

References

Bailey, R. G., Mc Dowell, I., & Nursten, H. E. (1990). Use of HPLC photodiode array detection in a study of the nature of black tea liquor. *Journal of the Science of Food and Agriculture, 52*, 509–525.

Biswas, A. K., Biswas, N., & Sarkar, A. R. (1971). Biological and chemical factors affecting the valuations of North East Indian plain teas. II. Statistical evaluation of the biological constituents and their effects on briskness, quality and cash evaluation of black tea. *Journal of the Science of Food and Agriculture, 22*, 191–195.

Cloughley, J. B. (1980). The effect of fermentation temperature on the quality parameters and price evaluation of Central African black teas. *Journal of the Science of Food and Agriculture, 31*, 911–919.

Dev Choudhury, M. N., & Goswami, M. R. (1983). A rapid method for determination of total polyphenolic matter in tea (*Camellia sinensis* L. (O.) Kuntze). *Two and a Bud, 30*, 59–61.

ISO/CD 14502–2 (Committee Draft Number). Determination of substances characteristic of green and black tea. Part 2: determination of catechins in green tea-method using high performance liquid chromatography.

Kato, C., Uritani, I., Saijo, R., & Takeo, T. (1976). Cellular localization of particulate-bound polyphenol oxidase in tea leaves. *Plant Cell Physiology, 17*, 1042–1052.

Madanhire, J., Whittle, A. M., & Khumalo, F. R. B. (1996). Catechin variation in tea clones and the effect on liquor composition and quality. In *Proceedings of the first regional tea research seminar, Blantyre, Malawi* (pp. 99–110)..

Mahanta, P. K. (1988). Biochemical basis of colour and flavour of black tea. In: *Proceedings of the 32nd Tocklai conference* (pp. 124–134). Tocklai Experimental Station, Jorhat, Assam: Tea Research Association.

Mahanta, P. K., & Hazarika, M. (1985). Chlorophyll degradation products in orthodox and CTC black teas and their influence on shade of colour and sensory quality in relation to thearubigins. *Journal of the Science of Food and Agriculture, 36*, 1133–1139.

Magoma, G. N., Wachira, F. N., Obanda, M., Imbuga, M., & Agong, S. G. (2000). The use of catechins as biochemical markers in diversity studies of tea (*Camellia sinensis*). *Genetic Resource and Crop Evolution, 47*, 107–114.

Mayer, A. M., & Harel, E. (1991). Polyphenol oxidases in plants. *Phytochemistry, 34*, 193–215.

Millin, D. J., & Rustridge, D. W. (1967). Tea manufacture process. *Biochemistry, 2*, 9–13.

Molla, M. M. (1992). Effect on polyphenol oxidase activity in extracts of tea leaf prepared by maceration at different temperature. *Sri Lankan Journal of Tea Science, 61*, 15–19.

Owuor, P. O., & Obanda, M. (1998). The use of chemical parameters as criteria for selecting for quality in clonal black tea in Kenya. *Tea, 19*(1), 49–58.

Oparin, A. I., Shubert, T. A. (1950). Oxidative respiratory systems in the tea leaf. *Biokhim. Chicago Proizvodstva Akad. Nauk S.S.S.R. Inst. Biokhim. im. A.N.Bakha Sbornik* 6, 82.Chem. Abstr. 49, 7064.

Ramakrishnan, M., Raj Kumar, R., Marimuthu, S., & Joseph Lopez, S. (2002). Prediction of black tea quality by using fresh leaf constituents as indicators. *Proceedings of Placrosym XIV*, 400–404.

Roberts, E. A. H. (1962). Economic importance of food substances tea fermentation. In T. A. Geissmann (Ed.), *The chemistry of flavanoid substances* (pp. 468–512).

Roberts, E. A. H., & Wood, D. J. (1950). Fermentation process in tea manufacture XI Oxidation of substrates by tea oxidase. *Biochemistry Journal, 47*, 175, Abstr. 45, 783.

Rzepecki, L. M., & Waite, J. H. A. (1989). Chromatographic assay for catechol oxidases based on the addition of L-proline to quinine. *Analytical Biochemistry, 179*, 375–381.

Sanderson, G. W. (1972). The chemistry of tea and tea manufacturing. In V. C. Runeckles (Ed.), *Structural and functional aspects of phytochemistry* (pp. 247–316). New York: Academic Press.

Sarma, S. (1999). Biosynthesis of precursors for liquor and flavour characteristics in black tea. *Global Advances in Tea*, 723–732.

Selvendran, R. R., & King, N. R. (1976). Ultra structural changes in tea leaves during "orthodox" black tea manufacture. *Annals of Applied Biology, 83*, 463–473.

Swain, T., & Hillis, W. E. (1959). The phenolic constituents of *Prunus domestica*. I. The quantitative analysis of phenolic constituents. *Journal of the Science of Food and Agriculture, 10*, 63–68.

Thanaraj, S. N. S., & Seshadri, R. (1990). Influence of polyphenol oxidase activity and polyphenol content of tea shoot on quality of black tea. *Journal of the Science of Food and Agriculture, 35*, 57–69.

Wickramasinge, R. L., Roberts, G. R., & Perera, B. P. M. (1967). The localization of the polyphenol oxidase of tea leaf. *Tea Quarterly, 38*, 309–310.