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Analysis of Selected Biochemical Constituents in Black Tea (*Camellia sinensis*) for Predicting the Quality of Tea Germplasm in Sri Lanka

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ABSTRACT. *Twenty two accessions from tea (Camellia sinensis) germplasm representing high, moderate and low quality of made tea were used for black tea production in wet and dry seasons. Total polyphenol content, total free amino acid content, total liquor colour and brightness were analysed by spectrophotometric methods and correlated with organoleptic parameters viz infused leaf colour, colour of liquor, strength and quality. Strong positive correlations were identified between infused leaf colour and total liquor colour with total quality score. It was also found that the polyphenol content had a positive correlation with brightness which was responsible for liquor quality. The resultant dendrogram of cluster analysis enabled separation of these accessions into high, moderate and low quality categories. Principal Component analysis (PCA) revealed that, of the 16 parameters studied, infused leaf colour has the highest contribution towards clustering of accessions. Group III represented 5 well established high quality accessions (DT1, DUN7, NAY3, TRI 777 and PK2) and group I represented moderate quality accessions including two accessions (N2 and TRI 4079) which were previously categorized in the high quality group. Therefore, polyphenol content, total liquor colour, brightness and infused leaf colour could be used as reliable quality parameters, whereas total amino acid and crude fibre contents cannot be considered as useful quality parameters. Furthermore, fermentation rate was determined for the 22 accessions and four groups were identified based on the fermentation rate. Results revealed that the fermentation rate could be used as an early selection criterion for determination of fermentation properties of tea accessions at progeny level.*

Keywords: *Brightness, polyphenols, tasters' scores, tea quality, total colour*

INTRODUCTION

The tea (*Camellia sinensis*) produced in Sri Lanka is popular as "Ceylon tea" and has a higher demand as 'best quality tea' in the international trade. Sri Lankan tea industry continues to maintain its creditability in terms of quality and cleanliness of the product. The quality of black tea refers to all the characters such as colour, brightness, appearance, liquoring properties, strength and aroma by which it may be judged for its market value. In general, quality of black tea is a summation of all its desirable attributes. Tea tasters while assessing the made tea using their palate usually measure the essential characteristics such as brightness, strength, flavour, colour of the brew and appearance of black tea as well as infused leaf (Keegel, 1959; Sanderson, 1972; Hazarika *et al.,* 2002).

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The tea manufacturing process is a biochemical one which is controlled to a certain extent by mechanical manipulation of the harvested shoots (tea flush). The traditional process of manufacture of black tea consisted of several stages viz. (a) harvesting, (b) withering, (c) leaf maceration or rolling, (d) fermentation, (e) drying and (f) sorting and packaging (Roberts, 2008; Sanderson, 1972). Most important chemical changes which result in development of desirable liquor characteristics such as liquor quality, strength and colour occur during fermentation. Polyphenols, the main group of chemical substances present in the tea flush, undergo oxidative changes during this stage.

In some countries biochemical constituents in tea have been used as discriminative markers to evaluate diversity and genetic potential warehoused in the germplasm. A review by Owuor and Obanda (1998) has suggested the possibility of using morphological features and various chemical constituents in selecting quality accessions. Therefore, it is timely to characterize and evaluate the available germplasm collection in Sri Lanka using biochemical methods to further support germplasm characterization to identify desired parents and elite germplasm for the tea breeding programme. Nonetheless, a pre-mediated selection among the germplasm of high quality has been lacking. Selection of accessions that can make good quality tea needs extensive evaluation of biochemical constituents which contribute towards the liquor characteristics. The most important chemical compounds that are directly responsible for tea quality are flavanols and their oxidation products (Saravanan *et al.,* 2005). However, many chemical constituents which are responsible for the quality have not been studied in detail using local germplasm accessions to identify desirable genotypes for breeding purposes or even for commercial production of tea in Sri Lanka.

Most of the previous studies carried out at the Tea Research Institute of Sri Lanka were focused only on individual biochemical properties in fresh leaf as well as in processed tea, such as total polyphenol content and total amino acids (Wickremasinghe *et al.,* 1966), composition of major fatty acids (Liyanage *et al.,* 1988), volatile compounds (Herath *et al.,* 1991), individual catechins content (Herath *et al.,* 1993), TF (theaflavins) and TR (thearubigins) composition and chlorophyll content (Wickremasinghe *et al.,* 1966; De Silva & Sivapalan, 1982). If a precise correlation can be established between chemical parameters in the fresh leaf and the final quality of the product, this could enhance the process of identifying quality tea accessions at an early stage in the tea breeding program. This would, in turn, facilitate germplasm characterization using biochemical markers to group the accessions into unique clusters.

In the present study, fermentation rate and crude fibre content of 22 tea accessions grown in high elevation (1400 m above) in Sri Lanka were investigated. Total polyphenol and amino acid content in the black tea manufactured from the 22 tea accessions were also determined. In addition, correlation between spectrophotometric quality parameters, such as total colour and brightness of liquor, and the tea taster scores were also determined.

MATERIALS AND METHODS

Twenty two germplasm accessions including TRI series, estate selections and introductions from other countries were selected based on their previously collected information as indicated in Table 1. Plants of those accessions which were conserved in the *ex situ* field gene bank at the Tea Research Institute of Sri Lanka, Talawakelle (latitude 6° 54'N, longitude 80° 42'E) were used to obtain the material. Average annual rainfall of the area is about 2500 mm with an annual average minimum, maximum, temperatures are 14.2 ºC and 22.8 ºC, respectively. Average elevation is 1394 m above sea level.

Table 1. Origin and descriptive characters of selected tea accessions

Accession	Origin*	Yield*	Quality†,*	Blister blight*	Shot hole borer*
CY9	Estate selection	Moderate	Low	Moderate resistant	Moderate resistant
DN	Estate selection	Moderate	Low	Susceptible	Resistant
DT1	Estate selection	High	High	Resistant	Susceptible
DT95	Estate selection	Moderate	Low	Susceptible	Moderate resistant
DUN7	Estate selection	Moderate	High	Susceptible	Moderate resistant
KEN16/3	Estate selection	High	Low	Resistant	Susceptible
N2	Estate selection	Moderate	High	Resistant	Resistant
NAY3	Estate selection	Moderate	Moderate	Resistant	Resistant
PK2	Estate selection	High	High	Resistant	Moderate resistant
TRI 2016	St. Coombs	Moderate	Low	Susceptible	Moderate resistant
TRI 2025	ASM 4/10 Seed	High	Moderate	Moderate resistant	Susceptible
TRI 2043	Shan Bansang	High	Low	Resistant	Moderate resistant
TRI 3013	TRI, Talawakelle	High	Low	Susceptible	Susceptible
TRI 3019	TRI, Talawakelle	High	Moderate	Moderate resistant	Susceptible
TRI 3072	TRI, Talawakelle	Moderate	Low	Resistant	Moderate resistant
TRI 4052	TRI, Talawakelle	High	Low	Resistant	Susceptible
TRI 4067	TRI, Talawakelle	Moderate	High	Resistant	Moderate resistant
TRI 4071	TRI, Talawakelle	High	Low	Moderate resistant	Susceptible
TRI 4078	TRI, Talawakelle	High	Moderate	Moderate resistant	Moderate resistant
TRI 4079	TRI, Talawakelle	High	High	Moderate resistant	Susceptible
TRI 62/5	ASM 4/10 Seed	Moderate	Moderate	Susceptible	Susceptible
TRI 777	Shan Cho Lang	Moderate	High	Susceptible	Resistant

Reference: † Kirthisinghe *et al.,* 1968; *Anon, 2002

Fermentation rate

The fermentation rate of 22 tea accessions was studied by conducting the chloroform test described initially by Sanderson (1963) and modified by Samaraweera and Ranaweera (1988). The tests were carried out in a glass tank fitted with a lid and chloroform-soaked cotton wool kept at the bottom. Second leaf of the tea shoot was hung on a wire and placed horizontally, so that the leaves were nearly equidistant from the soaked wool. The fermentation was considered to be complete when the leaf turned to a brown colour after a period of exposure to the chloroform vapour. The experiment was repeated six times.

Determination of crude fibre content

Crude fibre content of tea flush was determined and expressed as a percentage by mass of the sample on a dry basis according to the ISO standard (ISO - 15598).

Black tea manufacturing

Tea shoots consisting of two leaves and an active bud (500 g) were harvested from 20 bushes of each accession and black tea was produced in an environmental-controlled manufacturing system designed by Teacraft®. Harvested shoots were withered in a withering cabinet.

Withered leaves, after reaching the optimum withering stage (14 to 18 hours), were rolled for 15 minutes in a miniature roller and fed into a miniature spiral rotorvane to achieve a fine dhool. The macerated dhool was sieved through a 2.5 mm mesh and placed in a cabinet for a period of optimum fermentation time (2 ½ to 3 hours) at 20 º C. The inlet temperature of the miniature fluid bed dryer was set at 125 ºC and fermented dhool was fed to it. The drying step was terminated when the outlet temperature reached 98 ºC. The black tea production was repeated twice in wet (September to December, 2009) and dry seasons (January to April, 2010).

Sensory evaluation

Made tea samples (20 g from each accession) were sent for tasters' evaluation. Sensory evaluation made by two professional tea tasters with scores based on infused leaf colour, colour of liquor, strength and liquor quality was used to determine the parameters related to quality of made tea.

Determination of total colour and brightness of tea liquor

Nine grams of made tea samples were used for hot water tea extract and total colour and brightness of tea liquor was determined by measuring the absorbance at 380 nm and 460 nm using UV-VIS spectrophotometer (Roberts & Smith, 1963).

Preparation of tea stock extraction for biochemical analysis

Made tea samples were used for extracting tea infusion and 0.20 g of made tea was extracted in 70 % methanol and used as a stock extraction for all analysis as indicated below.

Determination of total polyphenols in black tea

The total polyphenol content in the tea extract was determined by measuring the colour development with Folin-Ciocalteu phenol reagent in alkaline medium (ISO 14502-1), at absorbance of 765 nm using UV-VIS spectrophotometer (CARY 510 Bio).

Determination of amino acids in black tea

Total free amino acid content in the tea extract was determined by measuring the absorbance at 570 nm using UV-VIS spectrophotometer (CARY 510 Bio) by the reaction with ninhydrin. The results were expressed as a percentage by mass of the black tea sample on a dry basis (Yemm & Cocking, 1955).

All the above experiments were repeated twice using 22 black tea samples processed in both wet and dry seasons.

Statistical analysis

The differences between mean values of each parameter were analyzed using Duncan's Multiple Range Test and cluster diagram was constructed using average linkage method. A principal component analysis was performed to determine the most contributory factors associated with grouping of accessions in the dendrogram. The Statistical analysis system (SAS, 1999) was used in the analysis.

RESULTS AND DISCUSSION

Rate of fermentation

According to the results obtained, (Table 2) the selected 22 accessions could be categorized into four groups based on the fermentation rate. TRI 777 was the most rapidly fermenting accession and TRI 2016 and TRI 3019 were the slowest-fermenting accessions. Samaraweera and Ranaweera (1988) used 8 accessions for estimating fermenting period and observed that the fermentation rate of TRI 777 and DT 1 was significantly higher than TRI 2025. A similar pattern was observed in the present study too, which grouped DT 1, NAY 3, TRI 2043 and CY 9 in the fast fermenting category. In contrast, high yielding accessions such as TRI 2025, TRI 3013, TRI 4052, TRI 4071 and TRI 4078 were found to be moderate fermenting accessions.

Table 2. Fermenting period of accessions from the chloroform test

Fermentation time (min)	Accession/s	Group
0-30	TRI 777	Rapid fermenting
30-50	NAY3, TRI 2043, DT 1, CY 9	Fast fermenting
50-70	KEN 16/3, TRI 4079, DUN 7, TRI 4052, DN, TRI 62/5, N2, TRI 4067, DT 95, TRI 3013, TRI 2025, TRI 3072, TRI 4078, PK 2, TRI 4071	Moderate fermenting
70-90	TRI 3019, TRI 2016	Slow fermenting

The period of fermentation widely practiced at present varies from 1 to 3 hours. During the fermentation period, polyphenols in tea oxidize into TF and TR. In general, fermentation is normally done for a shorter period to maximize the amount of TF. Prolonged oxidation results in deterioration of black tea quality. It was observed that TF reached a maximum at optimum oxidation time and showed a decline in quality if the processed dhool was over-oxidized during the longer period of fermentation (Cloughley, 1980; Lopez *et al.,* 2005). Therefore, optimum time of fermentation is a critical determinant of quality of black tea.

The results of the present study revealed that the rate of fermentation depends on the accession and facilitates to predict the optimum time for fermentation during black tea processing. These findings may be taken into consideration in planning, harvesting and processing of tea on a commercial scale to achieve best results. In general, 4-5 high yielding TRI accessions are frequently used for replanting or infilling purpose without considering the biochemical properties of the accessions. Therefore, attention should be paid to plant those accessions in separate blocks to avoid mixing of accessions between categories identified in this study.

Furthermore, fermentation rate could be used as a rapid means of evaluating the fermenting properties during the seedling tea selection programme and progeny trials at a very early stage of the breeding programme. This method was also used in early selection of potential quality accessions in tea breeding programs in Kenya (Seurei *et al.,* 1998) and Pakistan (Waheed *et al.,* 2001). On the other hand, green tea is a non-fermented form of tea and basically different from black tea. The present study indicated that those accessions with slow fermentation rate may be suitable for green tea production and hence, the suitability of accessions for green tea could be assessed in the selection programme of seedlings to generate potential tea accessions.

Polyphenol content of black tea

The present study revealed that the total polyphenol content largely varied and was in the range of 14.42 to 22.20 % in the wet season and 14.24 to 24.79 % in the dry season (Table 3).

Table 3. Mean ± SD values of the biochemical parameters in different tea accessions

High quality †,*

	Accession	PP_W (%)	PP_D (%)	TC_W	TC_D	BT_W (%)	BT_D (%)	Tqs_W	Tqs_D
High quality †,*	DT1	17.97± 0.16	18.51± 0.65	5.68± 0.26	5.31± 1.08	22.48± 0.22	27.32± 2.70	22± 1.41	24 ± 2.83
	DUN7	20.77± 0.53	24.79± 1.06	5.09± 0.06	3.73± 0.07	22.65± 0.70	30.06± 0.26	24± 1.41	23.5 ± 3.54
	N2	15.89± 0.37	20.54± 0.60	5.04± 0.14	4.46± 0.17	27.66± 2.18	31.32± 2.37	17.5± 2.12	17.5 ± 2.12
	PK2	17.83± 0.20	24.67± 0.18	5.44± 0.01	2.98± 0.03	28.33± 1.01	48.02± 3.69	20± 2.83	20.5 ± 2.12
	TRI 777	17.29± 1.47	20.32± 0.43	5.63± 0.19	5.18± 0.06	19.78± 0.51	30.74± 0.23	20± 1.41	21 ± 1.41
	TRI 4067	15.47± 0.14	16.52± 0.15	4.40± 0.25	4.43± 0.35	24.13± 1.28	23.00± 2.26	14± 0.00	17.5 ± 2.12
	TRI 4079	15.14± 0.25	20.67± 0.00	4.96± 0.18	4.74± 0.03	30.63± 2.89	27.22± 1.03	17.5± 0.71	19 ± 1.41
Moderate quality †,*	TRI 2025	19.68± 0.71	22.17± 0.06	4.42± 0.27	4.38± 0.75	26.20± 1.02	19.25± 4.01	16± 0.00	18.5 ± 0.71
	TRI 4078	17.54± 0.55	19.74± 0.04	4.84± 0.12	5.03± 0.17	21.57± 0.35	33.95± 0.27	18.5± 2.12	18 ± 1.41
	TRI 3019	18.71± 0.97	19.52± 1.57	4.28± 0.00	3.58± 0.06	15.78± 2.46	20.92± 0.84	13± 2.83	17.5 ± 0.71
	TRI 62/5	16.57± 0.56	19.67± 0.35	3.78± 0.19	3.13± 0.08	17.19± 1.26	23.47± 1.30	14± 2.83	14 ± 1.41
	NAY3	22.20± 1.52	19.45± 0.71	5.33± 0.50	5.63± 0.24	23.45± 1.37	28.27± 1.47	18.5± 0.71	22 ± 1.41
Low quality †,*	CY9	16.14± 0.04	15.35± 0.27	4.75± 0.04	4.54± 0.08	19.21± 0.42	23.63± 1.28	19± 1.41	17.5 ± 0.71
	DN	17.39± 0.13	18.02± 0.37	4.02± 0.00	5.23± 0.02	20.22± 0.00	27.67± 1.66	17.5± 0.71	16.5 ± 0.71
	DT95	14.45± 0.31	18.10± 0.26	4.70± 0.19	4.68± 0.31	14.89± 0.23	27.73± 0.78	18.5± 0.71	20.5 ± 2.12
	KEN16/3	16.83± 0.14	15.54± 0.18	4.69± 0.21	4.49± 0.15	17.96± 1.83	18.71± 0.63	17.5± 0.71	16.5 ± 0.71
	TRI 2016	16.98± 0.48	18.08± 0.15	4.06± 0.11	5.08± 0.08	13.40± 0.81	18.79± 1.43	15± 2.83	14.5 ± 2.12
	TRI 2043	16.29± 0.00	14.24± 0.13	4.35± 0.06	4.37± 0.00	15.50± 1.42	13.30± 1.63	18± 2.83	17 ± 1.41
	TRI 3013	15.09± 0.01	19.76± 0.90	4.79± 0.11	4.12± 0.06	21.32± 0.71	24.04± 1.25	20.5± 2.12	20 ± 0.00
	TRI 3072	16.22± 0.45	17.35± 0.17	3.73± 0.15	4.23± 0.01	17.68± 0.14	17.06± 2.44	14± 0.00	19.5 ± 2.12
	TRI 4052	14.42± 0.00	20.25± 1.73	4.04± 0.18	5.31± 0.07	28.12± 0.74	30.98± 1.83	14.5± 2.12	17 ± 1.41
	TRI 4071	14.70± 0.34	18.75± 2.13	3.82± 0.25	4.33± 0.13	27.51± 0.27	30.91± 2.17	14± 2.83	17.5 ± 2.12

Reference: Quality categorization of previous studies († Kirthisinghe *et al.,*1968; * Anon, 2002)
PP= polyphenol content in tea liquour, TC= total liquor colour, BT =brightness of liquor, Tqs = total quality score
W= wet season, D= dry season

Among the 22 accessions, the total polyphenol content of 7 well-known good quality accessions had increased during the dry season. The other 4 moderate quality accessions showed a similar pattern except NAY 3. It showed a slight decline of the total polyphenol content during the dry season, although other biochemical parameters such as brightness and

Low quality †,*

tasters' scores had increased (Table 3). In contrast, the total polyphenol content of low-quality accessions, except TRI 4052, showed a very little increment during the dry period. During the processing of black tea, about 90–95 % of the polyphenols undergo enzymatic oxidation and the majority of the polyphenols would convert into theaflavins and thearubigins, which contribute to the quality of black tea brew (Roberts and Smith, 1963 & Sanderson, 1972). The level of polyphenols is therefore highly attributable to the final quality of black teas.

Free amino acids and crude fiber content

Total free amino acid content of black tea varied from 0.72 to 2.67 % in the wet season and 0.86 – 2.00 % in the dry season. Range of the crude fiber content of the accessions was from 8.94 to 11.55 %. According to the results obtained in this study, a clear trend could not be discerned among the accessions in relation to quality and the crude fiber content (data not presented). As such, the total amino acid and crude fiber contents are not valid parameters for predicting the made-tea quality. Similar observations were noted by Sanderson and Kanapathipillai (1964), who stated that the total amino acid and crude fiber contents cannot be considered as useful quality parameters. Although those two parameters cannot be used to determine quality characteristics of an accession, the same could be used as biochemical descriptors in characterization of germplasm as there is a significant variation present among the accessions studied in relation to both parameters (crude fiber and amino acid content). Amino acids play an important role as precursors of some volatile flavor compounds in development of tea aroma (Sanderson, 1972). Therefore, further investigation on individual amino acid content and flavor profile of accessions would be more meaningful than total amino acid content. In addition, coarse plucking (mature leaf harvest) will have a tendency to produce off-grade teas with high values of crude fiber content.

Total color and brightness of tea liquor

Total liquor color and brightness of known quality accessions such as DT 1, N2, TRI 777 and TRI 4079 was higher than those of moderate quality accessions such as TRI 62/5, TRI 3019 and TRI 2025 in dry season. Present results also revealed that the accessions having higher values for brightness were considered as good quality accessions by tasters. Furthermore, TF contribute to brightness and long fermentation times produced more color in black tea at the expense of brightness (Owuor *et al.,* 2008). It indicates that the good quality teas should have high values of brightness. All high quality accessions except TRI 4067 followed the above pattern. On the other hand, brightness and overall tasters' score of NAY 3 showed higher values indicating that this accession may possibly be considered as a high quality accession. Therefore, it is necessary to study this in-depth for a proper re-evaluation of quality ranking of TRI 4067 and NAY3.

In addition to the biochemical parameters discussed above, liquor color and brightness were also considered as quality parameters by early scientists (Keegel, 1959; Kirthisinghe *et al.,* 1968) for evaluating made tea quality. Therefore, liquor color and brightness can also be considered as good quality parameters for determining the final quality of black teas.

Principal component analyses (PCA) on the biochemical and tasters' evaluation parameters are presented in Table 4. It shows that the first five principal components (PCs) account for 82 % of total variability. According to the PCs loadings for characters, infused leaf color and total quality scores in dry season (0.35) show the highest value indicating that they are the main contributing characters to the clustering order in the dendrogram. This suggests that the infused leaf color is highly correlated with the quality parameter of made tea. On the other hand, the second PC was correlated with brightness of liquor in wet and dry seasons (0.37). Parameters with high loadings for the third PC were total polyphenols in dry season (0.39) and tasters values for color of liquor in wet (0.39) and dry (0.37) seasons. In addition, total liquor colour in dry season (0.71) shows the highest loading value in the fifth PC.

According to the results of the average linkage cluster analysis, accessions studied were clustered into three main groups (Fig. 1). The group III comprised of DT 1, DUN 7, NAY 3, TRI 777 and PK 2. All those accessions were considered as high quality accessions and ranked similarly by earlier attempts (Kirthisinghe *et al.,* 1968; Anon, 2002). Most of the low quality accessions such as TRI 2016, TRI 2043, TRI 4052 and TRI 4071 were included in group II. Two moderate quality accessions (TRI 3019 and TRI 62/5) (Anon, 2002) also clustered in group II. Interestingly, the accession TRI 4067 has been clustered with the low quality group in the present study. In the current list of recommended tea accessions (Anon, 2002), TRI 4067 was rated as a high quality accession. Besides, most of moderate quality accessions such as DN, KEN 16/3, DT 95, and TRI 4078 (Kirthisinghe *et al.,* 1968; Anon, 2002) were clustered in group I with two high quality accessions such as N2 and TRI 4079. Quality of tea accessions were frequently assessed by professional tea taster's by sensory evaluation and present results revealed that it is worthwhile to incorporate biochemical parameters to derive more meaningful conclusions about black tea quality.

Table 4. Eigen values and individual and cumulative percentage of variation explained by the first five principal components loading of the sixteen quantitative characteristics.

Character	Pcs				
	PC1	**PC2**	**PC3**	**PC4**	**PC5**
Total polyphenols (W)	0.194	-0.243	-0.222	0.271	-0.178
Total polyphenols (D)	0.209	0.256	-0.386	-0.035	-0.208
Total colour (W)	0.341	0.032	0.079	0.081	0.146
Total colour (D)	0.034	-0.214	0.278	-0.235	0.712
Brightness of liquor (W)	0.123	0.366	-0.322	0.021	0.487
Brightness of liquor (D)	0.210	0.377	-0.251	-0.152	0.099
Infused leaf colour (W)	0.339	0.012	0.127	0.155	-0.039
Infused leaf colour (D)	0.351	-0.119	-0.021	-0.020	-0.033
Colour of liqour (W)	0.053	0.377	0.395	-0.317	-0.259
Colour of liqour (D)	-0.083	0.297	0.373	0.492	0.158
Strength of liqour (W)	0.196	-0.291	-0.028	0.504	0.062
Strength of liqour (D)	0.152	-0.444	-0.072	-0.433	-0.032
Liquor quality (W)	0.280	0.045	0.361	-0.130	-0.148
Liquor quality (D)	0.326	0.107	-0.101	-0.055	0.094
Total quality score (W)	0.342	0.028	0.302	0.068	-0.125
Total quality score (D)	0.357	-0.076	-0.001	-0.069	0.060
Eigen value	6.59	2.36	1.76	1.24	1.1
Difference	4.23	0.59	0.51	0.14	
Variance explained (%)	41.2	14.7	11	7.8	6.9
Variance cumulative (%)	41.2	55.9	66.9	74.7	81.6

W= wet season, D= dry season

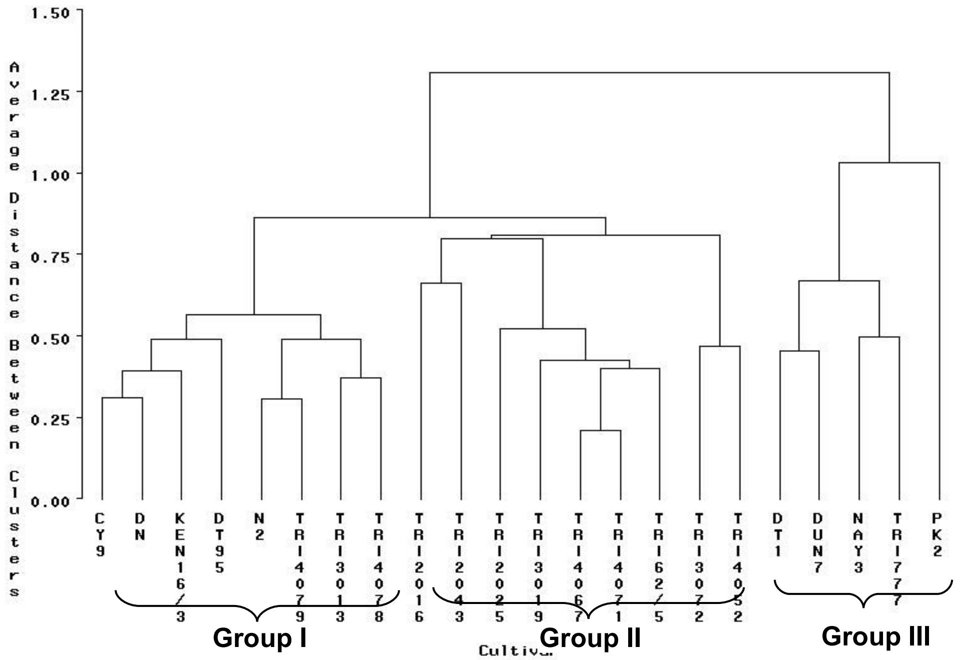

Fig. 1. Dendrogram of average linkage cluster analysis based on biochemical parameters

Table 5. Rank correlation coefficients of different biochemical parameters and tasters' scores

	PP_W	PP_D	TC_W	TC_D	BT_W	BT_D	Inf_W	Inf_D	Col_W	Col_D	Stn_W	Stn_D	Lq_W	Lq_D	Tqs_W	Tqs_D
PP_W	1.00															
PP_D	0.39	1.00														
TC_W	0.38	0.36	1.00													
TC_D	0.02	-0.38	0.18	1.00												
BT_W	-0.03	0.54*	0.28	0.03	1.00											
BT_D	0.06	0.66**	0.49*	-0.10	0.59*	1.00										
Inf_W	0.36	0.25	0.83**	0.01	0.17	0.42*	1.00									
Inf_D	0.39	0.39	0.69**	0.05	0.18	0.36	0.83**	1.00								
Col_W	-0.25	0.16	0.08	-0.05	0.04	0.22	0.10	0.01	1.00							
Col_D	-0.25	-0.12	-0.13	0.02	0.08	-0.14	-0.12	-0.28	0.29	1.00						
Stn_W	0.48*	0.14	0.43*	0.07	0.03	-0.01	0.43*	0.51*	-0.35	-0.01	1.00					
Stn_D	0.29	0.12	0.19	0.32	-0.19	-0.09	0.13	0.49*	-0.16	-0.61*	0.34	1.00				
Lq_W	0.22	0.32	0.64*	0.19	0.03	0.30	0.62*	0.55*	0.41	0.01	0.24	0.29	1.00			
Lq_D	0.39	0.45*	0.76**	0.01	0.42*	0.51*	0.70**	0.76**	0.16	-0.18	0.21	0.19	0.43*	1.00		
Tqs_W	0.34	0.33	0.79**	0.10	0.10	0.37	0.86**	0.75**	0.35	0.01	0.49*	0.25	0.89**	0.59*	1.00	
Tqs_D	0.42	0.42	0.74**	0.14	0.25	0.36	0.75**	0.94**	0.09	-0.19	0.45*	0.51*	0.57*	0.88**	0.73**	1.00

* significant at $p<0.05$, ** significant at $p<0.001$
Biochemical parameters (PP= polyphenols content in tea liqour, TC= total colour, BT =brightness of liquor)
Tasters' evaluation (Inf = Infused leaf colour, Col= colour of liqour, Stn= liquor strength, Lq= liquor quality, Tqs = total quality score), W= wet season, D= dry season

The correlation coefficient of different biochemical parameters and tasters' scores are given in Table 5. There was a strong positive correlation (0.94) observed between infused leaf colour and total quality score in dry season. Therefore, infused leaf colour could be considered as a good made tea quality parameter. In addition, a positive correlation (0.79) could be observed between total liquor colour and total quality score in wet season. There was a significant negative correlation (-0.61) between tasters' values for colour of liquor and liquor strength in dry season. On the other hand, liquor quality was positively correlated with total quality scores (0.90, 0.88) in wet and dry seasons respectively.

Overall quality of black tea should be evaluated by professional tea tasters and the prices will depend on the tasters' score. High market value is guaranteed with the opinion of tea tasters, whereas low tasters' scores indicate the poor quality of made tea. According to the tasters' quality score, DT 1, DUN 7, NAY 3, PK 2, and TRI 777 can be categorized as good quality accessions whereas CY 9, KEN 16/3, TRI 2016, TRI 2043, TRI 3019 and TRI 62/5 as low quality accessions. Those categorizations are in agreement with the results of previous studies to a greater extent (Kirthisinghe *et al.,* 1968; Anon, 2002), although certain accessions were not qualified to be in the same quality category.

In addition, several attempts have been made by various researchers to correlate biochemical compounds such as total polyphenols, amino acids etc., in assessing the quality of made tea, although the correlation between those compounds and the quality was found to be inconsistent in many cases (Wickremasighe *et al.,* 1966; Roberts & Fernando, 1981; Owuor and Obanda, 1998) to be able to use them as chemical markers in screening for made tea quality. In contrast, theaflavins and thearubigins contents, total colour and brightness (Owuor *et al.,* 2008), theaflavins and their fractions (Wright *et al.,* 2002), catechin fractions and ratio between polyphenol oxidase and catechins (Lopez *et al.,* 2005; Saravanan *et al.,* 2005) have been considered as reliable black tea quality parameters.

The results obtained in the present investigation on total polyphenol content, liquor colour and brightness can be considered as good quality parameters for determining the final quality of black tea. Further investigations of other biochemical parameters are vital to establish reliable parameters to screen large number of progenies and germplasm accessions.

CONCLUSIONS

The present study reveals that the fermentation rate could be used as a selection criterion to determine fermentation properties and identify potential quality accessions during the early stages of the tea breeding program. Polyphenol content in black tea, liquor color and brightness could be considered as reliable quality parameters whereas total amino acid and crude fiber content cannot be considered as useful criteria to determine quality of made tea. Furthermore, it is suggested to extend the study including other tea growing areas in different seasons with more accessions.

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REFERENCES

Anon, (2002). The suitability of tea clones for different regions. Tea Research Institute Advisory Circular No. PN 01- Serial No. 6/02. Tea Research Institute of Sri Lanka, Talawakelle.

Choughley, J.B. (1980). The effect of fermentation temperatures on the quality parameters and price evaluation of Central Africa black teas. J. of the Sci. of Food Agric. *31*, 911-919.

De Silva, M.J. and Sivapalan, K. (1982). The chlorophyll content of tea clones in relation to their yield classification and geographical location. Tea Quarterly *51*(2), 54-57.

Hazarika, M., Goswami, M.R., Tamuly, P., Sabhapondit, S., Baruah, S. and Gogoi, M.N. (2002). Quality management in tea – Biochemist's view. Two and a bud *49*, 3-8.

Herath, N.L., Amarakoon, A.M.T. and De Silva M.J. (1991). Chemical constituents in different parts of fresh tea flush and their contribution to overall quality in made tea. Sri Lanka Journal of Tea Science *60*(1), 36-42.

Herath, N.L., Punyasiri, P.A.N. and De Silva, M.J. (1993). Correlation of major flavanols of some tea clones with quality of tea. Sri Lanka Journal of Tea Science *62*(1)*,* 4-10.

ISO 14502-1:2005. Determination of substances characteristic of green and black tea. Part 1: content of total polyphenols in tea – Colorimetric method using Folin-Ciocalteu regent.

ISO 15598:1998. Determination of crude fibre content.

Keegel E.L. (1959). Tea made from clones. Tea Quarterly *30,* 134-141

Kirthisinghe, D., de Silva, W.A.C. and Samarasingham, S. (1968). Manufacturing properties of Ceylon tea clones. Tea Quarterly *39,* 29-36.

Liyanage, A.C., De Silva, M.J. and Ekanayake, A. (1988). Analysis of major fatty acids in tea. Sri Lanka Journal of Tea Science *57*(2), 46-49.

Lopez, S.J., Thomas J., Pius P.K., Kumar R.R. and Muraleedharan, N. (2005). A reliable technique to identify superior quality clones from tea germplasm. Food Chemistry *91*, 771-778.

Owuor, P.O. and Obanda, M. (1998). The use of chemical parameter as criteria for selecting for quality in clonal black tea in Kenya: Achievements, problems and prospects: Review. Tea, *19*(1) 49-58.

Owuor, P.O., Obanda, M., Nyirenda, H.E. and Mandala, W.L. (2008). Influence of region of production on clonal black tea chemical characteristics. Food Chemistry *108,* 263-271.

Roberts, G.R. (2008). Principles of tea manufacture. Hand Book on Tea. (Ed.) A. K. N. Zoysa. Tea Research Institute of Sri Lanaka, Talawakelle, Sri Lanka. 261-264.

Roberts, G.R. and Fernando, R.S.S. (1981). Some observation on the correlation of polyphenol content to the quality of tea clones. Tea Quarterly *50*(1), 30-34.
Roberts, E.H. and Smith, R.F. (1963). The phenolic substances of manufactured tea. Journal of the Science of Food and Agriculture. *14,* 689-700.

Samaraweera, D.S.A. and Ranaweera, A.S. (1988). Study of fermenting rates of clones using chloroform test. Sri Lanka Journal of Tea Science *57*(1), 24-29.

Sanderson, G.W. (1963) The Chloroform test – A study of its suitability as a means of rapidly evaluating fermentation properties of clones. Tea Quarterly *34*, 193-196.

Sanderson, G.W. (1972) The chemistry of tea and tea manufacturing. Structural and functional aspects of phytochemistry. Academic Press, London pp.247-316.

Sanderson, G.W. and Kanapathipillai, P. (1964) Further studies on the effect of climate on the chemical composition of fresh tea flush. Tea Quarterly *35*, 222-228.

Saravanan, M., John, K.M.M., Kumar, R.R., Pius, P.K. and Sasikumar, R. (2005). Genetic diversity of UPASI tea clones (*Camellia sinensis* (L.) O. Kuntze) on the basis of total catechins and their fractions. Phytochemistry, *66,* 561-565.

SAS Institute Inc. (1999). SAS/STAT user's guide. Version 8. SAS Institute Inc. Cary, NC, USA.

Seurei, P., Wachira, F.N., Obanda, M. and Owuor, P.O. (1998). Breeding and clonal selection; Preliminary results from a recent trial. Tea *19*(2), 71-76.

Waheed, A., Hamid, F.S. and Ahamad, N. (2001). Criteria used in selection of locally best tea bushes. Online Journal of Biological Sciences. *1*(1), 21-23.

Wickremasinghe, R.L., Perera, K.P.W.C., Perera, V.H. and Kanapathipillai, P. (1966). Analysis of polyphenols, amino acids and chlorophyll levels in tea flush at different seasons. Tea Quarterly *37*, 232-235.

Wright, L.P., Mphangwe, N.I.K., Nyirenda, H.E. and Apostolides, Z. (2002) Analysis of the theaflavin composition in black tea (*Camellia sinensis*) for predicting the quality of tea produce in Central and Southern Africa. Journal of the Science of Food and Agriculture *82*, 517–525.

Yemm, E.W. and Cocking, E.C., (1955). The determination of amino-acids with ninhydrin. The Analyst *80,* 209-213.